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REVIEW

Emerging roles of non-coding RNAs in colorectal cancer oxaliplatin resistance and liquid biopsy potential

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Abstract

Colorectal cancer (CRC) is one of the most common malignancies of the digestive tract, with the annual incidence and mortality increasing consistently. Oxaliplatin-based chemotherapy is a preferred therapeutic regimen for patients with advanced CRC. However, most patients will inevitably develop resistance to oxaliplatin. Many studies have reported that non-coding RNAs (ncRNAs), such as microRNAs, long non-coding RNAs, and circular RNAs, are extensively involved in cancer progression. Moreover, emerging evidence has revealed that ncRNAs mediate chemoresistance to oxaliplatin by transcriptional and post-transcriptional regulation, and by epigenetic modification. In this review, we summarize the mechanisms by which ncRNAs regulate the initiation and development of CRC chemoresistance to oxaliplatin. Furthermore, we investigate the clinical application of ncRNAs as promising biomarkers for liquid CRC biopsy. This review provides new insights into overcoming oxaliplatin resistance in CRC by targeting ncRNAs.

Key Words: Colorectal cancer; Non-coding RNAs; Oxaliplatin; Resistance; Liquid biopsy biomarkers

Core Tip: Oxaliplatin has served as a first-line chemotherapy option for colorectal cancer (CRC). However, owing to congenital or acquired resistance, treatment failure is common in some patients with CRC. Abundant evidence has revealed that non-coding RNAs (ncRNAs) are extensively involved in cancer progression, including drug resistance. Specifically, ncRNAs mediate resistance to oxaliplatin by mediating drug carriers, tumor microenvironment, resistance-related signaling pathways, and patterns of cell death. Importantly, we investigated the potential and clinical application values of these ncRNAs as liquid biopsy markers for CRC.

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INTRODUCTION

Colorectal cancer (CRC) is considered to be the leading cause of death associated with malignancy of the gastrointestinal tract, with approximately 1932 million new cases and 935000 deaths in 2020[1]. Currently, surgery is the preferred treatment option for early CRC patients; however, it has few clinical benefits for advanced patients due to high rates of postoperative metastasis and recurrence[2,3]. The annual survival rate for stage III CRC patients is about 30%-60%; this figure drops to just 10% for stage IV patients[4,5]. The combination of oxaliplatin, 5-fluorouracil, and leucovorin (FOLFOX) has significantly increased the overall survival of patients and now serves as a first-line standard regimen for metastatic CRC (mCRC)[6]. Oxaliplatin is a widely used third-generation platinum analog that functions by reacting with DNA to form hydration derivatives with intra- and inter-strand crosslinks eventually resulting in cell death[7]. However, oxaliplatin has not been satisfactory in improving the survival rate of some patients due to drug resistance[8]. Moreover, some toxic side effects persist after oxaliplatin treatment; these include peripheral neurotoxicity, which further hinders chemotherapy[9]. Fortunately, oxaliplatin-based chemotherapy retreatment strategies are being optimized to provide further personalized therapy for patients with mCRC[10]. Therefore, it is of great importance to understand the mechanism by which oxaliplatin resistance occurs, and to identify new biomarkers that can aid prediction of the outcomes for CRC patients treated with oxaliplatin-based therapy.

Although the processes of chemoresistance in CRC are intricate and inconclusive[11], ongoing research has revealed multiple oxaliplatin resistance mechanisms. Several studies have confirmed that chemoresistance is associated with the dysregulation of efflux proteins and drug-metabolizing enzymes, such as ABC transporters and GSTP1; these enzymes mediate drug uptake, transport, and toxicity[12,13]. Furthermore, accumulating evidence suggests that epithelial-mesenchymal transition (EMT)[14] and DNA damage repair[15] are driving factors that result in chemoresistance and tumor progression. Many studies have reported that various signaling pathways, such as the TGF-β/Smad[16], JNK/p38 MAPK[17], Wnt/β-catenin[18], and MEK/ERK/ELK1[19] pathways are closely associated with oxaliplatin resistance. As the main regulatory components of the tumor microenvironment (TME), cancer-associated fibroblasts (CAFs)[20] and tumor-associated macrophages[21] can modulate cancer chemotherapy resistance through complex mechanisms of crosstalk. Of note, emerging studies have confirmed that cell death mechanisms play a pivotal role in chemoresistance. It has been reported that apoptosis inhibition[22] and autophagy dysregulation[23] are underlying mechanisms leading to chemoresistance. Furthermore, numerous studies have identified that several additional death mechanisms can mediate CRC chemoresistance, including ferroptosis[24], pyroptosis[25], necroptosis[26], and others. However, the detailed molecular mechanisms by which these cell death modes lead to oxaliplatin resistance are unclear and require further investigation.

Non-coding RNAs (ncRNAs) refer to RNA molecules transcribed from genes that cannot encode proteins, accounting for more than 90% of human gene transcripts. ncRNAs can be divided into either small ncRNAs (< 200 nucleotides) or long non-coding RNAs (lncRNAs) (> 200 nucleotides) based on their length[27-29]. In the last decade, circular RNAs (circRNAs), a type of circular RNA generated in the splicing process of pre-mRNA, have emerged as a focus of research[30] (Figure 1). A growing number of studies have revealed that ncRNAs play critical roles in the occurrence and development of multiple human diseases *via* various mechanisms, such as epigenetics, transcription, and post-transcription[31,32]. Moreover, microRNA (miRNAs), lncRNAs, and circRNAs are three of the more widely studied types of ncRNAs and have been shown to mediate tumor progression by regulating tumor cell proliferation, aggression, metastasis, apoptosis, and drug resistance, amongst other pathways [33,34]. Drug resistance is one of the key factors in malignancy treatment failure, and whether this is mediated by ncRNAs has attracted increasing attention. In recent years, the rapid development of next-

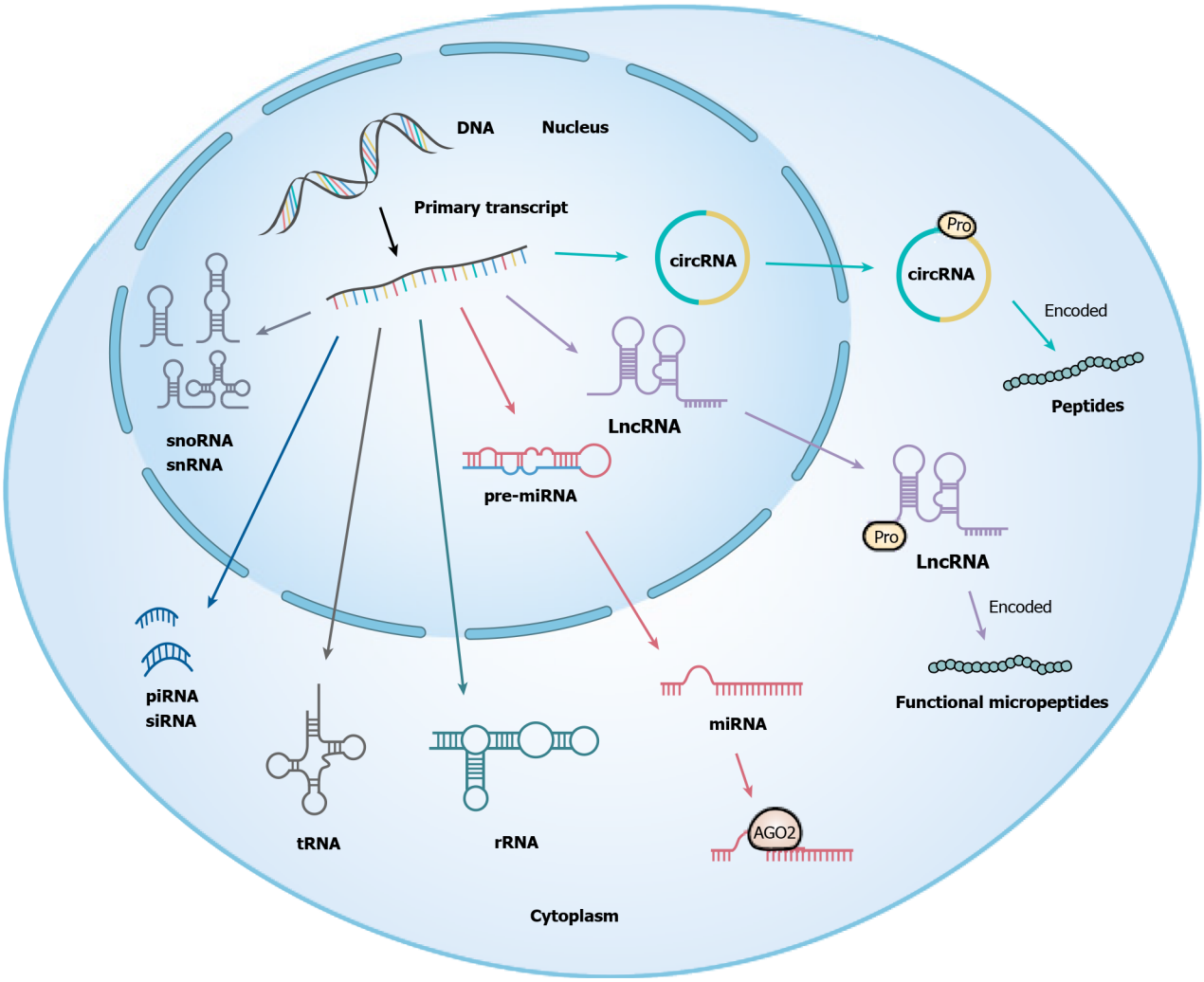

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Figure 1 Classification of non-coding RNAs. Non-coding RNAs can be divided into either small ncRNAs (< 200 nucleotides) or long non-coding RNAs (> 200 nucleotides) based on their length. Among these, small ncRNAs consist of ribosomal RNAs, transfer RNAs, small nucleosome/spliced RNAs, microRNAs, small interfering RNAs, PiWI-Interacting RNAs, and small nucleolar RNAs. Furthermore, the circular RNAs generated in the splicing process of pre-mRNA are also considered to be special ncRNAs. ncRNAs: Non-coding RNAs; lncRNAs: Long non-coding RNAs; rRNAs: Ribosomal RNAs; tRNAs: Transfer RNAs; snRNAs: Small nucleosome/spliced RNAs; miRNAs; MicroRNAs; siRNAs: Small interfering RNAs; piRNAs: PiWI-Interacting RNAs; snoRNAs: Small nucleolar RNAs.

generation sequencing technologies has expanded the understanding of tumor pathogenesis and drug resistance mechanisms, and an increasing number of tumor chemoresistance-associated differential ncRNAs have been identified[35-37]; these developments have provided the expansion of open datasets of candidate ncRNAs for basic research and clinical application. Currently, the targeting of ncRNAs as biomarkers in the clinic is being actively facilitated[38]. As such, a systematic understanding of the underlying roles of ncRNAs in malignancies provides valuable insights into overcoming chemoresistance in CRC patients.

Although tissue biopsy is the gold standard for malignancy diagnosis, the availability of clinical samples is often limited, and subsequently, tumor heterogeneity may not be reflected in analysis and is therefore not suitable for longitudinal clinical monitoring[39]. As a noninvasive approach, liquid biopsy has been extensively applied for the real-time monitoring of variations in tumor dynamics in body fluids including blood, ascites, and others. With the development of novel molecular detection technologies, growing evidence suggests that circulating tumor cells, circulating tumor DNA, circulating free nucleic acids, tumor-educated platelet, exosomes, *etc*[40,41] have gradually become prominent liquid biopsy hallmarks. Owing to their stability and high abundance, circulating ncRNAs can provide substantial details regarding tumor biology and therapeutic efficacy, and have become candidate biomarkers for earlier diagnosis, therapeutic monitoring, and prognosis evaluation of malignancies[42,43].

In this study, we performed a systematic literature review to identify the latent mechanisms of ncRNAs (miRNAs, lncRNAs, and circRNAs) in CRC oxaliplatin resistance. In particular, exosomal ncRNAs, as one of the most promising biomarkers in liquid biopsies, are expected to improve diagnostic, therapeutic, and drug monitoring in CRC patients.

OVERVIEW OF THE REGULATORY ROLES OF NCRNAS IN CRC OXALIPLATIN RESISTANCE

Classification and biological functions of miRNAs

MiRNAs are a class of small ncRNAs of approximately 22 nucleotides in length; they are produced by two RNase III proteins, Drosha and Dicer[44]. In general, miRNAs bind to the 3' untranslated region (UTR) of their target mRNAs resulting in cleavage or translational repression in the cytosol[45]. Intriguingly, numerous recent studies have demonstrated that some miRNAs activate gene transcription unconventionally in the nucleus by targeting enhancers, such miRNAs are known as nuclear activating miRNAs[46]. Moreover, enhancers (including super-enhancers) have been revealed to synergistically promote NamiRNA biogenesis and activate the expression of proximal genes[47,48]. Furthermore, studies have reported that the dysregulation of miRNAs may play an important role in CRC oxaliplatin resistance, as illustrated in Table 1.

Oncogenic miRNAs mediate oxaliplatin resistance in CRC

MiR-135b-5p has been confirmed to be upregulated in the serum of CRC patients, mechanistically this has been shown to induce protective autophagy *via* the MUL1/ULK1 signaling pathway, a process that contributes to oxaliplatin resistance[49]. Another miRNA, miR-454-3p, is highly expressed in oxaliplatin-resistant CRC cells compared to oxaliplatin-sensitive cells and promoted oxaliplatin resistance by inhibiting PTEN expression and activation of the AKT pathway[50]. Similarly, targeting miR-19a with high expression in oxaliplatin-resistant CRC cell lines could promote oxaliplatin sensitization of resistant cells through activation of the PTEN/PI3K/AKT pathway[51]. Compounding these findings, it has been revealed that dichloroacetate, a pyruvate dehydrogenase kinase inhibitor, can enhance the chemosensitivity of oxaliplatin-resistant CRC cells through the miR-543/PTEN/Akt/mTOR pathway and the miR-107/CAB39/AMPK/mTOR pathway[52,53]. In addition, an unbiased microRNA array demonstrated that miR-503-5p was up-regulated in oxaliplatin-resistant CRC cells; overexpression of miR-503-5p conferred resistance to oxaliplatin-induced apoptosis by inhibiting PUMA expression[54]. Further studies have revealed that by targeting BNI1, up-regulation of miR-744 enhanced the resistance of T84 and HCT116 cells to oxaliplatin[55]. Moreover, the oncogenic miR-5000-3p was upregulated in CRC tissues and oxaliplatin-resistant CRC cells, this miRNA negatively regulated USP9 to facilitate CRC chemoresistance[56]. Additionally, natural killer cells contributed to enhancing the sensitivity of oxaliplatin resistant CRC cells through the microRNA-146b-5p/WBSCR22 axis[57]. Remarkably, miR-19b-3p has been documented to be the most significantly upregulated candidate miRNA in colon cancer tissues, its expression correlates with tumorous histologic grading, staging, and poor prognosis in patients. Importantly, miR-19b-3p facilitated proliferation, curbed apoptosis, and induced oxaliplatin resistance in CRC cells by targeting SMAD4[58].

Tumor-suppressive miRNAs mediate oxaliplatin resistance in CRC

Interestingly, it has been demonstrated by some that tumor-suppressive miRNAs are associated with oxaliplatin resistance. Studies have identified that miR-1278 was significantly downregulated in CRC tissues, and that overexpression of miR-1278 inhibited CRC progression and enhanced oxaliplatin sensitivity through targeting of the KIF5B/BTG2 axis[59]. Similarly, miR-506 was weakly expressed in chemoresistant CRC cells, but its overexpression has been confirmed to impair the resistance of HCT116 cells to oxaliplatin *via* the Wnt/β-catenin pathway[60]. Moreover, miR-200b-3p was down-regulated in oxaliplatin-resistant CRC tissues and cells (HT29-OR and HCT116-OR), and studies revealed that miR-200b-3p reversed oxaliplatin resistance of CRC cells by targeting TUBB3[61]. Furthermore, miR-122, which was down-regulated in oxaliplatin-resistant SW480 and HT29 cells, has been demonstrated to be overexpressed in CRC cells, this could enhance the chemosensitivity of CRC by inhibiting the expression of XIAP[62]. As a tumor suppressor, miR-193a-5p can act directly upon CXCR4 to mitigate the chemosensitivity of CRC cells to 5-FU and oxaliplatin[63]. Using sequencing, Liang *et al*[64] discovered that miR-483-3p was negatively correlated with FAM171B expression, and targeting the miR-483-3p/FAM171B regulatory axis could enhance the sensitivity of CRC cells to oxaliplatin. Moreover, miR-325 mimics were observed to prevent the development of oxaliplatin resistance in CRC *via* interference with the HSPA12B/PI3K/AKT/Bcl-2 axis[65]. Finally, a proteomic analysis study reported that regulation of miR-195-5p and miR-497-5p is a potential strategy for alleviating oxaliplatin resistance in CRC cells[66].

As an additional factor, several groups showed that the interaction between ncRNAs and autophagy exerted significant roles in the therapeutic resistance of CRC[67]. Sun *et al*[68] demonstrated that miR-34a was expressed at low levels in oxaliplatin-resistant CRC patients and cells. Overexpression of miR-34a contributed to facilitating the sensitivity of CRC cells to oxaliplatin by regulating the TGF-β/Smad4 pathway resulting in the inhibition of macroautophagy. Furthermore, c-Myc has been shown to promote oxaliplatin resistance in CRC by integrating into the miR-27B promoter region and further mediating activation of the miR-27b-3p/ATG10 axis[69].

Table 1 Dysregulation of microRNAs and oxaliplatin resistance in colorectal cancer

MiRNAs	Expression[1]	Targets and pathways	Ref.
miR-135b-5p	↑	MUL1/ULK1	[49]
miR-454-3p	↑	PTEN	[50]
miR-19a	↑	PTEN/PI3K/AKT	[51]
miR-543	↑	PTEN/Akt/mTOR	[52]
miR-107	↑	CAB39/AMPK/mTOR	[53]
miR-503-5p	↑	PUMA	[54]
miR-744	↑	BIN1	[55]
miR-5000-3p	↑	USP49	[56]
miR-146b-5p	↑	WBSCR22	[57]
miR-19b-3p	↑	SMAD4	[58]
miR-1278	↓	-	[59]
miR-506	↓	Wnt/β-catenin	[60]
miR-200b-3p	↓	TUBB3	[61]
miR-122	↓	XIAP	[62]
miR-193a-5p	↓	CXCR4	[63]
miR-483-3p	↓	FAM171B	[64]
miR-325	↓	HSPA12B/PI3K/AKT/Bcl-2	[65]
miR-195-5p	↓	-	[66]
miR-497-5p	↓	-	
miR-34a	↓	TGF-β/Smad4	[68]
miR-27b-3p	↓	-	[69]

[1]Aberrant expression of miRNAs either up-regulated (↑) or down-regulated (↓) in CRC oxaliplatin resistance.
MiRNAs: MicroRNAs.

It is noteworthy that miR-181a could suppress the expression of BIRC6, a protein inhibitor of apoptosis, by directly targeting the 3'-UTR of BIRC6 mRNA[70]. Conversely, BIRC6 was observed to be significantly upregulated in acquired oxaliplatin-resistant CRC cells when compared to parental cells [71]. Considering that knockdown of BIRC6 helped enhance the chemosensitivity of CRC cells to oxaliplatin, targeting BIRC6 using miR-181a mimics may be a potential strategy to reverse oxaliplatin resistance in CRC.

Classification and biological functions of lncRNAs

LncRNAs are defined as a class of ncRNA molecules with transcript lengths of more than 200 nucleotides; they do not encode proteins due to their lack of an open reading frame[72]. LncRNAs can be classified into intergenic, intronic, sense, antisense, and bidirectional lncRNAs based on their genomic localization[73]. Alternatively, there are four categories of lncRNAs based on their functional mechanisms, including signal, decoy, guide, and scaffold lncRNAs[74,75]. It has been reported that lncRNAs can regulate transcription, translation, RNA stability, and alternative splicing[76-79]. It is noteworthy that numerous studies have revealed that aberrant lncRNAs (oncogenic and tumor suppressive lncRNAs) contribute to regulating the mechanism of oxaliplatin resistance through multiple cellular mechanisms, including but not limited to DNA damage repair, interference with drug influx and efflux, regulation of the cell cycle and apoptosis, and activation of signaling pathways[80,81]. Table 2 provides an overview of some aberrant lncRNAs and their target molecules in the context of oxaliplatin resistance in CRC.

Oncogenic lncRNAs as regulators of CRC oxaliplatin resistance

Studies discovered that high expression of the lncRNA GIHCG promoted the proliferation, migration, and invasion of tumor cells, and enhanced their resistance to 5-FU and oxaliplatin[82]. Similarly, overexpression of the lncRNA ARSR reduced cell apoptosis and induced oxaliplatin resistance[83]. Notably,

Table 2 Dysregulation of long non-coding RNAs and oxaliplatin resistance in colorectal cancer

LncRNAs	Expression[1]	Targets and pathways	Ref.
GIHCG	↑	-	[82]
lncARSR	↑	-	[83]
HOTAIR	↑	miR-1277-5p/ZEB1	[85]
MALAT1	↑	MALAT1/miR-218/EZH2	[86]
	↑	miR-324-3p/ADAM17	[88]
OIP5-AS1	↑	miR-137	[89]
Linc00152	↑	miR-193a-3p/ERBB4/AKT	[90]
CASC15	↑	miR-145/ABCC1	[92]
CBR3-AS1	↑	miR-145-5p	[93]
CRNDE	↑	miR-136/E2F1	[94]
LINC00460	↑	miR-149-5p/miR-150-5p/p53	[95]
KCNQ1OT1	↑	miR-34a/Atg4B	[96]
MIR155HG	↑	miR-650/Annexin A2	[91]
lnc-RP11-536 K7.3	↑	SOX2/HIF-1α/USP7	[97]
TUG1	↑	GATA6/BMP	[98]
LUCAT1	↑	UBA52/RPL40/MDM2/p53	[99]
PiHL	↑	EZH2/HMGA2/PI3K/Akt	[100]
ELFN1-AS1	↑	EZH2/DNMT3a/MEIS1	[102]
SNHG5	↑	STAU1	[103]
CCAT2	↑	BOP1/AURKA	[104]
NBAT-1	↓	WWC3/LATS1/YAP	[105]
MEG3	↓	-	[106]
		miR-141/PDCD4	[107]
lnc-AP	↓	pep-AP/TALDO1	[108]
lncRNA PVT1	-	hsa-miR-297/GSTA2	[109]

[1]Aberrant expression of lncRNAs either up-regulated (↑) or down-regulated (↓) in CRC oxaliplatin resistance.
LncRNAs: Long non-coding RNAs.

emerging evidence has revealed that lncRNAs could target mRNAs to promote chemoresistance[84]. For instance, upregulation of the lncRNA HOTAIR has been reported to facilitate EMT in a ZEB1-dependent manner by negatively regulating miR-1277-5p, a process that is involved in hypoxia-induced oxaliplatin resistance[85]. We previously discovered that metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was overexpressed in CRC tissues compared to paired noncancerous tissues. Correspondingly, the overexpression of MALAT1 suppressed E-cadherin expression and promoted oxaliplatin-induced EMT by interacting with EZH2. Furthermore, upregulated MALAT1 further inhibited miR-218 expression, resulting in poor response to oxaliplatin-based chemotherapy in CRC patients[86]. Numerous studies have demonstrated that some endogenous transcripts (such as endogenous pseudogenes, lncRNAs, and circRNAs) can complement miRNAs in sequences and inhibit their expression, resulting in the upregulation of target gene expression. This phenomenon is called the miRNA "sponge" effect[87]. Analogously, Fan *et al*[88] demonstrated that MALAT1 could function as a "sponge" for miR-324-3p and increase the expression of ADAM17 to facilitate resistance to oxaliplatin in CRC cells.

Moreover, lncRNA Opa-interacting protein 5 antisense RNA 1 can complement the sites of miR-137 in sequence, sponging miR-137 and inhibiting its expression, thus conferring oxaliplatin resistance in CRC cells[89]. It was reported that Linc00152 increased ERB-B2 receptor tyrosine kinase 4 expression by acting as a "sponge" for miR-193a-3p resulting in the promoted phosphorylation of AKT at Thr308 and Ser47; this process mediated oxaliplatin resistance in CRC cells[90]. In addition, researchers have illustrated that the lncRNA MIR155HG promoted M2 macrophage polarization and enhanced

oxaliplatin resistance in CRC cells by regulating the miR-650/Annexin A2 axis[91]. Similarly, other lncRNAs such as CASC15[92], CBR3-AS1[93], CRNDE[94], LINC00460[95], and KCNQ1OT1[96] have been reported to serve as "sponges" for miRNAs, thereby mediating oxaliplatin resistance in CRC.

Several research groups have revealed that lncRNAs could stabilize functional proteins, and mediate the chemoresistance of cancers. For example, lncRNA-RP11-536 K7.3 contributed to oxaliplatin resistance by recruiting SOX2 to activate deubiquitinase USP7 and stabilize the protein HIF-1α in CRC cells[97]. Furthermore, the lncRNA TUG1 induced oxaliplatin resistance in CRC stem cells and inhibited cell apoptosis by interacting with GATA6[98]. Moreover, two additional studies have further revealed that the lncRNAs LUCAT1 and PiHL mediated oxaliplatin resistance in CRC cells by interacting with UBA52 and EZH2 proteins, respectively[99,100].

LncRNAs have also been demonstrated to be regulators of gene expression by enhancing the stability of mRNA through epigenetic mechanisms[101]. Studies have elucidated that the lncRNA ELFN1-AS1 suppressed myeloid ecotype virus insertion site 1 transcription and promoted oxaliplatin resistance by interacting with EZH2 and DNA methyltransferase 3 alpha after localization to the promoter region of MEIS1 gene[102]. In addition, overexpression of the lncRNA SNHG 5 contributed to the proliferation of CRC cells and their resistance to oxaliplatin-induced apoptosis by blocking the degradation of SPATS 2 by STAU 1[103]. Of note, it has been identified that in microsatellite stable CRC, overexpression of the lncRNA CCAT2 could induce chromosomal instability, and enhance 5-FU and oxaliplatin-resistance by the upregulation of ribosomal biogenesis factor and the activation of aurora kinase A[104].

Tumor-suppressive lncRNAs as regulators of CRC oxaliplatin resistance

Previously, several tumor suppressor lncRNAs were reported to be associated with CRC oxaliplatin resistance. For instance, the expression levels of NBAT-1 and MEG3 in CRC tissues were low compared with normal adjacent tissues, especially in oxaliplatin-resistant patients[105,106]. Mechanistically, NBAT-1 was revealed to inhibit the growth of oxaliplatin-resistant CRC cells by activating the WWC 3/LATS 1/YAP pathway after acting as a "sponge" for miR-454[105]. Similarly, MEG3 promoted the sensitivity of CRC cells to oxaliplatin by regulating the miR-141/PDCD4 axis[107]. It was further reported that lnc-AP is a lncRNA with coding potential, and is highly associated with oxaliplatin resistance in CRC; lnc-AP encodes the short peptide pep-AP that enhances sensitization of CRC cells to oxaliplatin *via* the pep-AP/TALDO1 pathway[108]. Intriguingly, an epidemiological investigation demonstrated that an rs2278176 CT/TT mutation in the lncRNA PVT1 increased the chemosensitivity of CRC patients to FOLFOX compared to CRC patients carrying distinct genotypes[109].

Of note, when compared to parental cells, the lncRNA CRNDE was significantly down-regulated in oxaliplatin-resistant cells and can be considered a predictor of oxaliplatin treatment response and tumor prognosis[110]. Interestingly, this lncRNA can be combined with arginine-rich splicing factor 6 to reduce its stability, knockdown of the latter can inhibit autophagy and increase the sensitivity of cancer cells to oxaliplatin by the alternative splicing of PICALM[111]. Given that up-regulated expression of CRNDE aids an increase in the sensitivity of cancer cells to oxaliplatin, we speculate that increasing CRNDE expression and decreasing the stability of SRSF6 may be a promising strategy to ameliorate CRC resistance to oxaliplatin.

Classification and biological functions of circRNAs

Most circRNAs consist of covalently closed loop single-stranded RNAs produced by the back-splicing of exon precursor mRNAs that are more stable than their linear precursor gene[30]. Currently, circRNAs consist of four general categories: Exonic circRNAs, circular intronic RNAs, exon-intron circRNAs, and circRNA from other sources (such as antisense circular RNA or intergenic circular RNA) based on their structural domains and biogenesis features[112,113]. As vital biological regulators, circRNAs have been reported to widely participate in multiple oxaliplatin resistance-related mechanisms, such as apoptosis, autophagy, glycolysis, TME, EMT, DNA damage repair, *etc*[114]. Studies identified that hsa_circ_0040-809 was highly expressed in CRC cells and tissues; hsa_circ_0040809 upregulates DNMT1 expression by competitively combining with miR-515-5p, thus suppressing apoptosis and promoting cancer progression[115]. Conversely, as a tumor suppressor, circTADA2A was found to be down-regulated in cancer cells and tissues. Overexpression of circTADA2A elevated the expression of KLF14 by targeting miR-374a-3p, thereby promoting cell apoptosis and inhibiting tumor growth[116]. Moreover, has_circ_0001666 is a circRNA originating from exons 2, 3, and 4 of the host gene FAM120B. Studies have demonstrated that hsa_circ_0001666 competitively bound to miR-576-5p to increase PCDH10 expression, which in turn restrained cell stemness and EMT, and the Wnt/β-catenin pathway[117]. Zhang *et al*[118] revealed that the knockdown of circRNA_103948 contributed to enhanced autophagy of CRC cells by targeting the miR-1236-3p/TPT1 signaling pathway, inhibiting tumor growth. In addition, hypoxia facilitated circCCDC66 expression, which boosted the development of CRC by modulating the miR-3140/autophagy axis[119]. Furthermore, studies observed that hsa_circ_101555 was more highly expressed in CRC tissues than in normal tissues and was associated with adverse prognosis of patients. Inhibition of circ101555 resulted in the initiation of cell apoptosis and devastated DNA repair by activating the miR-597-5p/CDK6/RPA3 axis, thereby repressing the progression of CRC[120]. These studies suggested that circRNAs might be closely associated with the process leading to CRC oxaliplatin resistance.

CircRNAs regulate CRC oxaliplatin resistance

The majority of circRNAs containing miRNA response elements (MREs) can regulate gene expression and signaling pathways by functioning as ceRNAs, mediating tumor chemoresistance[121] (Table 3). Studies have identified that circular RNA protein tyrosine kinase 2 was significantly up-regulated in CRC tissues compared to normal tissues, and its high levels of expression promoted CRC progression and oxaliplatin resistance by regulation of the miR-136-5p/YTHDF1 axis[122]. Similarly, circ_0032833 was highly expressed in FOLFOX-resistant CRC cells, and knock-down of circ_0032833 could promote apoptosis and partially enhance the sensitivity of CRC cells to 5-FU and oxaliplatin through the activity of miR-125-5p/MSI1[123]. Lai *et al*[124] also demonstrated that hsa_circ_0079662 functioned as a "sponge" for hsa-mir-324-5p and activated HOXA9 through activation of the TNF-α pathway; this process induced oxaliplatin-resistance in CRC cells. Furthermore, our previous research revealed that circHIPK3 was highly expressed in chemoresistance CRC patients, and this circRNA was strongly associated with tumor size, regional lymph node metastasis, and distant metastasis. The following functional investigation revealed that circHIPK3 facilitated oxaliplatin resistance, but not 5-FU resistance, by restraining autophagy-related cell death. Mechanistically, circHIPK3 acted as a ceRNA and a "sponge" for miR-637 resulting in activation of the STAT3/Bcl-2/Beclin1 pathway, and subsequent induction of oxaliplatin-resistance in CRC cells[125].

Intriguingly, oxaliplatin could directly induce the overexpression of some oncogenes, further exacerbating chemoresistance. Circular CCDC66, derived from exons 6 to 11, was highly expressed in oxaliplatin-resistant CRC tissues and cells, a phenomenon caused by oxaliplatin-triggered cell stress through phosphoinositide 3-kinase related kinases-mediated phosphorylation of DHX9[126]. Despite extensive literature, many circRNAs remain to be explored in the study of oxaliplatin-resistant CRC. Abu *et al*[127] identified differential expression of circular RNAs when analyzing chemosensitive and chemoresistant CRC cells using microarray; hsa_circ_32883 and hsa_circ_0338 were screened as promising candidates. Subsequently, their study also identified that exosome-derived circRNAs (hsa_circ_0032883, hsa_circ_0002039, and hsa_circ_0000338) may play important roles in the manifestation of chemoresistance[128].

Studies have reported that the expression of exosomal miR-21-5p from oxaliplatin-resistant cells was significantly elevated, and therefore levels of this miRNA could act as a predictor of chemotherapy response in CRC patients[129]. Interestingly, miR-21-5p can be sponged by some tumor-suppressive circRNAs, such as circDDX17[35] and circEPB41L2[130], thus resulting in activation of the downstream PTEN/AKT pathway. Importantly, previous studies have confirmed that targeting the PTEN/PI3K/AKT/mTOR pathway partially reversed oxaliplatin resistance in CRC[51,52]. Given this, we speculate that targeting these suppressive circRNAs might contribute to the sensitization of CRC cells to oxaliplatin *via* the miR-21-5p/PTEN/AKT axis. Furthermore, hsa_circ_0001955 and hsa_circ_0000977, potential upstream targets for chemoresistance-associated miRNAs[49,93], were demonstrated to be dysfunctional in CRC tissue, a finding that suggests these circRNAs may be involved in the progression of CRC chemoresistance in a ceRNA-dependent manner[131]. Currently, studies regarding the roles of circRNAs in oxaliplatin resistance are less commonly reported, the precise and comprehensive mechanisms of circRNAs in CRC chemoresistance need to be explored by further study.

INTEGRATION, APPLICATION, AND CHALLENGES OF TARGETING EXOSOMAL NCRNAS IN CRC CHEMORESISTANCE

Exosomes biogenesis and characterization

Extracellular vesicles (EVs) are defined as a class of membranous vesicles that are released by cells to the extracellular matrix and play a key role in various physiological and pathological processes. According to the difference in their origin, size, content, and biological function, EVs can be roughly divided into three main subtypes—microbubbles, exosomes, and apoptotic bodies[132]. Exosomes are an emerging hallmark of liquid biopsy and have attracted much scientific attention of late, these small disc-shaped EVs have a diameter of approximately 40–150 nm and are enclosed by a lipid bilayer membrane[133]. Almost all cell types can secrete exosomes, and they also exist broadly within fluids including blood, urine, saliva, milk, ascites, cerebrospinal fluid, and others[40,134]. At present, ultracentrifugation remains the standard method for exosome extraction. However, with the emergence of novel technologies, exosome isolation and purification strategies are being continuously optimized, such as size exclusion chromatography, magnetic bead immune capture, and microfluidic-based chip technology techniques[135], these are aimed at efficiently obtaining exosomes with high abundance and quality. The characterization of exosomes has emerged as a prerequisite for their utilization, and the three basic characterization modes of morphological identification are particle size distribution and protein markers. However, more advanced features are gradually being included, in exosome characterization, such as the identification of the purity of exosomes and their uptake[135,136]. As signaling entities, numerous studies have demonstrated that exosomes exert biological effects in two ways: Firstly,

Table 3 Dysregulation of circular RNAs and oxaliplatin resistance in colorectal cancer

CircRNAs	Expression[1]	Targets and pathways	Ref.
circPTK2 (hsa_circ_0003221)	↑	miR-136-5P/YTHDF1	[122]
circ_0032833	↑	miR-125-5p/MSI1	[123]
hsa_circ_0079662	↑	hsa-mir-324-5p/TNF-α/HOXA9	[124]
circHIPK3	↑	miR-637/STAT3/Bcl-2/Beclin1	[125]
Circular CCDC66	↑	PI3KK/p-DHX9	[126]
hsa_circ_32883/ hsa_circ_0338	↑	-	[127,128]

[1]Aberrant expression of circRNAs either up-regulated (↑) or down-regulated (↓) in CRC oxaliplatin resistance.
CircRNAs: Circular RNAs.

exosomal membrane proteins or lipids act as ligands, directly activating receptors at the surface of target cells, generating cascade signal events and activating intracellular signaling pathways; Secondly, exosomes entrain and transport cellular signal-regulating molecules to target cells; these include DNA, lipids, proteins, and RNAs, which are extensively involved in tumorigenesis, metastasis, recurrence, and chemoresistance of cancers[137,138]. In particular, exosomal ncRNAs have received unprecedented appreciation in biomedical research and clinical application recently[139,140]. Several exosomal ncRNAs involved in CRC oxaliplatin resistance are listed in Table 4.

The latent mechanisms of exosomal ncRNAs in CRC oxaliplatin resistance

Increasing numbers of reports have revealed that exosomal ncRNAs can mediate chemoresistance *via* remodeling of the tumor microenvironment. Studies have identified that lncRNA H19 is highly expressed in CRC tissues compared to normal tissues. Moreover, lncRNA H19 derived from CAFs can act as a direct "sponge" for miR-141 and activate the β-catenin pathway, thus promoting stemness and oxaliplatin resistance[141]. Similarly, exosomal miR-92a-3p was transmitted from CAFs to cancer cells, promoting chemoresistance *via* the Wnt/β-catenin/apoptosis pathway[20]. Moreover, CAF-derived exosomes were revealed to deliver CRC-associated lncRNA to cancer cells, interacting with the mRNA of the stable protein human antigen R and increasing the expression of β-catenin; these processes promoted oxaliplatin resistance in CRC cells[142]. Qu *et al*[143] also found that exosomal cricN4BP2L2 secreted by CAFs can interact with EIF4A3 and modulate the PI3K/AKT/mTOR signaling pathway, thus promoting oxaliplatin resistance and the stemness of CRC cells. In addition, exosomal circ_0094343 derived from human colonic epithelial cells (NCM460 cells) was identified to be taken up by CRC cells in which it regulated cell apoptosis and glycolysis *via* the miR-766-5p/TRIM67 pathway, thereby enhancing the chemosensitivity of tumor cells[144].

Conversely, tumor cells have been revealed to generate exosomes in an autocrine or paracrine manner; they are taken up by themselves or delivered to the local microenvironment, thus exerting functional regulation. Ning *et al*[145] demonstrated that CRC cell-derived exosomal miR-208b could promote Treg expansion by directly inhibiting the expression of programmed cell death factor 4, resulting in promoted tumor progression and oxaliplatin resistance. Similarly, studies have revealed that exosomal miR-46146 conferred chemoresistance *via* the targeting of programmed cell death factor 10 in CRC cells[146]. Interestingly, one particular study revealed that miR-1915-3p was down-regulated in oxaliplatin-resistant CRC cells compared to oxaliplatin- sensitive cells and that EVs-derived miR-1915-3p increased the oxaliplatin sensitivity of drug-resistant cells by targeting PFKFB3/USP2[147]. Moreover, it has been reported that hsa_circ_0005963 can be transferred from oxaliplatin-resistant cells to sensitive cells by exosomes, directly acting as a miR-122 "sponge" and increasing the expression of PKM2, which promotes glycolysis and oxaliplatin-resistance in sensitive cells[148].

Exosomal ncRNAs as potential liquid biopsy biomarkers

Liquid biopsy, in which diseases are evaluated *via* sampling biological fluids, can avoid the influence of tissue heterogeneity on tumor molecular typing to some extent. More and more evidence show that abundant exosomes are enriched in body fluids and participate in many physiological and pathological processes. As an emerging hallmark of liquid biopsy, exosomes are of substantial value in cancer diagnosis, monitoring, and prediction[136,140]. Studies found that the ratio of exosomal miRNAs is an effective predictor of tumor response in peritoneal metastatic patients after repeated intraperitoneal chemotherapy, as patients with high ratios of miR-223-3p/miR-29b-3p or miR-21-5p/miR-29b-3p had inferior survival outcomes compared to patients with low ratios[149]. By analogy, exosomes derived

Table 4 Aberrant exosomal non-coding RNAs and their targets in the chemoresistance of colorectal cancer

Exosomal ncRNAs[1]	Donor cells	Recipient cells	Targets and pathways	Ref.
miR-21↓	THLG-293T/LG-293T cells	CRC cells	-	[156]
miR-208b↑	CRC cells	T cells	PDCD4	[145]
miR-92a-3p↑	CAFs cells	CRC cells	FBXW7/MOAP1	[20]
miR-46146↑	CRC-R cells	CRC-S cells	PDCD10	[146]
miR-1915-3p↓	FHC cells	CRC cells	PFKFB3/USP2	[147]
lncRNA H19↑	CAFs cells	CRC cells	-	[141]
lncRNA CCAL↑	CAFs cells	CRC cells	HuR/β-catenin	[142]
cricN4BP2L2	CAFs cells	CRC cells	EIF4A3/PI3K/AKT/mTOR	[143]
ciRS-122↑	CRC-R cells	CRC-S cells	miR-122/PKM2	[148]
circ_0094343↓	NCM460 cells	CRC cells	miR-766-5p/TRIM67	[144]

[1]Aberrant exosomal ncRNAs are either up-regulated (↑) or down-regulated (↓) in CRC oxaliplatin resistance. CRC-R cells and CRC-S cells represent oxaliplatin-resistant and oxaliplatin-sensitive cells, respectively.
ncRNAs: Non-coding RNAs; CRC: Colorectal cancer; CAFs: Cancer-associated fibroblasts.

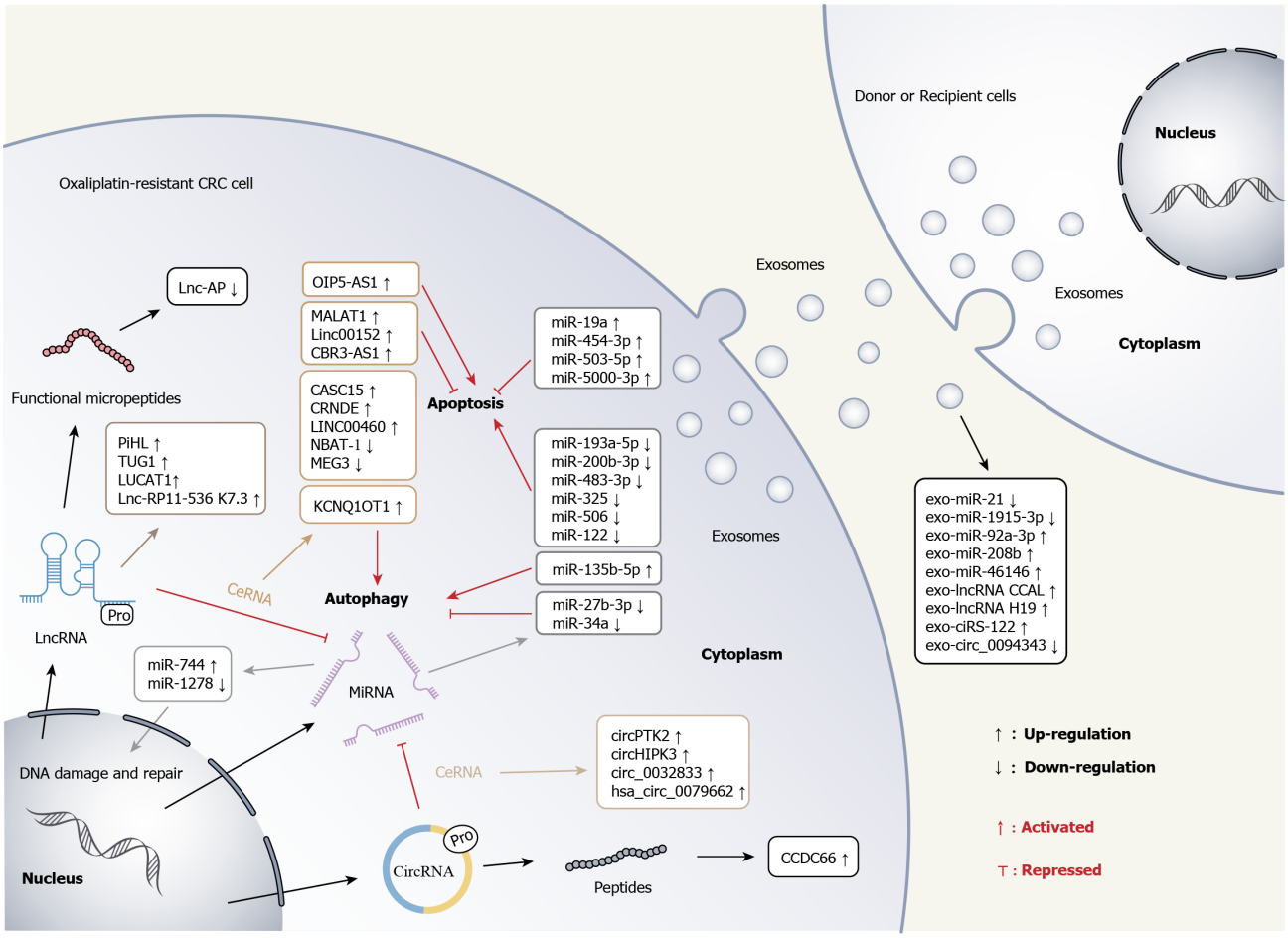
miR-21-5p, miR-1246, miR-1229-5p, and miR-96-5p were highly expressed in CRC patients and cancer cell lines resistant to 5-FU and oxaliplatin compared with those sensitive to chemotherapy. The area under the curve (AUC) of four combinations of exosomal miRNAs was 0.804, which is expected to be an effective predictor of chemotherapy[129]. In addition, researchers found that plasma-derived exosomal miRNA-125b was highly expressed in patients with progressive disease (PD) compared with the healthy control group or patients with stable disease. Importantly, some patients with PD who respond to modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) have obvious differences in the expression of plasma exosomal miRNA-125b before and after chemotherapy, and patients with low expression of exosomal miRNA-125b have higher progression-free survival (PFS)[150]. Furthermore, it was reported that miR-208b was highly expressed in the serum of FOLFOX-resistant CRC patients and the AUC of serum miRNA-208b was 0.771 [95% confidence interval (CI): 0.688-0.855], which is better than that of serum CEA of 0.493 (95%CI: 0.385-0.601). Their research suggests that this miRNA can be regarded as a promising liquid biopsy indicator to predict FOLFOX sensitivity in cancer patients[145]. Encouragingly, a panel of plasma exosomal miR-17-5p and miR-185-5p were successfully used to predict FOLFOX4/FOLFIRI responses in patients with advanced CRC[151].

Exosomal ncRNAs as delivery vehicles for chemosensitization

A large number of reports have revealed the prospects for the application of exosomes to overcome chemoresistance, their strong stability, intrinsic biocompatibility, low immunogenicity, and natural targeting ability making them favorable targets[152,153]. Novel research has exhibited an engineered exosome encapsulating oxaliplatin and PGM5 Antisense RNA 1 that were delivered into CRC cells, and these effectively relieved chemoresistance and inhibited tumor progression[154]. Furthermore, Pi *et al* [155] constructed engineered EVs enwrapped in folate nanoparticles and survivin siRNA, which significantly inhibited tumor growth in CRC xenograft mice. Similarly, miR-21 inhibitors have been co-incorporated into exosomes, resulting in enhanced chemosensitivity of CRC cells[156]. Taken together, these findings suggest that exogenous delivery of ncRNAs could serve as a novel therapeutic target for CRC.

CONCLUSION

A multitude of emerging studies have confirmed that dysfunctional ncRNAs contribute to the development of malignancies, including chemoresistance. In this review, we systematically summarized the multiple mechanisms by which ncRNAs function in CRC oxaliplatin resistance and discussed the biomedical prospects of exosomal ncRNAs as hallmarks for fluid biopsy and therapeutic targets. Figure 2 schematically illustrates the multiple regulatory roles of ncRNAs in CRC oxaliplatin resistance. Owing to the differential expression patterns of ncRNAs in cancer patients, it is considered they may be used as biomarkers for early detection, tumor staging, and clinical outcome. Although many mechanisms by which ncRNAs exert their biological effects remain to be deciphered, targeting these ncRNAs has emerged as a promising strategy to ameliorate chemoresistance.



DOI: 10.3748/wjg.v29.i1.1

Figure 2 Overview of the mechanisms by which several non-coding RNAs exert oxaliplatin resistance in colorectal cancer. Various microRNAs, long non-coding RNAs, and circular RNAs are up-regulated or down-regulated in colorectal cancer and mediate oxaliplatin resistance *via* a multitude of mechanisms. These mechanisms include acting as competing endogenous RNAs, generating encoded peptides, regulating autophagy, inducing apoptosis, and interfering with DNA damage and repair. In particular, several non-coding RNAs have been demonstrated to confer oxaliplatin resistance in colorectal cancer cells *via* exosomes. CRC: Colorectal cancer; lncRNAs: Long non-coding RNAs; miRNAs; MicroRNAs; circRNAs: Circular RNAs.

Nevertheless, the integration and application of ncRNAs in clinical practice are far from the existing research fervor and are currently being tested in experimental studies; there are still many challenges to be overcome before ncRNAs can be extensively implemented in clinics. Firstly, the results of existing research are confounding due to differing nomenclature being utilized for circRNAs. The circRNAs labeled using different naming standards make the subsequent genomic localization cumbersome. Secondly, the expression of ncRNAs in biological fluids is relatively low, and therefore accurate detection and quantification of these molecules is a prerequisite for the development of biomarkers. Furthermore, due to differences in the lengths and mechanisms by which ncRNAs function in distinct tumor types, it is a challenge to select appropriate targets for these multitudinous candidates. Exosomes may be a better intermediary for delivering ncRNAs therapies; this could be achieved in an endogenous or exogenous manner. However, it is crucial to increase the loading of ncRNAs delivered by endogenous exosomes and ensure the safety of engineered exosomes with complex structures. Measurements of the efficacy of exosomal ncRNAs in clinical applications cannot be limited to laboratory data alone. A series of large validation cohorts will be required to obtain reliable predictive models and accurate therapeutic doses. In the future, clinical translational trials utilizing target ncRNAs are expected to overcome oxaliplatin resistance and thus improve the clinical outcomes of CRC patients.

FOOTNOTES

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REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
2 **Dekker E**, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* 2019; **394**: 1467-1480 [PMID: 31631858 DOI: 10.1016/S0140-6736(19)32319-0]
3 **Biller LH**, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. *JAMA* 2021; **325**: 669-685 [PMID: 33591350 DOI: 10.1001/jama.2021.0106]
4 **Kanemitsu Y**, Shitara K, Mizusawa J, Hamaguchi T, Shida D, Komori K, Ikeda S, Ojima H, Ike H, Shiomi A, Watanabe J, Takii Y, Yamaguchi T, Katsumata K, Ito M, Okuda J, Hyakudomi R, Shimada Y, Katayama H, Fukuda H; JCOG Colorectal Cancer Study Group. Primary Tumor Resection Plus Chemotherapy Versus Chemotherapy Alone for Colorectal Cancer Patients With Asymptomatic, Synchronous Unresectable Metastases (JCOG1007; iPACS): A Randomized Clinical Trial. *J Clin Oncol* 2021; **39**: 1098-1107 [PMID: 33560877 DOI: 10.1200/JCO.20.02447]
5 **Grothey A**, Sobrero AF, Shields AF, Yoshino T, Paul J, Taieb J, Souglakos J, Shi Q, Kerr R, Labianca R, Meyerhardt JA, Vernerey D, Yamanaka T, Boukovinas I, Meyers JP, Renfro LA, Niedzwiecki D, Watanabe T, Torri V, Saunders M, Sargent DJ, Andre T, Iveson T. Duration of Adjuvant Chemotherapy for Stage III Colon Cancer. *N Engl J Med* 2018; **378**: 1177-1188 [PMID: 29590544 DOI: 10.1056/NEJMoa1713709]
6 **Hecht JR**, Lonardi S, Bendell J, Sim HW, Macarulla T, Lopez CD, Van Cutsem E, Muñoz Martin AJ, Park JO, Greil R, Wang H, Hozak RR, Gueorguieva I, Lin Y, Rao S, Ryoo BY. Randomized Phase III Study of FOLFOX Alone or With Pegilodecakin as Second-Line Therapy in Patients With Metastatic Pancreatic Cancer That Progressed After Gemcitabine (SEQUOIA). *J Clin Oncol* 2021; **39**: 1108-1118 [PMID: 33555926 DOI: 10.1200/JCO.20.02232]
7 **Martinez-Balibrea E**, Martínez-Cardús A, Ginés A, Ruiz de Porras V, Moutinho C, Layos L, Manzano JL, Bugés C, Bystrup S, Esteller M, Abad A. Tumor-Related Molecular Mechanisms of Oxaliplatin Resistance. *Mol Cancer Ther* 2015; **14**: 1767-1776 [PMID: 26184483 DOI: 10.1158/1535-7163.MCT-14-0636]
8 **Van der Jeught K**, Xu HC, Li YJ, Lu XB, Ji G. Drug resistance and new therapies in colorectal cancer. *World J Gastroenterol* 2018; **24**: 3834-3848 [PMID: 30228778 DOI: 10.3748/wjg.v24.i34.3834]
9 **Kang L**, Tian Y, Xu S, Chen H. Oxaliplatin-induced peripheral neuropathy: clinical features, mechanisms, prevention and treatment. *J Neurol* 2021; **268**: 3269-3282 [PMID: 32474658 DOI: 10.1007/s00415-020-09942-w]
10 **Mauri G**, Gori V, Bonazzina E, Amatu A, Tosi F, Bencardino K, Ruggieri L, Patelli G, Arena S, Bardelli A, Siena S, Sartore-Bianchi A. Oxaliplatin retreatment in metastatic colorectal cancer: Systematic review and future research opportunities. *Cancer Treat Rev* 2020; **91**: 102112 [PMID: 33091698 DOI: 10.1016/j.ctrv.2020.102112]
11 **Wei L**, Wang X, Lv L, Zheng Y, Zhang N, Yang M. The emerging role of noncoding RNAs in colorectal cancer chemoresistance. *Cell Oncol (Dordr)* 2019; **42**: 757-768 [PMID: 31359293 DOI: 10.1007/s13402-019-00466-8]
12 **Ghanbarian M**, Afgar A, Yadegarazari R, Najafi R, Teimoori-Toolabi L. Through oxaliplatin resistance induction in colorectal cancer cells, increasing ABCB1 level accompanies decreasing level of miR-302c-5p, miR-3664-5p and miR-129-5p. *Biomed Pharmacother* 2018; **108**: 1070-1080 [PMID: 30372807 DOI: 10.1016/j.biopha.2018.09.112]
13 **Cui J**, Li G, Yin J, Li L, Tan Y, Wei H, Liu B, Deng L, Tang J, Chen Y, Yi L. GSTP1 and cancer: Expression, methylation, polymorphisms and signaling (Review). *Int J Oncol* 2020; **56**: 867-878 [PMID: 32319549 DOI: 10.3892/ijo.2020.4979]
14 **Sreekumar R**, Al-Saihati H, Emaduddin M, Moutasim K, Mellone M, Patel A, Kilic S, Cetin M, Erdemir S, Navio MS, Lopez MA, Curtis N, Yagci T, Primrose JN, Price BD, Berx G, Thomas GJ, Tulchinsky E, Mirnezami A, Sayan AE. The ZEB2-dependent EMT transcriptional programme drives therapy resistance by activating nucleotide excision repair genes

ERCC1 and ERCC4 in colorectal cancer. *Mol Oncol* 2021; **15**: 2065-2083 [PMID: 33931939 DOI: 10.1002/1878-0261.12965]

15 **Xiao Y**, Lin FT, Lin WC. ACTL6A promotes repair of cisplatin-induced DNA damage, a new mechanism of platinum resistance in cancer. *Proc Natl Acad Sci U S A* 2021; **118** [PMID: 33408251 DOI: 10.1073/pnas.2015808118]

16 **Liu J**, Miao X, Xiao B, Huang J, Tao X, Zhang J, Zhao H, Pan Y, Wang H, Gao G, Xiao GG. Obg-Like ATPase 1 Enhances Chemoresistance of Breast Cancer *via* Activation of TGF-β/Smad Axis Cascades. *Front Pharmacol* 2020; **11**: 666 [PMID: 32528278 DOI: 10.3389/fphar.2020.00666]

17 **Kwak AW**, Park JW, Lee SO, Lee JY, Seo JH, Yoon G, Lee MH, Choi JS, Shim JH. Isolinderalactone sensitizes oxaliplatin-resistance colorectal cancer cells through JNK/p38 MAPK signaling pathways. *Phytomedicine* 2022; **105**: 154383 [PMID: 35987016 DOI: 10.1016/j.phymed.2022.154383]

18 **Liu X**, Su K, Sun X, Jiang Y, Wang L, Hu C, Zhang C, Lu M, Du X, Xing B. Sec62 promotes stemness and chemoresistance of human colorectal cancer through activating Wnt/β-catenin pathway. *J Exp Clin Cancer Res* 2021; **40**: 132 [PMID: 33858476 DOI: 10.1186/s13046-021-01934-6]

19 **Ma J**, Zeng S, Zhang Y, Deng G, Qu Y, Guo C, Yin L, Han Y, Cai C, Li Y, Wang G, Bonkovsky HL, Shen H. BMP4 promotes oxaliplatin resistance by an induction of epithelial-mesenchymal transition *via* MEK1/ERK/ELK1 signaling in hepatocellular carcinoma. *Cancer Lett* 2017; **411**: 117-129 [PMID: 28987388 DOI: 10.1016/j.canlet.2017.09.041]

20 **Hu JL**, Wang W, Lan XL, Zeng ZC, Liang YS, Yan YR, Song FY, Wang FF, Zhu XH, Liao WJ, Liao WT, Ding YQ, Liang L. CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial-mesenchymal transition in colorectal cancer. *Mol Cancer* 2019; **18**: 91 [PMID: 31064356 DOI: 10.1186/s12943-019-1019-x]

21 **Yin Y**, Yao S, Hu Y, Feng Y, Li M, Bian Z, Zhang J, Qin Y, Qi X, Zhou L, Fei B, Zou J, Hua D, Huang Z. The Immune-microenvironment Confers Chemoresistance of Colorectal Cancer through Macrophage-Derived IL6. *Clin Cancer Res* 2017; **23**: 7375-7387 [PMID: 28928161 DOI: 10.1158/1078-0432.CCR-17-1283]

22 **Rubio MF**, Lira MC, Rosa FD, Sambresqui AD, Salazar Güemes MC, Costas MA. RAC3 influences the chemoresistance of colon cancer cells through autophagy and apoptosis inhibition. *Cancer Cell Int* 2017; **17**: 111 [PMID: 29209153 DOI: 10.1186/s12935-017-0483-x]

23 **Zhang H**, Lu B. The Roles of ceRNAs-Mediated Autophagy in Cancer Chemoresistance and Metastasis. *Cancers (Basel)* 2020; **12** [PMID: 33050642 DOI: 10.3390/cancers12102926]

24 **Yang C**, Zhang Y, Lin S, Liu Y, Li W. Suppressing the KIF20A/NUAK1/Nrf2/GPX4 signaling pathway induces ferroptosis and enhances the sensitivity of colorectal cancer to oxaliplatin. *Aging (Albany NY)* 2021; **13**: 13515-13534 [PMID: 33819186 DOI: 10.18632/aging.202774]

25 **Hou X**, Xia J, Feng Y, Cui L, Yang Y, Yang P, Xu X. USP47-Mediated Deubiquitination and Stabilization of TCEA3 Attenuates Pyroptosis and Apoptosis of Colorectal Cancer Cells Induced by Chemotherapeutic Doxorubicin. *Front Pharmacol* 2021; **12**: 713322 [PMID: 34630087 DOI: 10.3389/fphar.2021.713322]

26 **Lan H**, Liu Y, Liu J, Wang X, Guan Z, Du J, Jin K. Tumor-Associated Macrophages Promote Oxaliplatin Resistance *via* METTL3-Mediated m^6A of TRAF5 and Necroptosis in Colorectal Cancer. *Mol Pharm* 2021; **18**: 1026-1037 [PMID: 33555197 DOI: 10.1021/acs.molpharmaceut.0c00961]

27 **Djebali S**, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Röder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakrabortty S, Chen X, Chrast J, Curado J, Derrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foissac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Robyr D, Sammeth M, Schaffer L, See LH, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigó R, Gingeras TR. Landscape of transcription in human cells. *Nature* 2012; **489**: 101-108 [PMID: 22955620 DOI: 10.1038/nature11233]

28 **ENCODE Project Consortium**. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012; **489**: 57-74 [PMID: 22955616 DOI: 10.1038/nature11247]

29 **Ali SA**, Peffers MJ, Ormseth MJ, Jurisica I, Kapoor M. The non-coding RNA interactome in joint health and disease. *Nat Rev Rheumatol* 2021; **17**: 692-705 [PMID: 34588660 DOI: 10.1038/s41584-021-00687-y]

30 **Liu CX**, Chen LL. Circular RNAs: Characterization, cellular roles, and applications. *Cell* 2022; **185**: 2016-2034 [PMID: 35584701 DOI: 10.1016/j.cell.2022.04.021]

31 **Kaikkonen MU**, Lam MT, Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovasc Res* 2011; **90**: 430-440 [PMID: 21558279 DOI: 10.1093/cvr/cvr097]

32 **Eidem TM**, Kugel JF, Goodrich JA. Noncoding RNAs: Regulators of the Mammalian Transcription Machinery. *J Mol Biol* 2016; **428**: 2652-2659 [PMID: 26920110 DOI: 10.1016/j.jmb.2016.02.019]

33 **Slack FJ**, Chinnaiyan AM. The Role of Non-coding RNAs in Oncology. *Cell* 2019; **179**: 1033-1055 [PMID: 31730848 DOI: 10.1016/j.cell.2019.10.017]

34 **Yan H**, Bu P. Non-coding RNA in cancer. *Essays Biochem* 2021; **65**: 625-639 [PMID: 33860799 DOI: 10.1042/EBC20200032]

35 **Li XN**, Wang ZJ, Ye CX, Zhao BC, Li ZL, Yang Y. RNA sequencing reveals the expression profiles of circRNA and indicates that circDDX17 acts as a tumor suppressor in colorectal cancer. *J Exp Clin Cancer Res* 2018; **37**: 325 [PMID: 30591054 DOI: 10.1186/s13046-018-1006-x]

36 **Zhu KP**, Zhang CL, Ma XL, Hu JP, Cai T, Zhang L. Analyzing the Interactions of mRNAs and ncRNAs to Predict Competing Endogenous RNA Networks in Osteosarcoma Chemo-Resistance. *Mol Ther* 2019; **27**: 518-530 [PMID: 30692017 DOI: 10.1016/j.ymthe.2019.01.001]

37 **Li Y**, Zhao J, Yu S, Wang Z, He X, Su Y, Guo T, Sheng H, Chen J, Zheng Q, Li Y, Guo W, Cai X, Shi G, Wu J, Wang L,

Wang P, Huang S. Extracellular Vesicles Long RNA Sequencing Reveals Abundant mRNA, circRNA, and lncRNA in Human Blood as Potential Biomarkers for Cancer Diagnosis. *Clin Chem* 2019; **65**: 798-808 [PMID: 30914410 DOI: 10.1373/clinchem.2018.301291]

38 **Chen B**, Dragomir MP, Yang C, Li Q, Horst D, Calin GA. Targeting non-coding RNAs to overcome cancer therapy resistance. *Signal Transduct Target Ther* 2022; **7**: 121 [PMID: 35418578 DOI: 10.1038/s41392-022-00975-3]

39 **Overman MJ**, Modak J, Kopetz S, Murthy R, Yao JC, Hicks ME, Abbruzzese JL, Tam AL. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? *J Clin Oncol* 2013; **31**: 17-22 [PMID: 23129736 DOI: 10.1200/JCO.2012.43.1718]

40 **Yu W**, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, Breakefield XO, Skog JK. Exosome-based liquid biopsies in cancer: opportunities and challenges. *Ann Oncol* 2021; **32**: 466-477 [PMID: 33548389 DOI: 10.1016/j.annonc.2021.01.074]

41 **Zhou H**, Zhu L, Song J, Wang G, Li P, Li W, Luo P, Sun X, Wu J, Liu Y, Zhu S, Zhang Y. Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer. *Mol Cancer* 2022; **21**: 86 [PMID: 35337361 DOI: 10.1186/s12943-022-01556-2]

42 **Anfossi S**, Babayan A, Pantel K, Calin GA. Clinical utility of circulating non-coding RNAs - an update. *Nat Rev Clin Oncol* 2018; **15**: 541-563 [PMID: 29784926 DOI: 10.1038/s41571-018-0035-x]

43 **Winkle M**, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics - challenges and potential solutions. *Nat Rev Drug Discov* 2021; **20**: 629-651 [PMID: 34145432 DOI: 10.1038/s41573-021-00219-z]

44 **Ha M**, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014; **15**: 509-524 [PMID: 25027649 DOI: 10.1038/nrm3838]

45 **Lu TX**, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol* 2018; **141**: 1202-1207 [PMID: 29074454 DOI: 10.1016/j.jaci.2017.08.034]

46 **Liang Y**, Zou Q, Yu W. Steering Against Wind: A New Network of NamiRNAs and Enhancers. *Genomics Proteomics Bioinformatics* 2017; **15**: 331-337 [PMID: 28882787 DOI: 10.1016/j.gpb.2017.05.001]

47 **Suzuki HI**, Young RA, Sharp PA. Super-Enhancer-Mediated RNA Processing Revealed by Integrative MicroRNA Network Analysis. *Cell* 2017; **168**: 1000-1014.e15 [PMID: 28283057 DOI: 10.1016/j.cell.2017.02.015]

48 **Odame E**, Chen Y, Zheng S, Dai D, Kyei B, Zhan S, Cao J, Guo J, Zhong T, Wang L, Li L, Zhang H. Enhancer RNAs: transcriptional regulators and workmates of NamiRNAs in myogenesis. *Cell Mol Biol Lett* 2021; **26**: 4 [PMID: 33568070 DOI: 10.1186/s11658-021-00248-x]

49 **Wang H**, Wang X, Zhang H, Deng T, Liu R, Liu Y, Li H, Bai M, Ning T, Wang J, Ge S, Ba Y. The HSF1/miR-135b-5p axis induces protective autophagy to promote oxaliplatin resistance through the MUL1/ULK1 pathway in colorectal cancer. *Oncogene* 2021; **40**: 4695-4708 [PMID: 34140641 DOI: 10.1038/s41388-021-01898-z]

50 **Qian XL**, Zhou F, Xu S, Jiang J, Chen ZP, Wang SK, Zuo Y, Ni C. MiR-454-3p Promotes Oxaliplatin Resistance by Targeting PTEN in Colorectal Cancer. *Front Oncol* 2021; **11**: 638537 [PMID: 34017681 DOI: 10.3389/fonc.2021.638537]

51 **Zhang Y**, Liu X, Zhang J, Xu Y, Shao J, Hu Y, Shu P, Cheng H. Inhibition of miR-19a partially reversed the resistance of colorectal cancer to oxaliplatin *via* PTEN/PI3K/AKT pathway. *Aging (Albany NY)* 2020; **12**: 5640-5650 [PMID: 32209726 DOI: 10.18632/aging.102929]

52 **Liang Y**, Zhu D, Zhu L, Hou Y, Hou L, Huang X, Li L, Wang Y, Zou H, Wu T, Yao M, Wang J, Meng X. Dichloroacetate Overcomes Oxaliplatin Chemoresistance in Colorectal Cancer through the miR-543/PTEN/Akt/mTOR Pathway. *J Cancer* 2019; **10**: 6037-6047 [PMID: 31762813 DOI: 10.7150/jca.34650]

53 **Liang Y**, Zhu D, Hou L, Wang Y, Huang X, Zhou C, Zhu L, Li L, Gu Y, Luo M, Wang J, Meng X. MiR-107 confers chemoresistance to colorectal cancer by targeting calcium-binding protein 39. *Br J Cancer* 2020; **122**: 705-714 [PMID: 31919406 DOI: 10.1038/s41416-019-0703-3]

54 **Xu K**, Chen G, Qiu Y, Yuan Z, Li H, Yuan X, Sun J, Xu J, Liang X, Yin P. miR-503-5p confers drug resistance by targeting PUMA in colorectal carcinoma. *Oncotarget* 2017; **8**: 21719-21732 [PMID: 28423513 DOI: 10.18632/oncotarget.15559]

55 **Zhou Y**, He A, Zhang L, Yi G. MiR-744 mediates the Oxaliplatin chemoresistance in colorectal cancer through inhibiting BIN1. *Neoplasma* 2020; **67**: 296-303 [PMID: 31884801 DOI: 10.4149/neo_2019_190508N411]

56 **Zhuang YY**, Zhong W, Xia ZS, Lin SZ, Chan MC, Jiang K, Li WF, Xu XY. miR-5000-3p confers oxaliplatin resistance by targeting ubiquitin-specific peptidase 49 in colorectal cancer. *Cell Death Discov* 2021; **7**: 129 [PMID: 34075026 DOI: 10.1038/s41420-021-00494-0]

57 **Zhao H**, Su W, Kang Q, Xing Z, Lin X, Wu Z. Natural killer cells inhibit oxaliplatin-resistant colorectal cancer by repressing WBSCR22 *via* upregulating microRNA-146b-5p. *Am J Cancer Res* 2018; **8**: 824-834 [PMID: 29888105]

58 **Jiang T**, Ye L, Han Z, Liu Y, Yang Y, Peng Z, Fan J. miR-19b-3p promotes colon cancer proliferation and oxaliplatin-based chemoresistance by targeting SMAD4: validation by bioinformatics and experimental analyses. *J Exp Clin Cancer Res* 2017; **36**: 131 [PMID: 28938919 DOI: 10.1186/s13046-017-0602-5]

59 **Lin W**, Zou H, Mo J, Jin C, Jiang H, Yu C, Jiang Z, Yang Y, He B, Wang K. Micro1278 Leads to Tumor Growth Arrest, Enhanced Sensitivity to Oxaliplatin and Vitamin D and Inhibits Metastasis *via* KIF5B, CYP24A1, and BTG2, Respectively. *Front Oncol* 2021; **11**: 637878 [PMID: 33791222 DOI: 10.3389/fonc.2021.637878]

60 **Zhou H**, Lin C, Zhang Y, Zhang X, Zhang C, Zhang P, Xie X, Ren Z. miR-506 enhances the sensitivity of human colorectal cancer cells to oxaliplatin by suppressing MDR1/P-gp expression. *Cell Prolif* 2017; **50** [PMID: 28217977 DOI: 10.1111/cpr.12341]

61 **Wu YZ**, Lin HY, Zhang Y, Chen WF. miR-200b-3p mitigates oxaliplatin resistance *via* targeting TUBB3 in colorectal cancer. *J Gene Med* 2020; **22**: e3178 [PMID: 32092782 DOI: 10.1002/jgm.3178]

62 **Hua Y**, Zhu Y, Zhang J, Zhu Z, Ning Z, Chen H, Liu L, Chen Z, Meng Z. miR-122 Targets X-Linked Inhibitor of Apoptosis Protein to Sensitize Oxaliplatin-Resistant Colorectal Cancer Cells to Oxaliplatin-Mediated Cytotoxicity. *Cell Physiol Biochem* 2018; **51**: 2148-2159 [PMID: 30522111 DOI: 10.1159/000495832]

63 **Azar MRMH**, Aghazadeh H, Mohammed HN, Sara MRS, Hosseini A, Shomali N, Tamjidifar R, Tarzi S, Mansouri M,

Sarand SP, Marofi F, Akbari M, Xu H, Shotorbani SS. miR-193a-5p as a promising therapeutic candidate in colorectal cancer by reducing 5-FU and Oxaliplatin chemoresistance by targeting CXCR4. *Int Immunopharmacol* 2021; **92**: 107355 [PMID: 33429333 DOI: 10.1016/j.intimp.2020.107355]

64 **Liang H**, Xu Y, Zhang Q, Yang Y, Mou Y, Gao Y, Chen R, Chen C, Dai P. MiR-483-3p regulates oxaliplatin resistance by targeting FAM171B in human colorectal cancer cells. *Artif Cells Nanomed Biotechnol* 2019; **47**: 725-736 [PMID: 30861353 DOI: 10.1080/21691401.2019.1569530]

65 **Zhang L**, Chen H, Song Y, Gu Q, Zhang L, Xie Q, Xu J, Zhang M. MiR-325 Promotes Oxaliplatin-Induced Cytotoxicity Against Colorectal Cancer Through the HSPA12B/PI3K/AKT/Bcl-2 Pathway. *Dig Dis Sci* 2021; **66**: 2651-2660 [PMID: 32914380 DOI: 10.1007/s10620-020-06579-7]

66 **Poel D**, Boyd LNC, Beekhof R, Schelfhorst T, Pham TV, Piersma SR, Knol JC, Jimenez CR, Verheul HMW, Buffart TE. Proteomic Analysis of miR-195 and miR-497 Replacement Reveals Potential Candidates that Increase Sensitivity to Oxaliplatin in MSI/P53wt Colorectal Cancer Cells. *Cells* 2019; **8** [PMID: 31546954 DOI: 10.3390/cells8091111]

67 **Chen L**, He M, Zhang M, Sun Q, Zeng S, Zhao H, Yang H, Liu M, Ren S, Meng X, Xu H. The Role of non-coding RNAs in colorectal cancer, with a focus on its autophagy. *Pharmacol Ther* 2021; **226**: 107868 [PMID: 33901505 DOI: 10.1016/j.pharmthera.2021.107868]

68 **Sun C**, Wang FJ, Zhang HG, Xu XZ, Jia RC, Yao L, Qiao PF. miR-34a mediates oxaliplatin resistance of colorectal cancer cells by inhibiting macroautophagy *via* transforming growth factor-β/Smad4 pathway. *World J Gastroenterol* 2017; **23**: 1816-1827 [PMID: 28348487 DOI: 10.3748/wjg.v23.i10.1816]

69 **Sun W**, Li J, Zhou L, Han J, Liu R, Zhang H, Ning T, Gao Z, Liu B, Chen X, Ba Y. The c-Myc/miR-27b-3p/ATG10 regulatory axis regulates chemoresistance in colorectal cancer. *Theranostics* 2020; **10**: 1981-1996 [PMID: 32104496 DOI: 10.7150/thno.37621]

70 **Liu XY**, Zhang FR, Shang JY, Liu YY, Lv XF, Yuan JN, Zhang TT, Li K, Lin XC, Liu X, Lei Q, Fu XD, Zhou JG, Liang SJ. Renal inhibition of miR-181a ameliorates 5-fluorouracil-induced mesangial cell apoptosis and nephrotoxicity. *Cell Death Dis* 2018; **9**: 610 [PMID: 29795190 DOI: 10.1038/s41419-018-0677-8]

71 **Van Houdt WJ**, Emmink BL, Pham TV, Piersma SR, Verheem A, Vries RG, Fratantoni SA, Pronk A, Clevers H, Borel Rinkes IH, Jimenez CR, Kranenburg O. Comparative proteomics of colon cancer stem cells and differentiated tumor cells identifies BIRC6 as a potential therapeutic target. *Mol Cell Proteomics* 2011; **10**: M111.011353 [PMID: 21788403 DOI: 10.1074/mcp.M111.011353]

72 **Chen LL**. Linking Long Noncoding RNA Localization and Function. *Trends Biochem Sci* 2016; **41**: 761-772 [PMID: 27499234 DOI: 10.1016/j.tibs.2016.07.003]

73 **Connerty P**, Lock RB, de Bock CE. Long Non-coding RNAs: Major Regulators of Cell Stress in Cancer. *Front Oncol* 2020; **10**: 285 [PMID: 32266130 DOI: 10.3389/fonc.2020.00285]

74 **Gao N**, Li Y, Li J, Gao Z, Yang Z, Liu H, Fan T. Long Non-Coding RNAs: The Regulatory Mechanisms, Research Strategies, and Future Directions in Cancers. *Front Oncol* 2020; **10**: 598817 [PMID: 33392092 DOI: 10.3389/fonc.2020.598817]

75 **Rai MI**, Alam M, Lightfoot DA, Gurha P, Afzal AJ. Classification and experimental identification of plant long non-coding RNAs. *Genomics* 2019; **111**: 997-1005 [PMID: 29679643 DOI: 10.1016/j.ygeno.2018.04.014]

76 **Sun Q**, Hao Q, Prasanth KV. Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression. *Trends Genet* 2018; **34**: 142-157 [PMID: 29249332 DOI: 10.1016/j.tig.2017.11.005]

77 **Postepska-Igielska A**, Giwojna A, Gasri-Plotnitsky L, Schmitt N, Dold A, Ginsberg D, Grummt I. LncRNA Khps1 Regulates Expression of the Proto-oncogene SPHK1 *via* Triplex-Mediated Changes in Chromatin Structure. *Mol Cell* 2015; **60**: 626-636 [PMID: 26590717 DOI: 10.1016/j.molcel.2015.10.001]

78 **Xu M**, Chen X, Lin K, Zeng K, Liu X, Pan B, Xu X, Xu T, Hu X, Sun L, He B, Pan Y, Sun H, Wang S. The long noncoding RNA SNHG1 regulates colorectal cancer cell growth through interactions with EZH2 and miR-154-5p. *Mol Cancer* 2018; **17**: 141 [PMID: 30266084 DOI: 10.1186/s12943-018-0894-x]

79 **Lan Z**, Yao X, Sun K, Li A, Liu S, Wang X. The Interaction Between lncRNA SNHG6 and hnRNPA1 Contributes to the Growth of Colorectal Cancer by Enhancing Aerobic Glycolysis Through the Regulation of Alternative Splicing of PKM. *Front Oncol* 2020; **10**: 363 [PMID: 32296635 DOI: 10.3389/fonc.2020.00363]

80 **Chen QN**, Wei CC, Wang ZX, Sun M. Long non-coding RNAs in anti-cancer drug resistance. *Oncotarget* 2017; **8**: 1925-1936 [PMID: 27713133 DOI: 10.18632/oncotarget.12461]

81 **Hu Y**, Zhu QN, Deng JL, Li ZX, Wang G, Zhu YS. Emerging role of long non-coding RNAs in cisplatin resistance. *Onco Targets Ther* 2018; **11**: 3185-3194 [PMID: 29881292 DOI: 10.2147/OTT.S158104]

82 **Jiang X**, Li Q, Zhang S, Song C, Zheng P. Long noncoding RNA GIHCG induces cancer progression and chemoresistance and indicates poor prognosis in colorectal cancer. *Onco Targets Ther* 2019; **12**: 1059-1070 [PMID: 30799935 DOI: 10.2147/OTT.S192290]

83 **Li T**, Jin X, Dong J, Deng H. Long noncoding RNA ARSR is associated with a poor prognosis in patients with colorectal cancer. *J Gene Med* 2020; **22**: e3241 [PMID: 32558022 DOI: 10.1002/jgm.3241]

84 **Yamamura S**, Imai-Sumida M, Tanaka Y, Dahiya R. Interaction and cross-talk between non-coding RNAs. *Cell Mol Life Sci* 2018; **75**: 467-484 [PMID: 28840253 DOI: 10.1007/s00018-017-2626-6]

85 **Weng X**, Liu H, Ruan J, Du M, Wang L, Mao J, Cai Y, Lu X, Chen W, Huang Y, Zhi X, Shan J. HOTAIR/miR-1277-5p/ZEB1 axis mediates hypoxia-induced oxaliplatin resistance *via* regulating epithelial-mesenchymal transition in colorectal cancer. *Cell Death Discov* 2022; **8**: 310 [PMID: 35798695 DOI: 10.1038/s41420-022-01096-0]

86 **Li P**, Zhang X, Wang H, Wang L, Liu T, Du L, Yang Y, Wang C. MALAT1 Is Associated with Poor Response to Oxaliplatin-Based Chemotherapy in Colorectal Cancer Patients and Promotes Chemoresistance through EZH2. *Mol Cancer Ther* 2017; **16**: 739-751 [PMID: 28069878 DOI: 10.1158/1535-7163.MCT-16-0591]

87 **Thomson DW**, Dinger ME. Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet* 2016; **17**: 272-283 [PMID: 27040487 DOI: 10.1038/nrg.2016.20]

88 **Fan C**, Yuan Q, Liu G, Zhang Y, Yan M, Sun Q, Zhu C. Long non-coding RNA MALAT1 regulates oxaliplatin-resistance *via* miR-324-3p/ADAM17 axis in colorectal cancer cells. *Cancer Cell Int* 2020; **20**: 473 [PMID: 33005106

DOI: 10.1186/s12935-020-01549-5]
89 **Liang J**, Tian XF, Yang W. Effects of long non-coding RNA Opa-interacting protein 5 antisense RNA 1 on colon cancer cell resistance to oxaliplatin and its regulation of microRNA-137. *World J Gastroenterol* 2020; **26**: 1474-1489 [PMID: 32308348 DOI: 10.3748/wjg.v26.i13.1474]
90 **Yue B**, Cai D, Liu C, Fang C, Yan D. Linc00152 Functions as a Competing Endogenous RNA to Confer Oxaliplatin Resistance and Holds Prognostic Values in Colon Cancer. *Mol Ther* 2016; **24**: 2064-2077 [PMID: 27633443 DOI: 10.1038/mt.2016.180]
91 **Zhou L**, Li J, Liao M, Zhang Q, Yang M. LncRNA MIR155HG induces M2 macrophage polarization and drug resistance of colorectal cancer cells by regulating ANXA2. *Cancer Immunol Immunother* 2022; **71**: 1075-1091 [PMID: 34562123 DOI: 10.1007/s00262-021-03055-7]
92 **Gao R**, Fang C, Xu J, Tan H, Li P, Ma L. LncRNA CACS15 contributes to oxaliplatin resistance in colorectal cancer by positively regulating ABCC1 through sponging miR-145. *Arch Biochem Biophys* 2019; **663**: 183-191 [PMID: 30639170 DOI: 10.1016/j.abb.2019.01.005]
93 **Xie L**, Cui G, Li T. Long Noncoding RNA CBR3-AS1 Promotes Stem-like Properties and Oxaliplatin Resistance of Colorectal Cancer by Sponging miR-145-5p. *J Oncol* 2022; **2022**: 2260211 [PMID: 35466320 DOI: 10.1155/2022/2260211]
94 **Gao H**, Song X, Kang T, Yan B, Feng L, Gao L, Ai L, Liu X, Yu J, Li H. Long noncoding RNA *CRNDE* functions as a competing endogenous RNA to promote metastasis and oxaliplatin resistance by sponging miR-136 in colorectal cancer. *Onco Targets Ther* 2017; **10**: 205-216 [PMID: 28115855 DOI: 10.2147/OTT.S116178]
95 **Meng X**, Sun W, Yu J, Zhou Y, Gu Y, Han J, Zhou L, Jiang X, Wang C. LINC00460-miR-149-5p/miR-150-5p-Mutant p53 Feedback Loop Promotes Oxaliplatin Resistance in Colorectal Cancer. *Mol Ther Nucleic Acids* 2020; **22**: 1004-1015 [PMID: 33251049 DOI: 10.1016/j.omtn.2020.10.018]
96 **Li Y**, Li C, Li D, Yang L, Jin J, Zhang B. lncRNA KCNQ1OT1 enhances the chemoresistance of oxaliplatin in colon cancer by targeting the miR-34a/ATG4B pathway. *Onco Targets Ther* 2019; **12**: 2649-2660 [PMID: 31040703 DOI: 10.2147/OTT.S188054]
97 **Li Q**, Sun H, Luo D, Gan L, Mo S, Dai W, Liang L, Yang Y, Xu M, Li J, Zheng P, Li X, Li Y, Wang Z. Lnc-RP11-536 K7.3/SOX2/HIF-1α signaling axis regulates oxaliplatin resistance in patient-derived colorectal cancer organoids. *J Exp Clin Cancer Res* 2021; **40**: 348 [PMID: 34740372 DOI: 10.1186/s13046-021-02143-x]
98 **Sun J**, Zhou H, Bao X, Wu Y, Jia H, Zhao H, Liu G. lncRNA TUG1 Facilitates Colorectal Cancer Stem Cell Characteristics and Chemoresistance by Enhancing GATA6 Protein Stability. *Stem Cells Int* 2021; **2021**: 1075481 [PMID: 34858502 DOI: 10.1155/2021/1075481]
99 **Zhou Q**, Hou Z, Zuo S, Zhou X, Feng Y, Sun Y, Yuan X. LUCAT1 promotes colorectal cancer tumorigenesis by targeting the ribosomal protein L40-MDM2-p53 pathway through binding with UBA52. *Cancer Sci* 2019; **110**: 1194-1207 [PMID: 30690837 DOI: 10.1111/cas.13951]
100 **Deng X**, Kong F, Li S, Jiang H, Dong L, Xu X, Zhang X, Yuan H, Xu Y, Chu Y, Peng H, Guan M. A KLF4/PiHL/EZH2/HMGA2 regulatory axis and its function in promoting oxaliplatin-resistance of colorectal cancer. *Cell Death Dis* 2021; **12**: 485 [PMID: 33986248 DOI: 10.1038/s41419-021-03753-1]
101 **Quinodoz S**, Guttman M. Long noncoding RNAs: an emerging link between gene regulation and nuclear organization. *Trends Cell Biol* 2014; **24**: 651-663 [PMID: 25441720 DOI: 10.1016/j.tcb.2014.08.009]
102 **Li Y**, Gan Y, Liu J, Li J, Zhou Z, Tian R, Sun R, Xiao Q, Li Y, Lu P, Peng Y, Shu G, Yin G. Downregulation of MEIS1 mediated by ELFN1-AS1/EZH2/DNMT3a axis promotes tumorigenesis and oxaliplatin resistance in colorectal cancer. *Signal Transduct Target Ther* 2022; **7**: 87 [PMID: 35351858 DOI: 10.1038/s41392-022-00902-6]
103 **Damas ND**, Marcatti M, Côme C, Christensen LL, Nielsen MM, Baumgartner R, Gylling HM, Maglieri G, Rundsten CF, Seemann SE, Rapin N, Thézenas S, Vang S, Ørntoft T, Andersen CL, Pedersen JS, Lund AH. SNHG5 promotes colorectal cancer cell survival by counteracting STAU1-mediated mRNA destabilization. *Nat Commun* 2016; **7**: 13875 [PMID: 28004750 DOI: 10.1038/ncomms13875]
104 **Chen B**, Dragomir MP, Fabris L, Bayraktar R, Knutsen E, Liu X, Tang C, Li Y, Shimura T, Ivkovic TC, De Los Santos MC, Anfossi S, Shimizu M, Shah MY, Ling H, Shen P, Multani AS, Pardini B, Burks JK, Katayama H, Reineke LC, Huo L, Syed M, Song S, Ferracin M, Oki E, Fromm B, Ivan C, Bhuvaneshwar K, Gusev Y, Mimori K, Menter D, Sen S, Matsuyama T, Uetake H, Vasilescu C, Kopetz S, Parker-Thornburg J, Taguchi A, Hanash SM, Girnita L, Slaby O, Goel A, Varani G, Gagea M, Li C, Ajani JA, Calin GA. The Long Noncoding RNA CCAT2 Induces Chromosomal Instability Through BOP1-AURKB Signaling. *Gastroenterology* 2020; **159**: 2146-2162.e33 [PMID: 32805281 DOI: 10.1053/j.gastro.2020.08.018]
105 **Li C**, Li X. Antitumor Activity of lncRNA NBAT-1 *via* Inhibition of miR-4504 to Target to WWC3 in Oxaliplatin-Resistant Colorectal Carcinoma. *J Healthc Eng* 2022; **2022**: 9121554 [PMID: 35494512 DOI: 10.1155/2022/9121554]
106 **Li L**, Shang J, Zhang Y, Liu S, Peng Y, Zhou Z, Pan H, Wang X, Chen L, Zhao Q. MEG3 is a prognostic factor for CRC and promotes chemosensitivity by enhancing oxaliplatin-induced cell apoptosis. *Oncol Rep* 2017; **38**: 1383-1392 [PMID: 28731151 DOI: 10.3892/or.2017.5828]
107 **Wang H**, Li H, Zhang L, Yang D. Overexpression of MEG3 sensitizes colorectal cancer cells to oxaliplatin through regulation of miR-141/PDCD4 axis. *Biomed Pharmacother* 2018; **106**: 1607-1615 [PMID: 30119236 DOI: 10.1016/j.biopha.2018.07.131]
108 **Wang X**, Zhang H, Yin S, Yang Y, Yang H, Yang J, Zhou Z, Li S, Ying G, Ba Y. lncRNA-encoded pep-AP attenuates the pentose phosphate pathway and sensitizes colorectal cancer cells to Oxaliplatin. *EMBO Rep* 2022; **23**: e53140 [PMID: 34779552 DOI: 10.15252/embr.202153140]
109 **Wu S**, Yang X, Tang W, Familiari G, Relucenti M, Aschner M, Li X, Chen R. Chemotherapeutic Risk lncRNA-PVT1 SNP Sensitizes Metastatic Colorectal Cancer to FOLFOX Regimen. *Front Oncol* 2022; **12**: 808889 [PMID: 35433465 DOI: 10.3389/fonc.2022.808889]
110 **Sun F**, Liang W, Qian J. The identification of CRNDE, H19, UCA1 and HOTAIR as the key lncRNAs involved in oxaliplatin or irinotecan resistance in the chemotherapy of colorectal cancer based on integrative bioinformatics analysis.

Mol Med Rep 2019; **20**: 3583-3596 [PMID: 31432188 DOI: 10.3892/mmr.2019.10588]
111 **Zhang F**, Wang H, Yu J, Yao X, Yang S, Li W, Xu L, Zhao L. LncRNA CRNDE attenuates chemoresistance in gastric cancer *via* SRSF6-regulated alternative splicing of PICALM. *Mol Cancer* 2021; **20**: 6 [PMID: 33397371 DOI: 10.1186/s12943-020-01299-y]
112 **Liu J**, Liu T, Wang X, He A. Circles reshaping the RNA world: from waste to treasure. *Mol Cancer* 2017; **16**: 58 [PMID: 28279183 DOI: 10.1186/s12943-017-0630-y]
113 **Huang A**, Zheng H, Wu Z, Chen M, Huang Y. Circular RNA-protein interactions: functions, mechanisms, and identification. *Theranostics* 2020; **10**: 3503-3517 [PMID: 32206104 DOI: 10.7150/thno.42174]
114 **Wang J**, Zhang Y, Liu L, Yang T, Song J. Circular RNAs: new biomarkers of chemoresistance in cancer. *Cancer Biol Med* 2021 [PMID: 33738995 DOI: 10.20892/j.issn.2095-3941.2020.0312]
115 **Mao G**, Zhou B, Xu W, Jiao N, Wu Z, Li J, Liu Y. Hsa_circ_0040809 regulates colorectal cancer development by upregulating methyltransferase DNMT1 *via* targeting miR-515-5p. *J Gene Med* 2021; **23**: e3388 [PMID: 34438465 DOI: 10.1002/jgm.3388]
116 **Li Z**, Yao H, Wang S, Li G, Gu X. CircTADA2A suppresses the progression of colorectal cancer *via* miR-374a-3p/KLF14 axis. *J Exp Clin Cancer Res* 2020; **39**: 160 [PMID: 32799891 DOI: 10.1186/s13046-020-01642-7]
117 **Zhou J**, Wang L, Sun Q, Chen R, Zhang C, Yang P, Tan Y, Peng C, Wang T, Jin C, Ji J, Jin K, Sun Y. Hsa_circ_0001666 suppresses the progression of colorectal cancer through the miR-576-5p/PCDH10 axis. *Clin Transl Med* 2021; **11**: e565 [PMID: 34841662 DOI: 10.1002/ctm2.565]
118 **Zhang N**, Zhang X, Xu W, Mu Z. CircRNA_103948 inhibits autophagy in colorectal cancer in a ceRNA manner. *Ann N Y Acad Sci* 2021; **1503**: 88-101 [PMID: 34480353 DOI: 10.1111/nyas.14679]
119 **Feng J**, Li Z, Li L, Xie H, Lu Q, He X. Hypoxiainduced circCCDC66 promotes the tumorigenesis of colorectal cancer *via* the miR3140/autophagy pathway. *Int J Mol Med* 2020; **46**: 1973-1982 [PMID: 33125087 DOI: 10.3892/ijmm.2020.4747]
120 **Chen Z**, Ren R, Wan D, Wang Y, Xue X, Jiang M, Shen J, Han Y, Liu F, Shi J, Kuang Y, Li W, Zhi Q. Hsa_circ_101555 functions as a competing endogenous RNA of miR-597-5p to promote colorectal cancer progression. *Oncogene* 2019; **38**: 6017-6034 [PMID: 31300733 DOI: 10.1038/s41388-019-0857-8]
121 **Hansen TB**, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013; **495**: 384-388 [PMID: 23446346 DOI: 10.1038/nature11993]
122 **Jiang Z**, Hou Z, Liu W, Yu Z, Liang Z, Chen S. Circular RNA protein tyrosine kinase 2 (circPTK2) promotes colorectal cancer proliferation, migration, invasion and chemoresistance. *Bioengineered* 2022; **13**: 810-823 [PMID: 34974791 DOI: 10.1080/21655979.2021.2012952]
123 **Li S**, Zheng S. Down-Regulation of Circ_0032833 Sensitizes Colorectal Cancer to 5-Fluorouracil and Oxaliplatin Partly Depending on the Regulation of miR-125-5p and MSI1. *Cancer Manag Res* 2020; **12**: 11257-11269 [PMID: 33177876 DOI: 10.2147/CMAR.S270123]
124 **Lai M**, Liu G, Li R, Bai H, Zhao J, Xiao P, Mei J. Hsa_circ_0079662 induces the resistance mechanism of the chemotherapy drug oxaliplatin through the TNF-α pathway in human colon cancer. *J Cell Mol Med* 2020; **24**: 5021-5027 [PMID: 32243061 DOI: 10.1111/jcmm.15122]
125 **Zhang Y**, Li C, Liu X, Wang Y, Zhao R, Yang Y, Zheng X, Zhang Y, Zhang X. circHIPK3 promotes oxaliplatin-resistance in colorectal cancer through autophagy by sponging miR-637. *EBioMedicine* 2019; **48**: 277-288 [PMID: 31631038 DOI: 10.1016/j.ebiom.2019.09.051]
126 **Lin YC**, Yu YS, Lin HH, Hsiao KY. Oxaliplatin-Induced DHX9 Phosphorylation Promotes Oncogenic Circular RNA CCDC66 Expression and Development of Chemoresistance. *Cancers (Basel)* 2020; **12** [PMID: 32187976 DOI: 10.3390/cancers12030697]
127 **Abu N**, Hon KW, Jeyaraman S, Yahaya A, Abdullah NM, Mustangin M, Sulaiman SA, Jamal R, Ab-Mutalib NS. Identification of differentially expressed circular RNAs in chemoresistant colorectal cancer. *Epigenomics* 2019; **11**: 875-884 [PMID: 31020847 DOI: 10.2217/epi-2019-0042]
128 **Hon KW**, Ab-Mutalib NS, Abdullah NMA, Jamal R, Abu N. Extracellular Vesicle-derived circular RNAs confers chemoresistance in Colorectal cancer. *Sci Rep* 2019; **9**: 16497 [PMID: 31712601 DOI: 10.1038/s41598-019-53063-y]
129 **Jin G**, Liu Y, Zhang J, Bian Z, Yao S, Fei B, Zhou L, Yin Y, Huang Z. A panel of serum exosomal microRNAs as predictive markers for chemoresistance in advanced colorectal cancer. *Cancer Chemother Pharmacol* 2019; **84**: 315-325 [PMID: 31089750 DOI: 10.1007/s00280-019-03867-6]
130 **Jiang Z**, Hou Z, Li L, Liu W, Yu Z, Chen S. Exosomal circEPB41L2 serves as a sponge for miR-21-5p and miR-942-5p to suppress colorectal cancer progression by regulating the PTEN/AKT signalling pathway. *Eur J Clin Invest* 2021; **51**: e13581 [PMID: 34022068 DOI: 10.1111/eci.13581]
131 **Ding B**, Yao M, Fan W, Lou W. Whole-transcriptome analysis reveals a potential hsa_circ_0001955/hsa_circ_0000977-mediated miRNA-mRNA regulatory sub-network in colorectal cancer. *Aging (Albany NY)* 2020; **12**: 5259-5279 [PMID: 32221048 DOI: 10.18632/aging.102945]
132 **Van Niel G**, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* 2018; **19**: 213-228 [PMID: 29339798 DOI: 10.1038/nrm.2017.125]
133 **Raposo G**, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013; **200**: 373-383 [PMID: 23420871 DOI: 10.1083/jcb.201211138]
134 **Kalluri R**. The biology and function of exosomes in cancer. *J Clin Invest* 2016; **126**: 1208-1215 [PMID: 27035812 DOI: 10.1172/JCI81135]
135 **Zhu L**, Sun HT, Wang S, Huang SL, Zheng Y, Wang CQ, Hu BY, Qin W, Zou TT, Fu Y, Shen XT, Zhu WW, Geng Y, Lu L, Jia HL, Qin LX, Dong QZ. Isolation and characterization of exosomes for cancer research. *J Hematol Oncol* 2020; **13**: 152 [PMID: 33168028 DOI: 10.1186/s13045-020-00987-y]
136 **Yu D**, Li Y, Wang M, Gu J, Xu W, Cai H, Fang X, Zhang X. Exosomes as a new frontier of cancer liquid biopsy. *Mol Cancer* 2022; **21**: 56 [PMID: 35180868 DOI: 10.1186/s12943-022-01509-9]
137 **Kalluri R**, LeBleu VS. The biology**,** function**,** and biomedical applications of exosomes. *Science* 2020; **367** [PMID: 32029601 DOI: 10.1126/science.aau6977]

138 **Yokoi A**, Ochiya T. Exosomes and extracellular vesicles: Rethinking the essential values in cancer biology. *Semin Cancer Biol* 2021; **74**: 79-91 [PMID: 33798721 DOI: 10.1016/j.semcancer.2021.03.032]

139 **He C**, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. *Theranostics* 2018; **8**: 237-255 [PMID: 29290805 DOI: 10.7150/thno.21945]

140 **Baassiri A**, Nassar F, Mukherji D, Shamseddine A, Nasr R, Temraz S. Exosomal Non Coding RNA in LIQUID Biopsies as a Promising Biomarker for Colorectal Cancer. *Int J Mol Sci* 2020; **21** [PMID: 32092975 DOI: 10.3390/ijms21041398]

141 **Ren J**, Ding L, Zhang D, Shi G, Xu Q, Shen S, Wang Y, Wang T, Hou Y. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. *Theranostics* 2018; **8**: 3932-3948 [PMID: 30083271 DOI: 10.7150/thno.25541]

142 **Deng X**, Ruan H, Zhang X, Xu X, Zhu Y, Peng H, Kong F, Guan M. Long noncoding RNA CCAL transferred from fibroblasts by exosomes promotes chemoresistance of colorectal cancer cells. *Int J Cancer* 2020; **146**: 1700-1716 [PMID: 31381140 DOI: 10.1002/ijc.32608]

143 **Qu Z**, Yang KD, Luo BH, Zhang F. CAFs-secreted exosomal cricN4BP2L2 promoted colorectal cancer stemness and chemoresistance by interacting with EIF4A3. *Exp Cell Res* 2022; **418**: 113266 [PMID: 35752345 DOI: 10.1016/j.yexcr.2022.113266]

144 **Li C**, Li X. Exosome-Derived Circ_0094343 Promotes Chemosensitivity of Colorectal Cancer Cells by Regulating Glycolysis *via* the miR-766-5p/TRIM67 Axis. *Contrast Media Mol Imaging* 2022; **2022**: 2878557 [PMID: 35854778 DOI: 10.1155/2022/2878557]

145 **Ning T**, Li J, He Y, Zhang H, Wang X, Deng T, Liu R, Li H, Bai M, Fan Q, Zhu K, Ying G, Ba Y. Exosomal miR-208b related with oxaliplatin resistance promotes Treg expansion in colorectal cancer. *Mol Ther* 2021; **29**: 2723-2736 [PMID: 33905821 DOI: 10.1016/j.ymthe.2021.04.028]

146 **Xu Y**, Zhu M. Novel exosomal miR-46146 transfer oxaliplatin chemoresistance in colorectal cancer. *Clin Transl Oncol* 2020; **22**: 1105-1116 [PMID: 31728833 DOI: 10.1007/s12094-019-02237-1]

147 **Xiao Z**, Liu Y, Li Q, Liu Q, Luo Y, Wei S. EVs delivery of miR-1915-3p improves the chemotherapeutic efficacy of oxaliplatin in colorectal cancer. *Cancer Chemother Pharmacol* 2021; **88**: 1021-1031 [PMID: 34599680 DOI: 10.1007/s00280-021-04348-5]

148 **Wang X**, Zhang H, Yang H, Bai M, Ning T, Deng T, Liu R, Fan Q, Zhu K, Li J, Zhan Y, Ying G, Ba Y. Exosome-delivered circRNA promotes glycolysis to induce chemoresistance through the miR-122-PKM2 axis in colorectal cancer. *Mol Oncol* 2020; **14**: 539-555 [PMID: 31901148 DOI: 10.1002/1878-0261.12629]

149 **Ohzawa H**, Kimura Y, Saito A, Yamaguchi H, Miyato H, Sakuma Y, Horie H, Hosoya Y, Lefor AK, Sata N, Kitayama J. Ratios of miRNAs in Peritoneal Exosomes are Useful Biomarkers to Predict Tumor Response to Intraperitoneal Chemotherapy in Patients with Peritoneal Metastases from Gastric Cancer. *Ann Surg Oncol* 2020; **27**: 5057-5064 [PMID: 32804324 DOI: 10.1245/s10434-020-09007-2]

150 **Yagi T**, Iinuma H, Hayama T, Matsuda K, Nozawa K, Tsukamoto M, Shimada R, Akahane T, Tsuchiya T, Ozawa T, Hashiguchi Y. Plasma exosomal microRNA-125b as a monitoring biomarker of resistance to mFOLFOX6-based chemotherapy in advanced and recurrent colorectal cancer patients. *Mol Clin Oncol* 2019; **11**: 416-424 [PMID: 31497299 DOI: 10.3892/mco.2019.1911]

151 **Sur D**, Balacescu L, Cainap SS, Visan S, Pop L, Burz C, Havasi A, Buiga R, Cainap C, Irimie A, Balacescu O. Predictive Efficacy of MiR-125b-5p, MiR-17-5p, and MiR-185-5p in Liver Metastasis and Chemotherapy Response Among Advanced Stage Colorectal Cancer Patients. *Front Oncol* 2021; **11**: 651380 [PMID: 34084747 DOI: 10.3389/fonc.2021.651380]

152 **Kim H**, Kim EH, Kwak G, Chi SG, Kim SH, Yang Y. Exosomes: Cell-Derived Nanoplatforms for the Delivery of Cancer Therapeutics. *Int J Mol Sci* 2020; **22** [PMID: 33374978 DOI: 10.3390/ijms22010014]

153 **Zhang Y**, Liu Q, Zhang X, Huang H, Tang S, Chai Y, Xu Z, Li M, Chen X, Liu J, Yang C. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. *J Nanobiotechnology* 2022; **20**: 279 [PMID: 35701788 DOI: 10.1186/s12951-022-01472-z]

154 **Hui B**, Lu C, Wang J, Xu Y, Yang Y, Ji H, Li X, Xu L, Tang W, Wang K, Gu Y. Engineered exosomes for co-delivery of PGM5-AS1 and oxaliplatin to reverse drug resistance in colon cancer. *J Cell Physiol* 2022; **237**: 911-933 [PMID: 34463962 DOI: 10.1002/jcp.30566]

155 **Pi F**, Binzel DW, Lee TJ, Li Z, Sun M, Rychahou P, Li H, Haque F, Wang S, Croce CM, Guo B, Evers BM, Guo P. Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression. *Nat Nanotechnol* 2018; **13**: 82-89 [PMID: 29230043 DOI: 10.1038/s41565-017-0012-z]

156 **Liang G**, Zhu Y, Ali DJ, Tian T, Xu H, Si K, Sun B, Chen B, Xiao Z. Engineered exosomes for targeted co-delivery of miR-21 inhibitor and chemotherapeutics to reverse drug resistance in colon cancer. *J Nanobiotechnology* 2020; **18**: 10 [PMID: 31918721 DOI: 10.1186/s12951-019-0563-2]

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REVIEW

Microbiota of the gastrointestinal tract: Friend or foe?

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Abstract

The gut microbiota is currently considered an external organ of the human body that provides important mechanisms of metabolic regulation and protection. The gut microbiota encodes over 3 million genes, which is approximately 150 times more than the total number of genes present in the human genome. Changes in the qualitative and quantitative composition of the microbiome lead to disruption in the synthesis of key bacterial metabolites, changes in intestinal barrier function, and inflammation and can cause the development of a wide variety of diseases, such as diabetes, obesity, gastrointestinal disorders, cardiovascular issues, neurological disorders and oncological concerns. In this review, I consider issues related to the role of the microbiome in the regulation of intestinal barrier function, its influence on physiological and pathological processes occurring in the body, and potential new therapeutic strategies aimed at restoring the gut microbiome. Herewith, it is important to understand that the gut microbiota and human body should be considered as a single biological system, where change of one element will inevitably affect its other components. Thus, the study of the impact of the intestinal microbiota on health should be considered only taking into account numerous factors, the role of which has not yet been fully elucidated.

Key Words: Gut microbiota; Bacterial metabolites; Intestinal barrier; Dysbiosis; Fecal microbiota transplantation; Probiotics

Core Tip: The gut microbiota affects the development and functioning of all body systems, providing metabolic, physiological, regulatory and protective functions. Violations in the qualitative and quantitative composition of the microbiome lead to the development of a wide variety of diseases, such as diabetes, obesity, cardiovascular issues, neurological disorders and oncological concerns. Considering that intestinal dysbiosis plays a key role in the development of a number of diseases, aim to normalize the microbiome seems to be a greatly perspective direction in their prevention and treatment.

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INTRODUCTION

Trillions of microorganisms, known as microbiota, colonize the human body. The human gastrointestinal tract harbours more than 1000 species of bacteria belonging to a relatively few known bacterial phyla[1]. Features of their mutual coexistence determine the nature of various physiological and pathological processes occurring in the human body. To discuss these issues, a search was made in the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) and *Reference Citation Analysis* (https://www.referencecitationanalysis.com/) for studies published up to July 1, 2022 using a combination of text keywords "gut microbiota", "bacterial metabolites", "intestinal barrier", "dysbiosis", "fecal microbiota transplantation", and "probiotics". A total of 846 unique results were identified, which were screened individually by title and abstract and were included based on the role of the microbiome in the regulation of intestinal barrier function, its influence on physiological and pathological processes occurring in the body, and new therapeutic strategies aimed at restoring the gut microbiome.

It is believed that bacteria begin to colonize the human intestine immediately after birth and, possibly, even in utero[2,3]. Breast milk plays a crucial role in the composition of the microbial community within newborns through transfer of the milk microbiota to the infant's gut[4]. In the first 6 mo of a child's life, there is a steady increase in the number of *Enterobacteriaceae*, *Bifidobacteriaceae* and *Clostridiaceae*. However, the microbiome of children differs depending on diet, gender, race and ethnicity[5,6]. In addition, the mode of delivery may affect the composition of the gut microbiota of early infants[3,7]. In particular, in children born by cesarean section, there is a high abundance of *Bifidobacterium*, and *Clostridium* genera and the family Enterobacteriaceae, along with a low abundance of *Streptococcus* and *Ruminococcus* genera[8]. Moreover, in children born by cesarean section, the *Bacteroides* genus is not detected in the feces until 6-18 mo after birth[9].

Microorganisms living in the gut of adults include bacteria, fungi, protozoa, archaea, and some viruses[10]. The total number of bacteria in a 70 kg "reference man" is estimated at 3.8×10^{13} cells, which is comparable to the number of human host cells (3.0×10^{13})[11]. The specific microbiota at the genus and species levels varies depending on geography, environment, diet, age, genotype, presence of diseases and lifestyle[12,13]. For instance, if the prevalence of proteins and animal fats in the diet exists, *Bacteroides* will predominate in the microbiota, and if there is a high level of carbohydrates, then *Prevotella* will ascendant. The gut microbiota encodes over 3 million genes, which is around 150 times more than the number of genes in the host genome[14]. Approximately 90% of the composition of the gut microbiome is represented by *Firmicutes* (79.4%), *Bacteroidetes* (16.9%), *Actinobacteria* (2.5%) and *Proteobacteria* (1%)[5,15]. The number of microorganisms increases from the proximal to the distal gastrointestinal tract and from the epithelial layer to the lumen. This difference can be explained by the presence of a more aggressive environment in the upper intestines due to the incoming gastric acid, action of digestive enzymes, rapid movement of chyme and the decrease in partial pressure of oxygen in the distal gastrointestinal tract. For this reason, aerobic bacteria predominate in the small intestine, while facultative and obligate anaerobes predominate in the lower gastrointestinal tract. Furthermore, the distribution and organization of the gut microbiota is determined by intestinal mucins, which protect intestinal epithelial cells (IECs) from bacterial colonization. At the same time, the presence of the gut microbiota is a necessary condition for normal functioning of the mucosal barrier[16]. For example, mice treated with antibiotics had a thinner layer of mucus[17,18].

GUT MICROBIOTA AND INTESTINAL BARRIER

Partition of the body's internal environment from the intestinal microbiota is carried out by three types of barriers: physical, chemical and immunological. The physical barrier consists of epithelial cells, glycocalyx and a layer of mucus covering the surface of the gastrointestinal wall[19].

Physical barrier

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. To maintain epithelial layer integrity, IECs are continuously replaced by proliferating progenitor cells derived from multipotent intestinal stem cells (ISCs) localized in the base of the crypts of Lieberkühn and the colon [20]. IECs differ in their proliferation ability, renewal rate and age. Aged IECs undergo apoptosis and are later ejected into the intestinal lumen, whereas Paneth cells leave the crypt bottom by cellular fragmentation and phagocytosis by macrophages infiltrating the lamina propria mucosae[21]. Under homeostatic conditions, the entire ileal crypt is replaced every 4-5 d[22].

It is customary to distinguish the following populations of the intestinal epithelium[22-24].

Columnar cells: Columnar cells (colonocytes) are the most numerous population of enterocytes. They participate in digestion due to the secretion of digestive enzymes, the absorption of digested products and transcellular transfer of dissociated monomers into the blood and lymph and take part in the exchange of bile acids and humoral immune response. Absorbent enterocytes produce polymeric immunoglobulin (Ig) receptor, which mediates transcytosis of dimeric IgA and polymeric IgM from the lamina propria through the epithelial barrier to the mucosal surface, ensuring the binding of bacteria and viruses on their surface and thereby preventing the penetration of pathogens into the internal environment of the body. In addition, during transcytosis of IgA through the epithelium, it can neutralize viruses that have entered cells, as well as bind and excrete proteins and other immune complexes on the surface of mucous[20,25].

Goblet cells: Goblet cells are a source of mucus. In addition, they can deliver small soluble antigens to dendritic cells (DCs) localized in the lamina propria and thus participate in the formation of immune tolerance to food antigens and the gut microbiome[20,26].

Enteroendocrine cells: Enteroendocrine cells secrete peptides and hormones (cholecystokinin, serotonin) to stimulate intestinal motility.

Tuft cells: Tuft cells participate in the clearance of parasites from the intestinal lumen due to the synthesis of interleukin (IL)-25, which is the key activator of type 2 innate lymphoid cells.

Paneth cells: Paneth cells in the small intestine or deep crypt secretory cells in the large intestine are the main regulators of microbial density in the intestines. When interacting with gram-negative bacteria, gram-positive bacteria or their products (lipopolysaccharides, lipoteichoic acids, lipid A, muramyl peptide), they secrete antimicrobial peptides (AMPs)[20,27,28]. Moreover, Paneth cells secrete important factors, such as epidermal growth factor, transforming growth factor-α, and Wnt ligands involved in stem cell maintenance[29].

Microfold cells: Microfold cells (M) are located in the follicle-associated epithelium overlying Peyer's patches and stimulate an immune response by binding luminal antigens for their further transport to subepithelial regions, where they are captured by DCs migrating to the mesenteric lymph nodes and stimulating the immune response[20]. The interaction of DCs with T cells stimulates an antigen-specific immune response directed against the pathogen or, conversely, leads to the induction of tolerance. Activation of B cells leads to the secretion of IgA, which plays an important role in the regulation of the gut microbiome. Immunoglobulin A, on the one hand, is involved in the binding and elimination of pathogenic bacteria, and on the other hand, it facilitates the translocation of commensals into Peyer's patches, activating the mechanisms of immunological tolerance and thereby stimulating the growth of intestinal symbionts[30,31].

It is worth noting that at the level of IECs, a structure composed of three junctions is formed: tight junctions (TJs), adherens junctions and desmosomes[20,32-34]. They provide mechanical strength between cells, intercellular adhesion and polarization and are also involved in cell signaling pathways [19]. The strength of the mechanical barrier also depends on the regeneration rate of the intestinal epithelium. It was established that the presence of intestinal microorganisms affects the number of Paneth cells and hence the integrity of the epithelial barrier, as Paneth cells regulate stem cell homeostasis[35]. Bacteria can directly damage TJ proteins or interfere with their synthesis *via* type 3 or type 4 secretion systems. The disruption of cell contacts can also be mediated by some bacterial enzymes and toxins, such as hemagglutinin/protease and ZO toxin, as well as bacterial metabolites, such as ethanol and acetaldehyde[13,36]. Apart from that, the gut microbiota can alter the mitochondrial metabolism of epithelial and immune cells, thereby activating inflammation and disrupting epithelial barrier function[37]. Integrity violation of the intestinal epithelium, the mucus barrier and cell contacts leads to the translocation of bacteria[38].

Recognition of the bacterial microbiota is carried out by TLR and NOD receptors, which are expressed by most IECs, including stem cells, enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and M cells[19]. Most TLRs (TLR2, TLR3, TLR4, TLR5, and TLR9) are present on the basolateral membrane, while TLR2, TLR3, and TLR9 are also expressed on the apical surface. TLR5 expression is limited to Paneth cells in the epithelium of the small intestine and proximal colon[39,40]. Activation of innate immune system receptors, including TLR and NOD receptors, as well as inflammasomes, leads to

a signaling cascade that triggers the secretion of cytokines and chemokines, including IL-1β, IL-6, IL-12, IL-18, tumor necrosis factor alpha (TNF-α), CXCL8 and CCL20, which activate immunocompetent cells localized in the lamina propria[41-43]. Mitochondrial reactive oxygen species (mtROS), produced by immune cells, play a key role in the eradication of invading pathogens through direct bactericidal action or indirect impact on the activation of the NLRP3 inflammasome and the production of proinflammatory cytokines. Invading bacteria, as well as gut microbiome fermentation products such as short-chain fatty acids (SCFAs), induce mtROS production in immune cells through increased mitochondrial respiration and increased oxidative phosphorylation[44]. Hypoxia inducible factor-1α is believed to be the main regulator of mitochondrial responses during bacterial infection[37]. At the same time, damage to the intestinal epithelium mitochondria by toxins of pathogenic bacteria leads to the accumulation of mtROS and disruption of the barrier function of the epithelium[45,46].

Intestinal mucus (mucins)

Intestinal mucus is the first barrier for microorganisms in the gastrointestinal tract. It regulates nutrient and drug delivery, regulates symbiosis with the gut microbiota and protects the epithelium from dietary antigens and food toxins[20,47,48]. The thickness of the mucus layer in the small and large intestine is not the same. The main function of the small intestine is food digestion and absorption of nutrients, so the small intestine has a loose, discontinuous layer of mucus that can be easily removed. In the large intestine, where the density of microorganisms is much higher than in the small intestine, the number of mucus-producing goblet cells is significantly larger[20]. In the large intestine, the mucus layer is organized into two layers: an inner, dense, microbiota-free mucus layer and an outer layer, which is friable and permeable for microorganisms[13,17,31,49,50].

More than 20 mucin subtypes have been identified in humans. The best known and most studied one, found in the small and large intestines, is MUC2[17,49]. MUC2 is a highly O-glycosylated gelling mucin that forms polymeric networks *via* C-terminal dimerization and N-terminal trimerization. MUC2 monomers are glycosylated in proline, threonine, and serine-rich domains[51,52].

Mucus is secreted by goblet cells, grows rapidly and forms a stratified, dense layer that adheres to the epithelium[53]. On the one hand, mucus is necessary for the normal functioning of the gut microbiome, but on the other hand, the presence of the microbiome is a necessary condition for the normal functioning of the mucus barrier[54]. As noted above, mice treated with antibiotics have a thinner layer of mucus[17,18].

The barrier function of the mucus is confirmed by the fact that mice genetically deficient in Muc2 (Muc2 -/-) have bacteria invading the normally sterile distal colon crypts, which results in the development of spontaneous colitis[55], adenomas arising in the small intestine and an invasive cancer [56]. However, intestinal bacteria can directly influence the production and quality of intestinal mucus and hence the intestinal barrier permeability[57]. Bacteria and their metabolites that enter crypts are endocytosed by specialized goblet cells known as 'sentinel' goblet cells. This leads to the activation of TLR2/1, TLR4, and TLR5 Ligands with activated ROS synthesis, triggering the formation of the NLRP6 inflammasome and Ca2+-dependent compound exocytosis of MUC2-containing granules[58,59]. Importantly, increased regulatory secretion leads to the secretion of large amounts of MUC2 and the physical removal of bacteria from the crypt opening, thereby protecting the inferior crypt and multipotent ISCs, located at the bottom of the crypts, from bacterial invasion[20,60].

Chemical barrier

An important function of the chemical barrier is to maintain the abundance and composition of the gut microbiome. The chemical barrier includes AMPs, gastric acid, digestive enzymes, mucopolysaccharides, glycoproteins, glycolipids, and other compounds[19,61,62]. In addition, the composition of the microbiota can be influenced by various factors, such as hygiene, diet (especially the "Western diet" low in fiber and high in sugar and fat), oxygen concentration, microbial adhesion, host stress and other factors[17,49,63,64]. It is believed that microbiome regulation in the small intestine is mainly carried out by antibacterial peptides, while in the large intestine, this regulation is carried out through pattern recognition receptors[5]. The microbiome population is maintained, either by preventing colonization or through direct killing mechanisms.

The production of antimicrobials that lyse target cells is one of the main mechanisms for regulating the homeostasis of the gut microbiome. The contact of bacteria with Toll (TLR2, TLR4, TLR7 and TLR9), NOD1 and NOD2 receptors activates adapter proteins (for example, MyD88) and genes responsible for the synthesis of cytokines and chemokines in IECs[65-68]. In turn, the synthesis of cytokines by immune cells activates the genes responsible for the synthesis of AMPs[69]. Thus, mice deficient in MyD88 exhibit a 100-fold higher bacterial load in the gut than wild-type mice, and this increase is correlated with a decrease in the antibacterial peptide RegIIIgγ[70]. Paneth cells expressing TLR5 are major producers of antimicrobials, many of which are cationic AMPs that interact with negatively charged bacterial membranes and destroy them[12]. Interestingly, TLR5 expression occurs predominantly in intestinal crypts and is genetically determined, as TLR5 expression does not require bacterial or immune signals. It is believed that their main function may be related to the protection of Paneth cells and stem cells[31,71].

Other AMPs are also involved in the regulation of the gut microbiome population. For example, protein 8 (Lypd8) is highly expressed by colonocytes and facilitates segregation of microorganisms in the colon *via* flagella binding[61]. The lectin-like protein ZG16 specifically binds the peptidoglycan of gram-positive bacteria and thereby inhibits their penetration into the inner layer of the colon[62]. Lectins RegIIIγ from IECs and beta-defensins from neutrophils have a bactericidal effect against a number of bacteria. However, *Firmicutes* and *Bacteroidetes* living in the small intestine are resistant to these antibacterial agents[72]. It is also worth noting that some bacteria can synthesize bacteriocins (for example, colicin and microcins), which inhibit the growth of competitors[73,74].

Immunological barrier

The gut immune barrier is represented by single lymphoid follicles and Peyer's patches — peripheral accumulations of lymphoid cells located in the lamina propria of the small intestine mucosa[20]. Within the follicles, there are various immune elements, including B and T lymphocytes, DCs, and neutrophils, that secrete cytokines and antibodies in response to antigen entry. Goblet cells are involved in the presentation of luminal antigens to the CD103+ DC complex of the intestinal mucosa lamina propria, forming antigenic complexes (goblet cell-associated antigen passages)[75]. Secretory IgA (SIgA), another component of the intestinal barrier, is produced by plasma cells (50 mg/kg SIgA daily in an adult) and localized predominantly in the lamina propria of the intestinal mucosa[31]. It is believed that SIgA is able to interact with commensal intestinal bacteria, mediating the formation of a bacterial biofilm. SIgA is resistant to the action of intestinal proteases, which provide protection for bacteria. SIgA can penetrate through the epithelial lining into the intestinal lumen, bind antigens and deliver them to the immune cells of the lymphoid tissue[76].

Elements of immune protection can also include an increase in "tolerance" to a microbe (or toxin of microbial origin)[52] and the death of infected cells. In particular, flagellins of pathogenic bacteria that have overcome the epithelial barrier are able to activate NAIP/NLRC4 in macrophages, which causes the death of infected epithelial cells and their expulsion into the intestinal lumen[77].

It is important to note that the intestine is the most important immune organ, which not only protects against external pathogens but also participates in the formation of immune tolerance to food substrates and the normal gut microbiome. The main cytokines involved in the formation of immunological tolerance are IL-10 and TGF-beta, which are produced by CD4+ T cells, some populations of macrophages and other cells and have an anti-inflammatory effect, limiting the expansion of effector cells and inducing the proliferation of regulatory T cells[78].

FUNCTIONS OF THE GUT MICROBIOTA AS AN EXTERNAL ORGAN OF THE HUMAN BODY

The microbiota are currently considered an external organ of the human body, which provides important mechanisms of metabolic regulation and protection, alongside the development and functioning of all organ systems[79]. It performs the following functions, the list of which is incomplete.

Digestion of plant polysaccharides. Approximately 17 carbohydrate-active enzymes are formed in the human body, while the microbiota provides around 89[80]. The gut microbiota actively digests dietary fiber, which the human body is unable to digest. These processes take place in the large intestine *via* the most actively involved enzymatic anaerobes, which decompose polysaccharides, particularly representatives of the *Bacteroidaceae* and *Clostridiaceae* families[12,81]. As a result of their digestion, compounds are produced that have a positive effect on the intestinal mucosa. In addition, the mucus layer is an alternative source of glycans for bacteria[12,51,81,82].

Participation in the metabolism of proteins, lipids and fatty acids[83-87]. In particular, gram-negative (*Bacteroides thetaiotamicron*) and gram-positive (*Lactobacillus rhamnosus gg*) bacteria are involved in the regulation of lipid absorption by activating cholecystokinin and secretin receptors expressed by epithelial endocrine cells of the proximal small intestine[88].

Energy supply of IECs[15,81,89] and regulation of their proliferative activity[90,91].

Modulation of goblet cell functions and mucin secretion[16].

The presence of intestinal microorganisms affects the number of Paneth cells and hence the integrity of the epithelial barrier, as Paneth cells regulate ISC homeostasis[35].

Stimulation of local and systemic immunity due to activation of the synthesis of IgA, interferons, and activation of immune cells (macrophages, lymphocytes, and DCs), influence on the development of the intestinal lymphoid apparatus in newborns[92-94].

Synthesis of group B, K vitamins, a number of coenzymes, for example, tocopherols[95-97].

Participation in the regulation of intestinal peristalsis[98-100].

Influence on bone metabolism and pathogenesis of osteoporosis[14,101,102]. The bacterial synthesis of SCFA leads to a decrease in pH in the intestinal lumen and an increase in calcium solubility, an increase in its absorption, and a decrease in bone resorption. *Bifidobacterium* and *Lactobacillus* are mainly involved in these processes. In addition, *Fusobacterium nucleatum* can enhance osteoclast differentiation through increased expression of IL-17A, TNF-alpha, and trimethylamine N-oxide (TMAO), while

Bacteroides, *Lactobacillus*, and *Bifidobacterium* can promote the development of Treg cells and thereby increase osteoblast activity[14,101,103,104].

Influence on the processes associated with the synthesis of neurotransmitters, myelination of neurons in the prefrontal cortex, with the development of the amygdala and hippocampus[105,106]. In dysbacteriosis, the response to antidepressant therapy may be impaired[107]. Germ-free mice show hyperactivity, memory and learning deficits and impaired expression of the serotonin 5-HT1A and NMDA receptors in the hippocampus[108,109].

Inhibition of the growth of pathogenic microorganisms is due to the activation of phagocytosis, the synthesis of antibacterial peptides or the synthesis of bacteriocins that inhibit the growth of competitors [69,73,74,81,100,110].

Influence the effectiveness of several drugs, in particular antibiotics, proton pump inhibitors, metformin, vitamin D and laxatives. It has been shown that the use of these drugs disrupts both the composition of the microbiota and its functional activity[89,111,112].

PRODUCTS OF BACTERIAL METABOLISM AND THEIR ROLE IN HEALTH AND DISEASE

As already noted, health and disease conditions are largely dependent on the functioning microbiome. Products of bacterial metabolism can be crucial for maintaining both the health of an organism and the development of various diseases[113].

SCFAs

The most important products of bacterial fermentation are SCFAs: butyrate, acetate and propionate. The main producers of SCFAs are *Firmicutes* and *Actinobacteria*.

Butyrate: Butyrate is the primary metabolite of *Firmicutes*. It can be synthesized through condensation of 2 molecules of acetyl-CoA, which are reduced to butyryl-CoA and then converted to butyric acid by phosphotransferase and butyrate kinase[114]. It can also be synthesized from butyryl-CoA, lactate and acetate using the acetyl-CoA transferase pathway[115] and from proteins using lysine[116]. It has anti-inflammatory, antitumour, antiproliferative and immunomodulatory properties and is involved in genetic/epigenetic regulation[117,118]. In particular: (1) Regulates antigen-specific adaptive immunity mediated by T- and B-cells: induce T-cells to produce IL-10; regulate the transcription of some cytokine genes, such as IFN-γ and TNF-α, and the activity of the nuclear factor kappa B (NF-κB) signaling pathway; reduce the production of proinflammatory mediators (TNF-α, IL-6, IFN-γ and NO); increase the production of antibodies by B-cells and promote the differentiation of B-cells into plasma B-cells [119-121]; (2) Participates in fat metabolism, reduces insulin resistance, hyperglycaemia, hyperinsulinaemia, and lipid concentrations in the liver and pancreas, thereby reducing the risk of obesity[122]; (3) Affects fatty acid receptors in epithelial, enteroendocrine, neuronal and glial cells, which leads to the production of serotonin by enterochromaffin cells. This may affect the peripheral and central nervous systems of experimental model organisms[50,123]; (4) Helps improve memory[124]; (5) Involved in maintaining the mechanical integrity of the intestinal barrier by inducing the expression of occludin, ZO-1 mRNA and claudin-1 mRNA, thereby reducing intestinal permeability and increasing intestinal villus growth[13,122,125,126]; and (6) Inhibits the rate of cancer cell migration and invasion by increasing the expression of antimetastatic genes (*e.g.*, metalloproteinases) and inhibiting the activation of prometastatic genes (*e.g.*, matrix metalloproteinases)[85,127].

Acetate: Acetate is a fermentation product of various bacteria and is produced from pyruvate using acetyl coenzyme A[128]. It performs the following functions: (1) Participates in the regulation of cholesterol synthesis and activation of local immunity[129]; (2) Helps increasing physical endurance [130]; (3) Influences cognitive functions by activating synaptophysin synthesis[131]; (4) Promotes appetite reduction, fat oxidation, increased levels of proinflammatory cytokines through activated secretion of intestinal hormones such as glucagon-like peptide-1 and peptide YY and increased insulin sensitivity[86]. Nonobese patients show higher production of acetate by gut microbiota than obese patients[132]; (5) Regulates the gut microbiome by increasing the production of IgA and its selective binding to certain microorganisms[86].

Propionate: Propionate is the primary metabolite of *Bacteroidetes* fermentation and is formed from the conversion of succinate to methylmalonyl-CoA by the succinate pathway or from acrylate by the acrylate pathway using lactate as a precursor[133]. Additionally, fucose and rhamnose can be used as substrates for the synthesis of propionic acid *via* the propanediol pathway[134]. Propionate performs the following functions: (1) Participates in maintaining the mechanical integrity of the intestinal barrier by increasing the expression of gut TJ proteins ZO-1, occludin and cadherin[135,136], as well as the synthesis of the antimicrobial protein Regenerating islet-derived protein type 3 (Reg3)[137]; (2) Reduces the risk of developing atherosclerosis and the development of cardiovascular disease by increasing insulin sensitivity and reducing the levels of proinflammatory IL-8[138] and IL-17[139], as well as by reducing the absorption of cholesterol in the intestine by an immune-mediated mechanism through an

increase in the number of regulatory T cells and the level of IL-10, which suppress the expression of C1-like 1 Niemann-Pick (Npc1 L1), the main cholesterol transporter in the intestine[140]; (3) Influences physical endurance[141] and motor functions[136]; (4) Promotes regeneration and functional recovery of sensory axons through an immune-mediated mechanism[142]; and (5) Involved in the regulation of the gut microbiome, possibly through direct suppression of the growth of pathogenic microorganisms[143].

Tryptophan derivatives

A number of other intestinal metabolites can also affect the health of individuals. Among these metabolites, a special place is occupied by tryptophan derivatives: serotonin, tryptamine, kynurenine, and indoles. They perform the following functions.

Act on the central nervous system through the brain-gut axis[144]. The influence is implemented due to the impact on the glutamatergic receptor N-methyl-d-aspartate (NMDA). It has been established that kynurenine, a breakdown product of tryptophan, easily penetrates the blood-brain barrier, where it is metabolized to form neuroactive glutamatergic compounds, kynurenic acid or quinoline acid, acting in the opposite way. Kynurenic acid acts as an NMDA receptor antagonist and has a neuroprotective effect, while quinolinic acid acts as an NMDA receptor agonist and exhibits a neurotoxic effect[145]. In major depressive disorder and bipolar disorder, a decrease in tryptophan and kynurenine was noted. In these mental disorders, there is a shift in tryptophan metabolism from the serotonin pathway to the kynurenine pathway[146]. Tryptophan metabolites are involved in the pathogenesis of various neurodegenerative disorders (Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease) as well as other diseases such as AIDS, cancer, cardiovascular disease, inflammation and irritable bowel syndrome[147,148].

Participate in the regulation of activation, proliferation and migration of immune surveillance cells (T- and B-lymphocytes, macrophages and natural killer cells) and the production of inflammatory signaling molecules, cytokines, nitric oxides and superoxides[149,150].

Influence the motility of the gastrointestinal tract. In particular, as a result of tryptamine action on the serotonin receptor 5-HT4R[151].

Indoles may contribute to the development of cardiovascular, metabolic and psychiatric diseases [152].

Secondary bile acids

It is worth noting that secondary bile acids play a special role in the development of inflammation and colorectal cancer (CRC). It is known that between 5% and 10% of nonreabsorbed bile acids can undergo biotransformation into secondary bile acids as a result of bacterial metabolism involving bacterial bile acid hydrolases (BSHs). Most BSH bacteria are gram-positive enteric bacteria, including *Clostridium*, *Enterococcus*, *Bifidobacterium*, and *Lactobacilli*. The only gram-negative bacteria with BSH activity are members of the genus *Bacteroides*[85,153]. Interestingly, secondary bile acids at high concentrations associated with Western diets can induce oxidative/nitrosative stress, ROS production, DNA damage, apoptosis and mutations[85,154,155] and induce proinflammatory macrophage M1 polarization[154] by binding secondary bile acids with Takeda G protein-coupled receptor 5 (TGR5)[156], thereby initiating inflammation[157]. Several studies have noted that through the activation of the epidermal growth factor receptor (EGFR), secondary bile acids can induce COX-2 expression, stimulate EGFR-MARK signaling[155,158], activate cellular β-catenin signaling and the NF-κB pathway[155,159], and provide transfer of extracellular signal-regulated kinase 1 and 2 *via* activator protein 1 and c-Myc, thereby stimulating the proliferation and invasiveness of colon cancer cells[85,160,161]. At the same time, at low and physiological concentrations, secondary bile acids can have an anti-inflammatory and antitumour effect through a reduction in proinflammatory cytokine levels[162,163]. In particular, an antitumour effect has been noted for lithocholic and ursodeoxycholic acid (UDCA), which are secondary bile acids produced by *Clostridium* species. Lithocholic acid (LCA) at concentrations corresponding to its tissue reference concentrations (< 1 μmol/L) has an antitumour effect on breast cancer cells by inhibiting the epithelial-mesenchymal transition, reducing the production of vascular endothelial growth factor, and activating antitumour immunity[164]. UDCA may prevent the development of CRC by regulating oxidative stress in colon cancer cells[165]. However, the preventive effect of UDCA on CRC is not universally accepted[166].

TMAO

TMAO is a molecule resulting from the oxidation in the liver of a microbial metabolism product, trimethylamine (TMA). TMA is formed in the colon from choline, betaine, and carnitine. The main food precursors of TMA are red meat, fish, poultry, and eggs. TMA from the colon is absorbed into the bloodstream, where it is oxidized by the hepatic enzyme flavin-containing monooxygenase-3 to TMAO, most of which is then excreted unchanged in the urine. Plasma TMAO levels are determined by several factors, including diet, age, gut microbiota, drug intake, and liver flavin monooxygenase activity. The main TMA producers are *Clostridia*, *Shigella*, *Proteus*, *Aerobacter*, and *Eubacterium sp.*[167,168]. Some bacterial enzymes are able to oxidize TMA to TMAO in the colon[169]. At the same time, TMAO can be metabolized into dimethylamine, formaldehyde, ammonia and methane by some methanogenic

bacteria, which leads to its depletion in the colon[170]. It cannot be excluded that the production of formaldehyde under oxidative stress conditions caused by TMAO metabolism may be one of the factors contributing to the induction of CRC. In the experiment, intragastric administration of a suspension of CaCO3 in a mixture with formaldehyde and hydrogen peroxide induced tumors of the stomach and cecum in rats[171].

It has now been established that elevated plasma levels of TMAO correlate with the risk of developing atherosclerosis[172-175], obesity[175,176], cardiovascular diseases[174,177,178], type 2 diabetes[175], chronic kidney disease[179,180] and CRC[181,182]. Elevated TMAO levels have been associated with endothelial dysfunction and inflammatory damage to the vascular endothelium[183, 184], an increase in the level of proinflammatory cytokines, and a decrease in the level of anti-inflammatory cytokines[185,186] with the activation of the MAPK and NF-κB transcriptional pathways[185], oxidative stress[177], cell proliferation and angiogenesis[187]. Xu *et al*[188] provided evidence that these risks may be genetically determined. Moreover, it is worth noting that the effects of TMAO may differ between healthy and diseased conditions. In healthy individuals, TMAO can demonstrate protective, antioxidant or anti-inflammatory effects, while in patients, especially under conditions of oxidative stress, it can have a negative impact on human health[189]. Further research is needed to elucidate the effects of TMAO on human health.

Hydrogen sulfide

Hydrogen sulfide is a metabolite of sulfate-reducing bacteria that metabolize dietary sulfates and other sulfur-containing compounds, including taurine[155]. It is produced by a wide range of *Enterobacteria*, primarily of the genus *γ-Proteobacteria*. The hydrogen sulfide concentration in the large intestine is more than that 5 times higher than in the small intestine[190]. Hydrogen sulfide, similarly to secondary bile acids and TMAO, can multidirectional affect inflammation, oxidative stress, and carcinogenesis[155, 191]. Various authors have demonstrated both its inflammatory[192] and anti-inflammatory effects[193, 194], as well as its carcinogenic[195,196] and anticancer properties[197,198]. For example, some researchers have demonstrated that hydrogen sulfide may be associated with the breakdown of disulfide bonds in the mucus double layer in the colon wall, leading to inflammation and translocation of bacteria and toxins[199]. At the same time, other researchers suggest that hydrogen sulfide can protect the mucus layer and repair this already destroyed, thereby preventing inflammation[190]. It is believed that the physiological and pathological effects of hydrogen sulfide are associated with its concentration[200]. Given that intracellular hydrogen sulfide has a significant impact on many cellular functions, such as TJs, autophagy, apoptosis, vesicle trafficking, cell signaling, epigenetics and inflammasomes[201], and can be used as a therapeutic agent, its further study may open new opportunities in understanding the mechanisms of the development of pathological conditions and their treatment.

Polyamines

Polyamines are versatile polyfunctional molecules involved in cell proliferation and differentiation, apoptosis, angiogenesis, immune response, signaling, and gene expression[202,203]. They can be supplied with food and can form as a result of endogenous synthesis, as well as a result of bacterial metabolism, such as putrescine, spermidine and cadaverine[204,205]. In particular, cadaverine is formed from lysine by decarboxylation with the participation of lysine decarboxylase (LDC). Putrescine is formed by decarboxylation of ornithine catalyzed by ornithine decarboxylase. Spermidine synthase is involved in the formation of spermidine from putrescine. These enzymes are produced by most gram-negative bacteria[205,206]. Polyamines synthesized by the intestinal microbiota are then transported into the bloodstream through the colonic mucosa[207].

It has been established that polyamines produced by intestinal bacteria suppress chronic inflammation and strengthen the intestinal barrier in the colon, contribute to a significant improvement in the host's cognitive functions and increase life expectancy in experimental animals and have a cardioprotective effect[203,208,209]. In a number of studies, the antitumour effect of cadaverine was noted. In an experiment, cadaverine caused a decrease in the proliferative activity and invasiveness of breast cancer cells and contributed to the induction of mesenchymal-epithelial transition, a decrease in the stemness of cancer cells and their ability to metastasize[210]. In breast cancer, a decrease in the intestinal biosynthesis of cadaverine was noted, especially in patients with carcinoma in situ and stage I of the disease. With a high expression of bacterial LDC in the gut contents, a significantly longer survival was noted than with a low expression[210].

At the same time, a number of studies have noted that high levels of polyamines may be associated with tumor transformation and cancer progression[211]. Thus, in CRC, an increase in the levels of bacterial cadaverine and putrescine in the feces was noted[212,213]. Huang *et al*[214] reported that increased spermine intake is associated with an increased risk of CRC, while a higher intake of total polyamine, putrescine and spermidine is significantly associated with a reduced risk of CRC. It is believed that the procarcinogenic effect of polyamines is associated with the activation of the PTEN-PI3K-mTOR (TORC1), WNT, and RAS pathways[211].

Microbiome and biotransformation of xenobiotics

The gut microbiome can influence the biotransformation of a number of xenobiotics with known carcinogenic properties, such as heterocyclic amines (HCAs). HCAs are formed during thermal processing (frying, baking, grilling, *etc.*) of various food products, including oils, grains and vegetables, but especially processed meat[215]. HCAs have pronounced genotoxic and mutagenic properties, contributing to the development of malignant neoplasms of the intestine, liver, lungs, breast and other tumors. The carcinogenicity of HCAs is associated with mutations in proto-oncogenes and tumor suppressor genes, including K-ras, Haras, Apc, β-catenin and TP53[216]. The intestinal microbiota can metabolize HCAs into molecules with increased mutagenic activity[217]. At the same time, the intestinal microbiota can bind or metabolize food-derived HCAs, facilitating their excretion with feces or conversion into less toxic compounds[215,218]. These processes involve bacterial beta-glucuronidase (B-GUS) and glycerol/diol dehydratase (GDH) produced by some lactic acid bacteria and probiotics. A decrease in the number of taxa with B-GUS and GDH activity was noted in patients with CRC[219].

DYSBIOSIS AND HUMAN DISEASES

According to the results of numerous studies, in many diseases, including inflammatory bowel diseases [113,220-224], chronic liver diseases[225-227], obesity[228,229], diabetes mellitus[230,231], osteoporosis [41,232], cardiovascular[233,234] and oncological diseases[212,213,235], a decrease in the microbiota diversity and an overgrowth of pathogenic and conditionally pathogenic flora are observed[50,236,237]. Interestingly, in CRC, *Fusobacterium nucleatum* was found not only in the primary tumor but also in metastatic lesions in the liver[238] and lung[239]. In addition, it has been noted that dysbiosis to a certain extent can influence the development of depression, bipolar depression and schizophrenia[240], as well as autism[241] and Parkinson's disease[242,243]. Figure 1 demonstrates the association of gut dysbiosis with various human diseases.

It should be emphasized that the diseases associated with dysbacteriosis are multifactorial in nature [50,236,237,244] and are associated with bacterial invasion through the physical and chemical barriers of the gastrointestinal tract[50]. It is hypothesized that diet and other environmental influences may change the microbiome and thus provoke an unstable basis of genetic predisposition, which may lead to disease development, at least in some patients. Dysbiosis may be related to heredity, use of antibiotics, proton pump inhibitors, certain types of chemotherapy, advanced age, diet, and other factors[245,246]. At the same time, it should be noted that numerous studies have not revealed typical changes in the microbiome for a particular pathology[247]. A detailed analysis of host metabolism (metabolic index) and habitual diet (including the consumption of plant and animal foods, and fermented milk probiotics, such as yogurt) allowed Asnicar *et al*[248] to establish consistent gut microbiome signatures, segregating favourable and unfavourable taxa with multiple measures of both dietary intake and cardiometabolic health. However, we believe the issue cannot be considered definitively resolved, since it is not completely clear what is primary: A violation of the microbiome that then contributes to the development of diseases or disorder can cause changes in the microbiome. These questions require further research.

MODERN APPROACHES TO THE REGULATION OF THE GUT MICROBIOME

Considering that intestinal dysbiosis plays an important role in the development of a number of diseases, normalizing the microbiome seems to be a promising direction for their treatment. There are several approaches to solve this problem.

"Mediterranean diet"

It is well known that a Mediterranean diet is rich in vegetables, fruits, whole grains and fish and thus creates favourable conditions for beneficial bacteria such as *Firmicutes*, which are involved in the production of butyrate necessary to maintain a healthy barrier between the colon and blood flow, preventing inflammation in the gut[64]. Unlike the Mediterranean diet, a “Western” high-fat, low-fiber diet contributes to colon inflammation and cancer[249,250].

Prebiotics

Prebiotics are substrates that are selectively utilized by host microorganisms to provide health benefits [251]. The use of prebiotics, such as dietary fiber, reduces obesity and has anti-inflammatory and anticancer effects[85,250,252,253]. Dietary fiber intake is associated with a lower incidence of colon cancer, since fiber reduces the concentration of intestinal carcinogens due to increased stool mass, intestinal motility and production of butyrate, which maintain colonocyte health, increase apoptosis and inhibit cancer cell proliferation. A long-term fiber-rich diet increases the density of *Firmicutes*, which have the ability to mediate an immunomodulatory and anti-inflammatory immune response[64].

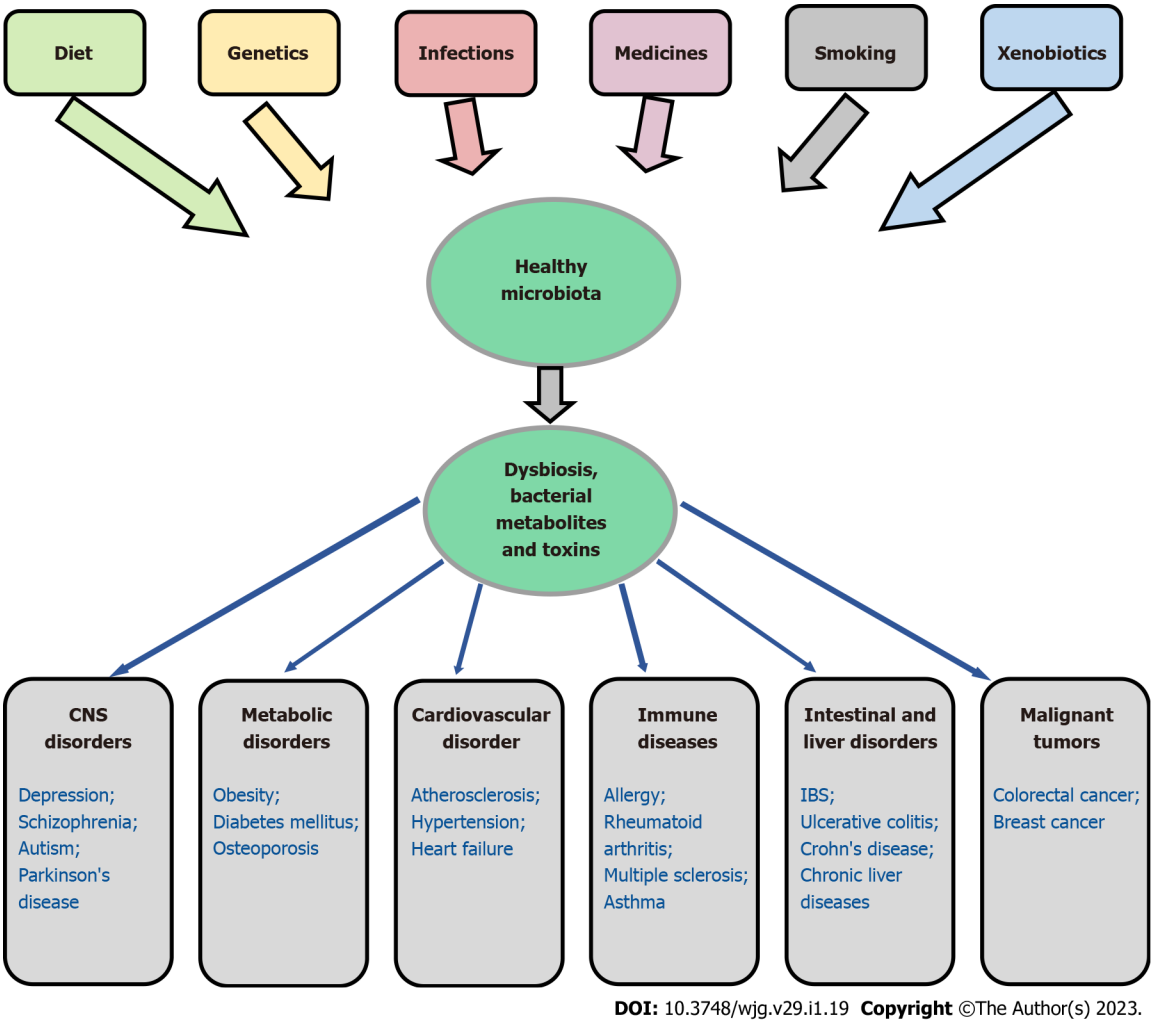

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Figure 1 Association of gut dysbiosis with various human diseases. CNS: Central nervous system; IBS: Irritable bowel syndrome.

Moreover, dietary fiber physically interferes with fatty acid reabsorption and cholesterol absorption, thereby reducing the risk of obesity, diabetes, and atherosclerosis[254,255].

Transplantation of fecal microbiota

The mechanism of transplantation of fecal microbiota (FMT) is to restore the fermentation activity, pH and redox potential of the microbiome habitat in the respective niches and restore normal gas production and synthesis of SCFA[245,256]. FMT has been used in the treatment of nosocomial diarrhea caused by *Clostridioides difficile*[245] and other intestinal diseases[257-259]. Scibelli *et al*[260] demonstrated the effectiveness of using FMT in the treatment of rectal fistula in a patient with a colostomy who received intensive antibiotic therapy for a long time due to trauma. Impressive results have also been obtained in the treatment of Crohn's disease by FMT: nearly 60% of patients achieved a clinical response to treatment, and more than 20% of patients experienced sustained clinical remission, including 2 of 6 patients with perianal fistula[261]. The use of FMT has been shown to be beneficial in hepatic encephalitis, metabolic diseases, neuropsychiatric disorders, autoimmune diseases, allergic disorders, tumors, Parkinson's disease, multiple sclerosis, myoclonus dystonia, chronic fatigue syndrome, and idiopathic thrombocytopenic purpura[257,262-264].

The disadvantages of FMT are frequent side effects such as constipation, diarrhea, bloating and possible transmission of potential pathogens[245,265]. Given the enormous promise of microbiota transplantation, the search for new methods and ways to use it continues[266]. Attempts have been made to replenish only bacteria that had certain characteristics and whose number was reduced[245,267,268], but reproducibility and standardization of preparations used for transplantation have been proven to be a problem[267]. Zhang *et al*[269] first revealed that washed microbiota transplantation is safer, more precise and more quality-controllable than crude FMT by manual. Currently, there are a series of clinical trials conducted for SER-109, which is a consortium from several species of *Firmicutes* isolated from the stool of healthy human donors and encapsulated. The use of the medication reduced the risk of recurrence of nosocomial diarrhea caused by *Clostridioides difficile* from 41.3% in the placebo group to 11.1% in the treatment group[270].

Probiotics

Probiotics are live strains of carefully selected microorganisms that, when administered in adequate amounts, confer a health benefit on the host[271]. Probiotics can be easily and economically prepared and given to patients daily in the form of yogurt, drinks, cheese or capsules[272]. They contribute to the maintenance of intestinal barrier function, have immunomodulatory, metabolic and antiproliferative effects[273,274], and regulate DC maturation by producing tolerogenic DCs, which can reduce inflammation[275] and synthesize antimicrobial substances[276]. The ability of *Bifidobacterium dentium* and its secreted factors to suppress endoplasmic reticulum stress genes and promote MUC2 secretion, as well as to secrete the antioxidant γ-glutamylcysteine, which reduces the formation of ROS and suppresses NF-kB activation and IL-8 secretion, has been established[277].

Currently, *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are used to treat hospital-acquired diarrhea caused by *Clostridioides difficile*[278]. Oral use of *Lactobacillus reuteri 6475* reduced the loss of total bone mineral density (BMD) in women aged 75 years to 80 years with low BMD[279]. Dietary supplementation with soluble corn fiber at doses of 10-20 g/d has also been associated with an increase in calcium absorption and a larger number of *Clostridium* and unclassified *Clostridiaceae* in feces[280]. These data suggest that both probiotics (live bacteria) and dietary supplements needed to feed the bacteria can be used as therapeutic agents to combat osteoporosis. It is possible that the use of their combination will be even more effective.

For therapeutic purposes, commensals (bacterial strains that are resistant to certain types of pathogenic bacteria), bacteriophages and fungi can be used. For example, CBM588 is a probiotic composed of the commensal *Clostridium butyricum*, which produces large amounts of butyrate and activates neutrophils and Th1 and Th17 cells[281]. Bacteriophages are viruses that can infect and multiply in pathogenic bacteria and eventually lyse them[245,282]. Yeasts such as *Saccharomyces boulardii* and *Candida albicans*, as well as fungal wall components such as β-glucans, can inhibit the growth of some enteric pathogens. *Saccharomyces boulardii* produces proteases or phosphatases that inactivate disease-causing toxins produced by gut bacteria and modulate multiple signaling pathways to suppress toxin-induced inflammation[245,283,284]. Because of the risk of fungemia, they should be used cautiously in debilitated patients[285].

CONCLUSION

The study of the influence of the gut microbiome on health and disease is one of the most relevant and interesting areas in modern science. The number of microorganisms inhabiting the human body is enormous, and their composition can vary significantly between individuals[248,286]. Dysbiosis has been found to play an important role in the development of a number of diseases, however, numerous studies have not revealed typical changes in the microbiome characteristic for a particular pathology [247]. It is likely that the impact of the microbiome and its metabolites on human health cannot be considered only in terms of health benefits or harms. Metabolites that perform important functions in the human body under certain conditions can have a negative impact on human health, while metabolites that are considered potentially dangerous can be beneficial[287].

As already noted, changes in the quantitative and qualitative microbiota composition can lead to an increase in the production of potentially toxic metabolites, such as secondary bile acids, TMAO and hydrogen sulfide, and an increase in the risk of developing intestinal, cardiovascular, neurological, oncological and other diseases. These changes may be related to diet, lifestyle, age, medications, and other factors. Thus, taking antibacterial drugs can increase sensitivity to viral infections[288], increase the risk of developing malignant neoplasms[289], and contribute to the development of resistance to chemotherapy drugs and immune checkpoint inhibitors in cancer patients[290]. Interestingly, some authors attribute an increase in the risk of malignant neoplasms with the use of antibiotics to a decrease in the synthesis of intestinal metabolites with antitumour activity, for example, LCA and cadaverine [289]. However, in some malignant neoplasms, the antitumour effect of antibacterial drugs was demonstrated. For example, in CRC, antibiotic therapy had a cytostatic effect due to the destruction of bacterial biofilms, the formation of which was associated with polyamines[291]. In an experiment, adding broad-spectrum antibiotics to drinking water for 3-4 wk reduced age-related oxidative stress and arterial dysfunction in mice[292]. It is believed that microbiota modulation using antibiotics, probiotics, fecal microbiota transplantation or nanotechnology can be effective in a variety of diseases, including enhancing the antitumour effect of chemotherapy drugs or immune checkpoint inhibitors [290].

It should be noted that despite the huge amount of research on the role of the microbiome in health and disease development, there are a number of issues that deserve further study. In particular, it is known that the microbiota affects the mood and behavior of a person, his physical activity, resistance to stress and diseases. At the same time, whether mental and physical health influence the human microbiome is not well understood. Many questions remain regarding the role of the gut microbiome in drug metabolism, the development of drug resistance and chemoresistance, and the role of the microbiome in cancer progression. Answers to these and other questions are still waiting for their

researchers.

FOOTNOTES

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REFERENCES

1 **Shreiner AB**, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol* 2015; **31**: 69-75 [PMID: 25394236 DOI: 10.1097/MOG.0000000000000139]
2 **Milani C**, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Delgado Palacio S, Arboleya Montes S, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiol Mol Biol Rev* 2017; **81** [PMID: 29118049 DOI: 10.1128/MMBR.00036-17]
3 **Barko PC**, McMichael MA, Swanson KS, Williams DA. The Gastrointestinal Microbiome: A Review. *J Vet Intern Med* 2018; **32**: 9-25 [PMID: 29171095 DOI: 10.1111/jvim.14875]
4 **Fernández L**, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, Rodríguez JM. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* 2013; **69**: 1-10 [PMID: 22974824 DOI: 10.1016/j.phrs.2012.09.001]
5 **Luo S**, Zhu H, Zhang J, Wan D. The Pivotal Role of Microbiota in Modulating the Neuronal-Glial-Epithelial Unit. *Infect Drug Resist* 2021; **14**: 5613-5628 [PMID: 34992388 DOI: 10.2147/IDR.S342782]
6 **Jha AR**, Davenport ER, Gautam Y, Bhandari D, Tandukar S, Ng KM, Fragiadakis GK, Holmes S, Gautam GP, Leach J, Sherchand JB, Bustamante CD, Sonnenburg JL. Gut microbiome transition across a lifestyle gradient in Himalaya. *PLoS Biol* 2018; **16**: e2005396 [PMID: 30439937 DOI: 10.1371/journal.pbio.2005396]
7 **Dominguez-Bello MG**, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010; **107**: 11971-11975 [PMID: 20566857 DOI: 10.1073/pnas.1002601107]
8 **Lundgren SN**, Madan JC, Emond JA, Morrison HG, Christensen BC, Karagas MR, Hoen AG. Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome* 2018; **6**: 109 [PMID: 29973274 DOI: 10.1186/s40168-018-0490-8]
9 **Yassour M**, Vatanen T, Siljander H, Hämäläinen AM, Härkönen T, Ryhänen SJ, Franzosa EA, Vlamakis H, Huttenhower C, Gevers D, Lander ES, Knip M; DIABIMMUNE Study Group, Xavier RJ. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med* 2016; **8**: 343ra81 [PMID: 27306663 DOI: 10.1126/scitranslmed.aad0917]
10 **Lazar V**, Ditu LM, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, Picu A, Petcu L, Chifiriuc MC. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. *Front Immunol* 2018; **9**: 1830 [PMID: 30158926 DOI: 10.3389/fimmu.2018.01830]
11 **Sender R**, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 2016; **14**: e1002533 [PMID: 27541692 DOI: 10.1371/journal.pbio.1002533]
12 **Donaldson GP**, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016; **14**: 20-32 [PMID: 26499895 DOI: 10.1038/nrmicro3552]
13 **Ma J**, Piao X, Mahfuz S, Long S, Wang J. The interaction among gut microbes, the intestinal barrier and short chain fatty acids. *Anim Nutr* 2022; **9**: 159-174 [PMID: 35573092 DOI: 10.1016/j.aninu.2021.09.012]
14 **Chen Y**, Wang X, Zhang C, Liu Z, Li C, Ren Z. Gut Microbiota and Bone Diseases: A Growing Partnership. *Front Microbiol* 2022; **13**: 877776 [PMID: 35602023 DOI: 10.3389/fmicb.2022.877776]
15 **Moszak M**, Szulińska M, Bogdański P. You Are What You Eat-The Relationship between Diet, Microbiota, and Metabolic Disorders-A Review. *Nutrients* 2020; **12** [PMID: 32326604 DOI: 10.3390/nu12041096]
16 **Engevik MA**, Luk B, Chang-Graham AL, Hall A, Herrmann B, Ruan W, Endres BT, Shi Z, Garey KW, Hyser JM,

Versalovic J. Bifidobacterium dentium Fortifies the Intestinal Mucus Layer *via* Autophagy and Calcium Signaling Pathways. *mBio* 2019; **10** [PMID: 31213556 DOI: 10.1128/mBio.01087-19]
17 **Paone P**, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut* 2020; **69**: 2232-2243 [PMID: 32917747 DOI: 10.1136/gutjnl-2020-322260]
18 **Goto Y**. Epithelial Cells as a Transmitter of Signals From Commensal Bacteria and Host Immune Cells. *Front Immunol* 2019; **10**: 2057 [PMID: 31555282 DOI: 10.3389/fimmu.2019.02057]
19 **Burgueño JF**, Abreu MT. Epithelial Toll-like receptors and their role in gut homeostasis and disease. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 263-278 [PMID: 32103203 DOI: 10.1038/s41575-019-0261-4]
20 **Gieryńska M**, Szulc-Dąbrowska L, Struzik J, Mielcarska MB, Gregorczyk-Zboroch KP. Integrity of the Intestinal Barrier: The Involvement of Epithelial Cells and Microbiota-A Mutual Relationship. *Animals (Basel)* 2022; **12** [PMID: 35049768 DOI: 10.3390/ani12020145]
21 **Gassler N**. Paneth cells in intestinal physiology and pathophysiology. *World J Gastrointest Pathophysiol* 2017; **8**: 150-160 [PMID: 29184701 DOI: 10.4291/wjgp.v8.i4.150]
22 **Allaire JM**, Crowley SM, Law HT, Chang SY, Ko HJ, Vallance BA. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends Immunol* 2018; **39**: 677-696 [PMID: 29716793 DOI: 10.1016/j.it.2018.04.002]
23 **Banerjee A**, McKinley ET, von Moltke J, Coffey RJ, Lau KS. Interpreting heterogeneity in intestinal tuft cell structure and function. *J Clin Invest* 2018; **128**: 1711-1719 [PMID: 29714721 DOI: 10.1172/JCI120330]
24 **Mabbott NA**, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol* 2013; **6**: 666-677 [PMID: 23695511 DOI: 10.1038/mi.2013.30]
25 **Turula H**, Wobus CE. The Role of the Polymeric Immunoglobulin Receptor and Secretory Immunoglobulins during Mucosal Infection and Immunity. *Viruses* 2018; **10** [PMID: 29751532 DOI: 10.3390/v10050237]
26 **Knoop KA**, Newberry RD. Goblet cells: multifaceted players in immunity at mucosal surfaces. *Mucosal Immunol* 2018; **11**: 1551-1557 [PMID: 29867079 DOI: 10.1038/s41385-018-0039-y]
27 **Kurashima Y**, Tokuhara D, Kamioka M, Inagaki Y, Kiyono H. Intrinsic Control of Surface Immune and Epithelial Homeostasis by Tissue-Resident Gut Stromal Cells. *Front Immunol* 2019; **10**: 1281 [PMID: 31275305 DOI: 10.3389/fimmu.2019.01281]
28 **Yokoi Y**, Nakamura K, Yoneda T, Kikuchi M, Sugimoto R, Shimizu Y, Ayabe T. Paneth cell granule dynamics on secretory responses to bacterial stimuli in enteroids. *Sci Rep* 2019; **9**: 2710 [PMID: 30804449 DOI: 10.1038/s41598-019-39610-7]
29 **Barreto E Barreto L**, Rattes IC, da Costa AV, Gama P. Paneth cells and their multiple functions. *Cell Biol Int* 2022; **46**: 701-710 [PMID: 35032139 DOI: 10.1002/cbin.11764]
30 **Abokor AA**, McDaniel GH, Golonka RM, Campbell C, Brahmandam S, Yeoh BS, Joe B, Vijay-Kumar M, Saha P. Immunoglobulin A, an Active Liaison for Host-Microbiota Homeostasis. *Microorganisms* 2021; **9** [PMID: 34683438 DOI: 10.3390/microorganisms9102117]
31 **Zhao Q**, Maynard CL. Mucus, commensals, and the immune system. *Gut Microbes* 2022; **14**: 2041342 [PMID: 35239459 DOI: 10.1080/19490976.2022.2041342]
32 **Raya-Sandino A**, Luissint AC, Kusters DHM, Narayanan V, Flemming S, Garcia-Hernandez V, Godsel LM, Green KJ, Hagen SJ, Conway DE, Parkos CA, Nusrat A. Regulation of intestinal epithelial intercellular adhesion and barrier function by desmosomal cadherin desmocollin-2. *Mol Biol Cell* 2021; **32**: 753-768 [PMID: 33596089 DOI: 10.1091/mbc.E20-12-0775]
33 **Garcia MA**, Nelson WJ, Chavez N. Cell-Cell Junctions Organize Structural and Signaling Networks. *Cold Spring Harb Perspect Biol* 2018; **10** [PMID: 28600395 DOI: 10.1101/cshperspect.a029181]
34 **Green KJ**, Jaiganesh A, Broussard JA. Desmosomes: Essential contributors to an integrated intercellular junction network. *F1000Res* 2019; **8** [PMID: 31942240 DOI: 10.12688/f1000research.20942.1]
35 **Schoenborn AA**, von Furstenberg RJ, Valsaraj S, Hussain FS, Stein M, Shanahan MT, Henning SJ, Gulati AS. The enteric microbiota regulates jejunal Paneth cell number and function without impacting intestinal stem cells. *Gut Microbes* 2019; **10**: 45-58 [PMID: 29883265 DOI: 10.1080/19490976.2018.1474321]
36 **Paradis T**, Bègue H, Basmaciyan L, Dalle F, Bon F. Tight Junctions as a Key for Pathogens Invasion in Intestinal Epithelial Cells. *Int J Mol Sci* 2021; **22** [PMID: 33801524 DOI: 10.3390/ijms22052506]
37 **Jackson DN**, Theiss AL. Gut bacteria signaling to mitochondria in intestinal inflammation and cancer. *Gut Microbes* 2020; **11**: 285-304 [PMID: 30913966 DOI: 10.1080/19490976.2019.1592421]
38 **Haderer M**, Neubert P, Rinner E, Scholtis A, Broncy L, Gschwendtner H, Kandulski A, Pavel V, Mehrl A, Brochhausen C, Schlosser S, Gülow K, Kunst C, Müller M. Novel pathomechanism for spontaneous bacterial peritonitis: disruption of cell junctions by cellular and bacterial proteases. *Gut* 2022; **71**: 580-592 [PMID: 33707230 DOI: 10.1136/gutjnl-2020-321663]
39 **Bugge M**, Bergstrom B, Eide OK, Solli H, Kjønstad IF, Stenvik J, Espevik T, Nilsen NJ. Surface Toll-like receptor 3 expression in metastatic intestinal epithelial cells induces inflammatory cytokine production and promotes invasiveness. *J Biol Chem* 2017; **292**: 15408-15425 [PMID: 28717003 DOI: 10.1074/jbc.M117.784090]
40 **Hamonic G**, Pasternak JA, Wilson HL. Recognizing conserved non-canonical localization patterns of toll-like receptors in tissues and across species. *Cell Tissue Res* 2018; **372**: 1-11 [PMID: 29330675 DOI: 10.1007/s00441-017-2767-9]
41 **Bouskra D**, Brézillon C, Bérard M, Werts C, Varona R, Boneca IG, Eberl G. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 2008; **456**: 507-510 [PMID: 18987631 DOI: 10.1038/nature07450]
42 **Próchnicki T**, Latz E. Inflammasomes on the Crossroads of Innate Immune Recognition and Metabolic Control. *Cell Metab* 2017; **26**: 71-93 [PMID: 28683296 DOI: 10.1016/j.cmet.2017.06.018]
43 **Uranga JA**, Martínez V, Abalo R. Mast Cell Regulation and Irritable Bowel Syndrome: Effects of Food Components with Potential Nutraceutical Use. *Molecules* 2020; **25** [PMID: 32962285 DOI: 10.3390/molecules25184314]
44 **West AP**, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 2011; **472**: 476-480

[PMID: 21525932 DOI: 10.1038/nature09973]
45 **Matarrese P**, Falzano L, Fabbri A, Gambardella L, Frank C, Geny B, Popoff MR, Malorni W, Fiorentini C. Clostridium difficile toxin B causes apoptosis in epithelial cells by thrilling mitochondria. Involvement of ATP-sensitive mitochondrial potassium channels. *J Biol Chem* 2007; **282**: 9029-9041 [PMID: 17220295 DOI: 10.1074/jbc.M607614200]
46 **Rao R**. Oxidative stress-induced disruption of epithelial and endothelial tight junctions. *Front Biosci* 2008; **13**: 7210-7226 [PMID: 18508729 DOI: 10.2741/3223]
47 **Sardelli L**, Pacheco DP, Ziccarelli A, Tunesi M, Caspani O, Fusari A, Briatico Vangosa F, Giordano C, Petrini P. Towards bioinspired *in vitro* models of intestinal mucus. *RSC Adv* 2019; **9**: 15887-15899 [PMID: 35521409 DOI: 10.1039/c9ra02368b]
48 **Nyström EEL**, Martinez-Abad B, Arike L, Birchenough GMH, Nonnecke EB, Castillo PA, Svensson F, Bevins CL, Hansson GC, Johansson MEV. An intercrypt subpopulation of goblet cells is essential for colonic mucus barrier function. *Science* 2021; **372** [PMID: 33859001 DOI: 10.1126/science.abb1590]
49 **Herath M**, Hosie S, Bornstein JC, Franks AE, Hill-Yardin EL. The Role of the Gastrointestinal Mucus System in Intestinal Homeostasis: Implications for Neurological Disorders. *Front Cell Infect Microbiol* 2020; **10**: 248 [PMID: 32547962 DOI: 10.3389/fcimb.2020.00248]
50 **Inczefi O**, Bacsur P, Resál T, Keresztes C, Molnár T. The Influence of Nutrition on Intestinal Permeability and the Microbiome in Health and Disease. *Front Nutr* 2022; **9**: 718710 [PMID: 35548572 DOI: 10.3389/fnut.2022.718710]
51 **Johansson ME**, Larsson JM, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. *Proc Natl Acad Sci U S A* 2011; **108** Suppl 1: 4659-4665 [PMID: 20615996 DOI: 10.1073/pnas.1006451107]
52 **Bergstrom K**, Xia L. The barrier and beyond: Roles of intestinal mucus and mucin-type O-glycosylation in resistance and tolerance defense strategies guiding host-microbe symbiosis. *Gut Microbes* 2022; **14**: 2052699 [PMID: 35380912 DOI: 10.1080/19490976.2022.2052699]
53 **Ambort D**, Johansson ME, Gustafsson JK, Nilsson HE, Ermund A, Johansson BR, Koeck PJ, Hebert H, Hansson GC. Calcium and pH-dependent packing and release of the gel-forming MUC2 mucin. *Proc Natl Acad Sci U S A* 2012; **109**: 5645-5650 [PMID: 22451922 DOI: 10.1073/pnas.1120269109]
54 **Bergstrom K**, Shan X, Casero D, Batushansky A, Lagishetty V, Jacobs JP, Hoover C, Kondo Y, Shao B, Gao L, Zandberg W, Noyovitz B, McDaniel JM, Gibson DL, Pakpour S, Kazemian N, McGee S, Houchen CW, Rao CV, Griffin TM, Sonnenburg JL, McEver RP, Braun J, Xia L. Proximal colon-derived O-glycosylated mucus encapsulates and modulates the microbiota. *Science* 2020; **370**: 467-472 [PMID: 33093110 DOI: 10.1126/science.aay7367]
55 **Van der Sluis M**, De Koning BA, De Bruijn AC, Velcich A, Meijerink JP, Van Goudoever JB, Büller HA, Dekker J, Van Seuningen I, Renes IB, Einerhand AW. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* 2006; **131**: 117-129 [PMID: 16831596 DOI: 10.1053/j.gastro.2006.04.020]
56 **Velcich A**, Yang W, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L. Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science* 2002; **295**: 1726-1729 [PMID: 11872843 DOI: 10.1126/science.1069094]
57 **Jakobsson HE**, Rodríguez-Piñeiro AM, Schütte A, Ermund A, Boysen P, Bemark M, Sommer F, Bäckhed F, Hansson GC, Johansson ME. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep* 2015; **16**: 164-177 [PMID: 25525071 DOI: 10.15252/embr.201439263]
58 **Yao D**, Dai W, Dong M, Dai C, Wu S. MUC2 and related bacterial factors: Therapeutic targets for ulcerative colitis. *EBioMedicine* 2021; **74**: 103751 [PMID: 34902790 DOI: 10.1016/j.ebiom.2021.103751]
59 **Birchenough GM**, Nyström EE, Johansson ME, Hansson GC. A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science* 2016; **352**: 1535-1542 [PMID: 27339979 DOI: 10.1126/science.aaf7419]
60 **Cornick S**, Kumar M, Moreau F, Gaisano H, Chadee K. VAMP8-mediated MUC2 mucin exocytosis from colonic goblet cells maintains innate intestinal homeostasis. *Nat Commun* 2019; **10**: 4306 [PMID: 31541089 DOI: 10.1038/s41467-019-11811-8]
61 **Okumura R**, Kurakawa T, Nakano T, Kayama H, Kinoshita M, Motooka D, Gotoh K, Kimura T, Kamiyama N, Kusu T, Ueda Y, Wu H, Iijima H, Barman S, Osawa H, Matsuno H, Nishimura J, Ohba Y, Nakamura S, Iida T, Yamamoto M, Umemoto E, Sano K, Takeda K. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. *Nature* 2016; **532**: 117-121 [PMID: 27027293 DOI: 10.1038/nature17406]
62 **Bergström JH**, Birchenough GM, Katona G, Schroeder BO, Schütte A, Ermund A, Johansson ME, Hansson GC. Gram-positive bacteria are held at a distance in the colon mucus by the lectin-like protein ZG16. *Proc Natl Acad Sci U S A* 2016; **113**: 13833-13838 [PMID: 27849619 DOI: 10.1073/pnas.1611400113]
63 **Schroeder BO**. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterol Rep (Oxf)* 2019; **7**: 3-12 [PMID: 30792861 DOI: 10.1093/gastro/goy052]
64 **Yang J**, Yu J. The association of diet, gut microbiota and colorectal cancer: what we eat may imply what we get. *Protein Cell* 2018; **9**: 474-487 [PMID: 29713943 DOI: 10.1007/s13238-018-0543-6]
65 **Cheng HY**, Ning MX, Chen DK, Ma WT. Interactions Between the Gut Microbiota and the Host Innate Immune Response Against Pathogens. *Front Immunol* 2019; **10**: 607 [PMID: 30984184 DOI: 10.3389/fimmu.2019.00607]
66 **Tyler CJ**, McCarthy NE, Lindsay JO, Stagg AJ, Moser B, Eberl M. Antigen-Presenting Human γδ T Cells Promote Intestinal CD4(+) T Cell Expression of IL-22 and Mucosal Release of Calprotectin. *J Immunol* 2017; **198**: 3417-3425 [PMID: 28330898 DOI: 10.4049/jimmunol.1700003]
67 **Abt MC**, Buffie CG, Sušac B, Becattini S, Carter RA, Leiner I, Keith JW, Artis D, Osborne LC, Pamer EG. TLR-7 activation enhances IL-22-mediated colonization resistance against vancomycin-resistant enterococcus. *Sci Transl Med* 2016; **8**: 327ra25 [PMID: 26912904 DOI: 10.1126/scitranslmed.aad6663]
68 **Semin I**, Ninnemann J, Bondareva M, Gimaev I, Kruglov AA. Interplay Between Microbiota, Toll-Like Receptors and Cytokines for the Maintenance of Epithelial Barrier Integrity. *Front Med (Lausanne)* 2021; **8**: 644333 [PMID: 34124086

DOI: 10.3389/fmed.2021.644333]
69 **Price AE**, Shamardani K, Lugo KA, Deguine J, Roberts AW, Lee BL, Barton GM. A Map of Toll-like Receptor Expression in the Intestinal Epithelium Reveals Distinct Spatial, Cell Type-Specific, and Temporal Patterns. *Immunity* 2018; **49**: 560-575.e6 [PMID: 30170812 DOI: 10.1016/j.immuni.2018.07.016]
70 **Vaishnava S**, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, Hooper LV. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science* 2011; **334**: 255-258 [PMID: 21998396 DOI: 10.1126/science.1209791]
71 **Nava GM**, Friedrichsen HJ, Stappenbeck TS. Spatial organization of intestinal microbiota in the mouse ascending colon. *ISME J* 2011; **5**: 627-638 [PMID: 20981114 DOI: 10.1038/ismej.2010.161]
72 **Cullen TW**, Schofield WB, Barry NA, Putnam EE, Rundell EA, Trent MS, Degnan PH, Booth CJ, Yu H, Goodman AL. Gut microbiota. Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. *Science* 2015; **347**: 170-175 [PMID: 25574022 DOI: 10.1126/science.1260580]
73 **Garcia-Gutierrez E**, Mayer MJ, Cotter PD, Narbad A. Gut microbiota as a source of novel antimicrobials. *Gut Microbes* 2019; **10**: 1-21 [PMID: 29584555 DOI: 10.1080/19490976.2018.1455790]
74 **Umu ÖC**, Bäuerl C, Oostindjer M, Pope PB, Hernández PE, Pérez-Martínez G, Diep DB. The Potential of Class II Bacteriocins to Modify Gut Microbiota to Improve Host Health. *PLoS One* 2016; **11**: e0164036 [PMID: 27695121 DOI: 10.1371/journal.pone.0164036]
75 **McDole JR**, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD, Miller MJ. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 2012; **483**: 345-349 [PMID: 22422267 DOI: 10.1038/nature10863]
76 **Mu Q**, Kirby J, Reilly CM, Luo XM. Leaky Gut As a Danger Signal for Autoimmune Diseases. *Front Immunol* 2017; **8**: 598 [PMID: 28588585 DOI: 10.3389/fimmu.2017.00598]
77 **Sellin ME**, Müller AA, Felmy B, Dolowschiak T, Diard M, Tardivel A, Maslowski KM, Hardt WD. Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict Salmonella replication in the intestinal mucosa. *Cell Host Microbe* 2014; **16**: 237-248 [PMID: 25121751 DOI: 10.1016/j.chom.2014.07.001]
78 **Mörbe UM**, Jørgensen PB, Fenton TM, von Burg N, Riis LB, Spencer J, Agace WW. Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal Immunol* 2021; **14**: 793-802 [PMID: 33753873 DOI: 10.1038/s41385-021-00389-4]
79 **Dietert RR**. Microbiome First Medicine in Health and Safety. *Biomedicines* 2021; **9** [PMID: 34572284 DOI: 10.3390/biomedicines9091099]
80 **Bhattacharya T**, Ghosh TS, Mande SS. Global Profiling of Carbohydrate Active Enzymes in Human Gut Microbiome. *PLoS One* 2015; **10**: e0142038 [PMID: 26544883 DOI: 10.1371/journal.pone.0142038]
81 **Zafar H**, Saier MH Jr. Gut Bacteroides species in health and disease. *Gut Microbes* 2021; **13**: 1-20 [PMID: 33535896 DOI: 10.1080/19490976.2020.1848158]
82 **Sommer F**, Bäckhed F. The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol* 2013; **11**: 227-238 [PMID: 23435359 DOI: 10.1038/nrmicro2974]
83 **Shah T**, Baloch Z, Shah Z, Cui X, Xia X. The Intestinal Microbiota: Impacts of Antibiotics Therapy, Colonization Resistance, and Diseases. *Int J Mol Sci* 2021; **22** [PMID: 34202945 DOI: 10.3390/ijms22126597]
84 **Schoeler M**, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. *Rev Endocr Metab Disord* 2019; **20**: 461-472 [PMID: 31707624 DOI: 10.1007/s11154-019-09512-0]
85 **Zeng H**, Umar S, Rust B, Lazarova D, Bordonaro M. Secondary Bile Acids and Short Chain Fatty Acids in the Colon: A Focus on Colonic Microbiome, Cell Proliferation, Inflammation, and Cancer. *Int J Mol Sci* 2019; **20** [PMID: 30862015 DOI: 10.3390/ijms20051214]
86 **Hernández MAG**, Canfora EE, Jocken JWE, Blaak EE. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients* 2019; **11** [PMID: 31426593 DOI: 10.3390/nu11081943]
87 **González-Bosch C**, Boorman E, Zunszain PA, Mann GE. Short-chain fatty acids as modulators of redox signaling in health and disease. *Redox Biol* 2021; **47**: 102165 [PMID: 34662811 DOI: 10.1016/j.redox.2021.102165]
88 **Martinez-Guryn K**, Hubert N, Frazier K, Urlass S, Musch MW, Ojeda P, Pierre JF, Miyoshi J, Sontag TJ, Cham CM, Reardon CA, Leone V, Chang EB. Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe* 2018; **23**: 458-469.e5 [PMID: 29649441 DOI: 10.1016/j.chom.2018.03.011]
89 **Kim S**, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev* 2017; **279**: 90-105 [PMID: 28856737 DOI: 10.1111/imr.12563]
90 **Chandrakesan P**, Jakkula LU, Ahmed I, Roy B, Anant S, Umar S. Differential effects of β-catenin and NF-κB interplay in the regulation of cell proliferation, inflammation and tumorigenesis in response to bacterial infection. *PLoS One* 2013; **8**: e79432 [PMID: 24278135 DOI: 10.1371/journal.pone.0079432]
91 **Sommer F**, Nookaew I, Sommer N, Fogelstrand P, Bäckhed F. Site-specific programming of the host epithelial transcriptome by the gut microbiota. *Genome Biol* 2015; **16**: 62 [PMID: 25887251 DOI: 10.1186/s13059-015-0614-4]
92 **Frei R**, Akdis M, O'Mahony L. Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence. *Curr Opin Gastroenterol* 2015; **31**: 153-158 [PMID: 25594887 DOI: 10.1097/MOG.0000000000000151]
93 **Takiishi T**, Fenero CIM, Câmara NOS. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. *Tissue Barriers* 2017; **5**: e1373208 [PMID: 28956703 DOI: 10.1080/21688370.2017.1373208]
94 **Ticinesi A**, Lauretani F, Tana C, Nouvenne A, Ridolo E, Meschi T. Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis. *Exerc Immunol Rev* 2019; **25**: 84-95 [PMID: 30753131]
95 **Rowland I**, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018; **57**: 1-24 [PMID: 28393285 DOI: 10.1007/s00394-017-1445-8]
96 **Pan WH**, Sommer F, Falk-Paulsen M, Ulas T, Best P, Fazio A, Kachroo P, Luzius A, Jentzsch M, Rehman A, Müller F, Lengauer T, Walter J, Künzel S, Baines JF, Schreiber S, Franke A, Schultze JL, Bäckhed F, Rosenstiel P. Exposure to the

gut microbiota drives distinct methylome and transcriptome changes in intestinal epithelial cells during postnatal development. *Genome Med* 2018; **10**: 27 [PMID: 29653584 DOI: 10.1186/s13073-018-0534-5]

97 **Huang R**, Xiang J, Zhou P. Vitamin D, gut microbiota, and radiation-related resistance: a love-hate triangle. *J Exp Clin Cancer Res* 2019; **38**: 493 [PMID: 31843023 DOI: 10.1186/s13046-019-1499-y]

98 **Tian Y**, Zuo L, Guo Q, Li J, Hu Z, Zhao K, Li C, Li X, Zhou J, Zhou Y, Li XA. Potential role of fecal microbiota in patients with constipation. *Therap Adv Gastroenterol* 2020; **13**: 1756284820968423 [PMID: 33193813 DOI: 10.1177/1756284820968423]

99 **Yang W**, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, Sun J, Pan F, Zhou J, Zhang W, Yao S, Maynard CL, Singh N, Dann SM, Liu Z, Cong Y. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat Commun* 2020; **11**: 4457 [PMID: 32901017 DOI: 10.1038/s41467-020-18262-6]

100 **He J**, Zhang P, Shen L, Niu L, Tan Y, Chen L, Zhao Y, Bai L, Hao X, Li X, Zhang S, Zhu L. Short-Chain Fatty Acids and Their Association with Signalling Pathways in Inflammation, Glucose and Lipid Metabolism. *Int J Mol Sci* 2020; **21** [PMID: 32887215 DOI: 10.3390/ijms21176356]

101 **Raveschot C**, Coutte F, Frémont M, Vaeremans M, Dugersuren J, Demberel S, Drider D, Dhulster P, Flahaut C, Cudennec B. Probiotic Lactobacillus strains from Mongolia improve calcium transport and uptake by intestinal cells in vitro. *Food Res Int* 2020; **133**: 109201 [PMID: 32466902 DOI: 10.1016/j.foodres.2020.109201]

102 **Lucas S**, Omata Y, Hofmann J, Böttcher M, Iljazovic A, Sarter K, Albrecht O, Schulz O, Krishnacoumar B, Krönke G, Herrmann M, Mougiakakos D, Strowig T, Schett G, Zaiss MM. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. *Nat Commun* 2018; **9**: 55 [PMID: 29302038 DOI: 10.1038/s41467-017-02490-4]

103 **Atarashi K**, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**: 232-236 [PMID: 23842501 DOI: 10.1038/nature12331]

104 **Ye X**, Wang R, Bhattacharya R, Boulbes DR, Fan F, Xia L, Adoni H, Ajami NJ, Wong MC, Smith DP, Petrosino JF, Venable S, Qiao W, Baladandayuthapani V, Maru D, Ellis LM. Fusobacterium Nucleatum Subspecies Animalis Influences Proinflammatory Cytokine Expression and Monocyte Activation in Human Colorectal Tumors. *Cancer Prev Res (Phila)* 2017; **10**: 398-409 [PMID: 28483840 DOI: 10.1158/1940-6207.CAPR-16-0178]

105 **Silva YP**, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front Endocrinol (Lausanne)* 2020; **11**: 25 [PMID: 32082260 DOI: 10.3389/fendo.2020.00025]

106 **Mirzaei R**, Bouzari B, Hosseini-Fard SR, Mazaheri M, Ahmadyousefi Y, Abdi M, Jalalifar S, Karimitabar Z, Teimoori A, Keyvani H, Zamani F, Yousefimashouf R, Karampoor S. Role of microbiota-derived short-chain fatty acids in nervous system disorders. *Biomed Pharmacother* 2021; **139**: 111661 [PMID: 34243604 DOI: 10.1016/j.biopha.2021.111661]

107 **Trzeciak P**, Herbet M. Role of the Intestinal Microbiome, Intestinal Barrier and Psychobiotics in Depression. *Nutrients* 2021; **13** [PMID: 33809367 DOI: 10.3390/nu13030927]

108 **Neufeld KM**, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 2011; **23**: 255-264, e119 [PMID: 21054680 DOI: 10.1111/j.1365-2982.2010.01620.x]

109 **Braniste V**, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, Korecka A, Bakocevic N, Ng LG, Kundu P, Gulyás B, Halldin C, Hultenby K, Nilsson H, Hebert H, Volpe BT, Diamond B, Pettersson S. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014; **6**: 263ra158 [PMID: 25411471 DOI: 10.1126/scitranslmed.3009759]

110 **Ratajczak W**, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). *Acta Biochim Pol* 2019; **66**: 1-12 [PMID: 30831575 DOI: 10.18388/abp.2018_2648]

111 **Vich Vila A**, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, Mujagic Z, Jonkers DMAE, Masclee AAM, Fu J, Kurilshikov A, Wijmenga C, Zhernakova A, Weersma RK. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* 2020; **11**: 362 [PMID: 31953381 DOI: 10.1038/s41467-019-14177-z]

112 **Feng W**, Liu J, Ao H, Yue S, Peng C. Targeting gut microbiota for precision medicine: Focusing on the efficacy and toxicity of drugs. *Theranostics* 2020; **10**: 11278-11301 [PMID: 33042283 DOI: 10.7150/thno.47289]

113 **Mars RAT**, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, Tang X, Sun Z, Kalari KR, Korem T, Bhattarai Y, Zheng T, Bar N, Frost G, Johnson AJ, van Treuren W, Han S, Ordog T, Grover M, Sonnenburg J, D'Amato M, Camilleri M, Elinav E, Segal E, Blekhman R, Farrugia G, Swann JR, Knights D, Kashyap PC. Longitudinal Multi-omics Reveals Subset-Specific Mechanisms Underlying Irritable Bowel Syndrome. *Cell* 2020; **183**: 1137-1140 [PMID: 33186523 DOI: 10.1016/j.cell.2020.10.040]

114 **Ait-Belgnaoui A**, Colom A, Braniste V, Ramalho L, Marrot A, Cartier C, Houdeau E, Theodorou V, Tompkins T. Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterol Motil* 2014; **26**: 510-520 [PMID: 24372793 DOI: 10.1111/nmo.12295]

115 **Duncan SH**, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microbiol* 2002; **68**: 5186-5190 [PMID: 12324374 DOI: 10.1128/AEM.68.10.5186-5190.2002]

116 **Vital M**, Howe AC, Tiedje JM. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *mBio* 2014; **5**: e00889 [PMID: 24757212 DOI: 10.1128/mBio.00889-14]

117 **O'Keefe SJ**. Diet, microorganisms and their metabolites, and colon cancer. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 691-706 [PMID: 27848961 DOI: 10.1038/nrgastro.2016.165]

118 **Perego S**, Sansoni V, Banfi G, Lombardi G. Sodium butyrate has anti-proliferative, pro-differentiating, and immunomodulatory effects in osteosarcoma cells and counteracts the TNFα-induced low-grade inflammation. *Int J Immunopathol Pharmacol* 2018; **32**: 394632017752240 [PMID: 29363375 DOI: 10.1177/0394632017752240]

119 **Melo AD**, Silveira H, Bortoluzzi C, Lara LJ, Garbossa CA, Preis G, Costa LB, Rostagno MH. Intestinal alkaline

phosphatase and sodium butyrate may be beneficial in attenuating LPS-induced intestinal inflammation. *Genet Mol Res* 2016; **15** [PMID: 27813594 DOI: 10.4238/gmr15048875]

120 **Simeoli R**, Mattace Raso G, Pirozzi C, Lama A, Santoro A, Russo R, Montero-Melendez T, Berni Canani R, Calignano A, Perretti M, Meli R. An orally administered butyrate-releasing derivative reduces neutrophil recruitment and inflammation in dextran sulphate sodium-induced murine colitis. *Br J Pharmacol* 2017; **174**: 1484-1496 [PMID: 27684049 DOI: 10.1111/bph.13637]

121 **Zhang H**, Du M, Yang Q, Zhu MJ. Butyrate suppresses murine mast cell proliferation and cytokine production through inhibiting histone deacetylase. *J Nutr Biochem* 2016; **27**: 299-306 [PMID: 26601598 DOI: 10.1016/j.jnutbio.2015.09.020]

122 **Matheus VA**, Monteiro L, Oliveira RB, Maschio DA, Collares-Buzato CB. Butyrate reduces high-fat diet-induced metabolic alterations, hepatic steatosis and pancreatic beta cell and intestinal barrier dysfunctions in prediabetic mice. *Exp Biol Med (Maywood)* 2017; **242**: 1214-1226 [PMID: 28504618 DOI: 10.1177/1535370217708188]

123 **Gershon MD**, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; **132**: 397-414 [PMID: 17241888 DOI: 10.1053/j.gastro.2006.11.002]

124 **Arnoldussen IAC**, Wiesmann M, Pelgrim CE, Wielemaker EM, van Duyvenvoorde W, Amaral-Santos PL, Verschuren L, Keijser BJF, Heerschap A, Kleemann R, Wielinga PY, Kiliaan AJ. Butyrate restores HFD-induced adaptations in brain function and metabolism in mid-adult obese mice. *Int J Obes (Lond)* 2017; **41**: 935-944 [PMID: 28220041 DOI: 10.1038/ijo.2017.52]

125 **Wang HB**, Wang PY, Wang X, Wan YL, Liu YC. Butyrate enhances intestinal epithelial barrier function *via* up-regulation of tight junction protein Claudin-1 transcription. *Dig Dis Sci* 2012; **57**: 3126-3135 [PMID: 22684624 DOI: 10.1007/s10620-012-2259-4]

126 **Huang C**, Song P, Fan P, Hou C, Thacker P, Ma X. Dietary Sodium Butyrate Decreases Postweaning Diarrhea by Modulating Intestinal Permeability and Changing the Bacterial Communities in Weaned Piglets. *J Nutr* 2015; **145**: 2774-2780 [PMID: 26491121 DOI: 10.3945/jn.115.217406]

127 **Zeng H**, Briske-Anderson M. Prolonged butyrate treatment inhibits the migration and invasion potential of HT1080 tumor cells. *J Nutr* 2005; **135**: 291-295 [PMID: 15671229 DOI: 10.1093/jn/135.2.291]

128 **Ragsdale SW**, Pierce E. Acetogenesis and the Wood-Ljungdahl pathway of CO(2) fixation. *Biochim Biophys Acta* 2008; **1784**: 1873-1898 [PMID: 18801467 DOI: 10.1016/j.bbapap.2008.08.012]

129 **Jugder BE**, Kamareddine L, Watnick PI. Microbiota-derived acetate activates intestinal innate immunity *via* the Tip60 histone acetyltransferase complex. *Immunity* 2021; **54**: 1683-1697.e3 [PMID: 34107298 DOI: 10.1016/j.immuni.2021.05.017]

130 **Okamoto T**, Morino K, Ugi S, Nakagawa F, Lemecha M, Ida S, Ohashi N, Sato D, Fujita Y, Maegawa H. Microbiome potentiates endurance exercise through intestinal acetate production. *Am J Physiol Endocrinol Metab* 2019; **316**: E956-E966 [PMID: 30860879 DOI: 10.1152/ajpendo.00510.2018]

131 **Zheng H**, Xu P, Jiang Q, Xu Q, Zheng Y, Yan J, Ji H, Ning J, Zhang X, Li C, Zhang L, Li Y, Li X, Song W, Gao H. Depletion of acetate-producing bacteria from the gut microbiota facilitates cognitive impairment through the gut-brain neural mechanism in diabetic mice. *Microbiome* 2021; **9**: 145 [PMID: 34172092 DOI: 10.1186/s40168-021-01088-9]

132 **Galuppo B**, Cline G, Van Name M, Shabanova V, Wagner D, Kien CL, Santoro N. Colonic Fermentation and Acetate Production in Youth with and without Obesity. *J Nutr* 2021; **151**: 3292-3298 [PMID: 34494088 DOI: 10.1093/jn/nxab277]

133 **Hetzel M**, Arslandemir C, König HH, Buck AK, Nüssle K, Glatting G, Gabelmann A, Hetzel J, Hombach V, Schirrmeister H. F-18 NaF PET for detection of bone metastases in lung cancer: accuracy, cost-effectiveness, and impact on patient management. *J Bone Miner Res* 2003; **18**: 2206-2214 [PMID: 14672356 DOI: 10.1359/jbmr.2003.18.12.2206]

134 **Koh A**, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* 2016; **165**: 1332-1345 [PMID: 27259147 DOI: 10.1016/j.cell.2016.05.041]

135 **Tong LC**, Wang Y, Wang ZB, Liu WY, Sun S, Li L, Su DF, Zhang LC. Propionate Ameliorates Dextran Sodium Sulfate-Induced Colitis by Improving Intestinal Barrier Function and Reducing Inflammation and Oxidative Stress. *Front Pharmacol* 2016; **7**: 253 [PMID: 27574508 DOI: 10.3389/fphar.2016.00253]

136 **Huang T**, Shi H, Xu Y, Ji L. The gut microbiota metabolite propionate ameliorates intestinal epithelial barrier dysfunction-mediated Parkinson's disease *via* the AKT signaling pathway. *Neuroreport* 2021; **32**: 244-251 [PMID: 33470765 DOI: 10.1097/WNR.0000000000001585]

137 **Bajic D**, Niemann A, Hillmer AK, Mejias-Luque R, Bluemel S, Docampo M, Funk MC, Tonin E, Boutros M, Schnabl B, Busch DH, Miki T, Schmid RM, van den Brink MRM, Gerhard M, Stein-Thoeringer CK. Gut Microbiota-Derived Propionate Regulates the Expression of Reg3 Mucosal Lectins and Ameliorates Experimental Colitis in Mice. *J Crohns Colitis* 2020; **14**: 1462-1472 [PMID: 32227170 DOI: 10.1093/ecco-jcc/jjaa065]

138 **Chambers ES**, Byrne CS, Morrison DJ, Murphy KG, Preston T, Tedford C, Garcia-Perez I, Fountana S, Serrano-Contreras JI, Holmes E, Reynolds CJ, Roberts JF, Boyton RJ, Altmann DM, McDonald JAK, Marchesi JR, Akbar AN, Riddell NE, Wallis GA, Frost GS. Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. *Gut* 2019; **68**: 1430-1438 [PMID: 30971437 DOI: 10.1136/gutjnl-2019-318424]

139 **Dupraz L**, Magniez A, Rolhion N, Richard ML, Da Costa G, Touch S, Mayeur C, Planchais J, Agus A, Danne C, Michaudel C, Spatz M, Trottein F, Langella P, Sokol H, Michel ML. Gut microbiota-derived short-chain fatty acids regulate IL-17 production by mouse and human intestinal γδ T cells. *Cell Rep* 2021; **36**: 109332 [PMID: 34233192 DOI: 10.1016/j.celrep.2021.109332]

140 **Haghikia A**, Zimmermann F, Schumann P, Jasina A, Roessler J, Schmidt D, Heinze P, Kaisler J, Nageswaran V, Aigner A, Ceglarek U, Cineus R, Hegazy AN, van der Vorst EPC, Döring Y, Strauch CM, Nemet I, Tremaroli V, Dwibedi C, Kränkel N, Leistner DM, Heimesaat MM, Bereswill S, Rauch G, Seeland U, Soehnlein O, Müller DN, Gold R, Bäckhed F, Hazen SL, Haghikia A, Landmesser U. Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. *Eur Heart J* 2022; **43**: 518-533 [PMID: 34597388 DOI: 10.1093/eurheartj/ehab644]

141 **Scheiman J**, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT, Yang Z, Hattab MW, Avila-Pacheco J, Clish CB, Lessard S, Church GM, Kostic AD. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions *via* lactate metabolism. *Nat Med* 2019; **25**: 1104-1109 [PMID: 31235964 DOI: 10.1038/s41591-019-0485-4]

142 **Zhang L**, Chu J, Hao W, Zhang J, Li H, Yang C, Yang J, Chen X, Wang H. Gut Microbiota and Type 2 Diabetes Mellitus: Association, Mechanism, and Translational Applications. *Mediators Inflamm* 2021; **2021**: 5110276 [PMID: 34447287 DOI: 10.1155/2021/5110276]

143 **Jacobson A**, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K, Bouley DM, Vilches-Moure JG, Smith M, Sonnenburg JL, Bhatt AS, Huang KC, Monack D. A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to Salmonella Infection. *Cell Host Microbe* 2018; **24**: 296-307.e7 [PMID: 30057174 DOI: 10.1016/j.chom.2018.07.002]

144 **Chen LM**, Bao CH, Wu Y, Liang SH, Wang D, Wu LY, Huang Y, Liu HR, Wu HG. Tryptophan-kynurenine metabolism: a link between the gut and brain for depression in inflammatory bowel disease. *J Neuroinflammation* 2021; **18**: 135 [PMID: 34127024 DOI: 10.1186/s12974-021-02175-2]

145 **Clarke G**, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, Dinan TG, Cryan JF. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* 2013; **18**: 666-673 [PMID: 22688187 DOI: 10.1038/mp.2012.77]

146 **Marx W**, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, Gomes-da-Costa S, Lane M, Sanches M, Diaz AP, Tseng PT, Lin PY, Berk M, Clarke G, O'Neil A, Jacka F, Stubbs B, Carvalho AF, Quevedo J, Soares JC, Fernandes BS. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Mol Psychiatry* 2021; **26**: 4158-4178 [PMID: 33230205 DOI: 10.1038/s41380-020-00951-9]

147 **Dehhaghi M**, Kazemi Shariat Panahi H, Guillemin GJ. Microorganisms, Tryptophan Metabolism, and Kynurenine Pathway: A Complex Interconnected Loop Influencing Human Health Status. *Int J Tryptophan Res* 2019; **12**: 1178646919852996 [PMID: 31258331 DOI: 10.1177/1178646919852996]

148 **Jamshed L**, Debnath A, Jamshed S, Wish JV, Raine JC, Tomy GT, Thomas PJ, Holloway AC. An Emerging Cross-Species Marker for Organismal Health: Tryptophan-Kynurenine Pathway. *Int J Mol Sci* 2022; **23** [PMID: 35682980 DOI: 10.3390/ijms23116300]

149 **Cervenka I**, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science* 2017; **357** [PMID: 28751584 DOI: 10.1126/science.aaf9794]

150 **Hajsl M**, Hlavackova A, Broulikova K, Sramek M, Maly M, Dyr JE, Suttnar J. Tryptophan Metabolism, Inflammation, and Oxidative Stress in Patients with Neurovascular Disease. *Metabolites* 2020; **10** [PMID: 32438592 DOI: 10.3390/metabo10050208]

151 **Bhattarai Y**, Williams BB, Battaglioli EJ, Whitaker WR, Till L, Grover M, Linden DR, Akiba Y, Kandimalla KK, Zachos NC, Kaunitz JD, Sonnenburg JL, Fischbach MA, Farrugia G, Kashyap PC. Gut Microbiota-Produced Tryptamine Activates an Epithelial G-Protein-Coupled Receptor to Increase Colonic Secretion. *Cell Host Microbe* 2018; **23**: 775-785.e5 [PMID: 29902441 DOI: 10.1016/j.chom.2018.05.004]

152 **Konopelski P**, Ufnal M. Indoles - Gut Bacteria Metabolites of Tryptophan with Pharmacotherapeutic Potential. *Curr Drug Metab* 2018; **19**: 883-890 [PMID: 29708069 DOI: 10.2174/1389200219666180427164731]

153 **Ridlon JM**, Harris SC, Bhowmik S, Kang DJ, Hylemon PB. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* 2016; **7**: 22-39 [PMID: 26939849 DOI: 10.1080/19490976.2015.1127483]

154 **Jia W**, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 111-128 [PMID: 29018272 DOI: 10.1038/nrgastro.2017.119]

155 **Fang Y**, Yan C, Zhao Q, Xu J, Liu Z, Gao J, Zhu H, Dai Z, Wang D, Tang D. The roles of microbial products in the development of colorectal cancer: a review. *Bioengineered* 2021; **12**: 720-735 [PMID: 33618627 DOI: 10.1080/21655979.2021.1889109]

156 **Cao W**, Tian W, Hong J, Li D, Tavares R, Noble L, Moss SF, Resnick MB. Expression of bile acid receptor TGR5 in gastric adenocarcinoma. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G322-G327 [PMID: 23238937 DOI: 10.1152/ajpgi.00263.2012]

157 **Vaughn BP**, Kaiser T, Staley C, Hamilton MJ, Reich J, Graiziger C, Singroy S, Kabage AJ, Sadowsky MJ, Khoruts A. A pilot study of fecal bile acid and microbiota profiles in inflammatory bowel disease and primary sclerosing cholangitis. *Clin Exp Gastroenterol* 2019; **12**: 9-19 [PMID: 30666146 DOI: 10.2147/CEG.S186097]

158 **Liang H**, Estes MK, Zhang H, Du G, Zhou Y. Bile acids target proteolipid nano-assemblies of EGFR and phosphatidic acid in the plasma membrane for stimulation of MAPK signaling. *PLoS One* 2018; **13**: e0198983 [PMID: 30169511 DOI: 10.1371/journal.pone.0198983]

159 **Shant J**, Cheng K, Marasa BS, Wang JY, Raufman JP. Akt-dependent NF-kappaB activation is required for bile acids to rescue colon cancer cells from stress-induced apoptosis. *Exp Cell Res* 2009; **315**: 432-450 [PMID: 19056378 DOI: 10.1016/j.yexcr.2008.11.003]

160 **Pai R**, Tarnawski AS, Tran T. Deoxycholic acid activates beta-catenin signaling pathway and increases colon cell cancer growth and invasiveness. *Mol Biol Cell* 2004; **15**: 2156-2163 [PMID: 15004225 DOI: 10.1091/mbc.e03-12-0894]

161 **Cheng K**, Raufman JP. Bile acid-induced proliferation of a human colon cancer cell line is mediated by transactivation of epidermal growth factor receptors. *Biochem Pharmacol* 2005; **70**: 1035-1047 [PMID: 16139803 DOI: 10.1016/j.bcp.2005.07.023]

162 **Ward JBJ**, Lajczak NK, Kelly OB, O'Dwyer AM, Giddam AK, Ní Gabhann J, Franco P, Tambuwala MM, Jefferies CA, Keely S, Roda A, Keely SJ. Ursodeoxycholic acid and lithocholic acid exert anti-inflammatory actions in the colon. *Am J Physiol Gastrointest Liver Physiol* 2017; **312**: G550-G558 [PMID: 28360029 DOI: 10.1152/ajpgi.00256.2016]

163 **West AC**, Jenkins BJ. Inflammatory and non-inflammatory roles for Toll-like receptors in gastrointestinal cancer. *Curr Pharm Des* 2015; **21**: 2968-2977 [PMID: 26004414 DOI: 10.2174/1381612821666150514104411]

164 **Mikó E**, Vida A, Kovács T, Ujlaki G, Trencsényi G, Márton J, Sári Z, Kovács P, Boratkó A, Hujber Z, Csonka T, Antal-Szalmás P, Watanabe M, Gombos I, Csoka B, Kiss B, Vígh L, Szabó J, Méhes G, Sebestyén A, Goedert JJ, Bai P.

Lithocholic acid, a bacterial metabolite reduces breast cancer cell proliferation and aggressiveness. *Biochim Biophys Acta Bioenerg* 2018; **1859**: 958-974 [PMID: 29655782 DOI: 10.1016/j.bbabio.2018.04.002]

165 **Kim EK**, Cho JH, Kim E, Kim YJ. Ursodeoxycholic acid inhibits the proliferation of colon cancer cells by regulating oxidative stress and cancer stem-like cell growth. *PLoS One* 2017; **12**: e0181183 [PMID: 28708871 DOI: 10.1371/journal.pone.0181183]

166 **Pearson T**, Caporaso JG, Yellowhair M, Bokulich NA, Padi M, Roe DJ, Wertheim BC, Linhart M, Martinez JA, Bilagody C, Hornstra H, Alberts DS, Lance P, Thompson PA. Effects of ursodeoxycholic acid on the gut microbiome and colorectal adenoma development. *Cancer Med* 2019; **8**: 617-628 [PMID: 30652422 DOI: 10.1002/cam4.1965]

167 **Carlström M**, Moretti CH, Weitzberg E, Lundberg JO. Microbiota, diet and the generation of reactive nitrogen compounds. *Free Radic Biol Med* 2020; **161**: 321-325 [PMID: 33131695 DOI: 10.1016/j.freeradbiomed.2020.10.025]

168 **Gatarek P**, Kaluzna-Czaplinska J. Trimethylamine N-oxide (TMAO) in human health. *EXCLI J* 2021; **20**: 301-319 [PMID: 33746664 DOI: 10.17179/excli2020-3239]

169 **Janeiro MH**, Ramírez MJ, Milagro FI, Martínez JA, Solas M. Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. *Nutrients* 2018; **10** [PMID: 30275434 DOI: 10.3390/nu10101398]

170 **Chhibber-Goel J**, Gaur A, Singhal V, Parakh N, Bhargava B, Sharma A. The complex metabolism of trimethylamine in humans: endogenous and exogenous sources. *Expert Rev Mol Med* 2016; **18**: e8 [PMID: 27126549 DOI: 10.1017/erm.2016.6]

171 **Senchukova M**, Tomchuk O, Shurygina E, Letuta S, Alidzhanov E, Nikiyan H, Razdobreev D. Calcium Carbonate Nanoparticles Can Activate the Epithelial–Mesenchymal Transition in an Experimental Gastric Cancer Model. *Biomedicines* 2019; **7** [PMID: 30893803 DOI: 10.3390/biomedicines7010021]

172 **Millard HR**, Musani SK, Dibaba DT, Talegawkar SA, Taylor HA, Tucker KL, Bidulescu A. Dietary choline and betaine; associations with subclinical markers of cardiovascular disease risk and incidence of CVD, coronary heart disease and stroke: the Jackson Heart Study. *Eur J Nutr* 2018; **57**: 51-60 [PMID: 27550622 DOI: 10.1007/s00394-016-1296-8]

173 **Senthong V**, Wang Z, Li XS, Fan Y, Wu Y, Tang WH, Hazen SL. Intestinal Microbiota-Generated Metabolite Trimethylamine-N-Oxide and 5-Year Mortality Risk in Stable Coronary Artery Disease: The Contributory Role of Intestinal Microbiota in a COURAGE-Like Patient Cohort. *J Am Heart Assoc* 2016; **5** [PMID: 27287696 DOI: 10.1161/JAHA.115.002816]

174 **Yang S**, Li X, Yang F, Zhao R, Pan X, Liang J, Tian L, Liu L, Xing Y, Wu M. Gut Microbiota-Dependent Marker TMAO in Promoting Cardiovascular Disease: Inflammation Mechanism, Clinical Prognostic, and Potential as a Therapeutic Target. *Front Pharmacol* 2019; **10**: 1360 [PMID: 31803054 DOI: 10.3389/fphar.2019.01360]

175 **Kuo CH**, Liu CH, Wang JH, Hsu BG. Serum Trimethylamine N-Oxide Levels Correlate with Metabolic Syndrome in Coronary Artery Disease Patients. *Int J Environ Res Public Health* 2022; **19** [PMID: 35886563 DOI: 10.3390/ijerph19148710]

176 **Randrianarisoa E**, Lehn-Stefan A, Wang X, Hoene M, Peter A, Heinzmann SS, Zhao X, Königsrainer I, Königsrainer A, Balletshofer B, Machann J, Schick F, Fritsche A, Häring HU, Xu G, Lehmann R, Stefan N. Relationship of Serum Trimethylamine N-Oxide (TMAO) Levels with early Atherosclerosis in Humans. *Sci Rep* 2016; **6**: 26745 [PMID: 27228955 DOI: 10.1038/srep26745]

177 **Chou RH**, Chen CY, Chen IC, Huang HL, Lu YW, Kuo CS, Chang CC, Huang PH, Chen JW, Lin SJ. Trimethylamine N-Oxide, Circulating Endothelial Progenitor Cells, and Endothelial Function in Patients with Stable Angina. *Sci Rep* 2019; **9**: 4249 [PMID: 30862856 DOI: 10.1038/s41598-019-40638-y]

178 **Yao ME**, Liao PD, Zhao XJ, Wang L. Trimethylamine-N-oxide has prognostic value in coronary heart disease: a meta-analysis and dose-response analysis. *BMC Cardiovasc Disord* 2020; **20**: 7 [PMID: 31918665 DOI: 10.1186/s12872-019-01310-5]

179 **Wiese GN**, Biruete A, Moorthi RN, Moe SM, Lindemann SR, Hill Gallant KM. Plant-Based Diets, the Gut Microbiota, and Trimethylamine N-Oxide Production in Chronic Kidney Disease: Therapeutic Potential and Methodological Considerations. *J Ren Nutr* 2021; **31**: 121-131 [PMID: 32616440 DOI: 10.1053/j.jrn.2020.04.007]

180 **Zixin Y**, Lulu C, Xiangchang Z, Qing F, Binjie Z, Chunyang L, Tai R, Dongsheng O. TMAO as a potential biomarker and therapeutic target for chronic kidney disease: A review. *Front Pharmacol* 2022; **13**: 929262 [PMID: 36034781 DOI: 10.3389/fphar.2022.929262]

181 **Bae S**, Ulrich CM, Neuhouser ML, Malysheva O, Bailey LB, Xiao L, Brown EC, Cushing-Haugen KL, Zheng Y, Cheng TY, Miller JW, Green R, Lane DS, Beresford SA, Caudill MA. Plasma choline metabolites and colorectal cancer risk in the Women's Health Initiative Observational Study. *Cancer Res* 2014; **74**: 7442-7452 [PMID: 25336191 DOI: 10.1158/0008-5472.CAN-14-1835]

182 **Liu X**, Liu H, Yuan C, Zhang Y, Wang W, Hu S, Liu L, Wang Y. Preoperative serum TMAO level is a new prognostic marker for colorectal cancer. *Biomark Med* 2017; **11**: 443-447 [PMID: 28621609 DOI: 10.2217/bmm-2016-0262]

183 **Ma G**, Pan B, Chen Y, Guo C, Zhao M, Zheng L, Chen B. Trimethylamine N-oxide in atherogenesis: impairing endothelial self-repair capacity and enhancing monocyte adhesion. *Biosci Rep* 2017; **37** [PMID: 28153917 DOI: 10.1042/BSR20160244]

184 **Liu Y**, Dai M. Trimethylamine N-Oxide Generated by the Gut Microbiota Is Associated with Vascular Inflammation: New Insights into Atherosclerosis. *Mediators Inflamm* 2020; **2020**: 4634172 [PMID: 32148438 DOI: 10.1155/2020/4634172]

185 **Seldin MM**, Meng Y, Qi H, Zhu W, Wang Z, Hazen SL, Lusis AJ, Shih DM. Trimethylamine N-Oxide Promotes Vascular Inflammation Through Signaling of Mitogen-Activated Protein Kinase and Nuclear Factor-κB. *J Am Heart Assoc* 2016; **5** [PMID: 26903003 DOI: 10.1161/JAHA.115.002767]

186 **Chen K**, Zheng X, Feng M, Li D, Zhang H. Gut Microbiota-Dependent Metabolite Trimethylamine N-Oxide Contributes to Cardiac Dysfunction in Western Diet-Induced Obese Mice. *Front Physiol* 2017; **8**: 139 [PMID: 28377725 DOI: 10.3389/fphys.2017.00139]

187 **Yang S**, Dai H, Lu Y, Li R, Gao C, Pan S. Trimethylamine N-Oxide Promotes Cell Proliferation and Angiogenesis in

Colorectal Cancer. *J Immunol Res* 2022; **2022**: 7043856 [PMID: 35832644 DOI: 10.1155/2022/7043856]
188 **Xu R**, Wang Q, Li L. A genome-wide systems analysis reveals strong link between colorectal cancer and trimethylamine N-oxide (TMAO), a gut microbial metabolite of dietary meat and fat. *BMC Genomics* 2015; **16** Suppl 7: S4 [PMID: 26100814 DOI: 10.1186/1471-2164-16-S7-S4]
189 **Chan CWH**, Law BMH, Waye MMY, Chan JYW, So WKW, Chow KM. Trimethylamine-N-oxide as One Hypothetical Link for the Relationship between Intestinal Microbiota and Cancer - Where We Are and Where Shall We Go? *J Cancer* 2019; **10**: 5874-5882 [PMID: 31737123 DOI: 10.7150/jca.31737]
190 **Wallace JL**, Motta JP, Buret AG. Hydrogen sulfide: an agent of stability at the microbiome-mucosa interface. *Am J Physiol Gastrointest Liver Physiol* 2018; **314**: G143-G149 [PMID: 29025733 DOI: 10.1152/ajpgi.00249.2017]
191 **Khattak S**, Rauf MA, Khan NH, Zhang QQ, Chen HJ, Muhammad P, Ansari MA, Alomary MN, Jahangir M, Zhang CY, Ji XY, Wu DD. Hydrogen Sulfide Biology and Its Role in Cancer. *Molecules* 2022; **27** [PMID: 35684331 DOI: 10.3390/molecules27113389]
192 **Mottawea W**, Chiang CK, Mühlbauer M, Starr AE, Butcher J, Abujamel T, Deeke SA, Brandel A, Zhou H, Shokralla S, Hajibabaei M, Singleton R, Benchimol EI, Jobin C, Mack DR, Figeys D, Stintzi A. Altered intestinal microbiota-host mitochondria crosstalk in new onset Crohn's disease. *Nat Commun* 2016; **7**: 13419 [PMID: 27876802 DOI: 10.1038/ncomms13419]
193 **Motta JP**, Flannigan KL, Agbor TA, Beatty JK, Blackler RW, Workentine ML, Da Silva GJ, Wang R, Buret AG, Wallace JL. Hydrogen sulfide protects from colitis and restores intestinal microbiota biofilm and mucus production. *Inflamm Bowel Dis* 2015; **21**: 1006-1017 [PMID: 25738373 DOI: 10.1097/MIB.0000000000000345]
194 **Liu Y**, Liao R, Qiang Z, Yang W, Cao J. Exogenous H2S promotes ion channel reconstruction to regulate colonic motility in rats with dinitrobenzene sulfonic acid-induced colitis. *Ann Transl Med* 2022; **10**: 681 [PMID: 35845545 DOI: 10.21037/atm-22-2126]
195 **Zhen Y**, Pan W, Hu F, Wu H, Feng J, Zhang Y, Chen J. Exogenous hydrogen sulfide exerts proliferation/anti-apoptosis/angiogenesis/migration effects *via* amplifying the activation of NF-κB pathway in PLC/PRF/5 hepatoma cells. *Int J Oncol* 2015; **46**: 2194-2204 [PMID: 25738635 DOI: 10.3892/ijo.2015.2914]
196 **Zhen Y**, Wu Q, Ding Y, Zhang W, Zhai Y, Lin X, Weng Y, Guo R, Zhang Y, Feng J, Lei Y, Chen J. Exogenous hydrogen sulfide promotes hepatocellular carcinoma cell growth by activating the STAT3-COX-2 signaling pathway. *Oncol Lett* 2018; **15**: 6562-6570 [PMID: 29725404 DOI: 10.3892/ol.2018.8154]
197 **Dong Q**, Yang B, Han JG, Zhang MM, Liu W, Zhang X, Yu HL, Liu ZG, Zhang SH, Li T, Wu DD, Ji XY, Duan SF. A novel hydrogen sulfide-releasing donor, HA-ADT, suppresses the growth of human breast cancer cells through inhibiting the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK signaling pathways. *Cancer Lett* 2019; **455**: 60-72 [PMID: 31042588 DOI: 10.1016/j.canlet.2019.04.031]
198 **Ma Y**, Yan Z, Deng X, Guo J, Hu J, Yu Y, Jiao F. Anticancer effect of exogenous hydrogen sulfide in cisplatinresistant A549/DDP cells. *Oncol Rep* 2018; **39**: 2969-2977 [PMID: 29658603 DOI: 10.3892/or.2018.6362]
199 **Ijssennagger N**, van der Meer R, van Mil SWC. Sulfide as a Mucus Barrier-Breaker in Inflammatory Bowel Disease? *Trends Mol Med* 2016; **22**: 190-199 [PMID: 26852376 DOI: 10.1016/j.molmed.2016.01.002]
200 **Rong F**, Wang T, Zhou Q, Peng H, Yang J, Fan Q, Li P. Intelligent polymeric hydrogen sulfide delivery systems for therapeutic applications. *Bioact Mater* 2023; **19**: 198-216 [PMID: 35510171 DOI: 10.1016/j.bioactmat.2022.03.043]
201 **Liu J**, Mesfin FM, Hunter CE, Olson KR, Shelley WC, Brokaw JP, Manohar K, Markel TA. Recent Development of the Molecular and Cellular Mechanisms of Hydrogen Sulfide Gasotransmitter. *Antioxidants (Basel)* 2022; **11** [PMID: 36139861 DOI: 10.3390/antiox11091788]
202 **Murray Stewart T**, Dunston TT, Woster PM, Casero RA Jr. Polyamine catabolism and oxidative damage. *J Biol Chem* 2018; **293**: 18736-18745 [PMID: 30333229 DOI: 10.1074/jbc.TM118.003337]
203 **Sagar NA**, Tarafdar S, Agarwal S, Tarafdar A, Sharma S. Polyamines: Functions, Metabolism, and Role in Human Disease Management. *Med Sci (Basel)* 2021; **9** [PMID: 34207607 DOI: 10.3390/medsci9020044]
204 **Hanus M**, Parada-Venegas D, Landskron G, Wielandt AM, Hurtado C, Alvarez K, Hermoso MA, López-Köstner F, De la Fuente M. Immune System, Microbiota, and Microbial Metabolites: The Unresolved Triad in Colorectal Cancer Microenvironment. *Front Immunol* 2021; **12**: 612826 [PMID: 33841394 DOI: 10.3389/fimmu.2021.612826]
205 **Tofalo R**, Cocchi S, Suzzi G. Polyamines and Gut Microbiota. *Front Nutr* 2019; **6**: 16 [PMID: 30859104 DOI: 10.3389/fnut.2019.00016]
206 **Pugin B**, Barcik W, Westermann P, Heider A, Wawrzyniak M, Hellings P, Akdis CA, O'Mahony L. A wide diversity of bacteria from the human gut produces and degrades biogenic amines. *Microb Ecol Health Dis* 2017; **28**: 1353881 [PMID: 28959180 DOI: 10.1080/16512235.2017.1353881]
207 **Kibe R**, Kurihara S, Sakai Y, Suzuki H, Ooga T, Sawaki E, Muramatsu K, Nakamura A, Yamashita A, Kitada Y, Kakeyama M, Benno Y, Matsumoto M. Upregulation of colonic luminal polyamines produced by intestinal microbiota delays senescence in mice. *Sci Rep* 2014; **4**: 4548 [PMID: 24686447 DOI: 10.1038/srep04548]
208 **Kurihara S**. Polyamine metabolism and transport in gut microbes. *Biosci Biotechnol Biochem* 2022; **86**: 957-966 [PMID: 35648468 DOI: 10.1093/bbb/zbac080]
209 **Ramos-Molina B**, Queipo-Ortuño MI, Lambertos A, Tinahones FJ, Peñafiel R. Dietary and Gut Microbiota Polyamines in Obesity- and Age-Related Diseases. *Front Nutr* 2019; **6**: 24 [PMID: 30923709 DOI: 10.3389/fnut.2019.00024]
210 **Kovács T**, Mikó E, Vida A, Sebő É, Toth J, Csonka T, Boratkó A, Ujlaki G, Lente G, Kovács P, Tóth D, Árkosy P, Kiss B, Méhes G, Goedert JJ, Bai P. Cadaverine, a metabolite of the microbiome, reduces breast cancer aggressiveness through trace amino acid receptors. *Sci Rep* 2019; **9**: 1300 [PMID: 30718646 DOI: 10.1038/s41598-018-37664-7]
211 **Casero RA Jr**, Murray Stewart T, Pegg AE. Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer* 2018; **18**: 681-695 [PMID: 30181570 DOI: 10.1038/s41568-018-0050-3]
212 **Yang Y**, Misra BB, Liang L, Bi D, Weng W, Wu W, Cai S, Qin H, Goel A, Li X, Ma Y. Integrated microbiome and metabolome analysis reveals a novel interplay between commensal bacteria and metabolites in colorectal cancer. *Theranostics* 2019; **9**: 4101-4114 [PMID: 31281534 DOI: 10.7150/thno.35186]
213 **Avuthu N**, Guda C. Meta-Analysis of Altered Gut Microbiota Reveals Microbial and Metabolic Biomarkers for

Colorectal Cancer. *Microbiol Spectr* 2022; **10**: e0001322 [PMID: 35766483 DOI: 10.1128/spectrum.00013-22]

214 **Huang CY**, Fang YJ, Abulimiti A, Yang X, Li L, Liu KY, Zhang X, Feng XL, Chen YM, Zhang CX. Dietary Polyamines Intake and Risk of Colorectal Cancer: A Case-Control Study. *Nutrients* 2020; **12** [PMID: 33266410 DOI: 10.3390/nu12113575]

215 **Nogacka AM**, Gómez-Martín M, Suárez A, González-Bernardo O, de Los Reyes-Gavilán CG, González S. Xenobiotics Formed during Food Processing: Their Relation with the Intestinal Microbiota and Colorectal Cancer. *Int J Mol Sci* 2019; **20** [PMID: 31027304 DOI: 10.3390/ijms20082051]

216 **Kobets T**, Smith BPC, Williams GM. Food-Borne Chemical Carcinogens and the Evidence for Human Cancer Risk. *Foods* 2022; **11** [PMID: 36140952 DOI: 10.3390/foods11182828]

217 **Abu-Ghazaleh N**, Chua WJ, Gopalan V. Intestinal microbiota and its association with colon cancer and red/processed meat consumption. *J Gastroenterol Hepatol* 2021; **36**: 75-88 [PMID: 32198788 DOI: 10.1111/jgh.15042]

218 **Zhang J**, Empl MT, Schwab C, Fekry MI, Engels C, Schneider M, Lacroix C, Steinberg P, Sturla SJ. Gut Microbial Transformation of the Dietary Imidazoquinoxaline Mutagen MelQx Reduces Its Cytotoxic and Mutagenic Potency. *Toxicol Sci* 2017; **159**: 266-276 [PMID: 28666384 DOI: 10.1093/toxsci/kfx132]

219 **Zhang J**, Lacroix C, Wortmann E, Ruscheweyh HJ, Sunagawa S, Sturla SJ, Schwab C. Gut microbial beta-glucuronidase and glycerol/diol dehydratase activity contribute to dietary heterocyclic amine biotransformation. *BMC Microbiol* 2019; **19**: 99 [PMID: 31096909 DOI: 10.1186/s12866-019-1483-x]

220 **Saulnier DM**, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, Weidler EM, Qin X, Coarfa C, Milosavljevic A, Petrosino JF, Highlander S, Gibbs R, Lynch SV, Shulman RJ, Versalovic J. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology* 2011; **141**: 1782-1791 [PMID: 21741921 DOI: 10.1053/j.gastro.2011.06.072]

221 **Pittayanon R**, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, Moayyedi P. Gut Microbiota in Patients With Irritable Bowel Syndrome-A Systematic Review. *Gastroenterology* 2019; **157**: 97-108 [PMID: 30940523 DOI: 10.1053/j.gastro.2019.03.049]

222 **Hugerth LW**, Andreasson A, Talley NJ, Forsberg AM, Kjellström L, Schmidt PT, Agreus L, Engstrand L. No distinct microbiome signature of irritable bowel syndrome found in a Swedish random population. *Gut* 2020; **69**: 1076-1084 [PMID: 31601615 DOI: 10.1136/gutjnl-2019-318717]

223 **Hirano A**, Umeno J, Okamoto Y, Shibata H, Ogura Y, Moriyama T, Torisu T, Fujioka S, Fuyuno Y, Kawarabayasi Y, Matsumoto T, Kitazono T, Esaki M. Comparison of the microbial community structure between inflamed and non-inflamed sites in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2018 [PMID: 29462845 DOI: 10.1111/jgh.14129]

224 **Gevers D**, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; **15**: 382-392 [PMID: 24629344 DOI: 10.1016/j.chom.2014.02.005]

225 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313 DOI: 10.1002/hep.26319]

226 **Wei X**, Yan X, Zou D, Yang Z, Wang X, Liu W, Wang S, Li X, Han J, Huang L, Yuan J. Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. *BMC Gastroenterol* 2013; **13**: 175 [PMID: 24369878 DOI: 10.1186/1471-230X-13-175]

227 **Boursier J**, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]

228 **Liu BN**, Liu XT, Liang ZH, Wang JH. Gut microbiota in obesity. *World J Gastroenterol* 2021; **27**: 3837-3850 [PMID: 34321848 DOI: 10.3748/wjg.v27.i25.3837]

229 **Zeng Q**, Li D, He Y, Li Y, Yang Z, Zhao X, Liu Y, Wang Y, Sun J, Feng X, Wang F, Chen J, Zheng Y, Yang Y, Sun X, Xu X, Wang D, Kenney T, Jiang Y, Gu H, Zhou K, Li S, Dai W. Discrepant gut microbiota markers for the classification of obesity-related metabolic abnormalities. *Sci Rep* 2019; **9**: 13424 [PMID: 31530820 DOI: 10.1038/s41598-019-49462-w]

230 **Zhou Z**, Sun B, Yu D, Zhu C. Gut Microbiota: An Important Player in Type 2 Diabetes Mellitus. *Front Cell Infect Microbiol* 2022; **12**: 834485 [PMID: 35242721 DOI: 10.3389/fcimb.2022.834485]

231 **Tang SS**, Liang CH, Liu YL, Wei W, Deng XR, Shi XY, Wang LM, Zhang LJ, Yuan HJ. Intermittent hypoxia is involved in gut microbial dysbiosis in type 2 diabetes mellitus and obstructive sleep apnea-hypopnea syndrome. *World J Gastroenterol* 2022; **28**: 2320-2333 [PMID: 35800187 DOI: 10.3748/wjg.v28.i21.2320]

232 **Ozaki D**, Kubota R, Maeno T, Abdelhakim M, Hitosugi N. Association between gut microbiota, bone metabolism, and fracture risk in postmenopausal Japanese women. *Osteoporos Int* 2021; **32**: 145-156 [PMID: 33241467 DOI: 10.1007/s00198-020-05728-y]

233 **Long D**, Mao C, Zhang X, Liu Y, Shangguan X, Zou M, Zhu Y, Wang X. Coronary heart disease and gut microbiota: A bibliometric and visual analysis from 2002 to 2022. *Front Cardiovasc Med* 2022; **9**: 949859 [PMID: 36158832 DOI: 10.3389/fcvm.2022.949859]

234 **Yu H**, Li L, Deng Y, Zhang G, Jiang M, Huang H, Li C, Lv Z, Zhou Y, Liu X. The relationship between the number of stenotic coronary arteries and the gut microbiome in coronary heart disease patients. *Front Cell Infect Microbiol* 2022; **12**: 903828 [PMID: 36093192 DOI: 10.3389/fcimb.2022.903828]

235 **Huo RX**, Wang YJ, Hou SB, Wang W, Zhang CZ, Wan XH. Gut mucosal microbiota profiles linked to colorectal cancer recurrence. *World J Gastroenterol* 2022; **28**: 1946-1964 [PMID: 35664963 DOI: 10.3748/wjg.v28.i18.1946]

236 **Bajaj JS**, Khoruts A. Microbiota changes and intestinal microbiota transplantation in liver diseases and cirrhosis. *J*

Hepatol 2020; **72**: 1003-1027 [PMID: 32004593 DOI: 10.1016/j.jhep.2020.01.017]
237 **Novakovic M**, Rout A, Kingsley T, Kirchoff R, Singh A, Verma V, Kant R, Chaudhary R. Role of gut microbiota in cardiovascular diseases. *World J Cardiol* 2020; **12**: 110-122 [PMID: 32431782 DOI: 10.4330/wjc.v12.i4.110]
238 **Bullman S**, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, Neuberg D, Huang K, Guevara F, Nelson T, Chipashvili O, Hagan T, Walker M, Ramachandran A, Diosdado B, Serna G, Mulet N, Landolfi S, Ramon Y Cajal S, Fasani R, Aguirre AJ, Ng K, Élez E, Ogino S, Tabernero J, Fuchs CS, Hahn WC, Nuciforo P, Meyerson M. Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. *Science* 2017; **358**: 1443-1448 [PMID: 29170280 DOI: 10.1126/science.aal5240]
239 **Chen S**, Su T, Zhang Y, Lee A, He J, Ge Q, Wang L, Si J, Zhuo W. Fusobacterium nucleatum promotes colorectal cancer metastasis by modulating KRT7-AS/KRT7. *Gut Microbes* 2020; **11**: 511-525 [PMID: 31910722 DOI: 10.1080/19490976.2019.1695494]
240 **Dickerson F**, Severance E, Yolken R. The microbiome, immunity, and schizophrenia and bipolar disorder. *Brain Behav Immun* 2017; **62**: 46-52 [PMID: 28003152 DOI: 10.1016/j.bbi.2016.12.010]
241 **van De Sande MM**, van Buul VJ, Brouns FJ. Autism and nutrition: the role of the gut-brain axis. *Nutr Res Rev* 2014; **27**: 199-214 [PMID: 25004237 DOI: 10.1017/S0954422414000110]
242 **Gerhardt S**, Mohajeri MH. Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases. *Nutrients* 2018; **10** [PMID: 29857583 DOI: 10.3390/nu10060708]
243 **Minato T**, Maeda T, Fujisawa Y, Tsuji H, Nomoto K, Ohno K, Hirayama M. Progression of Parkinson's disease is associated with gut dysbiosis: Two-year follow-up study. *PLoS One* 2017; **12**: e0187307 [PMID: 29091972 DOI: 10.1371/journal.pone.0187307]
244 **Li X**, He C, Li N, Ding L, Chen H, Wan J, Yang X, Xia L, He W, Xiong H, Shu X, Zhu Y, Lu N. The interplay between the gut microbiota and NLRP3 activation affects the severity of acute pancreatitis in mice. *Gut Microbes* 2020; **11**: 1774-1789 [PMID: 32529941 DOI: 10.1080/19490976.2020.1770042]
245 **Zhang Y**, Saint Fleur A, Feng H. The development of live biotherapeutics against Clostridioides difficile infection towards reconstituting gut microbiota. *Gut Microbes* 2022; **14**: 2052698 [PMID: 35319337 DOI: 10.1080/19490976.2022.2052698]
246 **Ianiro G**, Mullish BH, Kelly CR, Kassam Z, Kuijper EJ, Ng SC, Iqbal TH, Allegretti JR, Bibbò S, Sokol H, Zhang F, Fischer M, Costello SP, Keller JJ, Masucci L, van Prehn J, Quaranta G, Quraishi MN, Segal J, Kao D, Satokari R, Sanguinetti M, Tilg H, Gasbarrini A, Cammarota G. Reorganisation of faecal microbiota transplant services during the COVID-19 pandemic. *Gut* 2020; **69**: 1555-1563 [PMID: 32620549 DOI: 10.1136/gutjnl-2020-321829]
247 **Yu D**, Meng X, de Vos WM, Wu H, Fang X, Maiti AK. Implications of Gut Microbiota in Complex Human Diseases. *Int J Mol Sci* 2021; **22** [PMID: 34884466 DOI: 10.3390/ijms222312661]
248 **Asnicar F**, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, Leeming E, Gibson R, Le Roy C, Khatib HA, Francis L, Mazidi M, Mompeo O, Valles-Colomer M, Tett A, Beghini F, Dubois L, Bazzani D, Thomas AM, Mirzayi C, Khleborodova A, Oh S, Hine R, Bonnett C, Capdevila J, Danzanvilliers S, Giordano F, Geistlinger L, Waldron L, Davies R, Hadjigeorgiou G, Wolf J, Ordovás JM, Gardner C, Franks PW, Chan AT, Huttenhower C, Spector TD, Segata N. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nat Med* 2021; **27**: 321-332 [PMID: 33432175 DOI: 10.1038/s41591-020-01183-8]
249 **O'Neill AM**, Burrington CM, Gillaspie EA, Lynch DT, Horsman MJ, Greene MW. High-fat Western diet-induced obesity contributes to increased tumor growth in mouse models of human colon cancer. *Nutr Res* 2016; **36**: 1325-1334 [PMID: 27866828 DOI: 10.1016/j.nutres.2016.10.005]
250 **Zeng H**, Lazarova DL, Bordonaro M. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. *World J Gastrointest Oncol* 2014; **6**: 41-51 [PMID: 24567795 DOI: 10.4251/wjgo.v6.i2.41]
251 **Gibson GR**, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 491-502 [PMID: 28611480 DOI: 10.1038/nrgastro.2017.75]
252 **McNabney SM**, Henagan TM. Short Chain Fatty Acids in the Colon and Peripheral Tissues: A Focus on Butyrate, Colon Cancer, Obesity and Insulin Resistance. *Nutrients* 2017; **9** [PMID: 29231905 DOI: 10.3390/nu9121348]
253 **Makki K**, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe* 2018; **23**: 705-715 [PMID: 29902436 DOI: 10.1016/j.chom.2018.05.012]
254 **Gunness P**, Michiels J, Vanhaecke L, De Smet S, Kravchuk O, Van de Meene A, Gidley MJ. Reduction in circulating bile acid and restricted diffusion across the intestinal epithelium are associated with a decrease in blood cholesterol in the presence of oat β-glucan. *FASEB J* 2016; **30**: 4227-4238 [PMID: 27630168 DOI: 10.1096/fj.201600465R]
255 **Gunness P**, Williams BA, Gerrits WJ, Bird AR, Kravchuk O, Gidley MJ. Circulating triglycerides and bile acids are reduced by a soluble wheat arabinoxylan *via* modulation of bile concentration and lipid digestion rates in a pig model. *Mol Nutr Food Res* 2016; **60**: 642-651 [PMID: 26694900 DOI: 10.1002/mnfr.201500686]
256 **Brown JR**, Flemer B, Joyce SA, Zulquernain A, Sheehan D, Shanahan F, O'Toole PW. Changes in microbiota composition, bile and fatty acid metabolism, in successful faecal microbiota transplantation for Clostridioides difficile infection. *BMC Gastroenterol* 2018; **18**: 131 [PMID: 30153805 DOI: 10.1186/s12876-018-0860-5]
257 **Hassouneh R**, Bajaj JS. Gut Microbiota Modulation and Fecal Transplantation: An Overview on Innovative Strategies for Hepatic Encephalopathy Treatment. *J Clin Med* 2021; **10** [PMID: 33477417 DOI: 10.3390/jcm10020330]
258 **Costello SP**, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, Katsikeros R, Makanyanga J, Campaniello MA, Mavrangelos C, Rosewarne CP, Bickley C, Peters C, Schoeman MN, Conlon MA, Roberts-Thomson IC, Andrews JM. Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis: A Randomized Clinical Trial. *JAMA* 2019; **321**: 156-164 [PMID: 30644982 DOI: 10.1001/jama.2018.20046]
259 **Caldeira LF**, Borba HH, Tonin FS, Wiens A, Fernandez-Llimos F, Pontarolo R. Fecal microbiota transplantation in inflammatory bowel disease patients: A systematic review and meta-analysis. *PLoS One* 2020; **15**: e0238910 [PMID: 32946509 DOI: 10.1371/journal.pone.0238910]

260 **Scibelli N**, Singh P, Raynor K. Intestinal Dysbiosis Disguised as a Rectal Fistula Treated With Autologous Fecal Microbiota Transplantation. *Cureus* 2021; **13**: e14115 [PMID: 33927926 DOI: 10.7759/cureus.14115]

261 **Xiang L**, Ding X, Li Q, Wu X, Dai M, Long C, He Z, Cui B, Zhang F. Efficacy of faecal microbiota transplantation in Crohn's disease: a new target treatment? *Microb Biotechnol* 2020; **13**: 760-769 [PMID: 31958884 DOI: 10.1111/1751-7915.13536]

262 **Xu MQ**, Cao HL, Wang WQ, Wang S, Cao XC, Yan F, Wang BM. Fecal microbiota transplantation broadening its application beyond intestinal disorders. *World J Gastroenterol* 2015; **21**: 102-111 [PMID: 25574083 DOI: 10.3748/wjg.v21.i1.102]

263 **Vendrik KEW**, Ooijevaar RE, de Jong PRC, Laman JD, van Oosten BW, van Hilten JJ, Ducarmon QR, Keller JJ, Kuijper EJ, Contarino MF. Fecal Microbiota Transplantation in Neurological Disorders. *Front Cell Infect Microbiol* 2020; **10**: 98 [PMID: 32266160 DOI: 10.3389/fcimb.2020.00098]

264 **Chen D**, Wu J, Jin D, Wang B, Cao H. Fecal microbiota transplantation in cancer management: Current status and perspectives. *Int J Cancer* 2019; **145**: 2021-2031 [PMID: 30458058 DOI: 10.1002/ijc.32003]

265 **DeFilipp Z**, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, Turbett S, Chung RT, Chen YB, Hohmann EL. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019; **381**: 2043-2050 [PMID: 31665575 DOI: 10.1056/NEJMoa1910437]

266 **Wang X**, Zhao J, Feng Y, Feng Z, Ye Y, Liu L, Kang G, Cao X. Evolutionary Insights Into Microbiota Transplantation in Inflammatory Bowel Disease. *Front Cell Infect Microbiol* 2022; **12**: 916543 [PMID: 35811664 DOI: 10.3389/fcimb.2022.916543]

267 **Rode AA**, Chehri M, Krogsgaard LR, Heno KK, Svendsen AT, Ribberholt I, Helms M, Engberg J, Schønning K, Tvede M, Andersen CØ, Jensen US, Petersen AM, Bytzer P. Randomised clinical trial: a 12-strain bacterial mixture *vs* faecal microbiota transplantation *vs* vancomycin for recurrent Clostridioides difficile infections. *Aliment Pharmacol Ther* 2021; **53**: 999-1009 [PMID: 33694229]

268 **Ghimire S**, Roy C, Wongkuna S, Antony L, Maji A, Keena MC, Foley A, Scaria J. Identification of Clostridioides difficile-Inhibiting Gut Commensals Using Culturomics, Phenotyping, and Combinatorial Community Assembly. *mSystems* 2020; **5** [PMID: 32019832 DOI: 10.1128/mSystems.00620-19]

269 **Zhang T**, Lu G, Zhao Z, Liu Y, Shen Q, Li P, Chen Y, Yin H, Wang H, Marcella C, Cui B, Cheng L, Ji G, Zhang F. Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and *in vitro* screening. *Protein Cell* 2020; **11**: 251-266 [PMID: 31919742 DOI: 10.1007/s13238-019-00684-8]

270 **Khanna S**, Pardi DS, Kelly CR, Kraft CS, Dhere T, Henn MR, Lombardo MJ, Vulic M, Ohsumi T, Winkler J, Pindar C, McGovern BH, Pomerantz RJ, Aunins JG, Cook DN, Hohmann EL. A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent Clostridium difficile Infection. *J Infect Dis* 2016; **214**: 173-181 [PMID: 26908752 DOI: 10.1093/infdis/jiv766]

271 **Hill C**, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 506-514 [PMID: 24912386 DOI: 10.1038/nrgastro.2014.66]

272 **Shen NT**, Maw A, Tmanova LL, Pino A, Ancy K, Crawford CV, Simon MS, Evans AT. Timely Use of Probiotics in Hospitalized Adults Prevents Clostridium difficile Infection: A Systematic Review With Meta-Regression Analysis. *Gastroenterology* 2017; **152**: 1889-1900.e9 [PMID: 28192108 DOI: 10.1053/j.gastro.2017.02.003]

273 **Dos Reis SA**, da Conceição LL, Siqueira NP, Rosa DD, da Silva LL, Peluzio MD. Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutr Res* 2017; **37**: 1-19 [PMID: 28215310 DOI: 10.1016/j.nutres.2016.11.009]

274 **Commane D**, Hughes R, Shortt C, Rowland I. The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutat Res* 2005; **591**: 276-289 [PMID: 16095630 DOI: 10.1016/j.mrfmmm.2005.02.027]

275 **Baradaran Ghavami S**, Asadzadeh Aghdaei H, Sorrentino D, Shahrokh S, Farmani M, Ashrafian F, Dore MP, Keshavarz Azizi Raftar S, Mobin Khoramjoo S, Zali MR. Probiotic-Induced Tolerogenic Dendritic Cells: A Novel Therapy for Inflammatory Bowel Disease? *Int J Mol Sci* 2021; **22** [PMID: 34361038 DOI: 10.3390/ijms22158274]

276 **Sanders ME**, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 605-616 [PMID: 31296969 DOI: 10.1038/s41575-019-0173-3]

277 **Engevik MA**, Herrmann B, Ruan W, Engevik AC, Engevik KA, Ihekweazu F, Shi Z, Luck B, Chang-Graham AL, Esparza M, Venable S, Horvath TD, Haidacher SJ, Hoch KM, Haag AM, Schady DA, Hyser JM, Spinler JK, Versalovic J. Bifidobacterium dentium-derived y-glutamylcysteine suppresses ER-mediated goblet cell stress and reduces TNBS-driven colonic inflammation. *Gut Microbes* 2021; **13**: 1-21 [PMID: 33985416 DOI: 10.1080/19490976.2021.1902717]

278 **Lau CS**, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Int J Gen Med* 2016; **9**: 27-37 [PMID: 26955289 DOI: 10.2147/IJGM.S98280]

279 **Nilsson AG**, Sundh D, Bäckhed F, Lorentzon M. Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med* 2018; **284**: 307-317 [PMID: 29926979 DOI: 10.1111/joim.12805]

280 **Whisner CM**, Martin BR, Nakatsu CH, Story JA, MacDonald-Clarke CJ, McCabe LD, McCabe GP, Weaver CM. Soluble Corn Fiber Increases Calcium Absorption Associated with Shifts in the Gut Microbiome: A Randomized Dose-Response Trial in Free-Living Pubertal Females. *J Nutr* 2016; **146**: 1298-1306 [PMID: 27281813 DOI: 10.3945/jn.115.227256]

281 **Hayashi A**, Nagao-Kitamoto H, Kitamoto S, Kim CH, Kamada N. The Butyrate-Producing Bacterium Clostridium butyricum Suppresses Clostridioides difficile Infection *via* Neutrophil- and Antimicrobial Cytokine-Dependent but GPR43/109a-Independent Mechanisms. *J Immunol* 2021; **206**: 1576-1585 [PMID: 33597149 DOI: 10.4049/jimmunol.2000353]

282 **Nale JY**, Spencer J, Hargreaves KR, Buckley AM, Trzepiński P, Douce GR, Clokie MR. Bacteriophage Combinations Significantly Reduce Clostridium difficile Growth In Vitro and Proliferation In Vivo. *Antimicrob Agents Chemother* 2016;

60: 968-981 [PMID: 26643348 DOI: 10.1128/AAC.01774-15]
283 **Enaud R**, Prevel R, Ciarlo E, Beaufils F, Wieërs G, Guery B, Delhaes L. The Gut-Lung Axis in Health and Respiratory Diseases: A Place for Inter-Organ and Inter-Kingdom Crosstalks. *Front Cell Infect Microbiol* 2020; **10**: 9 [PMID: 32140452 DOI: 10.3389/fcimb.2020.00009]
284 **Markey L**, Shaban L, Green ER, Lemon KP, Mecsas J, Kumamoto CA. Pre-colonization with the commensal fungus Candida albicans reduces murine susceptibility to Clostridium difficile infection. *Gut Microbes* 2018; **9**: 497-509 [PMID: 29667487 DOI: 10.1080/19490976.2018.1465158]
285 **Cassone M**, Serra P, Mondello F, Girolamo A, Scafetti S, Pistella E, Venditti M. Outbreak of Saccharomyces cerevisiae subtype boulardii fungemia in patients neighboring those treated with a probiotic preparation of the organism. *J Clin Microbiol* 2003; **41**: 5340-5343 [PMID: 14605200 DOI: 10.1128/JCM.41.11.5340-5343.2003]
286 **El-Sayed A**, Aleya L, Kamel M. Microbiota's role in health and diseases. *Environ Sci Pollut Res Int* 2021; **28**: 36967-36983 [PMID: 34043164 DOI: 10.1007/s11356-021-14593-z]
287 **Zhang B**, Wang X, Xia R, Li C. Gut microbiota in coronary artery disease: a friend or foe? *Biosci Rep* 2020; **40** [PMID: 32352513 DOI: 10.1042/BSR20200454]
288 **Li N**, Ma WT, Pang M, Fan QL, Hua JL. The Commensal Microbiota and Viral Infection: A Comprehensive Review. *Front Immunol* 2019; **10**: 1551 [PMID: 31333675 DOI: 10.3389/fimmu.2019.01551]
289 **Mikó E**, Kovács T, Sebő É, Tóth J, Csonka T, Ujlaki G, Sipos A, Szabó J, Méhes G, Bai P. Microbiome-Microbial Metabolome-Cancer Cell Interactions in Breast Cancer-Familiar, but Unexplored. *Cells* 2019; **8** [PMID: 30934972 DOI: 10.3390/cells8040293]
290 **Cheng WY**, Wu CY, Yu J. The role of gut microbiota in cancer treatment: friend or foe? *Gut* 2020; **69**: 1867-1876 [PMID: 32759302 DOI: 10.1136/gutjnl-2020-321153]
291 **Johnson CH**, Dejea CM, Edler D, Hoang LT, Santidrian AF, Felding BH, Ivanisevic J, Cho K, Wick EC, Hechenbleikner EM, Uritboonthai W, Goetz L, Casero RA Jr, Pardoll DM, White JR, Patti GJ, Sears CL, Siuzdak G. Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metab* 2015; **21**: 891-897 [PMID: 25959674 DOI: 10.1016/j.cmet.2015.04.011]
292 **Brunt VE**, Gioscia-Ryan RA, Richey JJ, Zigler MC, Cuevas LM, Gonzalez A, Vázquez-Baeza Y, Battson ML, Smithson AT, Gilley AD, Ackermann G, Neilson AP, Weir T, Davy KP, Knight R, Seals DR. Suppression of the gut microbiome ameliorates age-related arterial dysfunction and oxidative stress in mice. *J Physiol* 2019; **597**: 2361-2378 [PMID: 30714619 DOI: 10.1113/JP277336]

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REVIEW

Current status and future perspectives of radiomics in hepatocellular carcinoma

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Abstract

Given the frequent co-existence of an aggressive tumor and underlying chronic liver disease, the management of hepatocellular carcinoma (HCC) patients requires experienced multidisciplinary team discussion. Moreover, imaging plays a key role in the diagnosis, staging, restaging, and surveillance of HCC. Currently, imaging assessment of HCC entails the assessment of qualitative characteristics which are prone to inter-reader variability. Radiomics is an emerging field that extracts high-dimensional mineable quantitative features that cannot be assessed visually with the naked eye from medical imaging. The main potential applications of radiomic models in HCC are to predict histology, response to treatment, genetic signature, recurrence, and survival. Despite the encouraging results to date, there are challenges and limitations that need to be overcome before radiomics implementation in clinical practice. The purpose of this article is to review the main concepts and challenges pertaining to radiomics, and to review recent studies and potential applications of radiomics in HCC.

Key Words: Radiomics; Hepatocellular carcinoma; Texture analysis; Radiology

Core Tip: Radiomics is an emerging field that extracts high-dimensional mineable quantitative features that cannot be assessed visually with the naked eye from medical imaging. The main potential applications of radiomic models in hepatocellular carcinoma (HCC) are to predict histology, predict response to treatment, predict genetic signature, predict recurrence, and predict survival. The purpose of this article is to review the main concepts and challenges pertaining to radiomics, and to review recent studies and potential applications of radiomics in HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide [1]. Liver cancer is especially common in Asia, where 72.5% of all new liver cancer cases worldwide are diagnosed[2]. HCC accounts for over 90% of all primary liver cancer cases[3]. The main risk factors for HCC in the West is viral hepatitis (hepatitis C virus in the West and hepatitis B virus in Asia and in developing countries) and alcohol intake. In addition, non-alcoholic steatohepatitis is becoming a common risk factor, particularly in the West[3,4]. HCC patient prognosis depends on the stage of HCC at the time of diagnosis[5]; and advanced-staged patients at the time of diagnosis have a poor prognosis [5-7].

The treatment of HCC is based on tumor burden, clinical performance of the patient, and liver function[8]. Given the frequent co-existence of an aggressive tumor and underlying chronic liver disease, the management of HCC requires experienced multidisciplinary team discussion[9]. Moreover, radiology plays a key role in the screening, diagnosis, staging, restaging, and surveillance of HCC. Currently, imaging assessment is based on qualitative characteristics, such as size and enhancement pattern, which are prone to inter-reader variability. Reliable tools that can potentially address this variability as well as deal with the vast amount of imaging data are warranted[10]. Over the last decade, radiomics has become a popular quantitative tool that can potentially address these challenges and provide information not previously available for precision decision-making[11].

Radiomics is an emerging field that extracts high-dimensional mineable quantitative features that cannot be assessed visually with the naked eye from medical imaging[12]. The main potential applications of radiomic models in HCC are to predict histology, response to treatment, genetic signature, recurrence, and survival[13]. Despite the encouraging results to date, there are several challenges and limitations that need to be overcome before the implementation of radiomics in clinical practice. The purpose of this study is to review the main concepts, challenges pertaining to radiomics and recent studies and potential applications of radiomics in HCC.

RADIOMICS

Main concepts

In the new era of precision medicine, artificial intelligence (AI) and in its various branches, such as machine learning (ML) and deep learning (DL), have provided new imaging biomarkers that can potentially provide new data that are useful for clinical decision-making. ML is related to a set of computational systems that improve with experience. DL is a subset of ML based on series of layers (trainable nonlinear operations), each of which transforms input data into a representation that facilitates pattern recognition[14].

Radiomics has recently emerged as a translational research field that proposes to discover new associations between clinical data and quantitative data extracted from medical images using conventional biostatistics or AI methods[12] and become popular, particularly in oncologic imaging. Radiomics involves mineable high-dimensional data extraction, characterizing intensity, shape, size, and/or texture from images to create big-data datasets that are then used to identify distinct sub-visual imaging

patterns[15]. Radiomics models usually use magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET) images data. Fundamentally, radiomics is motivated by the observation that these imaging characteristics reflect phenotype and genotype of underlying tissue and thus can help in clinical decision making[16].

Radiomic can be subdivided into texture, size and shape, and transformed based features. The most common radiomic features is texture. It can be subdivided into first-order, second-order and higher-order statistical features. First-order features reflect the distribution of values of individual voxels without concern for spatial relationships; they are generally histogram-based, such as mean (average intensity), entropy (quantify randomness of intensity), kurtosis (flatness) and skewness (asymmetry). Second-order features reflect the statistical interrelationships between voxels with similar (or dissimilar) contrast values[12] and some of the commonly used 2nd order features are: Grey level co-occurrence matrix, grey level run length matrix, and grey level size zone matrix features. Taking into account the repetitive patterns in radiological images, higher-order statistical methods use sophisticated filter grids on the images - such as Minkowski functionals (to evaluate voxels whose intensity is above a determined threshold), Wavelet and Laplacian transforms (to identify coarse texture patterns) and fractal analysis (to assess the irregularity of a surface)[12]. In practice, standard libraries with predefined feature configurations and validated reference values (such as PyRadiomics) are frequently used to increase the reproducibility of radiomic models.

Workflow

Radiomic analysis is a multistep process involving the processing of medical images to generate different features from segmented images. The typical radiomics workflow can be summarized in the following steps (Figure 1):

Image acquisition and preprocessing: Standardized imaging protocols should be used to avoid reproducibility issues related to noise and confounding. However, standardized imaging protocols also decrease the generalizability of the results. Once a patient dataset has been identified, images should be anonymized as well as exported as Digital Imaging and Communication in Medicine files[17]. De-noising and motion correction steps may be needed.

Segmentation: Segmentation involves the delineation of region of interests (ROIs) on the tumor or peritumoral zones. ROIs can be delineated manually, semiautomatically, or automatically (using ML tools) in either two-dimensional (2D) or three-dimensional (3D) views (Figure 2). Whenever possible, segmentations should be checked by a radiologist to ensure accuracy.

Feature extraction, feature selection and model building: A wide range of statistical models are commonly used to choose a subset of optimal features that correlate with the predenid outcome[15]. Many of the extracted features are in fact redundant and supervised or unsupervised approaches can be applied to achieve dimension reduction. ML and DL techniques are emerging as useful tools to achieve more accurate feature selection[18,19]. The features should be selected only based on the training data to avoid bias.

Of note, the number of extracted features is commonly larger than the study sample, which can contribute to overfitting of the model and to overoptimistic results. Some strategies can be done, for example, select the features in such a way to maintain the ratio or regularization methods are used to minimize the complexity of the respective models[20]. Once the optimal features are identified, a statistical model can be proposed to predict a specific clinical question using different classifiers such as generalized linear models, random forests, support vector machines, or neural networks[20,21].

Validation: Validation is essential to estimate model performance and can be done using subsets of the original training dataset (*i.e.*, cross-validation) or using a separate hold-out dataset containing either internal or external data[17].

Main challenges

To date, radiomic models reproducibility is often poor, due to insufficient reporting or limited open-source code and data, which undermines external validation and increases the subsequent risk of false-positive results[22]. Further, researchers often face great difficulty in acquiring unbiased and homogeneous datasets across multiple institutions, thus hampering multi-institutional collaborations involving large multi-institutional datasets for the training and validation of radiomic models[14]. For successful multi-institutional cooperation for building large multi-institutional datasets for radiomic models training and validation, radiomics workflow standardization, clear reporting of study methodology, and data sharing across different institutions are needed[17]. Additionally, an effective means to interpret the vast and varied data derived from radiomics analysis is another key obstacle to the clinical implementation of radiomic models. Therefore, a balanced interpretation of results and an increased focus on interpretable models are essential to their successful integration into clinical practice [23]. Finally, manual segmentation is a time-consuming process and one of the most common limitations that should be managed with automatic or semiautomatic strategies before widespread use of radiomics tools.

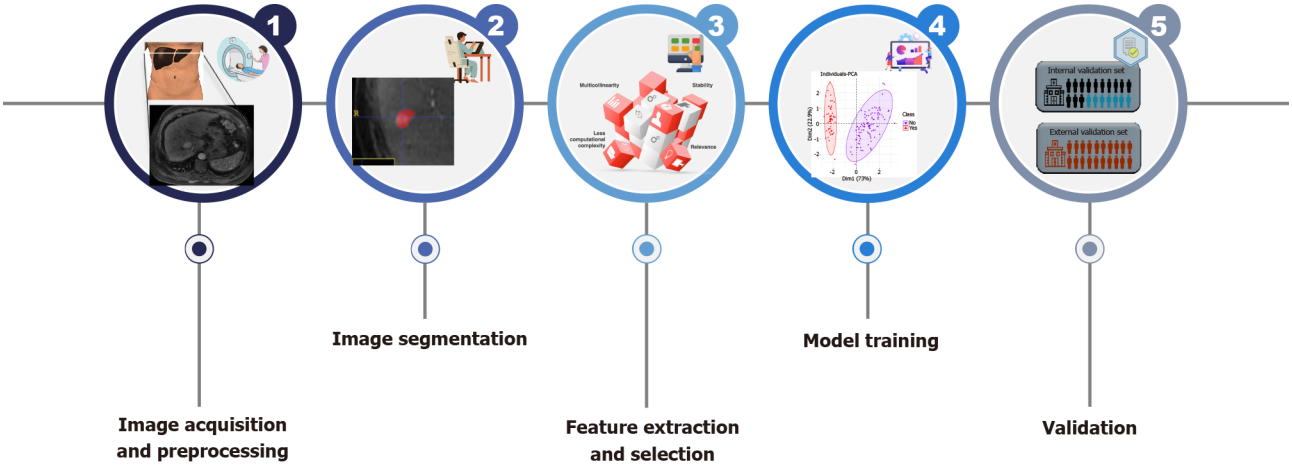

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Figure 1 Illustration summarizing radiomics workflow.

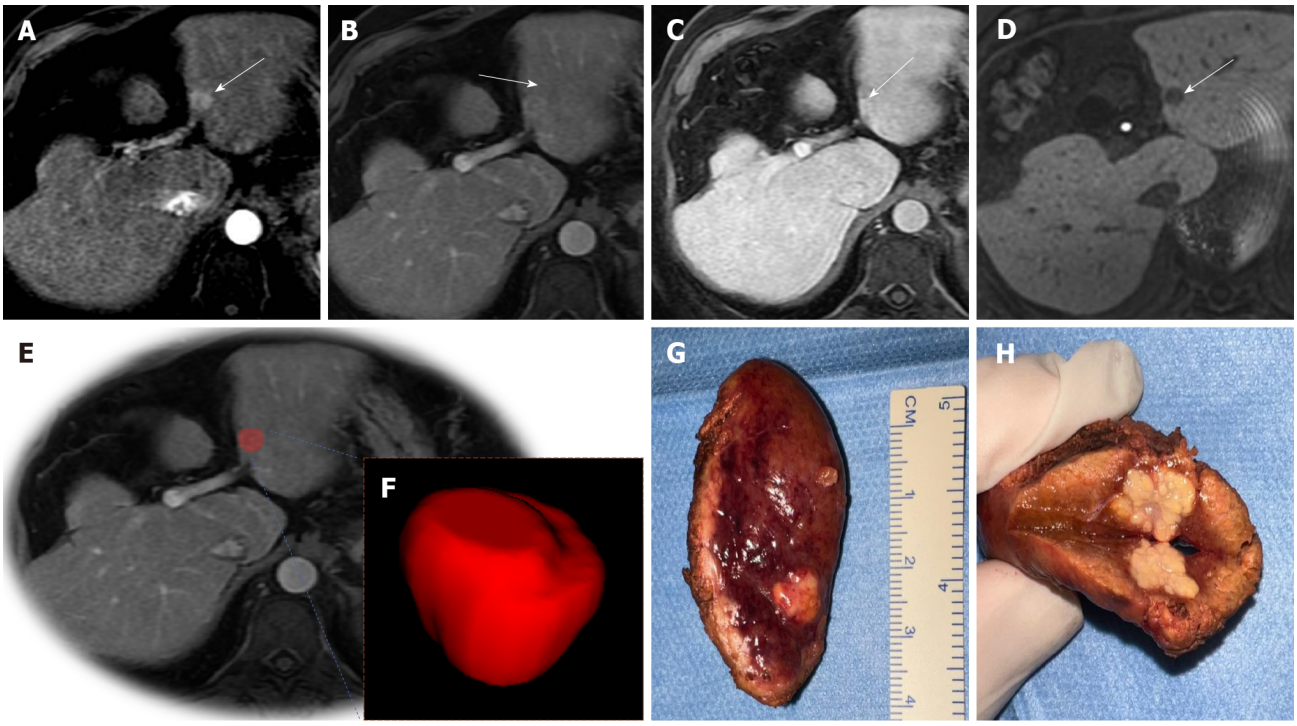

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Figure 2 Illustration of hepatocellular carcinoma segmentation. 72-year-old man with cirrhosis had a new liver lesion on computed tomography, indeterminate. Gadoxetic acid-enhanced T1-weighted images show a 1.3 cm (arrows) lesion. A: With arterial phase hyperenhancement; B: Questionable washout appearance on portal venous; C: Delayed phases; D: Hypointensity on during hepatobiliary phase (20 min); E and F: A tumor bed segmentation was exemplified, the portal venous phase (E) was used to manually segment the volume of interest (F); G and H: Note the gross findings after surgery. Histology confirmed hepatocellular carcinoma.

APPLICATIONS OF RADIOMICS IN HCC

Prediction of HCC histology

Table 1 summarizes the studies in the literature to date that have evaluated the use of radiomics to preoperatively predict HCC histology.

Distinguishing between HCC and other malignant or benign lesions: The distinction between HCC and other primary hepatobiliary malignancies can be challenging on imaging, because of the overlap of some features, especially for combined tumors[24]. In light of this, many studies have investigated radiomics performance in differentiating HCC from other malignant and benign hepatic lesions. For instance, Liu *et al*[24] studied the use of MRI- and CT-based radiomics to differentiate between HCC,

Table 1 Summary of the studies that evaluated radiomics to preoperatively predict hepatocellular cholangiocarcinoma histology

Ref.	Country	*n*	Imaging modality	Endpoint	Segmentation	ROI/VOI	No. of readers	Main results	Validation
Wang *et al* [92], 2022	China	196	MRI	cHCC-CC *vs* HCC	Manual, intratumoral	ROI	1	AUC (delayed phase MRI): 0.91	None
Liu *et al* [24], 2021	Canada	85	MRI and CT	cHCC-CC *vs* HCC *vs* CC	Manual, intratumoral	ROI	2	AUC (MRI): 0.77-0.81. AUC (CT): 0.71-0.81	Cross-validation
Lewis *et al* [25], 2019	United States	63	MRI	cHCC-CC *vs* HCC *vs* CC	Manual, intratumoral	VOI	2	AUC (LI-RADS and male gender): 0.90	None
Nie *et al* [27], 2020	China	156	CT	HCC *vs* FNH	Manual, intratumoral	ROI	2	AUC (radiomics): 0.96 training, 0.87 validation. AUC (radiomics + clinical factors): 0.98 training, 0.92 validation	None
Wu *et al* [28], 2019	China	369	MRI	HCC *vs* hemangioma	Manual, intratumoral	ROI	2	AUC: 0.86 training, 0.89 testing	None
Mokrane *et al*[29], 2020	United States	178	CT	HCC diagnosis	Manual, intratumoral	VOI	2	AUC: 0.70 training, 0.66 validation	External
Brancato *et al*[34], 2022	Italy	38	MRI	Tumor grade	Manual, intratumoral	VOI	1	AUC: 0.89	None
Gao *et al* [93], 2018	China	Training: 125. Validation: 45	MRI	Tumor grade	Manual, intratumoral	N/A	N/A	AUC: 0.83 training, 0.74 validation	None
Wu *et al* [30], 2019	China	Training: 125. Validation: 45	MRI	Tumor grade	Manual, intratumoral	ROI	1	AUC: 0.83 training, 0.74 validation	Internal
Zhou *et al* [94], 2017	China	46	MRI	Tumor grade	Manual, intratumoral	ROI	1	AUC: 0.83-0.92	None
Mao *et al* [31], 2022	China	Training: 85. Validation: 37	MRI	Tumor grade	Manual, intratumoral	ROI	2	AUC: 0.97 training, 0.94 validation	Internal
Chen *et al* [33], 2021	China	Training: 112. Validation: 49	CT	Tumor grade	Manual, intratumoral	VOI	2	AUC: 0.90 training, 0.94 validation	Internal
Yang *et al* [95], 2019	China	Training: 146. Validation: 62	Gadoxetic acid-enhanced MRI	MVI	Manual, intratumoral	ROI	2 (consensus)	AUC: 0.94 training, 0.86 validation	Internal
Xu *et al* [39], 2019	China	495	CT	MVI	Semi-automatic, intratumoral and peritumoral	VOI	3	AUC: 0.91 training, 0.89 validation	Internal
Feng *et al* [40], 2019	China	160	Gadoxetic acid-enhanced MRI	MVI	Manual, intratumoral and peritumoral	VOI	3	AUC: 0.85 training, 0.83 validation	Internal
Zheng *et al*[41], 2017	United States	120	CT	MVI	Semi-automatic	ROI	1	AUC: 0.80	None
Bakr *et al* [96], 2017	United States	28	CT	MVI	Manual, intratumoral	ROI	4	AUC: 0.76	None
Ma *et al* [97], 2019	China	157	CT	MVI	Manual, intratumoral	VOI	1	AUC (portal venous phase CT): 0.79	Cross-validation

AUC: Area under the curve; cHCC-CC: Combined hepatocellular cholangiocarcinoma; CT: Computed tomography; FNH: Focal nodular hyperplasia; HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging; MVI: Microvascular invasion; ROI: Region of interest; VOI: Volume of interest.

cholangiocarcinoma, and combined HCC-cholangiocarcinoma. Using MRI, radiomic features derived from contrast-enhanced phases demonstrated excellent performance to differentiate HCC from non-HCC [area under the curve (AUC) ≥ 0.79], with the highest AUC obtained from the arterial phase (AUC of 0.81); meanwhile, using CT, radiomic features derived from the pre-contrast and portal venous phase yielded AUC values of 0.81 and 0.71, respectively. In another study, Lewis *et al*[25] found that the combination of the apparent diffusion coefficient 5th percentile radiomic feature with Liver Imaging Reporting and Data System classification and male gender achieved an accuracy of 80%-81.5% in distinguishing HCC from intrahepatic cholangiocarcinoma (ICC) and combined HCC-ICC, and outperformed either measure alone. Other studies showed that radiomics is helpful to distinguish between HCC and benign tumors in non-cirrhotic livers, *e.g.*, from hepatocellular adenoma (AUC of 0.96 in the training set and 0.94 in the test set)[26], from focal nodular hyperplasia (AUC of 0.979 in the training set and 0.917 in the test set)[27], and from hemangioma (AUC: 0.86 in the training set and 0.89 in the test set)[28]. Mokrane *et al*[29] validated a radiomics signature to diagnose HCC in patients with cirrhosis and increased radiologists' confidence.

Prediction of histologic grade: Histologic grade is an important prognostic factor in patients with HCC and is only available preoperatively in patients who undergo biopsy. Therefore, studies have aimed to identify non-invasive imaging features such as radiomic features that could potentially predict the tumor grade. Wu *et al*[30] found that MRI-based radiomics can successfully categorize low-grade and high-grade HCC, with the radiomic model outperforming the clinical model (AUC 0.742 for the combined T1-weighted and T2-weighted MRI-based radiomic model *vs* AUC 0.6 for the clinical one) and the combined radiomic and clinical model (AUC 0.8) outperforming both models alone. Mao *et al* [31] also investigated MRI-based radiomic features, with Gd-EOB-DTPA contrast administered for the MRI exams, finding that the artificial neural network combining radiomic features from the contrast-enhanced arterial phase and hepatobiliary phase yielded the highest AUC of 0.944. Moreover, they found that the artificial neural network models were superior to the logistic regression models. In other studies, CT-based radiomics has been found to have high performance in distinguishing between low- and high-grade tumors[32-34]; for instance, Chen *et al*[33] found an AUC of 0.937 for a ML-based radiomics model based on the CT portal phase.

Prediction of microvascular invasion: Microvascular invasion (MVI) is found in about 15%-57% of patients with HCC who undergo surgery[35,36] and is associated with higher rates of recurrence and shorter survival after surgery[37]. Although imaging can be used to diagnose macrovascular invasion (or tumor in vein), preoperative imaging identification of MVI is difficult. Studies have evaluated the performance of radiomics as a tool to predict MVI, with most predictive models combining radiomics and clinical biomarkers[38]. For instance, Xu *et al*[39] proposed a model combining CT-based radiomic features with radiologic and clinical parameters; the model was not only an independent predictor of histologic MVI (AUC of 0.909 in the training/validation set and 0.889 in the test set) but was also an independent predictor of worse prognosis (disease-specific recurrence and disease-specific mortality). Of note, the radiomics-only model did not add significant value to radiologist scores alone. Since MVI occurs primarily at the tumor periphery (approximately 85% of MVI is located within one centimeter from the tumor margin), studies have investigated radiomic features derived from the peritumoral tissue. For instance, Feng *et al*[40] demonstrated that a model combining intratumoral and peritumoral radiomic features was superior in predicting MVI using Gd-EOB-DTPA-enhanced MRI compared to the model containing only intratumoral radiomics features. Additionally, Zheng *et al*[41] demonstrated that peritumoral textural features had an AUC of 0.80 and a multivariate model combining alfa-fetoprotein, tumor size, hepatitis status and quantitative features achieved an AUC of 0.88.

Prediction of HCC genetic expression

Compared to the prediction of histology, fewer researches in the literature have evaluated the use of radiomics to predict genetic expression in patients with HCC (Table 2). Overall, studies on the use of radiomics to predict genetic expression have focused on using radiomics to predict Ki67 expression as well as cytokeratin 19 (CK19), P53, and phosphatidylinositol-3 kinase (PI3K) status. Of note, in 2007, Segal *et al*[42] investigated for the first time the correlation between HCC genetic expression and CT imaging traits, finding 32 CT imaging traits that were correlated with the expression levels of 116 genetic markers.

Ki67 expression: High Ki-67 expression in HCC patients is associated with fast progression and poor prognosis[43]. To determine if radiomics can be useful to predict Ki67 expression, Wu *et al*[44] developed and validated a radiomic nomogram based on the combination of CT-based radiomic features and clinical factors. Using Gd-EOB-DTPA-enhanced MRI, Li *et al*[45] found that texture analysis of the hepatobiliary phase, arterial phase, and portal vein phase were helpful for predicting Ki67 expression. In their study, a single slice with the largest proportion of the lesion was delineated, and the predictive performance of models were compared by misclassification rate. In another study by Fan *et al* [46] using Gd-EOB-DTPA-enhanced MRI, the authors delineated the whole lesion, and the predictive performance of different models were compared using the receiver operating curve, calibration curve,

Table 2 Summary of the studies that evaluated radiomics models to predict genetic profile in patients with hepatocellular cholangiocarcinoma

Ref.	Country	*n*	Imaging modality	Endpoint	Segmentation	ROI/VOI	No. of readers	Main results	Validation
Xia *et al* [98], 2018	China	38	CT	Association with gene expression profile	Manual, intratumoral	ROI	1	Individual textural features predicted gene modules	No
Wu *et al*[44], 2022	China	Training: 120. Validation: 52	CT	Ki-67 expression	Manual, intratumoral	VOI	2	AUC: 0.85 (training), 0.74 (validation)	Internal
Li *et al* [45], 2019	China	83	MRI	Ki-67 expression	Manual, intratumoral	ROI	2	Some features were associated, no model	No
Ye *et al* [47], 2019	China	89	MRI	Ki-67 expression	Manual, intratumoral	VOI	2	C-index: 0.878	No
Fan *et al*[46], 2021	China	Training: 103. Validation: 48	MRI	Ki-67 expression	Manual, intratumoral	VOI	2	AUC: 0.88 (training), 0.80 (validation)	Internal
Hu *et al* [48], 2022	China	Training: 87. Validation: 21	MRI	Ki-67 expression	Manual, intratumoral	ROI	1	AUC: 0.90 (training), 0.83 (validation)	Internal
Wang *et al* [50], 2019	China	78	MRI	CK19 positivity	Manual, intra- and peritumoral	ROI	1	AUC: 0.76	No
Chen *et al*[51], 2021	China	Training: 102. Validation: 19	MRI	CK19 positivity	Manual, intratumoral	ROI	2	AUC: 0.82 (training), 0.78 (external validation)	Internal and external
Yang *et al*[52], 2021	China (multicenter)	Training: 143. Validation: 75	MRI	CK19 positivity	Manual, intratumoral	ROI	2	AUC: 0.85 (training), 0.79 (external validation)	Internal and external
Wu *et al*[55], 2019	China	63	CT	P53 mutation status	Manual, intratumoral	ROI	2	AUC: 0.62-0.79	No
Li *et al* [99], 2022	China	92	MRI	Gene signatures associated with disease recurrence	Manual, intratumoral	ROI	2	MRI radiomics features could help quantify GOLM1, SETD7, and RND1 expression levels	Internal
Liao *et al*[56], 2022	China	Training: 86. Validation: 46	CT	Somatic mutations of the PI3K signaling pathway	Manual, intratumoral and peritumoral	VOI	2	AUC: 0.74 (training), 0.73 (external validation)	Internal and external
Che *et al*[60], 2022	China	Training: 69. Validation: 30	CT	β-arrestin1 phosphorylation	Manual, intratumoral	ROI	1	AUC: 0.89 (training), 0.74 (validation)	Internal

AUC: Area under the curve; CT: Computed tomography; MRI: Magnetic resonance imaging; ROI: Region of interest; VOI: Volume of interest; CK19: Cytokeratin 19; PI3K: P53, and phosphatidylinositol-3 kinase.

and decision cure analysis. The optimal model combining the arterial phase radiomic score and serum alpha-fetoprotein (AFP) levels showed high AUCs (AUC of 0.922 and 0.863 in the training and validation cohorts, respectively) for the preoperative Ki-67 expression prediction. In yet another study using Gd-EOB-DTPA-enhanced MRI, Ye *et al*[47] showed that the nomogram combining the texture signature (using the segmentation of the whole lesion) and clinical factors demonstrated a high discrimination ability (C-index of 0.936) for predicting Ki-67 group (high *vs* low). Finally, Hu *et al*[48] explored the added value of viscoelasticity measured by magnetic resonance elastography to predict Ki-67 expression, showing that shear wave speed and phase angle significantly improved the performance of the radiomic model.

CK19 expression: CK19 expression is associated with aggressive tumor behavior, resistance to therapy, and poor outcomes including worse overall survival and recurrence[49]. To date, three studies have focused on developing radiomic models to predict CK19 expression[50-52], all using MRI. Wang *et al*[50] showed that their texture model independently predicted CK19-positive HCC cases and improved the diagnostic performance of AFP level ≥ 400 ng/mL and arterial rim enhancement. The two remaining studies developed a radiomics model based on Gd-EOB-DTPA-enhanced MRI, with external validation AUC varying from 0.78-0.79; of note, one of the studies was a multicenter study with over 250 patients [51,52].

P53, PI3K, and other genetic expression: P53 can be used as a tumor biomarker, since it plays an important role in the pathogenesis of HCC[53]. P53 mutation has also been suggested as a feasible target for antitumor therapy[54]. Wu *et al*[55] demonstrated a direct relationship between P53 mutations in patients with HCC and the gray-level co-occurrence matrix on CT. PI3K signaling is a key pathway regulating HCC aggressiveness and is associated with response to sorafenib. Liao *et al*[56] developed a CT-based radiomics model that yielded an AUC of 0.73 in the external validation set for prediction of PI3K status.

The phosphorylation status of β-arrestin1 is associated with sorafenib resistance[57-59]. Che *et al*[60] developed a model combining a CT-based radiomics score with clinico-radiological risk factors which yielded an AUC of 0.898 in predicting β-arrestin1 phosphorylation, and the predicted β-arrestin1 phosphorylation was in turn significantly associated with overall survival in both the training and validation cohorts ($P < 0.05$).

Prediction of recurrence, treatment response, and liver failure

Tumor recurrence, liver failure and treatment response rates are major concerns during HCC treatment. Radiomics has emerged as a promising tool to predict recurrence and treatment response beyond the current predictive criteria[61,62]. Table 3 summarizes the studies to date that have evaluated the use of radiomic models to predict recurrence and treatment response. Most of these studies were single-center studies performed in China, with only a few studies incorporating external validation[63,64]. Segmentation strategies were predominantly manual strategies, including manual segmentation of the tumor region or area of interest, with only a few studies involving the segmentation of the peritumoral liver parenchyma[63,65-67]. Overall, the radiomic models yielded an AUC between 0.59 and 0.94 (see Table 3).

Of the studies evaluating the use of radiomics to predict recurrence, most involved the prediction of recurrence after surgical resection on CT or MRI, demonstrating a validation AUC between 0.59 and 0.84 (Table 3). Zhou *et al*[68] demonstrated that combining the radiomic signature with conventional preoperative variables significantly improved clinical model accuracy in early recurrence prediction (AUC of 0.84). Ji *et al*[64] developed and externally validated a radiomic model with better prognostic ability (C index ≥ 0.77, AUC of 0.78), lower prediction error (Brier score ≤ 0.14), and better clinical use compared with other staging systems and models. A few other studies evaluating the use of radiomics to predict recurrence involved the prediction of recurrence after liver transplant[69], transarterial chemoembolization (TACE)[67,70], and radiofrequency ablation (RFA)[71], demonstrating a validation AUC between 0.71 and 0.82.

Of the studies evaluating the use of radiomics to predict treatment response, a few involved the prediction of treatment response post-TACE[63,72,73]. In Canada, Ivanics *et al*[73] developed a CT-based radiomic model and achieved an AUC of 0.87 on the internal validation set. A large multi-center Chinese study by Chen *et al*[63] evaluating treatment response after TACE performed semi-automatic segmentation of the tumor and of the peritumoral region on contrast-enhanced CT in 585 patients, and the validation AUC was 0.90. One small study by Horvat *et al*[74] assessed treatment response after RFA using tumor 3D volumes of interest on MRI, yielding an AUC of 0.76 for the radiomics model, although the model lacked validation. Finally, two studies from China evaluated the use of radiomics to predict liver failure after surgical resection[75,76].

Prediction of survival

Table 4 summarizes the studies to date that have evaluated the use of radiomics to predict survival in patients with HCC. Four studies evaluated the use of CT-based radiomics to predict survival after hepatic resection, demonstrating an AUC between 0.71 and 0.81, with two of the four studies performing internal validation[39,77-79]. A few other studies evaluated the use of radiomics to predict survival after TACE[80], TARE[81], and RFA[82], all without validation.

Of the studies that involved the prediction of survival after hepatic resection, Xu *et al*[39] had the largest sample size. In their study, a risk model integrating clinico-radiological factors and a high CT-based radiomic score was independently associated with long-term mortality and disease-specific recurrence. Kim *et al*[80] evaluated the use of CT-based radiomics in survival prediction in patients after TACE. They demonstrated a combined model integrating radiomic features and clinical data (HCC size, Child-Pugh score and AFP) outperformed the clinical sore model or the radiomic score model. Petukhova-Greenstein *et al*[82] found that a higher MRI-based radiomic signature based on nodular and

Table 3 Summary of the studies that assessed radiomics to predict recurrence and treatment response in patient with hepatocellular cholangiocarcinoma who underwent surgery, liver transplantation or locoregional treatment

Ref.	Country	*n*	Imaging modality	Endpoint	Treatment type	Segmentation	ROI/VOI	No. of readers	Main results	Validation
Hui *et al* [100], 2018	Singapore	50	MRI	Recurrence	Hepatic resection	Manual, intratumoral	ROI	3	AUC: 0.78-0.84	None
Kim *et al* [65], 2019	South Korea	Training: 128. Validation: 39	MRI	Recurrence	Hepatic resection	Semiautomatic, intra- and peritumoral	VOI	2	C-index: 0.716	Internal
Zhao *et al*[101], 2021	China	Training: 78. Validation: 35	MRI	Recurrence	Hepatic resection	Manual, intratumoral	VOI	2	AUC: 0.83 (training), 0.77 (validation)	Internal
Zhou *et al*[68], 2017	China	215	CT	Recurrence	Hepatic resection	Manual, intratumoral	ROI	2	AUC: 0.84 (combined model)	None
Ji *et al* [64], 2020	China	Internal: 177. External: 118	CT	Recurrence	Hepatic resection	Manual, intratumoral	VOI	1	AUC: 0.77 (internal), 0.78 (external)	External
Guo *et al* [69], 2019	China	Training: 93. Validation: 40	CT	Recurrence	Liver transplant	Semiautomatic, intratumoral	ROI	1	AUC: 0.79 (training), 0.79 (validation)	Internal
Shan *et al*[66], 2019	China	Training: 109. Validation: 47	CT	Recurrence	Hepatic resection or ablation	Manual, intra- and peritumoral	ROI	2	AUC: 0.80 (training), 0.79 (validation)	Internal
Zheng *et al*[79], 2018	China	Training: 212. Validation: 107	CT	Recurrence and survival	Hepatic resection	Manual, intratumoral	ROI	2	AUC: 0.64 (training), 0.59 (validation)	Internal
Song *et al*[67], 2020	China	Training: 110. Validation: 74	MRI	Recurrence	TACE	Semiautomatic, intra- and peritumoral	VOI	2	C-index: 0.82	Internal
Lv *et al* [71], 2021	China	Training: 40. Validation: 18	MRI	Recurrence	RFA	Semiautomatic, intratumoral	VOI	2	AUC: 0.94 (training), 0.82 (validation)	Internal
Sun *et al* [70], 2020	China	Training: 67. Validation: 17	MRI	Recurrence	TACE	Manual (intratumoral)	VOI	2	AUC: 0.71-0.79	Internal
Cai *et al* [75], 2019	China	Training: 80. Validation: 32	CT	Liver failure	Hepatic resection	Semiautomatic, intratumoral	ROI	2	AUC: 0.82 (training), 0.76 (validation)	Internal
Zhu *et al* [76], 2020	China	101	MRI	Liver failure	Hepatic resection	Manual, entire liver	ROI	2	AUC: 0.81-0.89	None
Ivanics *et al*[73], 2021	Canada	88	CT	Treatment response	TACE	Manual, intratumoral	VOI	1	AUC: 0.70-0.87	None
Kong *et al*[72], 2021	China	Training: 69. Validation: 30	MRI	Treatment response	TACE	Manual, intratumoral	VOI	2	AUC: 0.81 (training), 0.87 (validation)	Internal
Chen *et al*[63], 2021	China	Training: 355. Internal: 118. External: 122	CT	Treatment response	TACE	Semiautomatic, intra- and peritumoral	ROI	2	AUC: 0.94 (internal), 0.90 (external)	Internal and external
Horvat *et al*[74], 2021	Brazil	34	MRI	Treatment response	RFA	Manual, intratumoral	VOI	1	AUC: 0.76	None

AUC: Area under the curve; CT: Computed tomography; MRI: Magnetic resonance imaging; RFA: Radiofrequency ablation; ROI: Region of interest; TACE: Transarterial chemoembolization; VOI: Volume of interest.

perinodular radiomic features predicted poorer survival after RFA. A study evaluated the survival prediction after TARE, using 18-fuoro-deoxyglucose PET-based radiomics[81]. They observed that whole-liver radiomics textural features were an independent negative predictor of survival.

Table 4 Summary of the studies that evaluated radiomics to predict survival in patients with hepatocellular cholangiocarcinoma

Ref.	Country	*n*	Imaging modality	Endpoint	Treatment type	Segmentation	ROI/VOI	No. of readers	Main results	Validation
Kiryu *et al* [77], 2017	Japan	122	CT	Survival	Hepatic resection	Manual, intra- and peritumoral	ROI	1	OS and DFS were significantly different between 2 rad score groups	None
Xu *et al*[39], 2019	China	Training: 350. Validation: 145	CT	Survival	Hepatic resection	Semiautomatic, intratumoral	VOI	3	AUC: 0.91 (training), 0.81 (validation)	Internal
Akai *et al* [78], 2018	Japan	127	CT	Survival	Hepatic resection	Manual, intrat-umoral	ROI	1	OS and DFS were significantly different between 2 rad score groups	None
Kim *et al* [80], 2018	South Korea	88	CT	Survival	TACE	Manual, intrat-umoral	ROI	1	Combined clinical and radiomics score was a better predictor of survival	None
Blanc-Durand *et al* [81], 2018	Switzerland	47	^{18}F-FDG PET-CT	Survival	TARE	Semiautomatic, whole liver	VOI	N/A	PFS-Rad Score and OS-Rad Score were independent negative predictors	None
Petukhova-Greenstein *et al*[82], 2022	United States	65	MRI	Survival	RFA	Semiautomatic, intra- and peritumoral	VOI	2	OS was significantly different between 2 rad score groups	None
Zheng *et al* [79], 2018	China	Training: 212. Validation: 107	CT	Survival	Hepatic resection	Manual, intrat-umoral	ROI	2	AUC: 0.71 (training and validation)	Internal

AUC: Area under the curve; CT: Computed tomography; DFS: Disease-fee survival; MRI: Magnetic resonance imaging; OS: Overall survival; PFS: Progression-free survival; RFA: Radiofrequency ablation; ROI: Region of interest; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization; VOI: Volume of interest; PET: Positron emission tomography.

Furthermore, radiomic scoring system did not differ after stratification by tumor size and Barcelona Clinic Liver Cancer staging.

Other applications of radiomics in HCC

Immunotherapy represents a paradigm shift in the management of patients with advanced HCC. Preoperatively assessing the immune status can assist the multidisciplinary team to identify which patients are suitable for immunotherapy, potentially improving treatment efficiency and overall survival rate. A few studies have evaluated the use of radiomics to predict programmed cell death ligand 1 (PD-L1) expression[83], CD8+ T cell infiltration[84], immunoscore[85,86], and anti-PD-1 treatment efficacy[87] in patients with HCC, with none of them performing external validation. Tian *et al*[83] were the first group to explore the efficacy of MRI-based radiomics to predict PD-L1 status. They proposed a model integrating radiomic and DL features for the quick and accurate assessment of PD-L1 expression levels in HCC patients before immune checkpoint inhibitor therapy which yielded an AUC of 0.897. Chen *et al*[85] demonstrated in 207 patients that radiomic features including those from the peritumoural region were associated with a validated "immunoscore". This score characterizes the tumor infiltrating lymphocyte population and theoretically reflects the immune phenotype of the tumor microenvironment.

RADIOMICS REPRODUCIBILITY IN HCC

Reproducibility refers to variations of the same patient across different imaging scenarios (*e.g.*, scanner or imaging parameters), while repeatability refers to variations of the same patient using the same imaging protocol. Table 5 summarizes the 13 studies to date that have studied the reproducibility of radiomics in HCC patients. Most of these studies were conducted in China (8/13; 62%). Seven were performed using CT (54%), 5 using MRI (38%), and 1 using both CT scan and MRI (8%). Different software programs were used for segmentation and feature extraction. Most studies adopted manual segmentation (11/13; 85%), and most evaluated first- and second-order features, with a few including shape and higher-order features. In addition to intra and inter-reader reproducibility, some also assessed the repeatability of radiomic features obtained through two separate exams from the same scanner, different scanners from different vendors and centers, 3D *vs* 2D segmentation, different contrast imaging phases, injection rates and pixel resolutions on contrast-enhanced CT, and different *b*-values on diffusion-weighted imaging on MRI.

Of note, one study showed that intra-reader tumoral and peritumoral reproducibility were greatest in MRI[88]. Another study showed that for test-retest (same MRI system, 2 different MRI exams), the intraclass correlation coefficient varied from 0.53-0.99 and the inter-platform reproducibility (MRI systems from 2 different vendors) varied from 0.58-0.99[89]. Regarding different contrast phases, Ibrahim *et al*[90] showed that 25% of extracted features had a concordance correlation coefficient (CCC) > 0.9 across arterial and portal venous phases. Perrin *et al*[91] demonstrated that the number of reproducible features decreased with variations in contrast injection rate, pixel resolution, and scanner model.

FUTURE DIRECTIONS OF RADIOMICS IN HCC

Despite the increasing and encouraging results in the literature concerning radiomics in patients with HCC, there are challenges and limitations to be overcome before its clinical implementation, particularly related to reproducibility and repeatability, lesion segmentation, model overfitting, multidisciplinary acceptance, and multi-modal data integration[23].

Patient selection, imaging data, segmentation strategy, image processing, feature selection, and computational processing are some factors that may affect the reproducibility and repeatability. Transparent patient accrual, data normalization, standard image manipulation, and feature extraction data are some strategies that may improve these challenges. Additionally, multi-center studies are recommended to increase reproducibility of the results.

Overfitting occurs when the model performs better in the training set with limited generalization of the results. The main factors contributing to overfitting are the number of included features being higher than the number of events and overoptimistic feature selection. Multiple strategies can be implemented to decrease overfitting, such as increasing the number of patients and events, using regularization methods, and including external validation cohorts. Multidisciplinary acceptance may improve with clear methods and a close relationship between radiologists, surgeons, oncologists, statistician, and data scientists to improve the interpretability of the results and to make way for clinical translation.

Multi-omics data integration is an additional step to improve the clinical acceptance of radiomics. Radiomics requires a multistep workflow process using different software and expertise; technological investments to create integrated and user-friendly tools are necessary to facilitate its widespread use in clinical practice. Finally, segmentation is a time-consuming process, susceptible to intra and inter-observer variability. Automatic and semi-automatic segmentations are required, particularly using DL strategies to facilitate this crucial step.

Additionally, some heterogeneity related to patients with HCC should be take into consideration. Since pathological confirmation is not always performed, the definition of clear and reproducible endpoints, like the LI-RADS criteria, are relevant strategies. Combined data integrating imaging and clinical variables are important to address the issue that patients with HCC are also dealing with systemic consequences related to cirrhosis.

CONCLUSION

Radiomics is an evolving computer-assisted tool with the potential to improve the multidisciplinary management of patients with HCC and to provide personalized treatment optimizing the available resources. Multiple studies have evaluated the use of radiomics in HCC with promising applications, including the prediction of pre-surgical histology, genetic signature, recurrence, and treatment response, as well as survival rates. Although promising, several challenges need to be overcome before radiomics can achieve clinical translation, including workflow optimization, model validation in multi-center studies, and the development of integrated models to facilitate clinical use and acceptance.

Table 5 Summary of the studies that assessed reproducibility of hepatocellular cholangiocarcinoma textural features

Ref.	Country	*n*	Imaging modality	Segmentation	Segmentation software	ROI/VOI	No. of readers	Intra-reader reproducibility	Inter-reader reproducibility	Other reproducibility
Duan *et al* [88], 2022	China	19	CT, MRI	Manual, intra- and peritumoral	3D-Slicer	ROI	2 (1 radiologist and 1 radiation oncologist)	Features with ICC ≥ 0.75 in both tumoral and peritumoral tissue greatest in MR	Features with ICC ≥ 0.75 in both tumoral and peritumoral tissue greatest in MR	N/A
Zhang *et al* [102], 2022	China	90 (31 HCC)	MRI	Manual, intratumoral	ITK-SNAP	ROI and VOI	2 radiologists	N/A	ICC > 0.8 used	N/A
Carbonell *et al*[89], 2022	United States	55 (16 HCC)	MRI	Manual, intratumoral and liver parenchyma	Olea sphere 3.0, Olea Medical	ROI for normal liver, VOI for HCC	2 radiologists	N/A	CCC: 0.80-0.99	For test-retest (same MRI system, 2 different MRI exams): ICC: 0.53-0.99; and in liver parenchyma: ICC: 0.53-0.73. For inter-platform reproducibility (MRI systems from 2 different vendors): CCC: 0.58-0.99
Park *et al* [103], 2022	South Korea	249	CT	Manual followed by automatic segmentation, intratumoral	MEDIP PRO	ROI and VOI	1 radiologist	For VOI: Manual: ICC 0.594-0.998 for FO, 0.764-0.997 for shape, and 0.190-0.926 for SO; DL-AS: ICC > 0.75 for all. For ROI: Manual: 0.698-0.997 for FO, 0.556-0.997 for shape, and 0.341-0.935 for SO; DL-AS ICC > 0.75 for all	N/A	
Haniff *et al* [104], 2021	Malaysia	30	MRI	Manual and semi-automatic, intratumoral	3D-Slicer	VOI	Manual: 4 readers. Semi-automatic: 2 readers	N/A	Manual segmentation: ICC 0.897. Semi-automatic segmentation: ICC 0.952	NA
Ibrahim *et al*[90], 2021	Germany	61 patients, 104 lesions	CT	Manual, intratumoral	MIM software	ROI	1 nonradiologist revised by radiologist	N/A	N/A	Across different contrast imaging phases: 25% of extracted features had CCC > 0.9 across arterial and portal venous phases
Hu *et al* [105], 2021	China	30	CT	Manual, intratumoral	MaZda software	ROI	2 radiologists	ICC > 0.7	ICC > 0.7	N/A
Mao *et al* [32], 2020	China	30	CT	Manual, intratumoral	ITK-SNAP	ROI	2 radiologists	N/A	ICC ≥ 0.8	N/A
Hu *et al* [106], 2020	China	50	CT	Semi-automatic, peritumoral	Not mentioned	ROI	2 radiologists	N/A	ICC > 0.6	N/A
Qiu *et al* [107], 2019	China	26	CT	Manual and semi-automatic, intratumoral	GrowCut and GraphCut	ROI	Manual: 5 radiation oncologists. Semi-automatic: 2 radiation oncologists	N/A	ICC ≥ 0.75 in 69% of features extracted from manual segmentation, 73% from GraphCut, and 79% from GrowCut	Across different centers: Poor reproducibility of CT-based peritumoral-radiomics model
Zhang *et al* [108], 2019	China	46 (34 HCC)	MRI	Manual, intratumoral	MIM software	VOI	1 radiologist	N/A	N/A	Across different *b*-values: radiomic features extracted from *b* = 0, 20, 50,

										100, 200 s/mm^2 and b = 1000 s/mm^2 and nearby b-values DWIs showed a high reproducibility (ICC ≥ 0.8)
Feng *et al* [40], 2019	China	160 (110)	MRI	Manual, intra- and peritumoral	ITK-SNAP	VOI	3 radiologists	85% ICC ≥ 0.8	82% ICC ≥ 0.8	N/A
Perrin *et al* [91], 2018	United States	38 (6 HCC)	CT	Semi-automatic, intratumoral and liver parenchyma	Scout Liver	VOI	1 research fellow under supervision of radiologist	N/A	N/A	Across different contrast injection rates, pixel resolutions, and scanner models: Number of reproducible radiomic features (CCC > 0.9) decreased with variations in contrast injection rate, pixel resolution, and scanner model

CT: Computed tomography; MRI: Magnetic resonance imaging; ROI: Region of interest; VOI: Volume of interest; TACE: Transarterial chemoembolization; ICC: Intraclass correlation coefficient; DWI: Diffusion-weighted imaging; CCC: Concordance correlation coefficient; HCC: Hepatocellular carcinoma; N/A: Not applicable; FO: First order; SO: Second order; DL-AS: Deep learning-based auto-segmentation.

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REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **World Health Organization**. Cancer fact sheets. [cited 10 August 2022]. Available from: https://gco.iarc.fr/today/fact-sheets-cancers
3 **Llovet JM**, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021; **7**: 6 [PMID: 33479224 DOI: 10.1038/s41572-020-00240-3]
4 **Lopes Fde L**, Coelho FF, Kruger JA, Fonseca GM, Araujo RL, Jeismann VB, Herman P. Influence of hepatocellular carcinoma etiology in the survival after resection. *Arq Bras Cir Dig* 2016; **29**: 105-108 [PMID: 27438037 DOI: 10.1590/0102-6720201600020010]
5 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]
6 **Mittal S**, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]
7 **Venook AP**, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010; **15** Suppl 4: 5-13 [PMID: 21115576 DOI: 10.1634/theoncologist.2010-S4-05]
8 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
9 **Herman P**, Fonseca GM, Coelho FF, Kruger JAP, Makdissi FF, Jeismann VB, Carrilho FJ, D'Albuquerque LAC, Nahas SC. Two decades of liver resection with a multidisciplinary approach in a single institution: What has changed? *Clinics (Sao Paulo)* 2022; **77**: 100088 [PMID: 35901605 DOI: 10.1016/j.clinsp.2022.100088]
10 **Jiménez Pérez M**, Grande RG. Application of artificial intelligence in the diagnosis and treatment of hepatocellular carcinoma: A review. *World J Gastroenterol* 2020; **26**: 5617-5628 [PMID: 33088156 DOI: 10.3748/wjg.v26.i37.5617]
11 **Liu X**, Elbanan MG, Luna A, Haider MA, Smith AD, Sabottke CF, Spieler BM, Turkbey B, Fuentes D, Moawad A, Kamel S, Horvat N, Elsayes KM. Radiomics in Abdominopelvic Solid-Organ Oncologic Imaging: Current Status. *AJR Am J Roentgenol* 2022; **219**: 985-995 [PMID: 35766531 DOI: 10.2214/AJR.22.27695]
12 **Gillies RJ**, Kinahan PE, Hricak H. Radiomics: Images Are More than Pictures, They Are Data. *Radiology* 2016; **278**: 563-577 [PMID: 26579733 DOI: 10.1148/radiol.2015151169]
13 **Miranda Magalhaes Santos JM**, Clemente Oliveira B, Araujo-Filho JAB, Assuncao-Jr AN, de M Machado FA, Carlos Tavares Rocha C, Horvat JV, Menezes MR, Horvat N. State-of-the-art in radiomics of hepatocellular carcinoma: a review of basic principles, applications, and limitations. *Abdom Radiol (NY)* 2020; **45**: 342-353 [PMID: 31707435 DOI: 10.1007/s00261-019-02299-3]
14 **Bera K**, Braman N, Gupta A, Velcheti V, Madabhushi A. Predicting cancer outcomes with radiomics and artificial intelligence in radiology. *Nat Rev Clin Oncol* 2022; **19**: 132-146 [PMID: 34663898 DOI: 10.1038/s41571-021-00560-7]
15 **Rizzo S**, Botta F, Raimondi S, Origgi D, Fanciullo C, Morganti AG, Bellomi M. Radiomics: the facts and the challenges of image analysis. *Eur Radiol Exp* 2018; **2**: 36 [PMID: 30426318 DOI: 10.1186/s41747-018-0068-z]
16 **Wagner MW**, Namdar K, Biswas A, Monah S, Khalvati F, Ertl-Wagner BB. Radiomics, machine learning, and artificial intelligence-what the neuroradiologist needs to know. *Neuroradiology* 2021; **63**: 1957-1967 [PMID: 34537858 DOI: 10.1007/s00234-021-02813-9]
17 **Shur JD**, Doran SJ, Kumar S, Ap Dafydd D, Downey K, O'Connor JPB, Papanikolaou N, Messiou C, Koh DM, Orton MR. Radiomics in Oncology: A Practical Guide. *Radiographics* 2021; **41**: 1717-1732 [PMID: 34597235 DOI: 10.1148/rg.2021210037]
18 **Giger ML**. Machine Learning in Medical Imaging. *J Am Coll Radiol* 2018; **15**: 512-520 [PMID: 29398494 DOI: 10.1016/j.jacr.2017.12.028]
19 **Suzuki K**. Overview of deep learning in medical imaging. *Radiol Phys Technol* 2017; **10**: 257-273 [PMID: 28689314 DOI: 10.1007/s12194-017-0406-5]
20 **Larue RT**, Defraene G, De Ruysscher D, Lambin P, van Elmpt W. Quantitative radiomics studies for tissue characterization: a review of technology and methodological procedures. *Br J Radiol* 2017; **90**: 20160665 [PMID: 27936886 DOI: 10.1259/bjr.20160665]
21 **Thawani R**, McLane M, Beig N, Ghose S, Prasanna P, Velcheti V, Madabhushi A. Radiomics and radiogenomics in lung cancer: A review for the clinician. *Lung Cancer* 2018; **115**: 34-41 [PMID: 29290259 DOI: 10.1016/j.lungcan.2017.10.015]
22 **van Timmeren JE**, Cester D, Tanadini-Lang S, Alkadhi H, Baessler B. Radiomics in medical imaging-"how-to" guide and critical reflection. *Insights Imaging* 2020; **11**: 91 [PMID: 32785796 DOI: 10.1186/s13244-020-00887-2]
23 **Horvat N**, Miranda J, El Homsi M, Peoples JJ, Long NM, Simpson AL, Do RKG. A primer on texture analysis in abdominal radiology. *Abdom Radiol (NY)* 2022; **47**: 2972-2985 [PMID: 34825946 DOI: 10.1007/s00261-021-03359-3]
24 **Liu X**, Khalvati F, Namdar K, Fischer S, Lewis S, Taouli B, Haider MA, Jhaveri KS. Can machine learning radiomics provide pre-operative differentiation of combined hepatocellular cholangiocarcinoma from hepatocellular carcinoma and

cholangiocarcinoma to inform optimal treatment planning? *Eur Radiol* 2021; **31**: 244-255 [PMID: 32749585 DOI: 10.1007/s00330-020-07119-7]

25 **Lewis S**, Peti S, Hectors SJ, King M, Rosen A, Kamath A, Putra J, Thung S, Taouli B. Volumetric quantitative histogram analysis using diffusion-weighted magnetic resonance imaging to differentiate HCC from other primary liver cancers. *Abdom Radiol (NY)* 2019; **44**: 912-922 [PMID: 30712136 DOI: 10.1007/s00261-019-01906-7]

26 **Nie P**, Wang N, Pang J, Yang G, Duan S, Chen J, Xu W. CT-Based Radiomics Nomogram: A Potential Tool for Differentiating Hepatocellular Adenoma From Hepatocellular Carcinoma in the Noncirrhotic Liver. *Acad Radiol* 2021; **28**: 799-807 [PMID: 32386828 DOI: 10.1016/j.acra.2020.04.027]

27 **Nie P**, Yang G, Guo J, Chen J, Li X, Ji Q, Wu J, Cui J, Xu W. A CT-based radiomics nomogram for differentiation of focal nodular hyperplasia from hepatocellular carcinoma in the non-cirrhotic liver. *Cancer Imaging* 2020; **20**: 20 [PMID: 32093786 DOI: 10.1186/s40644-020-00297-z]

28 **Wu J**, Liu A, Cui J, Chen A, Song Q, Xie L. Radiomics-based classification of hepatocellular carcinoma and hepatic haemangioma on precontrast magnetic resonance images. *BMC Med Imaging* 2019; **19**: 23 [PMID: 30866850 DOI: 10.1186/s12880-019-0321-9]

29 **Mokrane FZ**, Lu L, Vavasseur A, Otal P, Peron JM, Luk L, Yang H, Ammari S, Saenger Y, Rousseau H, Zhao B, Schwartz LH, Dercle L. Radiomics machine-learning signature for diagnosis of hepatocellular carcinoma in cirrhotic patients with indeterminate liver nodules. *Eur Radiol* 2020; **30**: 558-570 [PMID: 31444598 DOI: 10.1007/s00330-019-06347-w]

30 **Wu M**, Tan H, Gao F, Hai J, Ning P, Chen J, Zhu S, Wang M, Dou S, Shi D. Predicting the grade of hepatocellular carcinoma based on non-contrast-enhanced MRI radiomics signature. *Eur Radiol* 2019; **29**: 2802-2811 [PMID: 30406313 DOI: 10.1007/s00330-018-5787-2]

31 **Mao Y**, Wang J, Zhu Y, Chen J, Mao L, Kong W, Qiu Y, Wu X, Guan Y, He J. Gd-EOB-DTPA-enhanced MRI radiomic features for predicting histological grade of hepatocellular carcinoma. *Hepatobiliary Surg Nutr* 2022; **11**: 13-24 [PMID: 35284527 DOI: 10.21037/hbsn-19-870]

32 **Mao B**, Zhang L, Ning P, Ding F, Wu F, Lu G, Geng Y, Ma J. Preoperative prediction for pathological grade of hepatocellular carcinoma *via* machine learning-based radiomics. *Eur Radiol* 2020; **30**: 6924-6932 [PMID: 32696256 DOI: 10.1007/s00330-020-07056-5]

33 **Chen W**, Zhang T, Xu L, Zhao L, Liu H, Gu LR, Wang DZ, Zhang M. Radiomics Analysis of Contrast-Enhanced CT for Hepatocellular Carcinoma Grading. *Front Oncol* 2021; **11**: 660509 [PMID: 34150628 DOI: 10.3389/fonc.2021.660509]

34 **Brancato V**, Garbino N, Salvatore M, Cavaliere C. MRI-Based Radiomic Features Help Identify Lesions and Predict Histopathological Grade of Hepatocellular Carcinoma. *Diagnostics (Basel)* 2022; **12** [PMID: 35626241 DOI: 10.3390/diagnostics12051085]

35 **Du M**, Chen L, Zhao J, Tian F, Zeng H, Tan Y, Sun H, Zhou J, Ji Y. Microvascular invasion (MVI) is a poorer prognostic predictor for small hepatocellular carcinoma. *BMC Cancer* 2014; **14**: 38 [PMID: 24460749 DOI: 10.1186/1471-2407-14-38]

36 **Ueda K**, Tokugawa K. Rubella vaccination and congenital rubella syndrome in Japan. *Acta Paediatr Jpn* 1988; **30**: 163-166 [PMID: 3149850 DOI: 10.1111/j.1442-200X.1988.tb02514.x]

37 **Lauwers GY**, Terris B, Balis UJ, Batts KP, Regimbeau JM, Chang Y, Graeme-Cook F, Yamabe H, Ikai I, Cleary KR, Fujita S, Flejou JF, Zukerberg LR, Nagorney DM, Belghiti J, Yamaoka Y, Vauthey JN; International Cooperative Study Group on Hepatocellular Carcinoma. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. *Am J Surg Pathol* 2002; **26**: 25-34 [PMID: 11756766 DOI: 10.1097/00000478-200201000-00003]

38 **Zhong X**, Long H, Su L, Zheng R, Wang W, Duan Y, Hu H, Lin M, Xie X. Radiomics models for preoperative prediction of microvascular invasion in hepatocellular carcinoma: a systematic review and meta-analysis. *Abdom Radiol (NY)* 2022; **47**: 2071-2088 [PMID: 35364684 DOI: 10.1007/s00261-022-03496-3]

39 **Xu X**, Zhang HL, Liu QP, Sun SW, Zhang J, Zhu FP, Yang G, Yan X, Zhang YD, Liu XS. Radiomic analysis of contrast-enhanced CT predicts microvascular invasion and outcome in hepatocellular carcinoma. *J Hepatol* 2019; **70**: 1133-1144 [PMID: 30876945 DOI: 10.1016/j.jhep.2019.02.023]

40 **Feng ST**, Jia Y, Liao B, Huang B, Zhou Q, Li X, Wei K, Chen L, Li B, Wang W, Chen S, He X, Wang H, Peng S, Chen ZB, Tang M, Chen Z, Hou Y, Peng Z, Kuang M. Preoperative prediction of microvascular invasion in hepatocellular cancer: a radiomics model using Gd-EOB-DTPA-enhanced MRI. *Eur Radiol* 2019; **29**: 4648-4659 [PMID: 30689032 DOI: 10.1007/s00330-018-5935-8]

41 **Zheng J**, Chakraborty J, Chapman WC, Gerst S, Gonen M, Pak LM, Jarnagin WR, DeMatteo RP, Do RKG, Simpson AL; Hepatopancreatobiliary Service in the Department of Surgery of the Memorial Sloan Kettering Cancer Center; Research Staff in the Department of Surgery at Washington University School of Medicine. Preoperative Prediction of Microvascular Invasion in Hepatocellular Carcinoma Using Quantitative Image Analysis. *J Am Coll Surg* 2017; **225**: 778-788.e1 [PMID: 28941728 DOI: 10.1016/j.jamcollsurg.2017.09.003]

42 **Segal E**, Sirlin CB, Ooi C, Adler AS, Gollub J, Chen X, Chan BK, Matcuk GR, Barry CT, Chang HY, Kuo MD. Decoding global gene expression programs in liver cancer by noninvasive imaging. *Nat Biotechnol* 2007; **25**: 675-680 [PMID: 17515910 DOI: 10.1038/nbt1306]

43 **Luo Y**, Ren F, Liu Y, Shi Z, Tan Z, Xiong H, Dang Y, Chen G. Clinicopathological and prognostic significance of high Ki-67 labeling index in hepatocellular carcinoma patients: a meta-analysis. *Int J Clin Exp Med* 2015; **8**: 10235-10247 [PMID: 26379815]

44 **Wu C**, Chen J, Fan Y, Zhao M, He X, Wei Y, Ge W, Liu Y. Nomogram Based on CT Radiomics Features Combined With Clinical Factors to Predict Ki-67 Expression in Hepatocellular Carcinoma. *Front Oncol* 2022; **12**: 943942 [PMID: 35875154 DOI: 10.3389/fonc.2022.943942]

45 **Li Y**, Yan C, Weng S, Shi Z, Sun H, Chen J, Xu X, Ye R, Hong J. Texture analysis of multi-phase MRI images to detect expression of Ki67 in hepatocellular carcinoma. *Clin Radiol* 2019; **74**: 813.e19-813.e27 [PMID: 31362887 DOI: 10.1016/j.crad.2019.06.024]

46 **Fan Y**, Yu Y, Wang X, Hu M, Hu C. Radiomic analysis of Gd-EOB-DTPA-enhanced MRI predicts Ki-67 expression in hepatocellular carcinoma. *BMC Med Imaging* 2021; **21**: 100 [PMID: 34130644 DOI: 10.1186/s12880-021-00633-0]

47 **Ye Z**, Jiang H, Chen J, Liu X, Wei Y, Xia C, Duan T, Cao L, Zhang Z, Song B. Texture analysis on gadoxetic acid enhanced-MRI for predicting Ki-67 status in hepatocellular carcinoma: A prospective study. *Chin J Cancer Res* 2019; **31**: 806-817 [PMID: 31814684 DOI: 10.21147/j.issn.1000-9604.2019.05.10]

48 **Hu X**, Zhou J, Li Y, Wang Y, Guo J, Sack I, Chen W, Yan F, Li R, Wang C. Added Value of Viscoelasticity for MRI-Based Prediction of Ki-67 Expression of Hepatocellular Carcinoma Using a Deep Learning Combined Radiomics (DLCR) Model. *Cancers (Basel)* 2022; **14** [PMID: 35681558 DOI: 10.3390/cancers14112575]

49 **Zhuo JY**, Lu D, Tan WY, Zheng SS, Shen YQ, Xu X. CK19-positive Hepatocellular Carcinoma is a Characteristic Subtype. *J Cancer* 2020; **11**: 5069-5077 [PMID: 32742454 DOI: 10.7150/jca.44697]

50 **Wang HQ**, Yang C, Zeng MS, Rao SX, Ji Y, Weng X, Wang JY, Sheng RF. Magnetic resonance texture analysis for the identification of cytokeratin 19-positive hepatocellular carcinoma. *Eur J Radiol* 2019; **117**: 164-170 [PMID: 31307643 DOI: 10.1016/j.ejrad.2019.06.016]

51 **Chen Y**, Chen J, Zhang Y, Lin Z, Wang M, Huang L, Huang M, Tang M, Zhou X, Peng Z, Huang B, Feng ST. Preoperative Prediction of Cytokeratin 19 Expression for Hepatocellular Carcinoma with Deep Learning Radiomics Based on Gadoxetic Acid-Enhanced Magnetic Resonance Imaging. *J Hepatocell Carcinoma* 2021; **8**: 795-808 [PMID: 34327180 DOI: 10.2147/JHC.S313879]

52 **Yang F**, Wan Y, Xu L, Wu Y, Shen X, Wang J, Lu D, Shao C, Zheng S, Niu T, Xu X. MRI-Radiomics Prediction for Cytokeratin 19-Positive Hepatocellular Carcinoma: A Multicenter Study. *Front Oncol* 2021; **11**: 672126 [PMID: 34476208 DOI: 10.3389/fonc.2021.672126]

53 **He X**, Liu F, Yan J, Zhang Y, Shang H, Dou Q, Zhao Q, Song Y. Trans-splicing repair of mutant p53 suppresses the growth of hepatocellular carcinoma cells in vitro and in vivo. *Sci Rep* 2015; **5**: 8705 [PMID: 25732051 DOI: 10.1038/srep08705]

54 **Mantovani F**, Walerych D, Sal GD. Targeting mutant p53 in cancer: a long road to precision therapy. *FEBS J* 2017; **284**: 837-850 [PMID: 27808469 DOI: 10.1111/febs.13948]

55 **Wu H**, Chen X, Chen J, Luo Y, Jiang X, Wei X, Tang W, Liu Y, Liang Y, Liu W, Guo Y. Correlations between P53 Mutation Status and Texture Features of CT Images for Hepatocellular Carcinoma. *Methods Inf Med* 2019; **58**: 42-49 [PMID: 31163452 DOI: 10.1055/s-0039-1688758]

56 **Liao H**, Jiang H, Chen Y, Duan T, Yang T, Han M, Xue Z, Shi F, Yuan K, Bashir MR, Shen D, Song B, Zeng Y. Predicting Genomic Alterations of Phosphatidylinositol-3 Kinase Signaling in Hepatocellular Carcinoma: A Radiogenomics Study Based on Next-Generation Sequencing and Contrast-Enhanced CT. *Ann Surg Oncol* 2022 [PMID: 35286532 DOI: 10.1245/s10434-022-11505-4]

57 **Ezzoukhry Z**, Louandre C, Trécherel E, Godin C, Chauffert B, Dupont S, Diouf M, Barbare JC, Mazière JC, Galmiche A. EGFR activation is a potential determinant of primary resistance of hepatocellular carcinoma cells to sorafenib. *Int J Cancer* 2012; **131**: 2961-2969 [PMID: 22514082 DOI: 10.1002/ijc.27604]

58 **Ma Y**, Xu R, Liu X, Zhang Y, Song L, Cai S, Zhou S, Xie Y, Li A, Cao W, Tang X. LY3214996 relieves acquired resistance to sorafenib in hepatocellular carcinoma cells. *Int J Med Sci* 2021; **18**: 1456-1464 [PMID: 33628103 DOI: 10.7150/ijms.51256]

59 **Negri FV**, Dal Bello B, Porta C, Campanini N, Rossi S, Tinelli C, Poggi G, Missale G, Fanello S, Salvagni S, Ardizzoni A, Maria SE. Expression of pERK and VEGFR-2 in advanced hepatocellular carcinoma and resistance to sorafenib treatment. *Liver Int* 2015; **35**: 2001-2008 [PMID: 25559745 DOI: 10.1111/liv.12778]

60 **Che F**, Xu Q, Li Q, Huang ZX, Yang CW, Wang LY, Wei Y, Shi YJ, Song B. Radiomics signature: A potential biomarker for β-arrestin1 phosphorylation prediction in hepatocellular carcinoma. *World J Gastroenterol* 2022; **28**: 1479-1493 [PMID: 35582676 DOI: 10.3748/wjg.v28.i14.1479]

61 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]

62 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

63 **Chen M**, Cao J, Hu J, Topatana W, Li S, Juengpanich S, Lin J, Tong C, Shen J, Zhang B, Wu J, Pocha C, Kudo M, Amedei A, Trevisani F, Sung PS, Zaydfudim VM, Kanda T, Cai X. Clinical-Radiomic Analysis for Pretreatment Prediction of Objective Response to First Transarterial Chemoembolization in Hepatocellular Carcinoma. *Liver Cancer* 2021; **10**: 38-51 [PMID: 33708638 DOI: 10.1159/000512028]

64 **Ji GW**, Zhu FP, Xu Q, Wang K, Wu MY, Tang WW, Li XC, Wang XH. Radiomic Features at Contrast-enhanced CT Predict Recurrence in Early Stage Hepatocellular Carcinoma: A Multi-Institutional Study. *Radiology* 2020; **294**: 568-579 [PMID: 31934830 DOI: 10.1148/radiol.2020191470]

65 **Kim S**, Shin J, Kim DY, Choi GH, Kim MJ, Choi JY. Radiomics on Gadoxetic Acid-Enhanced Magnetic Resonance Imaging for Prediction of Postoperative Early and Late Recurrence of Single Hepatocellular Carcinoma. *Clin Cancer Res* 2019; **25**: 3847-3855 [PMID: 30808773 DOI: 10.1158/1078-0432.CCR-18-2861]

66 **Shan QY**, Hu HT, Feng ST, Peng ZP, Chen SL, Zhou Q, Li X, Xie XY, Lu MD, Wang W, Kuang M. CT-based peritumoral radiomics signatures to predict early recurrence in hepatocellular carcinoma after curative tumor resection or ablation. *Cancer Imaging* 2019; **19**: 11 [PMID: 30813956 DOI: 10.1186/s40644-019-0197-5]

67 **Song W**, Yu X, Guo D, Liu H, Tang Z, Liu X, Zhou J, Zhang H, Liu Y. MRI-Based Radiomics: Associations With the Recurrence-Free Survival of Patients With Hepatocellular Carcinoma Treated With Conventional Transcatheter Arterial Chemoembolization. *J Magn Reson Imaging* 2020; **52**: 461-473 [PMID: 31675174 DOI: 10.1002/jmri.26977]

68 **Zhou Y**, He L, Huang Y, Chen S, Wu P, Ye W, Liu Z, Liang C. CT-based radiomics signature: a potential biomarker for preoperative prediction of early recurrence in hepatocellular carcinoma. *Abdom Radiol (NY)* 2017; **42**: 1695-1704 [PMID: 28180924 DOI: 10.1007/s00261-017-1072-0]

69 **Guo D**, Gu D, Wang H, Wei J, Wang Z, Hao X, Ji Q, Cao S, Song Z, Jiang J, Shen Z, Tian J, Zheng H. Radiomics analysis enables recurrence prediction for hepatocellular carcinoma after liver transplantation. *Eur J Radiol* 2019; **117**: 33-

40 [PMID: 31307650 DOI: 10.1016/j.ejrad.2019.05.010]
70 **Sun Y**, Bai H, Xia W, Wang D, Zhou B, Zhao X, Yang G, Xu L, Zhang W, Liu P, Xu J, Meng S, Liu R, Gao X. Predicting the Outcome of Transcatheter Arterial Embolization Therapy for Unresectable Hepatocellular Carcinoma Based on Radiomics of Preoperative Multiparameter MRI. *J Magn Reson Imaging* 2020; **52**: 1083-1090 [PMID: 32233054 DOI: 10.1002/jmri.27143]
71 **Lv X**, Chen M, Kong C, Shu G, Meng M, Ye W, Cheng S, Zheng L, Fang S, Chen C, Wu F, Weng Q, Tu J, Zhao Z, Ji J. Construction of a novel radiomics nomogram for the prediction of aggressive intrasegmental recurrence of HCC after radiofrequency ablation. *Eur J Radiol* 2021; **144**: 109955 [PMID: 34600237 DOI: 10.1016/j.ejrad.2021.109955]
72 **Kong C**, Zhao Z, Chen W, Lv X, Shu G, Ye M, Song J, Ying X, Weng Q, Weng W, Fang S, Chen M, Tu J, Ji J. Prediction of tumor response *via* a pretreatment MRI radiomics-based nomogram in HCC treated with TACE. *Eur Radiol* 2021; **31**: 7500-7511 [PMID: 33860832 DOI: 10.1007/s00330-021-07910-0]
73 **Ivanics T**, Salinas-Miranda E, Abreu P, Khalvati F, Namdar K, Dong X, Deniffel D, Gorgen A, Erdman L, Jhaveri K, Haider M, Veit-Haibach P, Sapisochin G. A Pre-TACE Radiomics Model to Predict HCC Progression and Recurrence in Liver Transplantation: A Pilot Study on a Novel Biomarker. *Transplantation* 2021; **105**: 2435-2444 [PMID: 33982917 DOI: 10.1097/TP.0000000000003605]
74 **Horvat N**, Araujo-Filho JAB, Assuncao-Jr AN, Machado FAM, Sims JA, Rocha CCT, Oliveira BC, Horvat JV, Maccali C, Puga ALBL, Chagas AL, Menezes MR, Cerri GG. Radiomic analysis of MRI to Predict Sustained Complete Response after Radiofrequency Ablation in Patients with Hepatocellular Carcinoma - A Pilot Study. *Clinics (Sao Paulo)* 2021; **76**: e2888 [PMID: 34287480 DOI: 10.6061/clinics/2021/e2888]
75 **Cai W**, He B, Hu M, Zhang W, Xiao D, Yu H, Song Q, Xiang N, Yang J, He S, Huang Y, Huang W, Jia F, Fang C. A radiomics-based nomogram for the preoperative prediction of posthepatectomy liver failure in patients with hepatocellular carcinoma. *Surg Oncol* 2019; **28**: 78-85 [PMID: 30851917 DOI: 10.1016/j.suronc.2018.11.013]
76 **Zhu WS**, Shi SY, Yang ZH, Song C, Shen J. Radiomics model based on preoperative gadoxetic acid-enhanced MRI for predicting liver failure. *World J Gastroenterol* 2020; **26**: 1208-1220 [PMID: 32231424 DOI: 10.3748/wjg.v26.i11.1208]
77 **Kiryu S**, Akai H, Nojima M, Hasegawa K, Shinkawa H, Kokudo N, Yasaka K, Ohtomo K. Impact of hepatocellular carcinoma heterogeneity on computed tomography as a prognostic indicator. *Sci Rep* 2017; **7**: 12689 [PMID: 28978930 DOI: 10.1038/s41598-017-12688-7]
78 **Akai H**, Yasaka K, Kunimatsu A, Nojima M, Kokudo T, Kokudo N, Hasegawa K, Abe O, Ohtomo K, Kiryu S. Predicting prognosis of resected hepatocellular carcinoma by radiomics analysis with random survival forest. *Diagn Interv Imaging* 2018; **99**: 643-651 [PMID: 29910166 DOI: 10.1016/j.diii.2018.05.008]
79 **Zheng BH**, Liu LZ, Zhang ZZ, Shi JY, Dong LQ, Tian LY, Ding ZB, Ji Y, Rao SX, Zhou J, Fan J, Wang XY, Gao Q. Radiomics score: a potential prognostic imaging feature for postoperative survival of solitary HCC patients. *BMC Cancer* 2018; **18**: 1148 [PMID: 30463529 DOI: 10.1186/s12885-018-5024-z]
80 **Kim J**, Choi SJ, Lee SH, Lee HY, Park H. Predicting Survival Using Pretreatment CT for Patients With Hepatocellular Carcinoma Treated With Transarterial Chemoembolization: Comparison of Models Using Radiomics. *AJR Am J Roentgenol* 2018; **211**: 1026-1034 [PMID: 30240304 DOI: 10.2214/AJR.18.19507]
81 **Blanc-Durand P**, Van Der Gucht A, Jreige M, Nicod-Lalonde M, Silva-Monteiro M, Prior JO, Denys A, Depeursinge A, Schaefer N. Signature of survival: a (18)F-FDG PET based whole-liver radiomic analysis predicts survival after (90)Y-TARE for hepatocellular carcinoma. *Oncotarget* 2018; **9**: 4549-4558 [PMID: 29435123 DOI: 10.18632/oncotarget.23423]
82 **Petukhova-Greenstein A**, Zeevi T, Yang J, Chai N, DiDomenico P, Deng Y, Ciarleglio M, Haider SP, Onyiuke I, Malpani R, Lin M, Kucukkaya AS, Gottwald LA, Gebauer B, Revzin M, Onofrey J, Staib L, Gunabushanam G, Taddei T, Chapiro J. MR Imaging Biomarkers for the Prediction of Outcome after Radiofrequency Ablation of Hepatocellular Carcinoma: Qualitative and Quantitative Assessments of the Liver Imaging Reporting and Data System and Radiomic Features. *J Vasc Interv Radiol* 2022; **33**: 814-824.e3 [PMID: 35460887 DOI: 10.1016/j.jvir.2022.04.006]
83 **Tian Y**, Komolafe TE, Zheng J, Zhou G, Chen T, Zhou B, Yang X. Assessing PD-L1 Expression Level *via* Preoperative MRI in HCC Based on Integrating Deep Learning and Radiomics Features. *Diagnostics (Basel)* 2021; **11** [PMID: 34679573 DOI: 10.3390/diagnostics11101875]
84 **Liao H**, Zhang Z, Chen J, Liao M, Xu L, Wu Z, Yuan K, Song B, Zeng Y. Preoperative Radiomic Approach to Evaluate Tumor-Infiltrating CD8(+) T Cells in Hepatocellular Carcinoma Patients Using Contrast-Enhanced Computed Tomography. *Ann Surg Oncol* 2019; **26**: 4537-4547 [PMID: 31520208 DOI: 10.1245/s10434-019-07815-9]
85 **Chen S**, Feng S, Wei J, Liu F, Li B, Li X, Hou Y, Gu D, Tang M, Xiao H, Jia Y, Peng S, Tian J, Kuang M. Pretreatment prediction of immunoscore in hepatocellular cancer: a radiomics-based clinical model based on Gd-EOB-DTPA-enhanced MRI imaging. *Eur Radiol* 2019; **29**: 4177-4187 [PMID: 30666445 DOI: 10.1007/s00330-018-5986-x]
86 **Hectors SJ**, Lewis S, Besa C, King MJ, Said D, Putra J, Ward S, Higashi T, Thung S, Yao S, Laface I, Schwartz M, Gnjatic S, Merad M, Hoshida Y, Taouli B. MRI radiomics features predict immuno-oncological characteristics of hepatocellular carcinoma. *Eur Radiol* 2020; **30**: 3759-3769 [PMID: 32086577 DOI: 10.1007/s00330-020-06675-2]
87 **Yuan G**, Song Y, Li Q, Hu X, Zang M, Dai W, Cheng X, Huang W, Yu W, Chen M, Guo Y, Zhang Q, Chen J. Development and Validation of a Contrast-Enhanced CT-Based Radiomics Nomogram for Prediction of Therapeutic Efficacy of Anti-PD-1 Antibodies in Advanced HCC Patients. *Front Immunol* 2020; **11**: 613946 [PMID: 33488622 DOI: 10.3389/fimmu.2020.613946]
88 **Duan J**, Qiu Q, Zhu J, Shang D, Dou X, Sun T, Yin Y, Meng X. Reproducibility for Hepatocellular Carcinoma CT Radiomic Features: Influence of Delineation Variability Based on 3D-CT, 4D-CT and Multiple-Parameter MR Images. *Front Oncol* 2022; **12**: 881931 [PMID: 35494061 DOI: 10.3389/fonc.2022.881931]
89 **Carbonell G**, Kennedy P, Bane O, Kirmani A, El Homsi M, Stocker D, Said D, Mukherjee P, Gevaert O, Lewis S, Hectors S, Taouli B. Precision of MRI radiomics features in the liver and hepatocellular carcinoma. *Eur Radiol* 2022; **32**: 2030-2040 [PMID: 34564745 DOI: 10.1007/s00330-021-08282-1]
90 **Ibrahim A**, Widaatalla Y, Refaee T, Primakov S, Miclea RL, Öcal O, Fabritius MP, Ingrisch M, Ricke J, Hustinx R, Mottaghy FM, Woodruff HC, Seidensticker M, Lambin P. Reproducibility of CT-Based Hepatocellular Carcinoma Radiomic Features across Different Contrast Imaging Phases: A Proof of Concept on SORAMIC Trial Data. *Cancers*

(Basel) 2021; **13** [PMID: 34572870 DOI: 10.3390/cancers13184638]

91 **Perrin T**, Midya A, Yamashita R, Chakraborty J, Saidon T, Jarnagin WR, Gonen M, Simpson AL, Do RKG. Short-term reproducibility of radiomic features in liver parenchyma and liver malignancies on contrast-enhanced CT imaging. *Abdom Radiol (NY)* 2018; **43**: 3271-3278 [PMID: 29730738 DOI: 10.1007/s00261-018-1600-6]

92 **Wang X**, Wang S, Yin X, Zheng Y. MRI-based radiomics distinguish different pathological types of hepatocellular carcinoma. *Comput Biol Med* 2022; **141**: 105058 [PMID: 34836622 DOI: 10.1016/j.compbiomed.2021.105058]

93 **Gao F**, Yan B, Chen J, Wu M, Shi D. Pathological grading of Hepatocellular Carcinomas in MRI using a LASSO algorithm. *J Physic Confer Series* 2018; **1053**: 012095 [DOI: 10.1088/1742-6596/1053/1/012095]

94 **Zhou W**, Zhang L, Wang K, Chen S, Wang G, Liu Z, Liang C. Malignancy characterization of hepatocellular carcinomas based on texture analysis of contrast-enhanced MR images. *J Magn Reson Imaging* 2017; **45**: 1476-1484 [PMID: 27626270 DOI: 10.1002/jmri.25454]

95 **Yang L**, Gu D, Wei J, Yang C, Rao S, Wang W, Chen C, Ding Y, Tian J, Zeng M. A Radiomics Nomogram for Preoperative Prediction of Microvascular Invasion in Hepatocellular Carcinoma. *Liver Cancer* 2019; **8**: 373-386 [PMID: 31768346 DOI: 10.1159/000494099]

96 **Bakr S**, Echegaray S, Shah R, Kamaya A, Louie J, Napel S, Kothary N, Gevaert O. Noninvasive radiomics signature based on quantitative analysis of computed tomography images as a surrogate for microvascular invasion in hepatocellular carcinoma: a pilot study. *J Med Imaging (Bellingham)* 2017; **4**: 041303 [PMID: 28840174 DOI: 10.1117/1.JMI.4.4.041303]

97 **Ma X**, Wei J, Gu D, Zhu Y, Feng B, Liang M, Wang S, Zhao X, Tian J. Preoperative radiomics nomogram for microvascular invasion prediction in hepatocellular carcinoma using contrast-enhanced CT. *Eur Radiol* 2019; **29**: 3595-3605 [PMID: 30770969 DOI: 10.1007/s00330-018-5985-y]

98 **Xia W**, Chen Y, Zhang R, Yan Z, Zhou X, Zhang B, Gao X. Radiogenomics of hepatocellular carcinoma: multiregion analysis-based identification of prognostic imaging biomarkers by integrating gene data-a preliminary study. *Phys Med Biol* 2018; **63**: 035044 [PMID: 29311419 DOI: 10.1088/1361-6560/aaa609]

99 **Li X**, Cheng L, Li C, Hu X, Tan L, Li Q, Liu C, Wang J. Associating Preoperative MRI Features and Gene Expression Signatures of Early-stage Hepatocellular Carcinoma Patients using Machine Learning. *J Clin Transl Hepatol* 2022; **10**: 63-71 [PMID: 35233374 DOI: 10.14218/JCTH.2021.00023]

100 **Hui TCH**, Chuah TK, Low HM, Tan CH. Predicting early recurrence of hepatocellular carcinoma with texture analysis of preoperative MRI: a radiomics study. *Clin Radiol* 2018; **73**: 1056.e11-1056.e16 [PMID: 30213434 DOI: 10.1016/j.crad.2018.07.109]

101 **Zhao Y**, Wu J, Zhang Q, Hua Z, Qi W, Wang N, Lin T, Sheng L, Cui D, Liu J, Song Q, Li X, Wu T, Guo Y, Cui J, Liu A. Radiomics Analysis Based on Multiparametric MRI for Predicting Early Recurrence in Hepatocellular Carcinoma After Partial Hepatectomy. *J Magn Reson Imaging* 2021; **53**: 1066-1079 [PMID: 33217114 DOI: 10.1002/jmri.27424]

102 **Zhang H**, Guo D, Liu H, He X, Qiao X, Liu X, Liu Y, Zhou J, Zhou Z, Fang Z. MRI-Based Radiomics Models to Discriminate Hepatocellular Carcinoma and Non-Hepatocellular Carcinoma in LR-M According to LI-RADS Version 2018. *Diagnostics (Basel)* 2022; **12** [PMID: 35626199 DOI: 10.3390/diagnostics12051043]

103 **Park S**, Kim JH, Kim J, Joseph W, Lee D, Park SJ. Development of a deep learning-based auto-segmentation algorithm for hepatocellular carcinoma (HCC) and application to predict microvascular invasion of HCC using CT texture analysis: preliminary results. *Acta Radiol* 2022; 2841851221100318 [PMID: 35570797 DOI: 10.1177/02841851221100318]

104 **Haniff NSM**, Abdul Karim MK, Osman NH, Saripan MI, Che Isa IN, Ibahim MJ. Stability and Reproducibility of Radiomic Features Based Various Segmentation Technique on MR Images of Hepatocellular Carcinoma (HCC). *Diagnostics (Basel)* 2021; **11** [PMID: 34573915 DOI: 10.3390/diagnostics11091573]

105 **Hu MJ**, Yu YX, Fan YF, Hu CH. CT-based radiomics model to distinguish necrotic hepatocellular carcinoma from pyogenic liver abscess. *Clin Radiol* 2021; **76**: 161.e11-161.e17 [PMID: 33267948 DOI: 10.1016/j.crad.2020.11.002]

106 **Hu HT**, Shan QY, Chen SL, Li B, Feng ST, Xu EJ, Li X, Long JY, Xie XY, Lu MD, Kuang M, Shen JX, Wang W. CT-based radiomics for preoperative prediction of early recurrent hepatocellular carcinoma: technical reproducibility of acquisition and scanners. *Radiol Med* 2020; **125**: 697-705 [PMID: 32200455 DOI: 10.1007/s11547-020-01174-2]

107 **Qiu Q**, Duan J, Duan Z, Meng X, Ma C, Zhu J, Lu J, Liu T, Yin Y. Reproducibility and non-redundancy of radiomic features extracted from arterial phase CT scans in hepatocellular carcinoma patients: impact of tumor segmentation variability. *Quant Imaging Med Surg* 2019; **9**: 453-464 [PMID: 31032192 DOI: 10.21037/qims.2019.03.02]

108 **Zhang J**, Qiu Q, Duan J, Gong G, Jiang Q, Sun G, Yin Y. Variability of radiomic features extracted from multi-b-value diffusion-weighted images in hepatocellular carcinoma. *Transl Cancer Res* 2019; **8**: 130-140 [PMID: 35116742 DOI: 10.21037/tcr.2019.01.14]

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REVIEW

Evolution of care in cirrhosis: Preventing hepatic decompensation through pharmacotherapy

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Abstract

Cirrhosis is a leading cause of morbidity and mortality, impacting more than 120 million people worldwide. Although geographic differences exist, etiologic factors such as alcohol use disorder, chronic viral hepatitis infections, and non-alcoholic fatty liver disease are prevalent in nearly every region. Historically, significant effort has been devoted to modifying these risks to prevent disease progression. Nevertheless, more than 11% of patients with compensated cirrhosis experience hepatic decompensation each year. This transition signifies the most important prognostic factor in the natural history of the disease, corresponding to a decline in median survival to below 2 years. Over the past decade, the need for pharmacotherapies aimed at reducing the risk for hepatic decompensation has been emphasized, and non-selective beta-blockers have emerged as the most effective option to date. However, a critical therapeutic gap still exists, and additional therapies have been proposed, including statins, rifaximin, and sodium-glucose cotransporter-2 inhibitors. Based on the results of innovative retrospective analyses and small-scale prospective trials, these pharmacotherapies represent promising options, but further studies, including randomized controlled trials, are necessary before they can be incorporated into clinical use. This report highlights the potential impact of these agents and others in preventing hepatic decompensation and discusses how this paradigm shift may pave the way for guideline-directed medical therapy in cirrhosis.

Key Words: Cirrhosis; Hepatic decompensation; Beta-blockers; Statins; Sodium-glucose cotransporter-2 inhibitors; Rifaximin

Core Tip: Hepatic decompensation is the most important clinical predictor of morbidity and mortality among patients with cirrhosis. New pharmacotherapies aimed at preventing hepatic decompensation in high-risk patients are emerging, augmenting traditional management strategies. These treatments represent safe, accessible, and effective options that may improve quality of life and prolong transplant-free survival, regardless of the etiologic factors involved.

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INTRODUCTION

Cirrhosis reflects the end stage of all chronic liver diseases. It impacts more than 120 million people globally, largely in the context of common risk factors such as alcohol use disorder, chronic viral hepatitis, and non-alcoholic fatty liver disease (NAFLD)[1]. It represents the 8th leading cause of mortality in the United States and 13th in the world, with the number of attributable deaths worldwide having increased by approximately 50% between 1990 and 2013[2]. Regardless of etiology, the most important prognostic factor for survival is the presence or absence of hepatic decompensation, which includes complications such as ascites, variceal hemorrhage, hepatic encephalopathy, and jaundice[3,4]. Although patients with cirrhosis may remain compensated for extended periods of time, especially if their underlying risk factors are mitigated, approximately 11% of those with compensated disease experience new decompensations each year[5], and the progression from compensated to decompensated cirrhosis is associated with a decline in median survival from 12 years to less than 2 years[6]. Among individuals with compensated disease, the 10-year risks of developing ascites, gastrointestinal hemorrhage, hepatic encephalopathy, and jaundice are 40%, 25%, 28%, and 33%, respectively[6].

Beyond its burden on patient health and quality of life, cirrhosis also represents a critical healthcare challenge. According to the Global Burden of Disease Study 2017[1], there are 112 million and 10.6 million cases of compensated and decompensated cirrhosis worldwide, respectively. In the United States, this is associated with significant healthcare costs, including approximately $2.5 billion annually for hospitalization and treatment and $10.6 billion annually for losses in work productivity and health-related quality of life[7]. These costs are disproportionately borne in the management of complications among those with decompensated disease. Despite the significant health and socioeconomic burdens, it is only recently that management strategies have pivoted from focusing primarily on risk factor modification and the treatment of complications towards the prevention of hepatic decompensation in high-risk patients. Unlike the management of other common chronic diseases such as congestive heart failure, in which the implementation of guideline-directed medical therapy has resulted in significant reductions in morbidity and mortality, the long-term management of cirrhosis has been historically limited by a lack of robust chemoprevention[8,9]. In this report, we discuss the management of cirrhosis, focusing on pharmacotherapies aimed at preventing hepatic decompensation.

TRADITIONAL TREATMENT STRATEGIES

Chronic hepatitis C virus (HCV) infection, NAFLD, and alcohol-related liver disease are the three leading causes of cirrhosis in the United States[10]. For each of these etiologies, a critical component of the long-term management involves risk factor modification. Among patients with chronic HCV infection and compensated disease, the advent of direct-acting antiviral agents represents a landmark achievement that has been shown to reduce the risk for liver-related complications[11-14]. Unfortunately, more than 40% of HCV infections are diagnosed after hepatic decompensation has already occurred[10], at which point antiviral treatment is less effective and associated with a higher risk for adverse events[15]. For those with NAFLD, a growing body of literature supports the efficacy of a multimodal approach which integrates dietary changes, weight reduction, and restoring insulin sensitivity[16]. Glucagon-like peptide 1 receptor agonists such as semaglutide have an evolving role in the treatment in of NAFLD, offering both weight reduction and possible attenuation of steatohepatitis, and a number of other drugs aimed at reducing fibrosis progression and the risk for complications are currently being evaluated in phase II and phase III clinical trials[17]. However, to date, disease-specific pharmacologic treatments have yet to be approved. The prevalence of NAFLD and its associated complications continue to increase[18] such that it is currently the second leading cause of cirrhosis among patients awaiting liver transplantation in the United States[14] and is expected to overtake HCV-

related cirrhosis as the most frequent indication for liver transplantation[19]. Finally, alcohol-related liver disease remains highly prevalent worldwide[20] with increasing cirrhosis-related mortality in regions with high alcohol consumption[21]. Abstaining from alcohol significantly improves survival among patients with cirrhosis, but pharmacologic options for alcohol use disorder remain limited, partially due to concerns about hepatotoxicity[22]. For patients with less common causes of chronic liver disease, risk factor modification may include therapies such as immunosuppression (autoimmune hepatitis), ursodeoxycholic acid (primary biliary cholangitis), and phlebotomy or chelation (hemochromatosis). Unfortunately, the impact of some of these treatments is generally diminished in the context of cirrhosis.

Once hepatic decompensation has occurred, treatment strategies may be implemented to address specific complications. This includes pharmacotherapy to eliminate ascites (diuretics), reduce the risk of variceal rupture [non-selective beta-blockers (NSBBs)], and prevent recurrent encephalopathy (lactulose and rifaximin)[23-25]. Among patients with refractory ascites or variceal hemorrhage, transjugular intrahepatic portosystemic shunting (TIPS) reduces the risk of further decompensation and may improve survival in a subset of patients[26-28]. Finally, liver transplantation is the only durable curative option for patients with decompensated cirrhosis. However, resource limitations and host factors restrict the use of TIPS and liver transplantation to a relatively small number of patients in resource-rich settings. Furthermore, despite advances in the care of decompensated cirrhosis, the 30-d mortality following hospital discharges for hepatic decompensation remains largely unchanged[29]. Thus, significant interest exists in preventing the progression to decompensated cirrhosis.

CURRENT THERAPIES TO PREVENT HEPATIC DECOMPENSATION

NSBBs are currently the only agents recommended for the long-term management of portal hypertension in patients with compensated cirrhosis[30]. Traditional NSBBs such as propranolol and nadolol inhibit β1 and β2 receptors, mitigating the hyperdynamic response and splanchnic arteriolar vasodilation that occur in the context of cirrhosis. Carvedilol has additional α1 inhibition that reduces intrahepatic vascular resistance[31,32]. Together, these hemodynamic effects attenuate portal hypertension, reducing the risk for hepatic decompensation. In addition, NSBBs impact the risk for liver-related complications through other mechanisms. For example, NSBBs reduce abnormal gastrointestinal permeability and bacterial translocation[33] and upregulate the phagocytic activity of monocytes and granulocytes after exposure to bacterial DNA[34]. As such, a recent prospective study demonstrated that NSBBs reduce the risk for bacterial infections [odds ratio = 0.46, 95% confidence interval (CI): 0.3-0.7; $P = 0.001$][35].

The landmark trial demonstrating the utility of NSBBs in preventing decompensation was the study on Beta-Blockers to Prevent Decompensation of Cirrhosis with Portal Hypertension (PREDESCI)[36]. In this multicenter, double-blind, randomized controlled trial (RCT), 201 patients with compensated cirrhosis and clinically-significant portal hypertension (CSPH) without high-risk varices were randomized to receive NSBBs (propranolol up to 160 mg twice daily or carvedilol up to 25 mg daily) or placebo. Over a median follow-up of 37 mo, the risk of hepatic decompensation, including ascites, variceal hemorrhage, or hepatic encephalopathy, was significantly lower among patients receiving NSBBs relative to those receiving placebo [hazard ratio (HR) = 0.51, 95%CI: 0.26-0.97; $P = 0.041$]. This difference was driven primarily by a reduction in ascites (HR = 0.42, 95%CI: 0.19-0.92; $P = 0.03$), although non-significant trends towards decreased progression to high-risk varices and improved survival were also observed. The number needed to treat for the prevention of a decompensation event over the follow-up period was 9, and the incidence of adverse events was similar between the treatment and placebo groups.

Prior to PREDESCI, other RCTs consistently demonstrated that propranolol and nadolol were effective in preventing variceal hemorrhage among patients with cirrhosis and large esophageal varies but without prior bleeding episodes. Pascal and Cales[37] found that patients receiving propranolol up to 320 mg daily were less likely to develop bleeding episodes compared to those in the placebo group (74% *vs* 39%; $P < 0.05$); Idéo *et al*[38] also observed significantly lower rates among those receiving nadolol up to 120 mg daily (94.4%) relative to those receiving placebo (70.2%). Other studies have reported similar risk reductions in initial[39,40] and subsequent[41] variceal bleeds, although one reported a significant difference only among patients who lacked ascites[42]. Given these data, NSBBs are currently recommended for the primary and secondary prophylaxis of variceal hemorrhage[37,41]. Notably, however, the Prevention of Esophageal Varices by Beta-Adrenergic Blockers trial observed no differences in the use of timolol up to 80 mg daily *vs* placebo in the development of varices among patients with compensated cirrhosis[43].

The potential benefits of carvedilol beyond those of traditional NSBBs have also been of great interest. Four studies have evaluated the impact of carvedilol in preventing hepatic decompensation or disease progression among patients with compensated cirrhosis and CSPH. A subgroup analysis of the PREDESCI trial[36] found a non-significant reduction in the risk for hepatic decompensation or death (HR = 0.39, 95%CI: 0.10-1.49; $P = 0.16$) and Bhardwaj *et al*[44] observed a significantly higher likelihood

of non-progression from small to large esophageal varices (79.4% *vs* 61.4%; P = 0.04). In comparing carvedilol to variceal band ligation, one study found carvedilol 12.5 mg daily to be associated with significantly lower rates of initial variceal hemorrhage (HR = 0.41, 95%CI: 0.19-0.96; P = 0.04) but similar rates of bleeding-related and overall mortality[45], while another reported comparable rates across all three outcomes with the same dosing[46]. Additionally, a recent meta-analysis of these four studies observed a significantly improved hazard ratio for decompensation among patients receiving carvedilol compared to control therapy (HR = 0.506, 95%CI: 0.289-0.887; P = 0.017)[47]. Finally, in comparison to propranolol, multiple studies, including PREDESCI, have suggested that carvedilol may be superior in reducing the hepatic venous pressure gradient[36,48]. However, it remains unclear whether this finding consistently translates into a difference in the risk for hepatic decompensation.

Although NSBBs have been extensively studied and represent the current treatment of choice for the prevention of hepatic decompensation, there is significant interest in identifying additional therapies for chemoprevention for a variety of reasons. First, most commonly, patients may have contraindications or intolerance to NSBBs. Common adverse effects include bradycardia, hypotension, and fatigue. Second, pharmacotherapy may be leveraged for pleiotropic benefits, and alternative therapies may provide an opportunity for enhanced personalized care. Finally, therapies may have additive or synergistic effects that may provide additional clinical benefit for high-risk patients. As such, a number of agents are currently under evaluation (Table 1).

EMERGING THERAPIES TO PREVENT HEPATIC DECOMPENSATION

Statins

These cholesterol-lowering drugs are a mainstay for the treatment of dyslipidemia and atherosclerotic cardiovascular disease but have diverse pleiotropic effects that may impact a wide range of other disease processes. Concerns regarding hepatotoxicity have historically limited their use among patients with chronic liver disease[49], but emerging evidence indicates that their anti-fibrotic, immunomodulatory, and antioxidant effects may attenuate portal hypertension and limit disease progression[50-53] without posing an excess safety risk among patients with compensated disease[54-56].

Several retrospective studies have evaluated the role of statins in preventing hepatic decompensation. In one case-control study of patients with predominantly early-stage cirrhosis, statin use was associated with a decreased risk of hepatic decompensation over 36 mo (HR = 0.58; P = 0.04)[57]. Similar findings have been reported in patients with cirrhosis due to chronic viral hepatitis; among statin users, the HR for hepatic decompensation was 0.39 (95%CI: 0.25-0.62)[58] for hepatitis B virus-related cirrhosis and 0.51 (95%CI: 0.29-0.93)[58] to 0.55 (95%CI: 0.39-0.77)[59] for HCV-related cirrhosis. A trend towards decreased hepatic decompensation was observed with among patients with alcohol-related cirrhosis (HR = 0.69, 95%CI: 0.45-1.07)[58]. A recent meta-analysis of these three observational studies found the pooled HR for hepatic decompensation to be 0.54 (95%CI: 0.46-0.65) with minimal heterogeneity (I^2 = 0%)[60]. A similar meta-analysis using these studies also demonstrated that the association between statin use and an improved decompensation was independent of cirrhosis etiology[61].

In light of the encouraging findings reported in observational studies, RCTs evaluating the impact of statins in preventing hepatic decompensation are currently in progress. In the United States, the Statins and Cirrhosis: Reducing Events of Decompensation trial is studying simvastatin at a dose of 40 mg daily (ClinicalTrials.gov, NCT03654053)[62]. In Denmark, the Statins for Prevention of Disease Progression and Hospitalization in Liver Cirrhosis trial is studying atorvastatin at doses of 10-20 mg daily (ClinicalTrials.gov, NCT04072601).

Rifaximin

Patients with cirrhosis can experience increased bacterial translocation secondary to elevated portal pressures, increased gastrointestinal permeability, and altered gut microbiota, thereby contributing to the inflammatory milieu. Rifaximin is a safe poorly-absorbed oral antibiotic with broad gut-selective antimicrobial activity against gram-positive and gram-negative bacteria. Beyond its impacts on the intestinal microbiome, it may also attenuate inflammation, decrease bacterial interactions with enterocytes, and improve intestinal epithelial integrity[63,64]. Furthermore, the combination of propranolol and rifaximin compared to propranolol alone is associated with a more significant reduction in portal pressure[65]. Although rifaximin is currently approved for the prevention of recurrent hepatic encephalopathy[66], it may also have a role in preventing other hepatic decompensations.

A number of studies have evaluated the association between rifaximin use and liver-related complications, demonstrating that rifaximin may reduce the risk of further decompensation in patients with decompensated cirrhosis[63]. However, little is known about its role in those with high-risk compensated disease. Most notably, in a *post-hoc* analysis of a RCT comparing rifaximin 550 mg twice daily to placebo, Flamm *et al*[67] demonstrated that rifaximin reduces the risk for further hepatic decompensation (HR = 0.41, 95%CI: 0.25-0.67; P < 0.001). This finding was corroborated by Zeng *et al*[68] in a prospective randomized open-labelled study. Currently, there are no ongoing trials investigating

Table 1 Chemoprevention for hepatic decompensation in cirrhosis: Current and emerging therapies

Agents	Mechanism of action	Primary benefits	Potential adverse effects	Other limitations	Supported by RCT
NSBBs	β1 and β2 blockade; α1 blockade (carvedilol)	Decreased portal pressure	Hypotension, bradycardia, fatigue	Dosing frequency (propranolol)	Yes
Statins	Inhibition of HMG-CoA reductase	Decreased inflammation and endothelial dysfunction	Myopathy, hepatitis, diabetes		Ongoing
Rifaximin	Broad-spectrum, gut-specific antibiotic	Reduced dysbiosis and bacterial translocation	Gastrointestinal upset	Cost	Included patients with prior decompensation
Anticoagulants	Inactivation of clotting factors	Reduced endothelial dysfunction	Hemorrhage	SQ injection (enoxaparin), dosing (warfarin)	Included patients with prior decompensation
ACE inhibitors	Inhibition of angiotensin II production	Decreased portal pressure[1]	Hypotension, AKI, electrolyte derangements, angioedema		No
ARBs	Inhibition of angiotensin II type 1 receptor	Decreased portal pressure	Hypotension, AKI, electrolyte derangements		No
MRAs	Inhibition of the aldosterone receptor in the distal nephron	Decreased portal pressure	Hypotension, AKI, electrolyte derangements	Gynecomastia (spirono-lactone)	Only in combination with NSBB
SGLT2 inhibitors	Inhibition of proximal tubule sodium-glucose cotransporter	Decreased portal pressure	Electrolyte derangements, mycotic genital infections	Cost, risk of ketoacidosis in AUD	No
Albumin	Anionic carrier protein with pleiotropic properties	Reduced inflammation; increased effective circulating volume	Volume overload	Cost, intravenous administration	Included patients with prior decompensation

[1]Not demonstrated in prior clinical studies.

ACE: Angiotensin converting enzyme; AKI: Acute kidney injury; ARB: Angiotensin receptor blocker; AUD: Alcohol use disorder; BP: Blood pressure; HMG-CoA: Hydroxy β-methylglutaryl-CoA; MRA: Mineralocorticoid receptor antagonist; NSBB: Non-selective beta-blocker; SGLT2: Sodium-glucose cotransporter 2; SQ: Subcutaneous; RCT: Randomized controlled trial.

the impact of rifaximin in patients with compensated disease, although the Simvastatin Plus Rifaximin in Decompensated Cirrhosis study is examining the role of statins and rifaximin among patients with pre-existing decompensated disease (ClinicalTrials.gov, NCT03780673). Historically, a critical barrier to the study and use of rifaximin has been cost.

Anticoagulants

In light of the impaired synthesis of clotting factors and the presence of thrombocytopenia associated with cirrhosis, the risk of bleeding has historically been prioritized over thrombosis[69]. However, more recent evidence suggests that a new state of rebalanced hemostasis is achieved among those with stable cirrhosis in which decompensation can lead to increased risks of both hemorrhage and thrombosis, which in turn, can increase the risk for further decompensation[69]. Thrombin has been proposed to activate hepatic stellate cells and lead to upregulation of hepatic fibrosis, suggesting a potential role for anticoagulation in slowing cirrhosis disease progression[70].

Several studies have evaluated the efficacy and safety of anticoagulation in preventing or managing venous thrombotic events in patients with cirrhosis, but only two have specifically addressed its association with preventing decompensation[71-75]. In a RCT of patients with advanced cirrhosis randomized to receive enoxaparin 4000 IU/d or no treatment, decompensation was significantly less common among those receiving enoxaparin (11.7% *vs* 59.4%; $P < 0.0001$)[75]. Enoxaparin was independently associated with a reduced risk for hepatic decompensation (HR = 0.331, 95%CI: 0.148-0.741; $P = 0.007$). Notably, following cessation of enoxaparin receipt, rates of hepatic decompensation were similar between those in the control and treatment groups. In contrast, in a retrospective study of patients with cirrhosis and portal vein thrombosis, the 1-year probability of hepatic decompensation was not significantly different between patients who did or did not receive warfarin (15.6% *vs* 17.9%; $P = 0.847$)[72]. The ongoing CIRROXABAN phase III RCT (ClinicalTrials.gov, NCT02643212) aims to evaluate the effect of rivaroxaban in the development of decompensation among patients with cirrhosis.

Renin-angiotensin-aldosterone system antagonists

The renin-angiotensin-aldosterone system (RAAS) has a central role in the pathogenesis and progression of portal hypertension. Splanchnic and peripheral vasodilation in response to excess nitric

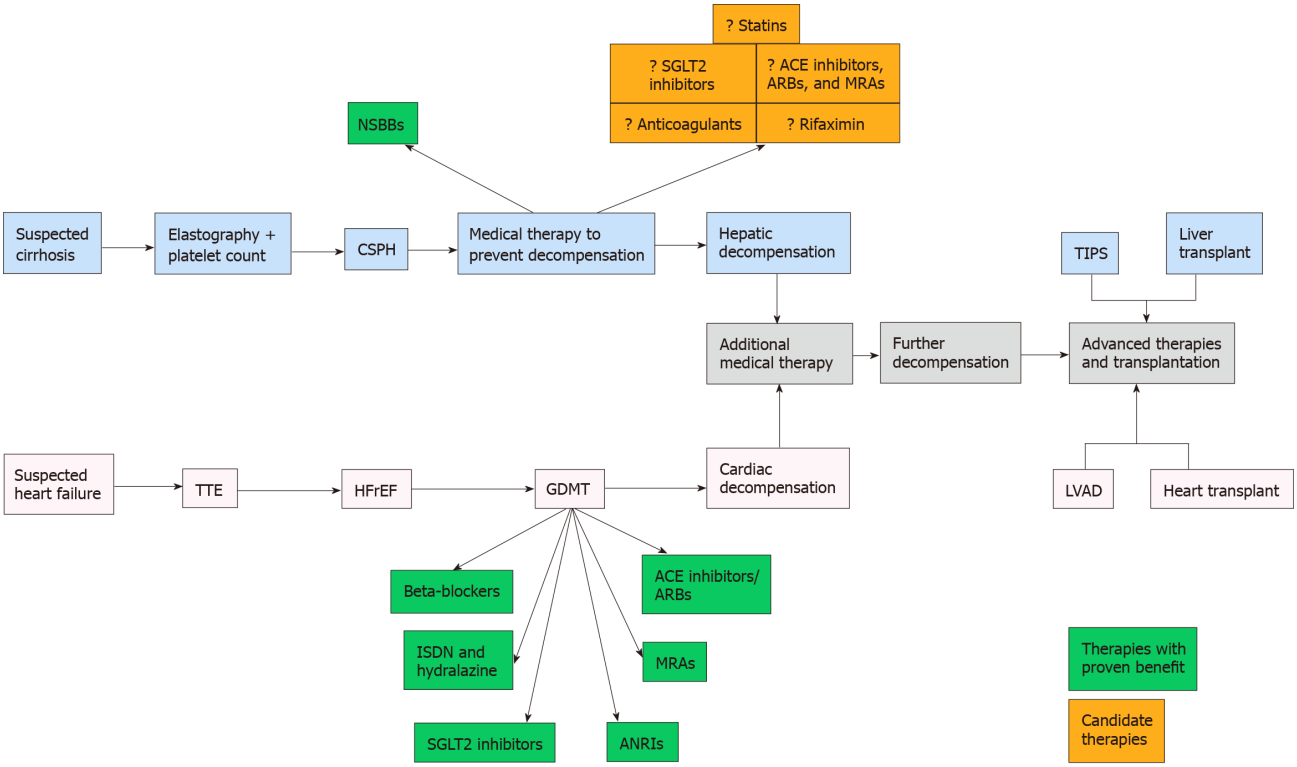

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Figure 1 Management of cirrhosis in comparison to congestive heart failure. ACE: Angiotensin converting enzyme; ANRI: Angiotensin receptor-neprilysin inhibitor; ARB: Angiotensin receptor blocker; CSPH: Clinically-significant portal hypertension; GDMT: Guideline-directed medical therapy; HFrEF: Heart failure with reduced ejection fraction; LVAD: Left ventricular assist device; MRA: Mineralocorticoid receptor antagonist; NSBB: Non-selective beta-blocker; SGLT2: Sodium-glucose cotransporter 2; TIPS: Transjugular intrahepatic portosystemic shunting; TTE: Transthoracic echocardiogram.

oxide stimulate the renin-angiotensin-aldosterone and sympathetic nervous systems, triggering a number of mechanisms that further exacerbate portal hypertension. Mediators of these pathways have been directly linked to mortality in patients with cirrhosis[76,77]. Thus, a number of drugs which attenuate the RAAS have been evaluated with mixed results. These include angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), mineralocorticoid receptor antagonists such as spironolactone, and sodium-glucose cotransporter-2 (SGLT2) inhibitors.

Both ACE inhibitors and ARBs have been studied in portal hypertension, but their impact in preventing hepatic decompensation remains unclear. Current evidence suggests that ARBs may reduce portal pressures[78-84], but they have yet to be evaluated in RCTs. In contrast, ACE inhibitors have not been shown to reduce portal pressures[85], but a recent large retrospective analysis suggest that they may reduce the risk for liver-related complications in patients with NAFLD[86]. Regardless, while both agents may be safe and potentially beneficial in patients with early-stage disease, their side-effect profile may be deleterious among patients with CSPH.

Spironolactone is critical in the management of cirrhotic ascites. However, the drug has been investigated as an adjunctive agent to NSBBs in the prevention of variceal hemorrhage. A prospective trial demonstrated that the addition of spironolactone 100 mg daily to nadolol reduced the risk of a combined endpoint of variceal hemorrhage and ascites (39% *vs* 20%; $P < 0.04$)[87]. Further trials exploring the utility of spironolactone in preventing hepatic decompensation among compensated patients have not been pursued, and thus the agent is currently not recommended for chemoprevention.

Initially introduced as antidiabetic drugs, SGLT2 inhibitors have become mainstays in the management of cardiovascular disease and chronic kidney disease among diabetic and nondiabetic patients due to their broad pleiotropic effects[88-93]. By inhibiting sodium and glucose reabsorption in the proximal convoluted tubule, SGLT2 inhibitors restore tubuloglomerular feedback, attenuate overactivation of the RAAS and the sympathetic nervous system, and promote natriuresis, thereby overcoming key mechanisms that are also implicated in the pathogenesis of portal hypertension in cirrhosis[9]. Unlike ACE inhibitors, ARBs, and spironolactone, SGLT2 inhibitors have limited effects on systemic blood pressure and may be better tolerated among patients with CSPH. Although RCTs in cirrhosis are currently lacking, retrospective studies suggest that these agents, namely empagliflozin, are likely safe and warrant further investigation[94-98].

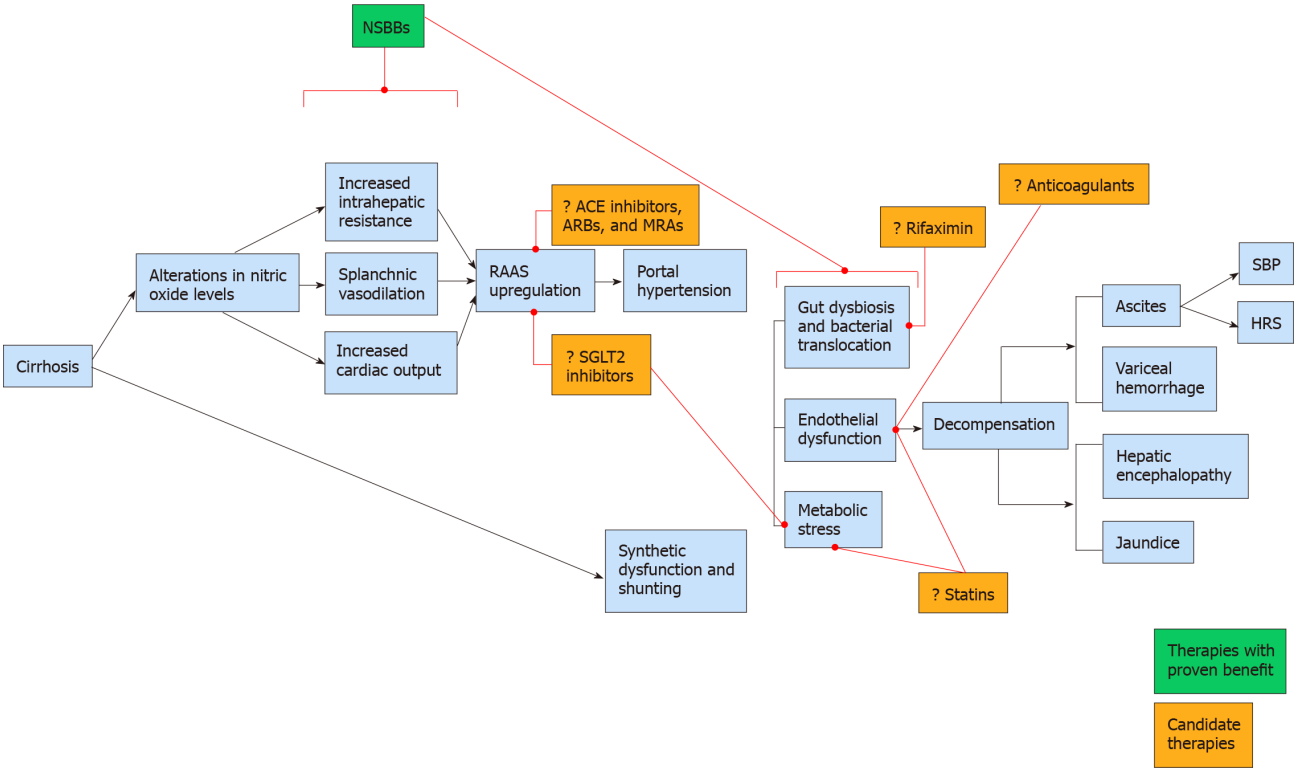

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Figure 2 Therapeutic targets in the prevention of hepatic decompensation. ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker; HRS: Hepatorenal syndrome; MRA: Mineralocorticoid receptor antagonist; NSBB: Non-selective beta-blocker; RAAS: Renin-angiotensin-aldosterone system; SBP: Spontaneous bacterial peritonitis; SGLT2: Sodium-glucose cotransporter 2.

Albumin

Albumin has versatile anti-inflammatory and plasma expansion properties that may also mitigate mechanisms implicated in hepatic decompensation. To our knowledge, no study has investigated the role of albumin in preventing hepatic decompensation among patients with compensated disease. However, the Human Albumin for the Treatment of Ascites in Patients with Hepatic Cirrhosis study assessed the impact of human albumin in patients with uncomplicated ascites[99]. Although the primary outcome was mortality, secondary analyses demonstrated that weekly 40-gram albumin infusions reduced the risk for refractory ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, and hepatic encephalopathy in comparison to standard medical therapy alone. Notably, the study was limited by the fact that the treatment group received more frequent outpatient evaluations, allowing providers to address potential impending complications in a more proactive manner. Because of accessibility and cost limitations, in addition to other factors that restrict widespread use, it is unlikely that albumin will receive significant consideration as a potential therapeutic option in patients with compensated cirrhosis.

Other candidate pharmacotherapies

Numerous other agents hypothesized to decrease the risk of hepatic decompensation have been evaluated. Despite early studies suggesting indirect benefits, there is a paucity of consistent, rigorous clinical evidence demonstrating a direct role for nitrates[100-103], endothelin-A antagonists[104,105], farnesoid X receptor agonists[106], phosphodiesterase-5 inhibitors[107-111], serelaxin[112], sorafenib [113-115], taurine[116], and thalidomide[117] in the prevention of hepatic decompensation. Furthermore, it has been postulated that aspirin and metformin could be attractive targets. However, at this time, a number of prospective and retrospective studies have only demonstrated that these agents may reduce the risk of hepatocellular carcinoma without necessarily impacting the risk for hepatic decompensation[118-120].

A NEW PARADIGM: CHEMOPREVENTION IN THE MODERN ERA

Until PREDESCI validated the use of NSBBs for chemoprevention, the pharmacologic management of cirrhosis had experienced little progress over the preceding half century. In contrast, over the same period of time, incremental advances in the medical management of systolic heart failure, a condition

that mechanistically mimics portal hypertension, led to the widespread implementation of guideline-directed medical therapy, which significantly improved survival (Figure 1). In the past, the lack of non-invasive tools to identify high-risk patients hindered the development and application of chemoprevention in cirrhosis. However, with the advent of elastography and the validation of clinical prediction tools that incorporate laboratory markers such as platelet count, clinicians are now able to risk-stratify patients more efficiently, paving the way for more robust medical management. Based on the findings of the ANTICIPATE study and the guidelines proposed in the Baveno VII workshop[121, 122], patients who have a high likelihood for CSPH based on a combination of liver stiffness measurements and platelet count should be initiated on appropriate chemoprevention with a NSBB in the absence of contraindications to therapy. As additional agents for chemoprevention are evaluated over the coming years, a diverse multi-targeted strategy (Figure 2) may become feasible, mimicking the approach currently utilized for congestive heart failure. Although there are a number of candidate drugs, statins, rifaximin, and SGLT2 inhibitors currently offer the most promise, combining potential efficacy with other important considerations such as safety, accessibility, and systemic benefits.

CONCLUSION

The development of hepatic decompensation represents the most important prognostic factor in the natural history of cirrhosis. Treatments that mitigate this risk have an important role in the management of patients with CSPH. In light of the development of robust non-invasive tools that allow for the timely and accurate risk stratification of patients with cirrhosis, the application of chemoprevention is becoming increasingly feasible. NSBBs are currently the mainstays of treatment in this regard, but emerging therapies such as statins, rifaximin, and SGLT2 inhibitors, may offer hope for personalized multimodal strategies in the future. This paradigm shift may ultimately reduce liver-related morbidity and mortality, improve quality of life, and limit the socioeconomic burden of cirrhosis regardless of the etiologic factors involved.

FOOTNOTES

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REFERENCES

1 **GBD 2017 Cirrhosis Collaborators**. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 245-266 [PMID: 31981519 DOI: 10.1016/S2468-1253(19)30349-8]

2 **GBD 2013 Mortality and Causes of Death Collaborators**. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **385**: 117-171 [PMID: 25530442 DOI: 10.1016/S0140-6736(14)61682-2]

3 **D'Amico G**, Pasta L, Morabito A, D'Amico M, Caltagirone M, Malizia G, Tinè F, Giannuoli G, Traina M, Vizzini G, Politi F, Luca A, Virdone R, Licata A, Pagliaro L. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients. *Aliment Pharmacol Ther* 2014; **39**: 1180-1193 [PMID: 24654740 DOI: 10.1111/apt.12721]

4 **Ripoll C**, Bari K, Garcia-Tsao G. Serum Albumin Can Identify Patients With Compensated Cirrhosis With a Good Prognosis. *J Clin Gastroenterol* 2015; **49**: 613-619 [PMID: 25203362 DOI: 10.1097/MCG.0000000000000207]

5 **Fleming KM**, Aithal GP, Card TR, West J. The rate of decompensation and clinical progression of disease in people with cirrhosis: a cohort study. *Aliment Pharmacol Ther* 2010; **32**: 1343-1350 [PMID: 21050236 DOI:

10.1111/j.1365-2036.2010.04473.x]
6 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191 DOI: 10.1002/hep.1840070124]
7 **Neff GW**, Duncan CW, Schiff ER. The current economic burden of cirrhosis. *Gastroenterol Hepatol (N Y)* 2011; **7**: 661-671 [PMID: 22298959]
8 **Heidenreich PA**, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, Deswal A, Drazner MH, Dunlay SM, Evers LR, Fang JC, Fedson SE, Fonarow GC, Hayek SS, Hernandez AF, Khazanie P, Kittleson MM, Lee CS, Link MS, Milano CA, Nnacheta LC, Sandhu AT, Stevenson LW, Vardeny O, Vest AR, Yancy CW. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation* 2022; **145**: e895-e1032 [PMID: 35363499 DOI: 10.1161/CIR.0000000000001063]
9 **Saffo S**, Taddei T. SGLT2 inhibitors and cirrhosis: A unique perspective on the comanagement of diabetes mellitus and ascites. *Clin Liver Dis (Hoboken)* 2018; **11**: 141-144 [PMID: 30992805 DOI: 10.1002/cld.714]
10 **Berzigotti A**. Advances and challenges in cirrhosis and portal hypertension. *BMC Med* 2017; **15**: 200 [PMID: 29121925 DOI: 10.1186/s12916-017-0966-6]
11 **Fried MW**, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**: 1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]
12 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
13 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]
14 **Goldberg D**, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, Charlton M. Changes in the Prevalence of Hepatitis C Virus Infection, Nonalcoholic Steatohepatitis, and Alcoholic Liver Disease Among Patients With Cirrhosis or Liver Failure on the Waitlist for Liver Transplantation. *Gastroenterology* 2017; **152**: 1090-1099.e1 [PMID: 28088461 DOI: 10.1053/j.gastro.2017.01.003]
15 **Fernández Carrillo C**, Lens S, Llop E, Pascasio JM, Crespo J, Arenas J, Fernández I, Baliellas C, Carrión JA, de la Mata M, Buti M, Castells L, Albillos A, Romero M, Turnes J, Pons C, Moreno-Planas JM, Moreno-Palomares JJ, Fernández-Rodriguez C, García-Samaniego J, Prieto M, Fernández Bermejo M, Salmerón J, Badia E, Salcedo M, Herrero JI, Granados R, Blé M, Mariño Z, Calleja JL. Treatment of hepatitis C virus infection in patients with cirrhosis and predictive value of model for end-stage liver disease: Analysis of data from the Hepa-C registry. *Hepatology* 2017; **65**: 1810-1822 [PMID: 28170112 DOI: 10.1002/hep.29097]
16 **Mundi MS**, Velapati S, Patel J, Kellogg TA, Abu Dayyeh BK, Hurt RT. Evolution of NAFLD and Its Management. *Nutr Clin Pract* 2020; **35**: 72-84 [PMID: 31840865 DOI: 10.1002/ncp.10449]
17 **Tapper EB**, Ufere NN, Huang DQ, Loomba R. Review article: current and emerging therapies for the management of cirrhosis and its complications. *Aliment Pharmacol Ther* 2022; **55**: 1099-1115 [PMID: 35235219 DOI: 10.1111/apt.16831]
18 **Estes C**, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018; **67**: 123-133 [PMID: 28802062 DOI: 10.1002/hep.29466]
19 **Ge PS**, Runyon BA. Treatment of Patients with Cirrhosis. *N Engl J Med* 2016; **375**: 767-777 [PMID: 27557303 DOI: 10.1056/NEJMra1504367]
20 **Rehm J**, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. *J Hepatol* 2013; **59**: 160-168 [PMID: 23511777 DOI: 10.1016/j.jhep.2013.03.007]
21 **Leon DA**, McCambridge J. Liver cirrhosis mortality rates in Britain, 1950 to 2002. *Lancet* 2006; **367**: 645 [PMID: 16503452 DOI: 10.1016/s0140-6736(06)68250-0]
22 **Veldt BJ**, Lainé F, Guillygomarc'h A, Lauvin L, Boudjema K, Messner M, Brissot P, Deugnier Y, Moirand R. Indication of liver transplantation in severe alcoholic liver cirrhosis: quantitative evaluation and optimal timing. *J Hepatol* 2002; **36**: 93-98 [PMID: 11804670 DOI: 10.1016/S0168-8278(01)00228-8]
23 **Biggins SW**, Angeli P, Garcia-Tsao G, Ginès P, Ling SC, Nadim MK, Wong F, Kim WR. Diagnosis, Evaluation, and Management of Ascites, Spontaneous Bacterial Peritonitis and Hepatorenal Syndrome: 2021 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2021; **74**: 1014-1048 [PMID: 33942342 DOI: 10.1002/hep.31884]
24 **Garcia-Tsao G**, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. *Hepatology* 2017; **65**: 310-335 [PMID: 27786365 DOI: 10.1002/hep.28906]
25 **Vilstrup H**, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology* 2014; **60**: 715-735 [PMID: 25042402 DOI: 10.1002/hep.27210]
26 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abraldes JG, Nevens F, Vinel JP, Mössner J, Bosch J; Early TIPS (Transjugular Intrahepatic Portosystemic Shunt) Cooperative Study Group. Early use of TIPS in

patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379 [PMID: 20573925 DOI: 10.1056/NEJMoa0910102]

27 **Lv Y**, Yang Z, Liu L, Li K, He C, Wang Z, Bai W, Guo W, Yu T, Yuan X, Zhang H, Xie H, Yao L, Wang J, Li T, Wang Q, Chen H, Wang E, Xia D, Luo B, Li X, Yuan J, Han N, Zhu Y, Niu J, Cai H, Xia J, Yin Z, Wu K, Fan D, Han G; AVB-TIPS Study Group. Early TIPS with covered stents versus standard treatment for acute variceal bleeding in patients with advanced cirrhosis: a randomised controlled trial. *Lancet Gastroenterol Hepatol* 2019; **4**: 587-598 [PMID: 31153882 DOI: 10.1016/S2468-1253(19)30090-1]

28 **Bureau C**, Thabut D, Oberti F, Dharancy S, Carbonell N, Bouvier A, Mathurin P, Otal P, Cabarrou P, Péron JM, Vinel JP. Transjugular Intrahepatic Portosystemic Shunts With Covered Stents Increase Transplant-Free Survival of Patients With Cirrhosis and Recurrent Ascites. *Gastroenterology* 2017; **152**: 157-163 [PMID: 27663604 DOI: 10.1053/j.gastro.2016.09.016]

29 **Kanwal F**, Tansel A, Kramer JR, Feng H, Asch SM, El-Serag HB. Trends in 30-Day and 1-Year Mortality Among Patients Hospitalized With Cirrhosis From 2004 to 2013. *Am J Gastroenterol* 2017; **112**: 1287-1297 [PMID: 28607480 DOI: 10.1038/ajg.2017.175]

30 **de Franchis R**; Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

31 **Bañares R**, Moitinho E, Piqueras B, Casado M, García-Pagán JC, de Diego A, Bosch J. Carvedilol, a new nonselective beta-blocker with intrinsic anti- Alpha1-adrenergic activity, has a greater portal hypotensive effect than propranolol in patients with cirrhosis. *Hepatology* 1999; **30**: 79-83 [PMID: 10385642 DOI: 10.1002/hep.510300124]

32 **Bañares R**, Moitinho E, Matilla A, García-Pagán JC, Lampreave JL, Piera C, Abraldes JG, De Diego A, Albillos A, Bosch J. Randomized comparison of long-term carvedilol and propranolol administration in the treatment of portal hypertension in cirrhosis. *Hepatology* 2002; **36**: 1367-1373 [PMID: 12447861 DOI: 10.1053/jhep.2002.36947]

33 **Reiberger T**, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, Lammert F, Trauner M, Peck-Radosavljevic M, Vogelsang H; Vienna Hepatic Hemodynamic Lab. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J Hepatol* 2013; **58**: 911-921 [PMID: 23262249 DOI: 10.1016/j.jhep.2012.12.011]

34 **Gimenez P**, Garcia-Martinez I, Francés R, Gonzalez-Navajas JM, Mauri M, Alfayate R, Almenara S, Miralles C, Palazon JM, Carnicer F, Pascual S, Such J, Horga JF, Zapater P. Treatment with non-selective beta-blockers affects the systemic inflammatory response to bacterial DNA in patients with cirrhosis. *Liver Int* 2018; **38**: 2219-2227 [PMID: 29802788 DOI: 10.1111/liv.13890]

35 **Merli M**, Lucidi C, Di Gregorio V, Giannelli V, Giusto M, Ceccarelli G, Riggio O, Venditti M. The chronic use of beta-blockers and proton pump inhibitors may affect the rate of bacterial infections in cirrhosis. *Liver Int* 2015; **35**: 362-369 [PMID: 24836902 DOI: 10.1111/liv.12593]

36 **Villanueva C**, Albillos A, Genescà J, Garcia-Pagan JC, Calleja JL, Aracil C, Bañares R, Morillas RM, Poca M, Peñas B, Augustin S, Abraldes JG, Alvarado E, Torres F, Bosch J. β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2019; **393**: 1597-1608 [PMID: 30910320 DOI: 10.1016/S0140-6736(18)31875-0]

37 **Pascal JP**, Cales P. Propranolol in the prevention of first upper gastrointestinal tract hemorrhage in patients with cirrhosis of the liver and esophageal varices. *N Engl J Med* 1987; **317**: 856-861 [PMID: 3306385 DOI: 10.1056/nejm198710013171403]

38 **Idéo G**, Bellati G, Fesce E, Grimoldi D. Nadolol can prevent the first gastrointestinal bleeding in cirrhotics: a prospective, randomized study. *Hepatology* 1988; **8**: 6-9 [PMID: 3276591 DOI: 10.1002/hep.1840080103]

39 **Poynard T**, Calès P, Pasta L, Ideo G, Pascal JP, Pagliaro L, Lebrec D. Beta-adrenergic-antagonist drugs in the prevention of gastrointestinal bleeding in patients with cirrhosis and esophageal varices. An analysis of data and prognostic factors in 589 patients from four randomized clinical trials. Franco-Italian Multicenter Study Group. *N Engl J Med* 1991; **324**: 1532-1538 [PMID: 1674104 DOI: 10.1056/nejm199105303242202]

40 **Groszmann RJ**, Bosch J, Grace ND, Conn HO, Garcia-Tsao G, Navasa M, Alberts J, Rodes J, Fischer R, Bermann M. Hemodynamic events in a prospective randomized trial of propranolol versus placebo in the prevention of a first variceal hemorrhage. *Gastroenterology* 1990; **99**: 1401-1407 [PMID: 2210246 DOI: 10.1016/0016-5085(90)91168-6]

41 **Lebrec D**, Poynard T, Hillon P, Benhamou JP. Propranolol for prevention of recurrent gastrointestinal bleeding in patients with cirrhosis: a controlled study. *N Engl J Med* 1981; **305**: 1371-1374 [PMID: 7029276 DOI: 10.1056/nejm198112033052302]

42 Propranolol prevents first gastrointestinal bleeding in non-ascitic cirrhotic patients. Final report of a multicenter randomized trial. The Italian Multicenter Project for Propranolol in Prevention of Bleeding. *J Hepatol* 1989; **9**: 75-83 [PMID: 2671121 DOI: 10.1016/0168-8278(89)90078-0]

43 **Groszmann RJ**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R; Portal Hypertension Collaborative Group. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; **353**: 2254-2261 [PMID: 16306522 DOI: 10.1056/NEJMoa044456]

44 **Bhardwaj A**, Kedarisetty CK, Vashishtha C, Bhadoria AS, Jindal A, Kumar G, Choudhary A, Shasthry SM, Maiwall R, Kumar M, Bhatia V, Sarin SK. Carvedilol delays the progression of small oesophageal varices in patients with cirrhosis: a randomised placebo-controlled trial. *Gut* 2017; **66**: 1838-1843 [PMID: 27298379 DOI: 10.1136/gutjnl-2016-311735]

45 **Tripathi D**, Ferguson JW, Kochar N, Leithead JA, Therapondos G, McAvoy NC, Stanley AJ, Forrest EH, Hislop WS, Mills PR, Hayes PC. Randomized controlled trial of carvedilol versus variceal band ligation for the prevention of the first variceal bleed. *Hepatology* 2009; **50**: 825-833 [PMID: 19610055 DOI: 10.1002/hep.23045]

46 **Shah HA**, Azam Z, Rauf J, Abid S, Hamid S, Jafri W, Khalid A, Ismail FW, Parkash O, Subhan A, Munir SM. Carvedilol vs. esophageal variceal band ligation in the primary prophylaxis of variceal hemorrhage: a multicentre randomized controlled trial. *J Hepatol* 2014; **60**: 757-764 [PMID: 24291366 DOI: 10.1016/j.jhep.2013.11.019]

47 **Villanueva C**, Torres F, Sarin SK, Shah HA, Tripathi D, Brujats A, Rodrigues SG, Bhardwaj A, Azam Z, Hayes PC,

Jindal A, Abid S, Alvarado E, Bosch J; Carvedilol-IPD-MA-group and the Baveno Cooperation: an EASL Consortium. Carvedilol reduces the risk of decompensation and mortality in patients with compensated cirrhosis in a competing-risk meta-analysis. *J Hepatol* 2022; **77**: 1014-1025 [PMID: 35661713 DOI: 10.1016/j.jhep.2022.05.021]

48 **Sinagra E**, Perricone G, D'Amico M, Tinè F, D'Amico G. Systematic review with meta-analysis: the haemodynamic effects of carvedilol compared with propranolol for portal hypertension in cirrhosis. *Aliment Pharmacol Ther* 2014; **39**: 557-568 [PMID: 24461301 DOI: 10.1111/apt.12634]

49 **Vargas JI**, Arrese M, Shah VH, Arab JP. Use of Statins in Patients with Chronic Liver Disease and Cirrhosis: Current Views and Prospects. *Curr Gastroenterol Rep* 2017; **19**: 43 [PMID: 28752475 DOI: 10.1007/s11894-017-0584-7]

50 **Cabrera L**, Abraldes JG. Statins: the Panacea of Cirrhosis? *Curr Hepatol Rep* 2016; **15**: 1-7 [DOI: 10.1007/s11901-016-0287-9]

51 **Zafra C**, Abraldes JG, Turnes J, Berzigotti A, Fernández M, Garca-Pagán JC, Rodés J, Bosch J. Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis. *Gastroenterology* 2004; **126**: 749-755 [PMID: 14988829 DOI: 10.1053/j.gastro.2003.12.007]

52 **Abraldes JG**, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. *Gastroenterology* 2009; **136**: 1651-1658 [PMID: 19208350 DOI: 10.1053/j.gastro.2009.01.043]

53 **Pollo-Flores P**, Soldan M, Santos UC, Kunz DG, Mattos DE, da Silva AC, Marchiori RC, Rezende GF. Three months of simvastatin therapy vs. placebo for severe portal hypertension in cirrhosis: A randomized controlled trial. *Dig Liver Dis* 2015; **47**: 957-963 [PMID: 26321186 DOI: 10.1016/j.dld.2015.07.156]

54 **Lewis JH**, Mortensen ME, Zweig S, Fusco MJ, Medoff JR, Belder R; Pravastatin in Chronic Liver Disease Study Investigators. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: Results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial. *Hepatology* 2007; **46**: 1453-1463 [PMID: 17668878 DOI: 10.1002/hep.21848]

55 **Chalasani N**, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. *Gastroenterology* 2004; **126**: 1287-1292 [PMID: 15131789 DOI: 10.1053/j.gastro.2004.02.015]

56 **Moctezuma-Velázquez C**, Abraldes JG, Montano-Loza AJ. The Use of Statins in Patients With Chronic Liver Disease and Cirrhosis. *Curr Treat Options Gastroenterol* 2018; **16**: 226-240 [PMID: 29572618 DOI: 10.1007/s11938-018-0180-4]

57 **Kumar S**, Grace ND, Qamar AA. Statin use in patients with cirrhosis: a retrospective cohort study. *Dig Dis Sci* 2014; **59**: 1958-1965 [PMID: 24838495 DOI: 10.1007/s10620-014-3179-2]

58 **Chang FM**, Wang YP, Lang HC, Tsai CF, Hou MC, Lee FY, Lu CL. Statins decrease the risk of decompensation in hepatitis B virus- and hepatitis C virus-related cirrhosis: A population-based study. *Hepatology* 2017; **66**: 896-907 [PMID: 28318053 DOI: 10.1002/hep.29172]

59 **Mohanty A**, Tate JP, Garcia-Tsao G. Statins Are Associated With a Decreased Risk of Decompensation and Death in Veterans With Hepatitis C-Related Compensated Cirrhosis. *Gastroenterology* 2016; **150**: 430-40.e1 [PMID: 26484707 DOI: 10.1053/j.gastro.2015.10.007]

60 **Kamal S**, Khan MA, Seth A, Cholankeril G, Gupta D, Singh U, Kamal F, Howden CW, Stave C, Nair S, Satapathy SK, Ahmed A. Beneficial Effects of Statins on the Rates of Hepatic Fibrosis, Hepatic Decompensation, and Mortality in Chronic Liver Disease: A Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2017; **112**: 1495-1505 [PMID: 28585556 DOI: 10.1038/ajg.2017.170]

61 **Kim RG**, Loomba R, Prokop LJ, Singh S. Statin Use and Risk of Cirrhosis and Related Complications in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 1521-1530.e8 [PMID: 28479502 DOI: 10.1016/j.cgh.2017.04.039]

62 **Kaplan DE**, Mehta R, Garcia-Tsao G, Albrecht J, Aytaman A, Baffy G, Bajaj J, Hernaez R, Hunt K, Ioannou G, Johnson K, Kanwal F, Lee TH, Monto A, Pandya P, Schaubel D, Taddei TH. SACRED: Effect of simvastatin on hepatic decompensation and death in subjects with high-risk compensated cirrhosis: Statins and Cirrhosis: Reducing Events of Decompensation. *Contemp Clin Trials* 2021; **104**: 106367 [PMID: 33771685 DOI: 10.1016/j.cct.2021.106367]

63 **Caraceni P**, Vargas V, Solà E, Alessandria C, de Wit K, Trebicka J, Angeli P, Mookerjee RP, Durand F, Pose E, Krag A, Bajaj JS, Beuers U, Ginès P; Liverhope Consortium. The Use of Rifaximin in Patients With Cirrhosis. *Hepatology* 2021; **74**: 1660-1673 [PMID: 33421158 DOI: 10.1002/hep.31708]

64 **Bajaj JS**. Review article: potential mechanisms of action of rifaximin in the management of hepatic encephalopathy and other complications of cirrhosis. *Aliment Pharmacol Ther* 2016; **43** Suppl 1: 11-26 [PMID: 26618922 DOI: 10.1111/apt.13435]

65 **Baik SK**, Lim YL, Cho YZ, Kim MY, Jang YO, Suk KT, Cheon GJ, Kim YD, Choi DH. G03: Rifaximin and propranolol combination therapy is more effective than propranolol monotherapy in the hepatic venous pressure gradient response and propranolol dose reduction – A pilot study. *J Hepatol* 2015; **62**: S188 [DOI: 10.1016/S0168-8278(15)30004-0]

66 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]

67 **Flamm SL**, Mullen KD, Heimanson Z, Sanyal AJ. Rifaximin has the potential to prevent complications of cirrhosis. *Therap Adv Gastroenterol* 2018; **11**: 1756284818800307 [PMID: 30283499 DOI: 10.1177/1756284818800307]

68 **Zeng X**, Sheng X, Wang PQ, Xin HG, Guo YB, Lin Y, Zhong JW, He CZ, Yin J, Liu TT, Ma WJ, Xiao X, Shi PM, Yuan ZL, Yang L, Ma X, Xu JM, Shen XZ, Yang CQ, Zhu X, Lv NH, Xie WF. Low-dose rifaximin prevents complications and improves survival in patients with decompensated liver cirrhosis. *Hepatol Int* 2021; **15**: 155-165 [PMID: 33385299 DOI: 10.1007/s12072-020-10117-y]

69 **Leonardi F**, Maria N, Villa E. Anticoagulation in cirrhosis: a new paradigm? *Clin Mol Hepatol* 2017; **23**: 13-21 [PMID: 28288507 DOI: 10.3350/cmh.2016.0110]

70 **Bitto N**, Liguori E, La Mura V. Coagulation, Microenvironment and Liver Fibrosis. *Cells* 2018; **7** [PMID: 30042349 DOI: 10.3390/cells7080085]

71 **Senzolo M**, M Sartori T, Rossetto V, Burra P, Cillo U, Boccagni P, Gasparini D, Miotto D, Simioni P, Tsochatzis E, A Burroughs K. Prospective evaluation of anticoagulation and transjugular intrahepatic portosystemic shunt for the

management of portal vein thrombosis in cirrhosis. *Liver Int* 2012; **32**: 919-927 [PMID: 22435854 DOI: 10.1111/j.1478-3231.2012.02785.x]

72 **Chen H**, Liu L, Qi X, He C, Wu F, Fan D, Han G. Efficacy and safety of anticoagulation in more advanced portal vein thrombosis in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* 2016; **28**: 82-89 [PMID: 26513611 DOI: 10.1097/MEG.0000000000000482]

73 **Delgado MG**, Seijo S, Yepes I, Achécar L, Catalina MV, García-Criado A, Abraldes JG, de la Peña J, Bañares R, Albillos A, Bosch J, García-Pagán JC. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. *Clin Gastroenterol Hepatol* 2012; **10**: 776-783 [PMID: 22289875 DOI: 10.1016/j.cgh.2012.01.012]

74 **Amitrano L**, Guardascione MA, Menchise A, Martino R, Scaglione M, Giovine S, Romano L, Balzano A. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. *J Clin Gastroenterol* 2010; **44**: 448-451 [PMID: 19730112 DOI: 10.1097/MCG.0b013e3181b3ab44]

75 **Villa E**, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012; **143**: 1253-1260.e4 [PMID: 22819864 DOI: 10.1053/j.gastro.2012.07.018]

76 **Ruiz-del-Arbol L**, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218 [PMID: 14578859 DOI: 10.1053/jhep.2003.50447]

77 **Osawa L**, Nakanishi H, Kurosaki M, Kirino S, Inada K, Yamashita K, Hayakawa Y, Sekiguchi S, Wang W, Okada M, Higuchi M, Komiyama Y, Takaura K, Takada H, Kaneko S, Maeyashiki C, Tamaki N, Yasui Y, Tsuchiya K, Itakura J, Takahashi Y, Enomoto N, Izumi N. Plasma Renin Activity Predicts Prognosis and Liver Disease-Related Events in Liver Cirrhosis Patients with Ascites Treated by Tolvaptan. *Dig Dis* 2022; **40**: 479-488 [PMID: 34348262 DOI: 10.1159/000518099]

78 **González-Abraldes J**, Albillos A, Bañares R, Del Arbol LR, Moitinho E, Rodríguez C, González M, Escorsell A, García-Pagán JC, Bosch J. Randomized comparison of long-term losartan *vs* propranolol in lowering portal pressure in cirrhosis. *Gastroenterology* 2001; **121**: 382-388 [PMID: 11487547 DOI: 10.1053/gast.2001.26288]

79 **Schepke M**, Werner E, Biecker E, Schiedermaier P, Heller J, Neef M, Stoffel-Wagner B, Hofer U, Caselmann WH, Sauerbruch T. Hemodynamic effects of the angiotensin II receptor antagonist irbesartan in patients with cirrhosis and portal hypertension. *Gastroenterology* 2001; **121**: 389-395 [PMID: 11487548 DOI: 10.1053/gast.2001.26295]

80 **Castaño G**, Viudez P, Riccitelli M, Sookoian S. A randomized study of losartan *vs* propranolol: Effects on hepatic and systemic hemodynamics in cirrhotic patients. *Ann Hepatol* 2003; **2**: 36-40 [PMID: 15094704 DOI: 10.1016/S1665-2681(19)32156-8]

81 **De BK**, Bandyopadhyay K, Das TK, Das D, Biswas PK, Majumdar D, Mandal SK, Ray S, Dasgupta S. Portal pressure response to losartan compared with propranolol in patients with cirrhosis. *Am J Gastroenterol* 2003; **98**: 1371-1376 [PMID: 12818283 DOI: 10.1111/j.1572-0241.2003.07497.x]

82 **Debernardi-Venon W**, Martini S, Biasi F, Vizio B, Termine A, Poli G, Brunello F, Alessandria C, Bonardi R, Saracco G, Rizzetto M, Marzano A. AT1 receptor antagonist Candesartan in selected cirrhotic patients: effect on portal pressure and liver fibrosis markers. *J Hepatol* 2007; **46**: 1026-1033 [PMID: 17336417 DOI: 10.1016/j.jhep.2007.01.017]

83 **Hidaka H**, Kokubu S, Nakazawa T, Okuwaki Y, Ono K, Watanabe M, Shibuya A, Saigenji K. New angiotensin II type 1 receptor blocker olmesartan improves portal hypertension in patients with cirrhosis. *Hepatol Res* 2007; **37**: 1011-1017 [PMID: 17608670 DOI: 10.1111/j.1872-034X.2007.00165.x]

84 **Hidaka H**, Nakazawa T, Shibuya A, Minamino T, Takada J, Tanaka Y, Okuwaki Y, Watanabe M, Koizumi W. Effects of 1-year administration of olmesartan on portal pressure and TGF-beta1 in selected patients with cirrhosis: a randomized controlled trial. *J Gastroenterol* 2011; **46**: 1316-1323 [PMID: 21850387 DOI: 10.1007/s00535-011-0449-z]

85 **Vlachogiannakos J**, Tang AK, Patch D, Burroughs AK. Angiotensin converting enzyme inhibitors and angiotensin II antagonists as therapy in chronic liver disease. *Gut* 2001; **49**: 303-308 [PMID: 11454810 DOI: 10.1136/gut.49.2.303]

86 **Zhang X**, Wong GL, Yip TC, Tse YK, Liang LY, Hui VW, Lin H, Li GL, Lai JC, Chan HL, Wong VW. Angiotensin-converting enzyme inhibitors prevent liver-related events in nonalcoholic fatty liver disease. *Hepatology* 2022; **76**: 469-482 [PMID: 34939204 DOI: 10.1002/hep.32294]

87 **Abecasis R**, Kravetz D, Fassio E, Ameigeiras B, Garcia D, Isla R, Landeira G, Dominguez N, Romero G, Argonz J, Terg R. Nadolol plus spironolactone in the prophylaxis of first variceal bleed in nonascitic cirrhotic patients: A preliminary study. *Hepatology* 2003; **37**: 359-365 [PMID: 12540786 DOI: 10.1053/jhep.2003.50032]

88 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]

89 **Wanner C**, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, Johansen OE, Woerle HJ, Broedl UC, Zinman B; EMPA-REG OUTCOME Investigators. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. *N Engl J Med* 2016; **375**: 323-334 [PMID: 27299675 DOI: 10.1056/NEJMoa1515920]

90 **McMurray JJV**, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Ponikowski P, Sabatine MS, Anand IS, Bělohlávek J, Böhm M, Chiang CE, Chopra VK, de Boer RA, Desai AS, Diez M, Drozdz J, Dukát A, Ge J, Howlett JG, Katova T, Kitakaze M, Ljungman CEA, Merkely B, Nicolau JC, O'Meara E, Petrie MC, Vinh PN, Schou M, Tereshchenko S, Verma S, Held C, DeMets DL, Docherty KF, Jhund PS, Bengtsson O, Sjöstrand M, Langkilde AM; DAPA-HF Trial Committees and Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med* 2019; **381**: 1995-2008 [PMID: 31535829 DOI: 10.1056/NEJMoa1911303]

91 **Heerspink HJL**, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, Mann JFE, McMurray JJV, Lindberg M, Rossing P, Sjöström CD, Toto RD, Langkilde AM, Wheeler DC; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. *N Engl J Med* 2020; **383**: 1436-1446 [PMID: 32970396 DOI: 10.1056/NEJMoa2024816]

92 **Neal B**, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]

93 **Perkovic V**, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, Edwards R, Agarwal R, Bakris G, Bull S, Cannon CP, Capuano G, Chu PL, de Zeeuw D, Greene T, Levin A, Pollock C, Wheeler DC, Yavin Y, Zhang H, Zinman B, Meininger G, Brenner BM, Mahaffey KW; CREDENCE Trial Investigators. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* 2019; **380**: 2295-2306 [PMID: 30990260 DOI: 10.1056/NEJMoa1811744]

94 **Montalvo-Gordon I**, Chi-Cervera LA, García-Tsao G. Sodium-Glucose Cotransporter 2 Inhibitors Ameliorate Ascites and Peripheral Edema in Patients With Cirrhosis and Diabetes. *Hepatology* 2020; **72**: 1880-1882 [PMID: 32294260 DOI: 10.1002/hep.31270]

95 **Kalambokis GN**, Tsiakas I, Filippas-Ntekuan S, Christaki M, Despotis G, Milionis H. Empagliflozin Eliminates Refractory Ascites and Hepatic Hydrothorax in a Patient With Primary Biliary Cirrhosis. *Am J Gastroenterol* 2021; **116**: 618-619 [PMID: 33027081 DOI: 10.14309/ajg.0000000000000995]

96 **Miyamoto Y**, Honda A, Yokose S, Nagata M, Miyamoto J. Weaning from concentrated ascites reinfusion therapy for refractory ascites by SGLT2 inhibitor. *Clin Kidney J* 2022; **15**: 831-833 [PMID: 35371450 DOI: 10.1093/ckj/sfab266]

97 **Saffo S**, Garcia-Tsao G, Taddei T. SGLT2 inhibitors in patients with cirrhosis and diabetes mellitus: A tertiary center cohort study and insights about a potential therapeutic target for portal hypertension. *J Diabetes* 2021; **13**: 265-269 [PMID: 33210815 DOI: 10.1111/1753-0407.13136]

98 **Saffo S**, Kaplan DE, Mahmud N, Serper M, John BV, Ross JS, Taddei T. Impact of SGLT2 inhibitors in comparison with DPP4 inhibitors on ascites and death in veterans with cirrhosis on metformin. *Diabetes Obes Metab* 2021; **23**: 2402-2408 [PMID: 34227216 DOI: 10.1111/dom.14488]

99 **Caraceni P**, Riggio O, Angeli P, Alessandria C, Neri S, Foschi FG, Levantesi F, Airoldi A, Boccia S, Svegliati-Baroni G, Fagiuoli S, Romanelli RG, Cozzolongo R, Di Marco V, Sangiovanni V, Morisco F, Toniutto P, Tortora A, De Marco R, Angelico M, Cacciola I, Elia G, Federico A, Massironi S, Guarisco R, Galioto A, Ballardini G, Rendina M, Nardelli S, Piano S, Elia C, Prestianni L, Cappa FM, Cesarini L, Simone L, Pasquale C, Cavallin M, Andrealli A, Fidone F, Ruggeri M, Roncadori A, Baldassarre M, Tufoni M, Zaccherini G, Bernardi M; ANSWER Study Investigators. Long-term albumin administration in decompensated cirrhosis (ANSWER): an open-label randomised trial. *Lancet* 2018; **391**: 2417-2429 [PMID: 29861076 DOI: 10.1016/S0140-6736(18)30840-7]

100 **García-Pagán JC**, Feu F, Bosch J, Rodés J. Propranolol compared with propranolol plus isosorbide-5-mononitrate for portal hypertension in cirrhosis. A randomized controlled study. *Ann Intern Med* 1991; **114**: 869-873 [PMID: 2014947 DOI: 10.7326/0003-4819-114-10-869]

101 **García-Pagán JC**, Morillas R, Bañares R, Albillos A, Villanueva C, Vila C, Genescà J, Jimenez M, Rodriguez M, Calleja JL, Balanzó J, García-Durán F, Planas R, Bosch J; Spanish Variceal Bleeding Study Group. Propranolol plus placebo versus propranolol plus isosorbide-5-mononitrate in the prevention of a first variceal bleed: a double-blind RCT. *Hepatology* 2003; **37**: 1260-1266 [PMID: 12774003 DOI: 10.1053/jhep.2003.50211]

102 **García-Pagán JC**, Villanueva C, Vila MC, Albillos A, Genescà J, Ruiz-Del-Arbol L, Planas R, Rodriguez M, Calleja JL, González A, Solà R, Balanzó J, Bosch J; MOVE Group. Mononitrato Varices Esofágicas. Isosorbide mononitrate in the prevention of first variceal bleed in patients who cannot receive beta-blockers. *Gastroenterology* 2001; **121**: 908-914 [PMID: 11606504 DOI: 10.1053/gast.2001.27993]

103 **Borroni G**, Salerno F, Cazzaniga M, Bissoli F, Lorenzano E, Maggi A, Visentin S, Panzeri A, de Franchis R. Nadolol is superior to isosorbide mononitrate for the prevention of the first variceal bleeding in cirrhotic patients with ascites. *J Hepatol* 2002; **37**: 315-321 [PMID: 12175626 DOI: 10.1016/s0168-8278(02)00174-5]

104 **Tripathi D**, Therapondos G, Ferguson JW, Newby DE, Webb DJ, Hayes PC. Endothelin-1 contributes to maintenance of systemic but not portal haemodynamics in patients with early cirrhosis: a randomised controlled trial. *Gut* 2006; **55**: 1290-1295 [PMID: 16434427 DOI: 10.1136/gut.2005.077453]

105 **Zipprichi A**, Schenkel E, Gittinger F, Winkler M, Michl P, Ripoll C. Selective endothelin-a blockade decreases portal pressure in patients with cirrhosis. A pilot study combining a local intraarterial and systemic administration. *J Hepatol* 2016; **64**: S247 [DOI: 10.1016/S0168-8278(16)00258-0]

106 **Mookerjee R**, Rosselli M, Pieri G, Beecher-Jones T, Hooshmand-Rad R, Chouhan M, Mehta G, Jalan R, Shapiro D. Effects of the FXR agonist obeticholic acid on hepatic venous pressure gradient (HVPG) in alcoholic cirrhosis: a proof of concept phase 2a study. *J Hepatol* 2014; **60**: S7-S8 [DOI: 10.1016/S0168-8278(14)60017-9]

107 **Deibert P**, Schumacher YO, Ruecker G, Opitz OG, Blum HE, Rössle M, Kreisel W. Effect of vardenafil, an inhibitor of phosphodiesterase-5, on portal haemodynamics in normal and cirrhotic liver -- results of a pilot study. *Aliment Pharmacol Ther* 2006; **23**: 121-128 [PMID: 16393289 DOI: 10.1111/j.1365-2036.2006.02735.x]

108 **Clemmesen JO**, Giraldi A, Ott P, Dalhoff K, Hansen BA, Larsen FS. Sildenafil does not influence hepatic venous pressure gradient in patients with cirrhosis. *World J Gastroenterol* 2008; **14**: 6208-6212 [PMID: 18985812 DOI: 10.3748/wjg.14.6208]

109 **Lee KC**, Yang YY, Wang YW, Hou MC, Lee FY, Lin HC, Lee SD. Acute administration of sildenafil enhances hepatic cyclic guanosine monophosphate production and reduces hepatic sinusoid resistance in cirrhotic patients. *Hepatol Res* 2008; **38**: 1186-1193 [PMID: 18631254 DOI: 10.1111/j.1872-034X.2008.00388.x]

110 **Tandon P**, Inayat I, Tal M, Spector M, Shea M, Groszmann RJ, Garcia-Tsao G. Sildenafil has no effect on portal pressure but lowers arterial pressure in patients with compensated cirrhosis. *Clin Gastroenterol Hepatol* 2010; **8**: 546-549 [PMID: 20144739 DOI: 10.1016/j.cgh.2010.01.017]

111 **Kreisel W**, Deibert P, Kupcinskas L, Sumskiene J, Appenrodt B, Roth S, Neagu M, Rössle M, Zipprich A, Caca K, Ferlitsch A, Dilger K, Mohrbacher R, Greinwald R, Sauerbruch T. The phosphodiesterase-5-inhibitor udenafil lowers portal pressure in compensated preascitic liver cirrhosis. A dose-finding phase-II-study. *Dig Liver Dis* 2015; **47**: 144-150 [PMID: 25483910 DOI: 10.1016/j.dld.2014.10.018]

112 **Snowdon VK**, Lachlan NJ, Hoy AM, Hadoke PW, Semple SI, Patel D, Mungall W, Kendall TJ, Thomson A, Lennen RJ,

Jansen MA, Moran CM, Pellicoro A, Ramachandran P, Shaw I, Aucott RL, Severin T, Saini R, Pak J, Yates D, Dongre N, Duffield JS, Webb DJ, Iredale JP, Hayes PC, Fallowfield JA. Serelaxin as a potential treatment for renal dysfunction in cirrhosis: Preclinical evaluation and results of a randomized phase 2 trial. *PLoS Med* 2017; **14**: e1002248 [PMID: 28245243 DOI: 10.1371/journal.pmed.1002248]

113 **Pinter M**, Sieghart W, Reiberger T, Rohr-Udilova N, Ferlitsch A, Peck-Radosavljevic M. The effects of sorafenib on the portal hypertensive syndrome in patients with liver cirrhosis and hepatocellular carcinoma--a pilot study. *Aliment Pharmacol Ther* 2012; **35**: 83-91 [PMID: 22032637 DOI: 10.1111/j.1365-2036.2011.04896.x]

114 **Coriat R**, Gouya H, Mir O, Ropert S, Vignaux O, Chaussade S, Sogni P, Pol S, Blanchet B, Legmann P, Goldwasser F. Reversible decrease of portal venous flow in cirrhotic patients: a positive side effect of sorafenib. *PLoS One* 2011; **6**: e16978 [PMID: 21340026 DOI: 10.1371/journal.pone.0016978]

115 **Garcia-Tsao G**, Fallon MB, Reddy KR, Loo N, Bari K, Augustin S, Ciarleglio M, Deng Y, Taddei TH, Strazzabosco M. Placebo-controlled, randomized, pilot study of the effect of sorafenib on portal pressure in patients with cirrhosis, portal hypertension and ablated hepatocellular carcinoma (HCC). *Hepatology* 2015; **62**: 580A-581A

116 **Schwarzer R**, Kivaranovic D, Mandorfer M, Paternostro R, Wolrab D, Heinisch B, Reiberger T, Ferlitsch M, Gerner C, Trauner M, Peck-Radosavljevic M, Ferlitsch A. Randomised clinical study: the effects of oral taurine 6g/day *vs* placebo on portal hypertension. *Aliment Pharmacol Ther* 2018; **47**: 86-94 [PMID: 29105115 DOI: 10.1111/apt.14377]

117 **Austin AS**, Mahida YR, Clarke D, Ryder SD, Freeman JG. A pilot study to investigate the use of oxpentifylline (pentoxifylline) and thalidomide in portal hypertension secondary to alcoholic cirrhosis. *Aliment Pharmacol Ther* 2004; **19**: 79-88 [PMID: 14687169 DOI: 10.1046/j.1365-2036.2003.01809.x]

118 **Zhou X**, Zhang T, Sun Y, Li C, Ding X, Zhu Y, Li L, Fan Z. Systematic Review and Meta-analysis: Association of Aspirin With Incidence of Hepatocellular Carcinoma. *Front Pharmacol* 2022; **13**: 764854 [PMID: 35300300 DOI: 10.3389/fphar.2022.764854]

119 **Memel ZN**, Arvind A, Moninuola O, Philpotts L, Chung RT, Corey KE, Simon TG. Aspirin Use Is Associated with a Reduced Incidence of Hepatocellular Carcinoma: A Systematic Review and Meta-analysis. *Hepatol Commun* 2021; **5**: 133-143 [PMID: 33437907 DOI: 10.1002/hep4.1640]

120 **Kaplan DE**, Serper M, John BV, Tessiatore KM, Lerer R, Mehta R, Fox R, Aytaman A, Baytarian M, Hunt K, Albrecht J, Taddei TH; Veterans Outcomes and Cost Associated with Liver disease Study Group. Effects of Metformin Exposure on Survival in a Large National Cohort of Patients With Diabetes and Cirrhosis. *Clin Gastroenterol Hepatol* 2021; **19**: 2148-2160.e14 [PMID: 32798709 DOI: 10.1016/j.cgh.2020.08.026]

121 **Abraldes JG**, Bureau C, Stefanescu H, Augustin S, Ney M, Blasco H, Procopet B, Bosch J, Genesca J, Berzigotti A; Anticipate Investigators. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: The "Anticipate" study. *Hepatology* 2016; **64**: 2173-2184 [PMID: 27639071 DOI: 10.1002/hep.28824]

122 **de Franchis R**, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII - Renewing consensus in portal hypertension. *J Hepatol* 2022; **76**: 959-974 [PMID: 35120736 DOI: 10.1016/j.jhep.2021.12.022]

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REVIEW

Emerging novel targets for nonalcoholic fatty liver disease treatment: Evidence from recent basic studies

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Abstract

Nonalcoholic fatty liver disease (NAFLD), a leading chronic disease worldwide, affects approximately a quarter of the global population. Nonalcoholic steatohepatitis (NASH) is an advanced form of NAFLD and is more likely to progress to liver fibrosis than simple steatosis. NASH is also identified as the most rapidly growing cause of hepatocellular carcinoma. Although in the past decade, several phase II/III clinical trials have shown promising results in the use of novel drugs targeting lipid synthase, farnesoid X receptor signaling, peroxisome proliferator-activated receptor signaling, hepatocellular injury, and inflammatory signaling, proven pharmaceutical agents to treat NASH are still lacking. Thus, continuous exploration of the mechanism underlying the pathogenesis of NAFLD and the identification of novel therapeutic targets remain urgent tasks in the field. In the current review, we summarize studies reported in recent years that not only

provide new insights into the mechanisms of NAFLD development but also explore the possibility of treating NAFLD by targeting newly identified signaling pathways. We also discuss evidence focusing on the intrahepatic targets involved in the pathogenesis of NAFLD as well as extrahepatic targets affecting liver metabolism and function.

Key Words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Pharmaceutical strategies; Liver microenvironment; Gut-liver axis; Adipose tissue-liver axis

Core Tip: Because of the urgent need to develop therapeutic approaches to treat nonalcoholic fatty liver disease (NAFLD), a large body of basic research has focused on the mechanisms of NAFLD to explore the possibility of new approaches to treat the disease. The current review summarizes studies reported in recent years that not only provide new insights into the mechanisms of NAFLD development but also explore the possibility of treating NAFLD by targeting newly identified signaling pathways. Evidence focusing on the intrahepatic targets involved in the pathogenesis of NAFLD as well as extrahepatic targets affecting liver metabolism and function are discussed.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become a leading chronic disease worldwide, affecting approximately a quarter of the global population. Nonalcoholic steatohepatitis (NASH), the advanced form of NAFLD, is closely related to liver fibrosis and even cirrhosis[1]. NASH has also been identified as the most rapidly growing cause of hepatocellular carcinoma (HCC) in liver transplant candidates in the United States[2]. Multiple factors, including disturbed lipid homeostasis, insulin resistance, and inflammation, lead to metabolic stress in hepatocytes and subsequent hepatocyte injury in NAFLD. Hepatocyte injury further triggers the wound-healing response, which involves immune cells, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs) and cholangiocytes, eventually leading to liver inflammation and fibrosis[3].

Despite the increasing prevalence of NASH, the United States Food and Drug Administration (FDA) has not yet approved any pharmaceuticals to treat patients with NASH. The mainstay of the current clinical recommendation for patients with NASH is lifestyle modification, with optimization of dietary structure, control of excessive consumption of fructose and fats and increased physical exercise, which may alleviate the progression of NASH to a certain extent[4]. In addition, bariatric surgery may be indicated for some patients with NASH suffering from severe obesity[5]. Regarding the pharmacotherapy for NASH, several biological pathways critical for glycolipid and bile acid metabolism, inflammation, hepatocellular damage (oxidative stress) and liver fibrosis have been explored as drug targets. Pharmaceutical agents, including modulators of farnesoid X receptor, peroxisome proliferator-activated receptors (PPARs), fibroblast growth factor, acetyl-CoA carboxylase (ACC), and apoptosis signal-regulating kinase 1 (ASK1), have been shown to exhibit some positive effects against NAFLD/NASH with various limitations in multiple clinical trials[6]. Thus, there is still an unmet clinical need to identify and validate novel targets for the treatment of NAFLD/NASH.

Recently, numerous novel therapeutic targets for NAFLD have been explored through basic research. These studies have focused on different pathophysiologic processes in NAFLD, including metabolic stress, liver inflammation, liver fibrosis and NASH-associated HCC. Diverse liver cells have been studied, such as hepatocytes, liver immune cells, HSCs and LSECs (Figure 1). As a metabolic disorder, crosstalk between the liver and extrahepatic organs, including the gut, adipose tissue, and skeletal muscle, largely contributes to the pathogenesis of NAFLD[7,8]. Thus, targets outside the liver broaden potential approaches to ameliorate NAFLD (Figure 1). The drugs in phase II/III clinical trials were extensively discussed in a recently published review[6]. In the present review, we summarize recent findings that not only provide new insights into the mechanisms of NAFLD development but also explore the possibility of treating NAFLD by targeting novel signaling pathways.

APPROACHES TARGETING METABOLIC STRESS TO PREVENT NAFLD PROGRESSION

Lipid accumulation in hepatocytes is a major feature of NAFLD. Disturbance of lipid and glucose metabolism causes metabolic stress in the liver. Therapeutic candidates directly regulating fatty acid metabolism that are currently in clinical trials include ACC, fatty acid synthase and stearoyl-CoA desaturase-1 inhibitors[6,9]. In addition to the targets acting on these lipid metabolic enzymes, in-depth basic research has uncovered multiple mechanisms underlying the metabolic disturbance during NAFLD, including but not limited to lipid, glucose, lactate and fructose metabolism, and has provided novel targets for NAFLD treatment.

Sterol regulatory element-binding proteins (SREBPs), including SREBP1a, SREBP1c and SREBP2, are key regulators mediating free fatty acid, triglyceride (TG) and cholesterol synthesis. 25-Hydroxanoanosterol (25-HL) is a newly identified SREBP inhibitor that induces the SCAP-INSIG interaction, which retains SREBPs in the endoplasmic reticulum (ER). 25-HL reduces hepatic TG and cholesterol levels and improves liver inflammation and fibrosis in western diet-fed mice[10]. Orosomucoid (ORM) 2, a secreted protein, has been shown to inhibit SREBP1c by activating AMP-activated protein kinase (AMPK). Recombinant ORM2 protein or stabilized ORM2–FC fusion protein inhibits lipogenesis and improves steatohepatitis[11].

Citrate regulates lipid metabolism as the substrate for lipogenesis. In the cytoplasm, citrate is catabolized by ATP-citrate lyase (ACLY) to generate acetyl-CoA and oxaloacetate. Acetyl-CoA is then converted to malonyl-CoA by ACC to fuel lipogenesis. The mitochondrial citrate carrier Slc25al plays an important role in regulating cytoplasmic and mitochondrial citrate pools. Tan and colleagues revealed that an inhibitor of Slc25a1, CTPI-2, inhibits the lipogenic pathway and prevents hepatic lipid accumulation in high-fat diet (HFD)-fed mice. Additionally, CTPI-2 also showed beneficial effects on liver inflammation[12]. ACLY, the enzyme mediating the production of acetyl-CoA from citrate, has been identified to be upregulated in the transition from simple steatosis to NASH[13]. Hepatocyte-specific deletion of ACLY reduces liver fatty acid and sterol synthesis, increases fatty acid oxidation, and attenuates glucose intolerance, liver steatosis, and ballooning[14]. Consistent with the observations following genetic inhibition, bempedoic acid, a pharmacological inhibitor of ACLY, also reduces fibrosis [14]. During the de novo lipogenesis process, nicotinamide adenine dinucleotide phosphate (NADPH), as an electron donor, aids in the reduction of acetyl-CoA. Thus, fine-tuning the NADPH pool can regulate lipogenesis. One-carbon units come largely from serine catabolism by the enzyme serine hydroxymethyltransferase (SHMT)[15]. Zhang e*t al*[16] demonstrated that SHMT1-driven serine catabolism is a substantial NADPH source in the liver. They further found that inhibition of serine catabolism by Shmt1 gene knockout or pharmacological inhibition of SHMT1/2 enzymes significantly decreased hepatic lipogenesis[16]. Moreover, in sucrose-induced fatty liver, lipogenesis also requires NADPH from serine catabolism, which can be inhibited by the SHMT1/2 inhibitor SHIN2 IV. Therefore, SHMT1/2 inhibition has the potential to treat NAFLD by decreasing de novo lipogenesis.

As an energy source and an important glycolysis product, lactate is increased in the plasma and liver of NAFLD individuals. A recent study showed that the acetylation of lactate dehydrogenase B (LDHB) is markedly increased in the livers of mice with NAFLD, which leads to decreased LDHB activity and increased hepatic lactate levels. The authors further found that P300/CBP-associated factor (PCAF)-mediated LDHB acetylation at K82 exacerbates lipid accumulation and inflammatory responses in HFD-fed mice. Consistently, embelin, an inhibitor of PCAF, significantly improves hepatic steatosis and inflammation in NASH mice[17]. In addition, increasing evidence has demonstrated that overconsumption of fructose is associated with NAFLD. Fructose is metabolized to fructose-1-phosphate by ketohexokinase. PF-06835919 is a ketohexokinase inhibitor that showed protective effects against fructose-induced liver steatosis. Moreover, the safety of PF-06835919 has been verified in a phase I clinical trial[18].

Mitochondrial and ER functions are crucial in lipid and glucose metabolism, and mitochondrial dysfunction and ER stress are closely related to NAFLD[19]. Methylation-controlled J (MCJ) protein is located at the inner mitochondrial membrane and restrains mitochondrial respiration. Its expression level is found to be increased in NAFLD patients. MCJ deficiency reduces hepatic steatosis and improves liver fibrosis in methionine- and choline-deficient (MCD) diet-fed mice. In a recent study, lipid-nanoparticle-encapsulated (LNP) siRNA was employed to target MCJ *in vivo*. LNP-siMCJ increased the β-oxidation of fatty acids and ameliorated lipid accumulation and liver fibrosis in MCD diet- or high-fat/high-fructose diet-induced NASH mice. To further specifically target MCJ in hepatocytes, N-acetylgalactosamine (GalNAc)-modified siMCJ was used to treat NASH mice. Consistent with LNP-siMCJ, GalNAc-siMCJ also reduced liver steatosis and fibrosis[20]. In addition, low-dose sorafenib, the first small-molecule multi-kinase inhibitor, was reported to induce mitochondrial uncoupling and subsequently suppress free fatty acid-induced lipid accumulation and inflammation by activating AMPK in hepatocytes. Low-dose sorafenib protects high-fat/high-cholesterol diet-fed mice against liver steatosis, inflammation and fibrosis and prevents the onset of NASH-associated HCC in mice. Notably, the beneficial effects of low-dose sorafenib were also confirmed in NASH monkeys[21]. Moreover, cyclophilin D is located in the mitochondrial matrix and mediates mitochondrial permeability transition pore opening. Cyclophilin D is upregulated in HFD-fed mice, which induces mitochondrial stress and hepatic steatosis. Cyclosporine A, an inhibitor of

cyclophilin D, decreased liver TG levels in HFD-fed mice by decreasing SREBP1c expression[22]. ER stress also plays important roles in NAFLD. The expression of forkhead box A3 (FOXA3) is induced by ER stress, and FOXA3 mediates ER stress-induced liver steatosis. As expected, targeting FOXA3 *via* a siRNA-based approach attenuates liver steatosis in HFD-fed mice[23].

It has been previously reported that the expression of DEAD-box protein 5 (DDX5), an ATP-dependent RNA helicase, is reduced in the livers of patients and mouse models with NASH, as well as in rodent models with NASH-HCC[24]. Hepatic DDX5 improves lipid metabolism by inhibiting mammalian target of rapamycin complex 1 (mTORC1) activation *via* recruitment of the tuberous sclerosis complex (TSC)1/2 complex to mTOR[24]. Through screening a natural compound library, hyperforcinol K was found to upregulate DDX5 expression by blocking tripartite motif protein 5-mediated ubiquitinated degradation of DDX5. In an animal study, hyperforcinol K was found to be effective in attenuating lipid accumulation in the livers of NASH mice[24].

Sterile 20-type kinase serine/threonine kinase 25 (STK25) has been demonstrated to play important roles in liver lipid partitioning[25,26] and systemic glucose and insulin homeostasis[27]. The level of STK25 protein is positively correlated with the development of NASH in patients[28]. Moreover, STK25 deficiency can protect against liver steatosis, inflammation, and fibrosis in mouse models of NASH-HCC[29]. STK25 antisense oligonucleotides (ASOs) significantly improved the NASH phenotype, and this preclinical validation of the effective metabolic efficacy of pharmacological inhibition of STK25 merits recognition[28].

Many nuclear receptors play critical roles in metabolic regulation. In current phase II and III trials, PPARs, farnesoid X receptor, and thyroid hormone receptor-β are promising candidate targets for NASH treatment[6]. Based on recent basic studies, modulation of retinoic acid receptor-related orphan receptor alpha (RORa) provides a novel target to treat NASH. RS-2982, a newly identified agonist of RORa, exhibited beneficial effects on reducing body weight and hepatic steatosis in HFD-fed mice. Furthermore, RS-2982 decreased alanine aminotransferase (ALT) and aspartate aminotransferase levels and reduced liver inflammation and fibrosis in mice fed an atherogenic diet. Mechanistically, an increase in miR-122 expression may account for these beneficial effects of RS-2982 on obesity and NAFLD/NASH[30].

Arachidonic acid (ARA) is an ω-6 long-chain polyunsaturated fatty acid (PUFA) that can be catalyzed into a series of bioactive eicosanoids *via* cycloxygenases (COX-1 and COX-2) and microsomal prostaglandin E synthases (mPGES-1 and mPGES-2). It has been recently reported that mPGES-2 deficiency exhibits significant protective effects against diet-induced NASH-associated phenotypes, including hepatic steatosis, inflammation and fibrosis. However, the beneficial effect of mPGES-2 deficiency against NAFLD is dependent on decreased cytochrome P450 4A14 (CYP4A14) and increased acyl-CoA thioesterase 4 levels but not PGE2[31]. Mechanistically, mPGES-2 binds with heme, which is released after mPGES2 inhibition and in turn activates the heme receptor nuclear receptor subfamily 1 group D member 1 (NR1D1) to upregulate CYP4A14 and acyl-CoA thioesterase 4 expression[31]. CYP4A14 catalyzes omega-hydroxylation of medium-chain fatty acids and ARA in mice. Its expression was previously found to be significantly increased in the livers of patients and mice with NAFLD. Loss of CYP4A14 function also markedly attenuated liver damage, inflammation, and fibrosis in MCD diet-induced NASH[32]. Moreover, both the mPGES-2 inhibitor SZ0232 and the CYP4A inhibitor TS-011 are capable of ameliorating the NASH phenotype in MCD diet-fed mice[31-33]. Taken together, these findings demonstrate that ARA metabolic enzymes play critical roles in liver lipid homeostasis and represent attractive targets for developing therapeutic drugs for NAFLD/NASH.

Emerging evidence suggests that novel therapeutic targets can be developed based on the discovery of genetic variants related to liver lipid metabolism, such as *PNPLA3*, *TM6SF2*, *MBOAT7* and *HSD17B13* [34-37]. It has been reported that phosphorylation of HSD17B13 at serine 33 by PKA promotes lipolysis. Reproterol, a β2 agonist used for treating asthma, protects against the NASH phenotype *via* PKA-mediated Ser33 phosphorylation of 17β-HSD13[38]. Most recently, a variant in the *pleckstrin and Sec7 domain-containing 3* (*PSD3*) gene, rs71519934, was reported to reduce susceptibility to fatty liver disease, consistent with the finding that the expression level of *PSD3* is increased in patients with NAFLD and that knockdown of *PSD3* by siRNA decreases TG synthesis in hepatocytes. Furthermore, specific downregulation of *PSD3* in hepatocytes by GalNAc-conjugated ASOs resulted in decreased hepatic lipid content and plasma ALT levels and improved liver fibrosis in NASH mice[39], suggesting that *PSD3* may be a potential therapeutic target for the treatment of NAFLD/NASH.

STRATEGIES PROTECTING HEPATOCYTES FROM INJURY DURING NASH DEVELOPMENT

Lipid accumulation in hepatocytes can cause cytotoxicity (lipotoxicity) in NAFLD[40,41]. Hepatocyte injury is a key event during the development of NASH, and ballooned hepatocytes are an essential feature to diagnose NASH. In phase II/III clinical trials, pancaspase inhibitors and ASK1 inhibitors are currently under evaluation as therapeutic agents directly targeting apoptosis[6]. Studies focused on developing targets to prevent hepatocyte injury have also shown therapeutic effects in NASH.

Mitochondrial stress is a major factor driving hepatocyte injury and promotes the progression from simple steatosis to NASH. Mitochondrial matrix caseinolytic protease P (ClpP) is a protease component of the caseinolytic protease complex that maintains protein homeostasis in mitochondria. Its expression level is frequently downregulated in NASH, which induces mitochondrial stress and inflammation in hepatocytes. The ClpP activator A54556A is capable of greatly ameliorating the NASH phenotype in high-fat/high-fructose diet-fed mice[42]. SH3 homology-associated BTK binding protein (SAB) was originally identified as a phosphorylated c-Jun N-terminal kinase (p-JNK) docking protein and a substrate of JNK in the mitochondrial outer membrane. The interaction of JNK with SAB leads to increased mitochondrial reactive oxygen species (ROS) production and promotes liver injury. In established NASH, hepatocyte-targeted GalNAc-Sab-ASO treatment can reverse steatohepatitis and fibrosis by abrogating the adverse effects of the JNK-SAB-ROS activation loop[43]. Sirtuin activation plays an important role in maintaining mitochondrial homeostasis and shows protective effects on NAFLD[44]. Increased NAD^+ levels can activate sirtuin. The enzyme α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase controls NAD^+ levels, and its inhibitor TES-991 boosts de novo NAD^+ synthesis and protects mice against liver injury and steatosis by improving mitochondrial function[45]. In addition, the serine/threonine protein phosphatase 2A inhibitors LB100[46], allyl isothiocyanate[47], celastrol[48] and the heme oxygenase inducer cobalt protoporphyrin[49] have all been found to significantly ameliorate NASH *via* the Sirt1 pathway. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a well-known important transcription factor involved in the expression of antioxidant genes during oxidative stress. Targeted deletion of Nrf2 results in enhanced susceptibility to a variety of oxidative stress-induced liver injuries, including NASH. In contrast, pharmacological or genetic activation of hepatic Nrf2 leads to hepatic protection against oxidative damage. Studies have shown beneficial effects of hepatocyte Nrf2 activation in NASH[50]. Pharmacological activation of Nrf2 by the acetylenic tricyclic bis (cyano enone) compounds TBE-31[51], NK-252[52], and dimethyl fumarate[53] suppresses NASH. These findings demonstrate that strategies that increase Nrf2 expression and activity may be attractive strategies to limit the development of NAFLD/NASH by attenuating hepatocyte lipotoxicity and injury.

As mentioned above, apoptosis plays an important role in hepatocyte injury or loss during the development of NAFLD/NASH. BCL-2 belongs to the BCL-2 family, which is antiapoptotic. Through screening a small molecule library, acridone derivative A22 was found to increase BCL-2 expression by stabilizing the BCL-2 promoter i-motif. As expected, A22 can attenuate HFD-induced hepatocyte injury, hepatic steatosis and liver fibrosis[54]. In addition, necroptosis, another form of programmed cell death, was found to be increased in NASH mouse models and the livers of NAFLD patients. Receptor-interacting protein 3 (RIP3) and receptor-interacting protein kinase (RIPK) 1 are key mediators of necroptosis. Liver RIP3 deficiency can attenuate MCD diet-induced liver injury, steatosis, inflammation and fibrosis[55]. Similarly, the RIPK1 inhibitor RIPA-56 can reduce liver inflammation and fibrosis in HFD-fed mice by abrogating necroptosis. In addition, RIPA-56 is able to ameliorate hepatic steatosis by suppressing mixed lineage kinase domain-like protein levels[56].

Iron overload leads to hepatic oxidative stress and hepatocellular ballooning injury and plays a multifactorial role in the pathogenesis of NASH[57]. Approximately one-third of patients with NAFLD show interrupted iron homeostasis[58]. Ferroptosis is a nonapoptotic programmed cell death process characterized by iron-dependent and lipid peroxidation-associated cell death[59]. The ferroptosis inhibitors Trolox and deferiprone can protect hepatocytes from cell death and suppress the subsequent initiation of inflammation in fatty liver[60].

In contrast to hepatocytes, agents that induce HSC apoptosis show beneficial effects against NASH-related liver fibrosis[61]. Thus, when using pharmacological agents to prevent hepatocyte injury, strategies more accurately targeting hepatocytes may strengthen the therapeutic effects of these agents against NASH with limited side effects.

APPROACHES TARGETING THE INFLAMMATORY PATHWAY TO TREAT NAFLD/NASH

Inflammatory responses mediated by various immune cells and hepatocytes promote the onset of NASH and liver fibrosis. Infiltration of neutrophils and macrophages is the main pathological feature of NASH. Neutrophil depletion attenuates diet-induced NASH[62]. CXC chemokine receptor 2 (CXCR2) signaling is considered to play a pivotal role in the entry of neutrophils into peripheral tissues. Leslie and colleagues demonstrated that human and mouse livers with NASH-HCC have more $CXCR2^+$ neutrophils[63]. The expression of CXCR2 in neutrophils is induced in NASH in an autocrine manner involving the upregulation of neutrophil-derived lipocalin 2[64]. AZD5069 is a small-molecule inhibitor of CXCR2. AZD5069 can significantly improve liver pathology in NAFLD, with reduced lipid content and hepatic neutrophil accumulation and improved insulin sensitivity[65]. In addition, AZD5069-mediated CXCR2 inhibition induces reprogramming of the tumor immune microenvironment, which promotes immune checkpoint inhibition in NASH-HCC[63], suggesting that blocking infiltrating neutrophil CXCR2 can restore sensitivity to immunotherapy in the NASH liver[66].

It is well known that the expression of macrophage scavenger receptor 1 (MSR1) mediates lipid uptake in macrophages and subsequently induces inflammatory activation *via* the JNK pathway. MSR1 was found to be correlated with liver inflammation in NAFLD patients[67]. Notably, therapeutic inhibition of MSR1 with an anti-MSR1 antibody improved hepatic steatosis in a mouse model of NASH. The number of F4/80-positive cells and the expression level of tumor necrosis factor alpha (TNF-α) were also found to be reduced by the anti-MSR1 antibody therapy[67].

It has long been recognized that the NOD-like receptor protein 3 (NLRP3) inflammasome is activated in NASH livers, which leads to increased inflammation and programmed cell death. MCC950, an NLRP3 inhibitor, can improve NAFLD pathology and fibrosis in obese diabetic mice[68]. X-box binding protein-1 (XBP1) is a key factor regulating the unfolded protein response. Macrophage XBP1 is upregulated in NASH livers, which activates macrophage NLRP3 signaling and promotes hepatocyte steatosis and HSC activation. As expected, the XBP1 inhibitor toyocamycin exhibits protective effects against NASH[69].

Nuclear factor-kappaB (NF-κB) is an essential transcription factor that mediates the inflammatory response and proinflammatory cytokine production in NASH[70]. miR-378 markedly facilitates the NF-κB-TNF-α axis in NASH development by directly targeting the *Prkag2* gene, which encodes AMPK-γ2. Similarly, downregulation of miR-378 by ASO is also capable of alleviating NASH development[71].

In addition to neutrophils and macrophages, other immune cells, including dendritic cells (DCs) and lymphocytes, play pivotal roles in NASH[8]. DC recruitment to the liver promotes the inflammatory response in NAFLD progression. Reportedly, delivery of liposomal curcumin or calcitriol to lipid-rich inflammatory DCs shifted their inflammatory profile toward a regulatory phenotype and improved hepatic steatosis, inflammation and fibrosis in a NASH mouse model[72]. Inflammatory CX_3CR1^+ monocyte-derived inflammatory DCs (moDCs) are found to contribute to sustained inflammation and liver injury during NASH. Treating MCD-fed mice with the hydrogen sulfide donor *i.e.*, sodium hydrosulphide, prevented the accumulation of CX_3CR1^+ moDCs and ameliorated parenchymal injury [73]. Moreover, Toll-like receptor 7 (TLR7) signaling can induce proinflammatory cytokine production in Kupffer cells and DCs, subsequently suppressing regulatory T cells (Tregs) and leading to steatohepatitis. Notably, treatment with IRS-661, a TLR7 antagonist, could ameliorate NASH development[74]. It has been reported that α4β7-mediated homing of CD4 T cells to the intestine and liver facilitates NASH development, and α4β7 blockade by neutralizing monoclonal antibody can attenuate hepatic inflammation and fibrosis and improve metabolic dysfunction associated with NASH[75]. In addition, a high level of Tregs reportedly promotes the initiation and progression of cancer in NASH livers. In a NASH-HCC model, anti-CD25 antibodies, capable of alternatively depleting Tregs, decreases the tumor burden and increased survival time[76]. The interaction of B2 Lymphocytes with T cells contributes to NASH progression, B-cell activating factor-neutralizing monoclonal antibody Sandy-2 prevented hepatic B2 cell response and ameliorated the evolution of NASH in mice[77].

Bioactive lipids are important nodes in lipid metabolism and tissue homeostasis networks[78]. An imbalance between protective and deteriorative bioactive lipids contributes to NASH progression[79]. Pharmacological targeting of the synthesizing enzymes or receptors of these bioactive lipids has shown beneficial effects in NASH. Leukotriene B4 (LTB4), a proinflammatory metabolite derived from the ω-6 PUFA ARA, is significantly increased in patients with NAFLD[80]. Both *in vivo* and *in vitro* experiments have shown that LTB4 can promote hepatocyte lipogenesis, which is dependent on the RNase activity of IRE1α through leukotriene B4 receptor 1 (Ltb4r1). Furthermore, LTB4/Ltb4r1 stimulation increases intracellular cAMP and then promotes IRE1α Ser724 phosphorylation by PKA[80]. The Ltb4r1 inhibitor CP-105696 significantly alleviated ER stress and dyslipidemia in NAFLD mice[80]. Unlike ω-6 PUFAs, ω-3 PUFAs and their metabolites show anti-inflammatory effects. Fat-1 mice, a transgenic animal model in which tissues are endogenously enriched with ω-3 PUFAs[81], are protected from diet-induced metabolic dysfunction and fibrosis[82]. However, they are vulnerable to lipid peroxidation, which limits their clinical applications[83]. Fraser *et al*[84] developed a structurally modified ω-3 fatty acid, icosabutate, which was designed to resist oxidation and incorporation into hepatocytes and possesses the potential to activate free fatty acid receptor 4. They found that icosabutate, but not the ω-3 PUFA eicosapentaenoic acid, can ameliorate liver inflammation and fibrosis in NASH rats. Moreover, icosabutate treatment decreases liver injury in patients at high risk of NASH and cardiovascular disease [84].

APPROACHES TARGETING HSC ACTIVATION TO PREVENT NASH-RELATED LIVER FIBROSIS

In clinical trials for NASH drug development, improvement of liver fibrosis has been frequently employed as a primary or secondary endpoint. Given the central role of HSCs in liver fibrosis, targeting HSC activation has long been proposed as a therapeutic strategy to prevent NASH-related fibrosis progression.

The Notch, Hedgehog, Hippo and WNT/β-catenin signaling pathways in hepatocytes play important roles in NASH-related liver fibrosis by modulating the hepatic microenvironment. The production of osteopontin (OPN), as a paracrine factor, from hepatocytes or cholangiocytes increases in NASH, which leads to the activation of HSCs[85-87]. Increased Notch signaling activity has been found to be responsible for the induction of OPN[85]. Thus, several studies have targeted Notch signaling to treat NASH, including studies on Nicastrin ASO and nanoparticle-mediated delivery systems targeting Notch antagonism[85,88]. In addition, nuclear factor of activated T-cell 4 (NFATc4) was also reported to induce OPN expression by negatively regulating the transcriptional activity of PPARα. As expected, inhibition of NFATc4 decreased lipid content and improved inflammation and fibrosis in NASH mice [89]. Hepatocyte transcriptional co-activator with PDZ-binding motif (TAZ) was found to promote NASH-related liver fibrosis by increasing Indian hedgehog, which activates HSCs[90]. A recent study reported that stabilized GalNAc-siRNAs targeting hepatocyte TAZ can significantly ameliorate liver inflammation and fibrosis in mice with established NASH[91]. Furthermore, WNT1-inducible signaling pathway protein 1 (WISP1), a member of the cellular communication network family, has recently been identified as an extracellular activator of the myocardin-related transcription factor-cytoskeleton pathway in HSCs. Activation of the WISP1-MRIF signaling pathway induces HSC migration and promotes liver fibrosis progression. Notably, an anti-WISP1 antibody significantly ameliorated liver fibrosis in NASH mice[92].

Lnterleukin-11 (IL-11) has been demonstrated to activate HSCs. Deletion of IL-11 receptor subunit alpha (IL-11RA) protects mice from NASH diet-induced hepatocyte death, liver inflammation and fibrosis. Widjaja *et al*[93] developed a neutralizing anti-IL-11 antibody and neutralizing anti-IL-11RA antibody and found that both of them greatly decreased ALT levels and significantly improved hepatic steatosis and liver fibrosis in mouse models of NASH[93].

Activation of the JAK/signal transducer and activator of transcription (STAT) pathway in HSCs promotes liver fibrosis. Ruxolitinib, an effective small-molecule JAK1/2 selective inhibitor, has been approved by the FDA for myelofibrosis treatment. Recently, it has been reported that ruxolitinib can block HSC activation and attenuate liver fibrosis progression[94]. In addition, the JAK2 inhibitor pacritinib affords protection against NAFLD-related liver fibrosis by inhibiting HSC activation[95]. Rilpivirine, a nonnucleoside reverse transcriptase inhibitor, is widely used to treat HIV infection. Rilpivirine can also ameliorate liver fibrosis, possibly through selective STAT1-dependent induction of apoptosis in HSCs, and suppress HFD- and CCl4-induced liver fibrosis. In addition, rilpivirine enhances STAT3-dependent proliferation in hepatocytes as an effect secondary to its pro-apoptotic effect in HSCs [96].

Protease-activated receptor-2 (PAR2) is an emerging new target for NASH that regulates liver injury, inflammation and fibrosis and plays a critical role in regulating hepatic cholesterol and glucolipid metabolism[97-99]. Pharmacological inhibition of PAR2 with pepducin PZ-235, a full antagonist of PAR2, not only protects against the activation of HSCs and fibrosis but also promotes hepatocellular viability by inhibiting mitochondrial ROS production induced by PAR2 stimulation[97].

APPROACHES TARGETING LSECS AND CHOLANGIOCYTES TO TREAT NAFLD

LSECs are highly specialized endothelial cells with fenestrae and lack a basement membrane. LSECs interact with hepatocytes, liver immune cells and HSCs and play important roles in regulating liver function. LSEC dysfunction, including LSEC capillarization, disturbed nitric oxide release from LSECs, and increased expression of adhesion molecules, all contribute to NAFLD pathogenesis[100]. Vascular cell adhesion molecule 1 (VCAM-1) is an adhesion molecule upregulated in LSECs in NASH mouse livers. Anti-VCAM1 antibody attenuates hepatic inflammation in NASH mice[101]. Endothelial nitric oxide synthase (eNOS) expression in LSECs is decreased in NASH mice, which is likely the result of Notch activation. Pharmacological inhibition of Notch signaling using DAPT and LY3039478, as well as the eNOS activator YC-1, improved the NASH phenotype in MCD diet-fed mice[102]. Although the role of LESCs in NAFLD has been increasingly emphasized, the underlying mechanisms and therapeutic approaches targeting LESCs need more exploration.

Cholangiocytes also play an important role in NASH development. In addition to hepatocytes, OPN is strongly expressed in cholangiocytes in NASH livers. Thus, it is highly possible that cholangiocyte-derived OPN can promote liver fibrosis by activating HSCs[87]. Cardiotrophin-like cytokine factor 1 is another cholangiocyte-derived paracrine factor that exerts beneficial effects in NASH. Downregulation of a subunit of its receptor complex leukemia inhibitory factor receptor contributes to the progression of NASH[103]. Compared with other nonparenchymal cells, there are fewer studies addressing the effects of cholangiocytes on NASH, and a related therapeutic approach is still lacking.

POTENTIAL TARGETS OUTSIDE THE LIVER

It is well-established that multiple organs form a network to regulate whole-body energy metabolism.

Extrahepatic organs influence liver function in an endocrine manner by releasing hormones or inflammatory factors or by regulating systemic metabolic homeostasis such as energy expenditure and insulin sensitivity. Thus, targeting these extrahepatic organs provides additional options for treating NAFLD/NASH.

Targets affect systemic energy metabolism

Diets with reduced contents of sugars, refined carbohydrates and saturated fat are recommended to treat NAFLD[104]. Moreover, exercise is considered as an effective strategy for preventing and treating NAFLD *via* metabolic pathways and interorgan crosstalk[105]. Glucagon-like peptide (GLP)-1 receptor agonists, which enhance insulin secretion and reduce food intake, show beneficial effects against NASH in clinical trials[6]. In addition, fibroblast growth factor 21 analogs improved systemic energy metabolism and demonstrated potential in NASH therapy[6]. In a recent basic study, a novel Zn supplement, ulvan oligosaccharide (UO)-Zn, can activate AMPK, leading to improved metabolic pathways in HFD-fed mice[106]. The increased hepatic expression of branched chain ketoacid dehydrogenase kinase (BDK) partly induced high levels of branched-chain amino acids (BCAA) in metabolic diseases[107-109]. Moreover, 3,6-dichlorobrenzo(b)thiophene-2-carboxylic acid, a small-molecule allosteric inhibitor of BDK, lowered levels of circulating BCAAs, reduced hepatic steatosis, and improved glucose tolerance in rodent models[107,108].

The gut-liver axis

The crosstalk between the gut and liver is regarded as an important pathway in regulating hepatic functions. Intestinal inflammation and dysfunction of intestinal lipid and bile acid absorption, epithelial cell permeability and the microbiome affect liver function and contribute to NAFLD progression. The relationship between gut microbiota and potential therapeutic targets has been recently discussed in depth elsewhere[110,111]; thus, we only focus on other aspects of the gut-liver axis.

Serum ceramide levels are positively related to NAFLD, and intestine-derived ceramide has been demonstrated to promote NAFLD[112,113]. Intestinal hypoxia-inducible factor 2α (HIF-2α) and myelocytomatosis oncogene (MYC) affect NAFLD by regulating ceramide metabolism. Intestinal HIF-2α , but not HIF-1α, is activated in obese patients and mice. Intestine-specific HIF-2α knockout improves hepatic steatosis by decreasing intestine-derived ceramide levels. Mechanistically, HIF-2α transcriptionally upregulates the expression of Neu3, a key enzyme in the ceramide salvage pathway. Targeting HIF-2α with its inhibitor PT2385 significantly attenuated hepatic lipid accumulation and decreased plasma ALT levels in HFD-fed mice[112]. Moreover, oral administration of PT2385 contributes to reduced body weight and improved insulin sensitivity[112]. Intestinal MYC expression is positively related to body mass index and serum ALT levels in humans. Genetic ablation of MYC in the intestine suppressed obesity and hepatic steatosis and led to decreased ceramide levels in HFD-fed mice. Ceramide synthase 4 has been further demonstrated to be a target gene of MYC. In line with this finding, pharmacological inhibition of MYC with 10058-F4 was found to ameliorate the metabolic disorders and liver fibrosis induced by HFD feeding[114]. In addition, increased intestinal ceramide also meditates the acceleration of NASH induced by nicotine[115].

Gut-derived 5-hydroxytryptamine (5-HT) has been reported to promote lipogenesis in the liver. Moreover, liver-specific knockout of the 5-HT receptor 2α (*Htr2α*) gene significantly lowered liver lipid content in HFD-fed mice and decreased the expression of genes involved in lipogenesis. To evaluate the potential of targeting the gut-5-HT-liver Htr2α pathway as a therapeutic strategy for NAFLD, the 5-HT receptor HTR2A antagonist sarpogrelate was used to treat HFD-fed mice. The results showed that sarpogrelate greatly ameliorates hepatic steatosis in NAFLD mice[116].

Yan *et al*[117] recently clarified that gut PPARα activation promotes NASH development by inducing fatty acid binding protein 1 expression, thereby aggravating fatty acid absorption in the small intestine [117]. GW6471, a PPARα-specific antagonist, was found to accumulate in the small intestine at much higher levels than in the liver. Intestine-specific PPARα deficiency and gut PPARα antagonism by GW6471 both improve NASH phenotypes[117]. The pleotropic roles of PPARα in different tissues in modulating NASH are worth discussing. Hepatocyte PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD[118]. Therapeutic strategies to prevent or treat NAFLD by activating hepatocyte PPARα and/or inhibiting gut PPARα may help guide drug delivery. Monoacylglycerol acyltransferase 2 (MGAT2) mediates the conversion of monoacylglycerol to diacylglycerol and is critical for dietary fat absorption. In the liver, MGAT2 regulates TG synthesis. BMS-963272, a potent MGAT2 inhibitor, improves the NAFLD activity score and liver fibrosis in NAFLD/NASH mice[119]. Of note, its safety has been confirmed in phase 1 clinical trials[119].

In addition, a long-acting dual agonist of GLP-1 and GLP-2 receptors, GLP1/2-Fc, has been found to greatly alter the microbiome composition and exhibits positive effects on gastrointestinal volume and the intestinal barrier. More importantly, GLP1/2-Fc can significantly ameliorate NASH phenotypes, including hepatic fat accumulation, inflammation, fibrosis and insulin tolerance[120], and its effects are superior to those of liraglutide, which has minimal efficacy on inflammation or fibrosis[121].

The adipose tissue-liver axis

Recently, several novel adipokines have been identified and verified to play important roles in NASH development and progression by mediating the crosstalk between adipose tissue and the liver. Isthmin-1 (ISM1) was identified as an adipokine that can activate phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-protein kinase B (Akt) signaling independent of insulin. *ISM1* gene expression is positively correlated with body mass index, and its deficiency leads to glucose intolerance *via* inhibition of PI3K-Akt signaling. In addition, ISM1 suppressed de novo lipogenesis in the liver. Recombinant ISM1 protein exhibited beneficial effects on both glucose tolerance and hepatic steatosis in NAFLD mice[122]. Recently, Liu *et al*[123] demonstrated that secreted protein acidic and rich in cysteine-like protein 1 (Sparcl1), which is a white adipose tissue-secreted protein, is correlated with hepatic pathological features in NASH patients. The authors further found that sparcl1 can induce liver inflammation by increasing C-C motif chemokine ligand 2 expression in the liver. To further determine the role of sparcl1 in the pathogenesis of NASH, the authors developed a neutralizing antibody against sparcl1 and found that the sparcl1-neutralizing antibody markedly ameliorated liver inflammation and liver fibrosis in NASH mice[123]. Gremlin 1, a newly identified adipokine, is positively related to insulin resistance and NAFLD/NASH. Treatment with recombinant Gremlin 1 protein impairs insulin sensitivity in various cell types, including human primary adipocytes, skeletal muscle cells, and liver cells. The insulin-sensitizing effect of the neutralizing anti–Gremlin 1 antibody indicates its beneficial effects on insulin resistance and NAFLD/NASH[124]. Neuregulin 4 (NRG4) is an adipose tissue-derived endocrine factor that has been demonstrated to suppress NASH-associated HCC by restraining the tumor-prone liver immune microenvironment. A recombinant fusion protein comprising amino acids 1–55 of human NRG4 and the Fc domain of immunoglobulin G 1 was found to suppress HCC induced by a NASH diet plus oncogene overexpression[125].

SWELL1/LRRC8a, a leucine-rich repeat-containing transmembrane protein, functionally encodes an ion channel signaling complex on the adipocyte plasma membrane[126]. Adipocyte SWELL1 is dispensable for adipose development[127]. SWELL1 protein is reduced in the adipocytes of type 2 diabetic animals. The small molecule SN-401, which binds at a constriction point within the SWELL hexamer, can increase adipocyte SWELL1 protein expression and SWELL1-dependent insulin signaling. Similarly, SN-401 can normalize glucose tolerance by augmenting tissue glucose uptake, suppressing hepatic glucose production, increasing serum fibroblast growth factor 21 levels, and reducing hepatic steatosis and hepatocyte ballooning in obese type 2 diabetic mice[128]. Taken together, these findings demonstrate that targeting the adipose tissue-liver axis may be an attractive strategy to treat NAFLD/NASH.

The skeletal muscle-liver axis

The crosstalk between skeletal muscle and the liver also impacts NAFLD progression. As one of the energy metabolism organs, skeletal muscle can affect liver lipid and glucose metabolism by regulating systemic metabolic homeostasis. In addition, decreased skeletal muscle mass and altered myokine secretion are associated with exacerbation of NAFLD progression[129,130]. Myostatin signaling negatively regulates muscle mass and is positively related to liver fibrosis[131]. Irisin (encoded by fibronectin type III domain containing 5, Fndc5), a myokine induced by exercise, is also expressed in adipose tissue and the liver. Serum irisin levels are inversely associated with TG content in the livers of obese adults[132]. Recombinant Fndc5/irisin significantly improved the NASH phenotype in HFD-fed mice[133]. The administration of nicotinamide riboside can alleviate obesity and steatosis by increasing irisin levels in HFD-fed mice[133]. Collectively, therapeutic strategies to improve skeletal muscle quantity and quality or target myokine signaling may provide promising options to treat NASH, but basic studies to collect more evidence are warranted.

CONCLUSION

Because of the urgent need to develop therapeutic approaches to treat NAFLD/NASH and the complexity of the pathophysiologic process of NAFLD, a large body of basic research has focused on the mechanisms of NAFLD to explore the possibility of applying new approaches to treat the disease (Tables 1 and 2). Metabolic stress-induced hepatocyte injury is an initial factor driving NASH development. Thus, in hepatocytes, strategies for treating NASH aim to improve metabolic disturbance and protect hepatocytes from injury. Alteration of the liver microenvironment and the interaction between diverse liver cells promote liver inflammation and fibrosis. Therefore, the inflammatory response of hepatocytes and immune cells in the liver is also an important target for NASH treatment. Strategies targeting HSC activation, proliferation and migration show beneficial effects on liver fibrosis and may become a novel way to attenuate NASH outcomes (Figure 1). As an indispensable part of hepatic metabolic regulation, extrahepatic organs play an essential role in liver lipid homeostasis, and their dysfunction contributes to the development and progression of NAFLD/NASH. The gut, adipose tissue and skeletal muscle can functionally affect liver or whole-body energy metabolic homeostasis *via* various endocrine factors (Figure 1 and Table 2).

Table 1 Novel intrahepatic targets explored by recent basic study

Molecular target	Potential agent	Classification	Function	Effects on NAFLD	Ref.
SREBPs	25-HL	Inhibitor	Decrease TG and cholesterol synthesis	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[10]
ORM2	Recombinant ORM2; ORM2-FC fusion protein	Recombinant protein	Inhibit lipogenesis	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[11]
Slc25al	CTPI-2	Inhibitor	Regulating cytoplasmic and mitochondrial citrate pool	-Hepatic steatosis -Liver inflammation	[12]
ACLY	Bempedoic acid	Inhibitor	Inhibit ACLY activity	-Hepatic steatosis -Liver injury	[14]
SHMT1/2	SHIN2 IV	Inhibitor	Decrease serine catabolism derived NADPH	-Hepatic lipogenesis	[16]
PCAF	Embelin	Inhibitor	Inhibit acetylation of LDHB	-Hepatic steatosis -Liver inflammation	[17]
Ketohexokinase	PF-06835919	Inhibitor	Regulating fructose metabolism	-Fructose-induced liver steatosis	[18]
Mitochondrial uncoupling	Sorafenib	Small molecule	Induce mitochondrial uncoupling and activate AMPK	-Hepatic steatosis -Liver inflammation -Liver fibrosis -NASH-associated HCC	[21]
MCJ	LNP-siMCJ; GalNAc-siMCJ	Nucleic acid-based therapy	Maintain mitochondrial function	+β-oxidation -Hepatic steatosis -Liver fibrosis	[20]
Cyclophilin D	CsA	Inhibitor		-Hepatic steatosis	[22]
ClpP	A54556A	Activator		-Hepatic steatosis -Liver inflammation -Liver fibrosis	[42]
SAB	GalNAc-Sab ASO	Nucleic acid-based therapy		-Hepatic steatosis -Liver inflammation -Liver fibrosis	[43]
ACMSD	TES-991	Inhibitor		-Hepatic steatosis -Liver inflammation	[45]
FOXA3	FOXA3 siRNA	Nucleic acid-based therapy	Attenuate ER stress induced liver steatosis	-Hepatic steatosis	[23]
TRIM5-DDX5	Hyperforcinol K	N/A	Inhibit ubiquitinated degradation of DDX5	-Hepatic steatosis -Liver inflammation	[24]
STK25	STK25 ASO	Nucleic acid-based therapy	Regulating energy homeostasis	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[28]
RORa	RS-2982	Agonist	Increase miR-122 level	-Hepatic steatosis -Liver inflammation	[30]

				-Liver fibrosis	
HSD17B13	Reproterol	Small molecule	Induce the Ser33 phosphorylation of 17β-HSD13 protein	-Hepatic steatosis -Liver fibrosis	[38]
PSD3	GalNAc-Psd3 ASO	Nucleic acid-based therapy	Decrease TG synthesis	-Hepatic steatosis -Liver injury -Liver fibrosis	[39]
Nrf2	TBE-31; NK-252; Dimethyl fumarate	Activator	Reduce oxidative stress	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[51-53]
BCL-2	A22	Small molecule	Anti-apoptosis	-Liver injury -Liver fibrosis	[54]
RIPK1	RIPA-56	Inhibitor	Abrogating necroptosis	-Hepatic steatosis -Liver inflammation	[56]
CXCR2	AZD5069	Inhibitor	Inhibit neutrophil infiltration	-Hepatic steatosis -Liver inflammation -Liver fibrosis -NASH-associated HCC	[63,65]
MSR1	Anti-MSR1 antibody	Neutralizing antibody	Inhibit inflammatory response	-Hepatic steatosis -Liver inflammation	[67]
NLRP3	MCC950	Inhibitor	Inhibit NLRP3 activation	-Liver inflammation -Liver fibrosis	[68]
XBP1	Toyocamycin	Inhibitor		-Hepatic steatosis -Liver inflammation -Liver fibrosis	[69]
DCs	Curcumin; calcitriol	Small molecule	Shift hepatic DC inflammatory profile toward a regulatory phenotype	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[72]
Hydrogen sulfide	NaHS	Hydrogen sulfide donor	Prevent the accumulation of TNF-α-producing CX_3CR1^+ moDCs	-Liver injury	[73]
TLR7	IRS-661	Antagonist	Decrease proinflammatory cytokine production in Kupffer cells and DCs	-Hepatic steatosis -Liver inflammation	[74]
Integrin	Anti-α4β7 antibody	Neutralizing antibody	Decrease $\alpha4\beta7^+$ CD4 T-cell recruitment	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[75]
Tregs	Anti-CD25 antibodies	Neutralizing antibody	Deplete Tregs	-NASH-associated HCC	[76]
BAFF	Sandy-2	Neutralizing antibody	Prevent hepatic B2-cell responses	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[77]
Notch	Nicastrin ASO	Nucleic acid-based therapy	Inhibit Notch signaling	-Liver fibrosis	[85]
	NP- dibenzazepine	Inhibitor with target delivery system		-Liver fibrosis	[88]
TAZ	GalNAc-TAZ siRNA	Nucleic acid-based	Inhibit TAZ in hepatocyte	-Liver inflammation	[91]

WISP1	Anti-WISP1 antibody	therapy	and inhibit HSC activation	-Liver fibrosis	[92]
		Neutralizing antibody	Inhibit HSC migration	-Liver fibrosis	
IL-11	Anti-IL-11 antibody	Neutralizing antibody	Inhibit HSC activation; inhibit hepatocyte injury	-Liver injury -Liver fibrosis	[93]
JAK1/2	Ruxolitinib	Inhibitor	Inhibit HSC activation	-Liver fibrosis	[94]
JAK2	Pacritinib	Inhibitor	Inhibit HSC activation	-Liver fibrosis	[95]
STAT1	Rilpivirine	Inhibitor	Induce HSC apoptosis and promote hepatocyte proliferation	-Liver fibrosis	[96]
PAR2	Pepducin PZ-235	Antagonist	Inhibit HSC activation	-Liver injury -Liver fibrosis	[97]
VCAM-1	Anti-VCAM-1 antibody	Neutralizing antibody	Suppress monocyte adhesion to LSEC	-Liver inflammation -Liver fibrosis	[101]
eNOS	YC-1	Activator	Increase NO production from LSEC	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[102]
Ltb4r1	CP-105696	Inhibitor	Inhibit the effects of LTB4	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[80]
mPGES-2	SZ0232	Inhibitor	Regulating ARA metabolism	-Hepatic steatosis	[31]
CYP4A	TS-011	Inhibitor		-Hepatic steatosis -Liver inflammation -Liver fibrosis	[32]
N/A	Icosabutate	Structurally modified ω-3 fatty acid	Resist oxidation and activate free fatty acid receptor 4	-Liver inflammation -Liver fibrosis	[84]

-: Inhibit; +: Stimulate; SREBPs: Sterol regulatory element-binding proteins; TG: Triglyceride; 25-HL: 25-Hydroxanoanosterol; HSC: Hepatic stellate cell; LSEC: Liver sinusoidal endothelial cell; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NO: Nitric oxide; N/A: No application; ORM2: Orosomucoid 2; ACLY: ATP-citrate lyase; SHMT1/2: Serine hydroxymethyltransferase 1/2; NADPH: Nicotinamide adenine dinucleotide phosphate; PCAF: P300/CBP-associated factor; LDHB: Lactate dehydrogenase B; AMPK: AMP-activated protein kinase; HCC: Hepatocellular carcinoma; MCJ: Methylation-controlled J; LNP: Lipid-nanoparticle-encapsulated; GalNAc: N-acetylgalactosamine; ClpP: Caseinolytic protease P; SAB: SH3 homology-associated BTK binding protein; ASO: Antisense oligonucleotides; ACMSD: α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase; FOXA3: Forkhead box A3; STK25: Sterile 20-type kinase serine/threonine kinase 25; RORa: Receptor-related orphan receptor alpha; PSD3: Pleckstrin and Sec7 domain-containing 3; Nrf2: Nuclear factor erythroid 2–related factor 2; RIPK1: Receptor-interacting protein kinase 1; CXCR2: CXC chemokine receptor 2; MSR1: Macrophage scavenger receptor 1; NLRP3: NOD-like receptor protein 3; XBP1: X-box binding protein-1; DC: Dendritic cell; TLR7: Toll-like receptor 7; Tregs: Regulatory T cells; BAFF: B-cell activating factor; NP: Nanoparticle-encapsulated; TAZ: Transcriptional co-activator with PDZ-binding motif; WISP1: WNT1-inducible signaling pathway protein 1; IL-11: Interleukin-11; JAK: Janus kinase; STAT1: Signal transducer and activator of transcription 1; PAR2: Protease-activated receptor-2; VCAM: Vascular cell adhesion molecule; eNOS: Endothelial nitric oxide synthase; LTB4: Leukotriene B4; mPGES: Microsomal prostaglandin E synthases; ARA: Arachidonic acid; CYP4A: Cytochrome P450 4A14; CsA: Cyclosporine A; TRIM5: Tripartite motif protein 5; DDX5: DEAD-box protein 5.

With the rapid development of research techniques, single-cell sequencing and spatial transcriptomics, single-cell proteomics and metabolomics have been widely used and will undoubtedly accelerate the discovery of novel NAFLD/NASH mechanisms and therapeutic targets. Drug libraries enable us to quickly find effective small molecules to target specific pathways identified in the basic research field. Endocrine factors that mediate the crosstalk between the liver and extrahepatic tissues provide an ideal target for developing neutralization antibodies or recombinant proteins for the treatment of NAFLD/NASH. In addition to traditional small molecules, neutralizing antibodies and recombinant proteins, nucleic acid-based therapy has become an attractive approach for the development of drugs for NAFLD/NASH patients. Nucleic acid-based therapeutics include ASOs, siRNAs, miRNAs and mRNAs with modifications to improve their stability, immunogenicity, and diversity[134]. Through nucleic acid-based approaches, therapeutic target genes can be specifically silenced or overexpressed in the liver. As an example, the safety and efficiency of HSD17B13 RNAi[36] and DGAT2 ASOs have been examined in a few clinical trials, which showed a positive impact on liver

Table 2 Novel extrahepatic targets explored by recent basic study

Molecular target	Potential agent	Classification	Function	Effect	Ref.
AMPK	UO-Zn	Small molecule	Improve lipid metabolism	-Lipid accumulation in liver and circulation	[106]
BDK	BT2	Inhibitor	Regulating BCAA metabolism	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[107,108]
HIF-2α (gut)	PT2385	Inhibitor	Decrease intestine-derived ceramide	-Hepatic steatosis -Liver injury	[112]
MYC (gut)	10058-F4	Inhibitor	Decrease intestine-derived ceramide	-Hepatic steatosis -Liver injury -Liver fibrosis	[114]
5-HT(gut)/HTR2A	Sarpogrelate	Antagonist	Inhibit gut-5-HT-liver Htr2α pathway	-Hepatic steatosis	[116]
PPARα (gut)	GW6471	Antagonist	Inhibit fatty acid absorption by intestine	-Hepatic steatosis -Liver inflammation -Liver fibrosis	[117]
MGAT2	BMS-963272	Inhibitor	Regulating fat absorption and liver TG synthesis	-Liver inflammation -Liver fibrosis	[119]
ISM1 (AT)	Recombinant Ism1	Recombinant protein	Suppress lipogenesis in liver	-Hepatic steatosis +Glucose tolerance	[122]
Sparcl1 (AT)	Anti-Sparcl1 antibody	Neutralizing antibody	Decrease CCL2 expression in liver	-Liver inflammation -Liver fibrosis	[123]
Gremlin 1 (AT)	Anti-Gremlin 1 antibody	Neutralizing antibody	Increase insulin sensitivity	-Insulin resistance	[124]
NRG4	hNRG4-Fc	Recombinant protein	Restrain the tumor-prone liver immune microenvironment	-NASH-associated HCC	[125]
SWELL1/LRRC8a (AT)	SN-401	Small molecule	Enhance insulin sensitivity and secretion	+Insulin sensitivity -Hepatic steatosis -Hepatocyte damage	[128]

AT: Adipose tissue; -: Inhibit; +: Stimulate; AMPK: AMP-activated protein kinase; BDK: Branched chain ketoacid dehydrogenase kinase; BCAA: Branched-chain amino acids; HIF-2α: Hypoxia-inducible factor 2α; MYC: Myelocytomatosis oncogene; 5-HT: 5-hydroxytryptamine; PPAR: Peroxisome proliferator-activated receptor; MGAT2: Monoacylglycerol acyltransferase 2; TG: Triglyceride; ISM1: Isthmin-1; Sparcl1: Secreted protein acidic and rich in cysteine-like protein 1; CCL2: C-C motif chemokine ligand 2; NRG4: Neuregulin 4; hNRG4-Fc: Human NRG4 and the Fc domain of immunoglobulin G 1; NASH: Nonalcoholic steatohepatitis; HCC: Hepatocellular carcinoma; UO: Ulvan oligosaccharide; BT2: 3,6-dichlorobrenzo(b)thiophene-2-carboxylic acid.

function and stiffness in patients with NASH[135].

To avoid side effects, efforts have been made to better target the liver or more accurately target specific hepatic cell types by exploring novel drug delivery systems. Based on the hepatocyte-specific expression of asialoglycoprotein receptor, conjugation of GalNAc with nucleic acids or nanoparticles can provide better targeting to hepatocytes. To specifically target HSCs, nanoparticles integrated with vitamin A, cyclic peptides, mannose 6-phosphate, and antibodies against synaptophysin have shown high affinity for HSCs[136,137]. By using these approaches, drugs can be more precisely delivered to HSCs to alleviate NASH-related liver fibrosis. For hepatic macrophages, nanoparticles modified with the phospholipid serine to mimic apoptotic cells can enhance uptake of the nanoparticles by hepatic macrophages[137]. However, challenges remain in targeting other cell types.

Finally, novel preclinical and *in vitro* models are required for drug screening for NASH. A 3D human liver model constructed *via* coculture of hepatocytes with non-parenchymal cells in a 3D collagen matrix has been shown to mimic NASH features when treated with free fatty acids and TNF-α[138]. Liver organoids from pluripotent stem cells or induced pluripotent stem cells also provide unique preclinical platforms to fill the gap between animal studies and clinical trials for therapeutic target validation and

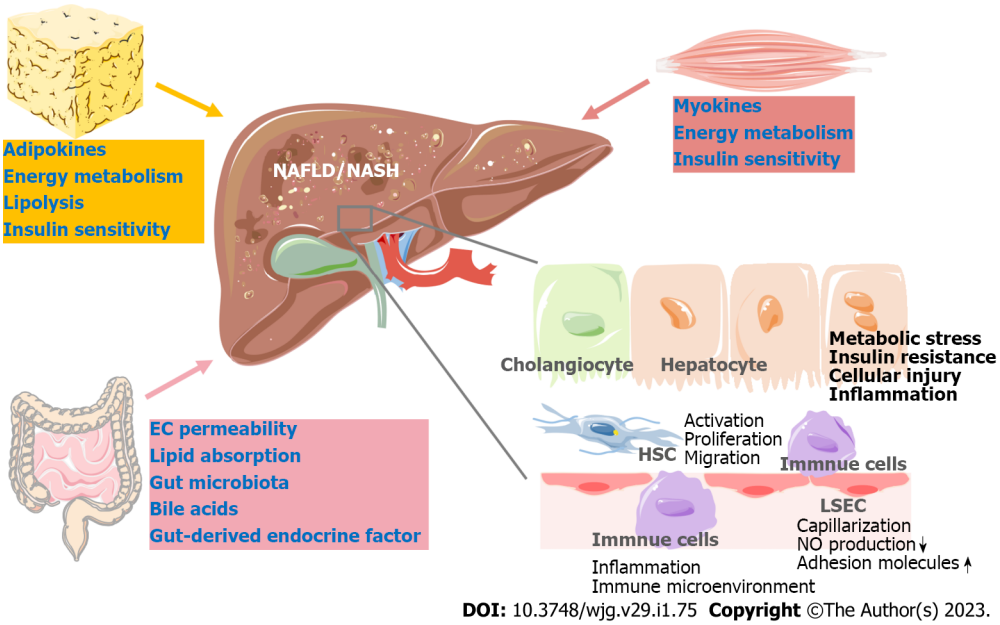

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Figure 1 An overview of intrahepatic and extrahepatic targets for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis treatment. In hepatocytes, strategies for treating nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) aim to improve metabolic disturbance and protect hepatocytes from injury. In the liver, the crosstalk network of nonparenchymal cells, including immune cells, hepatic stellate cells, liver sinusoidal endothelial cells and hepatocytes, is an important target for NAFLD/NASH. Outside the liver, the gut, adipose tissue and skeletal muscle interact with the liver *via* various endocrine factors or affect whole-body energy metabolic homeostasis, which has been verified to be an extrahepatic target for NAFLD/NASH treatment. EC: Epithelial cell; HSC: Hepatic stellate cell; LSEC: Liver sinusoidal endothelial cell; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NO: Nitric oxide.

drug development[139].

FOOTNOTES

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REFERENCES

1 **Schuster S**, Cabrera D, Arrese M, Feldstein AE. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 349-364 [PMID: 29740166 DOI: 10.1038/s41575-018-0009-6]
2 **Younossi Z**, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, Eguchi Y, Wong VW, Negro F, Yilmaz Y, Romero-Gomez M, George J, Ahmed A, Wong R, Younossi I, Ziayee M, Afendy A; Global Nonalcoholic Steatohepatitis

Council. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol* 2019; **17**: 748-755.e3 [PMID: 29908364 DOI: 10.1016/j.cgh.2018.05.057]

3 **Diehl AM**, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. *N Engl J Med* 2017; **377**: 2063-2072 [PMID: 29166236 DOI: 10.1056/NEJMra1503519]

4 **Sheka AC**, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. *JAMA* 2020; **323**: 1175-1183 [PMID: 32207804 DOI: 10.1001/jama.2020.2298]

5 **Lassailly G**, Caiazzo R, Ntandja-Wandji LC, Gnemmi V, Baud G, Verkindt H, Ningarhari M, Louvet A, Leteurtre E, Raverdy V, Dharancy S, Pattou F, Mathurin P. Bariatric Surgery Provides Long-term Resolution of Nonalcoholic Steatohepatitis and Regression of Fibrosis. *Gastroenterology* 2020; **159**: 1290-1301.e5 [PMID: 32553765 DOI: 10.1053/j.gastro.2020.06.006]

6 **Yang YY**, Xie L, Zhang NP, Zhou D, Liu TT, Wu J. Updates on novel pharmacotherapeutics for the treatment of nonalcoholic steatohepatitis. *Acta Pharmacol Sin* 2022; **43**: 1180-1190 [PMID: 35190696 DOI: 10.1038/s41401-022-00860-3]

7 **Arab JP**, Arrese M, Trauner M. Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Annu Rev Pathol* 2018; **13**: 321-350 [PMID: 29414249 DOI: 10.1146/annurev-pathol-020117-043617]

8 **Peiseler M**, Schwabe R, Hampe J, Kubes P, Heikenwälder M, Tacke F. Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J Hepatol* 2022; **77**: 1136-1160 [PMID: 35750137 DOI: 10.1016/j.jhep.2022.06.012]

9 **Loomba R**, Mohseni R, Lucas KJ, Gutierrez JA, Perry RG, Trotter JF, Rahimi RS, Harrison SA, Ajmera V, Wayne JD, O'Farrell M, McCulloch W, Grimmer K, Rinella M, Wai-Sun Wong V, Ratziu V, Gores GJ, Neuschwander-Tetri BA, Kemble G. TVB-2640 (FASN Inhibitor) for the Treatment of Nonalcoholic Steatohepatitis: FASCINATE-1, a Randomized, Placebo-Controlled Phase 2a Trial. *Gastroenterology* 2021; **161**: 1475-1486 [PMID: 34310978 DOI: 10.1053/j.gastro.2021.07.025]

10 **Jiang SY**, Yang X, Yang Z, Li JW, Xu MQ, Qu YX, Tang JJ, Li YF, Wang L, Shao YW, Meng XY, Hu H, Song BL, Rao Y, Qi W. Discovery of an insulin-induced gene binding compound that ameliorates nonalcoholic steatohepatitis by inhibiting sterol regulatory element-binding protein-mediated lipogenesis. *Hepatology* 2022; **76**: 1466-1481 [PMID: 35102596 DOI: 10.1002/hep.32381]

11 **Zhou B**, Luo Y, Ji N, Hu C, Lu Y. Orosomucoid 2 maintains hepatic lipid homeostasis through suppression of de novo lipogenesis. *Nat Metab* 2022; **4**: 1185-1201 [PMID: 36050503 DOI: 10.1038/s42255-022-00627-4]

12 **Tan M**, Mosaoa R, Graham GT, Kasprzyk-Pawelec A, Gadre S, Parasido E, Catalina-Rodriguez O, Foley P, Giaccone G, Cheema A, Kallakury B, Albanese C, Yi C, Avantaggiati ML. Inhibition of the mitochondrial citrate carrier, Slc25a1, reverts steatosis, glucose intolerance, and inflammation in preclinical models of NAFLD/NASH. *Cell Death Differ* 2020; **27**: 2143-2157 [PMID: 31959914 DOI: 10.1038/s41418-020-0491-6]

13 **Ryaboshapkina M**, Hammar M. Human hepatic gene expression signature of non-alcoholic fatty liver disease progression, a meta-analysis. *Sci Rep* 2017; **7**: 12361 [PMID: 28955037 DOI: 10.1038/s41598-017-10930-w]

14 **Morrow MR**, Batchuluun B, Wu J, Ahmadi E, Leroux JM, Mohammadi-Shemirani P, Desjardins EM, Wang Z, Tsakiridis EE, Lavoie DCT, Reihani A, Smith BK, Kwiecien JM, Lally JSV, Nero TL, Parker MW, Ask K, Scott JW, Jiang L, Paré G, Pinkosky SL, Steinberg GR. Inhibition of ATP-citrate lyase improves NASH, liver fibrosis, and dyslipidemia. *Cell Metab* 2022; **34**: 919-936.e8 [PMID: 35675800 DOI: 10.1016/j.cmet.2022.05.004]

15 **García-Cañaveras JC**, Lancho O, Ducker GS, Ghergurovich JM, Xu X, da Silva-Diz V, Minuzzo S, Indraccolo S, Kim H, Herranz D, Rabinowitz JD. SHMT inhibition is effective and synergizes with methotrexate in T-cell acute lymphoblastic leukemia. *Leukemia* 2021; **35**: 377-388 [PMID: 32382081 DOI: 10.1038/s41375-020-0845-6]

16 **Zhang Z**, TeSlaa T, Xu X, Zeng X, Yang L, Xing G, Tesz GJ, Clasquin MF, Rabinowitz JD. Serine catabolism generates liver NADPH and supports hepatic lipogenesis. *Nat Metab* 2021; **3**: 1608-1620 [PMID: 34845393 DOI: 10.1038/s42255-021-00487-4]

17 **Wang T**, Chen K, Yao W, Zheng R, He Q, Xia J, Li J, Shao Y, Zhang L, Huang L, Qin L, Xu M, Zhang Z, Pan D, Li Z, Huang F. Acetylation of lactate dehydrogenase B drives NAFLD progression by impairing lactate clearance. *J Hepatol* 2021; **74**: 1038-1052 [PMID: 33248168 DOI: 10.1016/j.jhep.2020.11.028]

18 **Gutierrez JA**, Liu W, Perez S, Xing G, Sonnenberg G, Kou K, Blatnik M, Allen R, Weng Y, Vera NB, Chidsey K, Bergman A, Somayaji V, Crowley C, Clasquin MF, Nigam A, Fulham MA, Erion DM, Ross TT, Esler WP, Magee TV, Pfefferkorn JA, Bence KK, Birnbaum MJ, Tesz GJ. Pharmacologic inhibition of ketohexokinase prevents fructose-induced metabolic dysfunction. *Mol Metab* 2021; **48**: 101196 [PMID: 33667726 DOI: 10.1016/j.molmet.2021.101196]

19 **Di Ciaula A**, Passarella S, Shanmugam H, Noviello M, Bonfrate L, Wang DQ, Portincasa P. Nonalcoholic Fatty Liver Disease (NAFLD). Mitochondria as Players and Targets of Therapies? *Int J Mol Sci* 2021; **22** [PMID: 34065331 DOI: 10.3390/ijms22105375]

20 **Barbier-Torres L**, Fortner KA, Iruzubieta P, Delgado TC, Giddings E, Chen Y, Champagne D, Fernández-Ramos D, Mestre D, Gomez-Santos B, Varela-Rey M, de Juan VG, Fernández-Tussy P, Zubiete-Franco I, García-Monzón C, González-Rodríguez Á, Oza D, Valença-Pereira F, Fang Q, Crespo J, Aspichueta P, Tremblay F, Christensen BC, Anguita J, Martínez-Chantar ML, Rincón M. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation. *Nat Commun* 2020; **11**: 3360 [PMID: 32620763 DOI: 10.1038/s41467-020-16991-2]

21 **Jian C**, Fu J, Cheng X, Shen LJ, Ji YX, Wang X, Pan S, Tian H, Tian S, Liao R, Song K, Wang HP, Zhang X, Wang Y, Huang Z, She ZG, Zhang XJ, Zhu L, Li H. Low-Dose Sorafenib Acts as a Mitochondrial Uncoupler and Ameliorates Nonalcoholic Steatohepatitis. *Cell Metab* 2020; **31**: 892-908.e11 [PMID: 32375062 DOI: 10.1016/j.cmet.2020.04.011]

22 **Wang X**, Du H, Shao S, Bo T, Yu C, Chen W, Zhao L, Li Q, Wang L, Liu X, Su X, Sun M, Song Y, Gao L, Zhao J. Cyclophilin D deficiency attenuates mitochondrial perturbation and ameliorates hepatic steatosis. *Hepatology* 2018; **68**: 62-77 [PMID: 29356058 DOI: 10.1002/hep.29788]

23 **Liu C**, Zhou B, Meng M, Zhao W, Wang D, Yuan Y, Zheng Y, Qiu J, Li Y, Li G, Xiong X, Bian H, Zhang H, Wang H, Ma X, Hu C, Xu L, Lu Y. FOXA3 induction under endoplasmic reticulum stress contributes to non-alcoholic fatty liver

disease. *J Hepatol* 2021; **75**: 150-162 [PMID: 33548387 DOI: 10.1016/j.jhep.2021.01.042]

24 **Zhang Y**, Ye S, Lu W, Zhong J, Leng Y, Yang T, Luo J, Xu W, Zhang H, Kong L. RNA helicase DEAD-box protein 5 alleviates nonalcoholic steatohepatitis progression *via* tethering TSC complex and suppressing mTORC1 signaling. *Hepatology* 2022 [PMID: 35796622 DOI: 10.1002/hep.32651]

25 **Amrutkar M**, Kern M, Nuñez-Durán E, Ståhlman M, Cansby E, Chursa U, Stenfeldt E, Borén J, Blüher M, Mahlapuu M. Protein kinase STK25 controls lipid partitioning in hepatocytes and correlates with liver fat content in humans. *Diabetologia* 2016; **59**: 341-353 [PMID: 26553096 DOI: 10.1007/s00125-015-3801-7]

26 **Amrutkar M**, Cansby E, Nuñez-Durán E, Pirazzi C, Ståhlman M, Stenfeldt E, Smith U, Borén J, Mahlapuu M. Protein kinase STK25 regulates hepatic lipid partitioning and progression of liver steatosis and NASH. *FASEB J* 2015; **29**: 1564-1576 [PMID: 25609431 DOI: 10.1096/fj.14-264937]

27 **Amrutkar M**, Chursa U, Kern M, Nuñez-Durán E, Ståhlman M, Sütt S, Borén J, Johansson BR, Marschall HU, Blüher M, Mahlapuu M. STK25 is a critical determinant in nonalcoholic steatohepatitis. *FASEB J* 2016; **30**: 3628-3643 [PMID: 27421788 DOI: 10.1096/fj.201600562R]

28 **Nuñez-Durán E**, Aghajan M, Amrutkar M, Sütt S, Cansby E, Booten SL, Watt A, Ståhlman M, Stefan N, Häring HU, Staiger H, Borén J, Marschall HU, Mahlapuu M. Serine/threonine protein kinase 25 antisense oligonucleotide treatment reverses glucose intolerance, insulin resistance, and nonalcoholic fatty liver disease in mice. *Hepatol Commun* 2018; **2**: 69-83 [PMID: 29404514 DOI: 10.1002/hep4.1128]

29 **Kurhe Y**, Caputo M, Cansby E, Xia Y, Kumari S, Anand SK, Howell BW, Marschall HU, Mahlapuu M. Antagonizing STK25 Signaling Suppresses the Development of Hepatocellular Carcinoma Through Targeting Metabolic, Inflammatory, and Pro-Oncogenic Pathways. *Cell Mol Gastroenterol Hepatol* 2022; **13**: 405-423 [PMID: 34624527 DOI: 10.1016/j.jcmgh.2021.09.018]

30 **Chai C**, Cox B, Yaish D, Gross D, Rosenberg N, Amblard F, Shemuelian Z, Gefen M, Korach A, Tirosh O, Lanton T, Link H, Tam J, Permyakova A, Ozhan G, Citrin J, Liao H, Tannous M, Hahn M, Axelrod J, Arretxe E, Alonso C, Martinez-Arranz I, Betés PO, Safadi R, Salhab A, Amer J, Tber Z, Mengshetti S, Giladi H, Schinazi RF, Galun E. Agonist of RORA Attenuates Nonalcoholic Fatty Liver Progression in Mice *via* Up-regulation of MicroRNA 122. *Gastroenterology* 2020; **159**: 999-1014.e9 [PMID: 32450149 DOI: 10.1053/j.gastro.2020.05.056]

31 **Zhong D**, Cai J, Hu C, Chen J, Zhang R, Fan C, Li S, Zhang H, Xu Z, Jia Z, Guo D, Sun Y. Inhibition of mPGES-2 ameliorates NASH by activating NR1D1 *via* heme. *Hepatology* 2022 [PMID: 35839302 DOI: 10.1002/hep.32671]

32 **Zhang X**, Li S, Zhou Y, Su W, Ruan X, Wang B, Zheng F, Warner M, Gustafsson JÅ, Guan Y. Ablation of cytochrome P450 omega-hydroxylase 4A14 gene attenuates hepatic steatosis and fibrosis. *Proc Natl Acad Sci U S A* 2017; **114**: 3181-3185 [PMID: 28270609 DOI: 10.1073/pnas.1700172114]

33 **Li S**, Su W, Zhang XY, Guan YF. [Arachidonic acid metabolism in liver glucose and lipid homeostasis]. *Sheng Li Xue Bao* 2021; **73**: 657-664 [PMID: 34405221]

34 **Su W**, Wang Y, Jia X, Wu W, Li L, Tian X, Li S, Wang C, Xu H, Cao J, Han Q, Xu S, Chen Y, Zhong Y, Zhang X, Liu P, Gustafsson JÅ, Guan Y. Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A* 2014; **111**: 11437-11442 [PMID: 25028495 DOI: 10.1073/pnas.1410741111]

35 **Kozlitina J**. Genetic Risk Factors and Disease Modifiers of Nonalcoholic Steatohepatitis. *Gastroenterol Clin North Am* 2020; **49**: 25-44 [PMID: 32033763 DOI: 10.1016/j.gtc.2019.09.001]

36 **Zhang HB**, Su W, Xu H, Zhang XY, Guan YF. HSD17B13: A Potential Therapeutic Target for NAFLD. *Front Mol Biosci* 2021; **8**: 824776 [PMID: 35071330 DOI: 10.3389/fmolb.2021.824776]

37 **Su W**, Mao Z, Liu Y, Zhang X, Zhang W, Gustafsson JA, Guan Y. Role of HSD17B13 in the liver physiology and pathophysiology. *Mol Cell Endocrinol* 2019; **489**: 119-125 [PMID: 30365983 DOI: 10.1016/j.mce.2018.10.014]

38 **Su W**, Wu S, Yang Y, Guo Y, Zhang H, Su J, Chen L, Mao Z, Lan R, Cao R, Wang C, Xu H, Zhang C, Li S, Gao M, Chen X, Zheng Z, Wang B, Liu Y, Liu Z, Wang Z, Liu B, Fan X, Zhang X, Guan Y. Phosphorylation of 17β-hydroxysteroid dehydrogenase 13 at serine 33 attenuates nonalcoholic fatty liver disease in mice. *Nat Commun* 2022; **13**: 6577 [PMID: 36323699 DOI: 10.1038/s41467-022-34299-1]

39 **Mancina RM**, Sasidharan K, Lindblom A, Wei Y, Ciociola E, Jamialahmadi O, Pingitore P, Andréasson AC, Pellegrini G, Baselli G, Männistö V, Pihlajamäki J, Kärjä V, Grimaudo S, Marini I, Maggioni M, Becattini B, Tavaglione F, Dix C, Castaldo M, Klein S, Perelis M, Pattou F, Thuillier D, Raverdy V, Dongiovanni P, Fracanzani AL, Stickel F, Hampe J, Buch S, Luukkonen PK, Prati D, Yki-Järvinen H, Petta S, Xing C, Schafmayer C, Aigner E, Datz C, Lee RG, Valenti L, Lindén D, Romeo S. PSD3 downregulation confers protection against fatty liver disease. *Nat Metab* 2022; **4**: 60-75 [PMID: 35102341 DOI: 10.1038/s42255-021-00518-0]

40 **Loomba R**, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 2021; **184**: 2537-2564 [PMID: 33989548 DOI: 10.1016/j.cell.2021.04.015]

41 **Ioannou GN**. The Role of Cholesterol in the Pathogenesis of NASH. *Trends Endocrinol Metab* 2016; **27**: 84-95 [PMID: 26703097 DOI: 10.1016/j.tem.2015.11.008]

42 **Choi SE**, Hwang Y, Lee SJ, Jung H, Shin TH, Son Y, Park S, Han SJ, Kim HJ, Lee KW, Lee G, Kemper JK, Song HK, Kang Y. Mitochondrial protease ClpP supplementation ameliorates diet-induced NASH in mice. *J Hepatol* 2022; **77**: 735-747 [PMID: 35421426 DOI: 10.1016/j.jhep.2022.03.034]

43 **Win S**, Min RWM, Zhang J, Kanel G, Wanken B, Chen Y, Li M, Wang Y, Suzuki A, Aung FWM, Murray SF, Aghajan M, Than TA, Kaplowitz N. Hepatic Mitochondrial SAB Deletion or Knockdown Alleviates Diet-Induced Metabolic Syndrome, Steatohepatitis, and Hepatic Fibrosis. *Hepatology* 2021; **74**: 3127-3145 [PMID: 34331779 DOI: 10.1002/hep.32083]

44 **Tang BL**. Sirt1 and the Mitochondria. *Mol Cells* 2016; **39**: 87-95 [PMID: 26831453 DOI: 10.14348/molcells.2016.2318]

45 **Katsyuba E**, Mottis A, Zietak M, De Franco F, van der Velpen V, Gariani K, Ryu D, Cialabrini L, Matilainen O, Liscio P, Giacchè N, Stokar-Regenscheit N, Legouis D, de Seigneux S, Ivanisevic J, Raffaelli N, Schoonjans K, Pellicciari R, Auwerx J. De novo NAD(+) synthesis enhances mitochondrial function and improves health. *Nature* 2018; **563**: 354-359 [PMID: 30356218 DOI: 10.1038/s41586-018-0645-6]

46 **Chen XY**, Cai CZ, Yu ML, Feng ZM, Zhang YW, Liu PH, Zeng H, Yu CH. LB100 ameliorates nonalcoholic fatty liver disease *via* the AMPK/Sirt1 pathway. *World J Gastroenterol* 2019; **25**: 6607-6618 [PMID: 31832001 DOI: 10.3748/wjg.v25.i45.6607]

47 **Li CX**, Gao JG, Wan XY, Chen Y, Xu CF, Feng ZM, Zeng H, Lin YM, Ma H, Xu P, Yu CH, Li YM. Allyl isothiocyanate ameliorates lipid accumulation and inflammation in nonalcoholic fatty liver disease *via* the Sirt1/AMPK and NF-κB signaling pathways. *World J Gastroenterol* 2019; **25**: 5120-5133 [PMID: 31558861 DOI: 10.3748/wjg.v25.i34.5120]

48 **Zhang Y**, Geng C, Liu X, Li M, Gao M, Fang F, Chang Y. Celastrol ameliorates liver metabolic damage caused by a high-fat diet through Sirt1. *Mol Metab* 2017; **6**: 138-147 [PMID: 28123944 DOI: 10.1016/j.molmet.2016.11.002]

49 **Liu X**, Gao Y, Li M, Geng C, Xu H, Yang Y, Guo Y, Jiao T, Fang F, Chang Y. Sirt1 mediates the effect of the heme oxygenase inducer, cobalt protoporphyrin, on ameliorating liver metabolic damage caused by a high-fat diet. *J Hepatol* 2015; **63**: 713-721 [PMID: 26026874 DOI: 10.1016/j.jhep.2015.05.018]

50 **Mohs A**, Otto T, Schneider KM, Peltzer M, Boekschoten M, Holland CH, Hudert CA, Kalveram L, Wiegand S, Saez-Rodriguez J, Longerich T, Hengstler JG, Trautwein C. Hepatocyte-specific NRF2 activation controls fibrogenesis and carcinogenesis in steatohepatitis. *J Hepatol* 2021; **74**: 638-648 [PMID: 33342543 DOI: 10.1016/j.jhep.2020.09.037]

51 **Sharma RS**, Harrison DJ, Kisielewski D, Cassidy DM, McNeilly AD, Gallagher JR, Walsh SV, Honda T, McCrimmon RJ, Dinkova-Kostova AT, Ashford MLJ, Dillon JF, Hayes JD. Experimental Nonalcoholic Steatohepatitis and Liver Fibrosis Are Ameliorated by Pharmacologic Activation of Nrf2 (NF-E2 p45-Related Factor 2). *Cell Mol Gastroenterol Hepatol* 2018; **5**: 367-398 [PMID: 29552625 DOI: 10.1016/j.jcmgh.2017.11.016]

52 **Shimozono R**, Asaoka Y, Yoshizawa Y, Aoki T, Noda H, Yamada M, Kaino M, Mochizuki H. Nrf2 activators attenuate the progression of nonalcoholic steatohepatitis-related fibrosis in a dietary rat model. *Mol Pharmacol* 2013; **84**: 62-70 [PMID: 23592516 DOI: 10.1124/mol.112.084269]

53 **Sano A**, Kakazu E, Hamada S, Inoue J, Ninomiya M, Iwata T, Tsuruoka M, Sato K, Masamune A. Steatotic Hepatocytes Release Mature VLDL Through Methionine and Tyrosine Metabolism in a Keap1-Nrf2-Dependent Manner. *Hepatology* 2021; **74**: 1271-1286 [PMID: 33724516 DOI: 10.1002/hep.31808]

54 **Li X**, Wang J, Gong X, Zhang M, Kang S, Shu B, Wei Z, Huang ZS, Li D. Upregulation of BCL-2 by acridone derivative through gene promoter i-motif for alleviating liver damage of NAFLD/NASH. *Nucleic Acids Res* 2020; **48**: 8255-8268 [PMID: 32710621 DOI: 10.1093/nar/gkaa615]

55 **Afonso MB**, Rodrigues PM, Carvalho T, Caridade M, Borralho P, Cortez-Pinto H, Castro RE, Rodrigues CM. Necroptosis is a key pathogenic event in human and experimental murine models of non-alcoholic steatohepatitis. *Clin Sci (Lond)* 2015; **129**: 721-739 [PMID: 26201023 DOI: 10.1042/CS20140732]

56 **Majdi A**, Aoudjehane L, Ratziu V, Islam T, Afonso MB, Conti F, Mestiri T, Lagouge M, Foufelle F, Ballenghien F, Ledent T, Moldes M, Cadoret A, Fouassier L, Delaunay JL, Aït-Slimane T, Courtois G, Fève B, Scatton O, Prip-Buus C, Rodrigues CMP, Housset C, Gautheron J. Inhibition of receptor-interacting protein kinase 1 improves experimental non-alcoholic fatty liver disease. *J Hepatol* 2020; **72**: 627-635 [PMID: 31760070 DOI: 10.1016/j.jhep.2019.11.008]

57 **Handa P**, Morgan-Stevenson V, Maliken BD, Nelson JE, Washington S, Westerman M, Yeh MM, Kowdley KV. Iron overload results in hepatic oxidative stress, immune cell activation, and hepatocellular ballooning injury, leading to nonalcoholic steatohepatitis in genetically obese mice. *Am J Physiol Gastrointest Liver Physiol* 2016; **310**: G117-G127 [PMID: 26564716 DOI: 10.1152/ajpgi.00246.2015]

58 **Datz C**, Müller E, Aigner E. Iron overload and non-alcoholic fatty liver disease. *Minerva Endocrinol* 2017; **42**: 173-183 [PMID: 27834478 DOI: 10.23736/S0391-1977.16.02565-7]

59 **Wang S**, Liu Z, Geng J, Li L, Feng X. An overview of ferroptosis in non-alcoholic fatty liver disease. *Biomed Pharmacother* 2022; **153**: 113374 [PMID: 35834990 DOI: 10.1016/j.biopha.2022.113374]

60 **Tsurusaki S**, Tsuchiya Y, Koumura T, Nakasone M, Sakamoto T, Matsuoka M, Imai H, Yuet-Yin Kok C, Okochi H, Nakano H, Miyajima A, Tanaka M. Hepatic ferroptosis plays an important role as the trigger for initiating inflammation in nonalcoholic steatohepatitis. *Cell Death Dis* 2019; **10**: 449 [PMID: 31209199 DOI: 10.1038/s41419-019-1678-y]

61 **Kawahara A**, Kanno K, Yonezawa S, Otani Y, Kobayashi T, Tazuma S, Ito M. Depletion of hepatic stellate cells inhibits hepatic steatosis in mice. *J Gastroenterol Hepatol* 2022; **37**: 1946-1954 [PMID: 35933582 DOI: 10.1111/jgh.15974]

62 **Ou R**, Liu J, Lv M, Wang J, Zhu L, Zhao L, Xu Y. Neutrophil depletion improves diet-induced non-alcoholic fatty liver disease in mice. *Endocrine* 2017; **57**: 72-82 [PMID: 28508193 DOI: 10.1007/s12020-017-1323-4]

63 **Leslie J**, Mackey JBG, Jamieson T, Ramon-Gil E, Drake TM, Fercoq F, Clark W, Gilroy K, Hedley A, Nixon C, Luli S, Laszczewska M, Pinyol R, Esteban-Fabró R, Willoughby CE, Haber PK, Andreu-Oller C, Rahbari M, Fan C, Pfister D, Raman S, Wilson N, Müller M, Collins A, Geh D, Fuller A, McDonald D, Hulme G, Filby A, Cortes-Lavaud X, Mohamed NE, Ford CA, Raffo Iraolagoitia XL, McFarlane AJ, McCain MV, Ridgway RA, Roberts EW, Barry ST, Graham GJ, Heikenwälder M, Reeves HL, Llovet JM, Carlin LM, Bird TG, Sansom OJ, Mann DA. CXCR2 inhibition enables NASH-HCC immunotherapy. *Gut* 2022; **71**: 2093-2106 [PMID: 35477863 DOI: 10.1136/gutjnl-2021-326259]

64 **Ye D**, Yang K, Zang S, Lin Z, Chau HT, Wang Y, Zhang J, Shi J, Xu A, Lin S. Lipocalin-2 mediates non-alcoholic steatohepatitis by promoting neutrophil-macrophage crosstalk *via* the induction of CXCR2. *J Hepatol* 2016; **65**: 988-997 [PMID: 27266617 DOI: 10.1016/j.jhep.2016.05.041]

65 **Phillips BE**, Lantier L, Engman C, Garciafigueroa Y, Singhi A, Trucco M, Mantzoros C, Wasserman D, Giannoukakis N. Improvement in insulin sensitivity and prevention of high fat diet-induced liver pathology using a CXCR2 antagonist. *Cardiovasc Diabetol* 2022; **21**: 130 [PMID: 35831885 DOI: 10.1186/s12933-022-01564-y]

66 **Pfister D**, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, Gupta R, Qiu M, Deczkowska A, Weiner A, Müller F, Sinha A, Friebel E, Engleitner T, Lenggenhager D, Moncsek A, Heide D, Stirm K, Kosla J, Kotsiliti E, Leone V, Dudek M, Yousuf S, Inverso D, Singh I, Teijeiro A, Castet F, Montironi C, Haber PK, Tiniakos D, Bedossa P, Cockell S, Younes R, Vacca M, Marra F, Schattenberg JM, Allison M, Bugianesi E, Ratziu V, Pressiani T, D'Alessio A, Personeni N, Rimassa L, Daly AK, Scheiner B, Pomej K, Kirstein MM, Vogel A, Peck-Radosavljevic M, Hucke F, Finkelmeier F, Waidmann O, Trojan J, Schulze K, Wege H, Koch S, Weinmann A, Bueter M, Rössler F, Siebenhüner A, De Dosso S, Mallm JP, Umansky V, Jugold M, Luedde T, Schietinger A, Schirmacher P, Emu B, Augustin HG, Billeter A, Müller-Stich B, Kikuchi H, Duda DG, Kütting F, Waldschmidt DT, Ebert MP, Rahbari N, Mei HE, Schulz AR, Ringelhan M,

Malek N, Spahn S, Bitzer M, Ruiz de Galarreta M, Lujambio A, Dufour JF, Marron TU, Kaseb A, Kudo M, Huang YH, Djouder N, Wolter K, Zender L, Marche PN, Decaens T, Pinato DJ, Rad R, Mertens JC, Weber A, Unger K, Meissner F, Roth S, Jilkova ZM, Claassen M, Anstee QM, Amit I, Knolle P, Becher B, Llovet JM, Heikenwalder M. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. *Nature* 2021; **592**: 450-456 [PMID: 33762733 DOI: 10.1038/s41586-021-03362-0]

67 **Govaere O**, Petersen SK, Martinez-Lopez N, Wouters J, Van Haele M, Mancina RM, Jamialahmadi O, Bilkei-Gorzo O, Lassen PB, Darlay R, Peltier J, Palmer JM, Younes R, Tiniakos D, Aithal GP, Allison M, Vacca M, Göransson M, Berlinguer-Palmini R, Clark JE, Drinnan MJ, Yki-Järvinen H, Dufour JF, Ekstedt M, Francque S, Petta S, Bugianesi E, Schattenberg JM, Day CP, Cordell HJ, Topal B, Clément K, Romeo S, Ratziu V, Roskams T, Daly AK, Anstee QM, Trost M, Härtlova A. Macrophage scavenger receptor 1 mediates lipid-induced inflammation in non-alcoholic fatty liver disease. *J Hepatol* 2022; **76**: 1001-1012 [PMID: 34942286 DOI: 10.1016/j.jhep.2021.12.012]

68 **Mridha AR**, Wree A, Robertson AAB, Yeh MM, Johnson CD, Van Rooyen DM, Haczeyni F, Teoh NC, Savard C, Ioannou GN, Masters SL, Schroder K, Cooper MA, Feldstein AE, Farrell GC. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J Hepatol* 2017; **66**: 1037-1046 [PMID: 28167322 DOI: 10.1016/j.jhep.2017.01.022]

69 **Wang Q**, Zhou H, Bu Q, Wei S, Li L, Zhou J, Zhou S, Su W, Liu M, Liu Z, Wang M, Lu L. Role of XBP1 in regulating the progression of non-alcoholic steatohepatitis. *J Hepatol* 2022; **77**: 312-325 [PMID: 35292349 DOI: 10.1016/j.jhep.2022.02.031]

70 **Franceschetti L**, Bonomini F, Rodella LF, Rezzani R. Critical Role of NFκB in the Pathogenesis of Non-alcoholic Fatty Liver Disease: A Widespread Key Regulator. *Curr Mol Med* 2021; **21**: 495-505 [PMID: 33106141 DOI: 10.2174/1566524020666201026162343]

71 **Zhang T**, Hu J, Wang X, Zhao X, Li Z, Niu J, Steer CJ, Zheng G, Song G. MicroRNA-378 promotes hepatic inflammation and fibrosis *via* modulation of the NF-κB-TNFα pathway. *J Hepatol* 2019; **70**: 87-96 [PMID: 30218679 DOI: 10.1016/j.jhep.2018.08.026]

72 **Maradana MR**, Yekollu SK, Zeng B, Ellis J, Clouston A, Miller G, Talekar M, Bhuyan ZA, Mahadevaiah S, Powell EE, Irvine KM, Thomas R, O'Sullivan BJ. Immunomodulatory liposomes targeting liver macrophages arrest progression of nonalcoholic steatohepatitis. *Metabolism* 2018; **78**: 80-94 [PMID: 28941597 DOI: 10.1016/j.metabol.2017.09.002]

73 **Sutti S**, Locatelli I, Bruzzì S, Jindal A, Vacchiano M, Bozzola C, Albano E. CX3CR1-expressing inflammatory dendritic cells contribute to the progression of steatohepatitis. *Clin Sci (Lond)* 2015; **129**: 797-808 [PMID: 26253086 DOI: 10.1042/CS20150053]

74 **Roh YS**, Kim JW, Park S, Shon C, Kim S, Eo SK, Kwon JK, Lim CW, Kim B. Toll-Like Receptor-7 Signaling Promotes Nonalcoholic Steatohepatitis by Inhibiting Regulatory T Cells in Mice. *Am J Pathol* 2018; **188**: 2574-2588 [PMID: 30125542 DOI: 10.1016/j.ajpath.2018.07.011]

75 **Rai RP**, Liu Y, Iyer SS, Liu S, Gupta B, Desai C, Kumar P, Smith T, Singhi AD, Nusrat A, Parkos CA, Monga SP, Czaja MJ, Anania FA, Raeman R. Blocking integrin α(4)β(7)-mediated CD4 T cell recruitment to the intestine and liver protects mice from western diet-induced non-alcoholic steatohepatitis. *J Hepatol* 2020; **73**: 1013-1022 [PMID: 32540177 DOI: 10.1016/j.jhep.2020.05.047]

76 **Wang H**, Zhang H, Wang Y, Brown ZJ, Xia Y, Huang Z, Shen C, Hu Z, Beane J, Ansa-Addo EA, Huang H, Tian D, Tsung A. Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. *J Hepatol* 2021; **75**: 1271-1283 [PMID: 34363921 DOI: 10.1016/j.jhep.2021.07.032]

77 **Bruzzì S**, Sutti S, Giudici G, Burlone ME, Ramavath NN, Toscani A, Bozzola C, Schneider P, Morello E, Parola M, Pirisi M, Albano E. B2-Lymphocyte responses to oxidative stress-derived antigens contribute to the evolution of nonalcoholic fatty liver disease (NAFLD). *Free Radic Biol Med* 2018; **124**: 249-259 [PMID: 29920340 DOI: 10.1016/j.freeradbiomed.2018.06.015]

78 **Wang C**, Yang J, Zhang X. Editorial: The Role of Bioactive Lipids in Homeostasis and Pathology. *Front Physiol* 2021; **12**: 773632 [PMID: 34880781 DOI: 10.3389/fphys.2021.773632]

79 **Musso G**, Cassader M, Paschetta E, Gambino R. Bioactive Lipid Species and Metabolic Pathways in Progression and Resolution of Nonalcoholic Steatohepatitis. *Gastroenterology* 2018; **155**: 282-302.e8 [PMID: 29906416 DOI: 10.1053/j.gastro.2018.06.031]

80 **Liu X**, Wang K, Wang L, Kong L, Hou S, Wan Y, Ma C, Chen J, Xing X, Xing C, Jiang Q, Zhao Q, Cui B, Huang Z, Li P. Hepatocyte leukotriene B4 receptor 1 promotes NAFLD development in obesity. *Hepatology* 2022 [PMID: 35931467 DOI: 10.1002/hep.32708]

81 **Kaliannan K**, Li XY, Wang B, Pan Q, Chen CY, Hao L, Xie S, Kang JX. Multi-omic analysis in transgenic mice implicates omega-6/omega-3 fatty acid imbalance as a risk factor for chronic disease. *Commun Biol* 2019; **2**: 276 [PMID: 31372515 DOI: 10.1038/s42003-019-0521-4]

82 **López-Vicario C**, Sebastián D, Casulleras M, Duran-Güell M, Flores-Costa R, Aguilar F, Lozano JJ, Zhang IW, Titos E, Kang JX, Zorzano A, Arita M, Clària J. Essential lipid autacoids rewire mitochondrial energy efficiency in metabolic dysfunction-associated fatty liver disease. *Hepatology* 2022 [PMID: 35788956 DOI: 10.1002/hep.32647]

83 **Duan J**, Song Y, Zhang X, Wang C. Effect of ω-3 Polyunsaturated Fatty Acids-Derived Bioactive Lipids on Metabolic Disorders. *Front Physiol* 2021; **12**: 646491 [PMID: 34113260 DOI: 10.3389/fphys.2021.646491]

84 **Fraser DA**, Wang X, Lund J, Nikolić N, Iruarrizaga-Lejarreta M, Skjaeret T, Alonso C, Kastelein JJP, Rustan AC, Kim YO, Schuppan D. A structurally engineered fatty acid, icosabutate, suppresses liver inflammation and fibrosis in NASH. *J Hepatol* 2022; **76**: 800-811 [PMID: 34915054 DOI: 10.1016/j.jhep.2021.12.004]

85 **Zhu C**, Kim K, Wang X, Bartolome A, Salomao M, Dongiovanni P, Meroni M, Graham MJ, Yates KP, Diehl AM, Schwabe RF, Tabas I, Valenti L, Lavine JE, Pajvani UB. Hepatocyte Notch activation induces liver fibrosis in nonalcoholic steatohepatitis. *Sci Transl Med* 2018; **10** [PMID: 30463916 DOI: 10.1126/scitranslmed.aat0344]

86 **Wang G**, Duan J, Pu G, Ye C, Li Y, Xiu W, Xu J, Liu B, Zhu Y, Wang C. The Annexin A2-Notch regulatory loop in hepatocytes promotes liver fibrosis in NAFLD by increasing osteopontin expression. *Biochim Biophys Acta Mol Basis Dis* 2022; **1868**: 166413 [PMID: 35413401 DOI: 10.1016/j.bbadis.2022.166413]

87 **Syn WK**, Choi SS, Liaskou E, Karaca GF, Agboola KM, Oo YH, Mi Z, Pereira TA, Zdanowicz M, Malladi P, Chen Y, Moylan C, Jung Y, Bhattacharya SD, Teaberry V, Omenetti A, Abdelmalek MF, Guy CD, Adams DH, Kuo PC, Michelotti GA, Whitington PF, Diehl AM. Osteopontin is induced by hedgehog pathway activation and promotes fibrosis progression in nonalcoholic steatohepatitis. *Hepatology* 2011; **53**: 106-115 [PMID: 20967826 DOI: 10.1002/hep.23998]

88 **Richter LR**, Wan Q, Wen D, Zhang Y, Yu J, Kang JK, Zhu C, McKinnon EL, Gu Z, Qiang L, Pajvani UB. Targeted Delivery of Notch Inhibitor Attenuates Obesity-Induced Glucose Intolerance and Liver Fibrosis. *ACS Nano* 2020; **14**: 6878-6886 [PMID: 32441510 DOI: 10.1021/acsnano.0c01007]

89 **Du M**, Wang X, Yuan L, Liu B, Mao X, Huang D, Yang L, Huang K, Zhang F, Wang Y, Luo X, Wang C, Peng J, Liang M. Targeting NFATc4 attenuates non-alcoholic steatohepatitis in mice. *J Hepatol* 2020; **73**: 1333-1346 [PMID: 32717288 DOI: 10.1016/j.jhep.2020.07.030]

90 **Wang X**, Zheng Z, Caviglia JM, Corey KE, Herfel TM, Cai B, Masia R, Chung RT, Lefkowitch JH, Schwabe RF, Tabas I. Hepatocyte TAZ/WWTR1 Promotes Inflammation and Fibrosis in Nonalcoholic Steatohepatitis. *Cell Metab* 2016; **24**: 848-862 [PMID: 28068223 DOI: 10.1016/j.cmet.2016.09.016]

91 **Wang X**, Sommerfeld MR, Jahn-Hofmann K, Cai B, Filliol A, Remotti HE, Schwabe RF, Kannt A, Tabas I. A Therapeutic Silencing RNA Targeting Hepatocyte TAZ Prevents and Reverses Fibrosis in Nonalcoholic Steatohepatitis in Mice. *Hepatol Commun* 2019; **3**: 1221-1234 [PMID: 31497743 DOI: 10.1002/hep4.1405]

92 **Xi Y**, LaCanna R, Ma HY, N'Diaye EN, Gierke S, Caplazi P, Sagolla M, Huang Z, Lucio L, Arlantico A, Jeet S, Brightbill H, Emson C, Wong A, Morshead KB, DePianto DJ, Roose-Girma M, Yu C, Tam L, Jia G, Ramalingam TR, Marsters S, Ashkenazi A, Kim SH, Kelly R, Wu S, Wolters PJ, Feldstein AE, Vander Heiden JA, Ding N. A WISP1 antibody inhibits MRTF signaling to prevent the progression of established liver fibrosis. *Cell Metab* 2022; **34**: 1377-1393.e8 [PMID: 35987202 DOI: 10.1016/j.cmet.2022.07.009]

93 **Widjaja AA**, Singh BK, Adami E, Viswanathan S, Dong J, D'Agostino GA, Ng B, Lim WW, Tan J, Paleja BS, Tripathi M, Lim SY, Shekeran SG, Chothani SP, Rabes A, Sombetzki M, Bruinstroop E, Min LP, Sinha RA, Albani S, Yen PM, Schafer S, Cook SA. Inhibiting Interleukin 11 Signaling Reduces Hepatocyte Death and Liver Fibrosis, Inflammation, and Steatosis in Mouse Models of Nonalcoholic Steatohepatitis. *Gastroenterology* 2019; **157**: 777-792.e14 [PMID: 31078624 DOI: 10.1053/j.gastro.2019.05.002]

94 **Song Z**, Liu X, Zhang W, Luo Y, Xiao H, Liu Y, Dai G, Hong J, Li A. Ruxolitinib suppresses liver fibrosis progression and accelerates fibrosis reversal *via* selectively targeting Janus kinase 1/2. *J Transl Med* 2022; **20**: 157 [PMID: 35382859 DOI: 10.1186/s12967-022-03366-y]

95 **Torres S**, Ortiz C, Bachtler N, Gu W, Grünewald LD, Kraus N, Schierwagen R, Hieber C, Meier C, Tyc O, Joseph Brol M, Uschner FE, Nijmeijer B, Welsch C, Berres ML, Garcia-Ruiz C, Fernandez-Checa JC, Trautwein C, Vogl TJ, Zeuzem S, Trebicka J, Klein S. Janus kinase 2 inhibition by pacritinib as potential therapeutic target for liver fibrosis. *Hepatology* 2022 [PMID: 35993369 DOI: 10.1002/hep.32746]

96 **Martí-Rodrigo A**, Alegre F, Moragrega ÁB, García-García F, Martí-Rodrigo P, Fernández-Iglesias A, Gracia-Sancho J, Apostolova N, Esplugues JV, Blas-García A. Rilpivirine attenuates liver fibrosis through selective STAT1-mediated apoptosis in hepatic stellate cells. *Gut* 2020; **69**: 920-932 [PMID: 31530714 DOI: 10.1136/gutjnl-2019-318372]

97 **Shearer AM**, Rana R, Austin K, Baleja JD, Nguyen N, Bohm A, Covic L, Kuliopulos A. Targeting Liver Fibrosis with a Cell-penetrating Protease-activated Receptor-2 (PAR2) Pepducin. *J Biol Chem* 2016; **291**: 23188-23198 [PMID: 27613872 DOI: 10.1074/jbc.M116.732743]

98 **Shearer AM**, Wang Y, Fletcher EK, Rana R, Michael ES, Nguyen N, Abdelmalek MF, Covic L, Kuliopulos A. PAR2 promotes impaired glucose uptake and insulin resistance in NAFLD through GLUT2 and Akt interference. *Hepatology* 2022; **76**: 1778-1793 [PMID: 35603482 DOI: 10.1002/hep.32589]

99 **Rana R**, Shearer AM, Fletcher EK, Nguyen N, Guha S, Cox DH, Abdelmalek M, Wang Y, Baleja JD, Covic L, Kuliopulos A. PAR2 controls cholesterol homeostasis and lipid metabolism in nonalcoholic fatty liver disease. *Mol Metab* 2019; **29**: 99-113 [PMID: 31668396 DOI: 10.1016/j.molmet.2019.08.019]

100 **Hammoutene A**, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *J Hepatol* 2019; **70**: 1278-1291 [PMID: 30797053 DOI: 10.1016/j.jhep.2019.02.012]

101 **Furuta K**, Guo Q, Pavelko KD, Lee JH, Robertson KD, Nakao Y, Melek J, Shah VH, Hirsova P, Ibrahim SH. Lipid-induced endothelial vascular cell adhesion molecule 1 promotes nonalcoholic steatohepatitis pathogenesis. *J Clin Invest* 2021; **131** [PMID: 33476308 DOI: 10.1172/JCI143690]

102 **Fang ZQ**, Ruan B, Liu JJ, Duan JL, Yue ZS, Song P, Xu H, Ding J, Xu C, Dou GR, Wang L. Notch-triggered maladaptation of liver sinusoidal endothelium aggravates nonalcoholic steatohepatitis through endothelial nitric oxide synthase. *Hepatology* 2022; **76**: 742-758 [PMID: 35006626 DOI: 10.1002/hep.32332]

103 **Liu T**, Wang Q, Zhou L, Zhang P, Mi L, Qiu X, Chen Z, Kuang H, Li S, Lin JD. Intrahepatic paracrine signaling by cardiotrophin-like cytokine factor 1 ameliorates diet-induced NASH in mice. *Hepatology* 2022 [PMID: 35950514 DOI: 10.1002/hep.32719]

104 **Yki-Järvinen H**, Luukkonen PK, Hodson L, Moore JB. Dietary carbohydrates and fats in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 770-786 [PMID: 34257427 DOI: 10.1038/s41575-021-00472-y]

105 **Gonzalez-Gil AM**, Elizondo-Montemayor L. The Role of Exercise in the Interplay between Myokines, Hepatokines, Osteokines, Adipokines, and Modulation of Inflammation for Energy Substrate Redistribution and Fat Mass Loss: A Review. *Nutrients* 2020; **12** [PMID: 32604889 DOI: 10.3390/nu12061899]

106 **Chi Y**, Wu Z, Du C, Zhang M, Wang X, Xie A, Wang P, Li R. Regulatory effects mediated by ulvan oligosaccharide and its zinc complex on lipid metabolism in high-fat diet-fed mice. *Carbohydr Polym* 2023; **300**: 120249 [PMID: 36372481 DOI: 10.1016/j.carbpol.2022.120249]

107 **White PJ**, McGarrah RW, Grimsrud PA, Tso SC, Yang WH, Haldeman JM, Grenier-Larouche T, An J, Lapworth AL, Astapova I, Hannou SA, George T, Arlotto M, Olson LB, Lai M, Zhang GF, Ilkayeva O, Herman MA, Wynn RM, Chuang DT, Newgard CB. The BCKDH Kinase and Phosphatase Integrate BCAA and Lipid Metabolism *via* Regulation of ATP-Citrate Lyase. *Cell Metab* 2018; **27**: 1281-1293.e7 [PMID: 29779826 DOI: 10.1016/j.cmet.2018.04.015]

108 **Bollinger E**, Peloquin M, Libera J, Albuquerque B, Pashos E, Shipstone A, Hadjipanayis A, Sun Z, Xing G, Clasquin M, Stansfield JC, Tierney B, Gernhardt S, Siddall CP, Greizer T, Geoly FJ, Vargas SR, Gao LC, Williams G, Marshall M, Rosado A, Steppan C, Filipski KJ, Zhang BB, Miller RA, Roth Flach RJ. BDK inhibition acts as a catabolic switch to mimic fasting and improve metabolism in mice. *Mol Metab* 2022; **66**: 101611 [PMID: 36220546 DOI: 10.1016/j.molmet.2022.101611]

109 **White PJ**, Newgard CB. Branched-chain amino acids in disease. *Science* 2019; **363**: 582-583 [PMID: 30733403 DOI: 10.1126/science.aav0558]

110 **Forlano R**, Sivakumar M, Mullish BH, Manousou P. Gut Microbiota-A Future Therapeutic Target for People with Non-Alcoholic Fatty Liver Disease: A Systematic Review. *Int J Mol Sci* 2022; **23** [PMID: 35955434 DOI: 10.3390/ijms23158307]

111 **Liu Q**, Liu S, Chen L, Zhao Z, Du S, Dong Q, Xin Y, Xuan S. Role and effective therapeutic target of gut microbiota in NAFLD/NASH. *Exp Ther Med* 2019; **18**: 1935-1944 [PMID: 31410156 DOI: 10.3892/etm.2019.7781]

112 **Xie C**, Yagai T, Luo Y, Liang X, Chen T, Wang Q, Sun D, Zhao J, Ramakrishnan SK, Sun L, Jiang C, Xue X, Tian Y, Krausz KW, Patterson AD, Shah YM, Wu Y, Gonzalez FJ. Activation of intestinal hypoxia-inducible factor 2α during obesity contributes to hepatic steatosis. *Nat Med* 2017; **23**: 1298-1308 [PMID: 29035368 DOI: 10.1038/nm.4412]

113 **Gorden DL**, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, Min J, Spann NJ, McDonald JG, Kelly SL, Duan J, Sullards MC, Leiker TJ, Barkley RM, Quehenberger O, Armando AM, Milne SB, Mathews TP, Armstrong MD, Li C, Melvin WV, Clements RH, Washington MK, Mendonsa AM, Witztum JL, Guan Z, Glass CK, Murphy RC, Dennis EA, Merrill AH Jr, Russell DW, Subramaniam S, Brown HA. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015; **56**: 722-736 [PMID: 25598080 DOI: 10.1194/jlr.P056002]

114 **Luo Y**, Yang S, Wu X, Takahashi S, Sun L, Cai J, Krausz KW, Guo X, Dias HB, Gavrilova O, Xie C, Jiang C, Liu W, Gonzalez FJ. Intestinal MYC modulates obesity-related metabolic dysfunction. *Nat Metab* 2021; **3**: 923-939 [PMID: 34211180 DOI: 10.1038/s42255-021-00421-8]

115 **Chen B**, Sun L, Zeng G, Shen Z, Wang K, Yin L, Xu F, Wang P, Ding Y, Nie Q, Wu Q, Zhang Z, Xia J, Lin J, Luo Y, Cai J, Krausz KW, Zheng R, Xue Y, Zheng MH, Li Y, Yu C, Gonzalez FJ, Jiang C. Gut bacteria alleviate smoking-related NASH by degrading gut nicotine. *Nature* 2022; **610**: 562-568 [PMID: 36261549 DOI: 10.1038/s41586-022-05299-4]

116 **Choi W**, Namkung J, Hwang I, Kim H, Lim A, Park HJ, Lee HW, Han KH, Park S, Jeong JS, Bang G, Kim YH, Yadav VK, Karsenty G, Ju YS, Choi C, Suh JM, Park JY. Serotonin signals through a gut-liver axis to regulate hepatic steatosis. *Nat Commun* 2018; **9**: 4824 [PMID: 30446669 DOI: 10.1038/s41467-018-07287-7]

117 **Yan T**, Luo Y, Yan N, Hamada K, Zhao N, Xia Y, Wang P, Zhao C, Qi D, Yang S, Sun L, Cai J, Wang Q, Jiang C, Gavrilova O, Krausz KW, Patel DP, Yu X, Wu X, Hao H, Liu W, Qu A, Gonzalez FJ. Intestinal peroxisome proliferator-activated receptor α-fatty acid-binding protein 1 axis modulates nonalcoholic steatohepatitis. *Hepatology* 2022 [PMID: 35460276 DOI: 10.1002/hep.32538]

118 **Montagner A**, Polizzi A, Fouché E, Ducheix S, Lippi Y, Lasserre F, Barquissau V, Régnier M, Lukowicz C, Benhamed F, Iroz A, Bertrand-Michel J, Al Saati T, Cano P, Mselli-Lakhal L, Mithieux G, Rajas F, Lagarrigue S, Pineau T, Loiseau N, Postic C, Langin D, Wahli W, Guillou H. Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. *Gut* 2016; **65**: 1202-1214 [PMID: 26838599 DOI: 10.1136/gutjnl-2015-310798]

119 **Cheng D**, Zinker BA, Luo Y, Shipkova P, De Oliveira CH, Krishna G, Brown EA, Boehm SL, Tirucherai GS, Gu H, Ma Z, Chu CH, Onorato JM, Kopcho LM, Ammar R, Smith J, Devasthale P, Lawrence RM, Stryker SA, Dierks EA, Azzara AV, Carayannopoulos L, Charles ED, Lentz KA, Gordon DA. MGAT2 inhibitor decreases liver fibrosis and inflammation in murine NASH models and reduces body weight in human adults with obesity. *Cell Metab* 2022; **34**: 1732-1748.e5 [PMID: 36323235 DOI: 10.1016/j.cmet.2022.10.007]

120 **Kim ER**, Park JS, Kim JH, Oh JY, Oh IJ, Choi DH, Lee YS, Park IS, Kim S, Lee DH, Cheon JH, Bae JW, Lee M, Cho JW, An IB, Nam EJ, Yang SI, Lee MS, Bae SH, Lee YH. A GLP-1/GLP-2 receptor dual agonist to treat NASH: Targeting the gut-liver axis and microbiome. *Hepatology* 2022; **75**: 1523-1538 [PMID: 34773257 DOI: 10.1002/hep.32235]

121 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]

122 **Jiang Z**, Zhao M, Voilquin L, Jung Y, Aikio MA, Sahai T, Dou FY, Roche AM, Carcamo-Orive I, Knowles JW, Wabitsch M, Appel EA, Maikawa CL, Camporez JP, Shulman GI, Tsai L, Rosen ED, Gardner CD, Spiegelman BM, Svensson KJ. Isthmin-1 is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis. *Cell Metab* 2021; **33**: 1836-1852.e11 [PMID: 34348115 DOI: 10.1016/j.cmet.2021.07.010]

123 **Liu B**, Xiang L, Ji J, Liu W, Chen Y, Xia M, Liu Y, Zhu P, Jin Y, Han Y, Lu J, Li X, Zheng M, Lu Y. Sparcl1 promotes nonalcoholic steatohepatitis progression in mice through upregulation of CCL2. *J Clin Invest* 2021; **131** [PMID: 34651580 DOI: 10.1172/JCI144801]

124 **Hedjazifar S**, Khatib Shahidi R, Hammarstedt A, Bonnet L, Church C, Boucher J, Blüher M, Smith U. The Novel Adipokine Gremlin 1 Antagonizes Insulin Action and Is Increased in Type 2 Diabetes and NAFLD/NASH. *Diabetes* 2020; **69**: 331-341 [PMID: 31882566 DOI: 10.2337/db19-0701]

125 **Zhang P**, Chen Z, Kuang H, Liu T, Zhu J, Zhou L, Wang Q, Xiong X, Meng Z, Qiu X, Jacks R, Liu L, Li S, Lumeng CN, Li Q, Zhou X, Lin JD. Neuregulin 4 suppresses NASH-HCC development by restraining tumor-prone liver microenvironment. *Cell Metab* 2022; **34**: 1359-1376.e7 [PMID: 35973424 DOI: 10.1016/j.cmet.2022.07.010]

126 **Gunasekar SK**, Xie L, Sah R. SWELL signalling in adipocytes: can fat 'feel' fat? *Adipocyte* 2019; **8**: 223-228 [PMID: 31112068 DOI: 10.1080/21623945.2019.1612223]

127 **Zhang Y**, Xie L, Gunasekar SK, Tong D, Mishra A, Gibson WJ, Wang C, Fidler T, Marthaler B, Klingelhutz A, Abel ED, Samuel I, Smith JK, Cao L, Sah R. SWELL1 is a regulator of adipocyte size, insulin signalling and glucose homeostasis. *Nat Cell Biol* 2017; **19**: 504-517 [PMID: 28436964 DOI: 10.1038/ncb3514]

128 **Gunasekar SK**, Xie L, Kumar A, Hong J, Chheda PR, Kang C, Kern DM, My-Ta C, Maurer J, Heebink J, Gerber EE, Grzesik WJ, Elliot-Hudson M, Zhang Y, Key P, Kulkarni CA, Beals JW, Smith GI, Samuel I, Smith JK, Nau P, Imai Y,

Sheldon RD, Taylor EB, Lerner DJ, Norris AW, Klein S, Brohawn SG, Kerns R, Sah R. Small molecule SWELL1 complex induction improves glycemic control and nonalcoholic fatty liver disease in murine Type 2 diabetes. *Nat Commun* 2022; **13**: 784 [PMID: 35145074 DOI: 10.1038/s41467-022-28435-0]

129 **Chakravarthy MV**, Siddiqui MS, Forsgren MF, Sanyal AJ. Harnessing Muscle-Liver Crosstalk to Treat Nonalcoholic Steatohepatitis. *Front Endocrinol (Lausanne)* 2020; **11**: 592373 [PMID: 33424768 DOI: 10.3389/fendo.2020.592373]

130 **Kuchay MS**, Martínez-Montoro JI, Kaur P, Fernández-García JC, Ramos-Molina B. Non-alcoholic fatty liver disease-related fibrosis and sarcopenia: An altered liver-muscle crosstalk leading to increased mortality risk. *Ageing Res Rev* 2022; **80**: 101696 [PMID: 35843589 DOI: 10.1016/j.arr.2022.101696]

131 **García PS**, Cabbabe A, Kambadur R, Nicholas G, Csete M. Brief-reports: elevated myostatin levels in patients with liver disease: a potential contributor to skeletal muscle wasting. *Anesth Analg* 2010; **111**: 707-709 [PMID: 20686014 DOI: 10.1213/ANE.0b013e3181eac1c9]

132 **Zhang HJ**, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW, Han CK, Zhuang XJ, Lu Y, Li XJ, Yang SY, Li XY. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. *J Hepatol* 2013; **59**: 557-562 [PMID: 23665283 DOI: 10.1016/j.jhep.2013.04.030]

133 **Li DJ**, Sun SJ, Fu JT, Ouyang SX, Zhao QJ, Su L, Ji QX, Sun DY, Zhu JH, Zhang GY, Ma JW, Lan XT, Zhao Y, Tong J, Li GQ, Shen FM, Wang P. NAD(+)-boosting therapy alleviates nonalcoholic fatty liver disease *via* stimulating a novel exerkine Fndc5/irisin. *Theranostics* 2021; **11**: 4381-4402 [PMID: 33754067 DOI: 10.7150/thno.53652]

134 **Gupta A**, Andresen JL, Manan RS, Langer R. Nucleic acid delivery for therapeutic applications. *Adv Drug Deliv Rev* 2021; **178**: 113834 [PMID: 34492233 DOI: 10.1016/j.addr.2021.113834]

135 **Loomba R**, Morgan E, Watts L, Xia S, Hannan LA, Geary RS, Baker BF, Bhanot S. Novel antisense inhibition of diacylglycerol O-acyltransferase 2 for treatment of non-alcoholic fatty liver disease: a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Gastroenterol Hepatol* 2020; **5**: 829-838 [PMID: 32553151 DOI: 10.1016/S2468-1253(20)30186-2]

136 **Li Y**, Pu S, Liu Q, Li R, Zhang J, Wu T, Chen L, Li H, Yang X, Zou M, Xiao J, Xie W, He J. An integrin-based nanoparticle that targets activated hepatic stellate cells and alleviates liver fibrosis. *J Control Release* 2019; **303**: 77-90 [PMID: 31004666 DOI: 10.1016/j.jconrel.2019.04.022]

137 **Kumar V**, Xin X, Ma J, Tan C, Osna N, Mahato RI. Therapeutic targets, novel drugs, and delivery systems for diabetes associated NAFLD and liver fibrosis. *Adv Drug Deliv Rev* 2021; **176**: 113888 [PMID: 34314787 DOI: 10.1016/j.addr.2021.113888]

138 **Duriez M**, Jacquet A, Hoet L, Roche S, Bock MD, Rocher C, Haussy G, Vigé X, Bocskei Z, Slavnic T, Martin V, Guillemot JC, Didier M, Kannt A, Orsini C, Mikol V, Fèvre AL. A 3D Human Liver Model of Nonalcoholic Steatohepatitis. *J Clin Transl Hepatol* 2020; **8**: 359-370 [PMID: 33447518 DOI: 10.14218/JCTH.2020.00015]

139 **Lee J**, Mun SJ, Shin Y, Lee S, Son MJ. Advances in liver organoids: model systems for liver disease. *Arch Pharm Res* 2022; **45**: 390-400 [PMID: 35661984 DOI: 10.1007/s12272-022-01390-6]

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MINIREVIEWS

Vibrational spectroscopy – are we close to finding a solution for early pancreatic cancer diagnosis?

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Abstract

Pancreatic cancer (PC) is an aggressive and lethal neoplasm, ranking seventh in the world for cancer deaths, with an overall 5-year survival rate of below 10%. The knowledge about PC pathogenesis is rapidly expanding. New aspects of tumor biology, including its molecular and morphological heterogeneity, have been reported to explain the complicated "cross-talk" that occurs between the cancer cells and the tumor stroma or the nature of pancreatic ductal adenocarcinoma-associated neural remodeling. Nevertheless, currently, there are no specific and sensitive diagnosis options for PC. Vibrational spectroscopy (VS) shows a promising role in the development of early diagnosis technology. In this review, we summarize recent reports about improvements in spectroscopic methodologies, briefly explain and highlight the drawbacks of each of them, and discuss available solutions. The important aspects of spectroscopic data evaluation with multivariate analysis and a convolutional neural network methodology are depicted. We conclude by presenting a study design for systemic verification of the VS-based methods in the diagnosis of PC.

Key Words: Spectroscopic cancer diagnosis; Raman spectroscopy; Pancreatic cancer diagnosis; DNA methylation; Liquid biopsy biomarkers; Convolutional neural networks

Core Tip: Vibrational spectroscopy (VS) may become a major player in the development of early diagnosis technology for pancreatic cancer. As with every technique, VS has promising attributes as well as drawbacks. We summarize recent reports about improvements in spectroscopic methodologies, briefly explain and highlight the drawbacks of each of them, and discuss available solutions. Additionally, the important aspects of spectroscopic data evaluation with multivariate analysis and a convolutional neural network methodology are depicted.

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INTRODUCTION

Pancreatic cancer (PC) is a very aggressive and lethal neoplasm, ranking seventh in the world for cancer deaths[1]. In 2020 there was an estimated number of 466003 new PC-related deaths worldwide[2]. Despite a better understanding of the nature of pancreatic tumors, 5-year survival rates have not improved and remain below 10%. Late-stage disease at diagnosis is a significant issue that contributes to poor overall survival rates. Another prognostically relevant, yet complex factor is related to the morphological and molecular heterogeneity of the tumor cells and the surrounding stroma. On one hand, the need for proper pathological evaluation with a detailed prognostic assessment is required, on the other, more importantly, the variability of PC leads to chemoresistance[3]. Moreover, differentiating PC from large tumors of the ampulla of Vater remains challenging[4]. Our previous article highlighted recent trends in PC pathology and research[3]. Vibrational spectroscopy (VS)-based methods will play prominent roles in the early diagnosis of PC (Figure 1). Detailed studies regarding the molecular nature of PC are required to reveal novel early and precise diagnostic technologies, thus improving survival rates.

EARLY DIAGNOSIS OF PC

The lack of specific and sensitive early diagnostic options for PC screening results in late-stage disease at the time of diagnosis and is one of the reasons for the poor overall PC survival rates. Out of the available options, serum antigen levels, such as carbohydrate antigen 19-9, are insufficient because of poor specificity and sensitivity for malignancy detection[5,6]. Some studies highlighted the usefulness of measuring interleukin-6 serum levels to differentiate pancreatic ductal adenocarcinoma (PDAC) from chronic or acute pancreatitis[7-9]. Recently, leukemia inhibitory factor was reported to be a promising serum biomarker of pancreatic malignancy[10], a monitoring indicator of treatment response[11-14], and a predictor of metastatic disease in PDAC patients[15].

LIQUID BIOPSY BIOMARKERS

Circulating tumor DNA

Recently, some new circulating biomarkers, particularly those associated with early-stage disease, have been intensely studied. These include a group of tumor nucleic acids in the patient's blood serum, such as circulating tumor DNA (ctDNA), cell-free DNA, cell-free RNA, circulating tumor cells, or extracellular vesicles (EVs) (Figure 2)[16]. Aberrant methylation markers specific for certain malignancies, including PDAC, were previously detected using genetic methods, such as next-generation sequencing and droplet digital polymerase chain reaction[17-20], but the sensitivity of these methods is still low[20].

EVs

Both tumor and healthy cells release lipid-membrane vesicles, termed “extracellular vesicles”, into the bloodstream. EVs include oncosomes, apoptotic bodies, microvesicles, and exosomes of different sizes and biogenesis[21,22]. They have an established role in cancer cell communication and metastasis[23,24]. EVs are released in a passive mode (apoptotic bodies) from dying cells (apoptosis, necrosis) or in an active mode (microvesicles, exosomes) from living cells[25]. They make up a large part of the ctDNA (single-strand DNA and double-strand DNA) in a liquid biopsy, but also carry other biomarkers, such as tumor protein antigens[26,27], or microRNA[28,29]. The cargo within an EV creates a unique spectro-

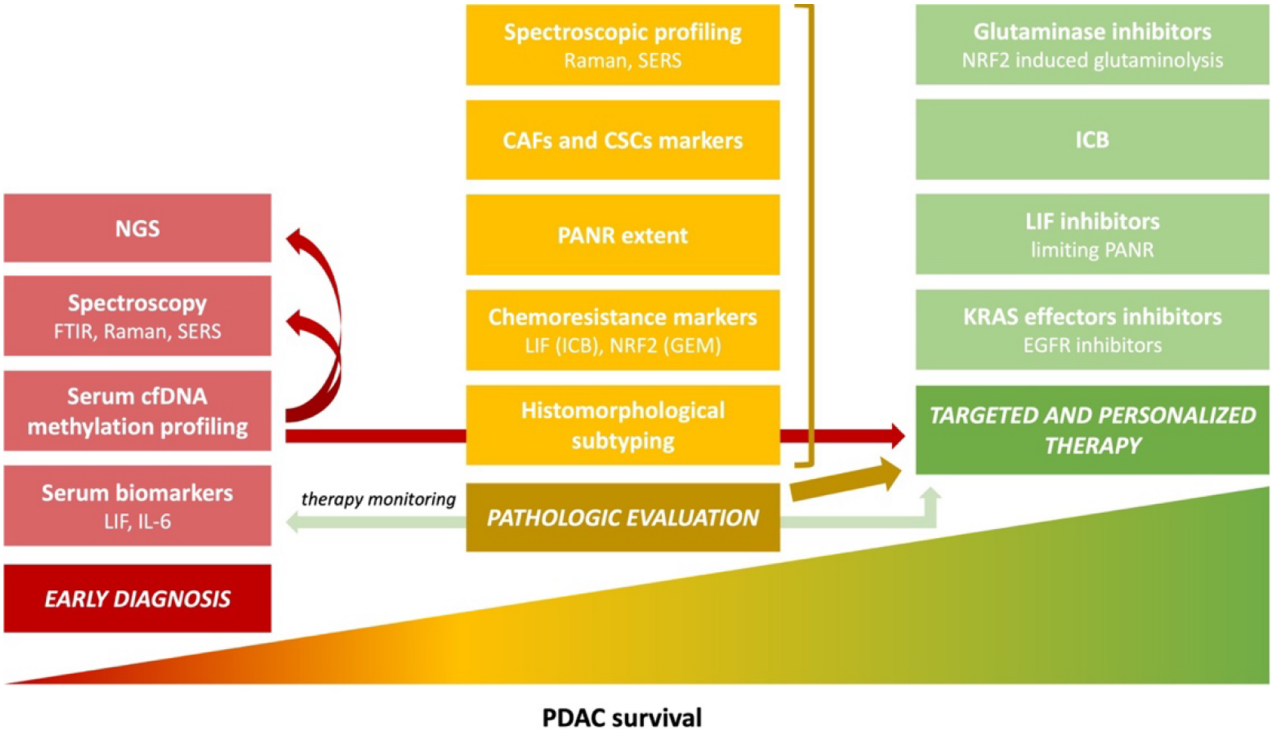

Figure 1 Main trends in pancreatic cancer pathology and research aim to improve survival[3]. Poor pancreatic ductal adenocarcinoma (PDAC) prognosis for a patient is multifactorial, and the lack of sensitive and specific early diagnostic methods is one of the reasons. Another is the resistance to available therapeutic options, which is caused, among others, by the tumor's molecular and morphological heterogeneity. Detailed pathological reporting is crucial for targeted and personalized therapy. The development of new diagnostic methods, combined with a proper pathologic evaluation and spectroscopic profiling, lead to effective treatment and supposedly will increase PDAC patients' survival rates; Adapted with permission from[3]. Citation: Szymoński K, Milian-Ciesielska K, Lipiec E, Adamek D. Current Pathology Model of Pancreatic Cancer. *Cancers (Basel)* 2022; 14: 2321. Copyright© The Authors 2020. Published by MDPI. The image may be redistributed without special permissions–source: https://www.mdpi.com/openaccess. PDAC: Pancreatic ductal adenocarcinoma; LIF: Leukemia inhibitory factor; IL-6: Interleukin-6; cfDNA: Cell-free DNA; FTIR: Fourier transform infrared spectroscopy; Raman: Raman spectroscopy; SERS: Surface-enhanced Raman spectroscopy; NGS: Next-generation sequencing; ICB: Immune checkpoint blockers; NRF2: Nuclear factor-erythroid factor 2-related factor 2; GEM: Gemcitabine; PANR: PDAC-associated neural remodeling; CAFs: Cancer-associated fibroblasts; CSCs: Cancer stem cells; EGFR: Epithelial growth factor receptor.

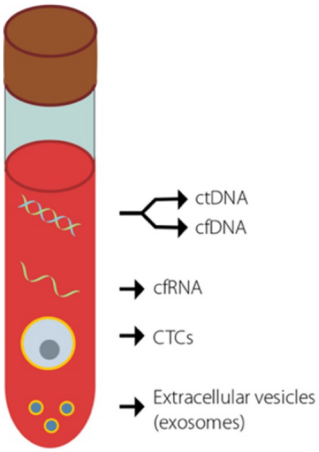

Figure 2 Potential cancer biomarkers that are detectable using liquid biopsies[16]. Key biomarkers that are currently used in an attempt to detect early-stage cancer. Adapted with permission from[16]. Citation: Jaworski JJ, Morgan RD, Sivakumar S. Circulating Cell-Free Tumour DNA for Early Detection of Pancreatic Cancer. *Cancers (Basel)* 2020; 12: 3704. Copyright© The Authors 2020. Published by MDPI. The image may be redistributed without special permissions–source: https://www.mdpi.com/openaccess. ctDNA: Circulating tumor DNA; cfDNA: Cell-free DNA; cfRNA: Cell-free RNA; CTCs: Circulating tumor cells.

scopic fingerprint specific for certain tumors, including PDAC. The molecular characteristics that were obtained using VS, when combined with the use of convolutional neural networks (CNN), can be specified to pinpoint valuable information from the general all-in-one data, thus creating a tool for successful diagnosis.

DNA methylation

DNA methylation plays an important role in the regulation of gene expression, and cellular differentiation. Aberrant methylation is associated with the development and progression of various cancers[16,30,31]. Moreover, these specific aberrations may act as cancer biomarkers, enabling early diagnosis. Standard methylation status evaluation techniques include bisulfite conversion assay, the sequencing or melting curve analysis, restricted enzymes-based assay, and affinity capture using methylated DNA binding proteins[31]. Some of these methods suffer from a laborious workflow and false positives. Others require specific reagents such as enzymes and binding proteins, which are costly and time-consuming. Therefore, we will discuss the usefulness of label-free spectroscopic methods, such as surface-enhanced Raman spectroscopy (SERS), in DNA methylation status detection, and we will address potential issues that arise when using these techniques.

PDAC HETEROGENEITY AND CHEMORESISTANCE

PC is well known to be very heterogeneous in molecular and morphological phenotype. It is one of the reasons, aside from the lack of adequate early diagnosis methods, for patients' poor prognosis, because current treatment options do not consider tumor heterogeneity and thus give insufficient results[32,33]. When designing studies based on PC diagnosis, one must differentiate the results concerning the histomorphological type of the tumor. This distinction is crucial for patient care, due to different molecular pathways governing the development and evolution of these tumors, as well as the prognosis assessment and potential treatment options[3]. In our previous study, we demonstrated that Raman spectroscopy (RS) is capable of detecting ampullary cancer in pancreatic tumor tissue slides[34]. A subsequent step could then differentiate between three groups of pancreatic tumors, specifically conventional PDAC (cPDAC), PDAC derived from intraductal papillary mucinous neoplasm (IPMC), and ampulla of Vater adenocarcinoma (AVAC). cPDAC is the most common form of PC developing *via* pancreatic intraepithelial neoplasia. It arises in the ductal epithelium localized in "normal" pancreatic tissue, sometimes with signs of chronic pancreatitis, or is combined with so-called "acinar-to-ductal metaplasia" regions. The KRAS mutation is the initiating event in this pathway of carcinogenesis[3]. IPMCs are carcinomas that arise from intraductal papillary mucinous neoplasms (IPMNs), cystic tumors that develop in the main or peripheral branches of pancreatic ducts. Guanine nucleotide-binding protein, alpha stimulating activity polypeptide proto-oncogene mutation, which is not found besides pancreatic tumors, plays a significant role in IPMN development[3]. The third relevant group, AVAC, is a cancer of the duodenal ampulla of Vater. AVAC tumors usually grow with large diameters and deeply infiltrate the pancreatic tissue. The histomorphological similarities of AVAC and cPDAC tumors often make them hard to distinguish from each other. Moreover, these tumors are treated clinically and diagnostically in the same way, but the latest reports suggest that they differ regarding the incidence of various prognostic factors, such as tumor differentiation, perineural invasion, venous invasion, or lymph node involvement[35]. The early occurrence of bile duct obstruction symptoms in IPMC or AVAC enables earlier diagnosis and thus may lead to a better prognosis. However, the reports supporting this are ambiguous[3]. Further subgrouping of PC tumors into morphologically distinct entities, such as large duct (cystic papillary), foamy glands, clear cell, adenosquamous, vacuolated-cell, or colloid, which are described in detail elsewhere[3], may benefit patients and clinicians, because of the different prognosis of some of these groups.

Recently, Mukhopadhyay *et al*[36] showed that the nuclear factor-erythroid 2–related factor 2 (NRF2) was responsible for gemcitabine chemoresistance, and the NRF2 expression level in PDAC tissues correlated with poor patient outcome. Another study by Patzak *et al*[37] described cytosolic 5′-nucleotidase 1A (NT5C1A) as a mediator of this resistance by reducing intracellular gemcitabine metabolites and limiting its efficacy. Gemcitabine is a standard chemotherapeutic agent for PDAC. The tumor's resistance to the therapy is among the main reasons for the drastically low 5-year survival rates of PC. Thus, recognizing this chemoresistance is of great importance. The utilization of VS is a step closer in this direction. VS may prove to be beneficial for identifying chemoresistant pancreatic tumors. More studies are required to evaluate NRF2 or/and NT5C1A expression levels in PDAC tissues, and compare them with spectroscopic data to identify spectral markers that correlate with gemcitabine chemoresistance. These studies will help select patients who might benefit from gemcitabine therapy.

Molecular spectroscopy can obtain all the information about the studied sample with a single measurement. There is no need for special labeling or selecting areas of interest that other genetic or biochemical methods may require. Analyzing spectral data plays a pivotal role. All data is ready for interpretation and provides information about the tumor, such as its subtype, differentiation level, specific chemoresistance, and other tumor-specific poor prognostic factors (hepatocyte nuclear factor-1B expression, or cancer stem cells). Whether or not we can decipher this information will determine the efficacy of this methodology in diagnostics.

MOLECULAR SPECTROSCOPY

Molecular VS was confirmed to play an important role in the characterization of the chemical structure and composition of malignant tissues[38-41] and the analysis of human blood serum[42]. Due to high chemical selectivity, RS and infrared spectroscopy (IR) might evolve into efficient pancreatic malignancy screening tools[43]. These methodologies provide information about various biologically significant molecules and functional groups in a tumor including phospholipids and triglycerides, proteins, nucleic acids, phosphates, and carbohydrates. Electromagnetic radiation (photons) can be absorbed and/or scattered by a sample because the energy of phonon excitations and vibrations, as well as oscillations of functional groups in molecules correspond to the energy in the infrared region of the electromagnetic radiation spectrum. Therefore, the presence of functional groups in the analyte can be detected *via* interaction with light, providing information about the molecular structure and composition of the investigated sample. IR and RS take advantage of absorption and inelastic scattering, respectively. The results obtained using these methods indicate differences in metabolic pathways typical for various neoplasms. The main advantage of the molecular spectroscopic approach is collecting information about samples in a label-free and noninvasive manner. The potential of spectroscopic methods has not yet been fully explored in the investigation of PC. However, preliminary results are promising[34,44].

Raman hyperspectral mapping

RS is a VS method that can deliver a detailed molecular fingerprint of a studied sample almost real-time, without the need for special labeling. A technique called Raman hyperspectral mapping (RHM) provides high-resolution imaging at a relatively low cost compared to other well-established medical imaging techniques, such as magnetic resonance imaging (MRI)[43]. RHM relies on multiple measurements of adjacent "pixels" of tissue and integrating the resulting spectra into a single map image. Such imaging allows the selection of points of interest in the studied sample and precise distinction between particular tissue elements, such as cancer cells (with nuclei and cytoplasm) or the stroma compartment (Figure 3).

The RHM methodology of tissue samples usually involves slicing 2.5 μm thick tissue sections with a microtome from a standard formalin-fixed paraffin-embedded tissue block. Because glass gives substantial interference in the Raman readings, for the slide mounting, special calcium fluoride (CaF_2) windows are used for the slide mounting instead. Another important aspect of RHM is the selection of the region of interest which is usually a part of the cancerous glands or stroma compartment. This should be completed by an experienced pathologist. Subsequently, a complete paraffin removal ought to be conducted involving a 12-h xylene bath and graded ethanol rehydration. On such preprocessed tissue slides, the Raman measurements can be performed. This methodology was already described in our previous paper on ampullary cancer detection with RHM[34].

RS is accurate, and the information obtained is characterized by good resolution. Nevertheless, RS requires a sophisticated methodology and equipment because the Raman effect is very weak in nature [43,45]. Another drawback of RS is the substantial sample pre-processing, manual selection of points of interest, and further data analysis.

SERS

To augment the Raman signal strength, another method called SERS is used[43,45]. SERS is a label-free, ultrasensitive tool, capable of DNA methylation analysis. SERS utilizes the same physical phenomenon as RS (Raman effect), but the effect is significantly enhanced using specially synthesized plasmonic nanoparticles[46] into which the molecules (such as DNA) are absorbed [31]. Furthermore, SERS is efficient for liquid biopsy measurements, and it does not require special labeling. However, the production of nanoparticles requires an experienced team and a proper methodology.

The unique ability for such sensitivity of SERS is achieved *via* the use of plasmonic nanostructures (SERS substrates), between which so-called "hot spots" are localized. The traditional methodology of SERS measurements involved mixing the sample with gold (Au) nanoparticles (20-50 nm diameter). One method of SERS substrate production prepares Au nanoparticles *via* chemical reduction of tetrachloroauric (III) acid using trisodium citrate under specific reaction conditions according to the procedure described by Frens[46]. However, this synthesis method is characterized by random and nonuniform hot spot distribution, which leads to the poor reproducibility of SERS[31,47,48]. Another issue, especially regarding DNA methylation studies, is the difficulty distinguishing between DNA methylation signals and the adjacent nucleotide signals due to their similarity[49]. Additionally, surfactants and/or capping agents can affect signal purity[50]. Overcoming these issues is crucial to utilizing SERS for diagnostics largely based on DNA methylation analysis. To achieve this, a methodology of proper SERS substrate development is required. Such a substrate is characterized by a large Raman enhancement, regularly arranged hot spots, and an open and easily accessible surface topology[31]. Luo *et al*[31] proposed the use of a plasmonic gold nanohole array (PGNA) as a SERS substrate. Originally, the authors described the use of electron beam lithography (EBL) for the PGNA substrates production, but a focused ion beam (FIB) might be even better, due to its higher resolution [51]. Both EBL and FIB can be used to obtain a periodic matrix of holes (plasmonic nano-holes array) in a

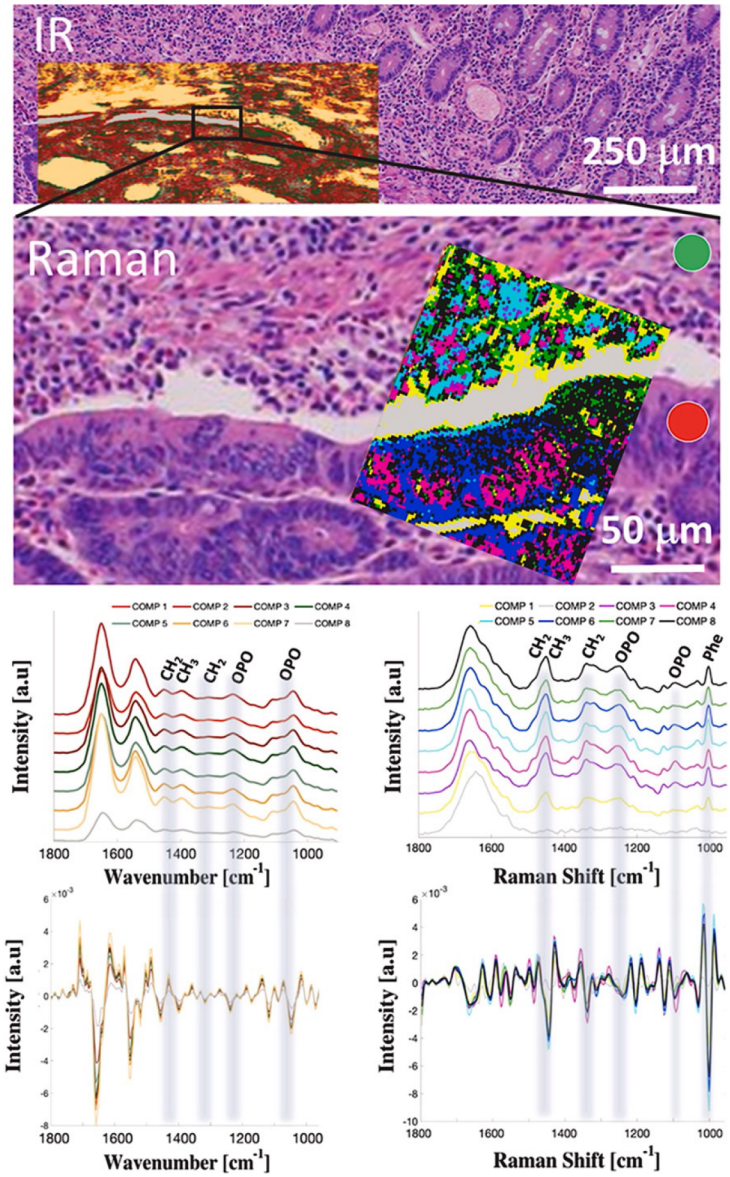

Figure 3 Spectroscopic mapping of ampullary adenocarcinoma[34]. A hematoxylin-eosin slide image of ampullary cancer tissue with superimposed Fourier transform infrared spectroscopy and Raman hyperspectral maps treated with hierarchical cluster analysis and averaged spectra with corresponding second derivatives from each cluster. Spectroscopic maps cover both cancerous (red circle) and noncancerous–stroma (green circle) tissue fragments. Adapted with permission from[34]. Citation: Szymoński K, Lipiec E, Sofińska K, Skirlińska-Nosek K, Milian-Ciesielska K, Szpor J, Czaja M, Seweryn S, Wilkosz N, Birarda G, Piccirilli F, Vaccari L, Szymoński M. Spectroscopic screening of pancreatic cancer. *Clin Spect* 2021; 3: 100016. Copyright© The Authors 2020. Published by ELSEVIER.

Au layer evaporated onto an atomically flat non-plasmonic substrate[31] (Figure 4).

SERS is characterized by a large amount of work required for substrate production, but the sample pre-processing is minimal. Raw blood serum samples can be analyzed, without requiring preselection by a specialist. This is a valuable asset in the search for a diagnostic option.

Attenuated total reflection Fourier transform infrared spectroscopy

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is a complementary method to SERS and RS, which measures absorption as an infrared spectrum of a biochemical fingerprint. It is widely accessible, inexpensive, and easy to use and implement, while still providing substantial information about the sample being analyzed. ATR-FTIR is characterized by high sensitivity in biomedical diagnostics[52] and can detect spectral markers of many pathologies in physiological liquids such as saliva or blood serum. ATR-FTIR was used to detect lung[53], bile duct[54], ovary[55, 56], breast[57], and brain[58,59] tumors in the blood serum samples. In a study by Butler *et al*[59], the team described the methodology of using ATR-FTIR in an early screening of brain tumor patients with sensitivity and specificity of 93.2% and 92.8%, respectively. The high sensitivity of ATR-FTIR can be achieved due to the design of the instrumental set-up. In the ATR-FTIR, a sample droplet is placed on an internal reflection element (IRE), also called the ATR crystal. IREs with high refractive indexes, such

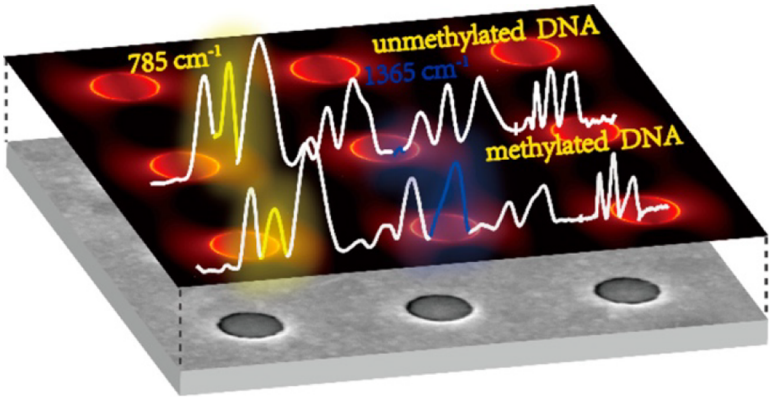

Figure 4 A schematic of a plasmonic gold nanohole array as a surface-enhanced Raman spectroscopy-active substrate used in the detection of DNA methylation[31]. Citation: Luo X, Xing Y, Galvan DD, Zheng E, Wu P, Cai C, Yu Q. Plasmonic Gold Nanohole Array for Surface-Enhanced Raman Scattering Detection of DNA Methylation. *ACS Sens* 2019; 4: 1534–1542. Copyright© The Authors 2020. Published by American Chemical Society.

as diamond, zinc selenide, germanium, or silicon are used. The incident IR beam is directed through the IRE and the resultant evanescent wave extends beyond the ATR crystal surface and penetrates the sample. As with SERS, there is no need for sample pre-processing or special labeling. No additional substrate production is required as well. However, achieving sensitive information about the samples, and their interpretation remains challenging. Employing CNNs to analyze ATR-FTIR data is a viable solution that has already shown success[59].

Molecular spectroscopy in diagnostics

Multiple studies have shown the usefulness of VS in the detection of DNA methylation status of cells, as well as other diagnostically important factors. For example, DNA methylation aberrations were successfully detected using SERS[31,49,60-62], and FTIR[63-69] methods. Recently, in an interesting study by Ho *et al*[70], the authors successfully utilized RS and deep neural networking that enabled culture-free serum bacteria identification and antibiotic susceptibility testing with about 97% accuracy. In other studies[71,72], RS and SERS were used to detect previously untraceable concentrations of biomarkers (matrix metalloproteinase 7, and mucin 4) in the serum of PDAC patients.

To date, no report has combined detecting aberrant DNA methylation markers of PDAC, obtained by analyzing patients' serum with the use of VS methods, specifically the ATR-FTIR combined with CNN classification. Although partial results are available and show promise, additional investigations are needed to support the combination of VS and CNN for PDAC detection.

Residual disease monitoring

Currently, there are no efficient methods of patient monitoring for minimal residual disease (MRD) in PC. Generally, postoperative surveillance methods, including monitoring of clinical symptoms, blood tumor markers, and computed tomography or MRI are used, but these methods lack sensitivity and specificity for MRD[73]. Recently, ctDNA detection using genetic methods in a liquid biopsy was highly advocated for MRD monitoring[73-76]. VS could be a better option for MRD monitoring compared to genetic mutation detection, because of VS's ability to identify DNA methylation aberrations[20]. However, there are currently no data supporting this. Notably, as highlighted by Henriksen *et al*[77], surgical trauma elevates the serum ctDNA levels up to 4 wk after the surgery. This should be taken into consideration when designing a MRD monitoring study using liquid biopsy analysis.

MULTIVARIATE DATA ANALYSIS

In spectroscopic data evaluation, it is very important to draw proper conclusions. Therefore, various methods of multivariate analysis are used to help with data interpretation[78]. Hyperspectral mapping with K-means clustering and principal component analysis (PCA) is commonly performed to explore spectral variation[79-81]. Pre-processing of the spectroscopic data involves cosmic rays removal, baseline correction, and smoothing (adaptive multi-round smoothing based on the Savitzky-Golay filter). Minimal necessary operations are performed to explore marker bands of significant aspects, such as DNA methylation while preventing the loss of important spectral information. Multivariate analysis is carried out to reduce data dimensionality and extract the most important parameters from the acquired information. Some of these methods are briefly described below.

Unsupervised hierarchical cluster analysis is a clustering algorithm designed to group the obtained spectra or to produce false-colored maps based on spectral similarity and variability.

PCA is based on a linear transformation of the data to a new space described by orthogonal axes, the so-called principal components. The most significant results are the "score" values, which represent the data in multidimensional space corresponding to the principal components, and the loading values, which identify the variables causing the data separation according to their influence on the scores. Additionally, the results of the PCA are complemented by the explained variance. The explained variance gives the percentage of the total variance of the original data set, which is explained by a certain principal component.

Partial least squares regression (PLSR) involves a linear transition of numerous original descriptors to a new variable space based on a small number of orthogonal factors (latent variables). In other words, PLSR allows the construction of predictive models when the factors are highly collinear. This analysis estimates unobservable variables as exact linear combinations of their empirical indicators. The estimated proxies are treated as substitutes for the latent variables. The selected case values capture most of the independent variables' variance. This variance is used for predicting the dependent variable.

Non-negative matrix factorization (NMF) is a useful tool for the analysis of high-dimensional data. Besides detecting a compressed representation, NMF provides insights into the structure and features of the given data by extracting easily interpretable factors. With the use of NMF, basic spectral components for proteins, lipids, phospholipids, or nucleic acids can be compared.

All of these methods depend on human decision-making, although minimal. As a result, these methods might disturb and lose seemingly irrelevant data. To overcome this and enhance the sensitivity and specificity of spectral data interpretation, deep neural networks are used.

CNNs

RS is characterized by a very low strength of the measured effect (Raman effect) and is thus very sensitive to distortion factors, such as fluorescence, thermal noise, the quality of the measuring equipment, and research team experience. Removing noise artifacts requires various pre-processing methods on acquired spectra (*i.e.*, cosmic rays removal, baseline correction, and smoothing). All of these depend on human input and definition. On one hand, this prevents automation, but on the other, some seemingly irrelevant data might be lost during the pre-processing. The successful use of CNNs in spectroscopic data evaluation and classification was shown in multiple studies[82,83] including those using RS[84], SERS[70,85], and ATR-FTIR[86]. As a source, the CNN is fed with raw spectral data, without human interaction-related pre-processing. This approach gives better results in classification than conventional methods[82] and makes the methodology more universal. CNN objectives should be clear and simple. Similarly, the proper selection of training data is of great importance. For each objective, different training sets should be created. All spectral results from each group are split (2:1) to form the training (two-thirds of data) and testing (one-third of data) datasets. Training datasets should include positive and negative cases, preferably with many variations. For example, when designing a training dataset for the CNN that will decide whether the results are from a malignant pancreatic tumor or not from a malignant pancreatic tumor, one might include cases from PC, but as a negative control also include malignancies of other sites (*i.e.*, colorectal or breast carcinomas), and benign pancreatic entities (*i.e.*, IPMN, mucinous cystic neoplasm, or groove pancreatitis).

There are some issues related to utilizing neural networks (NNs) with multiple layers (deep NNs), specifically very deep NNs. One obstacle is the vanishing/exploding gradient problem. A training process of a NN in simplest words usually involves updating the "weights" of the algorithm to better cope with the problem, that the NN is exposed to. A great benefit of a CNN is that it extracts features of the task on its own. Feature extraction is done using an optimization algorithm, such as "gradient descent", which simply finds values of a function's parameters to minimize the cost function. If gradients that update the weights shrink, the weights are no longer updated, and the learning stops. This is called a vanishing gradient problem. Similarly, if gradients grow, weights do not update reasonably, and the learning becomes unstable, resulting in the exploding gradient problem. The so-called "skip connection" technique is utilized to overcome the vanishing/exploding gradient problem [83,87]. This is the basis for a Residual Network CNN architecture[87]. Another issue of CNNs is data overfitting. When a model used to train the CNN is very complex, and there is a limited amount of learning data available, the CNN learns to know the training dataset well, but performs poorly against any new data (*i.e.*, validating/testing dataset or the implementation data). To improve this shortcoming, proper data augmentation techniques might be needed. Usually, in spectral data analysis, augmentation requires making additional artificial spectra by small spectral shifting, expanding spectral range, adding Gaussian noise, or superimposing the spectra for each real result[83].

Table 1 Drawbacks of spectroscopic-related methodologies, and proposed solutions

Methodology	Issue	Solution
Raman spectroscopy (random measurement points selection)	(1) Not differentiating the results with cancer subtype leads to mixed results; and (2) Mixing measurements of cancer cells or tumor stroma with pancreatitis, inflammation, necrosis, colloid, *etc*	(1) Results should be analyzed with regard to cancer subtype; (2) Raman hyperspectral mapping allows the selection of specific points of interest, whether cancer cells or tumor stroma areas; and (3) The selection should be done by an experienced pancreatic pathologist
SERS	(1) Poor reproducibility, due to random and nonuniform hot spots distribution in nanostructures production[31,47,48]; (2) The distinction between methylation signals and those from adjacent nucleotides is difficult, because of their similarity[49]; and (3) Signal purity is affected by the use of surfactants and/or capping agents[50]	(1) PGNA as a SERS substrate; and (2) The use of a FIB to obtain a periodic matrix of holes (plasmonic nano-holes array) in a gold layer evaporated on an atomically flat non-plasmonic substrate[31]
ATR-FTIR	(1) Low sensitivity and specificity; (2) Human-dependent pre-processing and analysis of spectral data; and (3) Not yet confirmed in pancreatic cancer diagnosis	To increase the sensitivity and specificity, and to limit the human intervention, convolutional neural networks are used
Multivariate data analysis	(1) Results might be disturbed by the human-dependant actions and seemingly irrelevant data will be lost; and (2) Losing data lowers the sensitivity and specificity of the method	Using of convolutional neural networks, which are fed with raw spectral data
CNN	(1) Very deep neural networks are characterized by a vanishing/exploding gradient problem; and (2) Overfitting of the CNN trained on a limited amount of data	(1) The use of ResNet CNN architecture, with the use of so-called "skip connections"; and (2) Proper data augmentation to prevent overfitting and sensitize the CNN to deal with various "scenarios"

PGNA: Plasmonic gold nanohole array; FIB: Focused ion beam; SERS: Surface-enhanced Raman spectroscopy; ResNet: Residual Network; CNN: Convolutional neural networks; ATR-FTIR: Attenuated total reflection Fourier transform infrared spectroscopy.

CONCLUSION

When designing a study investigating VS methods in PC diagnosis, it is important to bear in mind a couple of aspects. First, the study is required to address all the drawbacks of RS, SERS, and ATR-FTIR methodology, described briefly above. Another crucial issue is the proper cooperation of multidisciplinary teams, including medical specialists, such as pathologists and clinicians, and spectroscopic specialists, such as physicists and chemists. For example, comparing PC cases with pancreatic neuroendocrine neoplasms is not reasonable because the malignancies represent different entities with very distinct pathways governing their initiation and progression, as much as patient survival. When dealing with PC, detailed knowledge of the tumor's molecular and pathological nature is required. In particular, one should interpret the results of measurements in the context of PC subtypes and some other prognostic factors[3]. Proper analysis of measured spectroscopic data is impartant. For example, when conducting a RS on complex tissue samples, such as PC sections, the random spots of measurements will yield ambiguous results. The spectral data might be disturbed by numerous interfering phenomena, such as inflammation, tumor necrosis, or fibrosis. Additionally, the cytoplasmic and nuclear regions of cancer cells significantly differ as well. Interpretation of the PC stroma compartment, including the complicated cancer-stroma "cross-talk", is another aspect to address[3]. Careful selection of regions of interest is important too. This should be done by a specialized pancreatic pathologist familiar with the spectroscopic methodology. Finally, in the search for a PC diagnostic tool, we look for universality and automation. Thus, the interpretation of spectral data obtained from liquid biopsy, which relies on human-dependent pre-processing is not a good path to follow. CNNs are invaluable here, but proper design and training is the key to success. With these aims, we designed a study that will comprehensively evaluate the potential of VS methods used in diagnosing PC, by systemically evaluating liquid biopsy samples (Figure 5). In conclusion, VS seems to be leading the way in the race, with most of the methodology drawbacks resolved, at least partially (Table 1).

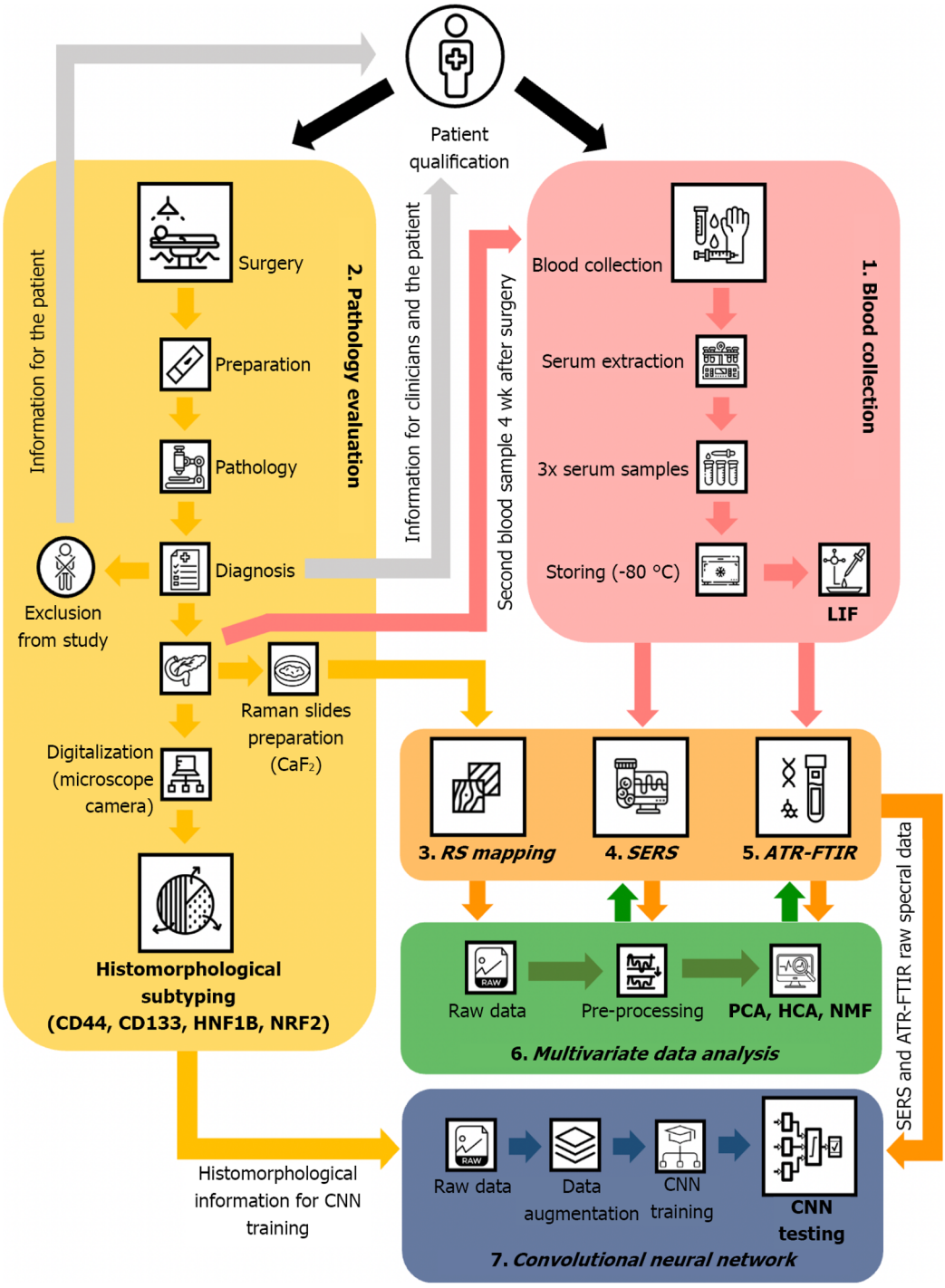

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Figure 5 A Designed study regarding the diagnostic potential of vibrational spectroscopic methods used for diagnosing pancreatic cancer by systemically evaluating liquid biopsy samples. LIF: Leukemia inhibitory factor; RS: Raman spectroscopy; SERS: Surface-enhanced Raman spectroscopy; ATR-FTIR: Attenuated total reflection Fourier transform infrared spectroscopy; CNN: Convolutional neural network; NRF2: Nuclear factor-erythroid 2–related factor 2; HNF1B: Hepatocyte nuclear factor-1B; PCA: Principal component analysis; HCA: Hierarchical clustering analysis; NMF: Non-negative matrix factorization.

FOOTNOTES

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REFERENCES

1 **Rawla P**, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World J Oncol* 2019; **10**: 10-27 [PMID: 30834048 DOI: 10.14740/wjon1166]
2 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
3 **Szymoński K**, Milian-Ciesielska K, Lipiec E, Adamek D. Current Pathology Model of Pancreatic Cancer. *Cancers (Basel)* 2022; **14** [PMID: 35565450 DOI: 10.3390/cancers14092321]
4 **Zimmermann C**, Wolk S, Aust DE, Meier F, Saeger HD, Ehehalt F, Weitz J, Welsch T, Distler M. The pathohistological subtype strongly predicts survival in patients with ampullary carcinoma. *Sci Rep* 2019; **9**: 12676 [PMID: 31481741 DOI: 10.1038/s41598-019-49179-w]
5 **Takaori K**, Bassi C, Biankin A, Brunner TB, Cataldo I, Campbell F, Cunningham D, Falconi M, Frampton AE, Furuse J, Giovannini M, Jackson R, Nakamura A, Nealon W, Neoptolemos JP, Real FX, Scarpa A, Sclafani F, Windsor JA, Yamaguchi K, Wolfgang C, Johnson CD; IAP/EPC study group on the clinical managements of pancreatic cancer. International Association of Pancreatology (IAP)/European Pancreatic Club (EPC) consensus review of guidelines for the treatment of pancreatic cancer. *Pancreatology* 2016; **16**: 14-27 [PMID: 26699808 DOI: 10.1016/j.pan.2015.10.013]
6 **Gui JC**, Yan WL, Liu XD. CA19-9 and CA242 as tumor markers for the diagnosis of pancreatic cancer: a meta-analysis. *Clin Exp Med* 2014; **14**: 225-233 [DOI: 10.1007/s10238-013-0234-9]
7 **Błogowski W**, Deskur A, Budkowska M, Sałata D, Madej-Michniewicz A, Dąbkowski K, Dołęgowska B, Starzyńska T. Selected cytokines in patients with pancreatic cancer: a preliminary report. *PLoS One* 2014; **9**: e97613 [PMID: 24849506 DOI: 10.1371/journal.pone.0097613]
8 **Talar-Wojnarowska R**, Gasiorowska A, Smolarz B, Romanowicz-Makowska H, Kulig A, Malecka-Panas E. Clinical significance of interleukin-6 (IL-6) gene polymorphism and IL-6 serum level in pancreatic adenocarcinoma and chronic pancreatitis. *Dig Dis Sci* 2009; **54**: 683-689 [PMID: 18661238 DOI: 10.1007/s10620-008-0390-z]
9 **Mroczko B**, Groblewska M, Gryko M, Kedra B, Szmitkowski M. Diagnostic usefulness of serum interleukin 6 (IL-6) and C-reactive protein (CRP) in the differentiation between pancreatic cancer and chronic pancreatitis. *J Clin Lab Anal* 2010; **24**: 256-261 [PMID: 20626020 DOI: 10.1002/jcla.20395]
10 **Bressy C**, Lac S, Nigri J, Leca J, Roques J, Lavaut MN, Secq V, Guillaumond F, Bui TT, Pietrasz D, Granjeaud S, Bachet JB, Ouaissi M, Iovanna J, Vasseur S, Tomasini R. LIF Drives Neural Remodeling in Pancreatic Cancer and Offers a New Candidate Biomarker. *Cancer Res* 2018; **78**: 909-921 [PMID: 29269518 DOI: 10.1158/0008-5472.CAN-15-2790]
11 **Shi Y**, Gao W, Lytle NK, Huang P, Yuan X, Dann AM, Ridinger-Saison M, DelGiorno KE, Antal CE, Liang G, Atkins AR, Erikson G, Sun H, Meisenhelder J, Terenziani E, Woo G, Fang L, Santisakultarm TP, Manor U, Xu R, Becerra CR, Borazanci E, Von Hoff DD, Grandgenett PM, Hollingsworth MA, Leblanc M, Umetsu SE, Collisson EA, Scadeng M, Lowy AM, Donahue TR, Reya T, Downes M, Evans RM, Wahl GM, Pawson T, Tian R, Hunter T. Targeting LIF-mediated paracrine interaction for pancreatic cancer therapy and monitoring. *Nature* 2019; **569**: 131-135 [PMID: 30996350 DOI: 10.1038/s41586-019-1130-6]
12 **Wrona E**, Potemski P, Sclafani F, Borowiec M. Leukemia Inhibitory Factor: A Potential Biomarker and Therapeutic Target in Pancreatic Cancer. *Arch Immunol Ther Exp (Warsz)* 2021; **69**: 2 [PMID: 33630157 DOI: 10.1007/s00005-021-00605-w]
13 **Vaddepally RK**, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. *Cancers (Basel)* 2020; **12** [PMID: 32245016 DOI: 10.3390/cancers12030738]
14 **Loriot Y**, Marabelle A, Guégan JP, Danlos FX, Besse B, Chaput N, Massard C, Planchard D, Robert C, Even C, Khettab M, Tselikas L, Friboulet L, André F, Nafia I, Le Loarer F, Soria JC, Bessede A, Italiano A. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune-checkpoint blockade resistance. *Ann Oncol* 2021; **32**: 1381-1390 [PMID: 34416362 DOI: 10.1016/j.annonc.2021.08.1748]
15 **Jiang W**, Bai W, Li J, Liu J, Zhao K, Ren L. Leukemia inhibitory factor is a novel biomarker to predict lymph node and distant metastasis in pancreatic cancer. *Int J Cancer* 2021; **148**: 1006-1013 [PMID: 32914874 DOI: 10.1002/ijc.33291]

16 **Jaworski JJ**, Morgan RD, Sivakumar S. Circulating Cell-Free Tumour DNA for Early Detection of Pancreatic Cancer. *Cancers (Basel)* 2020; **12** [PMID: 33317202 DOI: 10.3390/cancers12123704]

17 **Shinjo K**, Hara K, Nagae G, Umeda T, Katsushima K, Suzuki M, Murofushi Y, Umezu Y, Takeuchi I, Takahashi S, Okuno Y, Matsuo K, Ito H, Tajima S, Aburatani H, Yamao K, Kondo Y. A novel sensitive detection method for DNA methylation in circulating free DNA of pancreatic cancer. *PLoS One* 2020; **15**: e0233782 [PMID: 32520974 DOI: 10.1371/journal.pone.0233782]

18 **Gall TMH**, Belete S, Khanderia E, Frampton AE, Jiao LR. Circulating Tumor Cells and Cell-Free DNA in Pancreatic Ductal Adenocarcinoma. *Am J Pathol* 2019; **189**: 71-81 [PMID: 30558725 DOI: 10.1016/j.ajpath.2018.03.020]

19 **Imaoka H**, Sasaki M, Hashimoto Y, Watanabe K, Ikeda M. New Era of Endoscopic Ultrasound-Guided Tissue Acquisition: Next-Generation Sequencing by Endoscopic Ultrasound-Guided Sampling for Pancreatic Cancer. *J Clin Med* 2019; **8** [PMID: 31387310 DOI: 10.3390/jcm8081173]

20 **Huerta M**, Roselló S, Sabater L, Ferrer A, Tarazona N, Roda D, Gambardella V, Alfaro-Cervelló C, Garcés-Albir M, Cervantes A, Ibarrola-Villava M. Circulating Tumor DNA Detection by Digital-Droplet PCR in Pancreatic Ductal Adenocarcinoma: A Systematic Review. *Cancers (Basel)* 2021; **13** [PMID: 33673558 DOI: 10.3390/cancers13050994]

21 **Raposo G**, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013; **200**: 373-383 [PMID: 23420871 DOI: 10.1083/jcb.201211138]

22 **Colombo M**, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014; **30**: 255-289 [PMID: 25288114 DOI: 10.1146/annurev-cellbio-101512-122326]

23 **Becker A**, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell* 2016; **30**: 836-848 [PMID: 27960084 DOI: 10.1016/j.ccell.2016.10.009]

24 **Martins VR**, Dias MS, Hainaut P. Tumor-cell-derived microvesicles as carriers of molecular information in cancer. *Curr Opin Oncol* 2013; **25**: 66-75 [PMID: 23165142 DOI: 10.1097/CCO.0b013e32835b7c81]

25 **Amintas S**, Vendrely V, Dupin C, Buscail L, Laurent C, Bournet B, Merlio JP, Bedel A, Moreau-Gaudry F, Boutin J, Dabernat S, Buscail E. Next-Generation Cancer Biomarkers: Extracellular Vesicle DNA as a Circulating Surrogate of Tumor DNA. *Front Cell Dev Biol* 2020; **8**: 622048 [PMID: 33604335 DOI: 10.3389/fcell.2020.622048]

26 **Logozzi M**, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, Brambilla D, Venturi G, Lozupone F, Santinami M, Huber V, Maio M, Rivoltini L, Fais S. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS One* 2009; **4**: e5219 [PMID: 19381331 DOI: 10.1371/journal.pone.0005219]

27 **Hoshino A**, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, Singh S, Williams C, Soplop N, Uryu K, Pharmer L, King T, Bojmar L, Davies AE, Ararso Y, Zhang T, Zhang H, Hernandez J, Weiss JM, Dumont-Cole VD, Kramer K, Wexler LH, Narendran A, Schwartz GK, Healey JH, Sandstrom P, Labori KJ, Kure EH, Grandgenett PM, Hollingsworth MA, de Sousa M, Kaur S, Jain M, Mallya K, Batra SK, Jarnagin WR, Brady MS, Fodstad O, Muller V, Pantel K, Minn AJ, Bissell MJ, Garcia BA, Kang Y, Rajasekhar VK, Ghajar CM, Matei I, Peinado H, Bromberg J, Lyden D. Tumour exosome integrins determine organotropic metastasis. *Nature* 2015; **527**: 329-335 [PMID: 26524530 DOI: 10.1038/nature15756]

28 **Valadi H**, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; **9**: 654-659 [PMID: 17486113 DOI: 10.1038/ncb1596]

29 **Skog J**, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008; **10**: 1470-1476 [PMID: 19011622 DOI: 10.1038/ncb1800]

30 **Locke WJ**, Guanzon D, Ma C, Liew YJ, Duesing KR, Fung KYC, Ross JP. DNA Methylation Cancer Biomarkers: Translation to the Clinic. *Front Genet* 2019; **10**: 1150 [PMID: 31803237 DOI: 10.3389/fgene.2019.01150]

31 **Luo X**, Xing Y, Galvan DD, Zheng E, Wu P, Cai C, Yu Q. Plasmonic Gold Nanohole Array for Surface-Enhanced Raman Scattering Detection of DNA Methylation. *ACS Sens* 2019; **4**: 1534-1542 [PMID: 31074265 DOI: 10.1021/acssensors.9b00008]

32 **Sántha P**, Lenggenhager D, Finstadsveen A, Dorg L, Tøndel K, Amrutkar M, Gladhaug IP, Verbeke C. Morphological Heterogeneity in Pancreatic Cancer Reflects Structural and Functional Divergence. *Cancers (Basel)* 2021; **13** [PMID: 33672734 DOI: 10.3390/cancers13040895]

33 **Verbeke C**. Morphological heterogeneity in ductal adenocarcinoma of the pancreas - Does it matter? *Pancreatology* 2016; **16**: 295-301 [PMID: 26924665 DOI: 10.1016/j.pan.2016.02.004]

34 **Szymoński K**, Lipiec E, Sofińska K, Skirlińska-Nosek K, Milian-Ciesielska K, Szpor J, Czaja M, Seweryn S, Wilkosz N, Birarda G, Piccirilli F, Vaccari L, Szymoński M. Spectroscopic screening of pancreatic cancer. *Clin Spect* 2021; **3**: 100016 [DOI: 10.1016/j.clispe.2021.100016]

35 **Ferchichi M**, Jouini R, Koubaa W, Khanchel F, Helal I, Hadad D, Bibani N, Chadli-Debbiche A, BenBrahim E. Ampullary and pancreatic adenocarcinoma-a comparative study. *J Gastrointest Oncol* 2019; **10**: 270-275 [PMID: 31032094 DOI: 10.21037/jgo.2018.09.09]

36 **Mukhopadhyay S**, Goswami D, Adiseshaiah PP, Burgan W, Yi M, Guerin TM, Kozlov SV, Nissley DV, McCormick F. Undermining Glutaminolysis Bolsters Chemotherapy While NRF2 Promotes Chemoresistance in KRAS-Driven Pancreatic Cancers. *Cancer Res* 2020; **80**: 1630-1643 [PMID: 31911550 DOI: 10.1158/0008-5472.CAN-19-1363]

37 **Patzak MS**, Kari V, Patil S, Hamdan FH, Goetze RG, Brunner M, Gaedcke J, Kitz J, Jodrell DI, Richards FM, Pilarsky C, Gruetzmann R, Rümmele P, Knösel T, Hessmann E, Ellenrieder V, Johnsen SA, Neesse A. Cytosolic 5'-nucleotidase 1A is overexpressed in pancreatic cancer and mediates gemcitabine resistance by reducing intracellular gemcitabine metabolites. *EBioMedicine* 2019; **40**: 394-405 [PMID: 30709769 DOI: 10.1016/j.ebiom.2019.01.037]

38 **Krafft C**, Popp J. Micro-Raman spectroscopy in medicine. *Phy Sci Rev* 2019; **4** [DOI: 10.1515/psr-2017-0047]

39 **Krafft C**, Sergo V. Biomedical applications of Raman and infrared spectroscopy to diagnose tissues. *Spectroscopy* 2006; **20**: 195-218 [DOI: 10.1155/2006/738186]

40 **Diem M**, Miljković M, Bird B, Chernenko T, Schubert J, Marcsisin E, Mazur A, Kingston E, Zuser E, Papamarkakis K,

Laver N. Applications of infrared and raman microspectroscopy of cells and tissue in medical diagnostics: Present status and future promises. *Spectroscopy (New York)* 2012; **27**: 463-496 [DOI: 10.1155/2012/848360]

41 **Grzelak MM**, Wróbel PM, Lankosz M, Stęgowski Z, Chmura Ł, Adamek D, Hesse B, Castillo-Michel H. Diagnosis of ovarian tumour tissues by SR-FTIR spectroscopy: A pilot study. *Spectrochim Acta A Mol Biomol Spectrosc* 2018; **203**: 48-55 [PMID: 29859492 DOI: 10.1016/j.saa.2018.05.070]

42 **Acquarelli J**, van Laarhoven T, Gerretzen J, Tran TN, Buydens LMC, Marchiori E. Convolutional neural networks for vibrational spectroscopic data analysis. *Anal Chim Acta* 2017; **954**: 22-31 [PMID: 28081811 DOI: 10.1016/j.aca.2016.12.010]

43 **Kong K**, Kendall C, Stone N, Notingher I. Raman spectroscopy for medical diagnostics--From in-vitro biofluid assays to in-vivo cancer detection. *Adv Drug Deliv Rev* 2015; **89**: 121-134 [PMID: 25809988 DOI: 10.1016/j.addr.2015.03.009]

44 **Paraskevaidi M**, Matthew BJ, Holly BJ, Hugh BJ, Thulya CPV, Loren C, StJohn C, Peter G, Callum G, Sergei KG, Kamila K, Maria K, Kássio LMG, Pierre MHL, Evangelos P, Savithri P, John AA, Alexandra S, Marfran S, Josep SS, Gunjan T, Michael W, Bayden W. Clinical applications of infrared and Raman spectroscopy in the fields of cancer and infectious diseases. *App Spec Rev* 2021; **56**: 804-868 [DOI: 10.1080/05704928.2021.1946076]

45 **Bonifacio A**, Cervo S, Sergo V. Label-free surface-enhanced Raman spectroscopy of biofluids: fundamental aspects and diagnostic applications. *Anal Bioanal Chem* 2015; **407**: 8265-8277 [PMID: 25935674 DOI: 10.1007/s00216-015-8697-z]

46 **Frens G**. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature Phy Sci* 1973; **241**: 20-22 [DOI: 10.1038/physci241020a0]

47 **Klingsporn JM**, Jiang N, Pozzi EA, Sonntag MD, Chulhai D, Seideman T, Jensen L, Hersam MC, Van Duyne RP. Intramolecular insight into adsorbate-substrate interactions *via* low-temperature, ultrahigh-vacuum tip-enhanced Raman spectroscopy. *J Am Chem Soc* 2014; **136**: 3881-3887 [PMID: 24548252 DOI: 10.1021/ja411899k]

48 **Papadopoulou E**, Bell SE. DNA reorientation on Au nanoparticles: label-free detection of hybridization by surface enhanced Raman spectroscopy. *Chem Commun (Camb)* 2011; **47**: 10966-10968 [PMID: 21909525 DOI: 10.1039/c1cc13705k]

49 **Barhoumi A**, Halas NJ. Detecting Chemically Modified DNA Bases Using Surface Enhanced Raman Spectroscopy. *J Phys Chem Lett* 2011; **2**: 3118-3123 [PMID: 24427449 DOI: 10.1021/jz201423b]

50 **Xu LJ**, Lei ZC, Li J, Zong C, Yang CJ, Ren B. Label-free surface-enhanced Raman spectroscopy detection of DNA with single-base sensitivity. *J Am Chem Soc* 2015; **137**: 5149-5154 [PMID: 25835155 DOI: 10.1021/jacs.5b01426]

51 **Morimoto H**, Sasaki Y, Saitoh K, Watakabe Y, Kato T. Focused ion beam lithography and its application to submicron devices. *Microelectron Eng* 1986; **4**: 163-179 [DOI: 10.1016/0167-9317(86)90009-2]

52 **Baker MJ**, Trevisan J, Bassan P, Bhargava R, Butler HJ, Dorling KM, Fielden PR, Fogarty SW, Fullwood NJ, Heys KA, Hughes C, Lasch P, Martin-Hirsch PL, Obinaju B, Sockalingum GD, Sulé-Suso J, Strong RJ, Walsh MJ, Wood BR, Gardner P, Martin FL. Using Fourier transform IR spectroscopy to analyze biological materials. *Nat Protoc* 2014; **9**: 1771-1791 [PMID: 24992094 DOI: 10.1038/nprot.2014.110]

53 **Yang X**, Ou Q, Qian K, Yang J, Bai Z, Yang W, Shi Y, Liu G. Diagnosis of Lung Cancer by ATR-FTIR Spectroscopy and Chemometrics. *Front Oncol* 2021; **11**: 753791 [PMID: 34660320 DOI: 10.3389/fonc.2021.753791]

54 **Chatchawal P**, Wongwattanakul M, Tippayawat P, Kochan K, Jearanaikoon N, Wood BR, Jearanaikoon P. Detection of Human Cholangiocarcinoma Markers in Serum Using Infrared Spectroscopy. *Cancers (Basel)* 2021; **13** [PMID: 34680259 DOI: 10.3390/cancers13205109]

55 **Gajjar K**, Trevisan J, Owens G, Keating PJ, Wood NJ, Stringfellow HF, Martin-Hirsch PL, Martin FL. Fourier-transform infrared spectroscopy coupled with a classification machine for the analysis of blood plasma or serum: a novel diagnostic approach for ovarian cancer. *Analyst* 2013; **138**: 3917-3926 [PMID: 23325355 DOI: 10.1039/c3an36654e]

56 **Owens GL**, Gajjar K, Trevisan J, Fogarty SW, Taylor SE, Da Gama-Rose B, Martin-Hirsch PL, Martin FL. Vibrational biospectroscopy coupled with multivariate analysis extracts potentially diagnostic features in blood plasma/serum of ovarian cancer patients. *J Biophotonics* 2014; **7**: 200-209 [PMID: 24259229 DOI: 10.1002/jbio.201300157]

57 **Sitnikova VE**, Kotkova MA, Nosenko TN, Kotkova TN, Martynova DM, Uspenskaya MV. Breast cancer detection by ATR-FTIR spectroscopy of blood serum and multivariate data-analysis. *Talanta* 2020; **214**: 120857 [PMID: 32278436 DOI: 10.1016/j.talanta.2020.120857]

58 **Hands JR**, Clemens G, Stables R, Ashton K, Brodbelt A, Davis C, Dawson TP, Jenkinson MD, Lea RW, Walker C, Baker MJ. Brain tumour differentiation: rapid stratified serum diagnostics *via* attenuated total reflection Fourier-transform infrared spectroscopy. *J Neurooncol* 2016; **127**: 463-472 [PMID: 26874961 DOI: 10.1007/s11060-016-2060-x]

59 **Butler HJ**, Brennan PM, Cameron JM, Finlayson D, Hegarty MG, Jenkinson MD, Palmer DS, Smith BR, Baker MJ. Development of high-throughput ATR-FTIR technology for rapid triage of brain cancer. *Nat Commun* 2019; **10**: 4501 [PMID: 31594931 DOI: 10.1038/s41467-019-12527-5]

60 **Li L**, Lim SF, Puretzky A, Riehn R, Hallen HD. DNA Methylation Detection Using Resonance and Nanobowtie-Antenna-Enhanced Raman Spectroscopy. *Biophys J* 2018; **114**: 2498-2506 [PMID: 29874601 DOI: 10.1016/j.bpj.2018.04.021]

61 **Morla-Folch J**, Xie HN, Gisbert-Quilis P, Gómez-de Pedro S, Pazos-Perez N, Alvarez-Puebla RA, Guerrini L. Ultrasensitive Direct Quantification of Nucleobase Modifications in DNA by Surface-Enhanced Raman Scattering: The Case of Cytosine. *Angew Chem Int Ed Engl* 2015; **54**: 13650-13654 [PMID: 26447808 DOI: 10.1002/anie.201507682]

62 **Guerrini L**, Krpetić Ž, van Lierop D, Alvarez-Puebla RA, Graham D. Direct surface-enhanced Raman scattering analysis of DNA duplexes. *Angew Chem Int Ed Engl* 2015; **54**: 1144-1148 [PMID: 25414148 DOI: 10.1002/anie.201408558]

63 **Vidal Bde C**, Ghiraldini FG, Mello ML. Changes in liver cell DNA methylation status in diabetic mice affect its FT-IR characteristics. *PLoS One* 2014; **9**: e102295 [PMID: 25019512 DOI: 10.1371/journal.pone.0102295]

64 **Mello ML**, Vidal BC. Analysis of the DNA Fourier transform-infrared microspectroscopic signature using an all-reflecting objective. *Micron* 2014; **61**: 49-52 [PMID: 24792446 DOI: 10.1016/j.micron.2014.02.003]

65 **Mello ML**, Vidal BC. Changes in the infrared microspectroscopic characteristics of DNA caused by cationic elements, different base richness and single-stranded form. *PLoS One* 2012; **7**: e43169 [PMID: 22937023 DOI: 10.1371/journal.pone.0043169]

66 **Kelly JG**, Najand GM, Martin FL. Characterisation of DNA methylation status using spectroscopy (mid-IR versus Raman) with multivariate analysis. *J Biophotonics* 2011; **4**: 345-354 [PMID: 21520428 DOI: 10.1002/jbio.201000085]

67 **Kelly JG**, Martin-Hirsch PL, Martin FL. Discrimination of base differences in oligonucleotides using mid-infrared spectroscopy and multivariate analysis. *Anal Chem* 2009; **81**: 5314-5319 [PMID: 19499925 DOI: 10.1021/ac900546m]

68 **Banyay M**, Gräslund A. Structural effects of cytosine methylation on DNA sugar pucker studied by FTIR. *J Mol Biol* 2002; **324**: 667-676 [PMID: 12460569 DOI: 10.1016/s0022-2836(02)01104-x]

69 **Veronezi GMB**, Felisbino MB, Gatti MS v, Mello MLS, Vidal B de C. DNA Methylation Changes in Valproic Acid-Treated HeLa Cells as Assessed by Image Analysis, Immunofluorescence and Vibrational Microspectroscopy. *PLoS One* 2017; **12**: e0170740 [DOI: 10.1371/journal.pone.0170740]

70 **Ho CS**, Jean N, Hogan CA, Blackmon L, Jeffrey SS, Holodniy M, Banaei N, Saleh AAE, Ermon S, Dionne J. Rapid identification of pathogenic bacteria using Raman spectroscopy and deep learning. *Nat Commun* 2019; **10**: 4927 [PMID: 31666527 DOI: 10.1038/s41467-019-12898-9]

71 **Banaei N**, Foley A, Houghton JM, Sun Y, Kim B. Multiplex detection of pancreatic cancer biomarkers using a SERS-based immunoassay. *Nanotechnology* 2017; **28**: 455101 [PMID: 28937361 DOI: 10.1088/1361-6528/aa8e8c]

72 **Wang G**, Lipert RJ, Jain M, Kaur S, Chakraboty S, Torres MP, Batra SK, Brand RE, Porter MD. Detection of the potential pancreatic cancer marker MUC4 in serum using surface-enhanced Raman scattering. *Anal Chem* 2011; **83**: 2554-2561 [PMID: 21391573 DOI: 10.1021/ac102829b]

73 **Jiang J**, Ye S, Xu Y, Chang L, Hu X, Ru G, Guo Y, Yi X, Yang L, Huang D. Circulating Tumor DNA as a Potential Marker to Detect Minimal Residual Disease and Predict Recurrence in Pancreatic Cancer. *Front Oncol* 2020; **10**: 1220 [PMID: 32850360 DOI: 10.3389/fonc.2020.01220]

74 **Lee B**, Lipton L, Cohen J, Tie J, Javed AA, Li L, Goldstein D, Burge M, Cooray P, Nagrial A, Tebbutt NC, Thomson B, Nikfarjam M, Harris M, Haydon A, Lawrence B, Tai DWM, Simons K, Lennon AM, Wolfgang CL, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA as a potential marker of adjuvant chemotherapy benefit following surgery for localized pancreatic cancer. *Ann Oncol* 2019; **30**: 1472-1478 [PMID: 31250894 DOI: 10.1093/annonc/mdz200]

75 **Perets R**, Greenberg O, Shentzer T, Semenisty V, Epelbaum R, Bick T, Sarji S, Ben-Izhak O, Sabo E, Hershkovitz D. Mutant *KRAS* Circulating Tumor DNA Is an Accurate Tool for Pancreatic Cancer Monitoring. *Oncologist* 2018; **23**: 566-572 [PMID: 29371474 DOI: 10.1634/theoncologist.2017-0467]

76 **Montagut C**, Vidal J. ctDNA to detect minimal residual disease in pancreatic cancer: moving into clinical trials. *Ann Oncol* 2019; **30**: 1410-1413 [PMID: 31418008 DOI: 10.1093/annonc/mdz236]

77 **Henriksen TV**, Reinert T, Christensen E, Sethi H, Birkenkamp-Demtröder K, Gögenur M, Gögenur I, Zimmermann BG; IMPROVE Study Group, Dyrskjøt L, Andersen CL. The effect of surgical trauma on circulating free DNA levels in cancer patients-implications for studies of circulating tumor DNA. *Mol Oncol* 2020; **14**: 1670-1679 [PMID: 32471011 DOI: 10.1002/1878-0261.12729]

78 **Notarstefano V**, Sabbatini S, Conti C, Pisani M, Astolfi P, Pro C, Rubini C, Vaccari L, Giorgini E. Investigation of human pancreatic cancer tissues by Fourier Transform Infrared Hyperspectral Imaging. *J Biophotonics* 2020; **13**: e201960071 [PMID: 31648419 DOI: 10.1002/jbio.201960071]

79 **Gassner C**, Adegoke JA, Patel SK, Sharma VJ, Kochan K, Burrell LM, Raman J, Wood BR. Improved tissue preparation for multimodal vibrational imaging of biological tissues. *Clin Spect* 2022; **4**: 100021 [DOI: 10.1016/j.clispe.2022.100021]

80 **Meksiarun P**, Ishigaki M, Huck-Pezzei VA, Huck CW, Wongravee K, Sato H, Ozaki Y. Comparison of multivariate analysis methods for extracting the paraffin component from the paraffin-embedded cancer tissue spectra for Raman imaging. *Sci Rep* 2017; **7**: 44890 [PMID: 28327648 DOI: 10.1038/srep44890]

81 **Ali SM**, Bonnier F, Tfayli A, Lambkin H, Flynn K, McDonagh V, Healy C, Clive Lee T, Lyng FM, Byrne HJ. Raman spectroscopic analysis of human skin tissue sections ex-vivo: evaluation of the effects of tissue processing and dewaxing. *J Biomed Opt* 2013; **18**: 061202 [PMID: 23124057 DOI: 10.1117/1.JBO.18.6.061202]

82 **Liu J**, Osadchy M, Ashton L, Foster M, Solomon CJ, Gibson SJ. Deep convolutional neural networks for Raman spectrum recognition: a unified solution. *Analyst* 2017; **142**: 4067-4074 [PMID: 28993828 DOI: 10.1039/c7an01371j]

83 **Kazemzadeh M**, Hisey CL, Zargar-Shoshtari K, Xu W, Broderick NGR. Deep convolutional neural networks as a unified solution for Raman spectroscopy-based classification in biomedical applications. *Opt Commun* 2022; **510**: 127977 [DOI: 10.1016/j.optcom.2022.127977]

84 **Ma D**, Shang L, Tang J, Bao Y, Fu J, Yin J. Classifying breast cancer tissue by Raman spectroscopy with one-dimensional convolutional neural network. *Spectrochim Acta A Mol Biomol Spectrosc* 2021; **256**: 119732 [PMID: 33819758 DOI: 10.1016/j.saa.2021.119732]

85 **Weng S**, Yuan H, Zhang X, Li P, Zheng L, Zhao J, Huang L. Deep learning networks for the recognition and quantitation of surface-enhanced Raman spectroscopy. *Analyst* 2020; **145**: 4827-4835 [PMID: 32515435 DOI: 10.1039/d0an00492h]

86 **Berisha S**, Lotfollahi M, Jahanipour J, Gurcan I, Walsh M, Bhargava R, Van Nguyen H, Mayerich D. Deep learning for FTIR histology: leveraging spatial and spectral features with convolutional neural networks. *Analyst* 2019; **144**: 1642-1653 [PMID: 30644947 DOI: 10.1039/c8an01495g]

87 **He K**, Zhang X, Ren S, Sun J. Deep Residual Learning for Image Recognition. 2015. [cited 10 July 2022]. Available from: https://blog.csdn.net/wspba/article/details/57074389

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MINIREVIEWS

Unveiling the biological role of sphingosine-1-phosphate receptor modulators in inflammatory bowel diseases

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Abstract

Inflammatory bowel disease (IBD) is chronic inflammation of the gastrointestinal tract that has a high epidemiological prevalence worldwide. The increasing disease burden worldwide, lack of response to current biologic therapeutics, and treatment-related immunogenicity have led to major concerns regarding the clinical management of IBD patients and treatment efficacy. Understanding disease pathogenesis and disease-related molecular mechanisms is the most important goal in developing new and effective therapeutics. Sphingosine-1-phosphate (S1P) receptor (S1PR) modulators form a class of oral small molecule drugs currently in clinical development for IBD have shown promising effects on disease improvement. S1P is a sphingosine-derived phospholipid that acts by binding to its receptor S1PR and is involved in the regulation of several biological processes including cell survival, differentiation, migration, proliferation, immune response, and lymphocyte trafficking. T lymphocytes play an important role in regulating inflammatory responses. In inflamed IBD tissue, an imbalance between T helper (Th) and regulatory T lymphocytes and Th cytokine levels was found. The S1P/S1PR signaling axis and metabolism have been linked to inflammatory responses in IBD. S1P modulators targeting S1PRs and S1P metabolism have been developed and shown to regulate inflammatory responses by affecting lymphocyte trafficking, lymphocyte number, lymphocyte activity, cytokine production, and contributing to gut barrier function.

Key Words: Inflammatory bowel disease; Sphingosine-1-phosphate; Intestinal inflammation; T helper 1/T helper 17; Sphingosine 1 phosphate; Modulators; Immune responses

Core Tip: Recent literature has highlighted the use of novel oral small molecules, so-called sphingosine-1-phosphate (S1P) receptor modulators, in the therapeutic treatment of inflammatory bowel disease (IBD). Reviews and clinical trials have reported the safety profile and role of S1P modulators in alleviating IBD, but little information is available on their biological function. This is a comprehensive mini-review describing key biological mechanisms beyond the activity of S1P modulators reported in preclinical and clinical studies. The data from this study will contribute to the research field of developing therapeutic strategies in IBD based on the pathogenic biological background.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract that includes two main forms: ulcerative colitis (UC) and Crohn's disease (CD). IBD is thought to be mediated by dysregulation of the immune system, and its high epidemiological prevalence worldwide has led to great concern regarding the treatment and therapeutic management of patients[1]. Although current therapeutic agents have led to significant improvements in IBD in recent years, there is still a large percentage of patients who do not respond to these agents[2], and high immunogenicity has also been associated with monoclonal antibodies[3]. Sphingosine-1-phosphate (S1P) receptor modulators are novel oral small molecule drugs that have better prospects in terms of route of administration, pharmacokinetic properties, immunogenicity, manufacturing and cost compared to other biologic agents in IBD [4]. S1P is a highly bioactive molecule that regulates important biological processes such as inflammation, immune cell transport, and cell growth and transformation[5]. S1P level creates a gradient between blood and tissues, which contributes to the recruitment of immune cells and inflammatory mediators in tissues. S1P exerts its biological function by activating specific S1P receptors expressed by different cell types, initiating a signaling cascade that modulates lymphocyte migration, endothelial permeability, angiogenesis, cell proliferation, cell survival, apoptosis, and differentiation[6]. The components of S1P metabolism and S1P signaling have been associated with the regulation of immune responses and inflammation-related pathologies in the gastrointestinal tract. A number of agents targeting the inflammatory activity of sphingosine kinases (SphK1, SphK2) and S1P receptors are currently being tested in preclinical and clinical studies[5]. Amiselimod (MT-1303), etrasimod (APD-334), ozanimod (RPC-1063), and KRP-203 (2-amino-2-1,3-propanediol hydrochloride) belong to the class of S1P modulators currently in clinical development for IBD, and they help prevent lymphocyte migration into the gut[3]. Gut T lymphocyte colonization is a critical factor in chronic gut inflammation, considering the role they play in inflammatory immune responses and immune cell interactions. Overexpression of SphK kinases and the SphK/S1P axis are associated with the regulation of inflammation in the tumor microenvironment and mediate the development of gastric and colon cancer[7]. S1P receptor modulators (FTY720, ozanimod, etrasimod) have been proposed as therapeutics for gastrointestinal cancer due to their potential anti-inflammatory effects[8]. Elucidating the different mechanisms of action of therapeutics for IBD could be an important factor in selecting the right drug for the right patient.

In this brief review, we describe the main biological mechanisms of S1P receptor modulators in IBD and how these mechanisms might influence disease progression according to current experimental and clinical data.

ROLE OF T LYMPHOCYTES AND CYTOKINES IN INFLAMED IBD TISSUE

T helper (Th) lymphocytes play a key role in modulating immune responses in the intestinal mucosa by secreting cytokines and influencing the activity and function of other immune cells[9]. In the context of immune homeostasis, the intestinal mucosa has inflammatory immune responses under control, which are regulated by a delicate balance of Th1, Th2, Th3, Th9, Th17, and regulatory T cells (Tregs)[10-12]. Naive T cells circulate through secondary lymphoid organs until they interact with antigen-presenting cells (APCs) (*e.g.*, dendritic cells, macrophages, B cells) in the gut-associated lymphoid tissue, where they encounter their cognate antigen presented by APCs. This interaction leads to the activation and proliferation of T cells, which are also imprinted into a gut homing phenotype. Imprinted T cells express specific integrins and chemokine receptors to either settle in the small intestine or migrate to the colon [13-15]. The extravasation process of T cell homing begins with the binding and rolling of T cells across

the endothelium, which is mediated by the binding of selectins and integrins on T cells to their ligands on endothelial cells. This binding slows down T cells and activates them through tissue-activated chemokines [C-X-C motif chemokine ligand 10 (CXCL10), chemokine ligand 25 (CCL25)]. Conformational changes of integrins during the interaction between T cells and endothelium lead to the arrest of activated T cells, followed by transmigration through the endothelium into the intestinal tissue (Figure 1).

The development of IBD has been linked to the synergistic effects of Th cell activity, cytokines, antimicrobial peptides, and endoplasmic reticulum (ER) stress[16-18], which initiate signaling cascades leading to the activation of key pro-inflammatory transcription factors, including nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3), which further amplify and integrate signals from various stimuli[19].

In IBD, an imbalance has been observed between pro- and anti-inflammatory cytokines, which are released by the intestinal mucosa and influence the duration and intensity of inflammatory responses [20]. An accumulation of Th1 cells has been observed in IBD[21]. The proliferation and differentiation of Th1 cells is induced by interleukin 12 (IL-12)-secreting APCs[22]. Th1 cells produce interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) cytokines, which can exacerbate chronic epithelial damage of the intestinal mucosa, as they are thought to control beta-catenin signaling of intestinal epithelial cells and limit their differentiation and proliferation during intestinal inflammation[23]. IFN-γ production in colitis has been shown to induce other cells of the innate immune system to secrete inflammatory cytokines, thereby increasing chronic inflammation[24]. A specific IFN-γ+ cytotoxic CD4+ T cell subset directly promotes apoptosis of intestinal epithelial cells and intestinal enteroids in an *in vivo* colitis model[25].

In UC, both IL-10 and IL-13 cytokines are significantly increased, but the anti-inflammatory activity of IL-10 is not sufficient to reduce the activity of IL-13. The latter is restricted to the inflamed areas of the intestinal mucosa and is associated with epithelial barrier damage, cell apoptosis, decreased mucosal repair rate, and alteration of tight junctions, which negatively affects mucosal permeability[26,27].

Th17 cells are another important player in intestinal inflammation. They produce the cytokines IL-17 and IL-22, which are associated with the initiation of colitis, as they can trigger and amplify multiple inflammatory pathways. Th17 cells can also be converted to a Th1 cell phenotype in response to inflammatory cytokines (IL-12, IL-23)[28]. A disturbed Th17/Treg balance is thought to be responsible for the development of IBD. Th17 cells enhance inflammatory responses, while Tregs suppress intestinal inflammation and autoimmunity. Treg deficiency and an increase in Th17 activity have been observed in IBD[29].

The role of Th9 cells in mucosal inflammation has also been highlighted in experimental and human UC because expression of the transcription factor PU.1, a regulator of cellular communication, and secretion of IL-9 by Th9 cells can prevent the proliferation of intestinal epithelial cells and regulate the expression of several tight junction proteins. These factors promote the translocation of certain bacterial species to the intestine, leading to subsequent immune cell activation and inflammation of the intestinal mucosa[30].

Another pro-inflammatory type of T cells recently studied in CD are γδT cells[31]. The major subtype γδ2$^+$ of γδT cells has pro-inflammatory activity after migrating into inflamed intestinal tissue, where it produces the inflammatory cytokines TNF-α and IL-17A and induces the secretion of IFN-γ from αβT cells[32].

A pathogenic mechanism mediated by CD8+ T cells in the development of IBD has also been proposed. During chronic inflammation, cytotoxic CD8+ T cells can disrupt the intestinal epithelial barrier by recognizing peptides derived from commensal bacteria on the major histocompatibility complex class I of epithelial cells and releasing the inflammatory cytokines IFN-γ and TNFα, which destroy the tight junctions of intestinal epithelial cells. When the epithelial barrier is disrupted, bacteria can invade the lamina propria and trigger an immune response mediated by innate immune cells (*e.g.*, macrophages), which induces a strong pro-inflammatory milieu that drives CD8+ T cells toward Tc1 cells or IFN+ Tregs and further exacerbates tissue damage[33]. In parallel, the decreased apoptosis of intestinal lamina propria T cells observed in IBD patients favors their pro-inflammatory effect on tissues [34] (Figure 1).

BIOLOGY OF S1P METABOLISM AND SIGNALING IN IBD

S1P is a sphingosine-derived phospholipid found in high concentration in blood and in lower concentration in other tissues. Sphingolipids contain ceramides as a structural backbone with longer, amide-linked acyl chains ranging from 14 to 36 carbon atoms in length[35]. Ceramide synthesized in the ER is hydrolyzed to sphingosine, which is then phosphorylated by SphKs (SphK1 and SphK2) to form S1P, an important regulator of inflammation[36]. S1P comprises several types of sphingolipids (S1P1-5) that act by binding to 5 G protein-coupled receptors (S1PR1-5) and by affecting key intracellular molecules that regulate gene transcription, including activation of the pro-inflammatory transcription factor NF-κB and inhibition of histone deacetylases (HDACs), which are epigenetic regulators of gene expression.

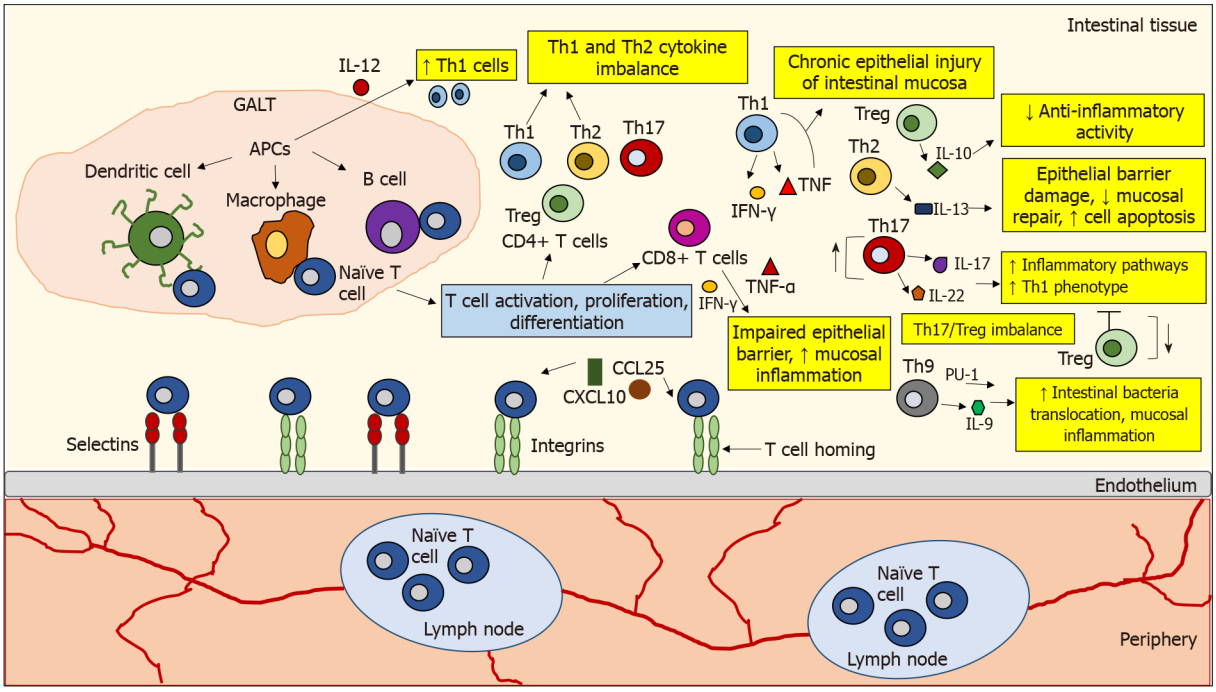

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Figure 1 The role of T lymphocytes in inflamed Inflammatory bowel disease tissue. Naive T cells circulate through secondary lymphoid organs until they interact with antigen-presenting cells (APCs) (*e.g.*, dendritic cells, macrophages, B cells) in gut-associated lymphoid tissue, where they encounter their cognate antigen presented by APCs. This interaction leads to T cell activation, proliferation, and differentiation. T cell homing begins with the binding and rolling of T cells across the endothelium, which is mediated by the binding of selectins and integrins on the T cells to their ligands expressed on the endothelial cells. This binding slows T cell activation by tissue-activated chemokines (C-X-C motif chemokine ligand 10, chemokine ligand 25). The development of inflammatory bowel disease (IBD) is associated with dysregulated activity of T helper (Th) cell subtypes. In IBD, an imbalance has been observed between pro- and anti-inflammatory cytokines released by the intestinal mucosa that influence the duration and intensity of inflammatory responses. In IBD, an accumulation of interleukin 12 (IL-12)-induced Th1 cells was observed. Th1 cells produce interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which may enhance chronic epithelial injury of the intestinal mucosa. IL-10 secreted by regulatory T cells (Tregs) is not sufficient to counteract inflammatory activity in IBD. IL-13 secreted by Th2 cells is associated with epithelial barrier damage, decreased mucosal repair, and increased cell apoptosis. Th17 cells produce IL-17 and IL-22 cytokines that trigger multiple inflammatory pathways. Th17 cells can also be converted to a Th1 cell phenotype. A disturbed Th17/Treg balance is thought to be responsible for the development of IBD. Th9 cells can promote the translocation of intestinal bacteria and inflammation of the intestinal mucosa by expressing the transcription factor PU.1 and the cytokine IL-9. CD8+ T cells can disrupt the intestinal epithelial barrier by releasing IFN-γ and TNF-α and increase mucosal inflammation. APCs: Antigen-presenting cells; CXCL10: C-X-C motif chemokine ligand 10; CCL25: Chemokine ligand 25; GALT: Gut-associated lymphoid tissue; IL: Interleukin; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-alpha; Th: T helper; Treg: T regulatory cells.

Abnormal S1P signaling has been demonstrated in preclinical colitis models, suggesting that targeting S1P production or interaction with S1PRs may alleviate colitis and reduce disease severity[37-39]. Metabolomic analysis of colon biopsies from IBD patients has revealed transcriptional and metabolic changes in sphingolipid metabolism that are thought to influence inflammation and intestinal mucosal integrity[40].

The SphK/S1PR network has been associated with the induction of inflammation-related transcription factors, including NF-κB[41] and forkhead box O[42]. The SphK/S1P axis has been shown to mediate inflammatory responses, induced by various pro-inflammatory effectors such as IL-1β and TNF-α[43,44]. Immune responses mediated by monocytes and macrophages also activate SphK/S1P signaling. Human monocytes express all five S1PRs[45], which have been shown to regulate monocyte chemotaxis and apoptosis[46]. Macrophage recruitment and manifestation of their anti-inflammatory properties have been shown to be modulated by increased activity of SphK1[47] (Figure 2). Increased expression of SphK1 has been demonstrated in IBD animal models and in human colon tissue from IBD patients, where it mediated colon damage during intestinal inflammation[48]. The SphK1/S1P/S1P1 axis was suggested to be an important signaling link between NF-κB and STAT3 transcription factors in a colitis animal model, which may have a significant impact on the relationship between chronic inflammation and colitis-associated cancer[49]. A possible role of sphingosine phosphate lyase (SPL), which degrades S1P, has been highlighted in colon carcinogenesis. SPL is expressed in differentiated enterocytes, Paneth cells, and inflammatory cells[50] and has been shown to be downregulated in colon carcinomas, resulting in increased S1P levels in neoplastic intestinal tissue[51].

S1P is a second messenger involved in the regulation of various biological processes and cellular activities, including cell survival, differentiation, migration, proliferation, immune response, trafficking of T and B cells, and cancer pathogenesis[52]. S1P also has a significant impact on the barrier function of the intestinal epithelium, as it has been shown *in vitro* to increase the concentration of E-cadherin, an

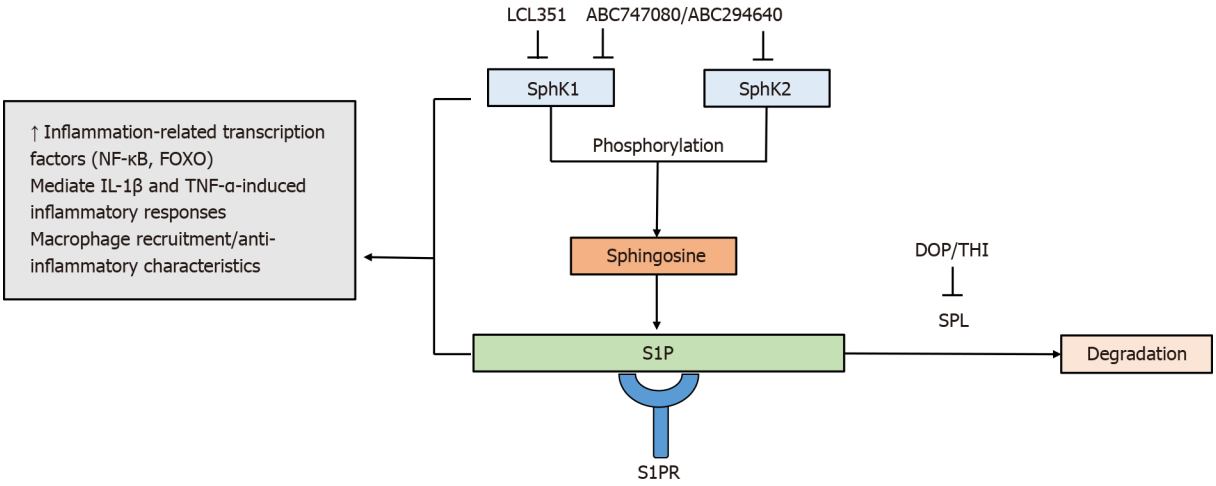

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Figure 2 Targeting sphingosine-1-phosphate metabolism by sphingosine-1-phosphate modulators. Sphingosine kinases (SphK1/SphK2) phosphorylate sphingosine to form sphingosine-1-phosphate (S1P). S1P degradation is mediated by sphingosine phosphate lyase. Components of S1P metabolism are involved in inflammatory responses. The SphK/S1P receptor network is associated with the induction of inflammation-related transcription factors, including nuclear factor-kappa B and forkhead box O. The SphK/S1P axis has been shown to mediate inflammatory responses induced by pro-inflammatory cytokines interleukin 1-beta and tumor necrosis factor-alpha. Increased activity of SphK1 modulates the recruitment of macrophages and the manifestation of their anti-inflammatory properties. S1P modulators targeting components of S1P metabolism contribute to the regulation of inflammatory immune responses. FOXO: Forkhead box O; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha; NF-Kβ: Nuclear factor-kappa B; S1P: Sphingosine-1-phosphate; SPL: Sphingosine phosphate lyase.

important adherens junctions protein, thereby improving barrier integrity[53]. *In vitro* studies have also shown that S1P can improve intestinal barrier integrity by reducing TNF-α-dependent disruption[54]. S1P1 binds to S1PR1 to regulate the extravasation and migration of lymphocytes from peripheral lymphoid organs to other tissues. The controlled S1P/S1PR1-dependent mechanism is also responsible for preventing extensive trafficking of T lymphocytes into inflamed tissues after antigen-mediated T cell activation. The increased level of S1P in blood leads to the internalization of S1PR1, and the decrease in S1PR1 expression in lymph nodes and tissues allows T cells to interact with APCs[55]. The re-expression of S1PR1 on the surface of T lymphocytes after several hours causes them to migrate from the lymph node or tissue into the blood, where S1PR1 senses the increased S1P and regulates the transport of immune cells into the bloodstream[3]. Elevated S1P levels in colitis *in vivo* have been shown to activate the transcription factor STAT3 and the production of the NF-κB-regulated cytokine IL-6, initiating a signaling cascade that leads to the upregulation of S1PR1[56]. Increased levels of S1PR1 and SphK1 have been detected in the inflamed intestinal mucosa of patients with UC[57].

Small molecules acting as S1PR modulators can prevent lymphocyte invasion of inflamed tissues by the process described above. S1PR1 agonists can induce persistent lymphopenia by leading to internalization and subsequent ubiquitination and proteasome degradation of the receptor. This prevents lymphocytes from leaving the lymph node because they cannot recognize S1P levels[55,58]. S1P2 acts as a pro-inflammatory factor and, together with S1P3, mediates vascular and intestinal vasoconstriction and fibrosis[59], and S1P2-S1P3 activity has been linked to NF-κB signaling, which is involved in cytokine production, cell survival, and inflammation[60]. *In vitro* and *in vivo* experiments have shown that the S1PR2 receptor can promote proliferation of intestinal epithelial cells and increase cell permeability, possibly by regulating the expression of SphK2, HDAC1, HDAC2, and the extracellular signalregulated protein kinase 1/2 (ERK1/2) pathway. Suppression of S1PR2 in the dextran sodium sulfate-induced colitis model has been shown to ameliorate pathological damage in the colon[61]. Rodent colitis induced by deoxycholic acid (DCA) has been associated with enhanced induction of S1PR2, which together with DCA-stimulated ERK1/2 protein kinases and released lysosomal cathepsin B, mediates the activation of NLR family pyrin domain containing 3 inflammasome formation[62]. S1P/S1PRs (1-3) can induce the expression of inflammatory mediators in rat intestinal smooth muscle cells, including IL-1 and cyclooxygenase-2 (COX-2) through activation of the transcription factor early growth response protein 1 and IL-6 through activation of the transcription factor STAT3[63]. S1P4 has an immunosuppressive function by inhibiting cytokine secretion and cytokine-driven proliferation of effector T lymphocytes and promoting secretion of the anti-inflammatory cytokine IL-10[64]. Expression of S1P4R4 on dendritic cells is associated with dendritic cell migration and cytokine production[65]. Expression of S1PR4 on dendritic cells is also involved in the regulation of Th17 cells and the production of IL-27, which promotes Treg-mediated suppression of CD8+ cytotoxic T cells. Migration of neutrophils from inflamed tissues to draining lymph nodes is promoted by S1PR4[66]. S1P5 may have an impact on immune regulation, considering its expression on endothelial cells within the blood-brain barrier[52] and the link between S1P5 expression and regulation of natural killer cell number[67].

S1PRs have been highlighted as important research targets in IBD because they regulate leukocyte migration and differentiation, endothelial function, and their interaction with immune cells, contributing to the development of intestinal inflammation[68]. S1P signaling triggered by S1P binding to S1PR, has been shown to mediate and regulate pro-inflammatory responses, including the pro-inflammatory TNF-α pathway, which is currently a therapeutic target in IBD[5]. Sphingolipid-metabolizing enzymes (SphK1, SphK2) are expressed by all cells of the gastrointestinal tract as well as immune cells, and the SphK/S1P/S1PRs signaling axis mediates both normal and pathogenic inflammatory responses by influencing lymphocyte trafficking and activation of cytokine signaling. Novel agents acting as SphK inhibitors and S1PRs antagonists are being evaluated in preclinical and clinical studies for their effect in ameliorating SphK/S1P/S1PRs-mediated exacerbation of inflammation in IBD [5]. Expression of S1PR1 and SP1R4 on T cells and expression of S1PR2 on lymphatic endothelial cells (LECs) regulate the migration of T cells through LECs and into lymphatic vessels and lymph nodes. S1PR1 and S1PR4 act differently in modulating T cell motility and binding to the adhesion molecule vascular cell adhesion protein 1 (VCAM-1). Deficiency of S1PR4 has been associated with disruption of the composition of peritoneal B cell populations and decreased immunoglobulin A levels in inflammatory colitis *in vivo*[69]. S1RP2 expression is increased in vascular endothelial cells in response to microbial components and the pro-inflammatory cytokine TNF-α[70]. S1PR2 regulates the layer structure and permeability of LECs as well as the expression of adherens junction proteins *via* the ERK signaling pathway[71]. Activated S1PR3 has been shown to induce the expression of the enzyme COX-2, an inflammatory mediator, in vascular smooth muscle cells through its interaction with calcium-dependent protein kinase C and Src tyrosines[72]. Increased activation of COX-2 and high inflammatory intensity were associated with increased S1P formation in a colitis animal model[39]. The effect of SphK/S1P/S1PR signaling on linking chronic inflammation and cancer development in the gastrointestinal tract has highlighted the important role of S1PR modulators in malignancies in IBD[7]. FTY720, a SphK1 and S1PR inhibitor, was proposed as an anticancer agent in gastrointestinal cancer cells because it led to the deactivation of cancer-related downstream signaling pathways (ERK1/2, Act, c-Myc, β-catenin), which enhanced pro-apoptotic activity and tumor regression. FTY720 regulates inflammation by inducing S1PR degradation, inhibiting SphK1 activity and expression, and disrupting pro-inflammatory NF-κB/IL-6/STAT3 signaling[7]. S1PRs may be mediators of inflammatory responses in the tumor microenvironment by exerting functions on the transport and activity of innate immune system cells[8]. S1PR1 is associated with macrophage recruitment, apoptosis and anti-inflammatory responses, dendritic cell transport and inhibition of IFN-α secretion, neutrophil and eosinophil/mast cell recruitment, monocyte transport and natural killer cell egress from the lymph nodes. S1PR2 enhances antibody-mediated phagocytosis by macrophages and regulates monocyte migration. S1PR3 and S1PR4 regulate monocyte and neutrophil recruitment. S1PR3 is involved in dendritic cell-mediated maturation, promotion of Th1 response, and suppression of Tregs. S1PR5 is related to natural killer cell exit from the bone marrow and monocyte trafficking[8]. S1PR modulators tested in preclinical and clinical studies (*e.g.*, JTE013, mocravimod, amiselimod, ozanimod, etrasimod, FTY720) have shown a promising role as immunomodulators in the prevention of chronic inflammation and the treatment of inflammatory gastrointestinal cancers[8].

S1P/S1PR MODULATORS IN IBD: BIOLOGICAL MECHANISMS AND EFFECTS ON DISEASE COURSE

The development of oral small molecules targeting S1P/S1PR signaling and metabolism has shown great promise in the therapeutic area of IBD, followed by two decades of monoclonal antibodies. S1P modulators are advantageous in IBD therapy due to their low molecular weight, oral administration, low immunogenicity, rapid action, and inexpensive production. An important mechanism of action of S1P receptor modulators is that they antagonize S1P1 receptors on lymphocytes, thereby inhibiting their migration from secondary lymphoid organs to the periphery, resulting in a decrease in the number of circulating lymphocytes, including autoreactive T cells, thus causing immunomodulation[73]. Another indirect function of S1P drugs in IBD is to affect S1P metabolism by being able to inhibit the activity of components of S1P metabolism (Figure 2). Several novel selective S1P modulators are currently in development and are being evaluated for efficacy and safety profile in preclinical and clinical studies (Table 1 and Figure 3).

BIOLOGICAL FUNCTION OF S1P/S1PR MODULATORS AND PHASE OF CLINICAL DEVELOPMENT IN IBD

Amiselimod (MT-1303)

Amiselimod (MT-1303) is an oral selective modulator of the S1P1 receptor that is converted *in vivo* by

Table 1 Sphingosine-1-phosphate modulators and mechanism of action in inflammatory bowel disease

S1P modulator	Mechanism of action in IBD	Developmental phase	Ref.
Amiselimod (MT-1303)	Modulator of the S1P1 receptor→ S1P1; Internalization into lymphocytes→↓migration to the periphery; ↓Infiltration of pro-inflammatory Th1/Th17 cells into the colon; ↑Tregs in mesenteric lymph nodes	Phase IIa	[73-75]
Fingolimod (FTY720)	Specific inhibitor of S1PR1; Interferes with S1P signaling→↓lymphocyte entry into lymph nodes; ↑CD4+CD25+FOXP3+ T cells, ↑CD25, FOXP3 expression, Treg activity; ↑ Immunosuppressive cytokines IL-10 and TGF-β and CTLA-4→↑Treg-mediated immunomodulation; ↓Pro-inflammatory signaling (IL-12p70, Th1 cytokines); ↓Pro-inflammatory signals on dendritic cells→↑activity of CD4+CD25+ Tregs	Preclinical studies, not tested in humans	[76-83]
Etrasimod (APD-334)	Agonist of S1P1, partial agonist of S1P4/S1P5; Lymphopenia, ↓mucosal thickness, immune cell infiltration, expression of T-cell and monocyte markers; ↓Pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17A, ↑anti-inflammatory IL-10	Phase II/III	NCT03996369, NCT03945188, NCT03950232, NCT04173273[84-86]
Ozanimod (RPC-1063)	Selective S1P1 and S1P5 receptor agonist; ↓T cell migration→↓peripheral lymphocytes↑S1P1 receptor internalization and degradation; ↓Circulating B and CCR7+ T lymphocytes→↓ inflammation; ↓Mononuclear cell infiltrate and mucosal thickness	Phase II/III	NCT03440385, NCT03464097, NCT03467958, NCT03440372[87-92]
KRP-203	Modulator of S1P1 receptor, partial agonist of S1PR3 receptor; Lymphopenia, ↑homing of lymphocytes to peripheral lymph nodes; ↓Infiltration of inflammatory cells in the lamina propria of the intestine; ↓CD4+ T and B220+ B cells in peripheral blood and in the lamina propria of the colon; ↓Peripheral naive and central memory CD4+ and CD8+ T cells and B cells; ↑Lymphocytes in mesenteric lymph nodes and spleen; ↓Pro-inflammatory cytokines IFN-γ, TNF-α, and IL-12 in the lamina propria of the colon	Phase II	[93-96]
LCL351	SphK1 inhibitor→↓S1P production; ↓Neutrophil infiltration into sites of inflammation, ↓ inflammatory marker TNF-α; Altered S1P levels and ↓neutrophil chemoattractants CXCL1 and CXCL2→↓leukocyte recruitment to sites of inflammation	Preclinical studies	[43,97]
ABC747080 and ABC294640	Inhibitors of SphKs→↓S1P formation, SphK activity; ↓TNF-α-induced activation of NF-Kβ; ↓ Effects of TNF-α on leukocyte recruitment and TNF-α- mediated increase in adhesion protein expression levels; ↓Pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-6) in colon tissue	Preclinical studies	[98]
DOP and THI	S1P lyase inhibitors; Peripheral lymphopenia, ↓CD4+ and CD8+ T cells; ↓Pro-inflammatory cytokines, including TNF-α, IL-6, IL-12, IFN-γ and IL-17; ↓S1PR1 expression on T lymphocytes; Depletion of late immature T cells (CD4+CD8+ double positive) and mature CD4+CD8- and CD4-CD8+ single positive cells	Preclinical studies	[99,100]
W-061	S1P receptor agonist; ↓Lymphocyte migration to the spleen and lamina propria, pro-inflammatory Th1 and Th17 cells in the lamina propria→ prevention of changes in intestinal mucosal architecture	Preclinical studies	[101]
SEW2871	S1P1 agonist; Mild inflammatory cell infiltration, ↓CD4+ T cells in the lamina propria of the colon; ↓Pro-inflammatory cytokines TNF-α and IFN-γ were significantly reduced in colonic tissues; Improved intestinal barrier function, ↑typical tight junction protein expression and distribution in the intestinal epithelium; ↓Apoptosis of intestinal epithelial cells→ restoration of colon tissue injury	Preclinical studies	[102,103]

CCR7: C-C chemokine receptor type 7; CTLA-4: Cytotoxic T-lymphocyte–associated antigen 4; CXCL: C-X-C motif chemokine ligand; FOXP3: Forkhead box P3; IBD: Inflammatory bowel disease; IFN-γ: Interferon-gamma; IL: Interleukin; NF-κB: Nuclear factor-kappa B; S1P: Sphingosine 1 phosphate; S1PR: Sphingosine 1 phosphate receptor; SphKs: Sphingosine kinases; TGF-β: Transforming growth factor-beta; Th: T helper; TNF-α: Tumor necrosis factor-alpha; Tregs: Regulatory T cells.

SphKs to its active metabolite MT-1303 phosphate (MT1303-P)[73]. The effect of MT-1303 on chronic colitis was studied in immunodeficient SCID mice induced by adoptive transfer of CD4+CD45RB^{high} T cells from BALB/c mice, an animal model for IBD. Oral administration of MT-1303 proved effective in the IBD mouse model and comparable to the effect of an anti-mTNF-α monoclonal antibody. MT-1303 significantly inhibited the infiltration of Th1 and Th17 cells into the colon by inducing the internalization of S1P1 into lymphocytes from lymph nodes and preventing their migration to the periphery. MT-1303 treatment also showed an effect on the migration of Tregs in normal mice by leading to an increased number of Tregs and an increased proportion of Tregs in mesenteric lymph nodes[74]. MT-1303 is currently enrolled and completed in a multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase IIa study comparing amiselimod 0.4 mg administration to placebo over a 14-wk treatment period to evaluate safety, tolerability, and efficacy in patients with moderate-to-severe active Crohn's disease. MT-1303 treatment proved no better than placebo in eliciting a clinical response and was well tolerated, with no new safety concerns[75].

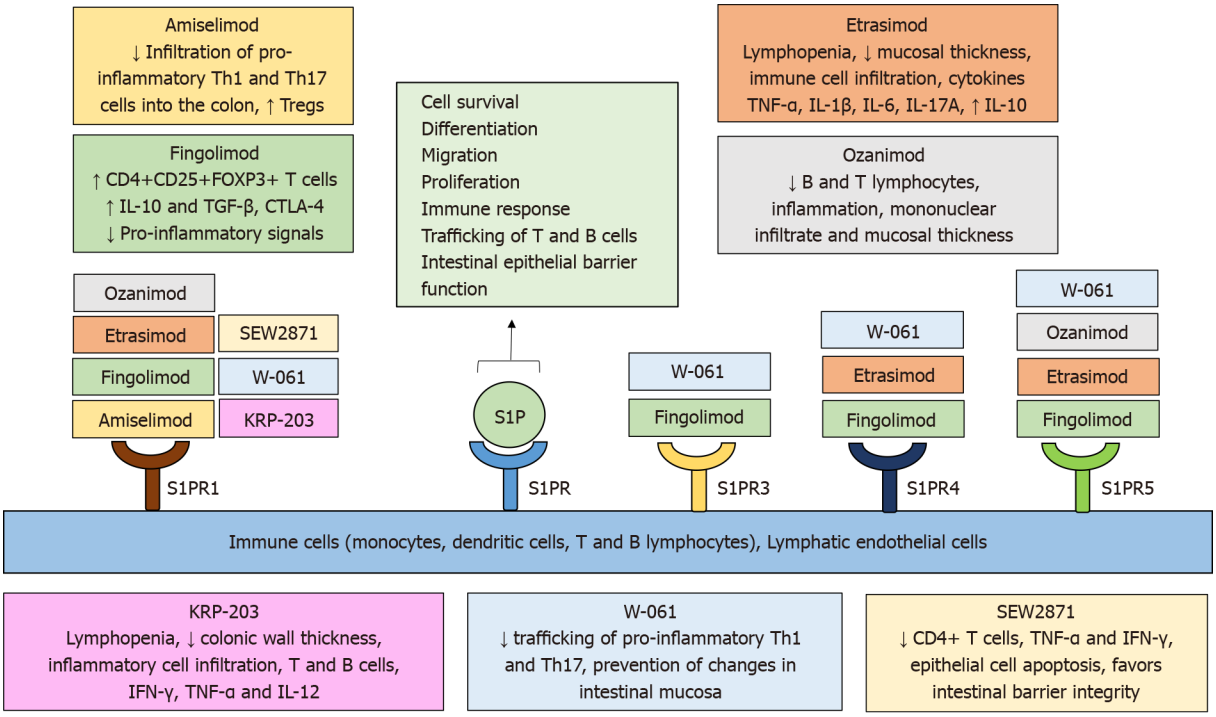

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Figure 3 Sphingosine-1-phosphate receptor modulators in preclinical and clinical studies in inflammatory bowel disease. Sphingosine-1-phosphate (S1P) receptors (S1PRs) have been highlighted as important research targets in inflammatory bowel disease (IBD). S1PRs are expressed on immune cells and lymphatic endothelial cells. Binding of S1P to S1PR regulates a variety of biological processes, including cell survival, differentiation, migration, proliferation, immune response, trafficking of T and B cells, and intestinal epithelial barrier function. Several novel, selective S1PR modulators are currently being tested in IBD and contribute to the amelioration of the inflammatory process in inflamed intestinal tissue by regulating immune cell concentration, activity, trafficking, and cytokine secretion, as well as intestinal barrier function and integrity. S1P: Sphingosine 1 phosphate; S1PR: Sphingosine 1 phosphate receptor; IBD: Inflammatory bowel disease; Th: T helper; Tregs: Regulatory T cells.

Fingolimod (FTY720)

Fingolimod (FTY720) is a specific inhibitor of S1PR1, which is a synthetic sphingosine analog (2-amino-2-2-(4-octylphenyl) ethyl-1,3-propanediol hydrochloride) of myriocin[76]. The main mechanism of action of FTY720 involves the induction of lymph node homing or sequestration of T cells. FTY720 is phosphorylated by SphK2[77] *in vivo*, and FTY720 phosphate can act as an S1P agonist that activates four of the five known G-protein-coupled S1PRs (S1P1, 3, 4, 5). S1PR1 and S1PR4 are mainly expressed on T and B lymphocytes. FTY720 interferes with S1P signaling, impeding lymphocyte entry into lymph nodes and thus delaying their subsequent return to the circulation[78]. FTY720 has not been shown to impair the activation, expansion, and differentiation of T cells to memory phenotypes, nor does it induce cell apoptosis[78-80]. FTY720 can effectively treat Th1-mediated colitis in mice by strongly affecting the activity of Tregs *in vivo*. Specifically, FTY720 administration in colitis mice was associated with increased numbers of CD4+CD25+FOXP3+ T cells and induction of CD25 and FOXP3 expression in CD4+ T cells. The immunosuppressive cytokines IL-10 and TGF-β, as well as CTLA-4, a receptor mediating the immunomodulatory function of Tregs, were also upregulated in the presence of FTY720. The therapeutic effect of FTY720 on ameliorating intestinal inflammation was associated with the downregulation of pro-inflammatory signals such as IL-12p70 and Th1 cytokines. It was suggested that FTY720-induced downregulation of pro-inflammatory signals on dendritic cells might contribute to the enhanced activity of CD4+CD25+ Tregs[81]. Although FTY720 has been approved by the Food and Drug Administration for the treatment of relapsing multiple sclerosis[82], it has not been tested in IBD patients because some adverse events (*e.g.*, elevated liver enzymes) have been noted[83].

Etrasimod (APD-334)

Etrasimod (APD-334) is a next-generation synthetic S1P receptor modulator that acts as a full agonist of human S1P1 and a partial agonist of S1P4 and S1P5[84]. Treatment with APD-334 in colitis mice resulted in dose-dependent lymphopenia, decreased mucosal thickness, and immune cell infiltration by significantly reducing the expression of T cell and monocyte markers. T cell and/or monocyte-derived proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17A were also significantly reduced in APD-334-treated mice, and there was a dose-dependent increase in the anti-inflammatory cytokine IL-10 after APD-334 administration[85]. APD-334 has been enrolled in phase II/III clinical trials in patients with UC. In the Phase-2 OASIS study in patients with moderately-to-severely active UC with prior failure or

intolerance of conventional or biologic therapy, administration of 2 mg APD-334 resulted in significant improvement in clinical symptoms, followed by clinical remission and endoscopic improvement, with no serious life-threatening adverse events or death[86]. Moderately to severely active UC patients are currently enrolled in the ELEVATE phase III trial (NCT03996369, NCT03945188, NCT03950232). An ongoing phase II/III trial (CULTIVATE, NCT04173273) is recruiting 1265 patients with CD to evaluate the safety and efficacy of APD-334.

Ozanimod (RPC-1063)

Ozanimod (RPC-1063) is a small molecule, selective S1P1 and S1P5 receptor agonist. RPC-1063 can bind to S1P1 and S1P5 receptors, limiting the migration of T cells from peripheral lymphoid organs and reducing the number of peripheral lymphocytes[87]. The exact mechanism of action in alleviating IBD has not yet been determined. Administration of RPC-1063 in three models of autoimmune disease (experimental autoimmune encephalitis, 2,4,6-trinitrobenzenesulfonic acid colitis, and CD4+CD45RBhi T-cell adoptive transfer colitis) has shown that it induces internalization and degradation of the S1P1 receptor, leading to a reduction in circulating B and CCR7+ T lymphocytes[88], which in turn leads to a reduction in inflammation. The potential benefit of RPC-1063 for CD patients was also highlighted in a spontaneous ileitis mouse model, in which treatment with RPC-1063 resulted in a reduction in mononuclear infiltrate and mucosal thickness[89]. RPC-1063 has completed phase II/III clinical trials in patients with moderate to severe UC in which it demonstrated improved clinical, endoscopic and histologic outcomes, including reduction in rectal bleeding scores, maintenance of clinical remission, mucosal healing, histologic and durable remission. Based on the good safety profile and efficacy results, RPC-1063 was approved by regulatory authorities for the treatment of moderate to severe UC[90,91]. Histologic improvements, endoscopic remission, and a good safety profile were also reported in a phase II trial evaluating RPC-1063 in moderate-to-severe CD patients[92], and placebo-controlled phase III trials of RPC-1063 in patients with moderate-to-severe active CD are ongoing (NCT03440385, NCT03464097, NCT03467958, NCT03440372).

KRP-203 (2-amino-2-1,3-propanediol hydrochloride)

KRP-203 is a selective S1P1 modulator with a molecular structure similar to that of FTY720. Like FTY720, it induces lymphopenia by decreasing the number of lymphocytes in peripheral blood and promoting lymphocyte homing to peripheral lymph nodes[93]. KRP-203 is phosphorylated *in vivo* by SphK2. The phosphate metabolite (KRP-203 phosphate) is the active molecule that targets the S1P1 receptor and acts as a partial agonist for the human S1P3 receptor[94]. The treatment efficacy of KRP-203 in chronic colitis was investigated using a IL-10 gene-deficient (IL-$10^{-/-}$) mouse model. Mice treated with KRP-203 showed significantly reduced severity of colitis, reduced thickness of the colonic wall, reduced expansion of glandular crypts, and reduced infiltration of inflammatory cells in the lamina propria of the intestine. Administration of KRP-203 to colitis mice significantly decreased the number of CD4+T and B220+B cells in the peripheral blood and lamina propria of the colon and increased the number of lymphocytes in the mesenteric lymph nodes and spleen. After treatment with KRP-203 in colitis mice, the production of the pro-inflammatory cytokines IFN-γ, TNF-α, and IL-12 by lymphocytes in the lamina propria of the colon was significantly reduced[95]. KRP-203 was tested for its safety, tolerability, and efficacy in patients with moderately active 5-aminosalicylate-refractory UC, where it resulted in significant reductions in peripheral naive and central memory CD4+ and CD8+ T cells and B cells. KRP-203 was also found to be safe and well tolerated and resulted in clinical remission (NCT01375179)[96].

LCL351 (L-erythro-2-N-(1'-carboxamidino)-sphingosine hydrochloride)

LCL351 is a selective inhibitor of SphK1, which plays a role in S1P production. SphK1 can be activated in response to TNF-α and induce the expression of COX-2 and the production of prostaglandin E2[43]. SphK inhibitors, including LCL351, act *via* a two-way mechanism. They can either cause competitive inhibition of kinase activity or lead to proteolysis of SphK1. LCL351 showed efficacy in reducing inflammation in DSS-induced colitis in mice. LCL351 had a longer residence time in colon tissue than in blood without causing cell death. Treatment of colitis mice with LC351 blocked the infiltration of neutrophils into inflammatory sites and slightly attenuated the induction of TNF-α. The altered S1P levels mediated by LCL351, together with the reduction of neutrophil chemoattractants CXCL1 and CXCL2, may help prevent leukocyte recruitment to sites of inflammation and keep immune cells in the circulation[97].

ABC747080 (4-2-4-(4-chlorophenyl)thiazol-2-ylcarbamoyl-vinyl-2-methoxy-phenyl ester) and ABC294640 (3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridin-4-ylmethyl)amide)

ABC747080 and ABC294640 are small molecules that act as selective inhibitors of SphKs and have been studied in mouse models of UC. These SphK inhibitors decreased cellular S1P formation in human endothelial cells and rat intestinal epithelial cells *in vitro* and caused a dose-dependent suppression of the activity of SphK. Treatment of fibroblasts with ABC294640 resulted in inhibition of TNF-α- induced NF-κB activation. Treatment with the SphK inhibitors ABC747080 or ABC294640 SphK *in vitro* was associated with attenuation of the effects of TNF-α on leukocyte recruitment, including TNF-α- mediated increases in adhesion protein (ICAM-1, VCAM-1) expression levels. Addition of ABC747080 or

ABC294640 to TNF-α-treated rat intestinal epithelial cells and human endothelial cell lines inhibited TNF-α-mediated induction of COX-2 activity, measured as production of PGE2. ABC294640 was also associated with decreased levels of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-6) in colonic tissue of mice with colitis. The favorable modulation of inflammatory mediators, including S1P, NF-κB, TNF-α, VCAM-1, ICAM-1, COX-2, IL-1β, IFN-γ, and IL-6 by SphK targeting, suggests that the anti-IBD activity of SphK modulators is associated with SphK inhibition and decreased S1P synthesis[98].

DOP (4-deoxypyridoxine hydrochloride) and THI (2-acetyl-4-(tetrahydroxybutyl)imidazole) SPL inhibitors

DOP (4-deoxypyridoxine hydrochloride) and THI (2-acetyl-4-(tetrahydroxybutyl)imidazole) are small molecules that target S1P lyase (SPL), an enzyme that, together with phosphatases, tightly regulates S1P levels and keeps them low in tissues[99]. The potential anti-inflammatory effects of these SPL inhibitors on IBD were investigated in a TNF-driven mouse model of chronic ileitis with CD features. Mice treated with SPL inhibitors DOP and THI showed peripheral lymphopenia characterized by decreased numbers of CD4+ and CD8+ T cells. DOP treatment was also associated with a reduction in ileal mRNA transcripts of pro-inflammatory cytokines, including TNF, IL-6, IL-12, IFN-γ, and IL-17, resulting in attenuation of active (granulocytic) inflammation, chronic (lymphocytic/monocytic) inflammation, and overall inflammatory indices. DOP SPL inhibitor treatment resulted in downregulated S1PR1 surface expression on lymphocytes. DOP treatment was associated with promotion of thymic atrophy, depletion of late immature T cells (CD4+CD8+ double-positive) and mature CD4+CD8- and CD4-CD8+ single positive cells. It has therefore been suggested that the impairment of T cell maturation and thymic activity mediated by SPL inhibitors may significantly impair the anti-inflammatory effects of SPL inhibitors in IBD[100].

W-061

W-061 is an S1P receptor agonist that has been shown to bind all human S1P receptors except S1PR2. The therapeutic effect of W-061 on IBD was tested in a mouse model of DSS-induced colitis. Administration of W-061 to colitis mice suppressed the migration of lymphocytes to the spleen and lamina propria and induced their homing to secondary lymphoid tissues (mesenteric lymph nodes, Peyer's patches). Specifically, W-061 inhibited the migration of pro-inflammatory Th1 and Th17 cells into the lamina propria, prevented changes in intestinal mucosal architecture, and ameliorated the acute exacerbation of colitis[101].

SEW2871

SEW2871, a selective S1P1 agonist, was administered to IL10$^{-/-}$ colitis mice to investigate its function in alleviating chronic inflammation in IBD, as its protective effect on the development of colitis was demonstrated[102]. Treatment with SEW2871 resulted in mild infiltration of inflammatory cells, a reduced inflammatory score, and a decrease in CD4+ T cells in the lamina propria of the colon. The levels of pro-inflammatory cytokines TNF-α and IFN-γ were significantly reduced in the colon tissue of SEW2871-treated mice. A beneficial effect of SEW2871 on gut barrier function was also highlighted in this study. SEW2871 treatment prevented colonic permeability in IL-10$^{-/-}$ mice and promoted the typical expression and distribution of tight junction proteins in the intestinal epithelium. A possible beneficial effect of SEW2871 on the healing of colon tissue injury was observed, as SEW2871 reduced apoptosis of intestinal epithelial cells[103].

CONCLUSION

The development of IBD is mediated by dysregulated immune responses that initiate and maintain a vicious cycle of chronic inflammation. Understanding the immunopathogenesis of IBD is of great clinical importance for the development of effective therapeutics targeting these immunological pathogenic mechanisms. S1P modulators are orally administered small molecules that show promise in alleviating chronic inflammation and clinical symptoms in IBD patients. Their therapeutic efficacy is based on the fact that they regulate immune cell trafficking at sites of inflammation and are involved in immune cell interaction, cytokine production and pro-inflammatory signaling, as well as contributing to gut barrier function. The S1P receptor agonists etrasimod and ozanimod have shown favorable efficacy and safety profiles in UC and CD, patients respectively, and have significantly improved the clinical and endoscopic characteristics of IBD patients[104]. Ozanimod is being studied in ongoing phase III trials for CD (NCT03440372, NCT03440385, and NCT03464097) and UC (NCT02435992 and NCT02531126). Phase III trials (NCT03996369, NCT03945188, NCT03950232) are currently ongoing to evaluate etrasimod in patients with UC and a phase II-III trial (NCT04173273) is ongoing for etrasimod in CD. Although ozanimod has been proposed as first-line therapy in IBD, its appropriate positioning in the therapeutic algorithm for the treatment of UC treatment has not yet been defined, while its potential therapeutic activity in CD remains to be clarified[105]. KRP-203 has been shown to be safe and well tolerated in UC

patients without reaching the relevant threshold for efficacy, indicating the need for improved study design and recruitment of a larger population[96]. Determining the appropriate treatment duration of S1P modulators may pose another problem in their therapeutic use in IBD, considering that an increased risk of progressive multifocal leukoencephalopathy was observed during treatment with ozanimod and fingolimod, which was associated with a longer treatment duration in patients with multiple sclerosis[104,106,107]. The satisfactory efficacy and safety profile of S1P modulators in clinical trials, combined with their structure and advantageous production characteristics underscores their novel perspective for the treatment of IBD. Ongoing studies need to further elucidate their positioning within current treatment algorithms, their potential as combination therapies, and potential complications associated with their treatment regimens.

FOOTNOTES

Author contributions: Triantos C conceived and coordinated the study; Tourkochristou E and Mouzaki A conducted the literature search and analysis and wrote the manuscript; Triantos C and Mouzaki A were responsible for revising the manuscript for important intellectual content; Tourkochristou E, Mouzaki A, and Triantos C approved the submitted version of the manuscript.

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REFERENCES

1 **Coward S**, Clement F, Benchimol EI, Bernstein CN, Avina-Zubieta JA, Bitton A, Carroll MW, Hazlewood G, Jacobson K, Jelinski S, Deardon R, Jones JL, Kuenzig ME, Leddin D, McBrien KA, Murthy SK, Nguyen GC, Otley AR, Panaccione R, Rezaie A, Rosenfeld G, Peña-Sánchez JN, Singh H, Targownik LE, Kaplan GG. Past and Future Burden of Inflammatory Bowel Diseases Based on Modeling of Population-Based Data. *Gastroenterology* 2019; **156**: 1345-1353.e4 [PMID: 30639677 DOI: 10.1053/j.gastro.2019.01.002]

2 **Singh S**, George J, Boland BS, Vande Casteele N, Sandborn WJ. Primary Non-Response to Tumor Necrosis Factor Antagonists is Associated with Inferior Response to Second-line Biologics in Patients with Inflammatory Bowel Diseases: A Systematic Review and Meta-analysis. *J Crohns Colitis* 2018; **12**: 635-643 [PMID: 29370397 DOI: 10.1093/ecco-jcc/jjy004]

3 **Olivera P**, Danese S, Peyrin-Biroulet L. Next generation of small molecules in inflammatory bowel disease. *Gut* 2017; **66**: 199-209 [PMID: 27856614 DOI: 10.1136/gutjnl-2016-312912]

4 **Argollo M**, Fiorino G, Hindryckx P, Peyrin-Biroulet L, Danese S. Novel therapeutic targets for inflammatory bowel disease. *J Autoimmun* 2017; **85**: 103-116 [PMID: 28711286 DOI: 10.1016/j.jaut.2017.07.004]

5 **Sukocheva OA**, Lukina E, McGowan E, Bishayee A. Sphingolipids as mediators of inflammation and novel therapeutic target in inflammatory bowel disease. *Adv Protein Chem Struct Biol* 2020; **120**: 123-158 [PMID: 32085881 DOI: 10.1016/bs.apcsb.2019.11.003]

6 **Danese S**, Furfaro F, Vetrano S. Targeting S1P in Inflammatory Bowel Disease: New Avenues for Modulating Intestinal Leukocyte Migration. *J Crohns Colitis* 2018; **12**: S678-S686 [PMID: 28961752 DOI: 10.1093/ecco-jcc/jjx107]

7 **Sukocheva OA**, Furuya H, Ng ML, Friedemann M, Menschikowski M, Tarasov VV, Chubarev VN, Klochkov SG, Neganova ME, Mangoni AA, Aliev G, Bishayee A. Sphingosine kinase and sphingosine-1-phosphate receptor signaling pathway in inflammatory gastrointestinal disease and cancers: A novel therapeutic target. *Pharmacol Ther* 2020; **207**: 107464 [PMID: 31863815 DOI: 10.1016/j.pharmthera.2019.107464]

8 **McGowan EM**, Lin Y, Chen S. Targeting Chronic Inflammation of the Digestive System in Cancer Prevention: Modulators of the Bioactive Sphingolipid Sphingosine-1-Phosphate Pathway. *Cancers (Basel)* 2022; **14** [PMID: 35158806 DOI: 10.3390/cancers14030535]

9 **Ma H**, Tao W, Zhu S. T lymphocytes in the intestinal mucosa: defense and tolerance. *Cell Mol Immunol* 2019; **16**: 216-

224 [PMID: 30787416 DOI: 10.1038/s41423-019-0208-2]
10 **Neurath MF**. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 2014; **14**: 329-342 [PMID: 24751956 DOI: 10.1038/nri3661]
11 **Neurath MF**, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. *Nat Med* 2002; **8**: 567-573 [PMID: 12042806 DOI: 10.1038/nm0602-567]
12 **Liu ZJ**, Yadav PK, Su JL, Wang JS, Fei K. Potential role of Th17 cells in the pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2009; **15**: 5784-5788 [PMID: 19998498 DOI: 10.3748/wjg.15.5784]
13 **Habtezion A**, Nguyen LP, Hadeiba H, Butcher EC. Leukocyte Trafficking to the Small Intestine and Colon. *Gastroenterology* 2016; **150**: 340-354 [PMID: 26551552 DOI: 10.1053/j.gastro.2015.10.046]
14 **Trivett MT**, Burke JD, Deleage C, Coren LV, Hill BJ, Jain S, Barsov EV, Breed MW, Kramer JA, Del Prete GQ, Lifson JD, Swanstrom AE, Ott DE. Preferential Small Intestine Homing and Persistence of CD8 T Cells in Rhesus Macaques Achieved by Molecularly Engineered Expression of CCR9 and Reduced *Ex Vivo* Manipulation. *J Virol* 2019; **93** [PMID: 31434738 DOI: 10.1128/JVI.00896-19]
15 **Dzutsev A**, Hogg A, Sui Y, Solaymani-Mohammadi S, Yu H, Frey B, Wang Y, Berzofsky JA. Differential T cell homing to colon vs. small intestine is imprinted by local CD11c$^+$ APCs that determine homing receptors. *J Leukoc Biol* 2017; **102**: 1381-1388 [PMID: 28951425 DOI: 10.1189/jlb.1A1116-463RR]
16 **Sun H**, Liu J, Zheng Y, Pan Y, Zhang K, Chen J. Distinct chemokine signaling regulates integrin ligand specificity to dictate tissue-specific lymphocyte homing. *Dev Cell* 2014; **30**: 61-70 [PMID: 24954024 DOI: 10.1016/j.devcel.2014.05.002]
17 **Festen EA**, Szperl AM, Weersma RK, Wijmenga C, Wapenaar MC. Inflammatory bowel disease and celiac disease: overlaps in the pathology and genetics, and their potential drug targets. *Endocr Metab Immune Disord Drug Targets* 2009; **9**: 199-218 [PMID: 19519468 DOI: 10.2174/187153009788452426]
18 **Grivennikov S**, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 2009; **15**: 103-113 [PMID: 19185845 DOI: 10.1016/j.ccr.2009.01.001]
19 **Bromberg J**, Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. *Cancer Cell* 2009; **15**: 79-80 [PMID: 19185839 DOI: 10.1016/j.ccr.2009.01.009]
20 **Neurath MF**, Fuss I, Kelsall BL, Presky DH, Waegell W, Strober W. Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. *J Exp Med* 1996; **183**: 2605-2616 [PMID: 8676081 DOI: 10.1084/jem.183.6.2605]
21 **Gwela A**, Siddhanathi P, Chapman RW, Travis S, Powrie F, Arancibia-Cárcamo CV, Geremia A. Th1 and Innate Lymphoid Cells Accumulate in Primary Sclerosing Cholangitis-associated Inflammatory Bowel Disease. *J Crohns Colitis* 2017; **11**: 1124-1134 [PMID: 28383652 DOI: 10.1093/ecco-jcc/jjx050]
22 **Hsieh CS**, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 1993; **260**: 547-549 [PMID: 8097338 DOI: 10.1126/science.8097338]
23 **Nava P**, Koch S, Laukoetter MG, Lee WY, Kolegraff K, Capaldo CT, Beeman N, Addis C, Gerner-Smidt K, Neumaier I, Skerra A, Li L, Parkos CA, Nusrat A. Interferon-gamma regulates intestinal epithelial homeostasis through converging beta-catenin signaling pathways. *Immunity* 2010; **32**: 392-402 [PMID: 20303298 DOI: 10.1016/j.immuni.2010.03.001]
24 **Geremia A**, Jewell DP. The IL-23/IL-17 pathway in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol* 2012; **6**: 223-237 [PMID: 22375527 DOI: 10.1586/egh.11.107]
25 **Chen L**, He Z, Reis BS, Gelles JD, Chipuk JE, Ting AT, Spicer JA, Trapani JA, Furtado GC, Lira SA. IFN-γ$^+$ cytotoxic CD4$^+$ T lymphocytes are involved in the pathogenesis of colitis induced by IL-23 and the food colorant Red 40. *Cell Mol Immunol* 2022; **19**: 777-790 [PMID: 35468944 DOI: 10.1038/s41423-022-00864-3]
26 **Heller F**, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Bürgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 2005; **129**: 550-564 [PMID: 16083712 DOI: 10.1016/j.gastro.2005.05.002]
27 **Szkaradkiewicz A**, Marciniak R, Chudzicka-Strugała I, Wasilewska A, Drews M, Majewski P, Karpiński T, Zwoździak B. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. *Arch Immunol Ther Exp (Warsz)* 2009; **57**: 291-294 [PMID: 19578817 DOI: 10.1007/s00005-009-0031-z]
28 **Monteleone I**, Sarra M, Pallone F, Monteleone G. Th17-related cytokines in inflammatory bowel diseases: friends or foes? *Curr Mol Med* 2012; **12**: 592-597 [PMID: 22515978 DOI: 10.2174/1566524012800620066]
29 **Yan JB**, Luo MM, Chen ZY, He BH. The Function and Role of the Th17/Treg Cell Balance in Inflammatory Bowel Disease. *J Immunol Res* 2020; **2020**: 8813558 [PMID: 33381606 DOI: 10.1155/2020/8813558]
30 **Gerlach K**, Hwang Y, Nikolaev A, Atreya R, Dornhoff H, Steiner S, Lehr HA, Wirtz S, Vieth M, Waisman A, Rosenbauer F, McKenzie AN, Weigmann B, Neurath MF. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis *via* IL-9 receptor signaling in intestinal epithelial cells. *Nat Immunol* 2014; **15**: 676-686 [PMID: 24908389 DOI: 10.1038/ni.2920]
31 **Catalan-Serra I**, Sandvik AK, Bruland T, Andreu-Ballester JC. Gammadelta T Cells in Crohn's Disease: A New Player in the Disease Pathogenesis? *J Crohns Colitis* 2017; **11**: 1135-1145 [PMID: 28333360 DOI: 10.1093/ecco-jcc/jjx039]
32 **McCarthy NE**, Bashir Z, Vossenkämper A, Hedin CR, Giles EM, Bhattacharjee S, Brown SG, Sanders TJ, Whelan K, MacDonald TT, Lindsay JO, Stagg AJ. Proinflammatory Vδ2+ T cells populate the human intestinal mucosa and enhance IFN-γ production by colonic αβ T cells. *J Immunol* 2013; **191**: 2752-2763 [PMID: 23904167 DOI: 10.4049/jimmunol.1202959]
33 **Casalegno Garduño R**, Däbritz J. New Insights on CD8$^+$ T Cells in Inflammatory Bowel Disease and Therapeutic Approaches. *Front Immunol* 2021; **12**: 738762 [PMID: 34707610 DOI: 10.3389/fimmu.2021.738762]
34 **Carrasco A**, Fernández-Bañares F, Pedrosa E, Salas A, Loras C, Rosinach M, Aceituno M, Andújar X, Forné M, Zabana Y, Esteve M. Regional Specialisation of T Cell Subsets and Apoptosis in the Human Gut Mucosa: Differences Between

Ileum and Colon in Healthy Intestine and Inflammatory Bowel Diseases. *J Crohns Colitis* 2016; **10**: 1042-1054 [PMID: 26995182 DOI: 10.1093/ecco-jcc/jjw066]
35 **Hannun YA**, Obeid LM. Many ceramides. *J Biol Chem* 2011; **286**: 27855-27862 [PMID: 21693702 DOI: 10.1074/jbc.R111.254359]
36 **Maceyka M**, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature* 2014; **510**: 58-67 [PMID: 24899305 DOI: 10.1038/nature13475]
37 **Al-Jarallah A**, Oriowo M. The effect of sphingosine-1-phosphate on colonic smooth muscle contractility: Modulation by TNBS-induced colitis. *PLoS One* 2017; **12**: e0170792 [PMID: 28493876 DOI: 10.1371/journal.pone.0170792]
38 **Deguchi Y**, Andoh A, Yagi Y, Bamba S, Inatomi O, Tsujikawa T, Fujiyama Y. The S1P receptor modulator FTY720 prevents the development of experimental colitis in mice. *Oncol Rep* 2006; **16**: 699-703 [PMID: 16969482 DOI: 10.3892/or.16.4.699]
39 **Snider AJ**, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Hannun YA, Obeid LM. A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. *FASEB J* 2009; **23**: 143-152 [PMID: 18815359 DOI: 10.1096/fj.08-118109]
40 **Suh JH**, Degagné É, Gleghorn EE, Setty M, Rodriguez A, Park KT, Verstraete SG, Heyman MB, Patel AS, Irek M, Gildengorin GL, Hubbard NE, Borowsky AD, Saba JD. Sphingosine-1-Phosphate Signaling and Metabolism Gene Signature in Pediatric Inflammatory Bowel Disease: A Matched-case Control Pilot Study. *Inflamm Bowel Dis* 2018; **24**: 1321-1334 [PMID: 29788359 DOI: 10.1093/ibd/izy007]
41 **Billich A**, Urtz N, Reuschel R, Baumruker T. Sphingosine kinase 1 is essential for proteinase-activated receptor-1 signalling in epithelial and endothelial cells. *Int J Biochem Cell Biol* 2009; **41**: 1547-1555 [PMID: 19162217 DOI: 10.1016/j.biocel.2009.01.001]
42 **Mahajan-Thakur S**, Sostmann BD, Fender AC, Behrendt D, Felix SB, Schrör K, Rauch BH. Sphingosine-1-phosphate induces thrombin receptor PAR-4 expression to enhance cell migration and COX-2 formation in human monocytes. *J Leukoc Biol* 2014; **96**: 611-618 [PMID: 24990321 DOI: 10.1189/jlb.3AB1013-567R]
43 **Pettus BJ**, Bielawski J, Porcelli AM, Reames DL, Johnson KR, Morrow J, Chalfant CE, Obeid LM, Hannun YA. The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. *FASEB J* 2003; **17**: 1411-1421 [PMID: 12890694 DOI: 10.1096/fj.02-1038com]
44 **Billich A**, Bornancin F, Mechtcheriakova D, Natt F, Huesken D, Baumruker T. Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1beta and TNF-alpha induced production of inflammatory mediators. *Cell Signal* 2005; **17**: 1203-1217 [PMID: 16038795 DOI: 10.1016/j.cellsig.2004.12.005]
45 **Duong CQ**, Bared SM, Abu-Khader A, Buechler C, Schmitz A, Schmitz G. Expression of the lysophospholipid receptor family and investigation of lysophospholipid-mediated responses in human macrophages. *Biochim Biophys Acta* 2004; **1682**: 112-119 [PMID: 15158762 DOI: 10.1016/j.bbalip.2004.03.002]
46 **Gude DR**, Alvarez SE, Paugh SW, Mitra P, Yu J, Griffiths R, Barbour SE, Milstien S, Spiegel S. Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a "come-and-get-me" signal. *FASEB J* 2008; **22**: 2629-2638 [PMID: 18362204 DOI: 10.1096/fj.08-107169]
47 **Furuya H**, Tamashiro PM, Shimizu Y, Iino K, Peres R, Chen R, Sun Y, Hannun YA, Obeid LM, Kawamori T. Sphingosine Kinase 1 expression in peritoneal macrophages is required for colon carcinogenesis. *Carcinogenesis* 2017; **38**: 1218-1227 [PMID: 29028945 DOI: 10.1093/carcin/bgx104]
48 **Maines LW**, Fitzpatrick LR, Green CL, Zhuang Y, Smith CD. Efficacy of a novel sphingosine kinase inhibitor in experimental Crohn's disease. *Inflammopharmacology* 2010; **18**: 73-85 [PMID: 20151210 DOI: 10.1007/s10787-010-0032-x]
49 **Yuza K**, Nagahashi M, Shimada Y, Nakano M, Tajima Y, Kameyama H, Nakajima M, Takabe K, Wakai T. Upregulation of phosphorylated sphingosine kinase 1 expression in colitis-associated cancer. *J Surg Res* 2018; **231**: 323-330 [PMID: 30278948 DOI: 10.1016/j.jss.2018.05.085]
50 **Aguilar A**, Saba JD. Truth and consequences of sphingosine-1-phosphate lyase. *Adv Biol Regul* 2012; **52**: 17-30 [PMID: 21946005 DOI: 10.1016/j.advenzreg.2011.09.015]
51 **Degagné E**, Pandurangan A, Bandhuvula P, Kumar A, Eltanawy A, Zhang M, Yoshinaga Y, Nefedov M, de Jong PJ, Fong LG, Young SG, Bittman R, Ahmedi Y, Saba JD. Sphingosine-1-phosphate lyase downregulation promotes colon carcinogenesis through STAT3-activated microRNAs. *J Clin Invest* 2014; **124**: 5368-5384 [PMID: 25347472 DOI: 10.1172/JCI74188]
52 **Peyrin-Biroulet L**, Christopher R, Behan D, Lassen C. Modulation of sphingosine-1-phosphate in inflammatory bowel disease. *Autoimmun Rev* 2017; **16**: 495-503 [PMID: 28279838 DOI: 10.1016/j.autrev.2017.03.007]
53 **Greenspon J**, Li R, Xiao L, Rao JN, Sun R, Strauch ED, Shea-Donohue T, Wang JY, Turner DJ. Sphingosine-1-phosphate regulates the expression of adherens junction protein E-cadherin and enhances intestinal epithelial cell barrier function. *Dig Dis Sci* 2011; **56**: 1342-1353 [PMID: 20936358 DOI: 10.1007/s10620-010-1421-0]
54 **Vetrano S**, Ploplis VA, Sala E, Sandoval-Cooper M, Donahue DL, Correale C, Arena V, Spinelli A, Repici A, Malesci A, Castellino FJ, Danese S. Unexpected role of anticoagulant protein C in controlling epithelial barrier integrity and intestinal inflammation. *Proc Natl Acad Sci U S A* 2011; **108**: 19830-19835 [PMID: 22109555 DOI: 10.1073/pnas.1107140108]
55 **Pérez-Jeldres T**, Tyler CJ, Boyer JD, Karuppuchamy T, Bamias G, Dulai PS, Boland BS, Sandborn WJ, Patel DR, Rivera-Nieves J. Cell Trafficking Interference in Inflammatory Bowel Disease: Therapeutic Interventions Based on Basic Pathogenesis Concepts. *Inflamm Bowel Dis* 2019; **25**: 270-282 [PMID: 30165490 DOI: 10.1093/ibd/izy269]
56 **Liang J**, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang WC, Hait NC, Allegood JC, Price MM, Avni D, Takabe K, Kordula T, Milstien S, Spiegel S. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer Cell* 2013; **23**: 107-120 [PMID: 23273921 DOI: 10.1016/j.ccr.2012.11.013]
57 **Montrose DC**, Scherl EJ, Bosworth BP, Zhou XK, Jung B, Dannenberg AJ, Hla T. $S1P_1$ localizes to the colonic vasculature in ulcerative colitis and maintains blood vessel integrity. *J Lipid Res* 2013; **54**: 843-851 [PMID: 23296878 DOI: 10.1194/jlr.M034108]
58 **Park SJ**, Im DS. Sphingosine 1-Phosphate Receptor Modulators and Drug Discovery. *Biomol Ther (Seoul)* 2017; **25**: 80-

90 [PMID: 28035084 DOI: 10.4062/biomolther.2016.160]
59 **Blankenbach KV**, Schwalm S, Pfeilschifter J, Meyer Zu Heringdorf D. Sphingosine-1-Phosphate Receptor-2 Antagonists: Therapeutic Potential and Potential Risks. *Front Pharmacol* 2016; **7**: 167 [PMID: 27445808 DOI: 10.3389/fphar.2016.00167]
60 **Yu OM**, Brown JH. G Protein-Coupled Receptor and RhoA-Stimulated Transcriptional Responses: Links to Inflammation, Differentiation, and Cell Proliferation. *Mol Pharmacol* 2015; **88**: 171-180 [PMID: 25904553 DOI: 10.1124/mol.115.097857]
61 **Chen T**, Gu K, Lin R, Liu Y, Shan Y. The function of Sphingosine-1-phosphate receptor 2 (S1PR2) in maintaining intestinal barrier and inducing ulcerative colitis. *Bioengineered* 2022; **13**: 13703-13717 [PMID: 35707833 DOI: 10.1080/21655979.2022.2076500]
62 **Zhao S**, Gong Z, Du X, Tian C, Wang L, Zhou J, Xu C, Chen Y, Cai W, Wu J. Deoxycholic Acid-Mediated Sphingosine-1-Phosphate Receptor 2 Signaling Exacerbates DSS-Induced Colitis through Promoting Cathepsin B Release. *J Immunol Res* 2018; **2018**: 2481418 [PMID: 29854830 DOI: 10.1155/2018/2481418]
63 **Gurgui M**, Broere R, Kalff JC, van Echten-Deckert G. Dual action of sphingosine 1-phosphate in eliciting proinflammatory responses in primary cultured rat intestinal smooth muscle cells. *Cell Signal* 2010; **22**: 1727-1733 [PMID: 20624458 DOI: 10.1016/j.cellsig.2010.06.013]
64 **Wang W**, Graeler MH, Goetzl EJ. Type 4 sphingosine 1-phosphate G protein-coupled receptor (S1P4) transduces S1P effects on T cell proliferation and cytokine secretion without signaling migration. *FASEB J* 2005; **19**: 1731-1733 [PMID: 16046470 DOI: 10.1096/fj.05-3730fje]
65 **Schulze T**, Golfier S, Tabeling C, Räbel K, Gräler MH, Witzenrath M, Lipp M. Sphingosine-1-phospate receptor 4 ($S1P_4$) deficiency profoundly affects dendritic cell function and TH17-cell differentiation in a murine model. *FASEB J* 2011; **25**: 4024-4036 [PMID: 21825036 DOI: 10.1096/fj.10-179028]
66 **Olesch C**, Ringel C, Brüne B, Weigert A. Beyond Immune Cell Migration: The Emerging Role of the Sphingosine-1-phosphate Receptor S1PR4 as a Modulator of Innate Immune Cell Activation. *Mediators Inflamm* 2017; **2017**: 6059203 [PMID: 28848247 DOI: 10.1155/2017/6059203]
67 **Walzer T**, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L, Jacques Y, Baratin M, Tomasello E, Vivier E. Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* 2007; **8**: 1337-1344 [PMID: 17965716 DOI: 10.1038/ni1523]
68 **Nielsen OH**, Li Y, Johansson-Lindbom B, Coskun M. Sphingosine-1-Phosphate Signaling in Inflammatory Bowel Disease. *Trends Mol Med* 2017; **23**: 362-374 [PMID: 28283249 DOI: 10.1016/j.molmed.2017.02.002]
69 **Kleinwort A**, Lührs F, Heidecke CD, Lipp M, Schulze T. S1P Signalling Differentially Affects Migration of Peritoneal B Cell Populations In Vitro and Influences the Production of Intestinal IgA In Vivo. *Int J Mol Sci* 2018; **19** [PMID: 29382132 DOI: 10.3390/ijms19020391]
70 **Du J**, Zeng C, Li Q, Chen B, Liu H, Huang X, Huang Q. LPS and TNF-α induce expression of sphingosine-1-phosphate receptor-2 in human microvascular endothelial cells. *Pathol Res Pract* 2012; **208**: 82-88 [PMID: 22244964 DOI: 10.1016/j.prp.2011.11.008]
71 **Xiong Y**, Piao W, Brinkman CC, Li L, Kulinski JM, Olivera A, Cartier A, Hla T, Hippen KL, Blazar BR, Schwab SR, Bromberg JS. CD4 T cell sphingosine 1-phosphate receptor (S1PR)1 and S1PR4 and endothelial S1PR2 regulate afferent lymphatic migration. *Sci Immunol* 2019; **4** [PMID: 30877143 DOI: 10.1126/sciimmunol.aav1263]
72 **Nodai A**, Machida T, Izumi S, Hamaya Y, Kohno T, Igarashi Y, Iizuka K, Minami M, Hirafuji M. Sphingosine 1-phosphate induces cyclooxygenase-2 *via* Ca2+-dependent, but MAPK-independent mechanism in rat vascular smooth muscle cells. *Life Sci* 2007; **80**: 1768-1776 [PMID: 17382352 DOI: 10.1016/j.lfs.2007.02.008]
73 **Sugahara K**, Maeda Y, Shimano K, Mogami A, Kataoka H, Ogawa K, Hikida K, Kumagai H, Asayama M, Yamamoto T, Harada T, Ni P, Inoue S, Kawaguchi A. Amiselimod, a novel sphingosine 1-phosphate receptor-1 modulator, has potent therapeutic efficacy for autoimmune diseases, with low bradycardia risk. *Br J Pharmacol* 2017; **174**: 15-27 [PMID: 27714763 DOI: 10.1111/bph.13641]
74 **Shimano K**, Maeda Y, Kataoka H, Murase M, Mochizuki S, Utsumi H, Oshita K, Sugahara K. Amiselimod (MT-1303), a novel sphingosine 1-phosphate receptor-1 functional antagonist, inhibits progress of chronic colitis induced by transfer of CD4+CD45RBhigh T cells. *PLoS One* 2019; **14**: e0226154 [PMID: 31805144 DOI: 10.1371/journal.pone.0226154]
75 **D'Haens G**, Danese S, Davies M, Watanabe M, Hibi T. A phase II, Multicentre, Randomised, Double-Blind, Placebo-controlled Study to Evaluate Safety, Tolerability, and Efficacy of Amiselimod in Patients with Moderate to Severe Active Crohn's Disease. *J Crohns Colitis* 2022; **16**: 746-756 [PMID: 34758080 DOI: 10.1093/ecco-jcc/jjab201]
76 **Kiuchi M**, Adachi K, Kohara T, Teshima K, Masubuchi Y, Mishina T, Fujita T. Synthesis and biological evaluation of 2,2-disubstituted 2-aminoethanols: analogues of FTY720. *Bioorg Med Chem Lett* 1998; **8**: 101-106 [PMID: 9925439 DOI: 10.1016/s0960-894x(97)10188-3]
77 **Kharel Y**, Lee S, Snyder AH, Sheasley-O'neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KS, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR. Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. *J Biol Chem* 2005; **280**: 36865-36872 [PMID: 16093248 DOI: 10.1074/jbc.M506293200]
78 **Brinkmann V**, Pinschewer D, Chiba K, Feng L. FTY720: a novel transplantation drug that modulates lymphocyte traffic rather than activation. *Trends Pharmacol Sci* 2000; **21**: 49-52 [PMID: 10664606 DOI: 10.1016/s0165-6147(99)01419-4]
79 **Pinschewer DD**, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM. FTY720 immunosuppression impairs effector T cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* 2000; **164**: 5761-5770 [PMID: 10820254 DOI: 10.4049/jimmunol.164.11.5761]
80 **Brinkmann V**, Wilt C, Kristofic C, Nikolova Z, Hof RP, Chen S, Albert R, Cottens S. FTY720: dissection of membrane receptor-operated, stereospecific effects on cell migration from receptor-independent antiproliferative and apoptotic effects. *Transplant Proc* 2001; **33**: 3078-3080 [PMID: 11750323 DOI: 10.1016/s0041-1345(01)02312-0]
81 **Daniel C**, Sartory N, Zahn N, Geisslinger G, Radeke HH, Stein JM. FTY720 ameliorates Th1-mediated colitis in mice by directly affecting the functional activity of CD4+CD25+ regulatory T cells. *J Immunol* 2007; **178**: 2458-2468 [PMID: 17277153 DOI: 10.4049/jimmunol.178.4.2458]

82 **Chaudhry BZ**, Cohen JA, Conway DS. Sphingosine 1-Phosphate Receptor Modulators for the Treatment of Multiple Sclerosis. *Neurotherapeutics* 2017; **14**: 859-873 [PMID: 28812220 DOI: 10.1007/s13311-017-0565-4]

83 **Verstockt B**, Vetrano S, Salas A, Nayeri S, Duijvestein M, Vande Casteele N; Alimentiv Translational Research Consortium (ATRC). Sphingosine 1-phosphate modulation and immune cell trafficking in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2022; **19**: 351-366 [PMID: 35165437 DOI: 10.1038/s41575-021-00574-7]

84 **Buzard DJ**, Kim SH, Lopez L, Kawasaki A, Zhu X, Moody J, Thoresen L, Calderon I, Ullman B, Han S, Lehmann J, Gharbaoui T, Sengupta D, Calvano L, Montalban AG, Ma YA, Sage C, Gao Y, Semple G, Edwards J, Barden J, Morgan M, Chen W, Usmani K, Chen C, Sadeque A, Christopher RJ, Thatte J, Fu L, Solomon M, Mills D, Whelan K, Al-Shamma H, Gatlin J, Le M, Gaidarov I, Anthony T, Unett DJ, Blackburn A, Rueter J, Stirn S, Behan DP, Jones RM. Discovery of APD334: Design of a Clinical Stage Functional Antagonist of the Sphingosine-1-phosphate-1 Receptor. *ACS Med Chem Lett* 2014; **5**: 1313-1317 [PMID: 25516790 DOI: 10.1021/ml500389m]

85 **Al-Shamma H**, Lehmann-Bruinsma K, Carroll C, Solomon M, Komori HK, Peyrin-Biroulet L, Adams J. The Selective Sphingosine 1-Phosphate Receptor Modulator Etrasimod Regulates Lymphocyte Trafficking and Alleviates Experimental Colitis. *J Pharmacol Exp Ther* 2019; **369**: 311-317 [PMID: 30872391 DOI: 10.1124/jpet.118.254268]

86 **Sandborn WJ**, Peyrin-Biroulet L, Zhang J, Chiorean M, Vermeire S, Lee SD, Kühbacher T, Yacyshyn B, Cabell CH, Naik SU, Klassen P, Panés J. Efficacy and Safety of Etrasimod in a Phase 2 Randomized Trial of Patients With Ulcerative Colitis. *Gastroenterology* 2020; **158**: 550-561 [PMID: 31711921 DOI: 10.1053/j.gastro.2019.10.035]

87 **D'Amico F**, Allocca M, Fiorino G. Positioning Ozanimod in Ulcerative Colitis: Restoring Leukocyte Traffic Under Control. *Gastroenterology* 2022; **162**: 1767-1769 [PMID: 34896116 DOI: 10.1053/j.gastro.2021.12.235]

88 **Scott FL**, Clemons B, Brooks J, Brahmachary E, Powell R, Dedman H, Desale HG, Timony GA, Martinborough E, Rosen H, Roberts E, Boehm MF, Peach RJ. Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br J Pharmacol* 2016; **173**: 1778-1792 [PMID: 26990079 DOI: 10.1111/bph.13476]

89 **Scott F**, Clemons B, Dedman H, Powell R, Martinborough E, Timony G. Pharmacokinetic properties of RPC1063, a selective S1P1 and S1P5 receptor agonist, significantly contribute to efficacy in animal models of inflammatory bowel disease. J Crohns Colitis 2015; 9: S93 [DOI: 10.1093/ecco-jcc/jju027.148]

90 **Sandborn WJ**, Feagan BG, Wolf DC, D'Haens G, Vermeire S, Hanauer SB, Ghosh S, Smith H, Cravets M, Frohna PA, Aranda R, Gujrathi S, Olson A; TOUCHSTONE Study Group. Ozanimod Induction and Maintenance Treatment for Ulcerative Colitis. *N Engl J Med* 2016; **374**: 1754-1762 [PMID: 27144850 DOI: 10.1056/NEJMoa1513248]

91 **Sandborn WJ**, Feagan BG, D'Haens G, Wolf DC, Jovanovic I, Hanauer SB, Ghosh S, Petersen A, Hua SY, Lee JH, Charles L, Chitkara D, Usiskin K, Colombel JF, Laine L, Danese S; True North Study Group. Ozanimod as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2021; **385**: 1280-1291 [PMID: 34587385 DOI: 10.1056/NEJMoa2033617]

92 **Feagan BG**, Sandborn WJ, Danese S, Wolf DC, Liu WJ, Hua SY, Minton N, Olson A, D'Haens G. Ozanimod induction therapy for patients with moderate to severe Crohn's disease: a single-arm, phase 2, prospective observer-blinded endpoint study. *Lancet Gastroenterol Hepatol* 2020; **5**: 819-828 [PMID: 32553149 DOI: 10.1016/S2468-1253(20)30188-6]

93 **Shimizu H**, Takahashi M, Kaneko T, Murakami T, Hakamata Y, Kudou S, Kishi T, Fukuchi K, Iwanami S, Kuriyama K, Yasue T, Enosawa S, Matsumoto K, Takeyoshi I, Morishita Y, Kobayashi E. KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. *Circulation* 2005; **111**: 222-229 [PMID: 15642767 DOI: 10.1161/01.CIR.0000152101.41037.AB]

94 **Fujishiro J**, Kudou S, Iwai S, Takahashi M, Hakamata Y, Kinoshita M, Iwanami S, Izawa S, Yasue T, Hashizume K, Murakami T, Kobayashi E. Use of sphingosine-1-phosphate 1 receptor agonist, KRP-203, in combination with a subtherapeutic dose of cyclosporine A for rat renal transplantation. *Transplantation* 2006; **82**: 804-812 [PMID: 17006328 DOI: 10.1097/01.tp.0000232687.78242.cd]

95 **Song J**, Matsuda C, Kai Y, Nishida T, Nakajima K, Mizushima T, Kinoshita M, Yasue T, Sawa Y, Ito T. A novel sphingosine 1-phosphate receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukin-10 gene-deficient mice. *J Pharmacol Exp Ther* 2008; **324**: 276-283 [PMID: 17898319 DOI: 10.1124/jpet.106.119172]

96 **Radeke HH**, Stein J, Van Assche G, Rogler G, Lakatos PL, Muellershausen F, Moulin P, Jarvis P, Colin L, Gergely P, Kruis W. A Multicentre, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Efficacy, Safety, and Tolerability of the S1P Receptor Agonist KRP203 in Patients with Moderately Active Refractory Ulcerative Colitis. *Inflamm Intest Dis* 2020; **5**: 180-190 [PMID: 33313070 DOI: 10.1159/000509393]

97 **Pulkoski-Gross MJ**, Uys JD, Orr-Gandy KA, Coant N, Bialkowska AB, Szulc ZM, Bai A, Bielawska A, Townsend DM, Hannun YA, Obeid LM, Snider AJ. Novel sphingosine kinase-1 inhibitor, LCL351, reduces immune responses in murine DSS-induced colitis. *Prostaglandins Other Lipid Mediat* 2017; **130**: 47-56 [PMID: 28377281 DOI: 10.1016/j.prostaglandins.2017.03.006]

98 **Maines LW**, Fitzpatrick LR, French KJ, Zhuang Y, Xia Z, Keller SN, Upson JJ, Smith CD. Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. *Dig Dis Sci* 2008; **53**: 997-1012 [PMID: 18058233 DOI: 10.1007/s10620-007-0133-6]

99 **Serra M**, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. *Adv Enzyme Regul* 2010; **50**: 349-362 [PMID: 19914275 DOI: 10.1016/j.advenzreg.2009.10.024]

100 **Karuppuchamy T**, Tyler CJ, Lundborg LR, Pérez-Jeldres T, Kimball AK, Clambey ET, Jedlicka P, Rivera-Nieves J. Sphingosine-1-Phosphate Lyase Inhibition Alters the S1P Gradient and Ameliorates Crohn's-Like Ileitis by Suppressing Thymocyte Maturation. *Inflamm Bowel Dis* 2020; **26**: 216-228 [PMID: 31807751 DOI: 10.1093/ibd/izz174]

101 **Sanada Y**, Mizushima T, Kai Y, Nishimura J, Hagiya H, Kurata H, Mizuno H, Uejima E, Ito T. Therapeutic effects of novel sphingosine-1-phosphate receptor agonist W-061 in murine DSS colitis. *PLoS One* 2011; **6**: e23933 [PMID: 21931623 DOI: 10.1371/journal.pone.0023933]

102 **Dong J**, Wang H, Wu G, Zhao J, Zhang L, Zuo L, Zhu W, Gong J, Li Y, Gu L, Li J. Oral treatment with SEW2871, a sphingosine-1-phosphate type 1 receptor agonist, ameliorates experimental colitis in interleukin-10 gene deficient mice.

Clin Exp Immunol 2014; **177**: 94-101 [PMID: 24611843 DOI: 10.1111/cei.12304]

103 **Dong J**, Wang H, Zhao J, Sun J, Zhang T, Zuo L, Zhu W, Gong J, Li Y, Gu L, Li J. SEW2871 protects from experimental colitis through reduced epithelial cell apoptosis and improved barrier function in interleukin-10 gene-deficient mice. *Immunol Res* 2015; **61**: 303-311 [PMID: 25588868 DOI: 10.1007/s12026-015-8625-5]

104 **Lucaciu LA**, Seicean R, Seicean A. Small molecule drugs in the treatment of inflammatory bowel diseases: which one, when and why? *Eur J Gastroenterol Hepatol* 2020; **32**: 669-677 [PMID: 32282548 DOI: 10.1097/MEG.0000000000001730]

105 **Becher N**, Swaminath A, Sultan K. A Literature Review of Ozanimod Therapy in Inflammatory Bowel Disease: From Concept to Practical Application. *Ther Clin Risk Manag* 2022; **18**: 913-927 [PMID: 36106049 DOI: 10.2147/TCRM.S336139]

106 **Sriwastava S**, Chaudhary D, Srivastava S, Beard K, Bai X, Wen S, Khalid SH, Lisak RP. Progressive multifocal leukoencephalopathy and sphingosine 1-phosphate receptor modulators used in multiple sclerosis: an updated review of literature. *J Neurol* 2022; **269**: 1678-1687 [PMID: 34800168 DOI: 10.1007/s00415-021-10910-1]

107 **Berger JR**, Cree BA, Greenberg B, Hemmer B, Ward BJ, Dong VM, Merschhemke M. Progressive multifocal leukoencephalopathy after fingolimod treatment. *Neurology* 2018; **90**: e1815-e1821 [PMID: 29669908 DOI: 10.1212/WNL.0000000000005529]

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MINIREVIEWS

Management of metabolic-associated fatty liver disease: The diabetology perspective

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Abstract

The metabolic syndrome as a consequence of the obesity pandemic resulted in a substantial increase in the prevalence of metabolic-associated fatty live disease (MAFLD) and type 2 diabetes mellitus (T2DM). Because of the similarity in pathobiology shared between T2DM and MAFLD, both disorders coexist in many patients and may potentiate the disease-related outcomes with rapid progression and increased complications of the individual diseases. In fact, awareness about this coexistence and the risk of complications are often overlooked by both hepatologists and diabetologists. Management of these individual disorders in a patient should be addressed wholistically using an appropriate multidisciplinary team approach involving both the specialists and, when necessary, liaising with dieticians and surgeons. This comprehensive review is to compile the current evidence from a diabetologist's perspective on MAFLD and T2DM and to suggest optimal management strategies.

Key Words: Metabolic syndrome; Metabolic-associated fatty liver disease; Type 2 diabetes mellitus; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Diabetology perspective; Obesity

Core Tip: The prevalence of metabolic-associated fatty live disease (MAFLD) and type 2 diabetes mellitus (T2DM) has increased exponentially as a consequence of the obesity pandemic across the globe. The pathobiology of T2DM and MAFLD are similar because both these disorders occur as a consequence of metabolic syndrome, and often coexist in many patients potentiating adverse outcomes and progression of individual diseases. However, the awareness about this coexistence is still inadequate even among hepatologists and diabetologists. A multidisciplinary team approach involving both the specialists is crucial in the optimal and wholistic management of both the disorders which is the theme of this comprehensive review.

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INTRODUCTION

The global prevalence of obesity is increasing daily because of adverse lifestyles such as overconsumption of a high-energy diet and lack of adequate physical activity. This is associated with various metabolic disorders, particularly metabolic-associated fatty liver disease (MAFLD) and type 2 diabetes mellitus (T2DM). MAFLD, previously termed as nonalcoholic fatty liver disease (NAFLD), is now identified as the most common cause of chronic liver disease in the world, affecting more than 30% of the global population[1].

Due to the similarity in pathogenesis, MAFLD is often associated with other lifestyle-related disorders such as hypertension, dyslipidemia, and most importantly T2DM, which significantly increases cardiovascular disease (CVD). MAFLD has also been found to be associated with excess risk of extrahepatic cancers, gallstones, gastro-oesophageal reflux disease, hypothyroidism, urolithiasis, chronic kidney disease (CKD), depression and worse maternal and foetal outcomes during pregnancy [2]. MAFLD is also associated with a substantial increase in cardiovascular morbidity and mortality, with a 3.5 times higher risk of heart failure and 1.93 times excess risk of all-cause CVD mortality as per recent study data in patients with T2DM[3].

In fact, the new definition for disease (MAFLD) was proposed in 2020 by replacing the old terminology NAFLD to reflect its highly remarkable link to metabolic syndrome (MetS) and the consequences such as T2DM[4]. While NAFLD was originally defined based on the imaging and/or histological evidence of steatosis in the absence of significant alcohol consumption and the exclusion of hepatitis from other etiologies, MAFLD diagnosis requires only the presence of metabolic dysfunction in persons with steatosis, and does not need excluding other etiologies of hepatitis. When MAFLD/NAFLD only involves liver steatosis without significant damage to the hepatocytes, nonalcoholic steatohepatitis (NASH) involves inflammation of liver cells with varying degrees of hepatocyte destruction, which may lead on the cirrhosis and end stage liver failure[5].

The prevalence of MAFLD in patients with T2DM varies widely in the published literature, with a recent study reporting a rate of 68% among those with obesity and T2DM[4]. The global epidemiologic data suggests that the pooled prevalence of T2DM in those with MAFLD is 22.5%, with 43.6% of those with advanced disease (NASH)[5]. Therefore, management strategies for each disease entity should definitely address the coexistence of MAFLD and T2DM, for optimal disease outcomes. In this evidence-based review, we update the management of MAFLD with due consideration of the interlink between MAFLD and diabetes to enable physicians to approach patients having better diagnostic and therapeutic perspectives.

To compile the best and most up-to-date evidence, we performed a PubMed search using the MeSH terms/key words: "metabolic-associated fatty liver disease/MAFLD", "nonalcoholic fatty liver disease/NAFLD", "type 2 diabetes mellitus/T2DM/T2D", "diabetes/diabetology/ diabetologist", "obesity" "nonalcoholic steatohepatitis/NASH", "metabolic syndrome/ MetS", "fatty liver", "steatosis", "pathophysiology", "lifestyle intervention", "exercise", "diet", "pharmacotherapy", "bariatric/metabolic surgery", "pregnancy", "children", and "elderly/ old age". The last date of search was 25th November 2022 and we used a Boolean search strategy using terms 'AND' or 'OR' where necessary to limit the search output. We limited the use of published literature in English language preferably from the most recent clinical guidelines, systematic reviews, randomised controlled trials, and high-quality review articles to procure the best available evidence on the topic of discussion while writing this narrative review paper.

MAFLD AND DIABETES: THE PATHOBIOLOGICAL INTERLINK

MetS, which encompasses central adiposity, insulin resistance, hypertension and atherogenic dyslipidemia, is the common consequence of obesity. MAFLD is considered as the hepatic manifestation of MetS, and has a bidirectional and solid relationship with other comorbidities[6]. T2DM, the predominant sequel of MetS, is therefore commonly associated with MAFLD and vice versa because of the similarity in pathogenesis. Various genetic predisposing factors, insulin resistance (IR), proatherogenic dyslipidemia [low levels of high-density lipoprotein cholesterol (HDL-C) as well as elevated concentrations of very low-density lipoproteins, triglycerides, and apolipoprotein B100], and alterations in gut microbiota resulting in intestinal dysbiosis are implicated in the development MAFLD[7].

IR, the key factor in the development of both disorders, grossly alters the hepatic glucose and lipid metabolism[7-9]. Fasting hyperglycemia as a consequence of IR and postprandial hyperglycemia from the inability of the liver to store glucose as glycogen are both implicated in the development of hepatic steatosis. IR also increases the de novo lipogenesis and triglyceride production in the liver as a result of carbohydrate-rich diet in experimental models[10]. Increased liver fat, in turn, reduces hepatic insulin clearance, insulin sensitivity and augments total body insulin resistance[11]. These factors aggravate the risk of T2DM, and in those with pre-existing T2DM, a worsening of the disease. Advanced stages of MAFLD such as NASH and cirrhosis can increase the risk of development and worsening of T2DM[5, 12-14]. Therefore, without addressing both the disease states, we cannot manage patients with MAFLD and T2DM. Figure 1 demonstrates the pathobiological interlink between T2DM and MAFLD.

DIAGNOSTIC EVALUATION FOR MAFLD IN THE PRESENCE OF METS AND DIABETES

By definition, MAFLD is associated with the accumulation of fat in > 5% of hepatocytes on histological evaluation or using imaging evidence of > 5.6% of liver fat on magnetic resonance spectroscopy[12-14]. Various professional bodies such as the American Association for the Study of Liver Diseases (AASLD) [13], European Association for the Study of the Liver (EASL)[12], the American Association of Clinical Endocrinology (AACE)[14], and the American Diabetes Association (ADA)[15], recommend screening for MAFLD in patients with diabetes. While the EASL guideline recommends screening patients using liver function tests (LFT) and/or ultrasound scan in all cases with obesity/MetS/T2DM, the AASLD suggests screening only those with a high index of suspicion for MAFLD or NASH because of the uncertainties about the diagnosis, treatment, and the natural history of the disease. The AASLD also recommends non-invasive screening strategies like NAFLD fibrosis score, fibrosis-4 (FIB-4) score, or transient elastography (TE) for the evaluation of risk of liver fibrosis.

Based on grade B, intermediate/high strength of evidence, the latest guideline by AACE in 2022 recommends that more rigorous screening strategy should be considered for patients with T2DM using FIB-4 score, even if they have normal LFTs[14]. This guideline also advises that clinicians should consider screening for MAFLD and advanced fibrosis in those with obesity and/or features of MetS, prediabetes, and those with liver steatosis on imaging study and/or high liver transaminase levels > 6 mo as they have "high risk" for advanced fibrosis. Those with intermediate to high FIB-4 scores are considered "high risk" and should be recommended a liver stiffness measurement by TE or enhanced lifer fibrosis (ELF) test[14].

The most validated and cost-effective screening test to assess the risk of fibrosis, to identify advanced disease and liver-related outcomes in MAFLD is the vibration controlled transient elastography[14]. The most accurate imaging technique, magnetic resonance elastography, should be reserved for selected cases because of the cost implications. Alternative tests such as shear wave elastography and steatosis, activity and fibrosis (SAF) score and NAFLD activity scoring (NAS) system are also useful for screening and risk stratification. The AACE guidelines (2022) also suggests considering screening for patients with type 1 diabetes mellitus (T1DM), using FIB-4 to exclude MAFLD with clinically significant fibrosis (stages F2-F4) in presence of obesity, features of MetS, deranged LFTs with transaminase levels (> 30 U/L), or liver steatosis on imaging studies.

Because of the close association of MAFLD with diabetes and the risk of worsening of either condition among the sufferers, all patients with one of these disease entities should be evaluated for the other disease and regularly monitored using clinical and biochemical markers for each condition. Patients with either T2DM or T1DM and cardiometabolic risk factors and/or elevated transaminases (> 30 U/L) should be further risk stratified using the FIB-4 scoring, TE, and/or ELF test to exclude advanced liver disease related to MAFLD[14]. Those with persistently elevated liver transaminases and/or the presence of steatosis upon imaging evaluation and categorized as intermediate or high risk based on biochemistry and/or imaging should be referred to a hepatologist for further assessment. Liver biopsy is now considered only rarely in the diagnostic evaluation of MAFLD because of the invasiveness of the test, the high risk of complications, and the availability of high quality non-invasive diagnostic strategies in recent years for all categories of patients[14,16-18]. Sequential testing for MAFLD using blood test and non-invasive testing does reduce the number of liver biopsies required for the confirmation. In the data from the STELLAR studies, sequential testing alone had a reasonable

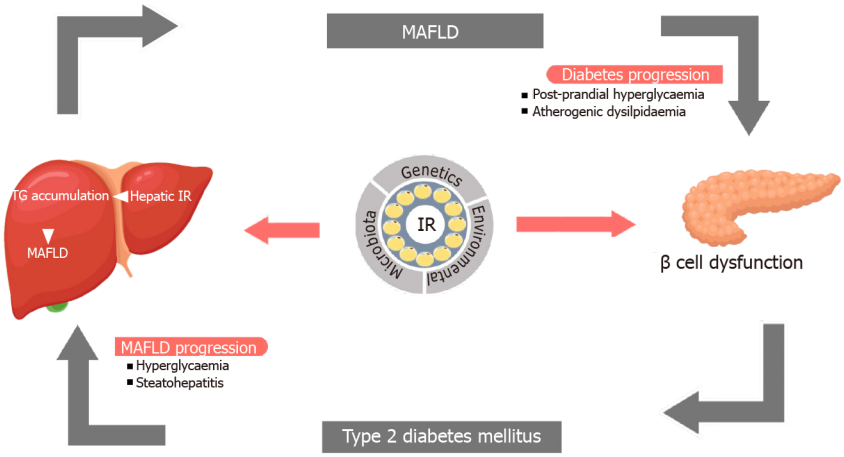

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Figure 1 Pathobiological link between metabolic-associated fatty live disease and type 2 diabetes mellitus. Metabolic-associated fatty live disease (MAFLD) may increase the risk for hyperglycemia and dyslipidemia and cause β-cell dysfunction leading to insulin resistance and type 2 diabetes which in turn may aggravate MAFLD progression to nonalcoholic steatohepatitis and fibrosis. IR: Insulin resistance; MAFLD: Metabolic-associated fatty live disease; TG: Triglycerides.

specificity and sensitivity with area under the curve between 0.75 to 0.80 to discriminate advanced fibrosis in MAFLD patients[19]. Our recommendations are in line with AACE/AASLD 2022 guidelines where liver biopsy is required only for intermediate to high-risk fibrosis patients or NASH diagnosis or exclude other coexisting diseases[14]. Bariatric surgery provides an opportunity to perform liver biopsy and can be potentially utilised, if the diagnosis of MAFLD is uncertain (with the other non-invasive tests) prior to the procedure, while being performed for the management of obesity.

MANAGEMENT APPROACH TO MAFLD

Lifestyle interventions

Being a metabolic disorder having significant pathogenic association with adverse lifestyles, including dietary energy imbalance and sedentary behaviors, main strategies in the management of MAFLD should be tailored around lifestyle interventions and behavioral adaptations. However, the likelihood of success with these measures is often modest because of lack of adherence and therefore, physicians are often forced to opt for pharmacotherapeutic interventions. Considering the lack of availability of many approved therapeutic agents for the treatment of MAFLD, and the well-proven additional benefits such as improvement of prediabetes/diabetes, cardiometabolic risks, and the improvement physical and emotional quality of life, the importance of lifestyle interventions cannot be underscored.

The most recent AACE guidelines (2022) reinforces the importance of lifestyle interventions as part of the standard care for patients with MAFLD and obesity, MetS, diabetes/prediabetes, hypertension, dyslipidemia, and CVD[14]. Lifestyle intervention-related weight loss of 3%-5% has shown to improve steatosis[13], with a reversal of hepatic IR[20]. The resolution of NASH in a proportion of cases with improved liver inflammation has been observed with 7%-10% weight reduction[21]. Further, fibrosis regression has been observed in those losing > 10% body weight by lifestyle changes[21,22]. However, approximately 70% of these clinical trials only achieved the target weight loss of 5% from lack of compliance and poor adherence to these weight management strategies.

Dietary interventions

Various nutritional interventions targeting a weight loss ≥ 5% has shown to be effective in the management of MAFLD[14,23,24]. Aim of all forms of nutritional interventions for MAFLD is to reduce the proportion of macronutrient content of the diet to achieve total energy deficit by restricting intake of simple carbohydrates, saturated fat, and added sugars, along with adoption of healthier eating options like a Mediterranean diet. Recent research data also suggests that intermittent fasting and time-restricted eating behavior are associated with improvement of MAFLD[25,26].

Nutritional intervention with energy restricted anti-inflammatory diets are also associated with reduction of inflammation along with better MAFLD outcomes in a recent randomized controlled trial [27]. The trial also showed significant improvements in body weight (-7.1%), visceral adiposity (-22.3%), metabolic parameters [homeostatic model assessment of IR (HOMA-IR): 15.5%; cholesterol: -5.3%; low-density lipoprotein cholesterol (LDL-C): -4.6%; and triglycerides: -12.2%], and various biomarkers of inflammation. Strict nutritional interventions for MAFLD have also been found to be associated with

improvements of CVD risk (blood pressure and QRISK2), metabolic health [fasting glucose, glycated hemoglobin (HbA1c), and insulin levels], body composition and quality of life along with improved liver-related outcomes[18,28]. However, the long-term effects and the sustainability of these nutritional interventions are still not clear in the absence of sufficiently long-term follow-up scientific research data. Although various dietary regimens are available starting from low carbohydrate/very low carbohydrate diet, ketogenic diet and Mediterranean diet, the ultimate goal for all these dietary regimens is to aim for calorie deficiency due to restriction of macronutrient (sugar and saturated fat). Of all the dietary regimens, the evidence base is strongest for the low glycaemic index Mediterranean diet which has shown to reduce the NAFLD score (median score: -4.14, 95%CI; -6.78, -1.49) when compared to the regular diet within six months duration[29]. Mediterranean diet also has cardiovascular protection property. Due to multiple health benefits with the Mediterranean diet, several professional societies recommend it as the preferred first line approach for MAFLD patients[12,14].

Exercise interventions

Physical activity is associated with improved muscle metabolism, resulting in energy dissipation and negative energy balance, especially with a calorie-restricted dietary intake. Exercise interventions have been demonstrated to significantly improve the liver transaminases regardless of the age, although more profound benefits have been observed in younger adults[30]. Along with improvements in MAFLD, various exercise programs have also been associated with improvements in metabolic parameters such as plasma levels of glucose, insulin, HbA1c, LDL-C, triglycerides, and HOMA-IR[31,32].

Apart from improvements in metabolic parameters, exercise interventions are also expected to improve all-cause morbidity and mortality associated with MAFLD. Both aerobic exercise and resistance training exercise result in reduction in the intrahepatic triglycerides (IHTG) content. In a randomised control trial from Thailand, a 12-wk regimen of moderate intensity aerobic exercise resulted in similar reduction of intrahepatic fat and improvement of insulin resistance in MAFLD patients when compared with resistance exercise and dietary modification[33]. A metanalysis involving six studies with 94 participants suggest that having a structured regimen results in greater reduction of IHTG and body weight[34]. Hence regular aerobic physical activity for 150-300 min/week of moderate-intensity or 75-150 min/week of vigorous-intensity coupled with some resistance training, aiming for a weight loss of > 5% in patients with MAFLD, can improve other comorbidities such as hypertension, diabetes, obstructive sleep apnea, dyslipidemia, CVD and possibly mortality[14,18,35]. Exercise interventions should ideally be coupled with nutritional changes to achieve hypocaloric energy balance to improve all these benefits. However, a recent Cochrane review concluded that there is considerable uncertainty in the evidence for benefits achieved by such interventions as the trials were of short follow-up duration of 2-24 mo[36]. Patients should be followed up for at least eight years to arrive at meaningful conclusions from these trials. Finally maintaining compliance in any chronic disorder is always challenging, different models like health behavioural model and protectional motivation theory have been proposed to improve the compliance, but all the models highlight the importance of explaining the effectiveness of the lifestyle changes to the patient and emphasising the importance of maintaining the target achieved for prevention of progression of MAFLD[37].

PHARMACOTHERAPY FOR MAFLD

Although multiple clinical trials on the pharmacotherapy for MAFLD have been completed to date, there are no drugs approved by the United States Food and Drug Administration (FDA) for the management of the disease. Most of the therapeutic agents used currently in clinical practice are based on low-moderate certainty of evidence or expert recommendations though we can expect some very promising molecules soon. Figure 2 shows the major sites of action of the drugs currently used in the management of MAFLD.

Insulin sensitizers

Pioglitazone: Pioglitazone belongs to the thiazolidinedione group of antidiabetic drugs, and acts as a peroxisome proliferator-activated receptor gamma agonist which modulates the insulin sensitivity in liver, muscles, and adipose tissues, having a marked improvement of IR[38]. Based on grade A and high strength evidence, pioglitazone is currently recommended for treatment of MAFLD when there is evidence of NASH (raised transaminases and/or suggestive non-invasive tests) in presence of T2DM[14,18,39]. Significant improvement in NASH without worsening of fibrosis was seen with the use of pioglitazone dose of 45 mg daily (relative risk: 2.64, 95%CI: 1.36; 5.12). An HbA1c reduction ranging from 1%-1.6% has been observed with neutral cardiovascular (CV) safety profile in patients with T2DM treated with pioglitazone though the use of the molecule is now less common owing to the availability of newer antidiabetic medications with better CV benefits[40]. Weight gain potential, probable risks of worsening heart failure and diabetic maculopathy from fluid retention and elevated fracture risk are the hindrances for widespread use of pioglitazone in clinical practice. The association between pioglitazone

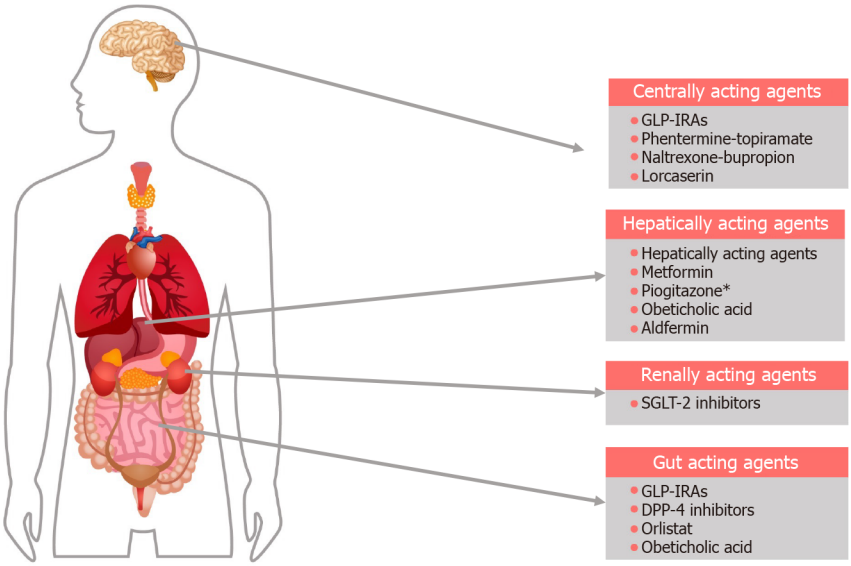

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Figure 2 Major sites of action of drugs currently used in the treatment of metabolic-associated fatty live disease. Various medications targeting comorbidities that coexist with metabolic-associated fatty live disease are used to treat symptomatic patients. The asterisk indicates adipocyte action is not shown. GLP-1RA: Glucagon-like insulinotropic peptide-1 receptor agonist; SGLT-2: Sodium glucose cotransporter-2.

and bladder cancer is controversial. FDA has issued safety warning and advise against the use of pioglitazone in patients with active bladder cancer and to exercise caution when used in patient with previous history of bladder cancer[41]. In a meta-analysis of 26 studies, the hazard ratio for developing bladder cancer in patient with type 2 diabetes with pioglitazone exposure was 1.07 (95%CI: 0.96-1.18) and was not statistically significant with the number needed to treat for one patient to develop bladder cancer was 899 to 6380 individuals[42]. Hence detailed history about the bladder symptoms if present, warrants further investigation before the start of the medication.

Metformin: Historically, metformin has been the drug of first choice in managing patients with T2DM over the past few decades and continues to be the first-line agent even today. By reducing hepatic gluconeogenesis and increasing skeletal muscle uptake of glucose, metformin improves insulin sensitivity and ameliorates IR. Metformin therapy in patients with MAFLD has been associated with therapeutic benefits such as alanine transaminase (ALT) and aspartate transaminase (AST) reductions of -2.84 (95%CI: -11.09 to 5.28) and -2.39 (95%CI: -7.55, 2.49), respectively, along with improvements in other biological indicators like lipid abnormalities and body mass index (BMI)[43]. In patients with T2DM and MAFLD, metformin therapy and lifestyle intervention were associated with a mean weight loss of 4.3%-7.9%, leading to improvements in hepatic IR and glycemic control[44], though the drug had no efficacy in improving NASH histology[7,45]. Due to the lack of evidence for benefits, the AACE guidelines (2022) do not recommend metformin therapy for patients with NASH though the treatment for diabetes with the drug may be continued in patients with MAFLD and NASH. In the absence of sufficient clinical data on the use of the drug in T1DM and MAFLD, metformin therapy may not be recommended in such patients though the drug may be continued in those already using it.

Incretin mimetics

Incretins are gastrointestinal (GI) mucosal hormones secreted in response to the presence of nutrient stimuli in the GI lumen. Glucagon-like insulinotropic peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two gut hormones in this class, and both these molecules possess profound multisystemic effects on human energy balance, insulin pharmacokinetic and dynamic equilibrium, and metabolic homeostasis. Pharmacological manipulation of incretin physiology has been an area of immense global scientific research input over the past three decades that revolutionized not only diabetes therapeutics but also the knowledge regarding mechanisms of origin and evolution of a variety of metabolic and multiorgan disorders affecting human health[46,47].

The endogenously secreted GLP-1 is rapidly degraded by the gastrointestinal luminal protease enzyme dipeptidyl peptidase-4 (DPP-4) with the termination of its biological effects soon after secretion [48], with a very short elimination half-life of ≈2 min[49]. To augment the biological effects (incretin effects), and thereby improve the actions of incretin hormones, several drug molecules (incretin mimetics) belonging to GLP-1 receptor agonist (GLP-1RA) and DPP-4 inhibitor classes were subsequently developed in the early part of twenty-first century making a major paradigm shift in the management of T2DM. By augmenting the endogenous insulin production and modulating satiety,

these molecules alter the pathobiology of T2DM with weight loss potential (GLP-1RAs) or weight neutrality (DPP-4 inhibitors) making them favorite drug choices to diabetologists. Cardiovascular morbidity and mortality benefits of some of the GLP-1RAs make them more attractive choices than other antidiabetic molecules, especially because of the CVD risks associated with diabetes.

GLP-1RAs: There are several molecules of this class currently available in the market, and newer drug molecules are under development. Exenatide, Liraglutide, Lixisenatide, Dulaglutide, Semaglutide, and Albiglutide are the widely available molecules in the GLP-1RA class. The new recent agent added to this group is Tirzepatide which also possesses GIP agonist property in addition to much higher weight loss potential and improvement of HbA1c in individuals with T2DM[50]. The mean HbA1c reduction achieved with different GLP-1RAs ranges from 0.66%-2.3%[50,51], while the mean weight loss attained varies from 2.0-11.4 kg (with Tirzepatide having maximum effect)[51,52].

Along with significant improvements in body weight and glycemic control, treatment with GLP-1RAs is also associated with improvement of cardiometabolic parameters like reduction of blood pressure, positive changes in adverse lipid profile (reduction of LDL-C and triglyceride and increase in HDL-C) and reduction of proteinuria, making this antidiabetic medication class a very desirable option for physicians[53]. Many of these benefits are also highly useful in managing patients with MAFLD, especially with diabetes.

Weight loss and the improvements in diabetes control and lipid abnormalities associated with GLP-1RA therapy may be the reasons for improvement of MAFLD. Based on grade A, high strength of evidence, the 2022 AACE guidelines recommends GLP-1RA therapy for patients with T2DM and biopsy-proven NASH, encouraging clinicians to consider the drug when there is high suspicion of NASH (high transaminases and positive non-invasive tests) and to offer these molecules for cardiometabolic benefits in presence of T2DM and MAFLD[14]. Judicious use of GLP-1RAs, in particular the long acting, once weekly molecules such as Dulaglutide and Semaglutide, are expected to improve the composite outcomes such as the activity scores, transaminases and progression of disease. A recent meta-analysis showed improvements in AST [weight mean difference (WMD) = -3.29 IU/L, 95%CI: -5.98, -0.61 IU/L, P = 0.02) , ALT (WMD = -9.92 IU/L, 95%CI: -19.89, 0.05 IU/L, P = 0.05), and GGT (WMD = -12.38 IU/L, 95%CI: -15.69, -9.07 IU/L, P < 0.00001) and reduction in FIB-4 score (WMD = -0.15, 95%CI: -0.29, 0.00, P = 0.05) when compared to placebo favoring use of GLP-1RAs for treatment of MAFLD[54]. Even though there is not enough data on the benefit of Tirzepatide in managing MAFLD, the drug must be associated with more profound effects considering remarkable improvements in cardiometabolic parameters, BMI, and diabetes control with this agent. Although GLP-1RA therapy is associated with improvements in body weight, total insulin requirements and cardiometabolic parameters in patients with T1DM[55], which might potentially benefit coexisting MAFLD, recommendations cannot be made in the absence of adequate scientific proof. GI intolerance in the form of nausea, vomiting and constipation (occasionally diarrhea and abdominal pain) are common side effects of this class of medications, with a discontinuation rate of 5%-10% within a few weeks of therapy (though clinical trials report only < 5%) observed in clinical practice[56]. Higher discontinuation rates of up to 47% being reported after 12 mo and 70% by 24 mo of treatment in some reports, mostly from lack of adequate benefit and cost implications[57].

DPP-4 inhibitors: DPP-4 inhibitors are useful for the management of T2DM with the added advantage of weight neutrality when compared to many other oral hypoglycemic agents. A recent meta-analysis showed an overall HbA1c reduction of -0.53% compared to placebo with slightly better benefit in Asians compared to Whites (-0.62% *vs* -0.49%) when this drug class was used as monotherapy in T2DM[58]. However, this class of medications is not recommended by the recent AACE guidelines for MAFLD because of lack of evidence on benefits except in managing patients with coexistent T2DM. Reinforcing these recommendations, a recent meta-analysis also did not show beneficial effects on the biochemical and imaging parameters of MAFLD in patients treated with DPP4 inhibitors[54].

Sodium glucose cotransporter-2 inhibitors

Sodium glucose cotransporter-2 inhibitors (SGLT-2i) are available for the management of T2DM for nearly a decade. Noninsulin dependent glucose lowering effect with the added advantage of energy deficit induced by glycosuria make this class of drugs unique with positive cardiometabolic outcomes and modest weight loss potential. Empagliflozin, Canagliflozin, Dapagliflozin and Ertugliflozin are the common agents in this class available widely in the global market, with few other molecules available in Asia and several ones under development now. Although the AACE guidelines (2022) suggested a lack of evidence of benefit in treating steatohepatitis[14], a more recent systematic review showed remarkable benefits with the use of SGLT-2i[54]. They observed improvements in AST (WMD = -2.31 IU/L, 95%CI: -3.16, -1.47 IU/L, P < 0.00001), ALT (WMD = -5.93 IU/L, 95%CI: -7.70, -4.16 IU/L, P < 0.00001), and GGT (WMD = -6.49 IU/L, 95%CI: -11.09, -1.89 IU/L, P = 0.006) and reduction in FIB-4 score (WMD = -0.21, 95%CI: -0.40, -0.03, P = 0.02) when compared to placebo favoring the use of SGLT-2i in the management of MAFLD especially in the presence of T2DM and cardiometabolic disease.

Other antidiabetic agents

The AACE guidelines (2022) do not recommend treatment of NASH patients with antidiabetic agents such as metformin, DPP-4 inhibitors, acarbose, and insulins because of the lack of evidence for their use on improving hepatocyte necrosis and inflammation[14]. However, these agents may be continued to manage T2DM optimally in these patients. Sulphonylureas are to be used with caution as some of these medications are metabolized by the liver.

Lipid lowering drugs

Dyslipidemia, whether it is primary or secondary, is associated with a significantly higher risk of worsening of MAFLD because of metabolic dysregulation and abnormal lipid handling by the liver. Hence, the treatment of dyslipidemia is part of the routine management of MAFLD. Statins are useful in patients for the improvement of cardiometabolic outcomes, and treatment may also slow down the disease process[59,60]. Most recent data suggests that statin treatment is associated with a reduction in the steatosis grade and NAS score[61]. Therefore, statin therapy should be considered in all patients, including those with compensated cirrhosis, except when the transaminase levels are > 3 times the upper limit of normal[60]. Although Omega-3 fatty acids and fenofibrate were found to offer potential benefits in patients with hypertriglyceridemia and MAFLD in a recent small clinical trial[62], firm conclusions cannot be reached without large-scale studies. Pro-protein convertase subtilisin/kexin type 9 inhibitors are used to treat hypercholesterolemia in patients with statin intolerance or in those unable to achieve target reduction of LDL-C with tolerated doses of statins. Although these drugs are very useful for CVD protection in patients with uncontrolled dyslipidemia on conventional therapy without affecting diabetes management[63], there is not enough data on their use in patients with MAFLD to suggest recommendations for use.

Vitamin E

α-tocopherol has been found to be useful in reducing liver transaminases with improvements in histological parameters of NASH, including ballooning, inflammation, and steatohepatitis in adults[64]. PIVEN study utilised a two by two factorial study design to evaluate the efficacy of α-tocopherol, at a dosage of 800 international units daily and pioglitazone in patients with biopsy proven NASH without diabetes[64]. At the end of 96 wk, patients on vitamin E therapy showed improvement in NASH as assessed by liver biopsy when compared to placebo (43% *vs* 19%, $P = 0.001$). The long-term efficacy of vitamin E to delay the progression of NASH is yet to be determined and there are some reports of increased all-cause mortality with high dose vitamin E but this is not proven and a meta-analysis have failed to confirm this association[65]. Also, we have to bear in mind that high dose vitamin E is associated with worsening insulin resistance; therefore, the drug is not recommended in patients with T2DM and those with advanced fibrosis[14].

Anti-obesity pharmacotherapy

Orlistat: Orlistat is a pancreatic and gastric lipase inhibitor that reduces dietary fat absorption and consequently causes energy malabsorption. The efficacy and side effects depend on the dietary fat content, and the discontinuation rate is high because of steatorrhea in those consuming high-fat diets. Conflicting results were observed in the review of clinical trials examining the efficacy of orlistat in a recent meta-analysis which showed improvements in transaminases and steatosis (on ultrasonographic imaging) in a few trials where drug use resulted in weight loss, between 5%-10%, with improvements in insulin resistance also[65]. The common drug dose is 120 mg three times daily, and steatorrhea may result in discontinuation in up to 25% of patients.

A meta-analysis of 330 patients with MAFLD treatment with orlistat showed marked improvements in the BMI (mean difference = -1.97; $P = 0.02$), reduction of ALT, and improvement of insulin resistance but with no significant changes to the liver fibrosis score[66]. A randomized, placebo-controlled clinical trial from Israel noted a higher reversal rate of fatty liver (24% *vs* 17%) in orlistat-treated group for 24 wk compared to the control group[67]. Similarly, orlistat treatment for 24 wk significantly reduced liver fat content as compared to the routine treatment. In an open label parallel group study of 170 patient who were either enrolled into orlistat or conventional care in 1:1.5 proportion, showed that the level of fat content decreased to a greater extent in the orlistat treated patients compared to the conventional care, which was [-5.45% *vs* -1.96%, where < 0.001 [intention to treat (ITT) analysis] and -6.66% *vs* -2.68%, $P < 0.001$ [per-protocol (PP) analysis][68]. Higher rates of improvement in steatosis grades were seen in the orlistat treatment group [45.6% *vs* 22.5% (ITT analysis), 57.4% *vs* 30.3% (PP analysis), both $P < 0.001$] [68]. In the same study, multivariate logistic regression analysis showed that the benefit of orlistat treatment was an independent source of improvement of steatosis. In experimental animal models, orlistat has shown protective and therapeutic effects against high-fat diet-induced MAFLD *via* modulation of signaling pathways involved in improved metabolism, especially the Nrf2 signaling pathway[69]. In experimental studies, orlistat treatment in high fat diet-fed rats up-regulated several antioxidant enzymes, down-regulated cell death and inflammation and apoptosis in the testis and thus, ameliorated testicular dysfunction[70]. Overall, these data suggest that orlistat could influence the metabolism of patients, and this may offer therapeutic benefits against MAFLD.

Lesser studied anti-obesity medications: The less studied anti-obesity medications in MAFLD are phentermine-topiramate, naltrexone-bupropion and lorcaserin. These agents have predominant central action on pathways to control appetite and craving for food. Although weight loss benefit with these agents is similar or, in some cases, more than orlistat but these agents are fraught with cardiovascular complication. Hence, these agents are less preferred compared to the recent GLP-1RAs which have a better cardiovascular safety profile. In a post-hoc analysis, extended-release naltrexone-bupropion combination promoted improvement in FIB-4 score with no statistical change in the ALT when treated for six months[71]. In another study, lorcaserin treatment for six months improved fatty liver index and reduced energy intake without affecting lean mass[72]. More studies are needed specifically in MAFLD patients to clarify these agents' efficacy across various stages of MAFLD.

Other drugs used in the management of MAFLD

Obeticholic acid: Obeticholic acid (OCA), or 6α-ethyl-chenodeoxycholic acid, is a semisynthetic chemical compound that mimics bile acid (BA), chenodeoxycholic acid (CDCA) and is the first farnesoid X receptor (FXR) agonist to be approved by the FDA and European medicine agency (EMA) for primary biliary cholangitis also known as "Ocaliva"[73]. BA binds through G-protein-coupled BA receptor (TGR5/Gpbar-1) and activates the nuclear receptor (FXR), which regulates lipid and glucose metabolism. Hence, FXRs showcase an ideal target for managing MAFLD by modulating the homeostasis of cholesterol, triglyceride, glucose, energy, and BA synthesis[74]. OCA binds FXR with 100-fold affinity compared to its natural ligand CDCA and proves to be a practical analog of BA[75]. A phase 2 study on 64 humans showed that OCA administration (dose of 25 or 50 mg) for 6 wk increased insulin sensitivity, and reduced liver inflammation and fibrosis biomarkers in patients with MAFLD and T2DM[76]. In this double-blind, placebo-controlled, proof-of-concept study, patients were treated with either placebo (23 people), 25 mg OCA (20 people), or 50 mg OCA (21 people) once daily for 6 wk. Insulin sensitivity was measured by a 2-stage hyperinsulinemic-euglycemic insulin clamp before and after the 6-wk treatment period along with liver enzymes, fibroblast growth factor 19, lipid analytes, 7α-hydroxy-4-cholesten-3-one (a BA precursor), endogenous BAs, and markers of liver fibrosis. The insulin sensitivity increased by 28.0% and 20.1% from baseline in the group (20 people) treated with 25 mg OCA (P = 0.019) and in the group (21 people) treated with 50 mg OCA (P = 0.060) respectively. In the combined OCA groups, insulin sensitivity increased by 24.5% (P = 0.011) with a decrease of 5.5% in the placebo group[76]. In animal models, OCA treatment resulted in increased weight loss and improved insulin sensitivity in rabbits[77]. Similarly, OCA treatment improved the metabolic profile in MAFLD rats [Zucker (fa/fa) rats], while reducing visceral adiposity and hepatic steatosis[78]. Along similar lines, OCA treatment markedly affected the gene expression profile involved in hepatic lipotoxicity, including fatty acid synthesis, lipogenesis and gluconeogenesis genes[78]. In the FLINT trial, which involved 283 patients randomly assigned to receive OCA 25 mg daily or placebo for 72 wk showed 35% of patients treated with OCA showed marked reduction in fibrosis compared to 19% of patients treated with placebo[79]. These findings indicate that OCA would be a potential treatment option for patients with MAFLD, considering that OCA treatment improves the metabolic profile and reduces fatty liver and fibrosis. It should be noted that OCA is effective in obesity and type 2 diabetes patients, but the long-term safety data and also head-to-head comparison with other newer antidiabetic agents are still awaited.

Aldafermin: Aldafermin, also known as M70 or NGM282, is an analog of fibroblast growth factor 19 (FGF19), a human gut hormone secreted from the ileum in response to activation of FXR. This hormone is essential for bile acids, carbohydrates, and energy metabolism[80,81]. Elevations of primary and secondary bile acid in circulation are linked with primary liver disease[82]. It has been reported that FGF19, upon FXR activation, acts on the FGFR1c-KLB and FGFR4-KLB receptor complex on hepatocytes to block bile acids synthesis. Aldafermin, the FGF19 analog, acts similar way on this receptor complex. Upon activation, FGFR1c-KLB receptor suppresses the *CYP7A1* expression. This gene encodes an enzyme, cholesterol 7a-hydroxylase, which is the first and a rate-limiting enzyme for the de novo synthesis of bile acids. In the phase 2 study of 176 patients with NASH and fibrosis (biopsy-confirmed) and increased fat in the liver (> 8% by magnetic resonance imaging-proton density fat fraction), it has been shown to have a significant reduction in the serum bile acid level[83]. These patients were administered with aldafermin 0.3 mg (23 patients), 1 mg (49 patients), 3 mg (49 patients), 6 mg (28 patients) or placebo (27 patients) for 12 wk. Similarly, 62 patients with PSC and > 1.5× upper limit of normally increased alkaline phosphatase were administered with 1 mg (21 patients), 3 mg (21 patients) aldafermin, or placebo (20 patients) for 12 wk. Serum bile acid profile and neoepitope-specific N-terminal pro-peptide of type III collagen or Pro-C3 (a direct measure of fibrogenesis) were measured for metabolic and cholestatic liver diseases. The results suggested that treatment with aldafermin caused dose-dependent reductions in serum bile acids, including deoxycholic acid, lithocholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid, and glycocholic acid in patients with metabolic and cholestatic liver diseases[84]. In addition, the treatment of aldafermin resulted in the reduction of glycine-conjugated bile acids but not the taurine-conjugated bile acids. Overall, these findings suggest the possibility of using aldafermin for 24 wk as a treatment option for fatty liver disease, fibrosis regression,

and resolution of NASH and stage 2 or stage 3 fibrosis.

Silymarin: Silymarin, extracted from the medicinal plant Silybum marianum, is a complex mixture of different flavonolignan isomers. Silymarin possesses an antioxidative effect and is mainly due to an isomer silybin diastereomers that is 50% of the mixture. Silybin undergoes biotransformation, forming glucuronide derivatives[85]. The mechanism of action of this drug on MAFLD involves antioxidative, choleretic, antifibrotic, regenerative, anti-inflammatory, and immunomodulatory effects[86]. Silymarin is now known as an antioxidant for reducing lipogenesis by downregulating the fatty acid synthase, peroxisome proliferator-activated receptor γ, and acetyl-CoA carboxylase[87,88]. MAFLD-induced insulin resistance and steatosis can be reduced by Silymarin which has shown the potential to restore the insulin action pathway through insulin receptor substrate-1/PI3K/Akt[89]. In a metanalysis involving 587 patients mostly from randomized controlled trials, results showed that Silymarin significantly decreased the AST and ALT levels more than the control group[90]. In a randomized, double-blind, placebo-controlled trial in NASH patients who had NAFLD activity scores of 4 or more, an intake of 700 mg per day of Silymarin for 48 wk caused a significant reduction in liver fibrosis[91]. Collectively, these studies have demonstrated that Silymarin is a safe drug, even at high doses, well tolerated, and it improves the functions of liver enzymes, activates liver metabolism, reduces oxidative stress, total cholesterol synthesis, and endothelial dysfunction in patients with MAFLD, but long-term data on different MAFLD stages are yet to come[92].

BARIATRIC PROCEDURES AND MAFLD

Metabolic surgery

One of the effective means of obtaining durable weight loss is through surgical intervention in obese people. It aims not only for weight loss but includes improvements in incretin profiles, insulin secretion, and insulin sensitivity; hence it is a promising tool for MAFLD management. Two commonly used bariatric or metabolic surgeries are Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG). The fundal portion of the stomach is surgically removed during SG, whereas RYGB entails making a small pouch; this new pouch is then connected to the small intestine. While both surgical procedures result in decreased stomach volume, decreased production of acid, and changed gut hormones, RYGB involves architectural reorganization of the gastrointestinal tract, potentially impacting bile acid reabsorption [93]. As the primary bile acids (PBA) are made and conjugated in the liver, excreted in the intestine, deconjugated and/or transformed to secondary bile acids (SBA) by the gut microbiota, and recycled through reabsorption in the terminal ileum, changes in stomach due to the RYGB leads reorganization of the microbiota leading to change in PBA and SBA composition[94]. It was shown that MAFLD patients' serum had more PBA/SBA ratio compared to healthy people[94]. According to the AACE recent guidelines, metabolic surgeries are reserved for patients with MAFLD and BMI 35 kg/m^2 especially if they have type 2 diabetes mellitus (level of recommendation grade B)[14]. It can be used as an opportunity for liver biopsy for histologic staging of liver fibrosis for further prognosis.

Endo-barrier treatment

Many bariatric surgeries come with certain complications and limitations, including anastomotic disruption leading to leaks and fistulae, stomal stenosis, dumping syndrome, *etc.* The endoscopically implanted and removably attached duodenal-jejunal sleeve bypass (DJSB) or EndoBarrier® (GI Dynamics Inc., Lexington MA) can also be used for the same purpose. It is anchored in the initial section of the duodenum, where it is connected to a 60-cm long polymer sleeve by a nitinol stent anchor. The sleeve shields the proximal upper small intestine's mucosa from food that has been swallowed. DJSB offers functional similarities with RYGB as it mimics some of the physiological effects. These include food excluded from the proximal small intestine, and pancreatic and biliary secretions mixed together after food has passed through the sleeve. Endo-barrier treatment has been shown to promote significant weight loss in a patient with obesity and type 2 diabetes who are otherwise eligible for bariatric surgery but declined due to personal choice. Over the 12-mo study period, the weight loss was 15 kg (95%CI: 0.62-29.38; $P < 0.05$) and BMI 4.9 kg/m^2 (95%CI: 1.1-8.7; $P < 0.005$), the confidence intervals are wide as expected for a small sample size of forty five patients[95]. The major limitation of the endo-barrier therapy is the attended complication like gastrointestinal intolerance, bleeding, and liver abscess. Although this can be reduced by improving the dietary compliance following the procedure and there are ongoing trials on how these complications can be effectively reduced so that endo-barrier can be a safe option for MAFLD management who fail medical therapy and decline surgery.

Bariatric endoscopy

Obese patient who undergoes bariatric surgery may need to demonstrate initial weight loss for pre-operative surgery optimization of metabolic profile for safe surgical outcome. Most of these patients can get benefit from endoscopic therapy. In a study of individuals with obesity and MAFLD, bariatric

endoscopy improved analytical and ultrasound parameters of insulin resistance, hepatic fat, and hypertriglyceridemia[96]. It can be suggested during short-term follow-up as an efficient and secure option, but the long-term benefits are yet to come as these are relatively newer modalities.

A broad approach for the management and referral system pathway for patients with MAFLD is shown in the Figure 3.

SPECIAL POPULATIONS WITH MAFLD

Pregnancy and MAFLD

Pregnancy-related liver diseases occur in a trimester-specific fashion, as there is biotransformation of almost the whole body in pregnant women throughout the pregnancy. Developments of primary liver diseases are common during pregnancy. MAFLD in pregnancy can result in the development of various health issues in the mother, such as complications of hypertension, postpartum hemorrhage, and premature birth of the baby[97]. In a multicentric prospective study from Korea, Lee *et al*[98] showed that 18.4% (112/608) women had MAFLD during their early pregnancy, and MALFD is an independent risk factor in the development of gestational diabetes mellitus (GDM; 36/608). The participants with GDM development showed higher prevalence of radiological steatosis (55.6% *vs* 16.1%; $P < 0.001$) and higher fatty liver index (40.0 *vs* 10.7; $P < 0.001$) and hepatic steatosis index (35.5 *vs* 29.0; $P < 0.001$)[98]. In utero, MAFLD exposed children have a greater risk of obesity at an early age and pediatric MAFLD development[99].

Management of MAFLD during pregnancy is a multi-model approach to control complications, which should recognize the population at high risk and control hyperglycemia, prevent excessive weight gain, and avoid the development of GDM. To our best knowledge, there is no single specific medication for the treatment of MAFLD during pregnancy. Managing weight and lifestyle during the postpartum period is mandatory for reversing the MAFLD effects and avoiding complications during the next pregnancy. Study conducted in 316 individuals (not only pregnant women), exercise without loss of weight significantly lowered intrahepatic lipid content (SMD: -0.76, 95%CI: -1.04, -0.48) and ALT concentration (SMD: -0.52, 95%CI: -0.90, -0.14), AST content (SMD: -0.68, 95%CI: -1.21, -0.15), LDL-C concentration (SMD: -0.34, 95%CI: -0.66, -0.02), and triglycerides content (SMD: -0.59, 95%CI: -1.16, -0.02)[100]. Hence, pregnant women with MAFLD should be encouraged to exercise regularly within the safe limit to improve the metabolic profile with proper guidance from the expert obstetric team.

Children and MAFLD

Due to a lack of recognition, screening, and appreciation of complications associated with MAFLD, the disease often remains undiagnosed among children. In a study of 2256 children with 715 children overweight, MAFLD was diagnosed in 23% among 715 with overweight ($P < 0.01$)[101]. As pediatric treatment options are limited due to inadequate number of clinical trials and insufficient knowledge, the overall goal is to improve the life quality of children and reduce liver morbidity and mortality. Lifestyle management is one of the best ways to manage MAFLD in children. In a study of 84 children of the age group between 3 and 18.8 years, over two years, with proper diet and physical exercise, participants who lost 5% or more of body weight had more significant improvements in the level of ALT when compared with the participants with < 5% weight loss (-35 ± 33 *vs* -20 ± 20 IU/L, respectively; $P < 0.05$)[102].

Elderly individuals and MAFLD

Between 40-50 years of age in males and 60-69 years in females, the prevalence of fatty liver is high and seems slightly low in older (> 70 years) cohorts. Hypertension, diabetes, hyperlipidemia, and obesity risk factors for MAFLD development are higher in elderly individuals. The diagnosis and management strategies are challenging in the elderly with other age-related comorbidities[103]. For the management of MAFLD, the underlying etiology must be treated parallel to other diseases including obesity, hyperlipidemia, and IR. Lifestyle modifications and pharmacological treatment are the two methods considered safe for managing MAFLD in elderly individuals. In a study with 261 elderly individuals for 52 wk, 72 (25%) achieved steatohepatitis resolution, 138 (47%) reduced NAS score, and 56 (19%) achieved fibrosis regression. During last week, 88 subjects (30%) reduced ≥ 5% of their weight. Degree of weight loss and improvements in all NASH-related histologic parameters are independently associated (odds ratios = 1.1-2.0; $P < 0.01$). A major proportion of participants with ≥ 5% loss in weight had [51 of 88 (58%)] NASH resolution and a 2-point reduction in NAS [72 of 88 (82%)] than participants with < 5% of loss in their weight ($P < 0.001$)[104]. In some elderly patients, these modifications might be incompatible. So, it is necessary to ensure that the introduced dietary instructions are not excessively aggressive and provide adequate nutrition to the elderly. Currently, no particular drug is approved for the treatment of MAFLD in the elderly. Therefore, there are no specific recommendations for drug usage in elderly individuals with MAFLD alone, but if they have concomitant T2DM, metformin, GLP-1RAs and SGLT-2i are the best available options.

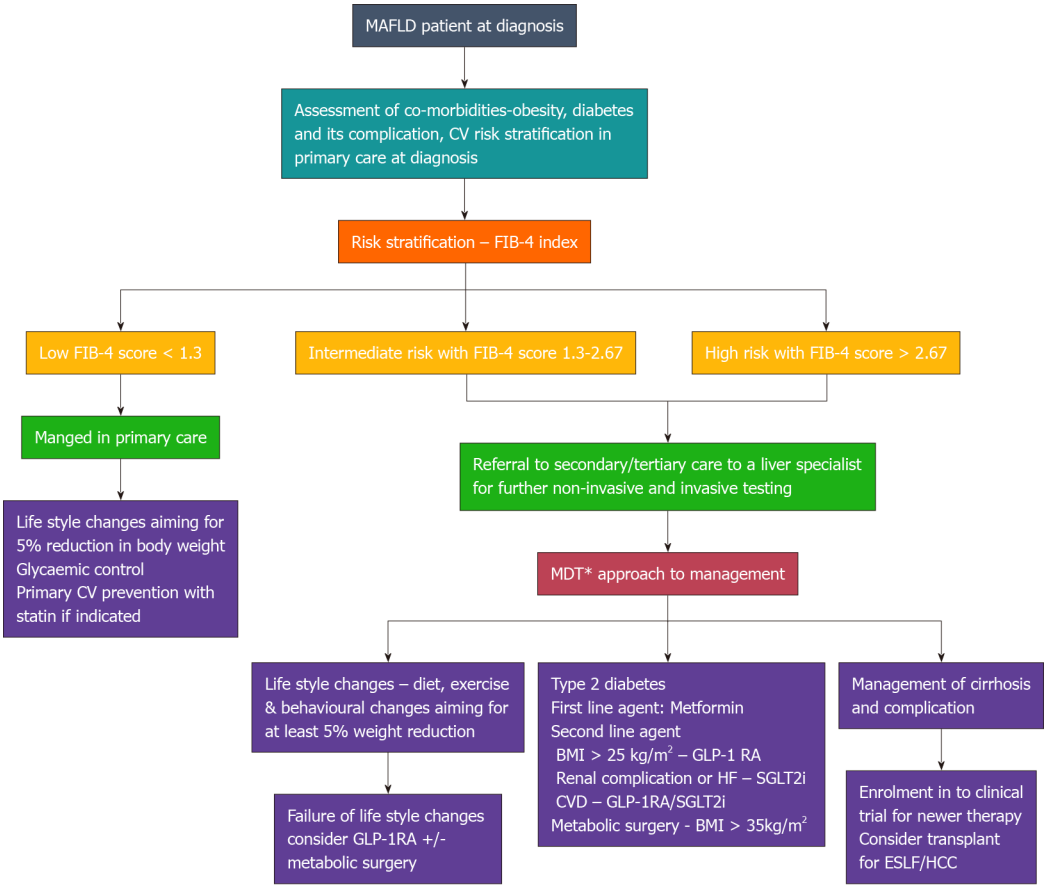

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Figure 3 Management and referral system pathway for patient with metabolic associated fatty liver disease. Multidisciplinary treatment approach involving dietician, physiotherapist, psychologist, diabetologist and hepatologist. In resource constrained setting, referral to individual specialist based on the predominant effect due to metabolic-associated fatty live disease as per the pathway. GLP-1RA: Glucagon-like insulinotropic peptide-1 receptor agonist; SGLT-2i: Sodium glucose cotransporter-2 inhibitors; CVD: Cardiovascular disease; HF: Heart failure; BMI: Body mass index.

EMERGING CONCEPTS

MAFLD is a significant cause of chronic liver disease with reported increase in hepatocellular carcinoma (HCC), and a leading contributor to various systemic complications such as T2DM, CVD, and CKD. A certain degree of uncertainty is present regarding the natural history and prognosis of MAFLD. In some patients, the long evolution time for MAFLD progression from steatohepatitis to fibrosis is noted, but some progress faster from cirrhosis to hepatocellular carcinoma[105]. The exact reason for the development of HCC among MAFLD patients remains uncertain to date. When compared with chronic hepatitis C infection, the cumulative incidence of MAFLD patient developing HCC is lower (4% *vs* 2.5%) at 1 year and (30% *vs* 11%) at 5 years[106]. Moreover, patients with MAFLD has a tendency to develop HCC even without cirrhosis and the risk is five times when compared those with chronic hepatitis C infection[107]. As these patients without cirrhosis are not in the surveillance program, they tend to present with bigger tumours with reduced median survival[108]. Recent data suggest that patients who develop HCC due to MAFLD cirrhosis live longer than hepatitis C related HCC after curative treatment [109]. Liver transplantation is used either for end stage liver disease or HCC due to MAFLD. The transplant related mortality and morbidity is high compared to the HCC due to other aetiologies such as very high BMI and MAFLD related cardiovascular complications[110]. But it is interesting to note that post-transplant 5-year survival rate is not different between MAFLD and non-MALFD aetiology because the lower risk of graft failure balances the higher risk of sepsis and cardiovascular disease in MAFLD patients when compared to other aetiology[111].

More clinical trials involving different ethnicity having divergent gene pools are required to get insights into the contribution of the genetic basis of MAFLD and the genetic basis of successful treatment outcomes and failure in treatment. The effect of environmental factors should also be a primary focus of work in the next decade. The growing human population and the environmental impact have dramatically changed human life. Especially lifestyle diseases are emerging in this decade due to the impact of the environment on human life. Future studies also need to be focused on understanding this phenomenon.

Table 1 Management of metabolic-associated fatty liver disease directed against the risk factors

No	Disease/condition	Directed therapy	Supportive therapies
1	Overweight and obesity	Anti-obesity drugs, Bariatric surgery, lifestyle intervention (Calorie-restriction, dietary pattern, *etc.*)	MAFLD: Newer agents targeting on cellular inflammation and oxidative stress.
2	T2DM	Hypoglycemic agents like Metformin, GLP-1RAs, SGLT-2i, Thiazolidinediones and DPP-4 inhibitors	Fibrotic MAFLD: Potential future anti-fibrotic agents.
3	> 2 metabolic risks	Modulators of metabolism (Farnesoid X receptor agonsit, Peroxisome proliferator-activated receptor, fibroblast growth receptors, statins, aspirin)	Cirrhosis Complications: Control portal hypertension and bacterial peritonitis prophylaxis. End-stage liver diseases: Liver transplantation.

T2DM: Type 2 diabetes mellitus; GLP-1RA: Glucagon-like insulinotropic peptide-1 receptor agonist; SGLT-2i: Sodium glucose cotransporter-2 inhibitors; DPP-4: Dipeptidyl peptidase-4; MAFLD: Metabolic-associated fatty live disease.

Different pharmacological approaches are tried to reduce the risk of MAFLD. However, current strategies remain ineffective. The asymptomatic time intervals in MAFLD and NASH progression to cirrhosis and ultimately a liver failure and gaps in knowledge regarding modifiers of the disease contribute to significant challenges in the drug design for the clinical trials. The application of nanoparticles for drug delivery demonstrated potentially promising for enhancing drug bioavailability for MAFLD treatment. Various types of liver-targeting nanoparticles are exploited for MAFLD management[112].

Table 1 shows a summary of therapeutic strategy for MAFLD targeting risk factors.

CONCLUSION

MAFLD is a growing global health burden among the population with high susceptibility to obesity and insulin resistance. Notably, MAFLD is the most common cause of chronic liver disease in children and adolescents, exhibited by fatty liver disease and severe fibrosis. The most effective prevention strategy for MAFLD is lifestyle modification. Considering the prevalence and its impact, several novel therapies, including several novel therapeutic and surgical approaches, are currently under investigation. Pharmacological interventions, including treatment with antidiabetic medications, and anti-obesity drugs, can be considered for MAFLD patients but should be chosen wisely on a case-to-case basis. In addition, bariatric surgeries aimed to obtain durable weight loss are options for MAFLD patients who either fail medical management or have associated comorbidities. Novel approaches in drug delivery would be ideal for managing MAFLD in the future. We need to improve the knowledge of the personalized treatment options with targeted drugs, which would be effective in that subset of patients who fail or have difficulty adhering to the routine management pathways.

FOOTNOTES

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REFERENCES

1 **Le MH**, Yeo YH, Li X, Li J, Zou B, Wu Y, Ye Q, Huang DQ, Zhao C, Zhang J, Liu C, Chang N, Xing F, Yan S, Wan ZH, Tang NSY, Mayumi M, Liu X, Rui F, Yang H, Yang Y, Jin R, Le RHX, Xu Y, Le DM, Barnett S, Stave CD, Cheung R, Zhu Q, Nguyen MH. 2019 Global NAFLD Prevalence: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2022; **20**: 2809-2817.e28 [PMID: 34890795 DOI: 10.1016/j.cgh.2021.12.002]

2 **Yi M**, Peng W, Feng X, Teng F, Tang Y, Kong Q, Chen Z. Extrahepatic morbidities and mortality of NAFLD: an umbrella review of meta-analyses. *Aliment Pharmacol Ther* 2022; **56**: 1119-1130 [PMID: 35989292 DOI: 10.1111/apt.17165]

3 **Minhas AMK**, Jain V, Maqsood MH, Pandey A, Khan SS, Fudim M, Fonarow GC, Butler J, Khan MS. Non-Alcoholic Fatty Liver Disease, Heart Failure, and Long-Term Mortality: Insights From the National Health and Nutrition Examination Survey. *Curr Probl Cardiol* 2022; **47**: 101333 [PMID: 35901855 DOI: 10.1016/j.cpcardiol.2022.101333]

4 **Rojas YAO**, Cuellar CLV, Barrón KMA, Arab JP, Miranda AL. Non-alcoholic fatty liver disease prevalence in Latin America: A systematic review and meta-analysis. *Ann Hepatol* 2022; **27**: 100706 [PMID: 35427804 DOI: 10.1016/j.aohep.2022.100706]

5 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

6 **Powell EE**, Wong VW, Rinella M. Non-alcoholic fatty liver disease. *Lancet* 2021; **397**: 2212-2224 [PMID: 33894145 DOI: 10.1016/S0140-6736(20)32511-3]

7 **Diaconu CT**, Guja C. Nonalcoholic Fatty Liver Disease and Its Complex Relation with Type 2 Diabetes Mellitus-From Prevalence to Diagnostic Approach and Treatment Strategies. *J Clin Med* 2022; **11** [PMID: 36079070 DOI: 10.3390/jcm11175144]

8 **Loria P**, Lonardo A, Anania F. Liver and diabetes. A vicious circle. *Hepatol Res* 2013; **43**: 51-64 [PMID: 23332087 DOI: 10.1111/j.1872-034X.2012.01031.x]

9 **Basu R**, Noureddin M, Clark JM. Nonalcoholic Fatty Liver Disease: Review of Management for Primary Care Providers. *Mayo Clin Proc* 2022; **97**: 1700-1716 [PMID: 36058582 DOI: 10.1016/j.mayocp.2022.04.005]

10 **Rabøl R**, Petersen KF, Dufour S, Flannery C, Shulman GI. Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. *Proc Natl Acad Sci U S A* 2011; **108**: 13705-13709 [PMID: 21808028 DOI: 10.1073/pnas.1110105108]

11 **Utzschneider KM**, Kahn SE, Polidori DC. Hepatic Insulin Extraction in NAFLD Is Related to Insulin Resistance Rather Than Liver Fat Content. *J Clin Endocrinol Metab* 2019; **104**: 1855-1865 [PMID: 30566676 DOI: 10.1210/jc.2018-01808]

12 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

13 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

14 **Cusi K**, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, Kashyap S, Mechanick JI, Mouzaki M, Nadolsky K, Rinella ME, Vos MB, Younossi Z. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). *Endocr Pract* 2022; **28**: 528-562 [PMID: 35569886 DOI: 10.1016/j.eprac.2022.03.010]

15 **Vieira Barbosa J**, Lai M. Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting. *Hepatol Commun* 2021; **5**: 158-167 [PMID: 33553966 DOI: 10.1002/hep4.1618]

16 **Popa SL**, Ismaiel A, Cristina P, Cristina M, Chiarioni G, David L, Dumitrascu DL. Non-Alcoholic Fatty Liver Disease: Implementing Complete Automated Diagnosis and Staging. A Systematic Review. *Diagnostics (Basel)* 2021; **11** [PMID: 34204822 DOI: 10.3390/diagnostics11061078]

17 **Yu Q**, Liu Y, Hu P, Gao F, Huang G. Performance of Imaging Techniques in Non-invasive Diagnosis of Non-alcoholic Fatty Liver Disease in Children: A Systematic Review and Meta-Analysis. *Front Pediatr* 2022; **10**: 837116 [PMID: 35899133 DOI: 10.3389/fped.2022.837116]

18 **Long MT**, Noureddin M, Lim JK. AGA Clinical Practice Update: Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Lean Individuals: Expert Review. *Gastroenterology* 2022; **163**: 764-774.e1 [PMID: 35842345 DOI: 10.1053/j.gastro.2022.06.023]

19 **Anstee QM**, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T, Trauner M, Kersey K, Li G, Han L, Jia C, Wang L, Chen G, Subramanian GM, Myers RP, Djedjos CS, Kohli A, Bzowej N, Younes Z, Sarin S, Shiffman ML, Harrison SA, Afdhal NH, Goodman Z, Younossi ZM. Noninvasive Tests Accurately Identify Advanced Fibrosis due to NASH: Baseline Data From the STELLAR Trials. *Hepatology* 2019; **70**: 1521-1530 [PMID: 31271665 DOI: 10.1002/hep.30842]

20 **Petersen KF**, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005; **54**: 603-608 [PMID: 15734833 DOI: 10.2337/diabetes.54.3.603]

21 **Vilar-Gomez E**, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L,

Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015; **149**: 367-78.e5; quiz e14 [PMID: 25865049 DOI: 10.1053/j.gastro.2015.04.005]

22 **Kasper P**, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen HM. NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021; **110**: 921-937 [PMID: 32696080 DOI: 10.1007/s00392-020-01709-7]

23 **Varkaneh HK**, Poursoleiman F, Al Masri MK, Alras KA, Shayah Y, Masmoum MD, Alangari FA, Alras AA, Rinaldi G, Day AS, Hekmatdoost A, Abu-Zaid A, Kutbi E. Low fat diet *vs* low carbohydrate diet for management of non-alcohol fatty liver disease: A systematic review. *Front Nutr* 2022; **9**: 987921 [PMID: 36051903 DOI: 10.3389/fnut.2022.987921]

24 **Haigh L**, Kirk C, El Gendy K, Gallacher J, Errington L, Mathers JC, Anstee QM. The effectiveness and acceptability of Mediterranean diet and calorie restriction in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis. *Clin Nutr* 2022; **41**: 1913-1931 [PMID: 35947894 DOI: 10.1016/j.clnu.2022.06.037]

25 **Różański G**, Pheby D, Newton JL, Murovska M, Zalewski P, Słomko J. Effect of Different Types of Intermittent Fasting on Biochemical and Anthropometric Parameters among Patients with Metabolic-Associated Fatty Liver Disease (MAFLD)-A Systematic Review. *Nutrients* 2021; **14** [PMID: 35010966 DOI: 10.3390/nu14010091]

26 **Yin C**, Li Z, Xiang Y, Peng H, Yang P, Yuan S, Zhang X, Wu Y, Huang M, Li J. Effect of Intermittent Fasting on Non-Alcoholic Fatty Liver Disease: Systematic Review and Meta-Analysis. *Front Nutr* 2021; **8**: 709683 [PMID: 34322514 DOI: 10.3389/fnut.2021.709683]

27 **Kendel Jovanović G**, Mrakovcic-Sutic I, Pavičić Žeželj S, Benjak Horvat I, Šuša L, Rahelić D, Klobučar Majanović S. Metabolic and Hepatic Effects of Energy-Reduced Anti-Inflammatory Diet in Younger Adults with Obesity. *Can J Gastroenterol Hepatol* 2021; **2021**: 6649142 [PMID: 33628758 DOI: 10.1155/2021/6649142]

28 **Scragg J**, Avery L, Cassidy S, Taylor G, Haigh L, Boyle M, Trenell MI, Anstee QM, McPherson S, Hallsworth K. Feasibility of a Very Low Calorie Diet to Achieve a Sustainable 10% Weight Loss in Patients With Nonalcoholic Fatty Liver Disease. *Clin Transl Gastroenterol* 2020; **11**: e00231 [PMID: 33094956 DOI: 10.14309/ctg.0000000000000231]

29 **Misciagna G**, Del Pilar Díaz M, Caramia DV, Bonfiglio C, Franco I, Noviello MR, Chiloiro M, Abbrescia DI, Mirizzi A, Tanzi M, Caruso MG, Correale M, Reddavide R, Inguaggiato R, Cisternino AM, Osella AR. Effect of a Low Glycemic Index Mediterranean Diet on Non-Alcoholic Fatty Liver Disease. A Randomized Controlled Clinici Trial. *J Nutr Health Aging* 2017; **21**: 404-412 [PMID: 28346567 DOI: 10.1007/s12603-016-0809-8]

30 **Hong F**, Liu Y, Lebaka VR, Mohammed A, Ye W, Chen B, Korivi M. Effect of Exercise Training on Serum Transaminases in Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Front Physiol* 2022; **13**: 894044 [PMID: 35837021 DOI: 10.3389/fphys.2022.894044]

31 **Ghaffari M**, Sadeghiyan S, Faramarzi M, Moghaddam M, Baghurst T. The effect of aerobic exercise on metabolic parameters of patients with non-alcoholic fatty liver disease: systematic review and meta-analysis. *J Sports Med Phys Fitness* 2022; Epub ahead of print [PMID: 35816139 DOI: 10.23736/S0022-4707.22.13801-6]

32 **Zhou BJ**, Huang G, Wang W, Zhu LH, Deng YX, He YY, Ma FH. Intervention effects of four exercise modalities on nonalcoholic fatty liver disease: a systematic review and Bayesian network meta-analysis. *Eur Rev Med Pharmacol Sci* 2021; **25**: 7687-7697 [PMID: 34982430 DOI: 10.26355/eurrev_202112_27615]

33 **Charatcharoenwitthaya P**, Kuljiratitikal K, Aksornchanya O, Chaiyasoot K, Bandidniyamanon W, Charatcharoenwitthaya N. Moderate-Intensity Aerobic *vs* Resistance Exercise and Dietary Modification in Patients With Nonalcoholic Fatty Liver Disease: A Randomized Clinical Trial. *Clin Transl Gastroenterol* 2021; **12**: e00316 [PMID: 33939383 DOI: 10.14309/ctg.0000000000000316]

34 **Sargeant JA**, Gray LJ, Bodicoat DH, Willis SA, Stensel DJ, Nimmo MA, Aithal GP, King JA. The effect of exercise training on intrahepatic triglyceride and hepatic insulin sensitivity: a systematic review and meta-analysis. *Obes Rev* 2018; **19**: 1446-1459 [PMID: 30092609 DOI: 10.1111/obr.12719]

35 **Younossi ZM**, Corey KE, Lim JK. AGA Clinical Practice Update on Lifestyle Modification Using Diet and Exercise to Achieve Weight Loss in the Management of Nonalcoholic Fatty Liver Disease: Expert Review. *Gastroenterology* 2021; **160**: 912-918 [PMID: 33307021 DOI: 10.1053/j.gastro.2020.11.051]

36 **Buzzetti E**, Linden A, Best LM, Madden AM, Roberts D, Chase TJG, Freeman SC, Cooper NJ, Sutton AJ, Fritche D, Milne EJ, Wright K, Pavlov CS, Davidson BR, Tsochatzis E, Gurusamy KS. Lifestyle modifications for nonalcohol-related fatty liver disease: a network meta-analysis. *Cochrane Database Syst Rev* 2021; **6**: CD013156 [PMID: 34114650 DOI: 10.1002/14651858.CD013156.pub2]

37 **Zelber-Sagi S**, Bord S, Dror-Lavi G, Smith ML, Towne SD Jr, Buch A, Webb M, Yeshua H, Nimer A, Shibolet O. Role of illness perception and self-efficacy in lifestyle modification among non-alcoholic fatty liver disease patients. *World J Gastroenterol* 2017; **23**: 1881-1890 [PMID: 28348495 DOI: 10.3748/wjg.v23.i10.1881]

38 **Miyazaki Y**, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 2002; **87**: 2784-2791 [PMID: 12050251 DOI: 10.1210/jcem.87.6.8567]

39 **Kovalic AJ**. Pharmacotherapeutic Impact on Nonalcoholic Steatohepatitis Histology: A Systematic Review and Network Meta-analysis. *J Clin Exp Hepatol* 2022; **12**: 1057-1068 [PMID: 35814516 DOI: 10.1016/j.jceh.2022.01.011]

40 **Raveendran AV**, Fernandez CJ, Jacob K. Efficacy and Cardiovascular Safety of Thiazolidinediones. *Curr Drug Saf* 2021; **16**: 233-249 [PMID: 33106148 DOI: 10.2174/1574886315666201026125530]

41 **Tang H**, Shi W, Fu S, Wang T, Zhai S, Song Y, Han J. Pioglitazone and bladder cancer risk: a systematic review and meta-analysis. *Cancer Med* 2018; **7**: 1070-1080 [PMID: 29476615 DOI: 10.1002/cam4.1354]

42 **Filipova E**, Uzunova K, Kalinov K, Vekov T. Pioglitazone and the Risk of Bladder Cancer: A Meta-Analysis. *Diabetes Ther* 2017; **8**: 705-726 [PMID: 28623552 DOI: 10.1007/s13300-017-0273-4]

43 **Lian J**, Fu J. Efficacy of Various Hypoglycemic Agents in the Treatment of Patients With Nonalcoholic Liver Disease With or Without Diabetes: A Network Meta-Analysis. *Front Endocrinol (Lausanne)* 2021; **12**: 649018 [PMID: 33841337 DOI: 10.3389/fendo.2021.649018]

44 **Musso G**, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic

fatty liver disease. *Hepatology* 2010; **52**: 79-104 [PMID: 20578268 DOI: 10.1002/hep.23623]
45 **Li Y**, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomed Rep* 2013; **1**: 57-64 [PMID: 24648894 DOI: 10.3892/br.2012.18]
46 **Turton MD**, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996; **379**: 69-72 [PMID: 8538742 DOI: 10.1038/379069a0]
47 **Jastreboff AM**, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, Kiyosue A, Zhang S, Liu B, Bunck MC, Stefanski A; SURMOUNT-1 Investigators. Tirzepatide Once Weekly for the Treatment of Obesity. *N Engl J Med* 2022; **387**: 205-216 [PMID: 35658024 DOI: 10.1056/NEJMoa2206038]
48 **Deacon CF**, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 1995; **44**: 1126-1131 [PMID: 7657039 DOI: 10.2337/diab.44.9.1126]
49 **Deacon CF**, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 1996; **271**: E458-E464 [PMID: 8843738 DOI: 10.1152/ajpendo.1996.271.3.E458]
50 **Frías JP**, Davies MJ, Rosenstock J, Pérez Manghi FC, Fernández Landó L, Bergman BK, Liu B, Cui X, Brown K; SURPASS-2 Investigators. Tirzepatide *vs* Semaglutide Once Weekly in Patients with Type 2 Diabetes. *N Engl J Med* 2021; **385**: 503-515 [PMID: 34170647 DOI: 10.1056/NEJMoa2107519]
51 **Fonseca VA**, Alvarado-Ruiz R, Raccah D, Boka G, Miossec P, Gerich JE; EFC6018 GetGoal-Mono Study Investigators. Efficacy and safety of the once-daily GLP-1 receptor agonist lixisenatide in monotherapy: a randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes (GetGoal-Mono). *Diabetes Care* 2012; **35**: 1225-1231 [PMID: 22432104 DOI: 10.2337/dc11-1935]
52 **Nauck MA**, D'Alessio DA. Tirzepatide, a dual GIP/GLP-1 receptor co-agonist for the treatment of type 2 diabetes with unmatched effectiveness regrading glycaemic control and body weight reduction. *Cardiovasc Diabetol* 2022; **21**: 169 [PMID: 36050763 DOI: 10.1186/s12933-022-01604-7]
53 **Yoshiji S**, Minamino H, Tanaka D, Yamane S, Harada N, Inagaki N. Effects of glucagon-like peptide-1 receptor agonists on cardiovascular and renal outcomes: A meta-analysis and meta-regression analysis. *Diabetes Obes Metab* 2022; **24**: 1029-1037 [PMID: 35137511 DOI: 10.1111/dom.14666]
54 **Zafar Y**, Rashid AM, Siddiqi AK, Ellahi A, Ahmed A, Hussain HU, Ahmed F, Menezes RG, Siddiqi TJ, Maniya MT. Effect of novel glucose lowering agents on non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2022; **46**: 101970 [PMID: 35659603 DOI: 10.1016/j.clinre.2022.101970]
55 **Avgerinos I**, Manolopoulos A, Michailidis T, Kitsios K, Liakos A, Karagiannis T, Dimitrakopoulos K, Matthews DR, Tsapas A, Bekiari E. Comparative efficacy and safety of glucose-lowering drugs as adjunctive therapy for adults with type 1 diabetes: A systematic review and network meta-analysis. *Diabetes Obes Metab* 2021; **23**: 822-831 [PMID: 33300282 DOI: 10.1111/dom.14291]
56 **Sikirica MV**, Martin AA, Wood R, Leith A, Piercy J, Higgins V. Reasons for discontinuation of GLP1 receptor agonists: data from a real-world cross-sectional survey of physicians and their patients with type 2 diabetes. *Diabetes Metab Syndr Obes* 2017; **10**: 403-412 [PMID: 29033597 DOI: 10.2147/DMSO.S141235]
57 **Weiss T**, Carr RD, Pal S, Yang L, Sawhney B, Boggs R, Rajpathak S, Iglay K. Real-World Adherence and Discontinuation of Glucagon-Like Peptide-1 Receptor Agonists Therapy in Type 2 Diabetes Mellitus Patients in the United States. *Patient Prefer Adherence* 2020; **14**: 2337-2345 [PMID: 33273810 DOI: 10.2147/PPA.S277676]
58 **Gan S**, Dawed AY, Donnelly LA, Nair ATN, Palmer CNA, Mohan V, Pearson ER. Efficacy of Modern Diabetes Treatments DPP-4i, SGLT-2i, and GLP-1RA in White and Asian Patients With Diabetes: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care* 2020; **43**: 1948-1957 [PMID: 33534728 DOI: 10.2337/dc19-2419]
59 **Tzanaki I**, Agouridis AP, Kostapanos MS. Is there a role of lipid-lowering therapies in the management of fatty liver disease? *World J Hepatol* 2022; **14**: 119-139 [PMID: 35126843 DOI: 10.4254/wjh.v14.i1.119]
60 **Leoni S**, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J Gastroenterol* 2018; **24**: 3361-3373 [PMID: 30122876 DOI: 10.3748/wjg.v24.i30.3361]
61 **Boutari C**, Pappas PD, Anastasilakis D, Mantzoros CS. Statins' efficacy in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Clin Nutr* 2022; **41**: 2195-2206 [PMID: 36081293 DOI: 10.1016/j.clnu.2022.08.001]
62 **Camacho-Muñoz D**, Kiezel-Tsugunova M, Kiss O, Uddin M, Sundén M, Ryaboshapkina M, Lind L, Oscarsson J, Nicolaou A. Omega-3 carboxylic acids and fenofibrate differentially alter plasma lipid mediators in patients with non-alcoholic fatty liver disease. *FASEB J* 2021; **35**: e21976 [PMID: 34618982 DOI: 10.1096/fj.202100380RRR]
63 **Monami M**, Sesti G, Mannucci E. PCSK9 inhibitor therapy: A systematic review and meta-analysis of metabolic and cardiovascular outcomes in patients with diabetes. *Diabetes Obes Metab* 2019; **21**: 903-908 [PMID: 30485622 DOI: 10.1111/dom.13599]
64 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]
65 **Abner EL**, Schmitt FA, Mendiondo MS, Marcum JL, Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. *Curr Aging Sci* 2011; **4**: 158-170 [PMID: 21235492 DOI: 10.2174/1874609811104020158]
66 **Pan CS**, Stanley TL. Effect of Weight Loss Medications on Hepatic Steatosis and Steatohepatitis: A Systematic Review. *Front Endocrinol (Lausanne)* 2020; **11**: 70 [PMID: 32153507 DOI: 10.3389/fendo.2020.00070]
67 **Wang H**, Wang L, Cheng Y, Xia Z, Liao Y, Cao J. Efficacy of orlistat in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomed Rep* 2018; **9**: 90-96 [PMID: 29930810 DOI: 10.3892/br.2018.1100]
68 **Zelber-Sagi S**, Kessler A, Brazowsky E, Webb M, Lurie Y, Santo M, Leshno M, Blendis L, Halpern Z, Oren R. A

double-blind randomized placebo-controlled trial of orlistat for the treatment of nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2006; **4**: 639-644 [PMID: 16630771 DOI: 10.1016/j.cgh.2006.02.004]

69 **Ye J**, Wu Y, Li F, Wu T, Shao C, Lin Y, Wang W, Feng S, Zhong B. Effect of orlistat on liver fat content in patients with nonalcoholic fatty liver disease with obesity: assessment using magnetic resonance imaging-derived proton density fat fraction. *Therap Adv Gastroenterol* 2019; **12**: 1756284819879047 [PMID: 31632457 DOI: 10.1177/1756284819879047]

70 **Zakaria Z**, Othman ZA, Bagi Suleiman J, Jalil NAC, Ghazali WSW, Mohamed M. Protective and Therapeutic Effects of Orlistat on Metabolic Syndrome and Oxidative Stress in High-Fat Diet-Induced Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) in Rats: Role on Nrf2 Activation. *Vet Sci* 2021; **8** [PMID: 34822647 DOI: 10.3390/vetsci8110274]

71 **Suleiman JB**, Nna VU, Zakaria Z, Othman ZA, Bakar ABA, Mohamed M. Obesity-induced testicular oxidative stress, inflammation and apoptosis: Protective and therapeutic effects of orlistat. *Reprod Toxicol* 2020; **95**: 113-122 [PMID: 32450208 DOI: 10.1016/j.reprotox.2020.05.009]

72 **Bajaj HS**, Burrows M, Blavignac J, Paron E, Camacho F, Gould E, Barakat M. Extended-release naltrexone/bupropion and liver health: Pooled, post hoc analysis from four randomized controlled trials. *Diabetes Obes Metab* 2021; **23**: 861-865 [PMID: 33275326 DOI: 10.1111/dom.14284]

73 **Tuccinardi D**, Farr OM, Upadhyay J, Oussaada SM, Mathew H, Paschou SA, Perakakis N, Koniaris A, Kelesidis T, Mantzoros CS. Lorcaserin treatment decreases body weight and reduces cardiometabolic risk factors in obese adults: A six-month, randomized, placebo-controlled, double-blind clinical trial. *Diabetes Obes Metab* 2019; **21**: 1487-1492 [PMID: 30724455 DOI: 10.1111/dom.13655]

74 **Nevens F**, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, Drenth JP, Pockros PJ, Regula J, Beuers U, Trauner M, Jones DE, Floreani A, Hohenester S, Luketic V, Shiffman M, van Erpecum KJ, Vargas V, Vincent C, Hirschfield GM, Shah H, Hansen B, Lindor KD, Marschall HU, Kowdley KV, Hooshmand-Rad R, Marmon T, Sheeron S, Pencek R, MacConell L, Pruzanski M, Shapiro D; POISE Study Group. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med* 2016; **375**: 631-643 [PMID: 27532829 DOI: 10.1056/NEJMoa1509840]

75 **Thomas C**, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* 2008; **7**: 678-693 [PMID: 18670431 DOI: 10.1038/nrd2619]

76 **Adorini L**, Pruzanski M, Shapiro D. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today* 2012; **17**: 988-997 [PMID: 22652341 DOI: 10.1016/j.drudis.2012.05.012]

77 **Mudaliar S**, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, Adorini L, Sciacca CI, Clopton P, Castelloe E, Dillon P, Pruzanski M, Shapiro D. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 574-82.e1 [PMID: 23727264 DOI: 10.1053/j.gastro.2013.05.042]

78 **Vignozzi L**, Morelli A, Filippi S, Comeglio P, Chavalmane AK, Marchetta M, Toce M, Yehiely-Cohen R, Vannelli GB, Adorini L, Maggi M. Farnesoid X receptor activation improves erectile function in animal models of metabolic syndrome and diabetes. *J Sex Med* 2011; **8**: 57-77 [PMID: 20955313 DOI: 10.1111/j.1743-6109.2010.02073.x]

79 **Cipriani S**, Mencarelli A, Palladino G, Fiorucci S. FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats. *J Lipid Res* 2010; **51**: 771-784 [PMID: 19783811 DOI: 10.1194/jlr.M001602]

80 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]

81 **DePaoli AM**, Zhou M, Kaplan DD, Hunt SC, Adams TD, Learned RM, Tian H, Ling L. FGF19 Analog as a Surgical Factor Mimetic That Contributes to Metabolic Effects Beyond Glucose Homeostasis. *Diabetes* 2019; **68**: 1315-1328 [PMID: 30862680 DOI: 10.2337/db18-1305]

82 **Kliewer SA**, Mangelsdorf DJ. Bile Acids as Hormones: The FXR-FGF15/19 Pathway. *Dig Dis* 2015; **33**: 327-331 [PMID: 26045265 DOI: 10.1159/000371670]

83 **Ferslew BC**, Xie G, Johnston CK, Su M, Stewart PW, Jia W, Brouwer KL, Barritt AS 4th. Altered Bile Acid Metabolome in Patients with Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2015; **60**: 3318-3328 [PMID: 26138654 DOI: 10.1007/s10620-015-3776-8]

84 **Sanyal AJ**, Ling L, Beuers U, DePaoli AM, Lieu HD, Harrison SA, Hirschfield GM. Potent suppression of hydrophobic bile acids by aldafermin, an FGF19 analogue, across metabolic and cholestatic liver diseases. *JHEP Rep* 2021; **3**: 100255 [PMID: 33898959 DOI: 10.1016/j.jhepr.2021.100255]

85 **Javed S**, Kohli K, Ali M. Reassessing bioavailability of silymarin. *Altern Med Rev* 2011; **16**: 239-249 [PMID: 21951025]

86 **Abenavoli L**, Capasso R, Milic N, Capasso F. Milk thistle in liver diseases: past, present, future. *Phytother Res* 2010; **24**: 1423-1432 [PMID: 20564545 DOI: 10.1002/ptr.3207]

87 **Akhtar DH**, Iqbal U, Vazquez-Montesino LM, Dennis BB, Ahmed A. Pathogenesis of Insulin Resistance and Atherogenic Dyslipidemia in Nonalcoholic Fatty Liver Disease. *J Clin Transl Hepatol* 2019; **7**: 362-370 [PMID: 31915606 DOI: 10.14218/JCTH.2019.00028]

88 **Cui CX**, Deng JN, Yan L, Liu YY, Fan JY, Mu HN, Sun HY, Wang YH, Han JY. Silibinin Capsules improves high fat diet-induced nonalcoholic fatty liver disease in hamsters through modifying hepatic de novo lipogenesis and fatty acid oxidation. *J Ethnopharmacol* 2017; **208**: 24-35 [PMID: 28648927 DOI: 10.1016/j.jep.2017.06.030]

89 **Zhang Y**, Hai J, Cao M, Zhang Y, Pei S, Wang J, Zhang Q. Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway. *Int Immunopharmacol* 2013; **17**: 714-720 [PMID: 24036369 DOI: 10.1016/j.intimp.2013.08.019]

90 **Zhong S**, Fan Y, Yan Q, Fan X, Wu B, Han Y, Zhang Y, Chen Y, Zhang H, Niu J. The therapeutic effect of silymarin in the treatment of nonalcoholic fatty disease: A meta-analysis (PRISMA) of randomized control trials. *Medicine (Baltimore)* 2017; **96**: e9061 [PMID: 29245314 DOI: 10.1097/MD.0000000000009061]

91 **Wah Kheong C**, Nik Mustapha NR, Mahadeva S. A Randomized Trial of Silymarin for the Treatment of Nonalcoholic Steatohepatitis. *Clin Gastroenterol Hepatol* 2017; **15**: 1940-1949.e8 [PMID: 28419855 DOI: 10.1016/j.cgh.2017.04.016]

92 **Aghemo A**, Alekseeva OP, Angelico F, Bakulin IG, Bakulina NV, Bordin D, Bueverov AO, Drapkina OM, Gillessen A, Kagarmanova EM, Korochanskaya NV, Kucheryavii UA, Lazebnik LB, Livzan MA, Maev IV, Martynov AI, Osipenko MF, Sas EI, Starodubova A, Uspensky YP, Vinnitskaya EV, Yakovenko EP, Yakovlev AA. Role of silymarin as antioxidant in clinical management of chronic liver diseases: a narrative review. *Ann Med* 2022; **54**: 1548-1560 [PMID: 35635048 DOI: 10.1080/07853890.2022.2069854]

93 **Aron-Wisnewsky J**, Doré J, Clement K. The importance of the gut microbiota after bariatric surgery. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 590-598 [PMID: 22926153 DOI: 10.1038/nrgastro.2012.161]

94 **Talavera-Urquijo E**, Beisani M, Balibrea JM, Alverdy JC. Is bariatric surgery resolving NAFLD *via* microbiota-mediated bile acid ratio reversal? *Surg Obes Relat Dis* 2020; **16**: 1361-1369 [PMID: 32336663 DOI: 10.1016/j.soard.2020.03.013]

95 **Patel N**, Mohanaruban A, Ashrafian H, Le Roux C, Byrne J, Mason J, Hopkins J, Kelly J, Teare J. EndoBarrier®: a Safe and Effective Novel Treatment for Obesity and Type 2 Diabetes? *Obes Surg* 2018; **28**: 1980-1989 [PMID: 29450844 DOI: 10.1007/s11695-018-3123-1]

96 **Espinet Coll E**, Vila Lolo C, Díaz Galán P, Gómez Valero JA, Bacchiddu S, Quintana Tomás C, Irigoyen D, Gunnard K, Juan-Creix Comamala A. Bariatric and metabolic endoscopy in the handling of fatty liver disease. A new emerging approach? *Rev Esp Enferm Dig* 2019; **111**: 283-293 [PMID: 30896957 DOI: 10.17235/reed.2019.5949/2018]

97 **Sarkar M**, Grab J, Dodge JL, Gunderson EP, Rubin J, Irani RA, Cedars M, Terrault N. Non-alcoholic fatty liver disease in pregnancy is associated with adverse maternal and perinatal outcomes. *J Hepatol* 2020; **73**: 516-522 [PMID: 32531415 DOI: 10.1016/j.jhep.2020.03.049]

98 **Lee SM**, Kwak SH, Koo JN, Oh IH, Kwon JE, Kim BJ, Kim SM, Kim SY, Kim GM, Joo SK, Koo BK, Shin S, Vixay C, Norwitz ER, Park CW, Jun JK, Kim W, Park JS. Non-alcoholic fatty liver disease in the first trimester and subsequent development of gestational diabetes mellitus. *Diabetologia* 2019; **62**: 238-248 [PMID: 30470912 DOI: 10.1007/s00125-018-4779-8]

99 **Mosca A**, De Cosmi V, Parazzini F, Raponi M, Alisi A, Agostoni C, Nobili V. The Role of Genetic Predisposition, Programing During Fetal Life, Family Conditions, and Post-natal Diet in the Development of Pediatric Fatty Liver Disease. *J Pediatr* 2019; **211**: 72-77.e4 [PMID: 31128886 DOI: 10.1016/j.jpeds.2019.04.018]

100 **Babu AF**, Csader S, Lok J, Gómez-Gallego C, Hanhineva K, El-Nezami H, Schwab U. Positive Effects of Exercise Intervention without Weight Loss and Dietary Changes in NAFLD-Related Clinical Parameters: A Systematic Review and Meta-Analysis. *Nutrients* 2021; **13** [PMID: 34579012 DOI: 10.3390/nu13093135]

101 **Riley MR**, Bass NM, Rosenthal P, Merriman RB. Underdiagnosis of pediatric obesity and underscreening for fatty liver disease and metabolic syndrome by pediatricians and pediatric subspecialists. *J Pediatr* 2005; **147**: 839-842 [PMID: 16356443 DOI: 10.1016/j.jpeds.2005.07.020]

102 **Nobili V**, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Angulo P. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006; **44**: 458-465 [PMID: 16871574 DOI: 10.1002/hep.21262]

103 **Alqahtani SA**, Schattenberg JM. NAFLD in the Elderly. *Clin Interv Aging* 2021; **16**: 1633-1649 [PMID: 34548787 DOI: 10.2147/CIA.S295524]

104 **Mobasheri N**, Ghahremani L, Fallahzadeh Abarghooee E, Hassanzadeh J. Lifestyle Intervention for Patients with Nonalcoholic Fatty Liver Disease: A Randomized Clinical Trial Based on the Theory of Planned Behavior. *Biomed Res Int* 2022; **2022**: 3465980 [PMID: 36132088 DOI: 10.1155/2022/3465980]

105 **Nath P**, P Singh S. Nonalcoholic Fatty Liver Disease: Time to Take the Bull by the Horns. *Euroasian J Hepatogastroenterol* 2018; **8**: 47-51 [PMID: 29963461 DOI: 10.5005/jp-journals-10018-1257]

106 **Oda K**, Uto H, Mawatari S, Ido A. Clinical features of hepatocellular carcinoma associated with nonalcoholic fatty liver disease: a review of human studies. *Clin J Gastroenterol* 2015; **8**: 1-9 [PMID: 25575848 DOI: 10.1007/s12328-014-0548-5]

107 **Mittal S**, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2016; **14**: 124-31.e1 [PMID: 26196445 DOI: 10.1016/j.cgh.2015.07.019]

108 **Dyson J**, Jaques B, Chattopadyhay D, Lochan R, Graham J, Das D, Aslam T, Patanwala I, Gaggar S, Cole M, Sumpter K, Stewart S, Rose J, Hudson M, Manas D, Reeves HL. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014; **60**: 110-117 [PMID: 23978719 DOI: 10.1016/j.jhep.2013.08.011]

109 **Tan DJH**, Ng CH, Lin SY, Pan XH, Tay P, Lim WH, Teng M, Syn N, Lim G, Yong JN, Quek J, Xiao J, Dan YY, Siddiqui MS, Sanyal AJ, Muthiah MD, Loomba R, Huang DQ. Clinical characteristics, surveillance, treatment allocation, and outcomes of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a systematic review and meta-analysis. *Lancet Oncol* 2022; **23**: 521-530 [PMID: 35255263 DOI: 10.1016/S1470-2045(22)00078-X]

110 **Mantovani A**, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism* 2020; **111S**: 154170 [PMID: 32006558 DOI: 10.1016/j.metabol.2020.154170]

111 **Cholankeril G**, Wong RJ, Hu M, Perumpail RB, Yoo ER, Puri P, Younossi ZM, Harrison SA, Ahmed A. Liver Transplantation for Nonalcoholic Steatohepatitis in the US: Temporal Trends and Outcomes. *Dig Dis Sci* 2017; **62**: 2915-2922 [PMID: 28744836 DOI: 10.1007/s10620-017-4684-x]

112 **Moosavian SA**, Sathyapalan T, Jamialahmadi T, Sahebkar A. The Emerging Role of Nanomedicine in the Management of Nonalcoholic Fatty Liver Disease: A State-of-the-Art Review. *Bioinorg Chem Appl* 2021; **2021**: 4041415 [PMID: 34659388 DOI: 10.1155/2021/4041415]

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MINIREVIEWS

Role of gut microbiota in the pathogenesis and therapeutics of minimal hepatic encephalopathy *via* the gut-liver-brain axis

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Abstract

Minimal hepatic encephalopathy (MHE) is a frequent neurological and psychiatric complication of liver cirrhosis. The precise pathogenesis of MHE is complicated and has yet to be fully elucidated. Studies in cirrhotic patients and experimental animals with MHE have indicated that gut microbiota dysbiosis induces systemic inflammation, hyperammonemia, and endotoxemia, subsequently leading to neuroinflammation in the brain *via* the gut-liver-brain axis. Related mechanisms initiated by gut microbiota dysbiosis have significant roles in MHE pathogenesis. The currently available therapeutic strategies for MHE in clinical practice, including lactulose, rifaximin, probiotics, synbiotics, and fecal microbiota transplantation, exert their effects mainly by modulating gut microbiota dysbiosis. Microbiome therapies for MHE have shown promised efficacy and safety; however, several controversies and challenges regarding their clinical use deserve to be intensively discussed. We have summarized the latest research findings concerning the roles of gut microbiota dysbiosis in the pathogenesis of MHE *via* the gut-liver-brain axis as well as the potential mechanisms by which microbiome therapies regulate gut microbiota dysbiosis in MHE patients.

Key Words: Gut microbiota; Minimal hepatic encephalopathy; Gut-liver-brain axis; Pathogenesis; Therapeutics

Core Tip: Minimal hepatic encephalopathy (MHE) is a common neuropsychiatric complication of liver cirrhosis. Gut microbiota dysbiosis has an essential role in the pathogenesis of MHE *via* the gut-liver-brain axis. Current therapeutic strategies for MHE are based on the modulation of gut microbiota dysbiosis. This review presents the recent evidence on the roles of gut microbiota dysbiosis in the pathogenesis and treatment of MHE *via* the gut-liver-brain axis.

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INTRODUCTION

Hepatic encephalopathy (HE) is a central nervous system complication of chronic liver disease or portal systemic shunting that is characterized by a broad range of neuropsychiatric symptoms[1]. Depending on the severity of clinical manifestations, HE can be classified as overt or covert, such as minimal HE (MHE) and West Haven grade I HE[1]. Overt HE (OHE) exhibits obvious neurological and psychiatric manifestations, such as flapping tremors, drowsiness, and sometimes coma[2]. In contrast, MHE presents with slight cognitive deficits in the executive function, including psychomotor speed, response inhibition, and working memory, with no clinical evidence of OHE[3]. MHE is diagnosed using neurophysiological and psychometric tests, and its prevalence range from approximately 30% to 70% in different populations with liver cirrhosis[1,3,4]. MHE compromises daily functions, affects the health-related quality of life, and increases the risk of progression to OHE[5,6].

The exact pathogenesis of MHE is complex and not completely understood. Furthermore, the pathophysiological basis of MHE is multifactorial, with ammonia, inflammation, and endotoxins considered causative factors[7]. Recently, gut microbiota dysbiosis has been demonstrated to be associated with hyperammonemia, systemic inflammation, neuroinflammation, and endotoxemia in cirrhotic patients and experimental animals with MHE[8-11]. With decompensated liver cirrhosis and hepatic dysfunction, dysbiotic gut microbiota and its metabolites cross the impaired intestinal barrier and induce hyperammonemia, systemic inflammation, and endotoxemia, which influence the permeability of the blood-brain barrier (BBB), resulting in neuroinflammation and low-grade edema in the cerebrum and contributing to central nervous system dysfunction[7]. It has been increasingly recognized that gut microbiota dysbiosis is the predominant factor accounting for MHE pathogenesis *via* the gut-liver-brain axis[12,13]. Furthermore, an increasing number of clinical studies have shown that currently available therapies for MHE patients, including lactulose, probiotics, synbiotics, rifaximin, and fecal microbiota transplantation (FMT), improve cognitive dysfunction through the modulation of gut microbiota dysbiosis[14-17].

Although several published reviews have elucidated the involvement of gut microbiota dysbiosis in the pathogenesis and treatment of HE, no specific review has focused on this involvement in MHE[13,18-20]. Therefore, this review aimed to comprehensively elucidate the roles of gut microbiota in MHE pathogenesis *via* the gut-liver-brain axis and systematically analyze the underlying mechanisms linked with microbiome therapies to modulate gut microbiota dysbiosis in cirrhotic patients with MHE.

GUT MICROBIOTA DYSBIOSIS IN MHE

Small intestinal bacterial overgrowth (SIBO) is a pathological dysregulation of gut microbiota, characterized by excessive bacteria and/or abnormal bacterial composition in the small intestine. Approximately 48% to 73% of cirrhotic patients have SIBO[21]. Intestinal immune dysfunction, intestinal dysmotility, and decreased bile acid synthesis are implicated in the pathogenesis of SIBO[22]. SIBO is closely associated with the severity of advanced liver cirrhosis, and it has been validated as a significant risk factor for MHE[23,24]. In MHE patients, the gut microbiota dysbiosis resulting from the SIBO has been characterized by lower bacterial diversity, decreased autochthonous beneficial bacteria, and increased pathogenic Gram-negative bacteria[8,14,25]. The gut microbiota dysbiosis in cirrhotic patients with MHE is summarized in Table 1. Notably, Bajaj *et al*[26] found that MHE patients have higher abundances of *Enterococcus* and *Veillonella* and a lower abundance of *Roseburia* in the gut mucosal microbiota; these signatures are significantly different from those of the fecal microbiota. It is hypothesized that the adherence and overgrowth of pathogenic bacteria in the gut mucosal microbiota, rather than the fecal microbiota, might be implicated in the pathogenesis of bacterial translocation.

Table 1 Clinical studies of gut microbiota dysbiosis in cirrhotic patients with minimal hepatic encephalopathy

Ref.	Nationality	Number of patients	Etiology of cirrhosis	MHE diagnosis	Sample	Method	Microbiota alteration
Zhang *et al*[8]	China	51	AIH, HBV, PBC, alcohol	NCT-A DST	Stool	16S rRNA pyrosequencing	Enriched *Streptococcus salivarius*
Wang *et al*[14]	China	98	HBV, HCV, others	NCT-A DST	Stool	16S rRNA sequencing	Enriched *Proteobacteria*, especially *Pasteurellaceae Haemophilus* and *Alcaligenaceae Parasutterella*
Luo *et al* [29]	China	143	HBV	PHES	Stool	16S rRNA sequencing	Enriched *Streptococcus salivarius* and *Veillonella*
Liu *et al* [76]	China	55	HBV, HCV, alcohol, others	NCT-A BAEP	Stool	Stool bacterial culture	Overgrowth of *E. coli* and *Staphylococcus* spp.
Bajaj *et al* [27]	United States	97	HCV, alcohol, NASH, others	ICT PHES	Stool	Shotgun metagenomic sequencing	*Alistipes ihumii*, *Prevotella copri*, and *Eubacterium* spp. were higher, while *Enterococcus* spp. were uniquely lower in MHE diagnosed by ICT
Bajaj *et al* [28]	United States	247	HCV, alcohol, NASH, others	PHES ICT Stroop	Stool	Multi-tagged sequencing	Enriched *Lactobacillaceae*

AIH: Autoimmune hepatitis; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PBC: Primary biliary cirrhosis; NASH: Non-alcoholic steatohepatitis; NCT-A: Number connection test-A; DST: Digit symbol test; ICT: Inhibitory control test; PHES: Psychometric hepatic encephalopathy score; MHE: Minimal hepatic encephalopathy.

Because of the lack of uniform criteria for diagnosing MHE, the results of the currently available diagnostic methods for MHE are inconsistent in clinical practice. Specific signatures of fecal microbiota correspond to unique cognitive impairments determined by different diagnostic methods for MHE, including the psychometric hepatic encephalopathy score, inhibitory control test, and EncephalApp Stroop test (Table 1). For example, the abundances of *Enterococcus* and *Streptococcus* were higher in cirrhotic patients with MHE diagnosed by the psychometric hepatic encephalopathy score only; however, the abundances of *Prevotella copri*, *Eggerthela*, and *Alistipes* spp. were higher in those with MHE diagnosed by the inhibitory control test only[27]. Of note, the *Lactobacillaceae* abundance was also higher in fecal samples of MHE patients, regardless of MHE testing; therefore, this might be able to be used as a substitution for MHE testing[28].

Gut microbiota signatures of MHE patients vary depending on the etiology of liver cirrhosis. In a Chinese cohort with cirrhosis, the abundances of *Streptococcaceae* and *Veillonellaceae* were overrepresented in cirrhotic patients, and MHE patients had a higher abundance of *Streptococcus salivarius*[8]. Moreover, *Streptococcus salivarius* was also enriched in the gut microbiome of patients with MHE due to hepatitis B-associated liver cirrhosis, especially in those with sleep disturbances[29]. In contrast, in a cohort with cirrhosis in the United States, the fecal *Lactobacillaceae* abundance was higher in MHE patients; however, the abundance of fecal *Lachnospiraceae* genera, such as *Clostridium XIVb* and *Ruminococcus*, was correlated with better cognitive function independent of clinical variables[28]. Additionally, another study in the United States revealed that a higher *Veillonellaceae* abundance was found in the fecal microbiota of MHE patients, and that *Porphyromonadaceae* and *Alcaligeneceae* were positively associated with cognitive dysfunction in MHE[30]. The altered gut microbiota in Chinese MHE patients differs from that of MHE patients in the United States because the primary etiology of liver cirrhosis in the Chinese population is hepatitis B; however, in the United States, hepatitis C and excessive alcohol consumption are the predominant etiologies[31,32].

GUT-LIVER-BRAIN AXIS IN MHE

Bacterial translocation

In healthy individuals, the characteristic structure and immune system of the intestinal mucosa can prevent bacteria and their byproducts from entering the systemic circulation. In patients with liver cirrhosis, the SIBO decreases the synthesis of secondary bile acids by inhibiting the activation of Farnesoid X receptor and Takeda G protein-coupled receptor, which reduces intestinal immunoglobulin A levels and further compromises the immune function of the intestinal mucosa[33,34]. Moreover, the SIBO induces decreased synthesis of antimicrobial peptide and activates mucosal immune responses, resulting in intestinal inflammation and impaired intestinal epithelium integrity[35]. Furthermore, the SIBO increases the permeability of epithelial intercellular junctions *via* the down-regulation of tight junction protein expressions[36]. These potential mechanisms induce a “leaky gut” which facilitates the

transfer of pathogenic bacteria and their metabolites from the intestinal tract to the circulatory system, resulting in systemic inflammation (Figure 1).

Systemic inflammation

Translocated bacteria and their products, such as pathogen-associated molecular patterns, are transported to the liver through the portal vein. At the molecular level, pathogen-associated molecular patterns are recognized by Toll-like receptors and cytoplasmic nucleotide-binding oligomerization domain-like receptors, and primarily stimulate hepatic Kupffer cells through the activation of MyD88-dependent and NF-κB signaling pathways[37,38]. Innate immune responses in the liver are triggered, resulting in liver damage, with the release of damage-associated molecular patterns and the production of proinflammatory cytokines and chemokines (Figure 1)[37,38]. MHE patients present the systemic proinflammatory environment reflected by increased circulatory levels of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins (ILs), and interferon, and chemokines such as CCL20, CXCL13 and CX3CL1[39].

In experimental mice with MHE, higher abundances of *Staphylococcaceae*, *Enterobacteriaceae*, and *Lactobacillaceae* in the large intestine and of *Staphylococcaceae*, *Streptococcaceae*, and *Enterobacteriaceae* in the small intestine were associated with systemic inflammation along with higher circulating concentrations of TNF-α and IL-1β[10]. Similarly, the abundances of *Enterobacteriaceae*, *Fusobacteriaceae*, and *Veillonellaceae* were positively associated with higher serum concentrations of IL-2, IL-13, and IL-23 in cirrhotic patients with MHE, and these increased cytokines were significantly correlated with MHE severity[30]. Furthermore, the association between MHE severity and increased proinflammatory cytokines has been demonstrated to be independent of the severity of liver cirrhosis and ammonia levels, suggesting that systemic inflammation with its proinflammatory cytokines is potentially implicated in the development of MHE[40].

Blood-brain barrier permeability

The blood-brain barrier is composed of capillary endothelial cells surrounded by capillary basement membrane and astrocytic perivascular endfeet. The BBB separates the systemic circulation and brain, prevents the entry of potentially harmful substances into the brain, and maintains the homeostasis of the brain microenvironment. Circulating proinflammatory cytokines cannot directly cross the BBB and exert their effects on the brain. However, these cytokines, including TNF-α, ILs, and interferon, down-regulate the expression of endothelial tight junction proteins, compromise cerebrovascular endothelial cells, activate astrocytes to an inflammatory reactive state, and alter BBB receptor expression and transport pathways, which consequently impair BBB integrity and further increase BBB permeability (Figure 2)[41,42]. Through the aforementioned mechanisms, the proinflammatory signaling, which is initiated by systemic inflammation, crosses the damaged BBB and underlies the neuroinflammatory response that develops in the cerebrum.

Neuroinflammation

Neuroinflammation refers to a series of inflammatory response processes characterized by microglial activation and proinflammatory cytokine production in the cerebrum[43]. The proinflammatory signaling, originating from systemic inflammation and crossing the BBB, induces microglial activation, stimulates Toll-like receptors, and activates NF-κB and myeloid protein-dependent pathways to produce proinflammatory mediators in the cerebrum[41]. Neuroinflammation interferes with neurotransmission, affects neuronal function, and induces low-grade cerebral edema in concert with hyperammonemia (Figure 2)[43]. Balzano *et al*[44] found that MHE rats experienced not only increased serum levels of prostaglandin E2, IL-6, and IL-17 but also microglial activation with increased mRNA expression of TNF-α and IL-1β in the hippocampus, which indicated the existence of both systemic inflammation and neuroinflammation in MHE. Additionally, *Enterobacteriaceae* in the cecum and *Staphylococcaceae* in the small intestine are linked to serum proinflammatory cytokines and neuroinflammation in cirrhotic mice[10]. Moreover, germ-free mice colonized with feces from MHE patients containing high abundances of *Enterobacteriaceae*, *Staphylococcaceae*, and *Streptococcaceae* had remarkable microglial activation and neuroinflammation[9]. Therefore, neuroinflammation is closely associated with gut microbiota dysbiosis in experimental animal models of MHE.

In contrast, the neuroinflammation in MHE patients has not been extensively studied. Postmortem examination of cerebral specimens from MHE patients showed that mRNA expressions of TNF-α, IL-1β, and IL-6 remained unchanged in the cerebral cortex, although genes related to microglial activation were upregulated[45,46]. Current evidence of the involvement of gut microbiota dysbiosis in the pathogenesis of neuroinflammation has been derived from experimental animal models of MHE; however, related studies of MHE patients are lacking. Magnetic resonance imaging (MRI) has been successfully used to quantify the manganese deposition in the brain and noradrenaline in MHE rats[47,48]. It is presumed that cerebral MRI examinations of MHE patients could facilitate further research concerning the involvement of gut microbiota dysbiosis in the pathogenesis of neuroinflammation.

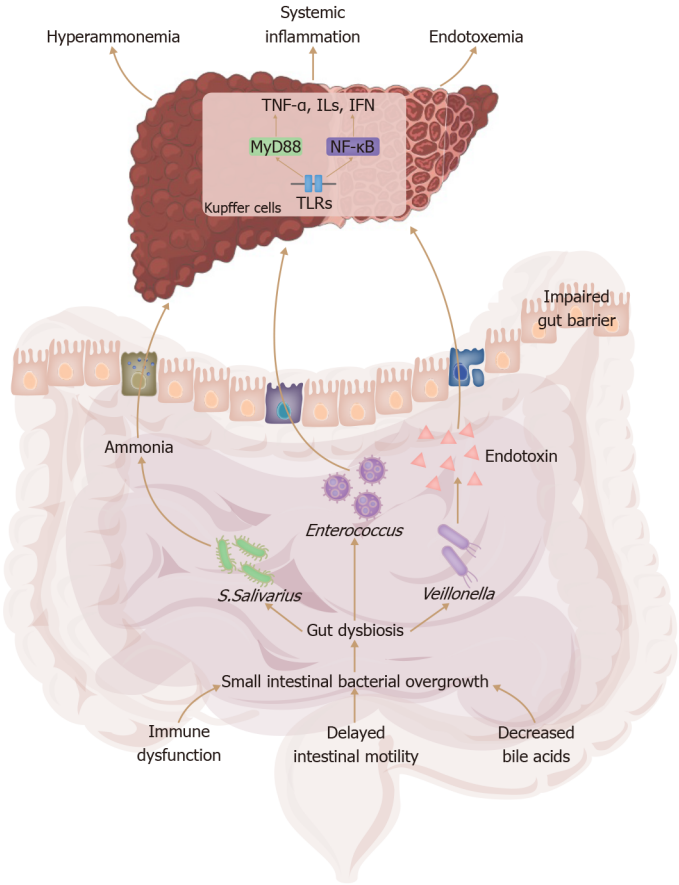

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Figure 1 On the background of liver cirrhosis with hepatic dysfunction, dysbiotic gut microbiota and its byproducts including ammonia and endotoxin cross the impaired intestinal barrier, stimulate innate immune responses in the liver, and lead to systemic inflammation, hyperammonemia, and endotoxemia. TNF-α: Tumor necrosis factor-alpha; ILs: Interleukins; IFN: Interferon.

Hyperammonemia

Ammonia, an important causative agent of MHE, is predominantly derived from the degradation of amino acids and urea by the gut bacteria. Urea hydrolysis is catalyzed by urease, an enzyme mainly produced by Gram-negative *Enterobacteriaceae*[49]. In MHE patients, an increased abundance of *Streptococcus salivarius* is associated with hyperammonemia because *Streptococcus salivarius* has a considerable number of urea catabolite genes that activate urease activity, facilitating ammonia production and accumulation, leading to further hyperammonemia[8,29,50]. *Streptococcus salivarius* might be a potential therapeutic target for ammonia-lowering strategies in MHE patients.

Hyperammonemia induces a leaky BBB, promotes glutamine accumulation in astrocytes, and leads to astrocyte swelling and subsequent low-grade cerebral edema that influences neurotransmission (Figure 2)[51,52]. Similar to systemic inflammation, chronic hyperammonemia induces microglial activation with the increased production of TNF-α, IL-1β, and IL-6 and impaired glutamatergic and GABAergic neurotransmission, resulting in cognitive deficits in MHE rats[53,54]. Moreover, treating MHE rats with anti-TNF-α, which does not cross the BBB, attenuated systemic inflammation, alleviated hyperammonemia-induced neuroinflammation, and ameliorated neurotransmission and cognitive function[44]. Experimental animal evidence indicated that hyperammonemia might be exerted in concert with systemic inflammation to drive the development of neuroinflammation.

Endotoxemia

Endotoxins, also known as lipopolysaccharides, are components of the outer membrane of Gram-negative bacteria. In patients with liver cirrhosis, serum endotoxin levels are increased and correlated with MHE severity, and functional modules associated with endotoxin production are abundant in the gut microbiome of MHE patients[11,29]. Several studies reported that increased endotoxin production was related to a higher *Veillonella* abundance in MHE patients[55,56]. Due to the impaired intestinal barrier and portal-systemic shunting, endotoxins enter the systemic circulation and cause endotoxemia with increased production of pro-inflammatory cytokines (Figure 1)[57]. Similar to pro-inflammatory cytokines, endotoxin is also unable to cross the BBB. Nevertheless, endotoxin stimulates microglia to release TNF-α, IL-1β, and reactive oxygen species, which increases the permeability of BBB tight

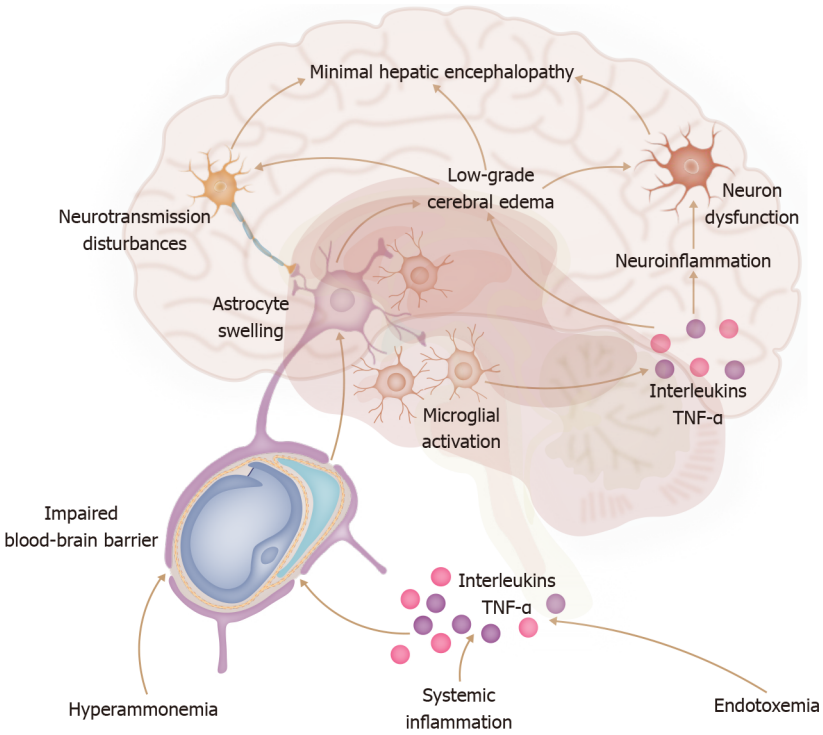

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Figure 2 Systemic inflammation, hyperammonemia, and endotoxemia influence the permeability of the blood-brain barrier, resulting in neuroinflammation and low-grade cerebral edema, contributing to the pathogenesis of minimal hepatic encephalopathy. TNF-α: Tumor necrosis factor-alpha.

junctions[58]. Peripheral lipopolysaccharide injection induces microglial hyperactivation, increases mRNA expressions of TNF-α, IL-1β, and IL-10 in the cerebral cortex, and impairs glutamate transmission, resulting in memory and learning deficits in mice[59,60]. Based on the synergistic effect of hyperammonemia, peripheral lipopolysaccharide injection induced cytotoxic brain swelling and a subsequent pre-coma status in cirrhotic rats[61]. However, the exact mechanism of the interaction between hyperammonemia and endotoxemia in the pathogenesis of MHE remains unclear and requires further research.

MICROBIOME THERAPEUTICS FOR MHE

The majority of current therapeutic strategies for MHE in clinical practice exert their effects through modulation of gut microbiota dysbiosis. These microbiome therapies, including lactulose, rifaximin, probiotics, synbiotics, and FMT, alter the composition and function of the gut microbiota, inhibit pathogenic bacterial overgrowth, increase the abundance of beneficial bacteria, and reduce the production and absorption of ammonia (Table 2).

Lactulose

Lactulose, the standard therapy for MHE, is considered a prebiotic. A multicenter, randomized, controlled trial in China suggested that lactulose reduces ammonia production and absorption by inhibiting the growth of ammonia-producing bacteria, such as *Streptococcus salivarius*, and facilitates the growth of beneficial saccharolytic bacteria, such as *Bifidobacterium* and *Lactobacillus*[14]. Moreover, several studies have revealed that lactulose reduces the serum concentrations of TNF-α, ILs, and endotoxins by inhibiting SIBO and bacterial translocation, thus improving cognitive dysfunction of MHE patients[11,62,63].

Despite lactulose treatment for MHE patients, there was still an increased gut microbiota dysbiosis with a lower cirrhosis dysbiosis ratio and enriched Gram-negative bacteria such as *Enterobacteriaceae* and *Bacteroidaceae*[64]. Similarly, Sarangi *et al*[65] indicated that lactulose did not significantly influence bacterial diversity, species richness, or taxa abundance in the gut microbiome of cirrhotic patients with MHE. Moreover, lactulose withdrawal only decreased the *Faecalibacterium* abundance and did not remarkably alter the gut microbiota composition[24]. These studies suggest that alterations in the gut microbiota function, rather than changes in the gut microbiota composition, may be associated with the therapeutic effects of lactulose in MHE patients.

Table 2 Clinical Studies of gut microbiota modulation in cirrhotic patients with minimal hepatic encephalopathy

Ref.	Design	Patients	Duration	Sample	Method	Microbiota alteration	Therapeutic effect
Lactulose							
Wang *et al* [14]	Multi-centre, open-label, randomized controlled trial	lactulose (n = 67), control (n = 31)	60 d	Stool	16S rRNA sequencing	Higher abundances of *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* were in non-responders for lactulose	Significantly ameliorated MHE
Rifaximin							
Bajaj *et al* [56]	Controlled clinical trial	MHE patients before/after rifaximin (n = 20)	8 wk	Stool	Multi-tagged pyrosequencing, GC/LC-MS	Modest decrease in *Veillonellaceae* and increase in *Eubacteriaceae*, with significant changes in metabolite correlations	Significant improvement in endotoxemia and cognition
Probiotics							
Lactobacillus GG							
Bajaj *et al* [74]	Randomized phase I, placebo-controlled trial	Probiotic (n = 14), placebo (n = 16)	8 wk	Stool	Multi-tagged pyrosequencing, GC/LC-MS	Decreased *Enterobacteriaceae* and increased *Lachnospiraceae* and *Clostridiales Incertae Sedis* XIV, with significant alterations in metabolite correlations with amino acid and secondary bile acid metabolism	Attenuated endotoxemia and decreased TNF-α without change in cognition
Probiotics							
Clostridium butyricum combined with *Bifidobacterium infantis*							
Xia *et al* [73]	Randomized controlled trial	Probiotic (n = 30), placebo (n = 37)	3 mo	Stool	16S rRNA sequencing	Increased *Clostridium cluster* I and *Bifidobacterium*, decreased *Enterobacteriaceae* and *Enterococcus*	Reduced ammonia and improved cognition
Escherichia coli Nissle 1917 strain							
Manzhalii *et al*[75]	Single-centre, open-label, randomized trial	Probiotic (n = 15), lactulose (n = 15), rifaximin (n = 15)	1 mo	Stool	16S rRNA sequencing	Normalized *Bifidobacterium* and *Lactobacilli* abundance	Reduced ammonia and pro-inflammatory cytokines and improved cognition
Rifaximin plus probiotic							
Zuo *et al* [84]	Controlled clinical trial	Rifaximin (n = 7), rifaximin plus probiotic (n = 7)	4 wk	Stool	16S rRNA sequencing	Both treatments alone reduced the overall microbiome diversity, with decreased *Streptococcus* and *Faecalibacterium*, *Clostridium* and increased *Lactobacillus*	Rifaximin plus probiotics showed a more apparent effect
Rifaximin plus lactulose							
Schulz *et al*[72]	Randomized controlled trial	Rifaximin (n = 1), rifaximin plus lactulose (n = 4)	3 mo	Stool	16S rRNA sequencing	Rifaximin with or without lactulose did not affect microbiota composition	MHE improvement with rifaximin lasted after the end of treatment
Synbiotics							
Probiotics plus fermentable fiber							
Liu *et al* [76]	Controlled clinical trial	Synbiotic (n = 20), fermentable fiber (n = 20), placebo (n = 15)	30 d	Stool	Stool quantitative bacteriological culture	Significant increase in non-urease-producing *Lactobacillus* species	Reduced ammonia and endotoxemia levels, reversal in 50% of MHE patients

GC/LC-MS: Gas chromatography/liquid chromatography-mass spectrometry; TNF-α: Tumor necrosis factor-alpha; MHE: Minimal hepatic encephalopathy.

Rifaximin

Rifaximin is an oral semisynthetic and nonsystemic antibiotic that inhibits transcription and RNA synthesis by binding to the β-subunit of bacterial RNA polymerase, with lower gastrointestinal absorption and better antimicrobial activity[66]. As an antibiotic, rifaximin also reduced pro-inflammatory cytokines and attenuated systemic and intestinal inflammation in a mouse model of MHE[10]. Similarly, rifaximin-α inhibited serum neutrophil TLR-4 expression, decreased TNF-α and IL levels, and ameliorated MHE in cirrhotic patients[67].

A systematic review and meta-analysis showed that rifaximin is an effective and safe therapy for SIBO with a higher overall eradication rate[68]. In a mouse model of MHE, rifaximin therapy decreased intestinal ammonia production and serum IL-1β and IL-6 Levels by altering the gut microbiota function with increased secondary bile acids and decreased deconjugation without altering the gut microbiota composition[69]. Similarly, several clinical studies revealed that rifaximin attenuated hyperammonemia and endotoxemia in patients with MHE and resulted in significant changes in gut metabolites with modest alterations in gut microbiota composition, such as decreased *Streptococcus* and *Veillonella* abundance[56,70,71]. Furthermore, long-term treatment with rifaximin with or without lactulose did not affect the gut microbiota composition over a period of 3 mo in cirrhotic patients with MHE[72]. The results of these studies further support the theory that rifaximin treats MHE by modulating the metabolic function of the gut microbiota rather than gut microbiota composition, similar to the mechanism of lactulose treatment for MHE.

Probiotics

Probiotics, which are added to yogurt or consumed as food supplements, are live bacteria with various health benefits. Treatment with probiotics containing *B. infantis* and *C. butyricum* increased *Bifidobacterium* and *Clostridium cluster* I abundances and decreased *Enterococcus* and *Enterobacteriaceae* abundances, thereby significantly lowering serum ammonia levels of patients with hepatitis B-associated liver cirrhosis[73]. Additionally, the probiotic *Lactobacillus* GG increased *Clostridiales* XIV and *Lachnospiraceae* abundances, decreased the *Enterobacteriaceae* abundance, and decreased serum endotoxemia and TNF-α levels, resulting in alterations in metabolites associated with amino acid and secondary bile acid metabolism[74]. Moreover, the probiotic *Escherichia coli Nissle* strain reduced the levels of ammonia and pro-inflammatory cytokines, normalized *Lactobacilli* and *Bifidobacterium* abundances, and improved the cognitive function of MHE patients[75]. A systematic review of 19 trials showed that probiotics increased beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, decreased SIBO and endotoxemia, and reversed MHE without affecting systemic inflammation[15]. Compared with other modalities, including lactulose, rifaximin, and L-ornithine-aspartate, probiotics have similar therapeutic effects on MHE reversal and OHE prevention, and no significant differences were observed in the gut microbiota composition when probiotics and lactulose were compared[15,73]. Probiotics are regarded as alternative therapies for MHE. In contrast to lactulose and rifaximin, probiotics have therapeutic effects on MHE by altering the gut microbiota composition. Because of the complicated interconnections within the gut microbiome, a single change in the gut microbiota composition may have an unexpected effect or no effect at all. The interactions among supplemental probiotics, autochthonous beneficial bacteria, and pathogenic bacteria in the intestinal tract remain to be determined.

Synbiotics

Synbiotics are a combination of probiotics and prebiotics. It is hypothesized that synbiotics improve the effectiveness of probiotics in the human intestine. A synbiotic containing probiotics and fermentable fibers significantly increased the nonurease-producing *Lactobacillus* abundance, decreased serum ammonia levels, and reversed MHE in cirrhotic patients[76]. Moreover, a combination of *Bifidobacterium longum* and fructo-oligosaccharide, which is another symbiotic, decreased serum ammonia levels and improved the cognitive function of MHE patients[77]. A systematic review revealed that synbiotic supplementation decreased SIBO, increased beneficial commensal bacteria such as *Lactobacillus* and *Bifidobacterium*, reduced blood ammonia and endotoxin levels, and decreased the risk of MHE recurrence[15]. Both a synbiotic and a prebiotic alone reduced ammonia and endotoxin levels, decreased the fecal *Escherichia coli* abundance, and reversed MHE; however, the synbiotic did not show better efficacy than the prebiotic alone[76]. Compared with prebiotics and probiotics used alone, the clinical benefits of synbiotics have yet to be demonstrated.

Fecal microbiota transplantation

FMT refers to the process of transferring fecal bacteria from healthy donors to patients with gut microbiota dysbiosis[78]. FMT is an effective therapy for *Clostridioides difficile* infection and inflammatory bowel diseases[78,79]. In germ-free mice colonized with feces from MHE patients, FMT modulated gut microbiota dysbiosis and ameliorated microglial activation and neuroinflammation independent of active liver inflammation[9]. In cirrhotic patients with MHE, oral FMT capsules increased *Ruminococcaceae* and *Bifidobacteriaceae* abundances, decreased *Streptococcaceae* and *Veillonellaceae* abundances, and reduced serum IL-6 and lipopolysaccharide-binding protein[17]. Furthermore, long-term treatment with FMT increased *Burkholderiaceae* abundance and decreased *Acidaminoccocaceae* abundance, which prevented HE recurrence and improved cognitive function during the follow-up[80]. The role of FMT in preventing OHE recurrence by modulating gut microbiota dysbiosis has been demonstrated; however, no clinical trial regarding the FMT for MHE treatment has been reported so far. It could be presumed that FMT is a potential and effective microbiome therapy for MHE; this would require rigorous clinical trials for verification.

Clinical studies of FMT for MHE have used different routes, doses, and dosing times. Owing to these differences, uniform criteria for selecting ideal FMT donors are lacking, and the optimal FMT dosing

regimen remains unclear. Moreover, the identification of pathogens in FMT donors is difficult. FMT is associated with Shigatoxin-producing *Escherichia coli*, extended-spectrum-β-lactamase-producing *Escherichia coli*, and enteropathogenic *Escherichia coli* infections due to the lack of donor screening[81,82]. Patients with liver cirrhosis are vulnerable to infection because of their weakened immune systems; therefore, rigorous screening and selection of FMT donors would improve FMT safety for patients with MHE.

Challenges and controversies

One major challenge of microbiome therapies for MHE is that host factors, dietary habits, and long-term medications may influence the gut microbiome of MHE. *Ruminococcus gnavus* and *Streptococcus salivarius* were the predictors of response to rifaximin treatment, and a higher abundance of *Bacteroidetes*, *Firmicutes*, *Actinobacteria,* and *Proteobacteria* could predict poor response to lactulose treatment[14,83]. Moreover, patients with MHE caused by non-alcoholic cirrhosis responded better to the treatment with rifaximin plus probiotics because they presented a significant decrease in ammonia-producing bacteria genera, such as *Clostridium* and *Streptococcus*[84]. Before the microbiome therapies, the baseline signatures of gut microbiota are identified to match the appropriate microbiome therapies, and gut microbiota biomarkers are explored to predict the therapeutic effects. Based on the different baseline compositions of gut microbiota, targeted and personalized microbiome therapies might be potentially effective strategies for MHE treatment.

Furthermore, cirrhosis is a chronic liver disease, and gut microbiota dysbiosis caused by liver cirrhosis may persist for a long time and require maintenance treatment. However, most microbiome therapeutics are currently single and short-term therapies. After probiotic yogurt supplementation for more than 2 mo, patients with liver cirrhosis had significant MHE reversal rates and excellent compliance; moreover, the potential for long-term compliance existed[85]. Additionally, MHE amelioration with rifaximin treatment for more than 3 mo lasted after the end of treatment, thus indicating a long-term effect on the metabolic function of the gut microbiota[72]. The efficacy and safety of long-term microbiome therapies for MHE require multicenter studies with large populations.

Another related challenge is that rifaximin, a validated antibiotic for MHE, potentially increases antibiotic resistance in liver cirrhosis. A large European cohort study of patients with liver cirrhosis revealed a significant increase in the prevalence of multidrug-resistant bacteria from 29% to 38% during the past decade[86]. Moreover, Chang *et al*[87] reported that rifampin-resistant *staphylococcal* isolates appeared after rifaximin treatment and disappeared during the short term in cirrhotic patients. Prophylactic use of rifaximin did not alter the diversity and composition of gut microbiota or the overall resistance over 12 wk[88]. Rifaximin has not induced significant bacterial resistance and has shown active antimicrobial activity against most bacteria. Multi-drug resistant bacteria should be monitored when using rifaximin in MHE patients, especially in cirrhotic patients previously treated with antibiotics.

CONCLUSION

Gut microbiota dysbiosis initiates the pathophysiological mechanisms of hyperammonemia, systemic inflammation, and endotoxemia, which contribute to neuroinflammation *via* the gut-liver-brain axis in MHE. Currently available strategies for MHE treatment mainly involve the modulation of gut microbiota dysbiosis. In the future, based on the specific microbial signatures identified, personalized and targeted microbiome therapies with optimal regimens and doses may improve the efficacy and safety of MHE treatments.

FOOTNOTES

Author contributions: Luo M and Xin RJ designed the outline, prepared the tables, and drafted the manuscript; Hu FR and Yao L summarized the data and plotted the figure; Hu SJ and Bai FH revised the manuscript. All authors approved the final version of the manuscript.

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REFERENCES

1 **Vilstrup H**, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology* 2014; **60**: 715-735 [PMID: 25042402 DOI: 10.1002/hep.27210]
2 **Patidar KR**, Bajaj JS. Covert and Overt Hepatic Encephalopathy: Diagnosis and Management. *Clin Gastroenterol Hepatol* 2015; **13**: 2048-2061 [PMID: 26164219 DOI: 10.1016/j.cgh.2015.06.039]
3 **Karanfilian BV**, Park T, Senatore F, Rustgi VK. Minimal Hepatic Encephalopathy. *Clin Liver Dis* 2020; **24**: 209-218 [PMID: 32245528 DOI: 10.1016/j.cld.2020.01.012]
4 **Elsaid MI**, Rustgi VK. Epidemiology of Hepatic Encephalopathy. *Clin Liver Dis* 2020; **24**: 157-174 [PMID: 32245524 DOI: 10.1016/j.cld.2020.01.001]
5 **Labenz C**, Baron JS, Toenges G, Schattenberg JM, Nagel M, Sprinzl MF, Nguyen-Tat M, Zimmermann T, Huber Y, Marquardt JU, Galle PR, Wörns MA. Prospective evaluation of the impact of covert hepatic encephalopathy on quality of life and sleep in cirrhotic patients. *Aliment Pharmacol Ther* 2018; **48**: 313-321 [PMID: 29863286 DOI: 10.1111/apt.14824]
6 **Hanai T**, Shiraki M, Nishimura K, Miwa T, Maeda T, Ogiso Y, Imai K, Suetsugu A, Takai K, Shimizu M. Usefulness of the Stroop Test in Diagnosing Minimal Hepatic Encephalopathy and Predicting Overt Hepatic Encephalopathy. *Hepatol Commun* 2021; **5**: 1518-1526 [PMID: 34510827 DOI: 10.1002/hep4.1738]
7 **Butterworth RF**. Hepatic Encephalopathy in Cirrhosis: Pathology and Pathophysiology. *Drugs* 2019; **79**: 17-21 [PMID: 30706423 DOI: 10.1007/s40265-018-1017-0]
8 **Zhang Z**, Zhai H, Geng J, Yu R, Ren H, Fan H, Shi P. Large-scale survey of gut microbiota associated with MHE Via 16S rRNA-based pyrosequencing. *Am J Gastroenterol* 2013; **108**: 1601-1611 [PMID: 23877352 DOI: 10.1038/ajg.2013.221]
9 **Liu R**, Kang JD, Sartor RB, Sikaroodi M, Fagan A, Gavis EA, Zhou H, Hylemon PB, Herzog JW, Li X, Lippman RH, Gonzalez-Maeso J, Wade JB, Ghosh S, Gurley E, Gillevet PM, Bajaj JS. Neuroinflammation in Murine Cirrhosis Is Dependent on the Gut Microbiome and Is Attenuated by Fecal Transplant. *Hepatology* 2020; **71**: 611-626 [PMID: 31220352 DOI: 10.1002/hep.30827]
10 **Kang DJ**, Betrapally NS, Ghosh SA, Sartor RB, Hylemon PB, Gillevet PM, Sanyal AJ, Heuman DM, Carl D, Zhou H, Liu R, Wang X, Yang J, Jiao C, Herzog J, Lippman HR, Sikaroodi M, Brown RR, Bajaj JS. Gut microbiota drive the development of neuroinflammatory response in cirrhosis in mice. *Hepatology* 2016; **64**: 1232-1248 [PMID: 27339732 DOI: 10.1002/hep.28696]
11 **Jain L**, Sharma BC, Srivastava S, Puri SK, Sharma P, Sarin S. Serum endotoxin, inflammatory mediators, and magnetic resonance spectroscopy before and after treatment in patients with minimal hepatic encephalopathy. *J Gastroenterol Hepatol* 2013; **28**: 1187-1193 [PMID: 23425082 DOI: 10.1111/jgh.12160]
12 **Ahluwalia V**, Betrapally NS, Hylemon PB, White MB, Gillevet PM, Unser AB, Fagan A, Daita K, Heuman DM, Zhou H, Sikaroodi M, Bajaj JS. Impaired Gut-Liver-Brain Axis in Patients with Cirrhosis. *Sci Rep* 2016; **6**: 26800 [PMID: 27225869 DOI: 10.1038/srep26800]
13 **Ding JH**, Jin Z, Yang XX, Lou J, Shan WX, Hu YX, Du Q, Liao QS, Xie R, Xu JY. Role of gut microbiota *via* the gut-liver-brain axis in digestive diseases. *World J Gastroenterol* 2020; **26**: 6141-6162 [PMID: 33177790 DOI: 10.3748/wjg.v26.i40.6141]
14 **Wang JY**, Bajaj JS, Wang JB, Shang J, Zhou XM, Guo XL, Zhu X, Meng LN, Jiang HX, Mi YQ, Xu JM, Yang JH, Wang BS, Zhang NP. Lactulose improves cognition, quality of life, and gut microbiota in minimal hepatic encephalopathy: A multicenter, randomized controlled trial. *J Dig Dis* 2019; **20**: 547-556 [PMID: 31448533 DOI: 10.1111/1751-2980.12816]
15 **Viramontes Hörner D**, Avery A, Stow R. The Effects of Probiotics and Symbiotics on Risk Factors for Hepatic Encephalopathy: A Systematic Review. *J Clin Gastroenterol* 2017; **51**: 312-323 [PMID: 28059938 DOI: 10.1097/MCG.0000000000000789]
16 **Schulz C**, Schütte K, Kropf S, Schmitt FC, Vasapolli R, Kliegis LM, Riegger A, Malfertheiner P. RiMINI - the influence of rifaximin on minimal hepatic encephalopathy (MHE) and on the intestinal microbiome in patients with liver cirrhosis: study protocol for a randomized controlled trial. *Trials* 2016; **17**: 111 [PMID: 26926775 DOI: 10.1186/s13063-016-1205-8]
17 **Bajaj JS**, Salzman NH, Acharya C, Sterling RK, White MB, Gavis EA, Fagan A, Hayward M, Holtz ML, Matherly S, Lee H, Osman M, Siddiqui MS, Fuchs M, Puri P, Sikaroodi M, Gillevet PM. Fecal Microbial Transplant Capsules Are Safe in Hepatic Encephalopathy: A Phase 1, Randomized, Placebo-Controlled Trial. *Hepatology* 2019; **70**: 1690-1703 [PMID: 31038755 DOI: 10.1002/hep.30690]

18 **Bloom PP**, Tapper EB, Young VB, Lok AS. Microbiome therapeutics for hepatic encephalopathy. *J Hepatol* 2021; **75**: 1452-1464 [PMID: 34453966 DOI: 10.1016/j.jhep.2021.08.004]

19 **Liu J**, Xu Y, Jiang B. Novel Insights Into Pathogenesis and Therapeutic Strategies of Hepatic Encephalopathy, From the Gut Microbiota Perspective. *Front Cell Infect Microbiol* 2021; **11**: 586427 [PMID: 33692964 DOI: 10.3389/fcimb.2021.586427]

20 **Fallahzadeh MA**, Rahimi RS. Hepatic Encephalopathy: Current and Emerging Treatment Modalities. *Clin Gastroenterol Hepatol* 2022; **20**: S9-S19 [PMID: 35940731 DOI: 10.1016/j.cgh.2022.04.034]

21 **Shah A**, Shanahan E, Macdonald GA, Fletcher L, Ghasemi P, Morrison M, Jones M, Holtmann G. Systematic Review and Meta-Analysis: Prevalence of Small Intestinal Bacterial Overgrowth in Chronic Liver Disease. *Semin Liver Dis* 2017; **37**: 388-400 [PMID: 29272899 DOI: 10.1055/s-0037-1608832]

22 **Ghosh G**, Jesudian AB. Small Intestinal Bacterial Overgrowth in Patients With Cirrhosis. *J Clin Exp Hepatol* 2019; **9**: 257-267 [PMID: 31024208 DOI: 10.1016/j.jceh.2018.08.006]

23 **Qin N**, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014; **513**: 59-64 [PMID: 25079328 DOI: 10.1038/nature13568]

24 **Gupta A**, Dhiman RK, Kumari S, Rana S, Agarwal R, Duseja A, Chawla Y. Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. *J Hepatol* 2010; **53**: 849-855 [PMID: 20675008 DOI: 10.1016/j.jhep.2010.05.017]

25 **Bajaj JS**, Torre A, Rojas ML, Fagan A, Nandez IE, Gavis EA, De Leon Osorio O, White MB, Fuchs M, Sikaroodi M, Gillevet PM. Cognition and hospitalizations are linked with salivary and faecal microbiota in cirrhosis cohorts from the USA and Mexico. *Liver Int* 2020; **40**: 1395-1407 [PMID: 32181561 DOI: 10.1111/liv.14437]

26 **Bajaj JS**, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, Monteith P, Noble NA, Sikaroodi M, Gillevet PM. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G675-G685 [PMID: 22821944 DOI: 10.1152/ajpgi.00152.2012]

27 **Bajaj JS**, Shamsaddini A, Fagan A, McGeorge S, Gavis E, Sikaroodi M, Brenner LA, Wade JB, Gillevet PM. Distinct gut microbial compositional and functional changes associated with impaired inhibitory control in patients with cirrhosis. *Gut Microbes* 2021; **13**: 1953247 [PMID: 34346283 DOI: 10.1080/19490976.2021.1953247]

28 **Bajaj JS**, Fagan A, White MB, Wade JB, Hylemon PB, Heuman DM, Fuchs M, John BV, Acharya C, Sikaroodi M, Gillevet PM. Specific Gut and Salivary Microbiota Patterns Are Linked With Different Cognitive Testing Strategies in Minimal Hepatic Encephalopathy. *Am J Gastroenterol* 2019; **114**: 1080-1090 [PMID: 30816877 DOI: 10.14309/ajg.0000000000000102]

29 **Luo M**, Hu FR, Xin RJ, Yao L, Hu SJ, Bai FH. Altered gut microbiota is associated with sleep disturbances in patients with minimal hepatic encephalopathy caused by hepatitis B-related liver cirrhosis. *Expert Rev Gastroenterol Hepatol* 2022; **16**: 797-807 [PMID: 35942803 DOI: 10.1080/17474124.2022.2111300]

30 **Bajaj JS**, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G168-G175 [PMID: 21940902 DOI: 10.1152/ajpgi.00190.2011]

31 **Scaglione S**, Kliethermes S, Cao G, Shoham D, Durazo R, Luke A, Volk ML. The Epidemiology of Cirrhosis in the United States: A Population-based Study. *J Clin Gastroenterol* 2015; **49**: 690-696 [PMID: 25291348 DOI: 10.1097/MCG.0000000000000208]

32 **Xu XY**, Ding HG, Li WG, Xu JH, Han Y, Jia JD, Wei L, Duan ZP, Ling-Hu EQ, Zhuang H. Chinese guidelines on the management of liver cirrhosis (abbreviated version). *World J Gastroenterol* 2020; **26**: 7088-7103 [PMID: 33362370 DOI: 10.3748/wjg.v26.i45.7088]

33 **Donaldson GP**, Ladinsky MS, Yu KB, Sanders JG, Yoo BB, Chou WC, Conner ME, Earl AM, Knight R, Bjorkman PJ, Mazmanian SK. Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 2018; **360**: 795-800 [PMID: 29724905 DOI: 10.1126/science.aaq0926]

34 **Wahlström A**, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab* 2016; **24**: 41-50 [PMID: 27320064 DOI: 10.1016/j.cmet.2016.05.005]

35 **Zong X**, Fu J, Xu B, Wang Y, Jin M. Interplay between gut microbiota and antimicrobial peptides. *Anim Nutr* 2020; **6**: 389-396 [PMID: 33364454 DOI: 10.1016/j.aninu.2020.09.002]

36 **Alaish SM**, Smith AD, Timmons J, Greenspon J, Eyvazzadeh D, Murphy E, Shea-Donahue T, Cirimotich S, Mongodin E, Zhao A, Fasano A, Nataro JP, Cross A. Gut microbiota, tight junction protein expression, intestinal resistance, bacterial translocation and mortality following cholestasis depend on the genetic background of the host. *Gut Microbes* 2013; **4**: 292-305 [PMID: 23652772 DOI: 10.4161/gmic.24706]

37 **Nakamoto N**, Kanai T. Role of toll-like receptors in immune activation and tolerance in the liver. *Front Immunol* 2014; **5**: 221 [PMID: 24904576 DOI: 10.3389/fimmu.2014.00221]

38 **Trivedi PJ**, Adams DH. Gut-liver immunity. *J Hepatol* 2016; **64**: 1187-1189 [PMID: 26686270 DOI: 10.1016/j.jhep.2015.12.002]

39 **Mangas-Losada A**, García-García R, Urios A, Escudero-García D, Tosca J, Giner-Durán R, Serra MA, Montoliu C, Felipo V. Minimal hepatic encephalopathy is associated with expansion and activation of CD(4+)CD28(-), Th22 and Tfh and B lymphocytes. *Sci Rep* 2017; **7**: 6683 [PMID: 28751644 DOI: 10.1038/s41598-017-05938-1]

40 **Shawcross DL**, Wright G, Olde Damink SW, Jalan R. Role of ammonia and inflammation in minimal hepatic encephalopathy. *Metab Brain Dis* 2007; **22**: 125-138 [PMID: 17260161 DOI: 10.1007/s11011-006-9042-1]

41 **Lukiw WJ**. Gastrointestinal (GI) Tract Microbiome-Derived Neurotoxins-Potent Neuro-Inflammatory Signals From the GI Tract *via* the Systemic Circulation Into the Brain. *Front Cell Infect Microbiol* 2020; **10**: 22 [PMID: 32117799 DOI: 10.3389/fcimb.2020.00022]

42 **Galea I**. The blood-brain barrier in systemic infection and inflammation. *Cell Mol Immunol* 2021; **18**: 2489-2501 [PMID: 34594000 DOI: 10.1038/s41423-021-00757-x]

43 **Butterworth RF**. The liver-brain axis in liver failure: neuroinflammation and encephalopathy. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 522-528 [PMID: 23817325 DOI: 10.1038/nrgastro.2013.99]

44 **Balzano T**, Dadsetan S, Forteza J, Cabrera-Pastor A, Taoro-Gonzalez L, Malaguarnera M, Gil-Perotin S, Cubas-Nuñez L, Casanova B, Castro-Quintas A, Ponce-Mora A, Arenas YM, Leone P, Erceg S, Llansola M, Felipo V. Chronic hyperammonemia induces peripheral inflammation that leads to cognitive impairment in rats: Reversed by anti-TNF-α treatment. *J Hepatol* 2020; **73**: 582-592 [PMID: 30654069 DOI: 10.1016/j.jhep.2019.01.008]

45 **Zemtsova I**, Görg B, Keitel V, Bidmon HJ, Schrör K, Häussinger D. Microglia activation in hepatic encephalopathy in rats and humans. *Hepatology* 2011; **54**: 204-215 [PMID: 21452284 DOI: 10.1002/hep.24326]

46 **Görg B**, Bidmon HJ, Häussinger D. Gene expression profiling in the cerebral cortex of patients with cirrhosis with and without hepatic encephalopathy. *Hepatology* 2013; **57**: 2436-2447 [PMID: 23325665 DOI: 10.1002/hep.26265]

47 **Zhang C**, Li Y, Lu J, Yang X, Wang J, Qiang J. MR T1 mapping for quantifying brain manganese deposition in type C hepatic encephalopathy rats. *Biometals* 2021; **34**: 841-854 [PMID: 33928475 DOI: 10.1007/s10534-021-00311-2]

48 **Yang X**, Liu W, Dang P, Wang Y, Ge X, Huang X, Wang M, Zheng J, Ding X, Wang X. Decreased brain noradrenaline in minimal hepatic encephalopathy is associated with cognitive impairment in rats. *Brain Res* 2022; **1793**: 148041 [PMID: 35944657 DOI: 10.1016/j.brainres.2022.148041]

49 **Collins CM**, D'Orazio SE. Bacterial ureases: structure, regulation of expression and role in pathogenesis. *Mol Microbiol* 1993; **9**: 907-913 [PMID: 7934918 DOI: 10.1111/j.1365-2958.1993.tb01220.x]

50 **Chen YY**, Weaver CA, Burne RA. Dual functions of Streptococcus salivarius urease. *J Bacteriol* 2000; **182**: 4667-4669 [PMID: 10913107 DOI: 10.1128/JB.182.16.4667-4669.2000]

51 **Liotta EM**, Kimberly WT. Cerebral edema and liver disease: Classic perspectives and contemporary hypotheses on mechanism. *Neurosci Lett* 2020; **721**: 134818 [PMID: 32035166 DOI: 10.1016/j.neulet.2020.134818]

52 **Jayakumar AR**, Rao KV, Murthy ChR, Norenberg MD. Glutamine in the mechanism of ammonia-induced astrocyte swelling. *Neurochem Int* 2006; **48**: 623-628 [PMID: 16517020 DOI: 10.1016/j.neuint.2005.11.017]

53 **Rodrigo R**, Cauli O, Gomez-Pinedo U, Agusti A, Hernandez-Rabaza V, Garcia-Verdugo JM, Felipo V. Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. *Gastroenterology* 2010; **139**: 675-684 [PMID: 20303348 DOI: 10.1053/j.gastro.2010.03.040]

54 **Hernández-Rabaza V**, Cabrera-Pastor A, Taoro-González L, Malaguarnera M, Agustí A, Llansola M, Felipo V. Hyperammonemia induces glial activation, neuroinflammation and alters neurotransmitter receptors in hippocampus, impairing spatial learning: reversal by sulforaphane. *J Neuroinflammation* 2016; **13**: 41 [PMID: 26883214 DOI: 10.1186/s12974-016-0505-y]

55 **Deng YD**, Peng XB, Zhao RR, Ma CQ, Li JN, Yao LQ. The intestinal microbial community dissimilarity in hepatitis B virus-related liver cirrhosis patients with and without at alcohol consumption. *Gut Pathog* 2019; **11**: 58 [PMID: 31788031 DOI: 10.1186/s13099-019-0337-2]

56 **Bajaj JS**, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, Fuchs M, Ridlon JM, Daita K, Monteith P, Noble NA, White MB, Fisher A, Sikaroodi M, Rangwala H, Gillevet PM. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013; **8**: e60042 [PMID: 23565181 DOI: 10.1371/journal.pone.0060042]

57 **Bellot P**, Francés R, Such J. Pathological bacterial translocation in cirrhosis: pathophysiology, diagnosis and clinical implications. *Liver Int* 2013; **33**: 31-39 [PMID: 23121656 DOI: 10.1111/liv.12021]

58 **Chastre A**, Bélanger M, Nguyen BN, Butterworth RF. Lipopolysaccharide precipitates hepatic encephalopathy and increases blood-brain barrier permeability in mice with acute liver failure. *Liver Int* 2014; **34**: 353-361 [PMID: 23910048 DOI: 10.1111/Liv.12252]

59 **Henry CJ**, Huang Y, Wynne AM, Godbout JP. Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. *Brain Behav Immun* 2009; **23**: 309-317 [PMID: 18814846 DOI: 10.1016/j.bbi.2008.09.002]

60 **Tanaka S**, Ide M, Shibutani T, Ohtaki H, Numazawa S, Shioda S, Yoshida T. Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. *J Neurosci Res* 2006; **83**: 557-566 [PMID: 16429444 DOI: 10.1111/liv.12252]

61 **Wright G**, Davies NA, Shawcross DL, Hodges SJ, Zwingmann C, Brooks HF, Mani AR, Harry D, Stadlbauer V, Zou Z, Williams R, Davies C, Moore KP, Jalan R. Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* 2007; **45**: 1517-1526 [PMID: 17523148 DOI: 10.1002/hep.21599]

62 **Moratalla A**, Ampuero J, Bellot P, Gallego-Durán R, Zapater P, Roger M, Figueruela B, Martínez-Moreno B, González-Navajas JM, Such J, Romero-Gómez M, Francés R. Lactulose reduces bacterial DNA translocation, which worsens neurocognitive shape in cirrhotic patients with minimal hepatic encephalopathy. *Liver Int* 2017; **37**: 212-223 [PMID: 27388776 DOI: 10.1111/liv.13200]

63 **Yuan Q**, Xin L, Han S, Su Y, Wu R, Liu X, Wuri J, Li R, Yan T. Lactulose Improves Neurological Outcomes by Repressing Harmful Bacteria and Regulating Inflammatory Reactions in Mice After Stroke. *Front Cell Infect Microbiol* 2021; **11**: 644448 [PMID: 34327147 DOI: 10.3389/fcimb.2021.644448]

64 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014; **60**: 940-947 [PMID: 24374295 DOI: 10.1016/j.jhep.2013.12.019]

65 **Sarangi AN**, Goel A, Singh A, Sasi A, Aggarwal R. Faecal bacterial microbiota in patients with cirrhosis and the effect of lactulose administration. *BMC Gastroenterol* 2017; **17**: 125 [PMID: 29179682 DOI: 10.1186/s12876-017-0683-9]

66 **Scarpignato C**, Pelosini I. Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. *Chemotherapy* 2005; **51** Suppl 1: 36-66 [PMID: 15855748 DOI: 10.1159/000081990]

67 **Patel VC**, Lee S, McPhail MJW, Da Silva K, Guilly S, Zamalloa A, Witherden E, Støy S, Manakkat Vijay GK, Pons N, Galleron N, Huang X, Gencer S, Coen M, Tranah TH, Wendon JA, Bruce KD, Le Chatelier E, Ehrlich SD, Edwards LA, Shoaie S, Shawcross DL. Rifaximin-α reduces gut-derived inflammation and mucin degradation in cirrhosis and

encephalopathy: RIFSYS randomised controlled trial. *J Hepatol* 2022; **76**: 332-342 [PMID: 34571050 DOI: 10.1016/j.jhep.2021.09.010]

68 **Gatta L**, Scarpignato C. Systematic review with meta-analysis: rifaximin is effective and safe for the treatment of small intestine bacterial overgrowth. *Aliment Pharmacol Ther* 2017; **45**: 604-616 [PMID: 28078798 DOI: 10.1111/apt.13928]

69 **Kang DJ**, Kakiyama G, Betrapally NS, Herzog J, Nittono H, Hylemon PB, Zhou H, Carroll I, Yang J, Gillevet PM, Jiao C, Takei H, Pandak WM, Iida T, Heuman DM, Fan S, Fiehn O, Kurosawa T, Sikaroodi M, Sartor RB, Bajaj JS. Rifaximin Exerts Beneficial Effects Independent of its Ability to Alter Microbiota Composition. *Clin Transl Gastroenterol* 2016; **7**: e187 [PMID: 27560928 DOI: 10.1038/ctg.2016.44]

70 **Kaji K**, Takaya H, Saikawa S, Furukawa M, Sato S, Kawaratani H, Kitade M, Moriya K, Namisaki T, Akahane T, Mitoro A, Yoshiji H. Rifaximin ameliorates hepatic encephalopathy and endotoxemia without affecting the gut microbiome diversity. *World J Gastroenterol* 2017; **23**: 8355-8366 [PMID: 29307995 DOI: 10.3748/wjg.v23.i47.8355]

71 **Kaji K**, Saikawa S, Takaya H, Fujinaga Y, Furukawa M, Kitagawa K, Ozutsumi T, Kaya D, Tsuji Y, Sawada Y, Kawaratani H, Moriya K, Namisaki T, Akahane T, Mitoro A, Yoshiji H. Rifaximin Alleviates Endotoxemia with Decreased Serum Levels of Soluble CD163 and Mannose Receptor and Partial Modification of Gut Microbiota in Cirrhotic Patients. *Antibiotics (Basel)* 2020; **9** [PMID: 32235367 DOI: 10.3390/antibiotics9040145]

72 **Schulz C**, Schütte K, Vilchez-Vargas R, Vasapolli R, Malfertheiner P. Long-Term Effect of Rifaximin with and without Lactulose on the Active Bacterial Assemblages in the Proximal Small Bowel and Faeces in Patients with Minimal Hepatic Encephalopathy. *Dig Dis* 2019; **37**: 161-169 [PMID: 30428474 DOI: 10.1159/000494216]

73 **Xia X**, Chen J, Xia J, Wang B, Liu H, Yang L, Wang Y, Ling Z. Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis. *J Int Med Res* 2018; **46**: 3596-3604 [PMID: 29806520 DOI: 10.1177/0300060518776064]

74 **Bajaj JS**, Heuman DM, Hylemon PB, Sanyal AJ, Puri P, Sterling RK, Luketic V, Stravitz RT, Siddiqui MS, Fuchs M, Thacker LR, Wade JB, Daita K, Sistrun S, White MB, Noble NA, Thorpe C, Kakiyama G, Pandak WM, Sikaroodi M, Gillevet PM. Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. *Aliment Pharmacol Ther* 2014; **39**: 1113-1125 [PMID: 24628464 DOI: 10.1111/apt.12695]

75 **Manzhalii E**, Moyseyenko V, Kondratiuk V, Molochek N, Falalyeyeva T, Kobyliak N. Effect of a specific Escherichia coli Nissle 1917 strain on minimal/mild hepatic encephalopathy treatment. *World J Hepatol* 2022; **14**: 634-646 [PMID: 35582294 DOI: 10.4254/wjh.v14.i3.634]

76 **Liu Q**, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441-1449 [PMID: 15122774 DOI: 10.1002/hep.20194]

77 **Malaguarnera M**, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Dig Dis Sci* 2007; **52**: 3259-3265 [PMID: 17393330 DOI: 10.1007/s10620-006-9687-y]

78 **Weingarden AR**, Vaughn BP. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. *Gut Microbes* 2017; **8**: 238-252 [PMID: 28609251 DOI: 10.1080/19490976.2017.1290757]

79 **Adelman MW**, Woodworth MH, Shaffer VO, Martin GS, Kraft CS. Critical Care Management of the Patient with Clostridioides difficile. *Crit Care Med* 2021; **49**: 127-139 [PMID: 33156122 DOI: 10.1097/CCM.0000000000004739]

80 **Bajaj JS**, Fagan A, Gavis EA, Kassam Z, Sikaroodi M, Gillevet PM. Long-term Outcomes of Fecal Microbiota Transplantation in Patients With Cirrhosis. *Gastroenterology* 2019; **156**: 1921-1923.e3 [PMID: 30664879 DOI: 10.1053/j.gastro.2019.01.033]

81 **Zellmer C**, Sater MRA, Huntley MH, Osman M, Olesen SW, Ramakrishna B. Shiga Toxin-Producing Escherichia coli Transmission *via* Fecal Microbiota Transplant. *Clin Infect Dis* 2021; **72**: e876-e880 [PMID: 33159210 DOI: 10.1093/cid/ciaa1486]

82 **DeFilipp Z**, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, Turbett S, Chung RT, Chen YB, Hohmann EL. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019; **381**: 2043-2050 [PMID: 31665575 DOI: 10.1056/NEJMoa1910437]

83 **Yukawa-Muto Y**, Kamiya T, Fujii H, Mori H, Toyoda A, Sato I, Konishi Y, Hirayama A, Hara E, Fukuda S, Kawada N, Ohtani N. Distinct responsiveness to rifaximin in patients with hepatic encephalopathy depends on functional gut microbial species. *Hepatol Commun* 2022; **6**: 2090-2104 [PMID: 35429147 DOI: 10.1002/hep4.1954]

84 **Zuo Z**, Fan H, Tang XD, Chen YM, Xun LT, Li Y, Song ZJ, Zhai HQ. Effect of different treatments and alcohol addiction on gut microbiota in minimal hepatic encephalopathy patients. *Exp Ther Med* 2017; **14**: 4887-4895 [PMID: 29201193 DOI: 10.3892/etm.2017.5141]

85 **Bajaj JS**, Saeian K, Christensen KM, Hafeezullah M, Varma RR, Franco J, Pleuss JA, Krakower G, Hoffmann RG, Binion DG. Probiotic yogurt for the treatment of minimal hepatic encephalopathy. *Am J Gastroenterol* 2008; **103**: 1707-1715 [PMID: 18691193 DOI: 10.1111/j.1572-0241.2008.01861.x]

86 **Fernández J**, Prado V, Trebicka J, Amoros A, Gustot T, Wiest R, Deulofeu C, Garcia E, Acevedo J, Fuhrmann V, Durand F, Sánchez C, Papp M, Caraceni P, Vargas V, Bañares R, Piano S, Janicko M, Albillos A, Alessandria C, Soriano G, Welzel TM, Laleman W, Gerbes A, De Gottardi A, Merli M, Coenraad M, Saliba F, Pavesi M, Jalan R, Ginès P, Angeli P, Arroyo V; European Foundation for the Study of Chronic Liver Failure (EF-Clif). Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe. *J Hepatol* 2019; **70**: 398-411 [PMID: 30391380 DOI: 10.1016/j.jhep.2018.10.027]

87 **Chang JY**, Kim SE, Kim TH, Woo SY, Ryu MS, Joo YH, Lee KE, Lee J, Lee KH, Moon CM, Jung HK, Shim KN, Jung SA. Emergence of rifampin-resistant staphylococci after rifaximin administration in cirrhotic patients. *PLoS One* 2017; **12**: e0186120 [PMID: 28982166 DOI: 10.1371/journal.pone.0186120]

88 **Yu X**, Jin Y, Zhou W, Xiao T, Wu Z, Su J, Gao H, Shen P, Zheng B, Luo Q, Li L, Xiao Y. Rifaximin Modulates the Gut Microbiota to Prevent Hepatic Encephalopathy in Liver Cirrhosis Without Impacting the Resistome. *Front Cell Infect Microbiol* 2021; **11**: 761192 [PMID: 35118004 DOI: 10.3389/fcimb.2021.761192]

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Abstract

The role of endoscopic ultrasound (EUS) in the last two decades has shifted from a diagnostic tool to an important therapeutic tool treating mainly pancreato-biliary disorders. In recent years, its applications for treating pancreatic diseases have broadened, including the implementation of radiofrequency ablation (RFA), which has been traditionally used for treating solid tumors. In this critical in-depth review, we summarized all the papers throughout the literature regarding EUS-RFA for pancreatic neuroendocrine neoplasms, adenocarcinoma, and pancreatic cystic lesions. Overall, for pancreatic neuroendocrine neoplasms we identified 16 papers that reported 96 patients who underwent EUS-RFA, with acceptable adverse events that were rated mild to moderate and a high complete radiological resolution rate of 90%. For pancreatic adenocarcinoma, we identified 8 papers with 121 patients. Adverse events occurred in 13% of patients, mostly rated mild. However, no clear survival benefit was demonstrated. For pancreatic cystic lesions, we identified 4 papers with 38 patients. The adverse events were mostly mild and occurred in 9.1% of patients, and complete or partial radiological resolution of the cysts was reported in 36.8%. Notably, the procedure was technically feasible for most of the patients. Nevertheless, a long road remains before this technique finds its definite place in guidelines due to several controversies. EUS-RFA for pancreatic tumors seems to be safe and effective, especially for pancreatic neuroendocrine neoplasms, but multicenter prospective trials are needed to consider this treatment as a gold standard.

Key Words: Endoscopic ultrasound; Radiofrequency ablation; Efficacy; Safety; Pancreas; Tumors

Core Tip: Endoscopic ultrasound guided radiofrequency ablation has been increasingly implemented in the treatment of pancreatic neoplasms. We reviewed the role of endoscopic ultrasound guided radiofrequency ablation in the treatment of pancreatic neuroendocrine tumors, unresectable pancreatic adenocarcinoma, and pancreatic cystic lesions, focusing on efficacy, safety, and controversies. We found that endoscopic ultrasound guided radiofrequency ablation was feasible with an excellent technical success, acceptable adverse events, and a beneficial effect for pancreatic neuroendocrine tumors, mainly on insulinoma. While its effect on pancreatic adenocarcinoma and cystic lesions is promising, more studies are needed to better explore its role.

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INTRODUCTION

In the recent years, endoscopic ultrasound (EUS) transformed from a diagnostic tool to an important therapeutic tool, especially for pancreato-biliary diseases[1]. Among the therapeutic options is radiofrequency ablation (RFA). RFA is a low-risk minimally invasive procedure that delivers heat waves (in the range of 350-500 kHz), with a high temperature ranging between 60 °C-100 °C that subsequently causes burning of the tumorous tissue. The effect is mediated *via* coagulation necrosis, leading to irreversible cellular damage and apoptosis, without significantly affecting the normal surrounding tissue[2]. Safety and effectiveness of EUS-RFA of the pancreatic tissue were evaluated in porcine models, which showed beneficial effects[3-6]. Moreover, RFA should have an anti-cancer effect induced by immunomodulatory activity[7]. RFA was shown previously to be a feasible and safe ablative therapeutic option for liver tumors[8]. With the invention of dedicated needles, RFA has recently been used more under EUS for the treatment of pancreatic neuroendocrine neoplasms (pNENs), pancreatic adenocarcinoma or pancreatic cystic lesions (PCL). However, the data in this field is still emerging. In this current review, we provided a critical in-depth overview of the most updated data of EUS-RFA for pancreatic tumors with a focus on safety, efficacy and controversies.

Literature search

A search for studies published before September 2022 was performed in the PubMed databases with the keywords EUS or endoscopic ultrasound with radiofrequency ablation and any of the following: Pancreatic neuroendocrine tumor or neoplasm, pancreatic functional neuroendocrine tumor or neoplasm, pancreatic non-functional neuroendocrine tumor or neoplasm, insulinoma, carcinoma or adenocarcinoma of pancreas, pancreatic tumor or neoplasm, pancreatic cystic lesions or neoplasms, pancreatic cysts, cysts of the pancreas, mucinous pancreatic cysts, pancreatic serous cystadenoma, intraductal papillary mucinous neoplasm, mucinous cyst, treatment or therapeutic and intervention. The search was restricted to articles in the English language and included prospective, retrospective, randomized controlled studies, case series and case reports. Moreover, the bibliographic section of the selected articles as well as the systematic and narrative articles on the topic were manually searched for further relevant articles. Review articles, presented abstracts and posters, position papers and guidelines were not included. Subsequently, we reviewed and summarized all the data on EUS-guided intervention for solid and cystic pancreatic neoplasms focusing on technical success, safety and efficacy.

Study definitions

Technical success was defined by the successful completion of the procedure (introduction of the RFA probe through EUS channel and induction of thermal current to the pancreatic lesions). Safety was defined by any adverse event (AE) that appeared during the procedure or after and that should be secondary to the EUS-RFA. Efficacy was defined as the complete or partial radiological resolution of the pancreatic tumor. Complete response was defined by total destruction of the lesion, while partial response was defined by 75%-90% destruction of the lesion. The longest follow-up period was used to report efficacy in studies that reported more than one follow-up time point. Pooled data for AE was calculated by the overall number of AE divided by the total number of EUS-RFA sessions. Procedure related AE was defined according to the American Society of Gastrointestinal Endoscopy classification [9] as follow: (1) Mild AE: Post-procedure medical consultation, unplanned hospitalization or hospital

stay prolongation for less than 3 nights; (2) Moderate AE: Unplanned anesthesia, unplanned hospitalization or hospital stay prolongation for 4-10 nights, admission to intensive care unit for 1 night, blood transfusion, interventional radiology or endoscopic treatment for AE secondary to the procedure; and (3) Severe AE: Unplanned admission or hospital stay prolongation for > 10 nights, intensive care stay for > 1 night, surgery needed for an AE related to the procedure, permanent disability and death related to the procedure[9]. The more recent AGREE classification was not used in these trials, and it was not possible retrospectively to find the data that would have been necessary to grade the AE. Pooled radiological response was calculated by the overall number of complete or partial radiological response divided by the total number of patients included. In cases where RFA session numbers were not provided by the original manuscript, we consider it the same as the number of patients included in the study[10-20].

EUS-RFA IN PNENS

To date, most of the studies assessed the role of RFA in the treatment of pNENs, in the form of case reports and case series. The first study was reported by Rossi *et al*[10] on 10 patients with pNENs. RFA was performed by the EUS route in 1 patient with non-functional pNEN. Lakhtakia *et al*[12] reported the first case series of 3 patients with functional pNENs (insulinoma), with rapid hypoglycemia relief in the same day. Similarly, Waung *et al*[21] and Bas-Cutrina *et al*[22] reported 2 successful cases of EUS-RFA for insulinoma. Pai *et al*[23] reported a study including 8 patients with complete resolution at 3-6 mo post-treatment and no procedure-related AEs.

Barthet *et al*[13] reported the first multicenter prospective study including 12 patients with 14 pNENs who underwent RFA. Two patients developed complications (16.7%), including acute pancreatitis with early infected necrosis and main pancreatic duct stenosis. Notably, the patient who developed infected necrosis had a cystic pNEN, and the cystic component was not aspirated before performing the RFA. Therefore, this AE was presumed to be secondary to the lack of cystic component suction. After this AE, the independent safety committee decided to administer antibioprophylaxis (2 g of intravenous amoxicillin and clavulanic acid intravenously) and to aspirate the main part of the fluid content prior to RFA in cystic pNEN in order to avoid excessive application of radiofrequency current into the liquid component[13]. The long-term follow-up data of the study by Barthet *et al*[24] was published recently; among the 12 patients with the 14 pNENs lesions, there was a complete disappearance in 12 pNENs lesions (85.7%) and 2 failures (14.3%) at a mean follow-up of 45.6 mo. The two failures were pNEN recurrence after disappearance at 1 year and a metastatic evolution at 3 years follow-up in a patient that had a persistence of the initial pancreatic tumor after two RFA sessions.

Another study by Choi *et al*[25] reported 8 patients with pNENs. Notably, the proliferative index Ki67% was reported in only 2 patients (1 patient with G1 and one patient with G2). Similarly, a prospective study by de Nucci *et al*[26] reported a complete radiological resolution rate at 6 and 12 mo following treatment among 10 patients with 11 pNEN lesions of G1 grading (< 5%), with only 2 mild AEs. Oleinikov *et al*[14] reported a retrospective study that included 18 patients with pNENs. Two patients with non-functional pNEN and one patient with insulinoma had multiple endocrine neoplasia syndrome type 1. Most of the lesions were G1 grading. Complete relief of hypoglycemia-related symptoms was achieved in the 7 patients with insulinoma within 1 h following the EUS-RFA[14].

Furthermore, recent case reports and prospective case series were published in patients with insulinoma who underwent EUS-RFA, with a complete clinical resolution of the hypoglycemic symptoms up to 1 d after the EUS-RFA and complete radiological resolution[27-29], with 1 case of acute necrotizing pancreatitis[28] and 2 cases of mild pancreatitis occurring 1 d after the procedure and 3 mo after[29]. Rossi *et al*[30] treated 3 elderly patients with insulinomas with one intraprocedural bleeding treated endoscopically.

Additionally, Marx *et al*[31] reported two recent trials. The first study included 7 patients with insulinoma, with a complete resolution rate reported in 6 patients (85.7%). Clinical success in terms of symptom relief was achieved immediately in all patients (100%). Notably, this study was associated with a safety signal, as 3 patients had mild to moderate AEs and 1 patient (aged 97 years) had a severe AE with retrogastric collection. He refused drainage, was symptomatically treated and died 2 wk later [15]. The second study retrospectively reported 27 patients with G1 non-functional pNENs. Nine out of the 27 lesions (33.3%) were cystic. Twenty-five patients (92.6%) had a complete radiological resolution at a mean follow-up time of 15.7 mo (range 2-41). Notably, procedure-related AEs occurred in 9 patients [31] (Table 1).

Pooling the available data, EUS-RFA was performed on 100 patients with 112 pNEN lesions that underwent 114 EUS-RFA sessions. Most of the data were published as case reports and small case series. The mean lesion size was 14.8 mm, ranging mostly from 10-20 mm. The procedure was technically feasible in all patients, and the AE rate was almost 21.9%, occurring in 25 of the 114 EUS-RFA sessions. Notably, most of the AE were mild and moderate according to the American Society of Gastrointestinal Endoscopy guideline[9] except for one fatal AE in a recent paper published by Marx *et al*[15]. Interestingly, the complete radiological resolution rate was approximately 90% during a follow-up

Table 1 Studies reporting endoscopic ultrasound-radiofrequency ablation for pancreatic neuroendocrine neoplasms

Ref.	Type of study (pNEN type)	Patients/lesions/RFA sessions, *n*	Location, *n*	Mean size (range) in mm	Histological grade (KI67%)	Technical success, *n* (%)	Adverse events, *n* (%)	Complete radiological/ clinical[1] resolution, *n* (%)	Mean follow-up in mo
Rossi *et al* [10]	Case report (nonfunctional)	1/1/1	Head (1)	10	NR	1 (100)	0	1 (100)	34
Armellini *et al*[11]	Case report (nonfunctional)	1/1/1	Tail (1)	20	G2 (> 5)	1 (100)	0	1 (100)	1
Lakhtakia *et al*[12]	Case series (insulinoma)	3/3/3	Body (2), diffuse (1)	17.7 (14-22)	NR	3 (100)	0	3 (100)/3 (100)	4.2
Waung *et al*[21]	Case report (insulinoma)	1/1/3	Uncinate (1)	18	NR	1 (100)	0	1 (100)/1 (100)	10
Bas-Cutrina *et al*[22]	Case report (insulinoma)	1/1/1	Body (1)	10	NR	1 (100)	0	1 (100)/1 (100)	10
Pai *et al* [23]	Prospective (nonfunctional)	2/2/3	Head (1)	27.5 (15-40)	NR	2 (100)	0	2 (100)	6
Barthet *et al*[13]	Prospective (nonfunctional)	12/14/12	Head (3), body (6), tail (5)	13.1 (10-20)	G1	12 (100)	2 (16.7)[2]	9 (75)	12
Choi *et al* [25]	Prospective (nonfunctional-7), (insulinoma-1)	8/8/14	Head (3), body (5)	19.25 (8-28)	Reported in 2 patients (G1 and G2)	8 (100)	2 (14.3)[3]	6 (75)/1 (100)	13
de Nucci *et al*[26]	Prospective (nonfunctional)	10/11/10	Head (3), body (8), tail (2)	14.5 (9-20)	G1 (< 4)	10 (100)	2 (20)[4]	10 (100)	12
Oleinikov *et al*[14]	Retrospective (nonfunctional-11), (insulinoma-7)	18/27/18	Head (10), body (8), tail (2), uncinate (5), metastasis (2)	14.8 (12-19)	G1 (< 5) in 15 patents, G3 (34-40) in 2 patients	18 (100)	2 (11.1)[5]	17 (94.4)/7 (100)	8.7
Rossi *et al* [30]	Case series (insulinoma)	3/3/4	NR	11.5 (9-14)	NR	3 (100)	1 (25)[6]	3 (100)/3 (100)	8.5
Chang *et al*[27]	Case report (insulinoma)	1/1/1	Head (1)	12	NR	1 (100)	0	1 (100)/1 (100)	18
Kluz *et al* [28]	Case report (insulinoma)	1/1/1	Head (1)	9	NR	1 (100)	1 (100)[7]	NR/1 (100)	NR
Furnica *et al*[29]	Case series (insulinoma)	4/4/4	Head (2), neck (1), tail (1)	12.9 (6.5-22.0)	G1 in 3 patients and G2 in 1 patient	4 (100)	2 (50)[8]	4 (100)/4 (100)	22
Marx *et al* [15]	Retrospective (insulinoma)	7/7/7	Head (1), body (1), neck (3), body-tail junction (2)	13.3 (8-20)	G1 (< 3) in 4 patient, G2 (4) in 1 patient	7 (100)	4 (57.1)[9]	6 (85.7)/7 (100)	20.3
Marx *et al* [31]	Retrospective (non-functional)	27/27/31	Head (6), body (3), tail (11), uncinate (2), body-tail junction (5)	14 (7-25)	G1 (< 3) in 25 patients, NR in 2 patients	27 (100)	9 (29)[10]	25 (92.6)	15.7
Pooled data	Case reports: 9. Prospective: 4. Retrospective: 3	100/112/114	Head and neck (33), body (34), tail (22), uncinate (8), metastasis and diffuse (3), junction (7)	14.8	Unable to pool due to data lacking	96 (100)	25 (21.9)	90 (90)/21 (100)	13

[1]Insulinoma.

[2]Infected pancreatic necrosis (1 patient, moderate), pancreatic duct stenosis (1 patient, moderate).
[3]Pancreatitis (1 patient, moderate), transient abdominal pain (1 patient, mild).
[4]Transient abdominal pain (2 patients, mild).
[5]Pancreatitis (2 patients, mild).
[6]Intraprocedural bleeding treated endoscopically (1 patient, moderate).
[7]Acute necrotizing pancreatitis (1 patient, moderate).
[8]Pancreatitis (2 patients, mild).
[9]Transient abdominal pain (1 patient, mild), pancreatitis (1 patient, moderate), coagulation necrosis (1 patient, moderate), retrogastric collection (1 patient, refused drainage, died 2 wk later, severe).
[10]Transient abdominal pain (3 patients, mild), pancreatitis (1 patient, moderate), periprocedural bleeding (2 patients, moderate), pancreatitis complicated by retrogastric or perisplenic collection managed by endoscopic drainage and antibiotic treatment (2 patients, moderate), pancreatitis with pancreatic fistula and paragastric collection drained by endoscopic cystgastrostomy (1 patient, moderate).
pNEN: Pancreatic neuroendocrine neoplasms; NR: Not reported; RFA: Radiofrequency ablation.

period of 13 mo (Table 1).

EUS-RFA IN PANCREATIC ADENOCARCINOMA

Recently, EUS-RFA was increasingly implemented in the treatment of pancreatic adenocarcinoma among patients who were not candidates for surgical resection. The first study was a feasibility study conducted by Arcidiacono *et al*[16] who reported 22 patients with locally advanced pancreatic adenocarcinoma who underwent EUS-RFA. Before the EUS-RFA treatment, all patients had received gemcitabine-based chemotherapy, and 6 patients had chemoradiation. Data regarding chemotherapeutic and radiation-induced response were available in 16 patients (3 patients had a partial response, whereas 13 had stable disease). The procedure was technically successfully completed in 16 patients (72.7%). For 6 patients, there was a failure to penetrate the gastric wall and the tumor. The number of procedure-related AEs was relatively high and noted in 8 patients (36.4%). However, most of them were mild. Neither clear survival benefit nor significant effect on tumor size was evidenced[16].

Later, Song *et al*[32] reported the safety among 6 patients with pancreatic adenocarcinoma (4 patients with locally advanced disease and 2 patients with metastatic disease). Three patients were on adjuvant chemotherapy with gemcitabine, whereas the other 3 patients did not receive concomitant chemotherapy. The procedure was successfully completed in all patients with only 2 mild procedure-related AEs (mild abdominal pain)[32]. Scopelliti *et al*[17] reported 10 patients with locally advanced pancreatic adenocarcinoma. All patients underwent systemic chemotherapy (4 patient received FOLFIRINOX, 2 patients received gemcitabine, 2 patients received GemOx and 2 patients received combined gemcitabine/nab-paclitaxel), and 5 patients underwent additional external radiation therapy. All patients had complete technical success, and mild pancreatitis occurred in 4 patients, with no major AEs[17].

Similarly, Crinò *et al*[18] reported 7 patients with locally advanced pancreatic adenocarcinoma that were previously treated with FOLFIRINOX + radiotherapy (3 patients), gemcitabine (2 patients), FOLFIRINOX (1 patient) and radiotherapy (1 patient) who underwent EUS-RFA with an excellent technical success rate and minor AE of mild abdominal pain in 3 patients. Mean tumor ablation was approximately 30% (5.8%-73.5%) at 30 d following the procedure. However, data regarding survival benefit were not reported[18]. Paiella *et al*[19] reported a genetic study of 30 patients with locally advanced adenocarcinoma. Thirteen patients received EUS-RFA before the chemotherapy, while 17 patients had EUS-RFA after treatment (FOLFIRINOX in 6 patients, gemcitabine/oxaliplatinum in 4 patients, nab paclitaxel/gemcitabine in 2 patients and data not available in 5 patients, with additional radiotherapy in 4 patients). The overall median disease specific survival for all patients was 15 mo. *SMAD4* mutation was diagnosed in 18 patients (60%). The estimated post-RFA disease specific survival of patients without and with *SMAD4* mutation was 22 mo and 12 mo, respectively, with complete technical success of EUS-RFA and only 1 AE of bleeding from a duodenal ulcer[19].

Moreover, a recent prospective randomized study by Bang *et al*[20] reported the yield of EUS-guided RFA (12 patients) *vs* celiac plexus neurolysis (14 patients) for palliation of pain in pancreatic adenocarcinoma. EUS-RFA guided treatment was associated with a significant improvement in pain associated with pancreatic cancer ($P < 0.05$). Procedure-related AE occurred in 10 out of 12 included patients (83.3%) but were always mild[20]. Another recent study by Wang *et al*[33] reported 11 patients with pancreatic adenocarcinoma (only 1 patient was on chemotherapy), with complete technical success and only 2 patients with minor AEs of abdominal pain. A decrease in tumor size was only notable in 2 patients (18.2%), without a significant benefit on survival[33]. A recent study by Oh *et al*[34] reported 22 patients with pancreatic adenocarcinoma (19 patients received systemic gemcitabine-based chemotherapy before, and 3 patients received chemotherapy) who underwent 107 EUS-RFA sessions. The overall survival rate was 24 mo, with 4 procedure-related AEs (3 patients had transient abdominal pain, and 1 had peritonitis)[34].

Overall, the pooled analysis showed that EUS-RFA was applied to date in 120 patients with pancreatic adenocarcinoma who underwent 222 EUS fine needle aspiration sessions, most of them with locally advanced disease. The mean lesion size was 37.4 mm. The procedure was successfully completed in 95% of the patients, and AE occurred in 29 EUS-RFA sessions (13%), most of them were mild in severity, including transient abdominal pain and gastrointestinal symptoms. Notably, any decrease in tumor size was reported in 4 studies, as it was recorded in 25 among 50 patients (50%). However, only 4 studies provided data regarding the post EUS-RFA survival. Two studies did not show a clear survival benefit[16,33], and the other two studies showed a potential survival benefit[19,34] (Table 2).

RFA IN PCL

In the last few years, EUS fine needle aspiration was also implemented in the treatment of PCL in a few human case series. The first case was reported by Wiersema *et al*[35] in a patient with bleeding remnant intraductal papillary mucinous neoplasm that was successfully treated with endoscopic intraductal RFA. Pai *et al*[23] prospectively reported 6 patients with PCL [4 mucinous cystic neoplasm, 1 intraductal papillary mucinous neoplasm and 1 serous cystadenoma (SCA)]. Two (33.3%) and four (66.7%) patients had complete and partial cyst resolution at 3-6 mo follow-up, respectively. Among the 4 patients with partial resolution, 2 patients (50%) had > 50% ablation of the cyst size. Only 2 patients (33.3%) had mild transient abdominal pain. Notably, no long follow-up data were provided to assess recurrence. Furthermore, Choi *et al*[25] reported 2 patients with solid pseudopapillary tumors who underwent EUS-RFA because they refused surgery. The procedure was successfully completed in both patients, without procedure-related AEs. At a median follow-up of 13 mo, 1 patient (50%) had complete radiological response, while the other patient had no response with a decrease of approximately 20% from its pre-ablation size[25].

Additionally, Barthet *et al*[13] reported the yield of EUS-RFA among 17 patients with PCL (16 patients with intraductal papillary mucinous neoplasm and 1 patient with mucinous cystic neoplasm), notably 12 patients (70.6%) and 4 patients (23.5%) had mural nodules and thick cystic walls, respectively. The follow-up was assessed at two time-points. At 6-mo, 8 patients (47.1%) had a complete disappearance and necrosis of the cysts, and 3 patients (17.6%) had > 50% decrease in cyst diameter. However, there were 6 patients (35.3%) with failure of the procedure. At 12-mo follow-up, 11 patients (64.7%) had a complete disappearance and necrosis of the cysts, and 1 patient (5.9%) had > 50% decrease in cyst diameter. However, there were 5 patients (29.4%) with procedure failure. Only 1 procedure-related AE was noted with fever and pneumoperitoneum due to a perforation of the jejunal loop surgically corrected[13]. The long-term follow-up in 15 patients was recently reported. At 42.6-mo follow-up, complete cyst disappearance was noticed in 6 patients (40%). Four patients (26.6%) had a partial radiological response (decrease > 50% of the initial cyst diameter). Failure was seen in 5 patients, as the cyst lesion decreased < 50%[24].

A recent study by Oh *et al*[36] reported 13 patients with SCA who underwent 19 EUS-RFA sessions. One patient (5.3%) had peri-procedural transient mild abdominal pain. Notably, none of the patients had complete radiological response at 9.2 mo of follow-up, while 8 patients (61.5%) had partial radiological response (more than 30% in the longest diameter with an estimated volume reduction more than 66%)[36].

Pooling the data, 4 studies assessed EUS-RFA for PCL, with 38 patients included who underwent 44 EUS-RFA sessions. The mean cyst size was 32.1 mm, and worrisome features were only reported in one study. The procedure was feasible in all patients, with mild AEs of transient abdominal pain in most studies. Notably, complete radiological cyst resolution was achieved in 14 patients (36.8%) at a follow-up of 10.2 mo (Table 3).

SAFETY AND EFFICACY OF EUS-RFA IN PANCREATIC TUMORS

Overall, 377 EUS-RFA sessions were performed in 255 patients. The rate of mild, moderate and severe AEs according to American Society of Gastrointestinal Endoscopy guidelines[9] were 10.1%, 4.2% and 0.5%, respectively. For pNENs, the rate of mild and moderate AEs was 8.2% and 11.8%, respectively. For pancreatic adenocarcinoma and pancreatic cystic tumors, most of the AE were mild in severity. Notably, the rate of severe AEs and mortality were extremely low in all pancreatic tumor categories (Table 4). Finally, the EUS-RFA treatment is technically feasible, with high clinical and radiological success rates for pNENs and PCL and an acceptable AE rate (Table 5). Nevertheless, some limitations and controversies must be underlined as those limitations might impact the interpretation of the published literature and should be considered when planning future studies.

Technical considerations

The studies reported different power setting and application number used (Table 6). Moreover, in

Table 2 Studies reporting endoscopic ultrasound-radiofrequency ablation for pancreatic adenocarcinoma

Ref.	Study type	Patients/RFA session, *n*	Tumor location (*n*)	Cancer stage (*n*)	Mean size (range) in mm	Any decrease in tumor size, *n* (%)	Technical success, *n* (%)	Adverse events, *n* (%)	Mean follow-up in mo	Survival after RFA in mo
Arcidiacono *et al*[16]	Prospective	22/22	Head (16), uncinate (2), body (4)	Locally advanced (22)	35.7 (23-54)	6 (37.5)	16 (72.7)	8 (36.4)[1]	3	5.6 (1-12)
Song *et al* [32]	Prospective	6/8	Head (4), body (2)	Locally advanced (4), metastasis (2)	48 (30-90)	NR	6 (100)	2 (25)[2]	4.2	NR
Scopelliti *et al*[17]	Prospective	10/10	Head (4), body (6)	Locally advanced (10)	49.2 (25-75)	10 (100)	10 (100)	4 (40)[3]	1	NR
Crinò *et al* [18]	Retrospective	7/7	Head (2), body (3), uncinate (2)	Locally advanced (7)	36 (22-67)	7 (100)	7 (100)	3 (42.8)[4]	6.1	NR
Paiella *et al* [19]	Retrospective	30/30	Head (23), body and tail (7)	Locally advanced (30)	35 (20-60)	NR	30 (100)	1 (3.3)[5]	15	15
Bang *et al* [20]	Prospective	12/12	Head and uncinate (8), body and tail (4)	Locally advanced (5), metastasis (7)	29.6 (22.5-35.0)	NR	12 (100)	5 (41.6)[6]	1	NR
Wang *et al* [33]	Retrospective	11/26	Head (4), neck (3), body (3), tail (1)	Locally advanced (7), metastasis (4)	28 (17.2-38)	2 (18.2)	11 (100)	2 (7.7)[7]	5.2	5.2
Oh *et al*[34]	Prospective	22/107	Head (14), body (4), tail (3), metastasis (1)	Locally advanced (14), metastatic (8)	38 (32.8-45.0)	NR	22 (100)	4 (3.7)[8]	21.2	24
Pooled data	Prospective: 5. Retrospective: 3	120/222	Head and uncinate (79). Body and tail (37), neck (3)	Locally advanced (100), metastasis (21)	37.4	Unable to pool due to data lacking	114 (95)	29 (13)	7.1	Unable to pool due to data lacking

[1]Transient abdominal pain (3 patients, mild), minor duodenal bleeding endoscopically treated (1 patient, moderate), transient amylase elevation (3 patients, mild), transient cystic fluid collection between pancreas and left hepatic lobe (1 patient, mild).
[2]Transient abdominal pain (2 patients, mild).
[3]Mild pancreatitis (4 patients, mild).
[4]Transient abdominal pain (3 patients, mild).
[5]Duodenal bleeding (1 patient, moderate).
[6]Nausea and vomiting (4 patients, mild), transient abdominal pain (1 patient, mild).
[7]Transient abdominal pain (2 patients, mild).
[8]Transient abdominal pain (3 patients, mild), peritonitis (1 patient, moderate).
NR: Not reported; RFA: Radiofrequency ablation.

several studies, the size of the tip of the needle was not considered or not detailed. Power setting, size of the active type, duration of the irradiation, size of the needle (18 G *vs* 19 G) can interfere in the final destruction. Therefore, uniform studies with similar technical aspects should be performed to better assess the treatment efficacy and safety.

Optimal size of the pNENs and PCL

To date, no data are available regarding the optimal size of the pNENs and cystic lesions that are amenable to EUS-RFA. Predictably, the RFA probe can induce a 3 cm ablation area with a single deployment, thus it is postulated that lesions up to 3 cm will achieve the best ablative results with a single application, and larger lesions may need more needle applications during the same session[6]. In fact, a lot of lesions had more than one needle application during the same session even in lesions < 2

Table 3 Studies reporting endoscopic ultrasound-radiofrequency ablation for pancreatic cystic tumors

Ref.	Type of study	Patients/RFA sessions, *n*	Type of cyst	Mean size (range) in mm	Worrisome features	Technical success, *n* (%)	Adverse events, *n* (%)	Complete/partial resolution, *n* (%)	Mean follow-up in mo
Pai *et al* [23]	Prospective	6/6	MCN (4), IPMN (1), MCA (1)	41 (24-70), 35, 20	NR	6 (100)	2 (33.3)[1]	2 (33.3)/4 (66.7)	6
Choi *et al*[25]	Prospective	2/2	SPT (2)	21.5 (20-23)	NR	2 (100)	0	1 (50)/1 (50)	13
Barthet *et al*[13]	Prospective	17/17	MCN (1), IPMN (16)	28 (9-60)	16 (94.1)	17 (100)	1 (5.9)[2]	11 (64.7)/1 (5.9)	12
Oh *et al* [36]	Retrospective	13/19	SCN (13)	50 (34-52.5)	NR	13 (100)	1 (5.3)[3]	0/8 (61.5)	9.2
Pooled data	Prospective: 3. Retrospective: 1	38/44	MCN (5), IPMN (17), MCA (1), SPT (2), SCN (13)	32.1	Unable to pool due to data lacking	38 (100)	4 (9.1)	14 (36.8)/14 (36.8)	10.2

[1]Transient abdominal pain (2 patients, mild).
[2]Fever and pneumoperitoneum with fluid collection and jejunal loop perforation needed surgery (1 patient, severe).
[3]Transient abdominal pain (1 patient, mild).
IPMN: Intraductal papillary mucinous neoplasm; MCA: Microcystic adenoma; MCN: Mucinous cystic neoplasm; NR: Not reported; RFA: Radiofrequency ablation; SPT: Solid pseudopapillary tumor; SCN: Serous cystic neoplasm.

Table 4 Pooled analysis of the adverse events

EUS-guided RFA for	Procedure-related adverse events according to ASGE[9][1]			
	Mild, *n* (%)	Moderate, *n* (%)	Severe, *n* (%)	Mortality, *n* (%)
Neuroendocrine neoplasms EUS-RFA sessions = 114	11 (9.6)	13 (11.4)	1 (0.9)	1 (0.9)
Adenocarcinoma EUS-RFA sessions = 223	26 (11.6)	3 (1.3)	0	0
Cystic tumors EUS-RFA sessions = 44	3 (6.8)	0	1 (2.3)	0
Pooled data	40 (10.5)	16 (4.2)	2 (0.5)	1 (0.26)

[1]Overall, 380 EUS-RFA sessions performed for all pancreatic tumors.
ASGE: American Society of Gastrointestinal Endoscopy; EUS-RFA: Endoscopic ultrasound-radiofrequency ablation.

cm.

Heterogenicity of reporting the histological grading and mitotic activity for the pNENs

EUS-RFA for pNENs should be reserved for patients with G1 (Ki67 < 3%) or low G2 (Ki67 < 5%). However, most of the reported studies did not address the histological and mitotic activity of the pNENs, and in one study by Oleinikov *et al*[14], 2 patients with G3 (Ki67% of 34%-40%) were included in their series. Therefore, identification of the optimal histological grading that will most benefit from EUS-RFA is needed.

Technical success

In the published papers, the technical success was almost complete. However, the data did not state how many patients failed to undergo the procedure due to technical difficulties. Thus, the pooled technical success rate should be carefully interpreted. Further prospective studies are warranted with inclusion of all patients referred for an EUS-RFA procedure in intention-to-treat.

AE rate

Most of the AEs that were reported in the literature were intra- and periprocedural AEs, mainly reported from retrospective and small series with scarce data on long-term AEs (follow-up of only 1 mo for some trials). Moreover, there was one death in an elderly patient who refused endoscopic intervention, which might bias the severity of AEs as well. Therefore, larger studies are needed with

Table 5 Summary of efficacy and safety of endoscopic ultrasound-guided radiofrequency ablation for pancreatic tumors

Procedure	EUS-guided RFA for pancreatic		
	Neuroendocrine tumors	Adenocarcinoma	Cystic tumors
Technical success	High	High	High
Safety, complications	Mild-moderate[1]	Mild	Mild
Efficacy			
Clinical improvement	Significant for insulinomas	None	-
Radiological partial/complete resolution	High	Modest	Encouraging
Palliation	-	Encouraging	-
Mortality	None	None	None

[1]When taking prophylactic measures (antibioprophylaxis, fluid component suction before radiofrequency ablation).
EUS-RFA: Endoscopic ultrasound-radiofrequency ablation.

Table 6 Technical considerations and imaging studies used in follow-up among patients with pancreatic neuroendocrine neoplasms

Ref.	Number of patients/sessions	Power setting in Watts	RFA application number in all sessions	Imaging study used in radiological follow-up
Rossi *et al*[10]	1/1	10-15	1	CECT or MRI
Armellini *et al*[11]	1/1	NR	2	CT and CE-EUS
Lakhtakia *et al*[12]	3/3	50	9	CECT (1 patient), CECT and EUS (2 patients)
Waung *et al*[21]	1/3	10	25	CT and gallium dotatate positron emission tomography
Bas-Cutrina *et al*[22]	1/1	10	3	NR
Pai *et al*[23]	2/3	20	10	Cross sectional imaging not stated which
Barthet *et al*[13]	12/12	50	NR	CT and EUS
Choi *et al*[25]	8/14	50	65	CECT and CE-EUS
de Nucci *et al*[26]	10/10	20	23	CT
Oleinikov *et al*[14]	18/18	50	3-10 in each EUS-RFA session	CECT (9 patients), NA (5 patients), CECT and somatostatin receptor imaging (3 patients)
Rossi *et al*[30]	3/4	30	14	EUS (1 patient), MRI (1 patient), refused follow-up (1 patient)
Chang *et al*[27]	1/1	50	2	CECT
Kluz *et al*[28]	1/1	50	3	NR
Furnica *et al*[29]	4/4	50	1-3 per each EUS-RFA	CT
Marx *et al*[15]	7/7	50	1-5 for each EUS-RFA session	CE-EUS or MRI
Marx *et al*[31]	27/31	50	1-5 for each EUS-RFA session	CT or MRI

CE-EUS: Contrast enhanced endoscopic ultrasound; CECT: Contrast enhanced computed tomography; CT: Computed tomography; EUS-RFA: Endoscopic ultrasound-radiofrequency ablation; MRI: Magnetic resonance imaging; NA: Not available; NR: Nor reported.

longer follow-up to better define the AEs in these procedures.

Antibioprophylaxis in cystic lesions

Antibioprophylaxis and liquid component suction of all the fluid composition of the lesions before performing the RFA procedure is a controversy that should be addressed for cystic pNENs and for PCL. In their study, Barthet *et al*[13] revised their prophylaxis protocol after an AE of infection, so they administered antibioprophylaxix and aspirated the major cystic liquid component in their subsequent patients. Antibioprophylaxis in a PCL patient who underwent EUS-guided fine needle aspiration has

been a long debated clinical indication, as there were conflicting results regarding this condition[35,37-39]. A recent meta-analysis showed no significant difference in the rate of pancreatic cyst infection rate after puncture irrespective of the administration of antibioprophylaxis[40]. Moreover, the advantage of emptying the cyst might be a double pitfall. It will be less evident to see the thickening or the mural nodule within the PCL undergoing EUS-RFA, and it will need two punctures (one for emptying the fluid, and one for the EUS-RFA procedure), which might increase the procedure-related AE.

Association between complete clinical and radiological resolution in insulinomas

The complete disappearance of the clinical symptoms of insulinoma occurred in all patients (100%) throughout the reported studies. However, it does not mean that the tumor was totally destroyed, as some patients with insulinoma will have normal insulin levels[41,42]. Among the nine studies that included patients with insulinoma, only three studies had almost similar clinical and radiological follow-up periods after EUS-RFA, while the other studies had a longer clinical than radiological follow-up (Table 7). Further prospective studies are needed with uniform clinical, biochemical and radiological long-term follow-up periods.

Radiological efficacy

According to the literature, a high complete radiological resolution rate was demonstrated after EUS-RFA. However, the studies reported different imaging modalities or combined imaging tools. Moreover, some studies did not specify which imaging tool was used. Notably, only three studies used a combination of contrast enhanced computed tomography (CT) and contrast enhanced EUS, while most of the other studies used only single imaging modality. Furthermore, in some studies, CT and EUS were used for follow-up. However; it was not stated whether contrast enhancement was implemented (Table 6).

Previous studies have shown that contrast enhanced magnetic resonance imaging including diffusion-weighted imaging is preferred over contrast enhanced CT for examination of the pancreas and the liver[43,44]. On the other hand, EUS has an important role in the diagnosis of small pNENs of < 2 cm and is now considered as the imaging study of choice to be performed where other non-invasive studies failed to diagnose the pNENs[45,46]. Previous systematic review and meta-analysis showed that EUS consistently increased the detection of pNENs by over 25% after performing CT scan[47]. PET-Dotatoc should also be proposed for the follow-up of non-functional pNENs. Therefore, a prospective study with uniform imaging study to be used at follow-up is mandatory to precisely assess the efficacy of EUS-RFA in pNENs.

Patient number and study designs

The small number of patients reported and the study designs, which were primarily case reports and small case series, with the lack of uniform and long-term follow-up should urge careful interpretation of the current literature. The follow-up is too short (only one trial has a follow-up longer than 3 years) to know the long-term result on the tumor and on the possible metastatic evolution.

RFA in PCL

The indication of RFA in cystic lesions remains debated. Oh *et al*[36] reported a study on 13 patients with SCA. However, the interest in this indication is debatable due to its very rare malignant potential [48]. Excluding SCA, only 25 patients with PCL were treated by EUS-RFA, which is too small of a sample size to enable good and precise data interpretation. Therefore, more studies are needed in patients with high-risk PCL.

RFA in pancreatic adenocarcinoma

Most of the studies did not report the additional survival benefit of EUS-RFA when added to standard chemotherapeutic regimens. Moreover, some studies included patients with metastatic disease. It is difficult to justify this treatment for metastatic disease. Prospective randomized trials with uniform disease stage and standard chemotherapeutic regimens are necessary to draw conclusions of the efficacy.

CONCLUSION

High and promising expectations are held for EUS-RFA. Taking advantage of the EUS transducer proximity to the pancreatic parenchyma, coupled with its excellent imaging resolution and the capability of avoiding major internal organs and vascular structures, makes this procedure safe. The current evidence of efficacy is weak, as most studies were case reports and series that included a small number and heterogenous groups of patients. Prospective and randomized studies are needed to establish the potential therapeutic role of EUS-RFA in pancreatic tumors. The available literature suggests a beneficial impact mainly on functional pNENs where RFA should replace surgery. In nonfunctional pNENs the data are encouraging. Its role for PCL treatment is still to be elucidated. For

Table 7 Clinical and radiological follow-up in pancreatic insulinomas studies

Ref.	Patients with insulinoma, *n*	Mean time (range) of clinical follow-up in mo	Mean time (range) of radiological follow-up in mo
Lakhtakia *et al*[12]	3	11.7 (11-12)	4.2 (1.5-8)
Waung *et al*[21]	1	10	3 d
Bas-Cutrina *et al*[22]	1	10	10
Choi *et al*[25]	1	NR	NR
Oleinikov *et al*[14]	7	9.7 (3-21)	8.7 (2-21)
Rossi *et al*[30]	3	22 (14-27)	5.7 (3-14)
Chang *et al*[27]	1	18	18
Kluz *et al*[28]	1	NR	NR
Furnica *et al*[29]	4	22 (13-28)	8 (3-14)
Marx *et al*[15]	7	21 (3-38)	20.3 (3-38)

NR: Not reported.

pancreatic adenocarcinoma, the data are lacking especially on the survival rate. Finally, EUS-RFA for pancreatic tumors is far from being adopted as a first-line treatment except for insulinomas. For grade 1 nonfunctional pNENs < 2 cm, EUS-RFA should be discussed as an alternative to surgery or follow-up. For PCL with worrisome features, EUS-RFA could be considered among patients who are not candidates or refuse surgical intervention. For pancreatic adenocarcinoma, randomized controlled trials are required to determine if EUS-RFA adds a survival benefit to chemotherapy in locally advanced pancreatic adenocarcinoma.

FOOTNOTES

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REFERENCES

1 **Sooklal S**, Chahal P. Endoscopic Ultrasound. *Surg Clin North Am* 2020; **100**: 1133-1150 [PMID: 33128884 DOI: 10.1016/j.suc.2020.07.003]
2 **Paiella S**, Salvia R, Ramera M, Girelli R, Frigerio I, Giardino A, Allegrini V, Bassi C. Local Ablative Strategies for Ductal Pancreatic Cancer (Radiofrequency Ablation, Irreversible Electroporation): A Review. *Gastroenterol Res Pract* 2016; **2016**: 4508376 [PMID: 26981115 DOI: 10.1155/2016/4508376]
3 **Goldberg SN**, Mallery S, Gazelle GS, Brugge WR. EUS-guided radiofrequency ablation in the pancreas: results in a

porcine model. *Gastrointest Endosc* 1999; **50**: 392-401 [PMID: 10462663 DOI: 10.1053/ge.1999.v50.98847]
4 **Kim HJ**, Seo DW, Hassanuddin A, Kim SH, Chae HJ, Jang JW, Park DH, Lee SS, Lee SK, Kim MH. EUS-guided radiofrequency ablation of the porcine pancreas. *Gastrointest Endosc* 2012; **76**: 1039-1043 [PMID: 23078928 DOI: 10.1016/j.gie.2012.07.015]
5 **Lee JM**, Han JK, Kim HC, Choi YH, Kim SH, Choi JY, Choi BI. Switching monopolar radiofrequency ablation technique using multiple, internally cooled electrodes and a multichannel generator: *ex vivo* and *in vivo* pilot study. *Invest Radiol* 2007; **42**: 163-171 [PMID: 17287646 DOI: 10.1097/01.rli.0000252495.44818.b3]
6 **Gaidhane M**, Smith I, Ellen K, Gatesman J, Habib N, Foley P, Moskaluk C, Kahaleh M. Endoscopic Ultrasound-Guided Radiofrequency Ablation (EUS-RFA) of the Pancreas in a Porcine Model. *Gastroenterol Res Pract* 2012; **2012**: 431451 [PMID: 23049547 DOI: 10.1155/2012/431451]
7 **Haen SP**, Pereira PL, Salih HR, Rammensee HG, Gouttefangeas C. More than just tumor destruction: immunomodulation by thermal ablation of cancer. *Clin Dev Immunol* 2011; **2011**: 160250 [PMID: 22242035 DOI: 10.1155/2011/160250]
8 **Tatli S**, Tapan U, Morrison PR, Silverman SG. Radiofrequency ablation: technique and clinical applications. *Diagn Interv Radiol* 2012; **18**: 508-516 [PMID: 22407695 DOI: 10.4261/1305-3825.DIR.5168-11.1]
9 **Cotton PB**, Eisen GM, Aabakken L, Baron TH, Hutter MM, Jacobson BC, Mergener K, Nemcek A Jr, Petersen BT, Petrini JL, Pike IM, Rabeneck L, Romagnuolo J, Vargo JJ. A lexicon for endoscopic adverse events: report of an ASGE workshop. *Gastrointest Endosc* 2010; **71**: 446-454 [PMID: 20189503 DOI: 10.1016/j.gie.2009.10.027]
10 **Rossi S**, Viera FT, Ghittoni G, Cobianchi L, Rosa LL, Siciliani L, Bortolotto C, Veronese L, Vercelli A, Gallotti A, Ravetta V. Radiofrequency ablation of pancreatic neuroendocrine tumors: a pilot study of feasibility, efficacy, and safety. *Pancreas* 2014; **43**: 938-945 [PMID: 24717825 DOI: 10.1097/MPA.0000000000000133]
11 **Armellini E**, Crinò SF, Ballarè M, Occhipinti P. Endoscopic ultrasound-guided radiofrequency ablation of a pancreatic neuroendocrine tumor. *Endoscopy* 2015; **47** Suppl 1 UCTN: E600-E601 [PMID: 26671543 DOI: 10.1055/s-0034-1393677]
12 **Lakhtakia S**, Ramchandani M, Galasso D, Gupta R, Venugopal S, Kalpala R, Reddy DN. EUS-guided radiofrequency ablation for management of pancreatic insulinoma by using a novel needle electrode (with videos). *Gastrointest Endosc* 2016; **83**: 234-239 [PMID: 26394384 DOI: 10.1016/j.gie.2015.08.085]
13 **Barthet M**, Giovannini M, Lesavre N, Boustiere C, Napoleon B, Koch S, Gasmi M, Vanbiervliet G, Gonzalez JM. Endoscopic ultrasound-guided radiofrequency ablation for pancreatic neuroendocrine tumors and pancreatic cystic neoplasms: a prospective multicenter study. *Endoscopy* 2019; **51**: 836-842 [PMID: 30669161 DOI: 10.1055/a-0824-7067]
14 **Oleinikov K**, Dancour A, Epshtein J, Benson A, Mazeh H, Tal I, Matalon S, Benbassat CA, Livovsky DM, Goldin E, Gross DJ, Jacob H, Grozinsky-Glasberg S. Endoscopic Ultrasound-Guided Radiofrequency Ablation: A New Therapeutic Approach for Pancreatic Neuroendocrine Tumors. *J Clin Endocrinol Metab* 2019; **104**: 2637-2647 [PMID: 31102458 DOI: 10.1210/jc.2019-00282]
15 **Marx M**, Trosic-Ivanisevic T, Caillol F, Demartines N, Schoepfer A, Pesenti C, Ratone JP, Robert M, Giovannini M, Godat S. EUS-guided radiofrequency ablation for pancreatic insulinoma: experience in 2 tertiary centers. *Gastrointest Endosc* 2022; **95**: 1256-1263 [PMID: 34902374 DOI: 10.1016/j.gie.2021.11.045]
16 **Arcidiacono PG**, Carrara S, Reni M, Petrone MC, Cappio S, Balzano G, Boemo C, Cereda S, Nicoletti R, Enderle MD, Neugebauer A, von Renteln D, Eickhoff A, Testoni PA. Feasibility and safety of EUS-guided cryothermal ablation in patients with locally advanced pancreatic cancer. *Gastrointest Endosc* 2012; **76**: 1142-1151 [PMID: 23021160 DOI: 10.1016/j.gie.2012.08.006]
17 **Scopelliti F**, Pea A, Conigliaro R, Butturini G, Frigerio I, Regi P, Giardino A, Bertani H, Paini M, Pederzoli P, Girelli R. Technique, safety, and feasibility of EUS-guided radiofrequency ablation in unresectable pancreatic cancer. *Surg Endosc* 2018; **32**: 4022-4028 [PMID: 29766302 DOI: 10.1007/s00464-018-6217-x]
18 **Crinò SF**, D'Onofrio M, Bernardoni L, Frulloni L, Iannelli M, Malleo G, Paiella S, Larghi A, Gabbrielli A. EUS-guided Radiofrequency Ablation (EUS-RFA) of Solid Pancreatic Neoplasm Using an 18-gauge Needle Electrode: Feasibility, Safety, and Technical Success. *J Gastrointestin Liver Dis* 2018; **27**: 67-72 [PMID: 29557417 DOI: 10.15403/jgld.2014.1121.271.eus]
19 **Paiella S**, Malleo G, Cataldo I, Gasparini C, De Pastena M, De Marchi G, Marchegiani G, Rusev B, Scarpa A, Girelli R, Giardino A, Frigerio I, D'Onofrio M, Secchettin E, Bassi C, Salvia R. Radiofrequency ablation for locally advanced pancreatic cancer: SMAD4 analysis segregates a responsive subgroup of patients. *Langenbecks Arch Surg* 2018; **403**: 213-220 [PMID: 28983662 DOI: 10.1007/s00423-017-1627-0]
20 **Bang JY**, Sutton B, Hawes RH, Varadarajulu S. EUS-guided celiac ganglion radiofrequency ablation versus celiac plexus neurolysis for palliation of pain in pancreatic cancer: a randomized controlled trial (with videos). *Gastrointest Endosc* 2019; **89**: 58-66.e3 [PMID: 30120957 DOI: 10.1016/j.gie.2018.08.005]
21 **Waung JA**, Todd JF, Keane MG, Pereira SP. Successful management of a sporadic pancreatic insulinoma by endoscopic ultrasound-guided radiofrequency ablation. *Endoscopy* 2016; **48** Suppl 1: E144-E145 [PMID: 27081874 DOI: 10.1055/s-0042-104650]
22 **Bas-Cutrina F**, Bargalló D, Gornals JB. Small pancreatic insulinoma: Successful endoscopic ultrasound-guided radiofrequency ablation in a single session using a 22-G fine needle. *Dig Endosc* 2017; **29**: 636-638 [PMID: 28295664 DOI: 10.1111/den.12866]
23 **Pai M**, Habib N, Senturk H, Lakhtakia S, Reddy N, Cicinnati VR, Kaba I, Beckebaum S, Drymousis P, Kahaleh M, Brugge W. Endoscopic ultrasound guided radiofrequency ablation, for pancreatic cystic neoplasms and neuroendocrine tumors. *World J Gastrointest Surg* 2015; **7**: 52-59 [PMID: 25914783 DOI: 10.4240/wjgs.v7.i4.52]
24 **Barthet M**, Giovannini M, Gasmi M, Lesavre N, Boustière C, Napoleon B, LaQuiere A, Koch S, Vanbiervliet G, Gonzalez JM. Long-term outcome after EUS-guided radiofrequency ablation: Prospective results in pancreatic neuroendocrine tumors and pancreatic cystic neoplasms. *Endosc Int Open* 2021; **9**: E1178-E1185 [PMID: 34447860 DOI: 10.1055/a-1479-2199]
25 **Choi JH**, Seo DW, Song TJ, Park DH, Lee SS, Lee SK, Kim MH. Endoscopic ultrasound-guided radiofrequency ablation for management of benign solid pancreatic tumors. *Endoscopy* 2018; **50**: 1099-1104 [PMID: 29727904 DOI: 10.1055/a-0583-8387]

26 **de Nucci G**, Imperatore N, Mandelli ED, di Nuovo F, d'Urbano C, Manes G. Endoscopic ultrasound-guided radiofrequency ablation of pancreatic neuroendocrine tumors: a case series. *Endosc Int Open* 2020; **8**: E1754-E1758 [PMID: 33269307 DOI: 10.1055/a-1261-9359]

27 **Chang JY**, Woo CS, Lui DT, Fung MM, Lee AC, Leung EK, Woo YC, Chow WS, Lam KS, Tan KC, Lee CH. Case Report: Insulinoma Co-Existing With Type 2 Diabetes - Advantages and Challenges of Treatment With Endoscopic Ultrasound-Guided Radiofrequency Ablation. *Front Endocrinol (Lausanne)* 2022; **13**: 957369 [PMID: 35942178 DOI: 10.3389/fendo.2022.957369]

28 **Kluz M**, Staroń R, Krupa Ł, Partyka M, Polkowski M, Gutkowski K. Successful endoscopic ultrasound-guided radiofrequency ablation of a pancreatic insulinoma. *Pol Arch Intern Med* 2020; **130**: 145-146 [PMID: 31820740 DOI: 10.20452/pamw.15100]

29 **Furnica RM**, Deprez P, Maiter D, Vandeleene B, Borbath I. Endoscopic ultrasound-guided radiofrequency ablation: An effective and safe alternative for the treatment of benign insulinoma. *Ann Endocrinol (Paris)* 2020; **81**: 567-571 [PMID: 33285105 DOI: 10.1016/j.ando.2020.11.009]

30 **Rossi G**, Petrone MC, Capurso G, Partelli S, Falconi M, Arcidiacono PG. Endoscopic ultrasound radiofrequency ablation of pancreatic insulinoma in elderly patients: Three case reports. *World J Clin Cases* 2022; **10**: 6514-6519 [PMID: 35979317 DOI: 10.12998/wjcc.v10.i19.6514]

31 **Marx M**, Godat S, Caillol F, Poizat F, Ratone JP, Pesenti C, Schoepfer A, Hoibian S, Dahel Y, Giovannini M. Management of non-functional pancreatic neuroendocrine tumors by endoscopic ultrasound-guided radiofrequency ablation: Retrospective study in two tertiary centers. *Dig Endosc* 2022; **34**: 1207-1213 [PMID: 34963025 DOI: 10.1111/den.14224]

32 **Song TJ**, Seo DW, Lakhtakia S, Reddy N, Oh DW, Park DH, Lee SS, Lee SK, Kim MH. Initial experience of EUS-guided radiofrequency ablation of unresectable pancreatic cancer. *Gastrointest Endosc* 2016; **83**: 440-443 [PMID: 26344883 DOI: 10.1016/j.gie.2015.08.048]

33 **Wang J**, Wang Y, Zhao Y, Wu X, Zhang M, Hou W, Chen Q, Cheng B. Endoscopic ultrasound-guided radiofrequency ablation of unresectable pancreatic cancer with low ablation power and multiple applications: a preliminary study of 11 patients. *Ann Palliat Med* 2021; **10**: 1842-1850 [PMID: 33440967 DOI: 10.21037/apm-20-1468]

34 **Oh D**, Seo DW, Song TJ, Park DH, Lee SK, Kim MH. Clinical outcomes of EUS-guided radiofrequency ablation for unresectable pancreatic cancer: A prospective observational study. *Endosc Ultrasound* 2022; **11**: 68-74 [PMID: 35083978 DOI: 10.4103/EUS-D-21-00049]

35 **Wiersema MJ**, Vilmann P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. *Gastroenterology* 1997; **112**: 1087-1095 [PMID: 9097990 DOI: 10.1016/s0016-5085(97)70164-1]

36 **Oh D**, Ko SW, Seo DW, Hong SM, Kim JH, Song TJ, Park DH, Lee SK, Kim MH. Endoscopic ultrasound-guided radiofrequency ablation of pancreatic microcystic serous cystic neoplasms: a retrospective study. *Endoscopy* 2021; **53**: 739-743 [PMID: 33063298 DOI: 10.1055/a-1250-7786]

37 **Williams DB**, Sahai AV, Aabakken L, Penman ID, van Velse A, Webb J, Wilson M, Hoffman BJ, Hawes RH. Endoscopic ultrasound guided fine needle aspiration biopsy: a large single centre experience. *Gut* 1999; **44**: 720-726 [PMID: 10205212 DOI: 10.1136/gut.44.5.720]

38 **O'Toole D**, Palazzo L, Arotçarena R, Dancour A, Aubert A, Hammel P, Amaris J, Ruszniewski P. Assessment of complications of EUS-guided fine-needle aspiration. *Gastrointest Endosc* 2001; **53**: 470-474 [PMID: 11275888 DOI: 10.1067/mge.2001.112839]

39 **Lee LS**, Saltzman JR, Bounds BC, Poneros JM, Brugge WR, Thompson CC. EUS-guided fine needle aspiration of pancreatic cysts: a retrospective analysis of complications and their predictors. *Clin Gastroenterol Hepatol* 2005; **3**: 231-236 [PMID: 15765442 DOI: 10.1016/s1542-3565(04)00618-4]

40 **Khoury T**, Gincul R, Mohammedi I, Sbeit W, Napoléon B. Antibioprophylaxis in endoscopic ultrasound guided fine needle aspiration in pancreatic cysts: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2022; **37**: 1685-1692 [PMID: 35912889 DOI: 10.1111/jgh.15972]

41 **Vezzosi D**, Bennet A, Fauvel J, Caron P. Insulin, C-peptide and proinsulin for the biochemical diagnosis of hypoglycaemia related to endogenous hyperinsulinism. *Eur J Endocrinol* 2007; **157**: 75-83 [PMID: 17609405 DOI: 10.1530/EJE-07-0109]

42 **Okabayashi T**, Shima Y, Sumiyoshi T, Kozuki A, Ito S, Ogawa Y, Kobayashi M, Hanazaki K. Diagnosis and management of insulinoma. *World J Gastroenterol* 2013; **19**: 829-837 [PMID: 23430217 DOI: 10.3748/wjg.v19.i6.829]

43 **Graziani R**, Brandalise A, Bellotti M, Manfredi R, Contro A, Falconi M, Boninsegna L, Pozzi Mucelli R. Imaging of neuroendocrine gastroenteropancreatic tumours. *Radiol Med* 2010; **115**: 1047-1064 [PMID: 20221711 DOI: 10.1007/s11547-010-0540-1]

44 **Foti G**, Boninsegna L, Falconi M, Mucelli RP. Preoperative assessment of nonfunctioning pancreatic endocrine tumours: role of MDCT and MRI. *Radiol Med* 2013; **118**: 1082-1101 [PMID: 23801403 DOI: 10.1007/s11547-013-0956-5]

45 **Falconi M**, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Klöppel G, Reed N, Kianmanesh R, Jensen RT; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for the Management of Patients with Functional Pancreatic Neuroendocrine Tumors and Non-Functional Pancreatic Neuroendocrine Tumors. *Neuroendocrinology* 2016; **103**: 153-171 [PMID: 26742109 DOI: 10.1159/000443171]

46 **Puli SR**, Kalva N, Bechtold ML, Pamulaparthy SR, Cashman MD, Estes NC, Pearl RH, Volmar FH, Dillon S, Shekleton MF, Forcione D. Diagnostic accuracy of endoscopic ultrasound in pancreatic neuroendocrine tumors: a systematic review and meta analysis. *World J Gastroenterol* 2013; **19**: 3678-3684 [PMID: 23801872 DOI: 10.3748/wjg.v19.i23.3678]

47 **Sundin A**, Arnold R, Baudin E, Cwikla JB, Eriksson B, Fanti S, Fazio N, Giammarile F, Hicks RJ, Kjaer A, Krenning E, Kwekkeboom D, Lombard-Bohas C, O'Connor JM, O'Toole D, Rockall A, Wiedenmann B, Valle JW, Vullierme MP; Antibes Consensus Conference participants. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Radiological, Nuclear Medicine & Hybrid Imaging. *Neuroendocrinology* 2017; **105**: 212-244 [PMID: 28355596 DOI: 10.1159/000471879]

48 **Chiang AL**, Lee LS. Clinical approach to incidental pancreatic cysts. *World J Gastroenterol* 2016; **22**: 1236-1245 [PMID:

26811661 DOI: 10.3748/wjg.v22.i3.1236]

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ORIGINAL ARTICLE

Basic Study

In vivo recognition of bioactive substances of *Polygonum multiflorum* for protecting mitochondria against metabolic dysfunction-associated fatty liver disease

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Abstract

BACKGROUND

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a severe threat to human health. *Polygonum multiflorum* (PM) has been proven to remedy mitochondria and relieve MAFLD, but the main pharmacodynamic ingredients for mitigating MAFLD remain unclear.

AIM

To research the active ingredients of PM adjusting mitochondria to relieve high-fat diet (HFD)-induced MAFLD in rats.

METHODS

Fat emulsion-induced L02 adipocyte model and HFD-induced MAFLD rat model were used to investigate the anti-MAFLD ability of PM and explore their action mechanisms. The adipocyte model was also applied to evaluate the activities of PM-derived constituents in liver mitochondria from HFD-fed rats (mitochondrial pharmacology). PM-derived constituents in liver mitochondria were confirmed by ultra-high-performance liquid chromatography/mass spectrometry (mitochondrial pharmacochemistry). The abilities of PM-derived monomer and monomer groups were evaluated by the adipocyte model and MAFLD mouse model, respectively.

RESULTS

PM repaired mitochondrial ultrastructure and prevented oxidative stress and

energy production disorder of liver mitochondria to mitigate fat emulsion-induced cellular steatosis and HFD-induced MAFLD. PM-derived constituents that entered the liver mitochondria inhibited oxidative stress damage and improved energy production against cellular steatosis. Eight chemicals were found in the liver mitochondria of PM-administrated rats. The anti-steatosis ability of one monomer and the anti-MAFLD activity of the monomer group were validated.

CONCLUSION

PM restored mitochondrial structure and function and alleviated MAFLD, which may be associated with the remedy of oxidative stress and energy production. The identified eight chemicals may be the main bioactive ingredients in PM that adjusted mitochondria to prevent MAFLD. Thus, PM provides a new approach to prevent MAFLD-related mitochondrial dysfunction. Mitochondrial pharmacology and pharmacochemistry further showed efficient strategies for determining the bioactive ingredients of Chinese medicines that adjust mitochondria to prevent diseases.

Key Words: Fatty liver; Mitochondria; Pharmacodynamic ingredients; *Polygonum multiflorum*

Core Tip: We found that *Polygonum multiflorum* (PM) protected the mitochondrial ultrastructure and prevented oxidative stress and energy production disorder in the liver mitochondria to mitigate metabolic dysfunction-associated fatty liver disease (MAFLD). Eight chemicals were identified from the liver mitochondria of the PM-treated rats using a novel strategy based on mitochondrial pharmacology and pharmacochemistry. The constituents identified regulated mitochondria to alleviate MAFLD. Our results indicate that PM restored mitochondrial structure and function and alleviated MAFLD, which may be related to oxidative stress and energy production. The eight substances may be the main pharmacodynamic ingredients in PM that regulate mitochondria to prevent MAFLD.

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) was considered a better definition of nonalcoholic fatty liver disease. In addition to causing cirrhosis and hepatocellular carcinoma, MAFLD is also closely related to the occurrence of a range of metabolic diseases. MAFLD affects the health of 30% of adults worldwide and represents a heavy clinical and economic burden on patients and society [1]. To date, no pharmacotherapy targeting MAFLD is available, except for preventative lifestyle improvements and physical exercise to lose weight[2,3]. Thus, it is urgent to find a cure for this complex liver disease[4].

The study found that mitochondrial dysfunction plays a significant role in the development of MAFLD. The oxidative damage of mitochondrial fatty acids and the decline of mitochondrial quality are considered to be extremely important motivations in the development of MAFLD[5]. Thus, modulation of mitochondrial function may be an effective strategy to alleviate MAFLD.

Polygonum multiflorum (PM) has been used as a traditional medicine for many centuries in China. Its main chemical components include anthraquinones, flavonoids, phenols, and phospholipids. Recent studies have shown that PM has many pharmacological activities, including hepatoprotective, antihyperlipidemic, anti-inflammatory, antioxidant, anticancer, immunomodulatory, and neuroprotective effects[6]. PM extracts can remedy mitochondrial dysfunction in the liver to alleviate MAFLD, such as the promotion of β-oxidation of fatty acids and the activity of carnitine palmitoyltransferase 1[7,8]. In addition, PM regulated lipid metabolism by reducing very low-density lipoprotein and diacylglycerol acyltransferase levels, downregulating hydroxyl methyl-glutaryl coenzyme A reductase, and upregulating cholesterol 7-alpha-hydroxylase or cytochrome P450 7A[9]. Although PM shows treatment efficacy against MAFLD through regulating mitochondria, the bioactive substances involved are still unclear, which acutely limits the development and utilization of PM.

In our previous study, a new strategy of mitochondrial pharmacology and pharmacochemistry for the investigation of pharmacodynamic substances in traditional Chinese medicines (TCMs) was proposed. Using this strategy, the main active ingredients in PM that adjust mitochondria to relieve MAFLD were identified. The results indicated that this strategy could be applied for the efficient search for *in vivo* mitochondria-regulated constituents in TCMs[10].

Here, we investigated the bioactive substances of PM extracts remedying mitochondria to alleviate MAFLD induced by a high-fat diet (HFD) through mitochondrial pharmacology and pharmacochemistry, after determining the efficacy and potential principle of PM on MAFLD and mitochondria *in vitro* and *in vivo* pharmacodynamic tests. The results showed that PM relieved HFD-induced MAFLD by improving oxidative stress and energy production. The five substances recognized by the proposed method may be the main active ingredients in PM that adjust mitochondria to relieve MAFLD. Thus, PM represents a promising drug for the treatment of MAFLD-related mitochondrial dysfunction to relieve MAFLD. Additionally, mitochondrial pharmacology and pharmacochemistry were efficient strategies for identifying the bioactive ingredients of TCMs regulating mitochondria to prevent human disorders.

MATERIALS AND METHODS

Reagents and chemicals

Standards including resveratrol [(RES), ≥ 98%], emodin [(ED), ≥ 98%], aloe-emodin [(AE), ≥ 98%], emodin 8-O-glucoside [(EG), ≥ 98%], 2,3,5,4′-tetrahydroxy stilbene 2-O-β-D-glucoside [(THSG), ≥ 98%], and physcion [(PN), ≥ 98%] were obtained from Chengdu Pufei De Biotech Co., Ltd. (Chengdu, China). Torachrysone [(TCS), ≥ 98%] was purchased from Yunnan Xili biology science and technology co., ltd (Kunming, China). The dried root of PM was collected from the Luoshiwan Chinese Medicine Market (Kunming, China). Dried PM decoction pieces were verified by Professor Jie Yu. PM credential specimens (No. 19080322) were stored in the Key Laboratory of Southern Medicine Utilization, Yunnan University of Chinese Medicine (Kunming, China). The other chemicals and reagents used were described in our previous report[10].

PM extract preparation

After PM was processed according to the method specified in the 2020 Chinese Pharmacopoeia and decocted with 10 times the amount of 90% ethanol for 110 min. The filtrate was collected after filtration. Dregs were decocted with 10 times 50% ethanol for 110 min and filtered. Then, two filtrates were mixed and condensed at 65 °C. Finally, the concentrate was freeze-dried into a powder.

The chemical characteristics of PM extract were assayed according to the regulation described in the 2020 Chinese Pharmacopoeia. PM was assayed by Shimadzu LC-30A ultra-high performance liquid chromatography (UHPLC). Analytical conditions were as follows: (1) Shimadzu UHPLC column (Shim-pack XR-ODS C8, 100 mm × 2.0 mm I.D., 2.2 μm); (2) Mobile phase for the separation of ED and PN: methanol and 0.1% aqueous phosphoric acid solution (80:20 v/v); (3) Mobile phase for the separation of THSG: Acetonitrile and water solution (25:75 v/v); (4) Detection wavelength: 254 nm for ED and PN; 320 nm for THSG; (5) Temperature: 30 °C; (6) Flow rate: 0.2 mL/min; and (7) Volume: 10 μL. References of ED, PN, and THSG were dissolved in methanol to yield contents of 0.08 mg/mL, 0.04 mg/mL, and 0.2 mg/mL, respectively. For the detection of THSG and for the detection of ED and PN, 0.1 g and 0.02 g, respectively, of PM powder was dissolved in 1.0 mL of methanol. Concentrations of free anthraquinone (ED and PN) and THSG in the PM extract were 0.57% and 0.71%, respectively.

In vitro assay of anti-steatosis activity of PM extract

L02 cells were grown in RPMI 1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C and 5% CO_2. After growing to about 80%, the cells were treated with lipid emulsion. Then, the cells were stimulated by PM extracts (10, 50, and 100 μg/mL) and fenofibrate capsules (150 μmol/L) or a media (control) for 24 h. Bicinchoninic acid (BCA) assay was used to analyze protein concentrations in cell lysates. Triglyceride (TG), total cholesterol (TC), aspartate transaminase (AST), alanine transaminase (ALT), superoxide dismutase (SOD), glutathione (GSH), ATP synthase (ATPase), complex I, and complex II levels were determined using the kits. All data were obtained by the microplate reader. Furthermore, for the detection of intracellular neutral lipid accumulation, L02 cells were seeded into 6-well chambers. The collected cells were washed twice with ice-cold phosphate-buffered saline and fixed with 2.5% paraformaldehyde for 30 min. Afterward, the cells were added with 60% isopropanol and cultured for 5 min. Then, cells were stained with 0.2% Oil red O solution for 30 min. After washing twice with phosphate-buffered saline, cells were stained with hematoxylin for 3 min. Finally, images were obtained using a CX31 Olympus Imaging System.

In vivo determination of PM extract against MAFLD

Experimental animals: Healthy male Sprague Dawley rats (200 ± 50 g) were provided by Dashuo

Biotech. Co., Ltd. (Chengdu, China). All animals were raised in conditions of 22 ± 1 °C, a humidity of 60% ± 10%, and a light/dark cycle of 12 h with free access to food and water. The experiments in this study were approved by the Institutional Ethical Committee on Animal Care and Experimentations of Yunnan University of Chinese Medicine (R-06201965).

Grouping and administration of treatment: After 5-7 d of adaptive feeding, the rats were randomly divided into six groups (n = 6): (1) Normal control group (NC); (2) Model group (MOD); (3) RES group (40 mg/kg); (4) Low-dose PM group (2 g/kg); (5) Middle-dose PM group (4 g/kg); and (6) High-dose PM group (8 g/kg). Rats were given the test samples by intragastric administration once a day for 3 mo. Except for the NC group, rats in all groups were fed an HFD (10% egg yolk, 10% refined lard, 79% basic diet, and 1% cholesterol) to induce MAFLD in rats for 3 mo.

Sample collection: The daily food intake of all rats was recorded, and weight was recorded once a week. At the end of the experiment, the rats were anesthetized by intraperitoneal injection with 10% sodium pentobarbital. Blood samples were obtained from the abdominal aorta and coagulated at 4 °C. Blood was then centrifuged at 3500 rpm for 15 min to acquire serum. The liver, kidneys, and spleen were weighed. A portion of the liver was applied for Oil red O staining and transmission electron microscopy examination as previously described[10], and the remaining portion was stored at -80 °C.

Measurement of biochemical indicators in the serum and liver: Serum levels of TC, TG, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), ALT, and AST were determined following the kit instructions. In addition, liver homogenates were prepared with nine volumes of normal saline and were centrifuged at 2000 r/min for 10 min. The levels of TC, TG, LDL-C, HDL-C, ALT, and AST in the liver supernatant were detected according to the instructions of the kits. BCA assay was used to determine protein concentration in the liver. All data were collected by a microplate reader.

Detection of liver mitochondrial indices: Liver mitochondria were obtained as in previous studies[11] and resuspended in saline. BCA assay was used to determine protein concentration. The levels of malondialdehyde (MDA), SOD, GSH, ATPase, and complex I and II in the suspension of mitochondria were detected according to the instructions of the kit. All data were collected by a microplate reader.

Activity evaluation and structural assignment of PM-derived constituents in mitochondria from HFD-induced rats

Production of liver mitochondrial extracts: The mitochondria of rat livers in the MOD group and HPM group were separated as previously reported[11]. The obtained mitochondria were suspended in chromatographic methanol and then ultrasonicated for 20 min followed by centrifugation at 10000 × g at 4 °C for 25 min. The supernatant was collected and dried with mild nitrogen, and mitochondrial extracts were stored at -80 °C.

***In vitro* assay of anti-steatosis ability of PM-derived constituents in mitochondria:** L02 cells were stimulated with 5% fat emulsion for 24 h and then cultured with fenofibrate capsules (150 μmol/L) and mitochondrial extracts (50, 100, and 200 μg/mL) of rats in the MOD group and HPM group for 24 h. Cells were collected after being washed twice with phosphate-buffered saline. BCA assay was used to determine the protein concentration in the cell lysate. TC, TG, SOD, GSH, complex I, complex II, and ATPase levels in the cell were detected according to the instructions of the kits. All data were collected by a microplate reader. Additionally, cells stained with Oil red O were examined as in the aforementioned method.

Structural assignment of PM-derived constituents in liver mitochondria: PM extracts and liver mitochondrial extracts isolated from the MOD and HPM groups were redissolved in HPLC-grade methanol and filtered through a 0.22 μm Millipore filter (Bedford, MA, United States). The filtrate was analyzed by a UHPLC Dionex Ultimate 3000 system coupled with a Thermo Scientific Q-Exactive TM hybrid quadrupole-orbitrap mass spectrometry (MS) (Thermo Fisher Scientific, San Jose, CA, United States). The detailed analytical conditions of UHPLC and MS were described in our previous report[10].

***In vitro* assay of anti-steatosis ability of PM-derived monomer in mitochondria:** The anti-steatosis activity of TCS, a PM-derived monomer, on L02 cells induced by fat emulsion was measured. First, the cell activity of TCS at different concentrations (0, 6.25, 12.5, 25, 50, 100, and 200 μmol/L) was measured according to the CCK-8 kit's instructions. Subsequently, fat emulsion-treated steatosis cells were introduced with TCS (12.5 and 25 μmol/L). BCA assay was used to analyze the protein concentration of the cell lysate. The levels of TG, TC, AST, ALT, SOD, GSH, and ATPase were measured on the microplate reader according to the instructions of the kits.

***In vivo* determination of anti-MAFLD activity of the PM-derived monomer group in liver mitochondria:** Effects of the PM-derived monomer group on MAFLD were evaluated on HFD-induced mice. The monomer group consisted of ED, AE, EG, and THSG in a ratio of 14.87:1:10.03:18.37. This ratio was the concentration ratio of these molecules in the PM extract counted according to the areas of the

chromatographic peak obtained in the UHPLC/MS assay of PM. Male C57BL/6J mice (20 ± 2 g) were provided from Hunan SJA Laboratory Animal Co., Ltd. (Hunan, China). After 5-7 d of adaptive feeding, mice were fed with a normal chow diet as the control group (n = 6), and other groups were fed an HFD for 8 wk to induce MAFLD. Mice in the treatment groups (n = 6) were intraperitoneally injected as follows: (1) A low-dose PM-derived monomer group in the mitochondria (30 mg/kg/d); (2) High-dose PM-derived monomer group in the mitochondria (90 mg/kg/d); and (3) RES (18 mg/kg/d). MAFLD was induced by an HFD for 3 mo. Starting from the 9th wk, mice received an intraperitoneal injection with the test samples once a day for 4 wk. The methods of sample collection and serum, liver, and mitochondrial indices detection were the same as the experiment of PC extract administered to rats described above.

Statistical analysis

All data were represented as the mean ± SD. Statistical analysis was performed on GraphPad Prism version 8.0 software for Windows (San Diego, CA, United States). One-way analysis of variance was used for comparison between groups. Statistical significance is displayed as $P < 0.05$.

RESULTS

Effects of PM extract on fat emulsion triggered L02 adipocytes

The inhibitory activity of PM extracts was assessed by 5% fat emulsion-induced intracellular lipid accumulation in L02 cells. Oil red O staining revealed that fat emulsion-treated cells had higher intracellular lipid levels compared to untreated L02 cells (NC). However, treatment with PM extracts greatly blocked fat emulsion-induced lipid accumulation (Figure 1A). As shown in Figures 1B-K, after 24 h of stimulation with fat emulsion, TG, TC, AST, and ALT levels remarkably increased, and GSH, SOD, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, complex I, and complex II levels declined. However, after PM incubation, ALT and AST levels decreased, and SOD, GSH, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, and complex II levels markedly increased. The levels of TC and TG showed a decreasing trend, and the level of complex I showed an increasing trend. Together, these findings suggested that PM extract relieved the steatosis of L02 cells.

Effects of PM extract on MAFLD rats induced by HFD-feeding

Effect of PM on body weight, food consumption, and organ indices of rats: As shown in Table 1, without affecting food intake, rats fed an HFD for 3 mo gained weight at the end of the study. Moreover, after 3 mo of HFD feeding, the liver index of rats increased remarkably, and body weight, kidney index, and spleen index showed an increasing trend. However, the administration of PM extract significantly decreased the spleen index, and body weight, liver index, and kidney index showed a decreasing trend. Thus, these data suggested that PM relieved HFD-induced weight gain and organ enlargement.

Effects of PM extract on serum parameters: As displayed in Figures 2A-F, after 3 mo of an HFD, ALT levels dramatically increased, while HDL-C levels markedly decreased. Additionally, the levels of TC, TG, AST, and LDL-C showed an increasing trend. After administration with PM extract, TG, ALT, and AST levels decreased obviously, and HDL-C level markedly increased. LDL-C levels showed a decreasing trend. Taken together, these data suggested that PM relieved HFD-induced dyslipidemia.

Effects of PM extract on liver status: Figure 3A shows the massive accumulation of lipid droplets, diffuse hepatocellular edema, and degeneration in the liver of rats after 3 mo of HFD feeding. However, after administration with PM extract and RES, the lipid droplets in the liver cells of rats reduced, and liver status tended to be normal. Additionally, Figures 3B-E shows that the levels of TC and TG improved obviously after 3 mo of HFD feeding. The level of AST showed an increasing trend, and the level of HDL-C showed a decreasing trend. After treatment with PM extract, TG and AST levels dramatically declined, and HDL-C levels dramatically increased. Thus, these findings suggested that PM extract reduced lipid accumulation in the liver of HFD-fed rats.

Effects of PM extract on mitochondrial status: Figure 4A shows that after the feeding of an HFD, the liver mitochondria swelled. The inner and outer membranes and ridges were blurred, and the matrix was unclear, as indicated by the red arrow in the MOD group. However, the ultrastructural appearance of mitochondria was reversed by PM treatment. Additionally, Figures 4B-H shows that after the feeding of an HFD, SOD, complex I, and complex II levels in the mitochondria decreased obviously. Furthermore, GSH, Na^+-K^+-ATPase, and Ca^{2+}-Mg^{2+}-ATPase levels in liver mitochondria showed a decreasing trend, and the MDA level in the liver mitochondria showed an increasing trend. After administration with PM extract, SOD, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, complex I, and complex II levels remarkably increased, and the level of MDA remarkably decreased. The level of GSH showed an increasing trend. Combined, these indices suggested that PM prevented damage to mitochondrial

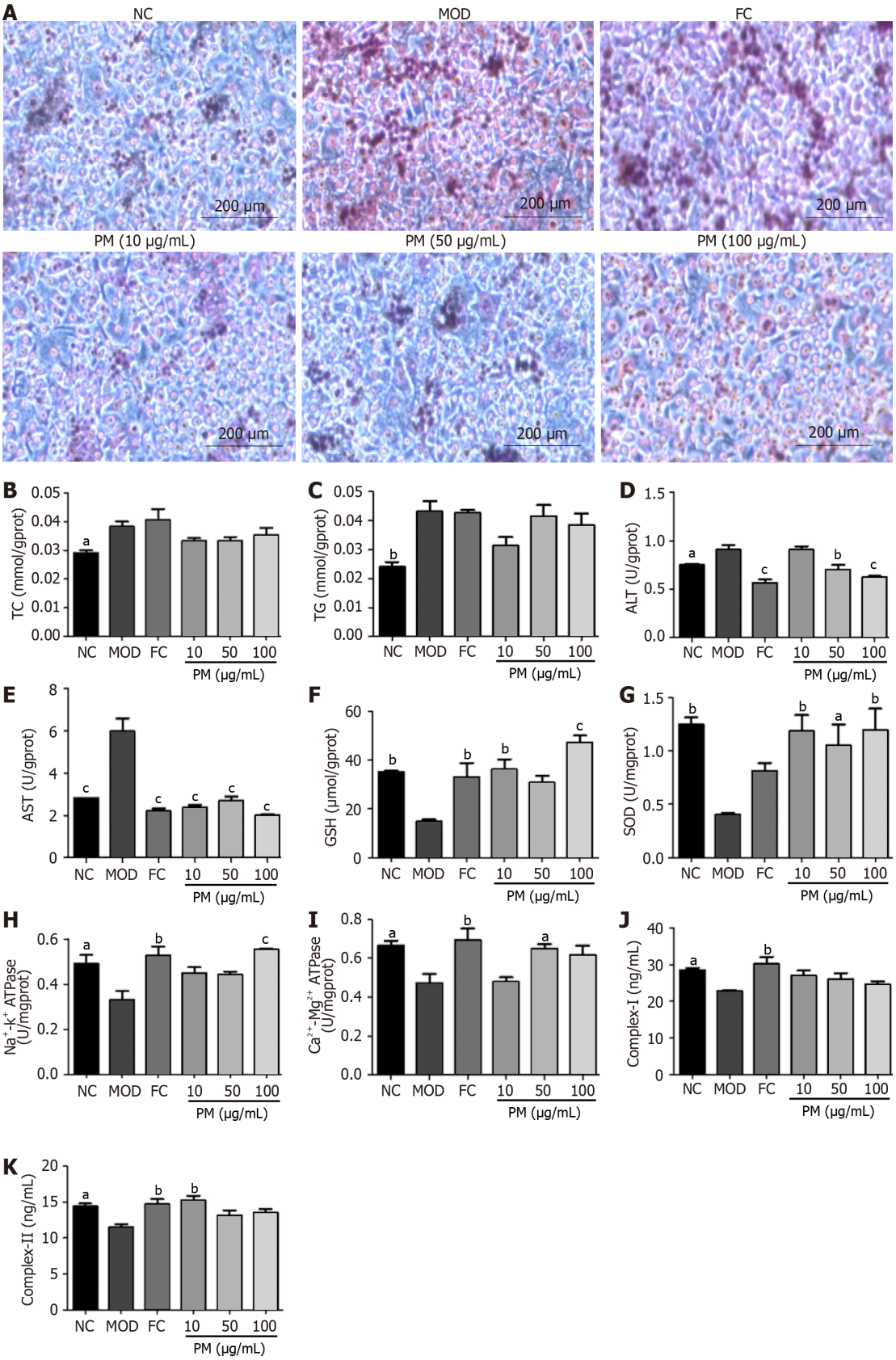

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Figure 1 Effects of *Polygonum multiflorum* extract on L02 adipocytes induced by fat emulsion. A: L02 cells were treated with 5% fat emulsion for 24 h followed by *Polygonum multiflorum* extract (10, 50, and 100 μg/mL) or fenofibrate capsules (150 μmol/L) for 24 h. Cells were harvested, and fat accumulation in cells was detected under a light microscope (× 200) after Oil red O staining; B: Levels of total cholesterol was determined using commercial kits; C: Levels of triglyceride was determined using a commercial kit; D: Levels of alanine transaminase were determined using a commercial kit; E: Levels of aspartate transaminase were determined using a commercial kit; F: Levels of glutathione were determined using a commercial kit; G: Levels of superoxide dismutase were determined using

a commercial kit; H: Levels of Na^+-K^+-ATPase were determined using a commercial kit; I: Levels of Ca^{2+}-Mg^{2+}-ATPase were determined using a commercial kit; J: Levels of complex I were determined using a commercial kit; K: Levels of complex II were determined using a commercial kit. Data were obtained from 3 independent measurements and are expressed as the mean ± SD. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* model group. ALT: Alanine transaminase; AST: Aspartate transaminase; ATPase: ATP synthase; Complex I/II: human mitochondrial respiratory chain complex I/II, FC: Fenofibrate capsules; GSH: Glutathione; MOD: Model; NC: Normal control; PM: *Polygonum multiflorum*; SOD: Superoxide dismutase; TC: Total cholesterol; TG: Triglyceride.

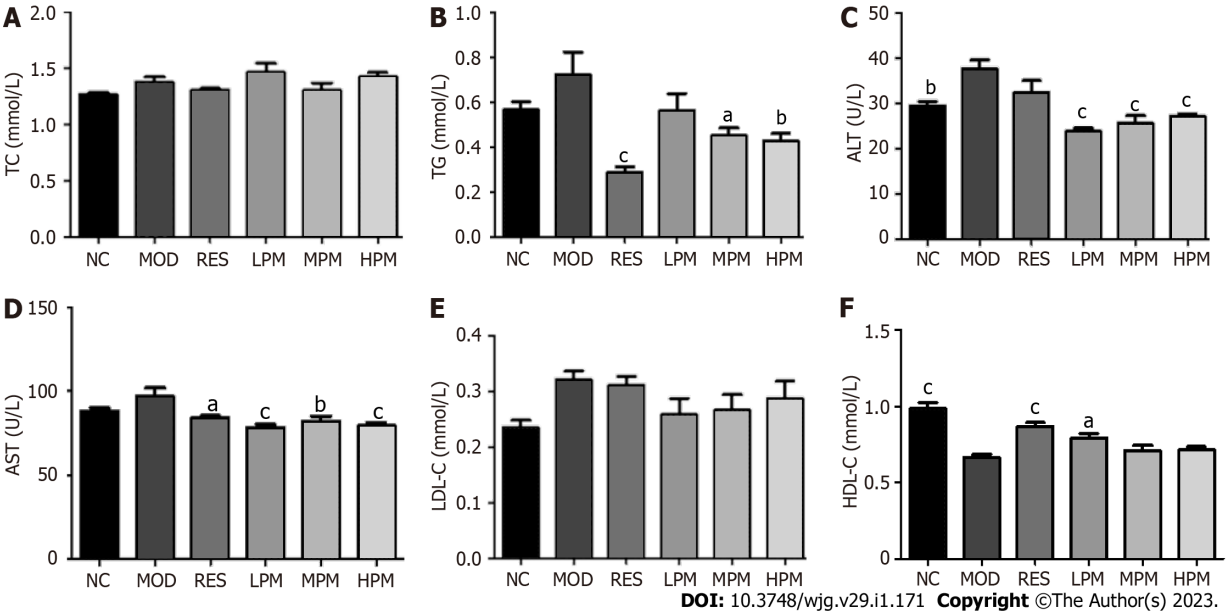

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Figure 2 Effects of *Polygonum multiflorum* extract on serum biochemical parameters of high-fat diet-fed rats. Sprague Dawley rats were administered saline, resveratrol, or *Polygonum multiflorum* extract (2, 4 or 8 g/kg/d). After 3 mo of high-fat diet feeding, rats were sacrificed, and serum was collected. A: Serum levels of total cholesterol were determined using a commercial kit; B: Serum levels of triglyceride were determined using a commercial kit; C: Serum levels of alanine transaminase were determined using a commercial kit; D: Serum levels of aspartate transaminase were determined using a commercial kit; E: Serum levels of low-density lipoprotein cholesterol were determined using a commercial kit; F: Serum levels of high-density lipoprotein cholesterol were determined using a commercial kit. Values represent the mean ± SD from six animals. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* model group. ALT: Alanine transaminase; AST: Aspartate transaminase; HDL-C: High-density lipoprotein cholesterol; HPM: High-dose *Polygonum multiflorum* extract; LDL-C: Low-density lipoprotein cholesterol; LPM: Low dose *Polygonum multiflorum* extract; MOD: Model; MPM: Middle-dose *Polygonum multiflorum* extract; NC: Normal control; RES: Resveratrol; TC: Total cholesterol; TG: Triglyceride.

structure and functions to alleviate MAFLD.

Effects of PM-derived constituents in mitochondria on adipocytes induced by fat emulsion

Oil red O staining revealed that fat emulsion-triggered L02 cells had higher intracellular lipid levels compared to untreated L02 cells (NC). After liver mitochondrial extracts from HPM treatment, intracellular lipid accumulation was prevented and was not prevented by liver mitochondrial extracts from MOD treatment (Figure 5A). As shown in Figures 5B-I, after incubation with fat emulsion, the levels of TC and TG increased, and GSH, SOD, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, complex I, and complex II levels decreased. After incubation with liver mitochondrial extracts from HPM, TC levels decreased, and GSH, SOD, Na^+-K^+-ATPase, and Ca^{2+}-Mg^{2+}-ATPase levels increased. Furthermore, the TG level showed a downward trend, and levels of complex I and complex II displayed an increasing trend. However, liver mitochondrial extracts from MOD treatment did not reverse the pathological changes induced by fat emulsion, except for the significantly reversed TC level. These results showed that PM-derived constituents in the liver mitochondrial extracts ameliorated cellular steatosis by regulating oxidative stress and energy production.

Structural assignment of PM-derived constituents in liver mitochondria

After analyzing accurate high-resolution MS_n data provided by UHPLC/MS (Table 2) and comparing the data with antecedently described[12-19] and standards, eight PM-derived monomers, including five prototype monomers and three of their metabolites (Figure 6), were screened in mitochondrial liver extracts of PM-treated rats. Among the identified monomers, there were one stilbene, five anthraquinones, and two naphthols. Prototype monomers consisted of ED, AE, EG, THSG, and TCS. The metabolites comprised emodin-glucuronide, torachrysone-glucuronide, and emodin-8-O-β-D-glucopyranoside reduction metabolite, which originated from ED, THCS, and EG, respectively.

Table 1 Effects of *Polygonum multiflorum* extract on body weight, food intake, and organ indices of high-fat diet-fed rats

	NC	MOD	RES	LPM	MPM	HPM
Initial body weight (g)	263.67 ± 23.43	263.83 ± 28.54	261.50 ± 16.99	275.83 ± 6.05	268.83 ± 12.42	246.00 ± 15.09
Final body weight (g)	458.67 ± 46.43	470.17 ± 89.14	424.67 ± 25.97	454.67 ± 16.98	479.83 ± 33.88	432.83 ± 47.94
Body weight gain (g)	195.00 ± 23.00	206.33 ± 60.60	163.17 ± 8.98	178.83 ± 10.93	211.00 ± 21.46	186.83 ± 32.86
Food intake (g)	15852.54	14023.10	14265.09	13828.25	13081.76	13645.74
Liver index (%)	2.15 ± 0.09[b]	2.64 ± 0.22	2.32 ± 0.12[a]	2.55 ± 0.11	2.49 ± 0.16	2.49 ± 0.20
Kidney index (%)	0.52 ± 0.02	0.54 ± 0.07	0.52 ± 0.02	0.50 ± 0.02	0.49 ± 0.03	0.54 ± 0.04
Spleen index (%)	0.1589 ± 0.01	0.1618 ± 0.02	0.1432 ± 0.01	0.1267 ± 0.01[b]	0.1427 ± 0.01	0.1345 ± 0.01[a]

[a]$P < 0.01$ *vs* model group.
[b]$P < 0.001$ *vs* model group.
Values represent the mean ± SD from six animals. HPM: High-dose *Polygonum multiflorum* extract; LPM: Low dose *Polygonum multiflorum* extract; MOD: Model; MPM: Middle-dose *Polygonum multiflorum* extract; NC: Normal control; RES: Resveratrol.

Table 2 Ultra-high performance liquid chromatography/mass spectrometry data and chemical structure assignment of *Polygonum multiflorum*-derived monomers from liver mitochondria

No	T_R (min)	(M-H)- (m/z)	Molecular formula	Predicted (m/z)	Measured (m/z)	Diff (ppm)	ESI-MSn(-), m/z (abundance)	Assigned identification	Ref.
1	18.87	269.0454	$C_{15}H_{10}O_5$	269.0450	269.0454	3.457	269.04541 (100.00), 241.05089 (2.18), 225.14899 (1.98)	Aloe-emodin[1]	[12-14]
2	15.90	431.0981	$C_{21}H_{20}O_{10}$	431.0978	431.0981	2.011	431.109811 (34.23), 311.05627 (4.32), 269.04553 (100.00), 240.04503 (0.69)	Emodin-8-O-glucoside[1]	[14-16]
3	33.91	269.0457	$C_{15}H_{10}O_5$	269.0450	269.0457	4.461	269.04553 (100), 241.05331 (0.18), 225.05482 (1.37)	Emodin[1]	[15-19]
4	18.80	445.0778	$C_{21}H_{18}O_{11}$	445.0771	445.0778	2.881	269.04553 (100.00), 225.05527 (0.19)	Emodin-glucuronide	[17]
5	9.06	405.1190	$C_{20}H_{22}O_9$	405.1186	405.1190	2.422	243.06613 (62.27)	2,3,5,4′-Tetrahydroxy stilbene-[1]	[17]
6	13.17	433.1136	$C_{21}H_{22}O_{10}$	433.1135	433.1136	1.539	257.08191 (100.00), 175.02435 (8.79), 152.99582 (3.58), 113.02423 (59.47)	Emodin-8-O-β-D-glucopyranoside reduction metabolite	[17]
7	13.46	245.0820	$C_{14}H_{14}O_4$	245.0814	245.0820	4.834	245.08208 (87.57), 230.05850 (9.07)	Torachrysone[1]	[17]
8	15.15	421.1142	$C_{20}H_{22}O_{10}$	421.1135	421.1142	3.032	245.08179 (76.39), 230.05852 (4.02)	Torachrysone-glucuronide	[17]

[1]Compared with reference substances.

Effects of PM-derived monomer in mitochondria of steatosis cells

As shown in Figure 7, after incubation with fat emulsion, the levels of TC, TG, ALT, and AST dramatically increased, and GSH, SOD, Na^+-K^+-ATPase, and Ca^{2+}-Mg^{2+}-ATPase levels dramatically decreased. After TCS stimulation, TC and AST levels decreased, and the levels of GSH, SOD, and Ca^{2+}-Mg^{2+}-ATPase increased. The levels of TG and ALT showed a decreasing trend. These data suggested that TCS mitigated fat emulsion-triggered cellular steatosis.

Effects of PM-derived monomer group in mitochondria on MAFLD mice induced by HFD-feeding

Effects of PM-derived monomer group on body weight, food consumption, and organ index of MAFLD mice: As shown in Table 3, without affecting food intake, mice fed an HFD for 3 mo gained weight at the end of the study. The liver and kidney index increased obviously, and the spleen index showed an increasing trend. After treatment with the PM-derived monomer group, the liver index decreased. Body weight and the kidney index showed a decreasing trend, whereas the spleen index level increased in the HMG group. Thus, these data indicated that the PM-derived monomer group reduced weight gain and organ index swelling caused by HFD.

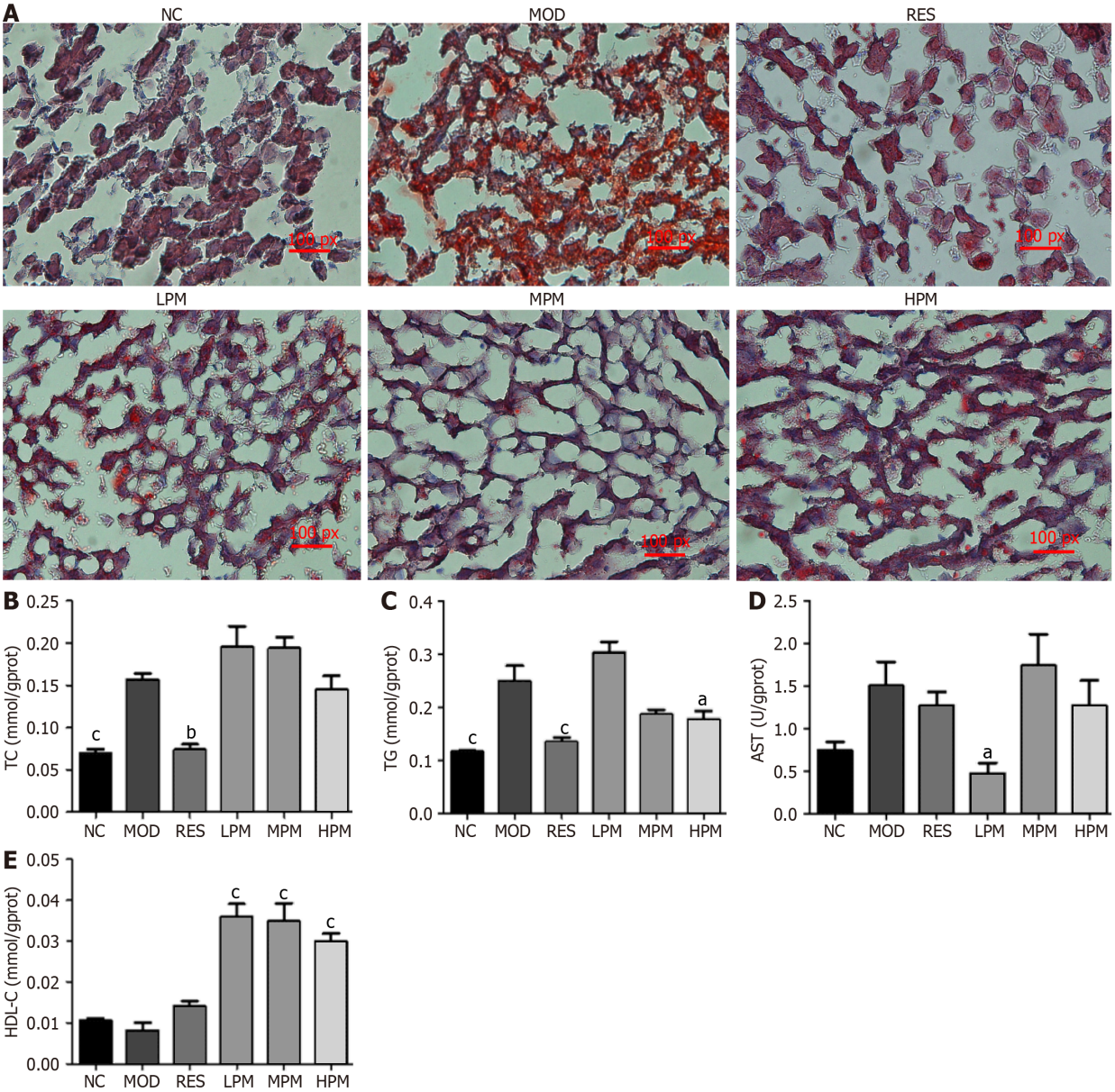

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Figure 3 Effects of *Polygonum multiflorum* extract on the liver lipid content of high-fat diet-fed rats. Sprague Dawley rats were administered saline, resveratrol, or *Polygonum multiflorum* extract (2, 4, or 8 g/kg/d). After 3 mo of high-fat diet feeding, rats were sacrificed, and livers were harvested. A: Liver histology after Oil Red O staining was observed under a light microscope (× 200); B: Liver levels of total cholesterol were determined using a commercial kit; C: Liver levels of triglyceride were determined using a commercial kit; D: Liver levels of aspartate transaminase were determined using a commercial kit; E: Liver levels of high-density lipoprotein cholesterol were determined using a commercial kit. Values represent the mean ± SD from six animals. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* the relevant model group. AST: Aspartate transaminase; HDL-C: High-density lipoprotein cholesterol; HPM: High-dose *Polygonum multiflorum* extract; LPM: Low dose *Polygonum multiflorum* extract; MOD: Model; MPM: Middle-dose *Polygonum multiflorum* extract; NC: Normal control; RES: Resveratrol; TC: Total cholesterol; TG: Triglyceride.

Effects of the PM-derived monomer group on serum indicators: Figures 8A-C show that after 3 mo of HFD feeding, TC and LDL-C levels increased obviously, and the level of AST showed an increasing trend. After treatment with the PM-derived monomer group, the levels of TC, AST, and LDL-C were decreased. Thus, the results show that the PM-derived monomer group alleviated HFD-induced dyslipidemia.

Effects of the PM-derived monomer group on the liver lipid content: As displayed in Figure 9A, after 3 mo of HFD feeding, the livers exhibited lipid accumulation, diffuse hepatocellular edema, and degeneration. However, this status of the livers was restored to normal after administration with PM-derived monomer groups. Figures 9B and 9D shows that after 3 mo of HFD consumption, ALT and AST levels increased, and the level of TC showed an increasing trend. After administration with the PM-derived monomer group, levels of TC, ALT, and AST decreased obviously. These data suggested that the PM-derived monomer group reduced lipid accumulation in the livers of HFD mice.

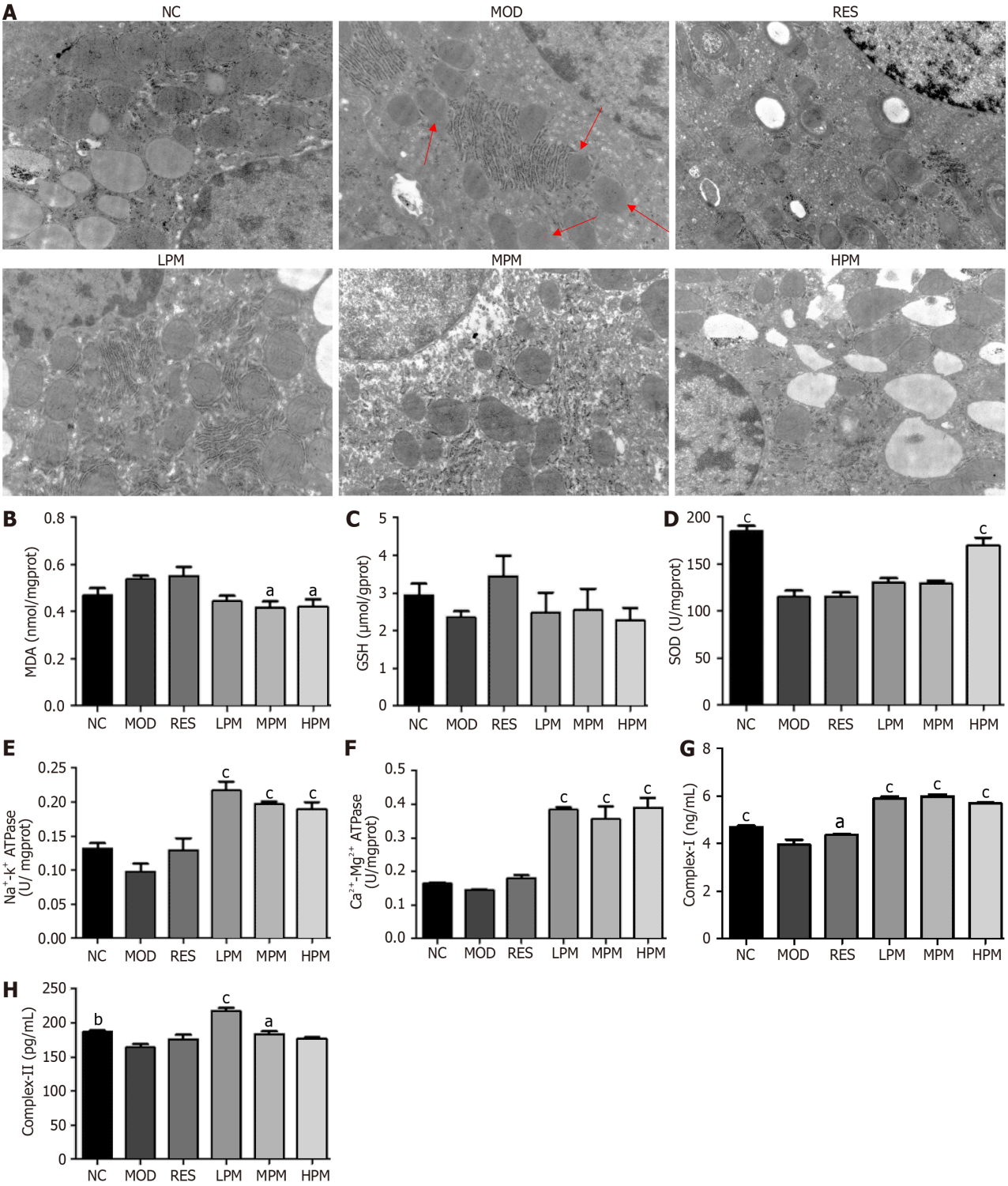

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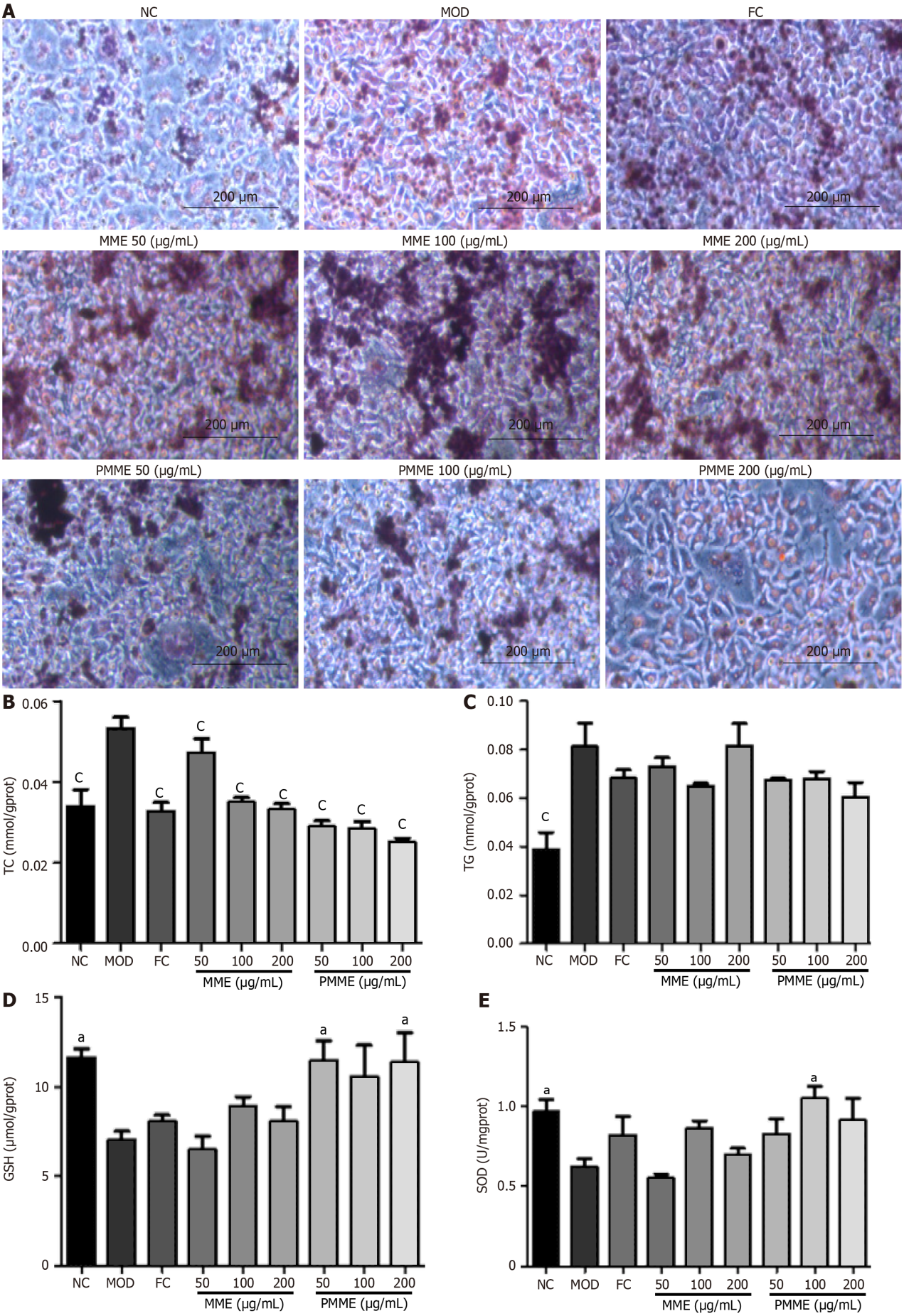
Figure 4 Effects of *Polygonum multiflorum* extract on the mitochondrial status of the liver from high-fat diet-fed rats. Sprague Dawley rats were administered saline, resveratrol, or *Polygonum multiflorum* extract (2, 4, or 8 g/kg/d). After 3 mo of high-fat diet feeding, rats were sacrificed, and livers were harvested for observation of the mitochondrial ultrastructure and mitochondrial isolations. A: Mitochondria were assessed by transmission electron microscope (original magnification × 30000; bar = 500 nm); B: Levels of malondialdehyde were determined using a commercial kit; C: Levels of glutathione were determined using a commercial kit; D: Levels of superoxide dismutase were determined using a commercial kit; E: Levels of Na^+-K^+-ATPase were determined using a commercial kit; F: Levels of Ca^{2+}-Mg^{2+}-ATPase were determined using a commercial kit; G: Levels of complex I were determined using a commercial kit; H: Levels of complex II in isolated liver mitochondria were determined using a commercial kit. Values represent the mean ± SD from six animals. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* model group. ATPase: ATP synthase; Complex I/II: rat mitochondrial respiratory chain complex I/II, GSH: Glutathione; HPM: High-dose *Polygonum multiflorum* extract; LPM: Low-dose *Polygonum multiflorum* extract; MDA: Malondialdehyde; MOD: Model; MPM: Middle-dose *Polygonum multiflorum* extract; NC: Normal control; RES: Resveratrol; SOD: Superoxide dismutase.


A
NC
MOD
FC
MME 50 (μg/mL)
MME 100 (μg/mL)
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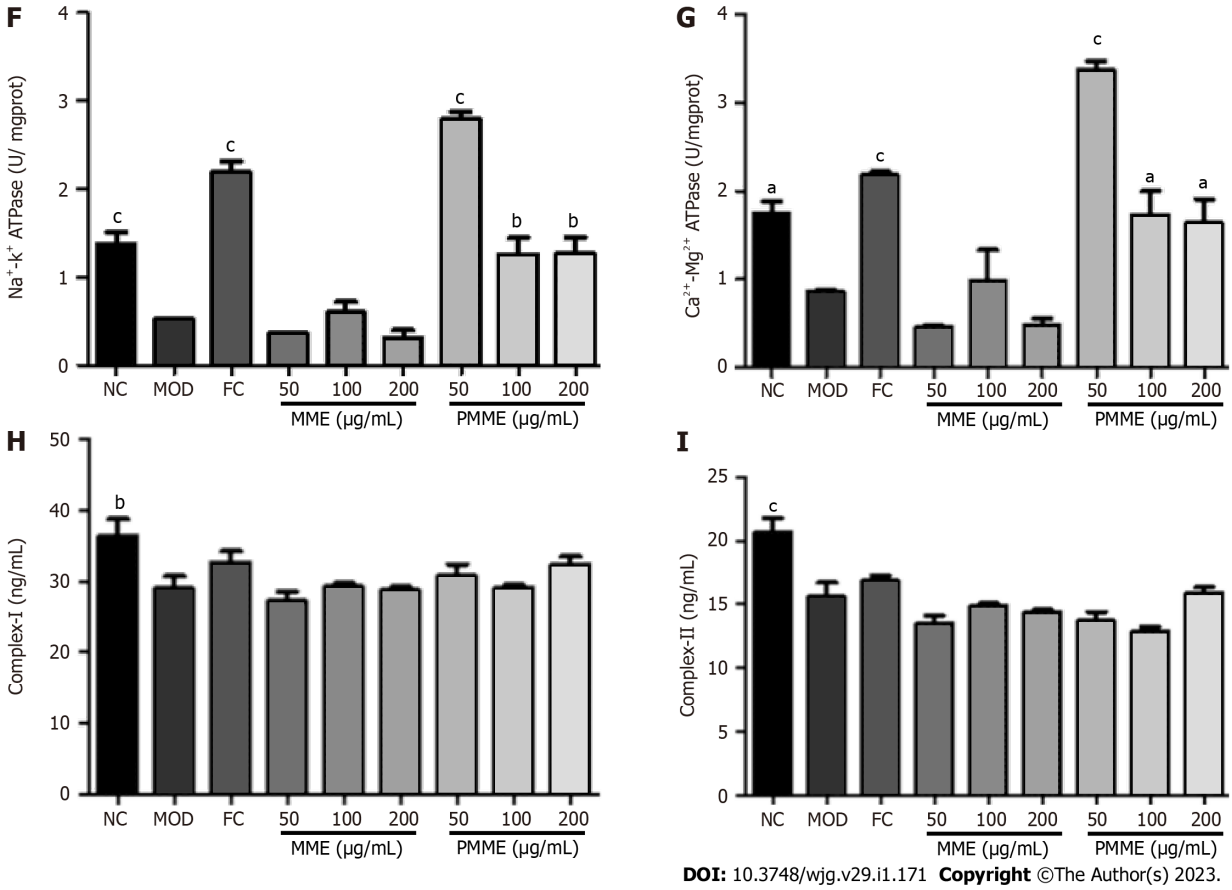

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Figure 5 Effects of *Polygonum multiflorum*-derived constituents in liver mitochondria on L02 adipocytes induced by fat emulsion. L02 cells were induced by fat emulsion for 24 h, then treated with liver mitochondrial extracts of the model group (50, 100, and 200 μg/mL), liver mitochondrial extract of high-dose of *Polygonum multiflorum* extract (50, 100, and 200 μg/mL), or fenofibrate capsules (150 μmol/L) for 24 h. Cells were harvested. A: Fat accumulation in cells was evaluated under a light microscope (× 200) after Oil Red O staining; B: Cell levels of total cholesterol were determined using a commercial kit; C: Cell levels of triglyceride were determined using a commercial kit; D: Cell levels of glutathione were determined using a commercial kit; E: Cell levels of superoxide dismutase were determined using a commercial kit; F: Cell levels of Na^+-K^+-ATPase were determined using a commercial kit; G: Cell levels of Ca^{2+}-Mg^{2+}-ATPase were determined using a commercial kit; H: Cell levels of complex I were determined using a commercial kit; I: Cell levels of complex II were determined using a commercial kit. Data were obtained from 3 independent measurements and are expressed as the mean ± SD. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* model group. ATPase: ATP synthase; Complex I/II: human mitochondrial respiratory chain complex I/II, FC: Fenofibrate capsules; GSH: Glutathione; MOD: Model; MME: Liver mitochondrial extracts from the model group; NC: Normal control; PMME: Liver mitochondrial extracts from the high-dose of *Polygonum multiflorum* extract group; SOD: Superoxide dismutase; TC: Total cholesterol; TG: Triglyceride.

Effects of PM-derived monomer group on the status of liver mitochondria: As shown in Figure 10A, after 3 mo of HFD feeding, the liver mitochondria swelled. The inner and outer membranes and ridges were blurred, and the matrix was unclear, as indicated by the red arrows in the MOD group. However, treatment with the PM-derived monomer group markedly improved the ultrastructure of mitochondria. Figures 10B and 10C displays that after 3 mo of HFD feeding, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase levels decreased obviously. However, after treatment with the PM-derived monomer group, the levels of Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase increased. The results showed that the PM-derived monomer group regulated the state of mitochondria to alleviate MAFLD.

DISCUSSION

In this study, fat emulsion-induced L02 adipocytes and HFD-induced MAFLD rats were established to evaluate the efficacy and underlying mechanism of action of PM on MAFLD and mitochondria. MAFLD primarily manifests as excessive liver fat accumulation, increased serum and liver TC, TG, ALT, AST, LDL-C levels, and decreased HDL-C level[20]. TC, TG, ALT, and AST in L02 cell levels were markedly increased by the induction of fat emulsion, which could be alleviated by PM extracts. Our data also showed that the contents of TC, TG, ALT, AST, and LDL-C in the serum and liver of rats fed with HFD increased, while the level of HDL-C decreased, which could be recovered after PM treatment. Moreover, significant lipid droplet accumulation was observed in the liver of rats fed an HFD, which could be recovered after PM treatment. Together, these findings indicated that PM effectively prevented lipid

Table 3 Effects of the *Polygonum multiflorum*-derived monomer group in liver mitochondria extract on body weight, food intake, and organ indices of rats

	NC	MOD	RES	LMG	HMG
Initial body weight (g)	20.03 ± 0.54	20.38 ± 0.32	20.40 ± 0.59	19.83 ± 0.92	20.33 ± 1.00
Final body weight (g)	22.90 ± 0.56[b]	25.68 ± 1.12	24.22 ± 1.29	25.20 ± 1.74	24.37 ± 1.50
Body weight gain (g)	2.87 ± 0.02	5.30 ± 0.79	3.82 ± 0.70	5.37 ± 0.82	4.03 ± 0.50
Food intake (g)	2274.20	2268.50	2655.80	2473.30	2290.60
Liver index (%)	3.52 ± 0.05[c]	4.24 ± 0.10	3.17 ± 0.11[c]	3.16 ± 0.11[c]	3.50 ± 0.21[c]
Kidney index (%)	1.00 ± 0.57[c]	1.20 ± 0.05	1.11 ± 0.08	1.15 ± 0.08	1.15 ± 0.08
Spleen index (%)	0.21 ± 0.03	0.31 ± 0.06	0.31 ± 0.04	0.30 ± 0.06	0.48 ± 0.22[a]

[a]$P < 0.05$ *vs* model group.
[b]$P < 0.01$ *vs* model group.
[c]$P < 0.001$ *vs* model group.
Values represent the mean ± SD from six animals. HMG: High-dose *Polygonum multiflorum*-derived monomer group; LMG: Low-dose *Polygonum multiflorum*-derived monomer group; MOD: Model; NC: Normal control; RES: Resveratrol.

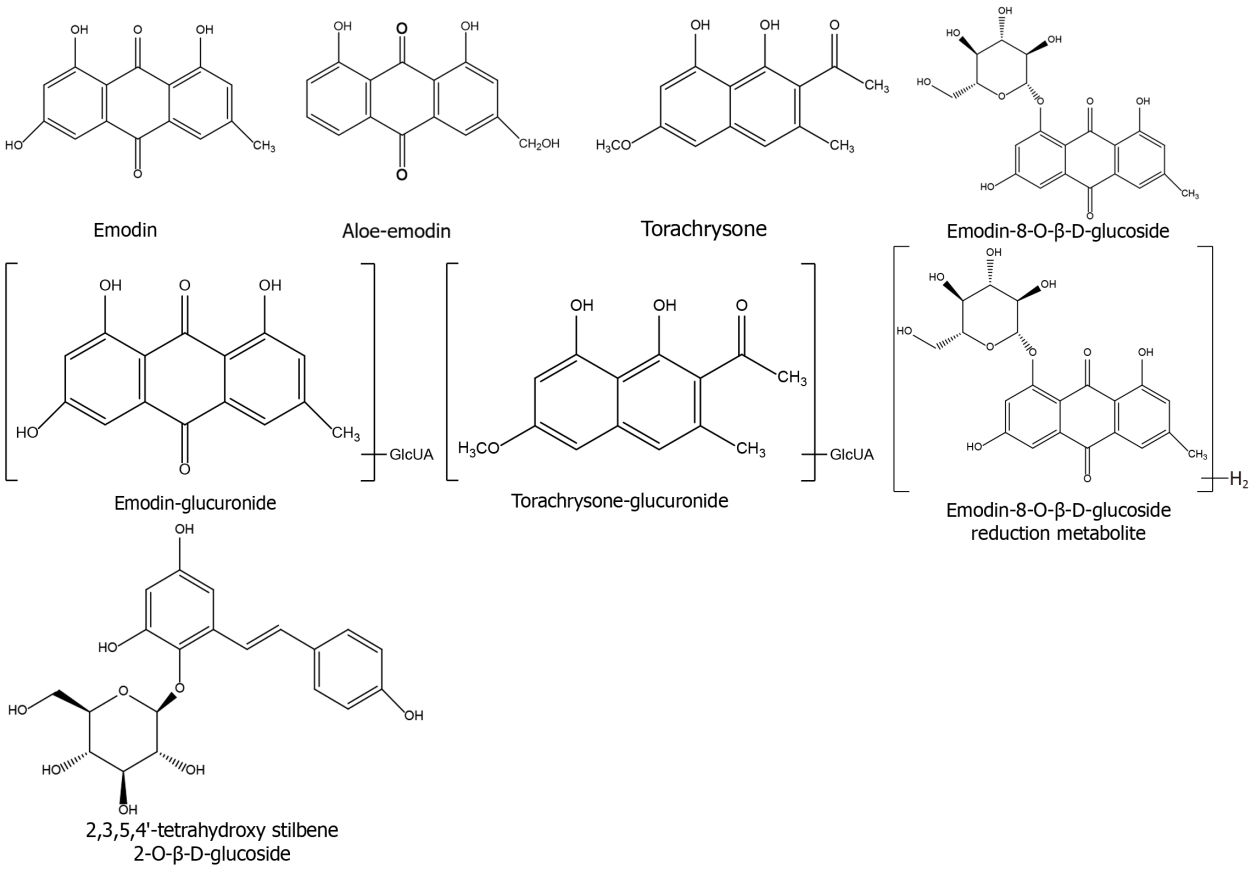

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Figure 6 Chemical structures of *Polygonum multiflorum*-derived constituents from the liver mitochondria extract. GlcUA: Glucuronidation.

metabolism disorders in MAFLD.

Mitochondria regulate a range of important physiological and biochemical cellular processes, such as energy production, apoptosis, oxidative stress, lipid metabolism, and calcium homeostasis. Multiple studies have shown evidence of molecular, biochemical, and biophysical mitochondrial abnormalities in MAFLD initiation and progression[21]. In this study, fat emulsion decreased GSH, SOD, Ca^{2+}-Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase, complex I, and complex II levels in hepatic cells, which could be recovered to a certain extent by PM treatment. Similarly, the mitochondria of MAFLD rats fed an HFD were swollen and damaged. The levels of SOD, GSH, ATPase, and complex I and II in liver mitochondria of MAFLD

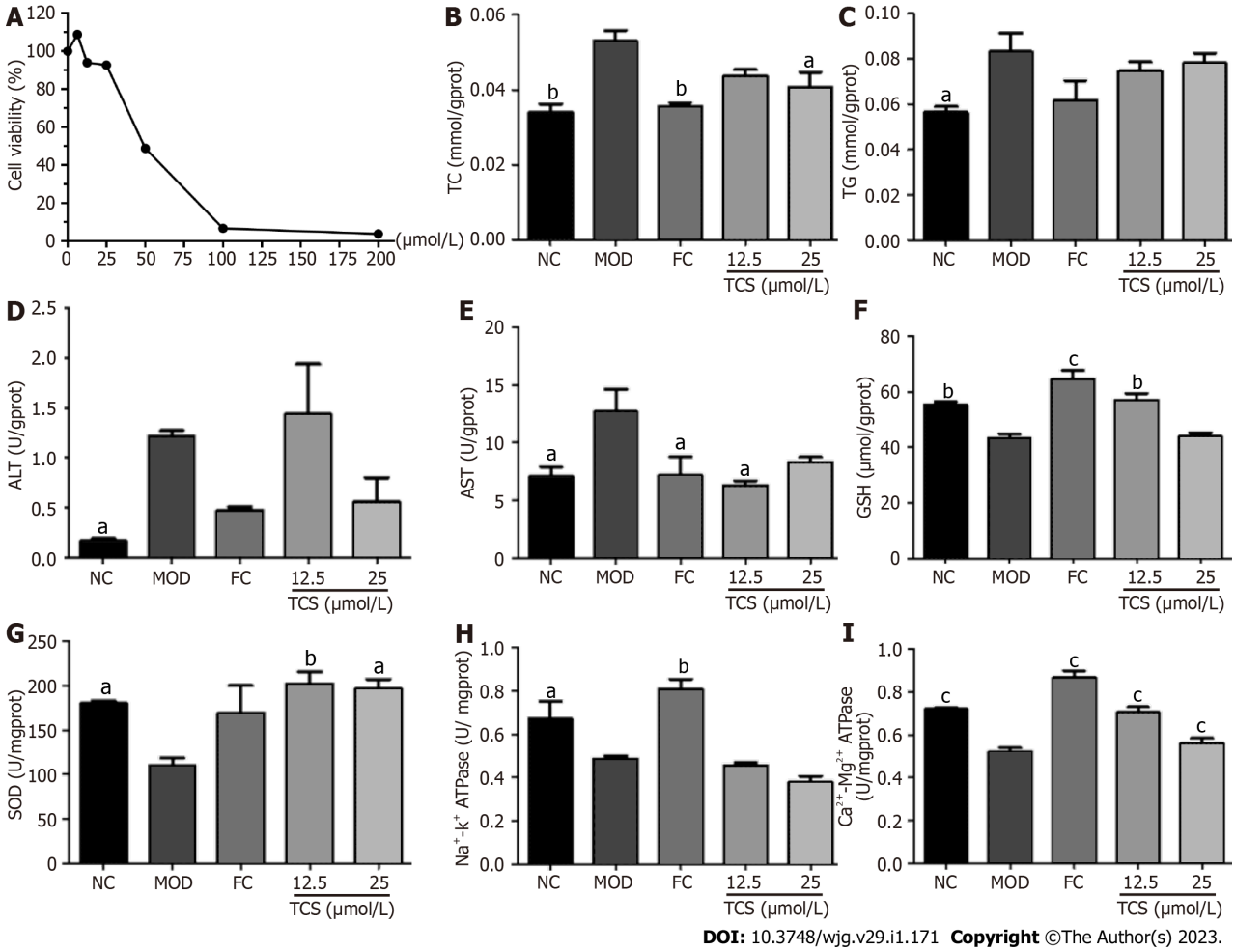

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Figure 7 Effects of *Polygonum multiflorum*-derived monomer in liver mitochondria on L02 adipocytes induced by fat emulsion. A: The cell counting kit-8 assay showed that torachrysone had no obvious cytotoxic effect on L02 cells at 12.5 and 25 μmol/L. These two concentrations were selected for *in vitro* anti-lipid activity detection. L02 cells were induced by fat emulsion for 24 h followed by stimulation with torachrysone (12.5 and 25 μmol/L) or fenofibrate capsules (150 μmol/L) for 24 h. Cells were harvested; B: Levels of total cholesterol were determined using a commercial kit; C: Levels of triglyceride were determined using a commercial kit; D: Levels of alanine transaminase were determined using a commercial kit; E: Levels of aspartate transaminase were determined using a commercial kit; F: Levels of glutathione were determined using a commercial kit; G: Levels of superoxide dismutase were determined using a commercial kit; H: Levels of Na^+-K^+-ATPase were determined using a commercial kit; I: Levels of Ca^{2+}-Mg^{2+}-ATPase were determined using a commercial kit. Data were obtained from 3 independent measurements and are expressed as the mean ± SD. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* model group. ALT: Alanine transaminase; AST: Aspartate transaminase; ATPase: ATP synthase; FC: Fenofibrate capsules; GSH: Glutathione; MOD: Model; NC: Normal control; SOD: Superoxide dismutase; TC: Total cholesterol; TG: Triglyceride; TCS: Torachrysone.

rats were greatly reduced, and the level of MDA increased, which could be reversed by PM treatment. These results suggested that PM extract significantly improved mitochondrial structure and function in the MAFLD process.

In our previous study, the strategy of mitochondrial pharmacology and pharmacochemistry was confirmed to possess the capability to screen the main active constituents of the TCMs regulating the mitochondria to prevent human disease[10]. In the present study, this strategy was used to reveal the pharmacodynamic substances in PM extract remedying mitochondria against MAFLD. Mitochondrial pharmacology confirmed that the PM-derived constituents that regulate oxidative stress and energy production to relieve steatosis of hepatocytes entered the liver mitochondria. Moreover, a total of eight PM-derived monomers that entered the mitochondria of MAFLD rats treated with PM extract were successfully identified by mitochondrial pharmacochemistry, including five prototype components (AE, ED, EG, THSG, and TCS), and three of their metabolites (emodin-glucuronide, emodin-8-O-β-D-glucopyranoside reduction metabolite, and torachrysone-glucuronide). Four monomers have been reported to cure MAFLD, and include AE[22,23], ED[22,24,25], EG[26], and THSG[27]. Our data also show that TCS improved oxidative stress and energy production to prevent steatosis in L02 adipocytes induced by fat emulsion. Furthermore, it was found that the PM-derived monomer group identified from liver mitochondria regulated the state of mitochondria to alleviate MAFLD in HFD-fed mice. Together, these results indicated that the eight substances were potentially major active constituents of PM against MAFLD, thereby validating the capability of mitochondrial pharmacology and pharmacochemistry for efficiently identifying *in vivo* mitochondria-regulated constituents in TCMs.

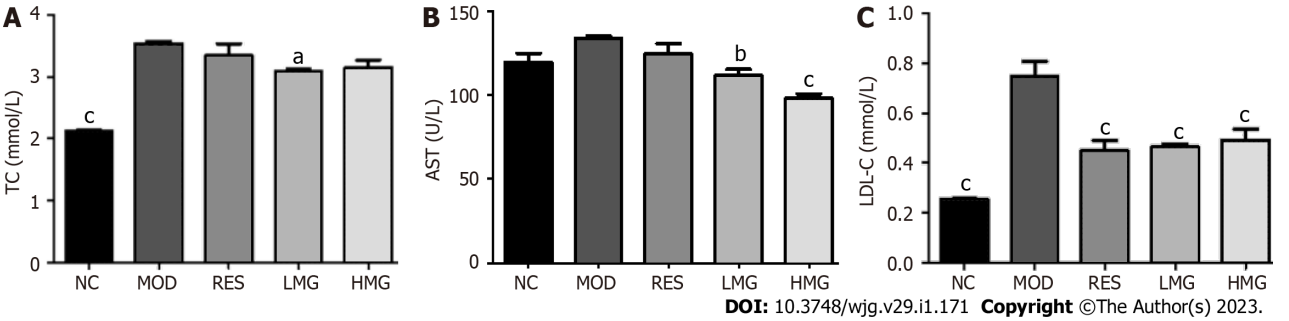

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Figure 8 Effects of *Polygonum multiflorum*-derived monomer group in liver mitochondria on serum biochemical parameters of high-fat diet-fed mice. Mice were administered saline, *Polygonum multiflorum*-derived monomer groups (30 and 90 mg/kg/d), or resveratrol (18 mg/kg/d). After 3 mo of high-fat diet feeding, mice were sacrificed, and serum was collected. A: Serum levels of total cholesterol were determined using a commercial kit; B: Serum levels of aspartate transaminase were determined using a commercial kit; C: Serum levels of low-density lipoprotein cholesterol were determined using a commercial kit. Values represent the mean ± SD from six animals. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* model group. AST: Aspartate transaminase; HMG: High-dose *Polygonum multiflorum*-derived monomer group; LDL-C: Low-density lipoprotein cholesterol; LMG: Low-dose *Polygonum multiflorum*-derived monomer group; MOD: Model; NC: Normal control; RES: Resveratrol; TC: Total cholesterol.

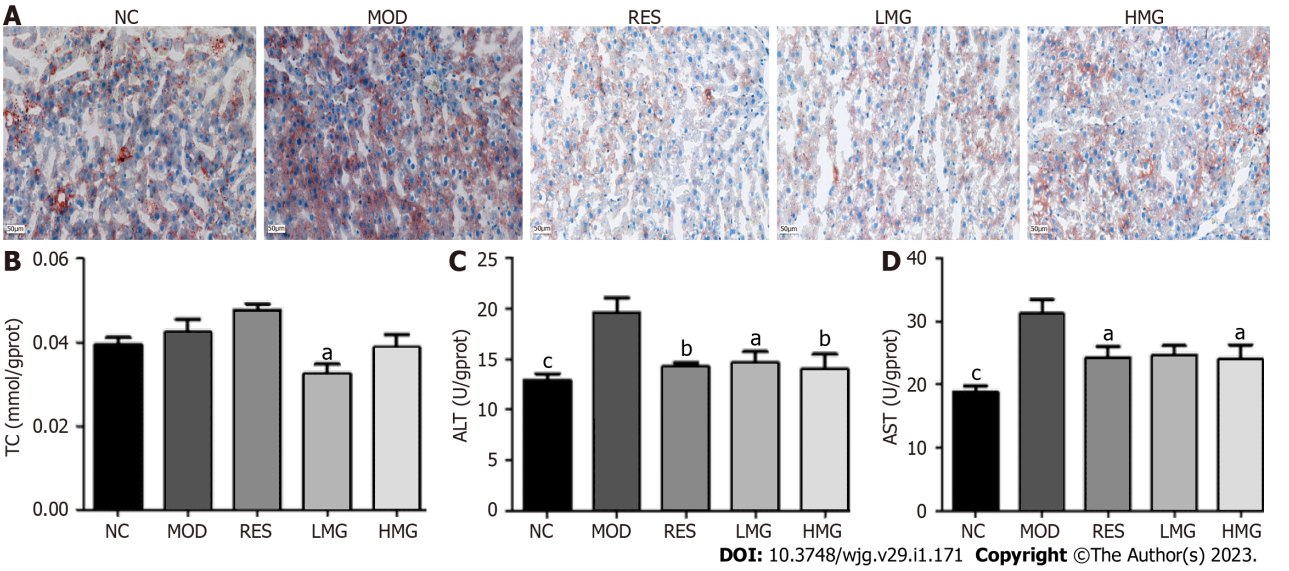

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Figure 9 Effects of *Polygonum multiflorum*-derived monomer group in liver mitochondria on the liver lipid content of high-fat diet-fed mice. Mice were administered saline, *Polygonum multiflorum*-derived monomer groups (30, and 90 mg/kg/d), or resveratrol (18 mg/kg/d). After 3 mo of high-fat diet feeding, mice were sacrificed, and livers were collected. A: Liver histology after Oil Red O staining was observed under a light microscope (× 200); B: Liver levels of total cholesterol were determined using a commercial kit; C: Liver levels of alanine transaminase were determined using a commercial kit; D: Liver levels of aspartate transaminase were determined using a commercial kit. Values represent the mean ± SD from six animals. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* the relevant model group. ALT: Alanine transaminase; AST: Aspartate transaminase; HMG: High-dose *Polygonum multiflorum*-derived monomer group; LMG: Low-dose *Polygonum multiflorum*-derived monomer group; MOD: Model; NC: Normal control; RES: Resveratrol; TC: Total cholesterol.

It is noted that the effects of PM extracts and their components on the detected indices did not show dose-dependence, which is similar to many other TCMs[20]. This may be caused by the complicated substances and action mechanisms of the TCMs, such as multi-components, multiple acting targets, and multiple ways[20]. Additionally, the doses of PM extract and its ingredients used in this study were likely not within the dose-dependent range. In addition, due to the insufficient liver samples, the depth analysis of mechanisms that PM extract and its monomer group regulated mitochondrial status to alleviate MAFLD in HFD-fed animals was not performed, which merits further investigation.

CONCLUSION

The data obtained in this study showed that PM extract restored mitochondrial structure and function and alleviated MAFLD caused by HFD, which may be related to the mitigation of oxidative stress-induced damage and the improvement of energy production. The eight substances obtained by the strategy may be the main bioactive ingredients in PM that regulate mitochondria to relieve MAFLD.

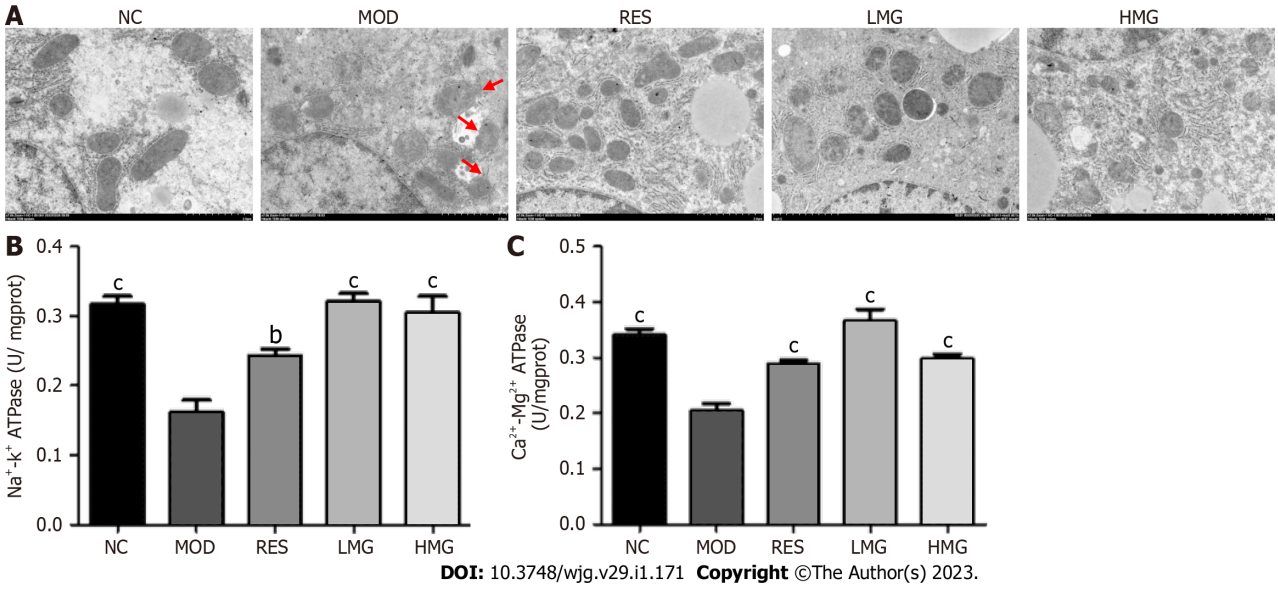

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Figure 10 Effects of *Polygonum multiflorum*-derived monomer group in liver mitochondria on mitochondrial status in the liver from high-fat diet-fed mice. Mice were administered saline, *Polygonum multiflorum*-derived monomer groups (30, and 90 mg/kg/d), or resveratrol (18 mg/kg/d). After 3 mo of high-fat diet feeding, mice were sacrificed, and livers were harvested for mitochondrial ultrastructure observations and mitochondrial isolations. A: Mitochondria were assessed by transmission electron microscope (original magnification × 30000; bar = 500 nm); B: Levels of Na^+-K^+-ATPase in isolated liver mitochondria were determined using a commercial kit; C: Levels of Ca^{2+}-Mg^{2+}-ATPase in isolated liver mitochondria were determined using a commercial kit. Values represent the mean ± SD from six animals. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ *vs* the relevant model group. ATPase: ATP synthase; HMG: High-dose *Polygonum multiflorum*-derived monomer group; LMG: Low-dose *Polygonum multiflorum*-derived monomer group; MOD: Model; NC: Normal control; RES: Resveratrol.

Thus, PM treatment provided a new method to prevent MAFLD-related mitochondrial dysfunction from alleviating MAFLD. Mitochondrial pharmacology and pharmacochemistry were shown to be efficient strategies for recognizing the bioactive ingredients of TCMs that alleviate mitochondria to prevent disease.

ARTICLE HIGHLIGHTS

Research background

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a serious threat to human health. Mitochondrial dysfunction is a mechanism involved in MAFLD. Modulation of mitochondrial function may become a novel strategy for the treatment of MAFLD. For centuries, *Polygonum multiflorum* (PM) has been used as a common traditional Chinese medicine and nutritional ingredient in China and has been proven to remedy mitochondria and further relieve MAFLD.

Research motivation

To date, the main pharmacodynamic ingredients of PM for regulating mitochondria against MAFLD remain unclear.

Research objectives

To investigate the pharmacodynamic ingredients for the mitochondrial remedy action of PM against high-fat diet (HFD)-induced MAFLD in rats.

Research methods

Fat emulsion-induced L02 adipocyte model and HFD-induced MAFLD rat model were used to evaluate the anti-MAFLD ability of PM and the mechanism of action involved. The adipocyte model was also used to determine the activities of PM-derived constituents in liver mitochondria from HFD-fed rats (mitochondrial pharmacology). PM-derived constituents in liver mitochondria were recognized by ultra-high performance liquid chromatography/mass spectrometry (mitochondrial pharmacochemistry). The abilities of the PM-derived monomer and the monomer group were evaluated by adipocyte model and MAFLD mouse model, respectively.

Research results

PM repaired mitochondrial ultrastructure and prevented oxidative stress and energy production disorder of mitochondria to mitigate fat emulsion-induced cellular steatosis and HFD-induced MAFLD. PM-derived constituents that entered the mitochondria inhibited oxidative stress damage and improved energy production against cellular steatosis. Eight chemicals were found in the mitochondria of PM-administrated rats. The anti-steatosis ability of one monomer and the anti-MAFLD activity of the monomer group were validated.

Research conclusions

PM restored mitochondrial structure and function and alleviated MAFLD, which may be associated with the remedy of oxidative stress and energy production. The identified eight chemicals may be the main bioactive ingredients in PM that adjust mitochondria to prevent MAFLD.

Research perspectives

PM treatment provided a new approach to prevent MAFLD-related mitochondrial dysfunction from alleviating MAFLD. Mitochondrial pharmacology and pharmacochemistry are efficient strategies for identifying the bioactive ingredients of traditional Chinese medicines regulating mitochondria to prevent disease.

FOOTNOTES

Author contributions: Yu LP, Li YJ, and Wang T contributed equally to this work; Li YJ wrote the manuscript; Li YJ, Yu LP, and Wang T performed the experiments; Tao YX and Zhang M provided technical support and suggestions; Yang XX, Yu LP, Zhang M, and Gu W participated in writing and modifying the manuscript; Yang XX and Yu J designed the study; and all authors approved the final manuscript.

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Institutional animal care and use committee statement: Approval was obtained from the Ethical Committee on Animal Care and Experimentation of the Yunnan University of Chinese Medicine (R-06201965).

Conflict-of-interest statement: All authors report no relevant conflicts of interest for this article.

Data sharing statement: All data used to support the findings of this study are available from the corresponding author upon reasonable request.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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REFERENCES

1 **Shao YL**, Fan JG. [Prevalence and harm of nonalcoholic fatty liver disease]. *Zhonghua Gan Zang Bing Za Zhi* 2019; **27**: 10-13 [PMID: 30685917 DOI: 10.3760/cma.j.issn.1007-3418.2019.01.004]
2 **Wang PX**, Ji YX, Zhang XJ, Zhao LP, Yan ZZ, Zhang P, Shen LJ, Yang X, Fang J, Tian S, Zhu XY, Gong J, Zhang X, Wei QF, Wang Y, Li J, Wan L, Xie Q, She ZG, Wang Z, Huang Z, Li H. Corrigendum: Targeting CASP8 and FADD-like apoptosis regulator ameliorates nonalcoholic steatohepatitis in mice and nonhuman primates. *Nat Med* 2017; **23**: 1241

[PMID: 28985205 DOI: 10.1038/nm1017-1241b]
3 **Xu X**, Poulsen KL, Wu L, Liu S, Miyata T, Song Q, Wei Q, Zhao C, Lin C, Yang J. Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). *Signal Transduct Target Ther* 2022; **7**: 287 [PMID: 35963848 DOI: 10.1038/s41392-022-01119-3]
4 **Chiang H**, Lu HF, Chen JC, Chen YH, Sun HT, Huang HC, Tien HH, Huang C. Adlay Seed (*Coix lacryma-jobi* L.) Extracts Exhibit a Prophylactic Effect on Diet-Induced Metabolic Dysfunction and Nonalcoholic Fatty Liver Disease in Mice. *Evid Based Complement Alternat Med* 2020; **2020**: 9519625 [PMID: 32595752 DOI: 10.1155/2020/9519625]
5 **Ramanathan R**, Ali AH, Ibdah JA. Mitochondrial Dysfunction Plays Central Role in Nonalcoholic Fatty Liver Disease. *Int J Mol Sci* 2022; **23** [PMID: 35806284 DOI: 10.3390/ijms23137280]
6 **Lin L**, Ni B, Lin H, Zhang M, Li X, Yin X, Qu C, Ni J. Traditional usages, botany, phytochemistry, pharmacology and toxicology of Polygonum multiflorum Thunb.: a review. *J Ethnopharmacol* 2015; **159**: 158-183 [PMID: 25449462 DOI: 10.1016/j.jep.2014.11.009]
7 **Fei-Fei HU**, Zhan-Xia H, Shao-Bo Z, Yu-Chen S, Li-Li JI. [Study on improvement provided by water extracts of Polygoni Multiflori Radix and Polygoni Multiflori Radix Praeparata on non-alcoholic steatohepatitis in mice induced by MCD]. *Zhongguo Zhong Yao Za Zhi* 2020; **45**: 4732-4739 [PMID: 33164440 DOI: 10.19540/j.cnki.cjcmm.20200610.401]
8 **Lin L**, Hao Z, Zhang S, Shi L, Lu B, Xu H, Ji L. Study on the protection of water extracts of Polygoni Multiflori Radix and Polygoni Multiflori Radix Praeparata against NAFLD and its mechanism. *J Ethnopharmacol* 2020; **252**: 112577 [PMID: 31953200 DOI: 10.1016/j.jep.2020.112577]
9 **Lin P**, He YR, Lu JM, Li N, Wang WG, Gu W, Yu J, Zhao RH. In vivo lipid regulation mechanism of polygoni multiflori radix in high-fat diet fed rats. *Evid Based Complement Alternat Med* 2014; **2014**: 642058 [PMID: 24876874 DOI: 10.1155/2014/642058]
10 **Yu LP**, Li YQ, Li YJ, Zi L, Tao YX, Hao JJ, Zhang M, Gu W, Zhang F, Yu J, Yang XX. In vivo identification of the pharmacodynamic ingredients of Polygonum cuspidatum for remedying the mitochondria to alleviate metabolic dysfunction-associated fatty liver disease. *Biomed Pharmacother* 2022; **156**: 113849 [PMID: 36252355 DOI: 10.1016/j.biopha.2022.113849]
11 **Yang XX**, Xu F, Wang D, Yang ZW, Tan HR, Shang MY, Wang X, Cai SQ. Development of a mitochondria-based centrifugal ultrafiltration/Liquid chromatography/mass spectrometry method for screening mitochondria-targeted bioactive constituents from complex matrixes: Herbal medicines as a case study. *J Chromatogr A* 2015; **1413**: 33-46 [PMID: 26306914 DOI: 10.1016/j.chroma.2015.08.014]
12 12.**Wang L**, Sang M, Liu E, Banahene PO, Zhang Y, Wang T, Han L, Gao X. Rapid profiling and pharmacokinetic studies of major compounds in crude extract from Polygonum multiflorum by UHPLC-Q-TOF-MS and UPLC-MS/MS. *J Pharm Biomed Anal* 2017; **140**: 45-61 [PMID: 28342304 DOI: 10.1016/j.jpba.2017.03.016]
13 **Wu Z**, Wang X, Chen M, Hu H, Cao J, Chai T, Wang H. A Study on Tissue-Specific Metabolite Variations in *Polygonum cuspidatum* by High-Resolution Mass Spectrometry-Based Metabolic Profiling. *Molecules* 2019; **24** [PMID: 30889850 DOI: 10.3390/molecules24061058]
14 **Wang X**, Qin Y, Li GQ, Chen S, Ma JQ, Guo YL, Luo WZ. Study on Chemical Constituents in Polygoni Cuspidati Folium and its Preparation by UPLC-ESI-Q-TOF-MS/MS. *J Chromatogr Sci* 2018; **56**: 425-435 [PMID: 29554228 DOI: 10.1093/chromsci/bmy017]
15 **Tang D**, Zhu JX, Wu AG, Xu YH, Duan TT, Zheng ZG, Wang RS, Li D, Zhu Q. Pre-column incubation followed by fast liquid chromatography analysis for rapid screening of natural methylglyoxal scavengers directly from herbal medicines: case study of Polygonum cuspidatum. *J Chromatogr A* 2013; **1286**: 102-110 [PMID: 23489496 DOI: 10.1016/j.chroma.2013.02.058]
16 **Xu H**, Qu J, Wang J, Han J, Han K, Li Q, Bi W, Liu R. Discovery of pulmonary fibrosis inhibitor targeting TGF-β RI in Polygonum cuspidatum by high resolution mass spectrometry with in silico strategy. *JPA* 2020 [DOI: 10.1016/j.jpha.2020.05.007]
17 **Guo ZH**, Jia ZX, Chen KK, Liu J, Xu WJ, Duan FP, Xian Z, Wei ZY, Chen LM, Xiao HB. [Studies on constituents of Polygonum multiflorum extract *in vivo* and *in vitro* based on UPLC-Q-TOF-MS]. *Zhongguo Zhong Yao Za Zhi* 2018; **43**: 2796-2805 [PMID: 30111034 DOI: 10.19540/j.cnki.cjcmm.20180419.002]
18 **Pan Z**, Liang H, Liang C, Xu W. [An ultra-high-pressure liquid chromatography/linear ion trap-Orbitrap mass spectrometry method coupled with a diagnostic fragment ions-searching-based strategy for rapid identification and characterization of chemical components in Polygonum cuspidatum]. *Se Pu* 2015; **33**: 22-28 [PMID: 25958663 DOI: 10.3724/sp.j.1123.2014.07006]
19 **Liang L**, Xu J, Zhou WW, Brand E, Chen HB, Zhao ZZ. Integrating Targeted and Untargeted Metabolomics to Investigate the Processing Chemistry of Polygoni Multiflori Radix. *Front Pharmacol* 2018; **9**: 934 [PMID: 30210339 DOI: 10.3389/fphar.2018.00934]
20 **Mu JK**, Zi L, Li YQ, Yu LP, Cui ZG, Shi TT, Zhang F, Gu W, Hao JJ, Yu J, Yang XX. Jiuzhuan Huangjing Pills relieve mitochondrial dysfunction and attenuate high-fat diet-induced metabolic dysfunction-associated fatty liver disease. *Biomed Pharmacother* 2021; **142**: 112092 [PMID: 34449316 DOI: 10.1016/j.biopha.2021.112092]
21 **Liang L**, Li FJ, Liu X, Mu JK, Wang X, Dong JC, Zeng LX, Gu W, Li JP, Yang XX, Yu J. Identification of Mitochondrial Ligands with Hepatoprotective Activity from Notopterygii Rhizoma et Radix Using Affinity Ultrafiltration/Liquid Chromatography/Mass Spectrometry. *Biomed Res Int* 2019; **2019**: 5729263 [PMID: 31950043 DOI: 10.1155/2019/5729263]
22 **Wu C**, Bian Y, Lu B, Wang D, Azami NLB, Wei G, Ma F, Sun M. Rhubarb free anthraquinones improved mice nonalcoholic fatty liver disease by inhibiting NLRP3 inflammasome. *J Transl Med* 2022; **20**: 294 [PMID: 35765026 DOI: 10.1186/s12967-022-03495-4]
23 **Su ZL**, Hang PZ, Hu J, Zheng YY, Sun HQ, Guo J, Liu KY, Du ZM. Aloe-emodin exerts cholesterol-lowering effects by inhibiting proprotein convertase subtilisin/kexin type 9 in hyperlipidemic rats. *Acta Pharmacol Sin* 2020; **41**: 1085-1092 [PMID: 32203084 DOI: 10.1038/s41401-020-0392-8]
24 **Shen C**, Pan Z, Wu S, Zheng M, Zhong C, Xin X, Lan S, Zhu Z, Liu M, Wu H, Huang Q, Zhang J, Liu Z, Si Y, Tu H,

Deng Z, Yu Y, Liu H, Zhong Y, Guo J, Cai J, Xian S. Emodin palliates high-fat diet-induced nonalcoholic fatty liver disease in mice *via* activating the farnesoid X receptor pathway. *J Ethnopharmacol* 2021; **279**: 114340 [PMID: 34171397 DOI: 10.1016/j.jep.2021.114340]

25 **Wang S**, Li X, Guo H, Yuan Z, Wang T, Zhang L, Jiang Z. Emodin alleviates hepatic steatosis by inhibiting sterol regulatory element binding protein 1 activity by way of the calcium/calmodulin-dependent kinase kinase-AMP-activated protein kinase-mechanistic target of rapamycin-p70 ribosomal S6 kinase signaling pathway. *Hepatol Res* 2017; **47**: 683-701 [PMID: 27492505 DOI: 10.1111/hepr.12788]

26 **Mishra SK**, Tiwari S, Shrivastava A, Srivastava S, Boudh GK, Chourasia SK, Chaturvedi U, Mir SS, Saxena AK, Bhatia G, Lakshmi V. Antidyslipidemic effect and antioxidant activity of anthraquinone derivatives from Rheum emodi rhizomes in dyslipidemic rats. *J Nat Med* 2014; **68**: 363-371 [PMID: 24343839 DOI: 10.1007/s11418-013-0810-z]

27 **Wang W**, He Y, Lin P, Li Y, Sun R, Gu W, Yu J, Zhao R. In vitro effects of active components of Polygonum Multiflorum Radix on enzymes involved in the lipid metabolism. *J Ethnopharmacol* 2014; **153**: 763-770 [PMID: 24680992 DOI: 10.1016/j.jep.2014.03.042]

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ORIGINAL ARTICLE

Observational Study

Impact of *Helicobacter pylori* virulence markers on clinical outcomes in adult populations

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Abstract

BACKGROUND

In recent years, associations between specific virulence markers of *Helicobacter pylori* (*H. pylori*) and gastrointestinal disorders have been suggested.

AIM

To investigate the presence of virulence factors including vacuolating cytotoxin A genotypes (*s1m1*, *s1m2*, *s2m1*, and *s2m2*), cytotoxin-associated gene A (*Cag*A), and urease activity in *H. pylori* strains isolated from Arab and Jewish populations in northern Israel and to assess associations between these factors and patients' demographics and clinical outcomes.

METHODS

Patients (n = 108) who underwent gastroscopy at the Baruch Padeh Medical Center, Poriya due to symptomatic gastroduodenal pathologies as part of *H. pylori* diagnosis were enrolled in the study. Gastric biopsy specimens were collected from the antrum of the stomach. Clinical condition was assessed by clinical pathology tests. Bacteria were isolated on modified BD Helicobacter Agar (BD Diagnostics, Sparks, MD, United States). Bacterial DNA was extracted, and PCR was performed to detect *Cag*A and vacuolating cytotoxin A genes. Urease activity was assessed using a rapid urease test.

RESULTS

A significant correlation was found between disease severity and patient ethnicity (P = 0.002). A significant correlation was found between *Cag*A presence and the *s1m1* genotype (P = 0.02), which is considered the most virulent genotype. Further, a higher level of urease activity was associated with isolates originating from the Jewish population. Moreover, higher urease activity levels were

measured among *Cag*A-/*s1m1* and *Cag*A-/*s2m2* isolates.

CONCLUSION
Our study highlights the importance of incorporating molecular methods for detection of virulence markers of *H. pylori* in order to tailor optimal treatments for each patient. Further investigation should be performed regarding associations between *H. pylori* virulence factors and ethnicity.

Key Words: *Helicobacter pylori*; Virulence factors; Vacuolating cytotoxin A; Cytotoxin-associated gene A; Urease activity

Core Tip: In recent years, associations have been found between virulence markers of *Helicobacter pylori* and gastrointestinal disorders. In parallel, several physicians in northern Israel noted a higher treatment failure rate among Arab patients compared to Jewish patients. This work found a significant correlation between disease severity and patient ethnicity ($P = 0.002$). Further, a higher level of urease activity was associated with isolates originating from the Jewish population. Moreover, higher urease activity levels were measured among *Cag*A-/*s1m1* and *Cag*A-/*s2m2* isolates. These findings are expected to advance personalization of treatment to specific strains based on their virulence factors.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a microaerophilic, Gram-negative bacterium that colonizes the gastric mucosa and infects the stomach epithelium, causing peptic ulcer disease[1]. Unrelenting *H. pylori* infections cause chronic inflammation, which can result in gastritis, intestinal metaplasia, and even gastric cancer[1].

The genetic variability of *H. pylori* and its host, combined with environmental factors, have been suggested to affect the clinical outcome[1-3]. *H. pylori* pathogenesis is mediated *via* distinct virulence factors, including the secreted vacuolating cytotoxin A (*Vac*A), cytotoxin-associated gene A (*Cag*A) protein, and urease[1,2]. VacA is one of the main toxins secreted by the bacterium; once bound to host cells and internalized, it causes "vacuolation," characterized by the accumulation of large vesicles that disrupt protein trafficking pathways. Two polymorphic regions in the *Vac*A gene sequence are the signal sequence region (s-region) and the mid region (m-region), with allelic variations classified as *s1* or *s2* and *m1* or *m2*, respectively[3]. It was suggested that isolates with a *s1/m1* genotype cause more severe chronic inflammation compared to the other *Vac*A genotypes[4].

H. pylori strains can be divided into two main subpopulations according to their ability to produce CagA, a 120-145 kDa protein associated with gastric carcinoma. The protein interferes with cellular signal transduction, provoking cellular dysfunction that can eventually lead to cell transformation and cancer[5].

Along with VacA and CagA, urease activity in *H. pylori* is an indicator of bacterial virulence. Urease is a metalloenzyme that requires nickel for its activity and consists of two components, UreA and UreB. The enzyme breaks down urea into ammonia and carbon dioxide, promoting bacterial survival in the acidic environment of the stomach. In addition, urease is involved in *H. pylori* colonization in the gastric tissue, prevention of phagocytosis, and induction of proinflammatory cytokines[6,7].

In addition to the virulence factors that may affect the efficacy of *H. pylori* eradication regimens, antibiotic resistance poses a major challenge to treatment success. Several physicians in northern Israel noted that the Arab population has a higher treatment failure rate compared to the Jewish population, a finding which triggered a study that examined antibiotic resistance of *H. pylori* among these two groups in Israel[8]. Indeed, isolates from the Arab population were more resistant to both clarithromycin and levofloxacin and exhibited simultaneous resistance to more antibiotics as compared to isolates from Jewish patients[8]. The current study further examined the characteristics of *H. pylori* in adult Arab and Jewish populations in northern Israel, with an emphasis on the three virulence markers of *H. pylori* and their correlation with clinical outcomes.

MATERIALS AND METHODS

Study population

The study group included 108 isolates of patients who underwent gastroscopy at the Baruch Padeh Medical Center, Poriya, due to symptomatic gastroduodenal pathologies as part of the *H. pylori* workup between November 2018 and December 2019. We included the first 108 biopsies from which *H. pylori* was successfully isolated at the microbiology laboratory of the medical center. The study was approved by the Helsinki Committee of the Baruch Padeh Medical Center, Poriya (Approval no. POR 0007-20). The Institutional Review Board committee waived the need for participant approval. Gastric biopsy specimens were collected from the antrum of the stomach. Clinical pathology tests were performed to assess the patients' clinical conditions. Demographic and clinical data were retrospectively collected from the patients' medical records.

Histology staining and pathology

Gastric biopsy specimens were stained using hematoxylin and eosin staining method in order to identify *H. pylori* and to evaluate the degree of inflammation. The bacterium has an actively dividing spiral shape that changes to coccoid morphology under stressful environments.

All histologic slides were reviewed by a single blinded gastrointestinal pathologist at the pathology laboratory of the Baruch Padeh Medical Center, Poriya and graded unremarkable (none), mild, moderate, or severe, based on the presence of acute (polymorphonuclear cells) or chronic (monocytes, lymphocytes, plasma cells) inflammation, lymphoid aggregates, and metaplasia.

Bacterial isolation and identification

H. pylori identification was carried out in accordance with the routine identification tests of the clinical microbiology laboratory including a Gram stain, oxidase, catalase, and urease tests.

Biopsy specimens were manually minced with a sterile scalpel, seeded on modified BD Helicobacter Agar plates (BD Diagnostics, Sparks, MD), and incubated for 7 d at 35 °C in a microaerobic atmosphere (5% O_2 and 10% CO_2) produced by a gas-generating system adapted for *Campylobacter* (CampyGen™; Gamidor Diagnostics, Petah Tikva, Israel).

Final identification of the bacteria was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry[9], using the Bruker Biotyper system (Bruker Daltonics, Bremen, Germany) with MALDI BIOTYPER 3.3 (Bruker Daltonics) software.

Molecular characterization of genes associated with bacterial virulence

DNA extraction: Tissue collected from gastroscopic biopsy was finely chopped with a sterile scalpel and then lysed by tissue lysis buffer supplemented with proteinase K enzyme (Bioneer, Daejeon, Korea). Total DNA was extracted using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions.

Multiplex PCR assay: DNA was amplified with a multiplex PCR, designed to detect the *Vac*A and *Cag*A genes in a single run, using specific primers (Table 1). For this purpose, 10 μL Taq ReadyMix2 (Hy labs, Rehovot, Israel) was added to 0.2 μL forward primer, 0.2 μL reverse primer, 4 μL template DNA, and 5.6 μL nuclease-free water. Reaction conditions were 35 cycles of: Denaturation of the pre-amplified templates at 95 °C for 1 min, followed by an annealing step at 72 °C for 1 min, an extension step at 25 °C for 1 min and one additional extension step for 7 min. PCR products were visualized by 1% agarose gel electrophoresis.

Urease activity

Urease activity was quantified using the rapid urease test in a 96-well plate. For this purpose, bacteria were placed in a sterile Eppendorf tube containing sterile physiological solution until 0.5 McFarland turbidity was reached. Rapid Urease Test Brute solution (100 μL; Novamed, Jerusalem, Israel), containing 2% urea, pH 6.8, was then added to each well, along with 100 μL of the bacterial stock. Then, absorbance at 570 nm (optical density, $O.D_{570}$) was measured after 1 min, 5 min and 10 min using the Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, United States)[10]. A change in the solution color from orange to pink was considered a positive result.

Statistical analysis

Continuous variables (age, urease activity) were presented as means and ranges with standard deviations and categorical variables (sex, disease severity, ethnicity, living area, genotypes) were presented as absolute numbers and percentages. *T* test or one-way analysis of variance was used to compare continuous variables of two or more groups and Fisher's exact test to assess the relationship between categorical variables. *P* value < 0.05 indicated statistical significance. Statistical analysis was performed using R Statistical Software (version 4.1; R Foundation for Statistical Computing, Vienna, Austria).

Table 1 List of genes and primer sequences used in the study

Primer	Gene	Primer sequence	Product size (bp)
CAGAF	*Cag*A	5′-GATAACAGGCAAGCTTTTGAGG-3′	349
CAGAR		5′-CTGCAAAAGATTGTTTGGCAGA-3′	
VA1-F	*Vac*A signal region	5′-ATGGAAATACAACAAACACAC-3′	259/286 (s1/s2)
VA1-R		5′-CTGCTTGAATGCGCCAAAC-3′	
VAG-F	*Vac*A middle region	5′-CAATCTGTCCAATCAAGCGAG-3′	567/642 (m1/m2)
VAG-R		5′-GCGTCTAAATAATTCCAAGG-3′	

*Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A.

RESULTS

Demographic characteristics

In total, 108 patients [average of 42.3 (18.0-88.0) years] were enrolled in this study, 24% of whom were males and 76% of whom were females (Table 2). Within the study group, 56 were Arabs and 52 were Jews; 61.1% lived in a city and 33.9% lived in a village. A larger percentage of the Arab *vs* Jewish cohort lived in villages (62.5% *vs* 13.5%, respectively).

VacA and CagA genotypes

To characterize the common *Cag*A and *Vac*A genotypes in the patient population, 108 isolates were randomly selected, of which 56 originated from the Arab cohort and 52 from the Jewish cohort (Table 3). *Cag*A was identified in 24 (22.2%) samples and was distributed equally between the two ethnic groups. However, it had a higher frequency among isolates from patients living in villages (67.7%) compared to isolates from those living in cities (33.3%) (P = 0.149). The most prevalent *Vac*A genotype was *s2m1*. No statistically significant associations were noted between the different genotypes and demographic characteristics.

A significant association was found between the presence of *Cag*A and specific *Vac*A genotypes (P = 0.002) (Figure 1); 33.3% of the *Cag*A-positive strains had the *s1m1* genotype, which is considered the most virulent genotype[7]. Additionally, 61.9% of the *Cag*A-negative strains had the *s2m1* genotype.

Urease activity

Urease activity was found to be faster in isolates from the Jewish population compared to isolates from the Arab population. This was observed at all tested time points (1 min, 5 min, and 10 min; $P < 0.05$ for all). The results of the first-minute measurements are presented in Figure 2. No significant associations were found between urease activity and patient's sex, place of residence and the presence of the *Cag*A gene.

In addition, urease activity in *Cag*A (-) *s2m2* strains (mean O.D 0.33) and in *Cag*A (-) *s1m1* isolates (mean O.D 0.32) were significantly higher than urease activity in *Cag*A (-) *s1m2* (mean O.D 0.28), (P = 0.013 and P = 0.016, respectively) (Figure 3).

Associations between virulence factors, patient ethnicity and disease severity

A significant association was found between disease severity and ethnicity (P =0.002) (Table 4); in patients with mild disease, 58% were Jews while 42% were Arabs. In patients with moderate disease, 75.7% were Arabs compared to 24.3% Jews. Finally, the severe group included 66.7% Arabs compared to 33.3% Jews. No significant links were found between disease severity and urease activity, *Cag*A gene occurrence, *Vac*A alleles, and genotype combinations.

DISCUSSION

The main purpose of this study was to evaluate and compare the virulence markers of *H. pylori* among two adult populations in northern Israel and their correlation with clinical outcomes. It should be noted that, due to its special growth requirements and slow growth, cultivation of *H. pylori* is difficult. As a result, the diagnosis of *H. pylori* infection is usually performed, as opposed to diagnosis of other bacterial infections, on the basis of indirect tests that do not require bacterial isolation[11]. This is the reason why there is limited data on the distribution of virulence factors and their associations with clinical outcomes among *H. pylori* isolates in Israel.

Table 2 Demographic characteristics of the study participants, *n* (%)

Characteristic	Arabs, *n* = 56	Jews, *n* = 52	Total, *n* = 108
Sex			
Male	14 (25)	39 (23)	26 (24)
Female	84 (75)	64 (77)	82 (76)
Age, yr			
Average (range)	40.93 (18-81)	43.65 (18-88)	42.30 (18-88)
Area of residence			
City	21 (37.5)	45 (86.5)	66 (61.1)
Village	35 (62.5)	7 (13.5)	42 (33.9)

Table 3 The prevalence of vacuolating cytotoxin A alleles and cytotoxin-associated gene A *vs* patient demographics

Characteristic	*CagA*-positive, *n* = 24	*CagA*-negative, *n* = 84	*P*	*VacA* genotype				*P*
				s1m1	*s1m2*	*s2m1*	*s2m2*	
Ethnicity			0.834					0.729
Arabs	12 (50.0)	40 (47.6)		11 (21.2)	11 (21.2)	27 (51.9)	3 (5.8)	
Jewish	12 (50.0)	44 (52.4)		8 (14.3)	15 (26.8)	31 (55.4)	2 (3.6)	
Sex			0.623					0.214
Male	7 (29.2)	29 (34.5)		6 (16.7)	5 (13.9)	22 (61.1)	3 (8.3)	
Female	17 (70.8)	55 (65.5)		13 (18.1)	21 (29.2)	36 (50.0)	2 (2.8)	
Residence			0.149					0.055
Village	16 (67.7)	42 (50.0)		8 (13.8)	17 (29.3)	28 (48.3)	5 (8.6)	
City	8 (33.3)	42 (50.0)		11 (22.0)	9 (18.0)	30 (60.0)	0 (0)	

*Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A.

*Cag*A was found in 22.2% of the detected strains. This rate is a bit lower than expected according to previous studies; the prevalence of the cytotoxin-associated gene pathogenicity island, which includes *Cag*A, varies between different geographic areas, ranging from 95% in Western and South Africa and East and Central Asia to 28% in Latin America. In Europe, approximately 58% of the *H. pylori* strains carry the *Cag*A gene, while in the Middle East, *CagA* was detected in approximately 50% of the strains [12,13]. As only a sample of our isolates were tested for the presence of *Cag*A, it is possible that a higher prevalence of this gene exists among *H. pylori* strains in Israel.

We did not find significant differences in the distribution of *Cag*A between the two populations. Interestingly, Muhsen *et al*[14], who also investigated *H. pylori* isolates in Arab and Jewish populations, demonstrated higher *Cag*A IgG antibodies in the Arab population. Another study, performed in Israeli children and adolescents, found higher *H. pylori* seroprevalence among Arab participants as compared to Jewish participants[15].

Regarding the virulence factor *Vac*A, *s2m1* genotype was the most common, present in 53.3% of the isolates, while only 17.6% carried the *s1m1* genotype. In a study conducted in South Africa, *s1m1* was the most common genotype (56.4%), while the *s2m1* genotype was present in only 10.3%[16]. In a similar study conducted in Iran, the most common genotype was *s2m2*, found in 50% of the isolates[17]. It should be noted that the frequency of the alleles in different populations is influenced by several factors, including evolution, natural selection, mutations, and genetic drift[18].

*Cag*A was identified most frequently together with the *Vac*A genotype *s1m1* (P = 0.02), which is considered the most virulent[4]. This result reinforces evidence from a previous study, which found that most *Vac*A s1 strains carry *Cag*A as well[19,20]. Previous studies suggested that the presence of *Cag*A together with certain genotypes of *Vac*A can indicate disease severity. For example, its appearance with the genotype *s1m2* or *s1m1* was correlated with the appearance of peptic ulcers, while its appearance with *s2m2* was correlated with gastritis[17]. In light of the above, it is important to profile the *Vac*A and *Cag*A variants in patients in order to assess disease progression. This may aid in optimizing medical treatment.

Table 4 Associations between virulence factors, patient ethnicity, and disease severity, *n* (%)

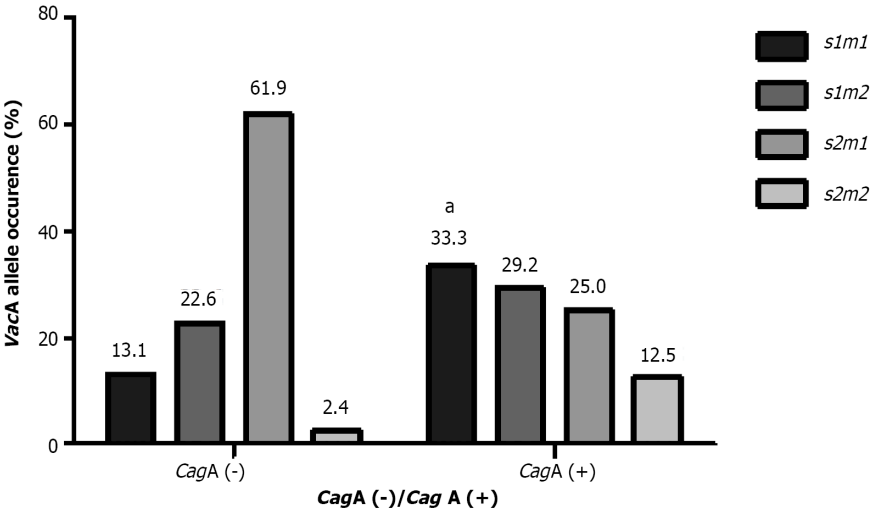
	Disease severity					*P* value
	Unremarkable, *n* = 18	Mild, *n* = 50	Moderate, *n* = 37	Severe, *n* = 3	Total, *n* = 108	
Ethnicity						0.002
Arab	5 (27.8)	21 (42.0)	28 (75.7)	2 (66.7)	56 (51.9)	
Jewish	13 (72.2)	29 (58.0)	9 (24.3)	1 (33.3)	52 (48.1)	
Urease activity						
t1	0.32 (0.05)	0.29 (0.06)	0.29 (0.07)	0.30 (0.06)	0.30 (0.06)	0.273
t5	0.32 (0.05)	0.30 (0.05)	0.29 (0.07)	0.34 (0.02)	0.30 (0.06)	0.174
t10	0.32 (0.05)	0.30 (0.06)	0.30 (0.06)	0.35 (0.04)	0.30 (0.06)	0.171
t15	0.33 (0.04)	0.31 (0.05)	0.31 (0.06)	0.36 (0.03)	0.31 (0.05)	0.245
*Cag*A gene						0.534
*Cag*A-	13 (72.2)	41 (82.0)	27 (73.0)	3 (100)	84 (77.8)	
*Cag*A+	5 (27.8)	9 (18.0)	10 (27.0)	0 (0)	24 (22.2)	
*Vac*A *s* allele						0.651
*Vac*A *s1*	5 (27.8)	22 (44.0)	16 (43.2)	1 (33.3)	44 (40.7)	
*Vac*A *s2*	13 (72.2)	28 (56.0)	21 (56.8)	2 (66.7)	64 (59.3)	
VacA *m* Allele						0.652
*Vac*A *m1*	3 (16.7)	14 (28.0)	7 (18.9)	1 (33.3)	25 (23.1)	
*Vac*A *m2*	15 (83.3)	36 (72.0)	30 (81.1)	2 (66.7)	83 (76.9)	
Genotype						0.926
*Cag*A+/*s1m1*	0 (0)	3 (6.0)	4 (10.8)	0 (0)	7 (6.5)	
*Cag*A+/*s1m2*	1 (5.6)	2 (4.0)	3 (8.1)	0 (0)	6 (5.6)	
*Cag*A+/*s2m2*	2 (11.1)	2 (4.0)	2 (5.4)	0 (0)	6 (5.6)	
*Cag*A+/*s2m1*	1 (5.6)	1 (2.0)	1 (2.7)	0 (0)	3 (2.8)	
*Cag*A-/*s1m1*	2 (11.1)	7 (14.0)	2 (5.4)	1 (33.3)	12 (11.1)	
*Cag*A-/*s1m2*	2 (11.1)	11 (22.0)	7 (18.9)	0 (0)	20 (18.5)	
*Cag*A-/*s2m2*	10 (55.6)	22 (44.0)	18 (48.6)	2 (66.7)	52 (48.1)	
*Cag*A-/*s2m1*	0 (0)	2 (4.0)	0 (0)	0 (0)	2 (1.9)	

*Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A.

Apart from *Vac*A and *Cag*A, urease activity is another indicator of *H. pylori* virulence. We found a higher urease activity among isolates from the Jewish population as compared to those from Arabs ($P < 0.005$). No previous study has investigated this issue. As urease activity is influenced by specific food ingredients such as isothiocyanates[10] and essential oils[21], variations in nutrition habits may explain the difference in urease activity between isolates from Arab and Jewish patients. Further studies are needed to confirm our result and investigate its meaning.

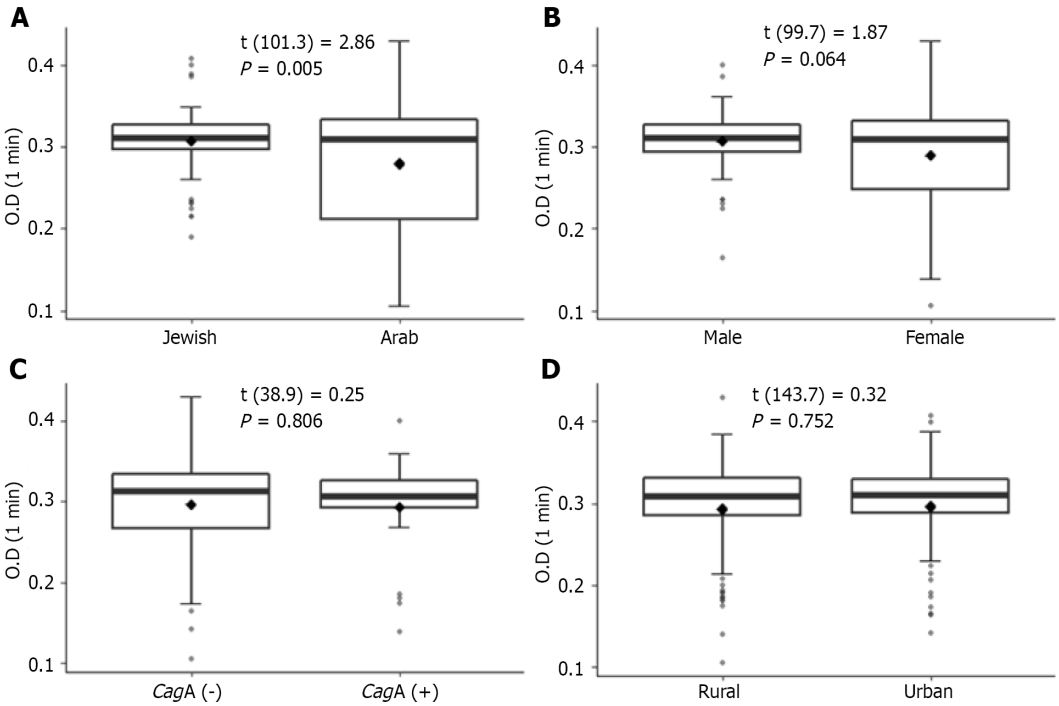
Significant differences in urease activity were noted among isolates with specific *Cag*A and *VacA* genotype combinations. Both *Cag*A (-) *s1m1* and *Cag*A (-) *s2m2* showed the highest urease activity. This result shows that the absence of *Cag*A may result in increased urease activity, especially when combined with the most virulent alleles of the *Vac*A gene, *s1m1*, as previously suggested[6].

Given that antibiotic resistance was found to be higher in the Arab population[8], we cautiously suggest that increased urease activity does not coincide with increased antibiotic resistance. In our preliminary analysis, no significant correlation was found between disease severity and urease activity. These results were quite surprising as previous studies did report on such associations[22]. It is possible that this contradiction is due to our relatively small sample size. However, we did find a significant correlation between ethnicity and disease severity ($P = 0.002$). This finding may be ascribable to differences in health-related lifestyle between different ethnic groups as well as differences in socioeconomic conditions along with cultural and social customs between groups related to their



DOI: 10.3748/wjg.v29.i1.190

Figure 1 Distribution of the different vacuolating cytotoxin A genotypes (percentage) among 108 isolates, according to cytotoxin-associated gene A presence. *Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A. [a]*P* < 0.05.



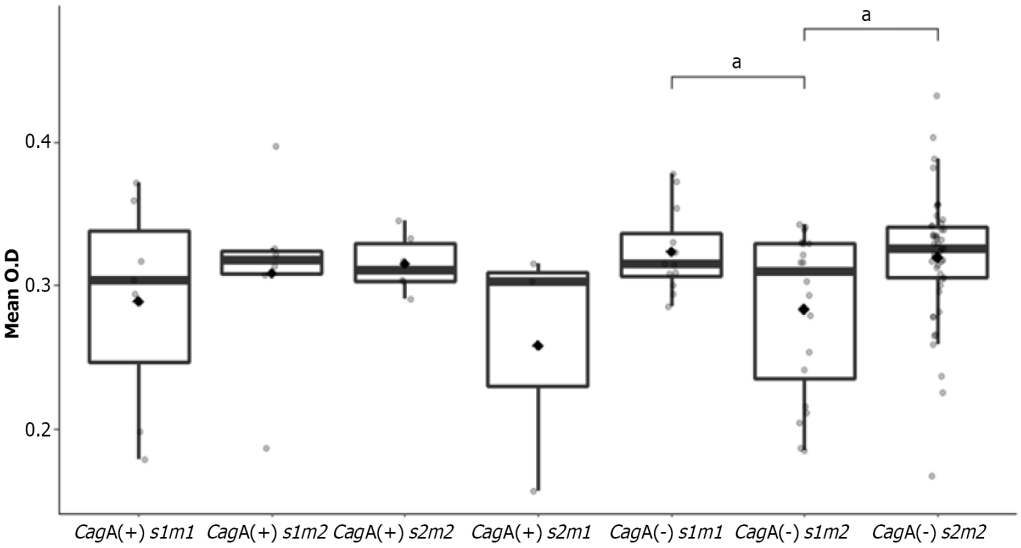
DOI: 10.3748/wjg.v29.i1.190

Figure 2 Urease activity in relation to ethnicity, sex, cytotoxin-associated gene occurrence, and place of residence. A: Ethnicity; B: Sex; C: Cytotoxin-associated A gene occurrence; D: Place of residence. Urease activity (indicated by O.D) was measured 1 min after incubation of bacteria with urea solution, as described in the Materials and Methods section. *Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A.

residential environments, which can undoubtedly affect morbidity. Furthermore, it can also be explained by the high antibiotic resistance found among the Arab population[8].

CONCLUSION

Our study highlighted the importance of incorporating molecular methods for detection of virulence markers of *H. pylori* in order to tailor optimal treatments for each patient. Further investigation should be performed regarding associations between *H. pylori* virulence factors and ethnicity.



DOI: 10.3748/wjg.v29.i1.190

Figure 3 Urease activity as a function of isolate genotype combination. Urease activity was measured 1 min, 5 min, and 10 min after incubation of bacteria with urea solution, as described in the Materials and Methods section. The minimal, maximal, and median of mean urease activity values are shown (the median is indicated by the line within the bar) per each group of isolates with a different genotype of cytotoxin-associated gene A and vacuolating cytotoxin A genes. O.D: Optical density; *Vac*A: Vacuolating cytotoxin A; *Cag*A: Cytotoxin-associated gene A. $^{a}P < 0.05$.

ARTICLE HIGHLIGHTS

Research background

Helicobacter pylori (*H. pylori*) is a microaerophilic, Gram-negative bacterium that colonizes the gastric mucosa and infects the stomach epithelium, causing peptic ulcer disease. The genetic variability of *H. pylori* and its host, combined with environmental factors, have been suggested to affect the clinical outcome. *H. pylori* pathogenesis is mediated *via* distinct virulence factors, including the secreted vacuolating cytotoxin A, cytotoxin-associated gene A, and urease.

Research motivation

In recent years, associations between specific virulence markers of *H. pylori* and gastrointestinal disorders have been suggested.

Research objectives

To investigate the distribution of three virulence factors among isolates from both Arab and Jewish populations and to assess their impact on clinical presentations.

Research methods

We enrolled 108 patients tested for the presence of vacuolating cytotoxin A and cytotoxin-associated gene A genes and evaluated the urease activity levels. We assessed the clinical state of the patients by hematoxylin and eosin staining of the gastric biopsies from which the bacteria were recovered.

Research results

We found associations between disease severity and ethnicity and between some of the virulence factors to ethnicity.

Research conclusions

Our study highlighted the importance of incorporating molecular methods for detection of virulence markers of *H. pylori* in order to tailor optimal treatments for each patient.

Research perspectives

Further investigation should be performed regarding associations between *H. pylori* virulence factors and ethnicity.

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FOOTNOTES

Author contributions: Roshrosh H, Rohana H, and Peretz A contributed to conceptualization; Roshrosh H, Rohana H, Azrad M, and Leshem T contributed to data curation; Roshrosh H, Rohana H, Azrad M, Leshem T, and Peretz A contributed to formal analysis; Roshrosh H, Rohana H, and Leshem T contributed to investigation and methodology; Leshem T, Azrad M, and Peretz A contributed to project administration and supervision; Roshrosh H, Rohana H, and Leshem T contributed to validation; Roshrosh H, Rohana H, and Leshem T contributed to visualization; Roshrosh H, Rohana H, Azrad M, and Peretz A contributed to writing the original draft; Roshrosh H, Rohana H, Azrad M, Leshem T, and Peretz A contributed to reviewing and editing; All authors read and agreed to the published version of the manuscript.

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REFERENCES

1 **Moreno-Ochoa MF**, Valencia ME, Morales-Figueroa GG, Moya-Camarena SY. Association of cagA+ Helicobacter pylori strains with high urease activity and dyspepsia in Mexican adults. *Rev Gastroenterol Mex (Engl Ed)* 2020; **85**: 404-409 [PMID: 32224002 DOI: 10.1016/j.rgmx.2019.09.002]
2 **Panayotopoulou EG**, Sgouras DN, Papadakos KS, Petraki K, Breurec S, Michopoulos S, Mantzaris G, Papatheodoridis G, Mentis A, Archimandritis A. CagA and VacA polymorphisms are associated with distinct pathological features in Helicobacter pylori-infected adults with peptic ulcer and non-peptic ulcer disease. *J Clin Microbiol* 2010; **48**: 2237-2239 [PMID: 20392907 DOI: 10.1128/JCM.00662-10]
3 **Palframan SL**, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for Helicobacter pylori pathogenesis. *Front Cell Infect Microbiol* 2012; **2**: 92 [PMID: 22919683 DOI: 10.3389/fcimb.2012.00092]
4 **Sheikh AF**, Yadyad MJ, Goodarzi H, Hashemi SJ, Aslani S, Assarzadegan MA, Ranjbar R. CagA and vacA allelic combination of Helicobacter pylori in gastroduodenal disorders. *Microb Pathog* 2018; **122**: 144-150 [PMID: 29908307 DOI: 10.1016/j.micpath.2018.06.023]
5 **Hatakeyama M**, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* 2005; **96**: 835-843 [PMID: 16367902 DOI: 10.1111/j.1349-7006.2005.00130.x]
6 **Ghalehnoei H**, Ahmadzadeh A, Farzi N, Alebouyeh M, Aghdaei HA, Azimzadeh P, Molaei M, Zali MR. Relationship between ureB Sequence Diversity, Urease Activity and Genotypic Variations of Different Helicobacter pylori Strains in Patients with Gastric Disorders. *Pol J Microbiol* 2016; **65**: 153-159 [PMID: 28517916]
7 **Shimoyama T**, Fukuda S, Liu Q, Nakaji S, Fukuda Y, Sugawara K. Helicobacter pylori water soluble surface proteins prime human neutrophils for enhanced production of reactive oxygen species and stimulate chemokine production. *J Clin Pathol* 2003; **56**: 348-351 [PMID: 12719454 DOI: 10.1136/jcp.56.5.348]
8 **Yeganeh M**, Paritsky M, On A, Azrad M, Roshrosh H, Moalem R, Peretz A. Characteristics of Antibiotic Resistance of Helicobacter pylori Among Adult Arab and Jewish Populations in Northern Israel. *Microb Drug Resist* 2019; **25**: 103-107

[PMID: 30129819 DOI: 10.1089/mdr.2018.0148]

9 **Singhal N**, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol* 2015; **6**: 791 [PMID: 26300860 DOI: 10.3389/fmicb.2015.00791]

10 **Fahey JW**, Stephenson KK, Wade KL, Talalay P. Urease from Helicobacter pylori is inactivated by sulforaphane and other isothiocyanates. *Biochem Biophys Res Commun* 2013; **435**: 1-7 [PMID: 23583386 DOI: 10.1016/j.bbrc.2013.03.126]

11 **Perez-Perez GI**. Accurate diagnosis of Helicobacter pylori. Culture, including transport. *Gastroenterol Clin North Am* 2000; **29**: 879-884 [PMID: 11190072 DOI: 10.1016/s0889-8553(05)70155-2]

12 **Olbermann P**, Josenhans C, Moodley Y, Uhr M, Stamer C, Vauterin M, Suerbaum S, Achtman M, Linz B. A global overview of the genetic and functional diversity in the Helicobacter pylori cag pathogenicity island. *PLoS Genet* 2010; **6**: e1001069 [PMID: 20808891 DOI: 10.1371/journal.pgen.1001069]

13 **Akeel M**, Shehata A, Elhafey A, Elmakki E, Aboshouk T, Ageely H, Mahfouz M. Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from southwestern region, Saudi Arabia: distribution and association with clinical outcomes and histopathological changes. *BMC Gastroenterol* 2019; **19**: 16 [PMID: 30683054 DOI: 10.1186/s12876-019-0934-z]

14 **Muhsen K**, Barak M, Shifnaidel L, Nir A, Bassal R, Cohen D. Helicobacter pylori infection is associated with low serum ferritin levels in Israeli Arab children: a seroepidemiologic study. *J Pediatr Gastroenterol Nutr* 2009; **49**: 262-264 [PMID: 19525869 DOI: 10.1097/MPG.0b013e31818f0a0d]

15 **Muhsen K**, Sinnereich R, Beer-Davidson G, Nassar H, Abu Ahmed W, Cohen D, Kark JD. Correlates of infection with *Helicobacter pylori* positive and negative cytotoxin-associated gene A phenotypes among Arab and Jewish residents of Jerusalem. *Epidemiol Infect* 2019; **147**: e276 [PMID: 31552815 DOI: 10.1017/S0950268819001456]

16 **Idowu A**, Mzukwa A, Harrison U, Palamides P, Haas R, Mbao M, Mamdoo R, Bolon J, Jolaiya T, Smith S, Ally R, Clarke A, Njom H. Detection of Helicobacter pylori and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. *BMC Gastroenterol* 2019; **19**: 73 [PMID: 31088381 DOI: 10.1186/s12876-019-0986-0]

17 **Pajavand H**, Alvandi A, Mohajeri P, Bakhtyari S, Bashiri H, Kalali B, Gerhard M, Najafi F, Abiri R. High Frequency of vacA s1m2 Genotypes Among Helicobacter pylori Isolates From Patients With Gastroduodenal Disorders in Kermanshah, Iran. *Jundishapur J Microbiol* 2015; **8**: e25425 [PMID: 26862378 DOI: 10.5812/jjm.25425]

18 **Chen N**, Juric I, Cosgrove EJ, Bowman R, Fitzpatrick JW, Schoech SJ, Clark AG, Coop G. Allele frequency dynamics in a pedigreed natural population. *Proc Natl Acad Sci U S A* 2019; **116**: 2158-2164 [PMID: 30598449 DOI: 10.1073/pnas.1813852116]

19 **Atherton JC**, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777 [PMID: 7629077 DOI: 10.1074/jbc.270.30.17771]

20 **Homan M**, Luzar B, Kocjan BJ, Orel R, Mocilnik T, Shrestha M, Kveder M, Poljak M. Prevalence and clinical relevance of cagA, vacA, and iceA genotypes of Helicobacter pylori isolated from Slovenian children. *J Pediatr Gastroenterol Nutr* 2009; **49**: 289-296 [PMID: 19525870 DOI: 10.1097/MPG.0b013e31818f09f2]

21 **Korona-Glowniak I**, Glowniak-Lipa A, Ludwiczuk A, Baj T, Malm A. The In Vitro Activity of Essential Oils against *Helicobacter Pylori* Growth and Urease Activity. *Molecules* 2020; **25** [PMID: 32013183 DOI: 10.3390/molecules25030586]

22 **Baj J**, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, Maciejewski R. *Helicobacter pylori* Virulence Factors-Mechanisms of Bacterial Pathogenicity in the Gastric Microenvironment. *Cells* 2020; **10** [PMID: 33375694 DOI: 10.3390/cells10010027]

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SYSTEMATIC REVIEWS

Liver pathology in COVID-19 related death and leading role of autopsy in the pandemic

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Abstract

BACKGROUND

Information on liver involvement in patients with coronavirus disease 2019 is currently fragmented.

AIM

To highlight the pathological changes found during the autopsy of severe acute respiratory syndrome coronavirus 2 positive patients.

METHODS

A systematic literature search on PubMed was carried out until June 21, 2022.

RESULTS

A literature review reveals that pre-existing liver disease and elevation of liver enzyme in these patients are not common; liver enzyme elevations tend to be seen in those in critical conditions. Despite the poor expression of viral receptors in the liver, it seems that the virus is able to infect this organ and therefore cause liver damage. Unfortunately, to date, the search for the virus inside the liver is not frequent (16% of the cases) and only a small number show the presence of the virus. In most of the autopsy cases, macroscopic assessment is lacking, while microscopic evaluation of livers has revealed the frequent presence of congestion (42.7%) and steatosis (41.6%). Less frequent is the finding of hepatic inflammation or necrosis (19%) and portal inflammation (18%). The presence of microthrombi, frequently found in the lungs, is infrequent in the liver, with only 12% of cases presenting thrombotic formations within the vascular tree.

CONCLUSION
To date, the greatest problem in interpreting these modifications remains the association of the damage with the direct action of the virus, rather than with the inflammation or alterations induced by hypoxia and hypovolemia in patients undergoing oxygen therapy and decompensated patients.

Key Words: Liver; COVID-19; Autopsy; Immunohistochemistry; *In situ* hybridization; Immunofluorescence

Core Tip: A literature review, about liver pathology in coronavirus disease 2019 (COVID-19) patients, demonstrates the presence of liver damage, which is represented mainly by congestion, steatosis, hepatic inflammation and necrosis, and portal inflammation. The problem to date is whether the damage is COVID-19 related (meaning from direct virus damage/inflammatory related/systemic pathology related) or drug induced. However, this demonstration involves the need to be careful during drug treatment in patients with altered liver enzyme values to prevent further clinical worsening.

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INTRODUCTION

The new coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been well studied in relation to pulmonary and cardiac histologic manifestations, but little is yet known regarding hepatic manifestations. COVID-19 has, in fact, to be considered a systemic infectious and inflammatory disease with histological changes also in other organs apart from its main target represented by the lungs. Liver involvement to date is recognized and defined as any liver damage occurring during the course of the disease or its treatment[1], meaning that liver damage can be caused by direct cytotoxicity or inflammatory response and hypoxic/cardio-vascular changes, or it may be drug-induced[2-4]. SARS-CoV-2 liver tropism is also well studied, with many authors demonstrating the presence of angiotensin converting enzyme 2 (ACE2) receptor and transmembrane serine protease 2 in the liver, mainly expressed on cholangiocytes, where the levels of expression are similar to those on alveolar cells, though they are only minimally expressed on hepatocytes. No ACE2 expression was demonstrated on sinusoidal endothelial cells or Kupffer cells, apart from Wanner *et al*[5] who demonstrated minimal expression of ACE2 on Kupffer cells through immunofluorescence and Pirisi *et al*[6] who demonstrated the presence of virus-like particles in endothelial cells of hepatic sinusoids. Curiously, in patients with liver fibrosis/cirrhosis and in cases of hypoxia, the expression of ACE2 is increased, therefore pre-existing liver injury or hypoxic conditions, common in patients with COVID-19, could favor SARS-CoV-2 liver tropism[4,7-9]. Liver infection could also be explained by its immunological role and the proximity to the digestive organs, which exhibit a strong SARS-CoV-2-tropism, that could favor the entry of the virus through the portal system. Hepatic macrophages (mainly Kupffer cells) and sinusoidal endothelial cells have a key role in the activation of the immune response through pathogen recognition receptors, thus favoring virus entry[10].

The incidence of liver injury in COVID-19 patients is seen in 14%-53% cases[9,11,12] mainly demonstrated through abnormal liver function enzymes. In the literature, only a small number of studies focus on liver damage and even fewer on histological changes in patients who died with or from COVID-19. The purpose of this review is to summarize the results of studies in the literature and evaluate the biochemical and histological changes in the liver, demonstrating that the execution of autopsies is not obsolete, but represents a fundamental tool to create a bridge between clinical manifest-ations and cytological damage.

MATERIALS AND METHODS

A systematic literature search on PubMed was carried out until June 21, 2022. No time restrictions were applied. The review was conducted using MeSH terms, Boolean operators, and free-text terms to broaden the research. Studies focusing on autopsies of COVID-19 deaths and in particular on liver

pathology were initially searched using the terms "((COVID-19) AND (autopsy) AND ((death) OR (liver))" in title, abstract, and keywords. Study design included case reports, case series, and retrospective and prospective studies. Reviews were excluded in order not to create duplication of data, but were analyzed to search for any studies not resulting from the search in the database. No unpublished or gray literature was searched. A total of 526 articles were found in the database. The evaluation of references during full text screening allowed the inclusion of further seven studies. After evaluation of abstracts and full text, 46 articles were included because of their compliance with the inclusion criteria. We also conducted a relevant search using Reference Citation Analysis (https://www.referencecitationanalysis.com/) database to supplement and improve the highlights of the latest cutting-edge research results. Data from each included study were extracted using Microsoft Excel spreadsheets, including information on authors, publishing year, nation, sample size, gender, age, type of autopsy, laboratory results, pre-existing liver disease, macroscopic and microscopic results, additional staining, cause of death, medications, and search of the virus in the liver (Table 1).

RESULTS

Demographics

A total of 11 case reports and 35 case series were analyzed, with a total of 994 autopsy cases of COVID-19 patients. Studies were from all over the world: One from Hungary, Romania, Japan, South Africa, and United States in association with Brazil each, two from Austria, Belgium, India, Iran, and Turkey each, three from the United Kingdom, four from Italy, five from Germany, Switzerland, and China each, and nine from the United States. Gender was specified in 882 cases, of whom 54% (540) were male and 35% (342) were female. Age ranged from 18 to 102 years with a mean age of 53 years. Age distribution is summarized in Figure 1.

Liver disease

Pre-existing liver diseases were described in 61 (6%) cases, comprising 28 cases of fatty liver disease, 19 cases of chronic liver disease, 11 cases of cirrhosis, and 1 case each of hepatitis B and C. In 161 cases, body mass index (BMI) was over 30 kg/m^2.

Laboratory findings

Laboratory values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were described in 350 cases, with only 1 case described with values within the ranges of normality. The description of the laboratory values differed somewhat between the various studies, with only 5 studies (55 cases) reporting AST and ALT values at admission and 8 (64 cases) reporting the maximum values during hospitalization. Additional 4 reports for AST (51 cases) and 5 papers for ALT (61 cases) described the laboratory values without specifying the timing of the sampling. Data is summarized in Table 2. Abnormal AST and ALT values were described in 105 and 91 additional cases, respectively.

Hospitalization and medications

For the subsequent analysis of the macroscopic and microscopic findings, it was decided to evaluate whether the patients were hospitalized and whether drug therapies capable of causing liver alterations, such as antibiotics, antivirals, and quinine, were administered. In 861 cases the place where the death took place was described. In 752 cases the patient was hospitalized and died in hospital, 76 cases died at home, 22 cases died in community settings, and 11 cases were not hospitalized and died in other circumstances such as car accidents and falls from a height. In 133 cases a hospital stay or the place of death was not described. Medication administration was described in 201 cases, of which 22 were administered with hydroxychloroquine only. In 41 cases quinine was administered together with an antibiotic or antiviral, in 17 cases antibiotics and antivirals were given, and in 56 only an antibiotic was administered. In 766 cases the administration of hepatotoxic drugs was not reported.

Type of autopsy

Autopsies were performed in all 994 cases; in 508 (51%) cases autopsies were complete, of which 2% (22) had a complete autopsy without the evaluation of the brain to avoid the risk of COVID-19 infection, in 38% (372) of the cases a core biopsy was performed, in 51 (5%) cases a partial autopsy was carried out, and in 41 cases information about the type of autopsy performed was not reported.

Macroscopic results

Macroscopic results were described in only 265 (27%) cases. The most frequent finding, in 79 cases, was the presence of congestion, followed by steatosis in 39 cases. A nutmeg or yellow aspect of the liver surface was seen in 16 cases, a fibrosis-indurated consistency in 6 cases, and only 1 case showed the macroscopic presence of cancer. Lastly, 11 livers were described of increased size (hepatomegaly) and 10 livers as normal. For 144 patients weight was reported; mean weight was 1805 g with a range from 520

Table 1 Literature review results

Ref.	Country	Cases (*n*)	Age (mean, range)	Sex	Type of autopsy	Pre-existing liver disease or other diseases	Laboratory findings	Macroscopi-c results	Microscopi-c results	Additional stainings	Cause of death	Medications	Hospitalizat-ion	Virus identificatio-n in liver
Aguiar *et al* [13], 2020	Switzerland	1	31	F	Complete	Obesity	NR	Nutmeg appearance	Microabs-cesses	None	Respiratory failure in COVID-19	None	Home death	No search
Arslan *et al* [14], 2021	Turkey	7	56, 43-68	M 6, F 1	Partial	3 obesity, 2 hypertension, 1 in hemodialysis	NR	NR	4 mild steatosis, 1 biliary microhamart-oma	None	Respiratory failure in COVID-19	NR	5 hospit-alized, 2 NR	No search
Barton *et al* [15], 2020	United States	2	59, 42-77	M 2	Complete	2 obesity, 1 hypertension and 1 myotonic muscular dystrophy	NR	Case 1: weight: 2232 g, steatosis. Case 2: weight: 1683 g, cirrhosis	Nr	None	1 respiratory failure in COVID-19, 1 complic-ations of hepatic cirrhosis	NR	1 hospit-alized and 1 home death	No search
Beigmo-hammadi *et al*[16], 2021	Iran	7	68, 46-84	M 5, F 2	Core-biopsy	4 hypertension, 1 immuno-compromised and 1 valvular hearth disease	NR	NR	7 congestion, 7 steatosis, 7 portal inflam-mation, 7 hepatitis, 4 ballooning degeneration of hepatocytes, 2 bile plugs, 7 focal confluent necrosis, 4 focal hepatocyte drop out	Masson's trichrome: 1 case of mild fibrosis	NR	7 were treated with hydroxychol-orquine and 6 with antivirals	All hospit-alized	No search
Bradley *et al* [17], 2020	United States	14	74, 42-84	M 6, F 8	7 partial and 7 complete	5 obesity, 8 hypertension, 4 heart failure, 8 CKD	NR	Congestion	10 congestion, 9 steatosis, 1 toxic or metabolic disease, 4 centrilobular necrosis, 3 periportal inflammation	None	12 respiratory failure in COVID-19, 2 cardiovascula-r failure	NR	All hospit-alized	2 positive and 1 negative PCR-test, 11 not tested. 14 negative IHC and TEM

Bryce *et al* [18], 2020	United States	92	NR	NR	Complete	28 fatty liver disease	NR	NR	8 cirrhosis, 57 early organizing thrombi in portal venules and terminal hepatic venules, 41 congestion with some cases showing hemophago-cytosis	None	NR	NR	NR	No search
Bösmüller *et al*[19], 2020	Germany	4	72, 59-79	M 3, F 1	Complete-no brain	2 HIV, 2 hypertension	3 NR 1 with normal values	1 hepato-spleno-megaly and 1 yellowish surface	Case 1: congestion and activation of Kupffer cells. Case 2: macrophages activation with signs hemophago-cytosis	None	1 respiratory failure in COVID-19, 3 MOF	1 had a MOF and was intubated, 1 was treated with meropenem, 1 was treated with ECMO	3 hospit-alized and 1 home death	Negative PCR-test
Bugra *et al* [20], 2021	Turkey	100	55, 7-98	M 80, F 20	Partial	NR	NR	NR	84 inflam-mation, 54 steatosis, 19 glycogen-isation, 9 centilobulary necrosis, 18 autolysis, 45 congestion, 7 endotheliitis, 1 cirrhosis, 2 fibrin thrombosis, 2 bridging necrosis, 1 granulo-matous inflam-mation, 23 cholestasis	CD3+ in portal space	74 respiratory failure in COVID-19 and 26 NR	NR	25 hospit-alized, 55 home dead, 6 falling from height, 5 car accidents and 9 NR	No search
Chornenkyy *et al*[21], 2021	United States	8	58, 18-81	M 3, F 5	Complete	2 chronic liver disease (1 HCV and 1 autoimmune hepatitis), 6 obesity, 4 hypertension	Peak AST: 146 (20-1470) and ALT: 214 (10-9961)	Yellowish surface and congestion	3 periportal fibrosis, 2 necrosis, 5 inflam-mation, 7 portal inflam-	NR	NR	NR	All hospit-alized	4 positive and 4 negative PCR-test

								mation, 6 congestion, 4 steatosis, 6 acute hepatitis					
Danics *et al* [22], 2021	Hungary	100	75, 40-102	M 50, F 50	Complete	36 obesity, 6 liver diseases, 85 hypertension	41 elevated AST values and 27 elevated ALT values	Average weight 1544 g (range 520-3046 g)	63 steatosis, 43 portal fibrosis, 4 cirrhosis, 11 centrolobular necrosis, 87 congestion, 52 hepato-cellular cholestasis	None	NR	NR	All hospit-alized	No search
Del Nonno *et al*[23], 2021	Italy	3	69, 63-76	M 2, F 1	Complete	NR	Admission AST: 63 (31-128) and ALT: 41 (19-84)	NR	All cases showed steatosis, portal inflam-mation, portal fibrosis, focal lobular inflam-mation, zonal necrosis and congestion	IHC: CD8+ in portal inflam-mation, CD34 positive staining in the portal tract vasculature and sinusoids, Perl's staining for iron demonstrate-d iron deposits into hepatocytes	All respiratory failure in COVID-19	1 NR, one with immunosup-pressor (tocilizumab) and one with antibiotics + morphine. All had O_2 therapy (1 CPAP and 2 venturi mask)	All hospit-alized	Negative PCR-test and IHC detection (nucleo-capsid and nucleo-protein)
Edler *et al* [24], 2020	Germany	80	79, 52-96	M 46, F 34	Complete	6 obesity, 4 cirrhosis	NR	NR	Congestion	None	76 respiratory failure in COVID-19, 1 pericardial tamponade, 1 sepsis and 2 cardiovascula-r failure	17 with NIV	51 hospit-alized, 13 in nursing care homes, 12 home deaths, 1 in a hotel and 3 NR	No search
Elsoukkary *et al*[25], 2020	United States	32	68, 30-100	M 22, F 10	Partial	17 hypertension, 12 obesity	AST: 567 (18-6000) and ALT: 387 (12-4885)	NR	9 steatosis, 6 portal inflam-mation, 3 bridging fibrosis and/or cirrhosis	None	NR	19 hydroxy-chloroquine and antibiotics, 9 only antibiotics	All hospit-alized	No search
Evert *et al*	Germany	8	62, 44-73	M 4, F 4	Complete	7 obesity, 1	NR	NR	7 cholestasis,	None	8 MOF	All did NIV,	All hospit-	3 positive

[26], 2021						liver cirrhosis, 5 hypertension			7 single-cell necrosis, 5 fatty degeneration with 2 showing marked steatosis, 2 mild fibrosis, 1 cirrhosis			dialysis and antibiotics. 5 had ECMO	alized	PCR-test
Falasca *et al* [27], 2020	Italy	22	68, 27-92	M 15, F 7	Complete	1 obesity	NR	Congestion	11 inflam-mation, 10 congestion, 12 steatosis	None	All respiratory failure in COVID-19	NR	All hospit-alized	No search
Fassan *et al* [28], 2020	Italy	26	82, 61-97	M 14, F 11	Complete	5 obesity, 1 HCV-related cirrhosis	NR	NR	1 cirrhosis, 22 congestion, 5 centrilobular parenchymal atrophy, 2 fibrosis, 5 sinusoidal diffuse microthromb-i, 3 portal vein thrombosis, 2 centroacinar necrosis, 26 activation of Kupffer cells, 1 portal inflam-mation, 9 steatosis	None	NR	NR	NR	Negative ISH
Greuel *et al* [29], 2021	Germany	6	35, 26-46	M 3 F 3	Complete	1 obesity, 2 right cardiac insufficiency, 1 Ewing sarcoma	4 elevated AST and ALT values	NR	1 severe cholestasis, 1 focal ischemic damage 2 steatosis	None	3 MOF, 1 acute mesenteric ischemia, 1 cardiovascula-r failure, 1 hemorrhagic shock	5 had ECMO and NIV	All hospit-alized	Negative PCR-test
Grosse *et al* [30], 2020	Austria	14	82, 55-94	M 9, F 5	Complete	1 liver cirrhosis, 8 hypertension	Admission AST: 49 (12-98) and ALT: 25 (7-87)	NR	13 steatosis, 14 congestion, 12 portal lymphoid infiltration, 4 portal fibrosis	None	2 bronchopneu-monia, 12 NR	12 had antibiotics	All hospit-alized	No search
Hanley *et al*	United	10	73, 52-79	M 7, F 3	Complete	5 obesity, 4	NR	Average	7 steatosis, 3	None	NR	4 NIV	All hospit-	3 positive

[31], 2020	Kingdom					hypertension		weight 1432 g (range 1012-2466) and 3 hepato-megaly	cirrhosis or bridging fibrosis				alized	PCR-test (e gene)
Hirayama *et al*[32], 2021	United Kingdom	19	71, 42-94	M 11, F 8	Complete	5 obesity, 8 hypertension	NR	NR	12 steatosis, 5 congestion, 4 cirrhosis, 3 portal inflammation	None	NR	NR	All hospit-alized	No search
Hooper *et al* [33], 2021	United States-Brazil	135	61	M 80, F 55	36 core-biopsy and 99 partial	34 obesity, 5 liver disease, 86 hypertension	NR	NR	41 necrosis, 37 steatosis, 19 inflam-mation, 7 fibrosis, 6 congestion, 5 cirrhosis, 3 cholestasis	None	101 respiratory failure in COVID-19, 6 cardiovascula -r failure, 28 NR	NR	All hospit-alized	No search
Ihlow *et al* [34], 2021	Germany	1	88	F	Complete	None	Peak AST: 1690 and ALT: 1632	Subtotal liver dystrophy	Necrosis, cirrhosis, portal inflammation	IHC for ACE2, TMPESS2 and cathepsin L: strong membranous signals in intrahepatic bile duct epithelium	Acute liver failure	Antibiotics	Hospitalized	ISH positive in the bile duct epithelium and positive PCR-test
Lacy *et al* [35], 2020	United States	1	58	F	Complete	Obesity	NR	Weight 1990 g	Steatosis and congestion	None	Respiratory failure in COVID-19	NR	Home death	No search
Lagana *et al* [36], 2020	United States	40	70, 66-80	M 28, F 12	NR	2 chronic liver disease, 1 alcohol-related cirrhosis, 1 liver transplant with acute rejection and 1 with anti-HBV core antibody positivity	*n* = 33 Admission AST: 63 (43-92) and ALT: 32 (19 - 55). Peak AST: 102 (54-294) and ALT: 68 (32-258)	2 fibrosis and 1 had abscesses, 37 with steatosis and congestion	20 lobular necroinflam-mation, 20 portal inflam-mation, 10 lobular apoptosis, 30 steatosis, 32 congestion, 16 centrilobular necrosis, 15 cholestasis	None	NR	22 steroids, 19 hydroxy-chloroquine, and 6 received tocilizumab	All hospit-alized	11 positive and 9 negative PCR-test
Lax *et al*[37], 2020	Austria	11	82, 75-91	M 8, F 3	Partial	2 obesity, 9 hypertension, 1 Hodgkin lymphoma	AST: 66 (17-189) and ALT: 41 (19-98)	NR	11 steatosis, 8 congestion, 7 necrosis, 10 Kupffer cell	None	Pulmonary arterial thrombosis	2 NIV, 9 AIRVO and 9 had antibiotics	All hospit-alized	No search

						and 1 bladder carcinoma			proliferation, 6 portal fibrosis, 8 inflammation, 8 ductular proliferation					
Malik *et al* [38], 2021	India	1	31	F	Complete	None	NR	Congestion	Congestion, mild chronic inflammatory infiltrate in some portal tract, and occasional lymphocytic aggregate adjacent to central vein	None	Respiratory failure in COVID-19	None	Hospitalized	Positive PCR-test
Menter *et al* [39], 2020	Switzerland	21	76, 53-96	M 17, F 4	17 complete and 4 partial	2 chronic liver disease, 21 hypertension, 6 obesity	*n* = 10 AST: 67.2 (22-214)	NR	7 steatosis, 5 necrosis, 3 ASH/NASH	None	Respiratory failure in COVID-19	NR	All hospitalized	No search
Nunes *et al* [40], 2021	South Africa	75	60, 49-68	M 29, F 46	Core- biopsy	41 hypertension, 20 HIV	NR	NR	33 portal inflammation, 24 steatosis, 40 sinusoidal inflammation, 10 lobular hepatitis, 9 Kupffer cell activation, 11 spotty necrosis, 4 confluent necrosis, 6 fibrosis, 26 congestion, 7 fibrin-platelet thrombi	None	NR	NR	All hospitalized	No search
Oprinca[41], 2020	Romania	3	59, 27-79	M 3	1 complete and 2 partial	1 choledochal preampular intraluminal obstruction	NR	Case 1: choledochal preampullary intraluminal obstruction, case 2: normal, case 3: hepatomegaly and cirrhosis	Case 1: congestion, steatosis, periportal fibrosis and portal inflammation, case 2: nothing, case 3:	None	2 respiratory failure in COVID-19, 1 shock hemorrhagic	Case 1: antibiotics, corticosteroids and assisted oxygenation. Case 2: none (home death). Case 3: none	2 hospitalized, 1 NR	No search

								bridging fibrosis and portal inflammation					
Rapkiewicz *et al*[42], 2020	United States	7	NR, 44-65	M 3, F 4	Complete	5 obesity and 7 hypertension	NR	NR	6 steatosis, 1 cirrhosis, 6 platelet-fibrin microthromb-i in sinusoids, 2 necrosis	None	Cardiovascular failure	5 azithro-mycina and hydroxy-chloroquine and O_2 NIV	5 hospit-alized, 2 home deaths	No search
Remmelink *et al*[43], 2020	Belgium	17	72, 62-77	M 12, F 5	Complete	2 cirrhosis, 1 liver transplant, 10 hypertension	NR	5 hepato-megaly	7 congestion, 1 steato-necrosis, 10 steatosis, 1 cholestasis, 3 chronic hepatitis, 2 cirrhosis, 1 centro-obular necrosis	None	9 respiratory failure in COVID-19, 7 MOF and 1 NR	11 had mechanical ventilation	All hospit-alized	14 positive and 3 negative PCR-test
Ren *et al*[44], 2021	China	1	53	F	Complete	None	Admission AST: 27 and ALT: 24. Peak AST: 83 and ALT: 93	Normal	Nothing remarkable	None	Respiratory failure with bacterial infection	She treated herself at home with Chinese herb medicine. In hospital intensive oxygen and supportive measurement-s, extensive antibiotics and antiviral	Hospitalized	Positive PCR-test
Schmit *et al* [45], 2020	Belgium	14	63, 50-83	M 10, F 4	Complete	1 HIV, 1 non-alcoholic steatohep-atitis, 1 HCV-hepatitis, 6 obesity	Admission AST: 54 (15-188) and ALT: 30 (7-62). Peak AST: 2610 (15-24176) and ALT: 854 (10-7245)	Average weight 1988 g (range 1280-3220 g). 8 cases yellowish appearance 6 nutmeg appearance, 2 indurated consistency, 1 hepato-cellular carcinoma, 1 normal	11 centrilobular necrosis, 9 steatosis, 8 lobular inflam-mation, 12 portal inflam-mation, 4 fibrosis, 5 bile duct proliferation, 5 cholestasis, 5 iron overload	None	13 NR and 1 acute mesenteric ischemia	8 hydroxy-chloroquine and antibiotics, 4 with antibiotics, 2 with hydroxy-chloroquine	All hospit-alized	No search
Schweitzer *et*	Switzerland	1	50	M	Complete	HIV	NR	Reduced	Steatosis and	None	Respiratory	None	Home death	No search

al[46], 2020							consistency	liver dystrophy		failure in COVID-19			
Shishido-Hara *et al* [47], 2021	Japan	1	75	M	Complete	None	NR	Normal	Portal inflammation	None	Severe hemorrhage	Anti-viral therapy, antibiotics, O_2 therapy	Hospitalized	No search
Sonzogni *et al* [48], 2020	Italy	48	71, 32-86	M 22, F 8	30 partial and 18 complete - no brain	7 obesity	47 elevated values	NR	24 lobular inflammation, 32 portal inflammation, 18 confluent necrosis 18, 26 steatosis, 48 vascular thrombosis (35 portal, 13 sinusoidal), 37 fibrosis	None	NR	NR	All hospitalized	No search
Suess *et al* [49], 2020	Switzerland	1	59	M	Complete	None	NR	NR	Steatosis and some single necrotic hepatocytes	None	Respiratory failure in COVID-19	NR	Home death	No search
Tehrani *et al* [50], 2022	Iran	5	71, 55-85	M 3, F 2	Partial	None	AST: 275 (106-528) and ALT: 392 (168-978)	NR	Congestion, hepatocytes mildly expanding and bile plugs	None	4 respiratory failure in COVID-19 and 1 cardiovascular failure	1 hydroxychloroquine and antibiotics, 2 with hydroxychloroquine and anti-viral therapy, 1 only anti-viral therapy, 1 anti-viral therapy + antibiotics	All hospitalized	No search
Tian *et al*[51], 2020	China	4	73, 59-81	M 3, F 1	Core-biopsy	1 cirrhosis and 1 hypertension	AST: 36,4 (30-48.8) and ALT: 16 (11-25.5)	NR	Case 1: congestion, glycogen accumulation and focal steatosis, case 2: regenerative nodules and fibrous bands, lobular inflammation	None	Respiratory failure in COVID-19	Antibiotics, antiviral therapy assisted oxygenation	All hospitalized	1 positive and 2 negative PCR-test, 1 was not tested

									and Kupffer cell activation, cases 3: Kupffer cell activation, case 4: periportal and centrilobular necrosis					
Varga *et al* [52], 2020	Switzerland	1	58	F	NR	Obesity and hypertension	NR	NR	Endotheliitis and necrosis	None	MOF	Dialysis	Hospitalized	No search
Wang *et al* [53], 2020	China	2	50 and 79	M 1, F 1	Core-biopsy	NR	Case 1 peak ALT and AST of 70 U/L and 111 U/L, respectively. Case 2 peak ALT and AST of 76 and 236 U/L	NR	Case 1: apoptotic hepatocytes, steatosis, lobular inflammation, portal inflammation, case 2: apoptotic bodies, steatosis, portal inflammation	IHC: case 1 increased CD68 + cells in hepatic sinusoids and infrequent CD4+. Case 2: many CD68+ cells in sinusoids	1 respiratory failure in COVID-19 and 1 septic shock	Both had antiviral therapy and antibiotics	All hospitalized	2 positive TEM (viral particles exist without membrane-bound vesicles)
Wang *et al* [54], 2020	China	1	75	F	Core-biopsy	Chronic cardiac insufficiency, hypertension	Elevated AST and ALT values	NR	Necrosis, activated histiocytes, occasional apoptotic hepatocytes, steatosis and cholestasis	None	MOF	NR	Hospitalized	Negative ISH
Xu *et al*[55], 2020	China	1	50	M	Core-biopsy	NR	NR	NR	Steatosis	None	Respiratory failure in COVID-19	Antibiotics, antiviral therapy and oxygenation	Hospitalized	No search
Yadav *et al* [56], 2022	India	21	61, 25-84	M 15, F 6	Complete	6 obesity, 1 hepatitis B, 1 multiple myeloma	Admission AST: 95.4 (18.9-760.4) and ALT: 52,1 (13,2-229,2). Peak AST: 162,6 (19,8-760,4) and ALT: 75 (21.8-229.2)	NR	20 portal inflammation, 17 steatosis, 9 lobular inflammation, 1 fibrosis, 1 vascular thrombosis, 1 necrosis	None	10 MOF, 1 multiple injuries, 6 septic shock, 3 cardiovascular failure, 1 respiratory failure in COVID-19	11 treated with antibiotics, 7 antibiotics and antiviral therapy	All hospitalized	11 positive, 9 negative PCR-test, 1 not tested

Youd *et al* [57], 2020	United Kingdom	9	72, 33-88	M 4, F 5	Complete	3 obesity	NR	4 congestion, 1 steatosis and 4 normal	NR	None	Respiratory failure in COVID-19	NR	9 deaths in community settings	No search
Zhao *et al* [58], 2020	United States	17	65, 44-85	M 10, F 7	Complete	5 hyperlipidemia, 1 cirrhosis	12 elevated AST and ALT values. Peak AST: 1903 (24-13592) and ALT 1059 (13-6136)	Weight 17694 g (1000-2600 g)	12 platelet-fibrin microthrombi, 5 histiocyte activation, 12 steatosis, 5 lobular inflammation, 8 portal inflammation, 10 necrosis	CD68 stain confirmed histiocytic hyperplasia	NR	NR	All hospitalized	5 positive IHC (spike protein) in the histiocytes in the portal tracts. Negative IHC in endothelial cells and hepatocytes

F: Female; Male: M; HCV: Hepatitis C virus; NIV: Non-invasive ventilation; ECMO: Extracorporeal membrane oxygenation; COVID-19: Coronavirus disease 2019; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ISH: *In situ* hybridization; MOF: Multi-organ failure; PCR: Polymerase chain reaction; TEM: Transmission electron microscopy; NR: Not reported; IHC: Immunohistochemistry.

to 3220 g.

Microscopic results

Microscopic results were described in 983 (99%) cases. The two most frequent findings were congestion, in 420 cases, and steatosis, in 409 cases. Four cases were described as normal. All findings are described in Table 3.

Cause of death

Cause of death was reported for 440 (44%) cases. The most frequent cause of death was respiratory failure in COVID-19, seen in 355 (81%) cases, followed by multi-organ failure in 33 cases, cardiovascular failure in 22, pulmonary thrombosis in 11, and sepsis in 8. The remaining 11 cases died respectively of hemorrhagic shock (3 cases), acute liver failure (2 cases), acute mesenteric ischemia (2 cases), bronchopneumonia (2 cases), and one case each of cardiac tamponade and multiple injuries.

Virus search

The search for the presence of SARS-CoV-2 was performed in only 162 (16%) cases. Of these 105 were tested by real-time reverse-transcription polymerase chain reaction (RT-PCR) and found positive in 53 cases, 34 cases were tested by immunohistochemistry (IHC) and all found negative, 28 were tested by *in situ* hybridization (ISH) and found negative in all cases, and lastly, 16 were tested by transmission electron microscopy and found positive in 2 cases.

Table 2 Laboratory findings

Laboratory findings	Mean (UI/L)	Range (UI/L)
Admission values (n = 53)		
AST	58	12-760
ALT	34	7-229
Peak values (n = 64)		
AST	868	15-24176
ALT	509	10-9961
Non specified		
AST (n = 61)	202	17-6000
ALT (n = 51)	209	11-4885

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Table 3 Microscopic findings

Microscopic findings	*n* (%)
Hepatic necrosis	190 (19)
Hepatic inflammation	190 (19)
Portal inflammation	178 (18)
Fibrosis	149 (15)
Microthrombi	121 (12)
Cholestasis	114 (11)
Hemophagocytosis	51 (5)
Bile plugs	2 (0.2)
Endotheliitis	7 (0.7)
Autolysis	20 (2)
Iron overload	5 (0.5)
Other (abscess, ductal proliferation and granulomatosis)	15 (1.5)

DISCUSSION

A total of 994 autopsy cases of COVID-19 patients with liver assessment were found in the literature. As expected, more than half of the deceased were males and age distribution was highly variable, with a predominance of subjects in the age group 60-90 (71.1%).

Pre-existing liver disease was rare (6%-literature data shows a frequency of 2%-11%), with only 16.2% of the cases presenting obesity (BMI > 30 kg/m^2)[7]. Obesity, in association with diabetes and hypertension, is a prominent risk factor for severe disease and could predispose to nonalcoholic fatty liver disease (NAFLD), a metabolic syndrome which is known to suppress the pro-inflammatory M1 macrophages favoring the progression of virus infection[2,8,11]. NAFLD seems to be identified with a higher prevalence in patients with severe COVID-19 and predisposes to higher liver enzymes at admission and at discharge[59]. To date the fact that pre-existing liver disease is an independent risk factor for poor outcome is still debated; for some authors patients with liver diseases are not over-represented in hospital casuistry[4,60-62], while for others the presence of a pre-exiting illness is index of a greater probability of a bad outcome[7,63-65]. This does not count in the case of cirrhosis, seen in only 1% in this review, which is known to be an important predictor of mortality, with a mortality rate of 31%[2,61]. It appears that in the case of cirrhosis those who survive the first insult have a re-admission rate in hospital similar to those with cirrhosis, but without COVID-19, indicating that beyond the acute phase SARS-CoV-2 does not change the natural history of the disease[4]. There are currently few data regarding the mortality rate associated with alcohol liver disease as an independent risk factor, mainly related to the difficulties of correlating liver damage or elevation of liver enzymes to alcohol

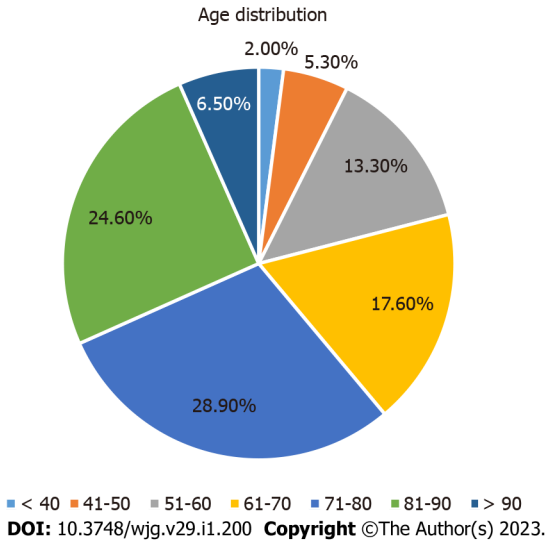

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Figure 1 Age distribution.

consumption. To date, it seems that alcohol liver disease increases the mortality risk by 1.8 fold[61].

Laboratory findings have not been collected in a homogeneous way, with 27 papers not reporting any data, 5 reporting AST and ALT values at admission, and 8 reporting the maximum values during hospitalization, and 4 reports for AST and 5 papers for ALT described the laboratory values without specifying the timing of the sampling. Abnormal values, without specifying the laboratory values, were described in 5 articles. From literature data it appears that liver enzyme abnormalities have a wide range, occurring in 14%-76% of the cases[4,5,7,11,66]. This great range, as Marjot *et al*[4] pointed out, could be attributed to different limits of the definition of normal values. It is still debated whether elevated liver enzymes are associated with a greater risk of mortality, because patients with worst outcomes tend to be monitored in intensive care units, while those with mild symptoms are not strictly monitored. Thus, the use of abnormal laboratory findings at admission as a predictor of poor outcome is still not sure. Liver enzyme elevation mainly affects AST and ALT, indicating hepatocellular damage rather than cholestatic, despite a greater expression of ACE2 receptor in cholangiocytes[3]. As the study of Wong *et al*[67] pointed out, the odd ratio of elevated AST and ALT levels in COVID-19 patients is 3.4 and 2.5, respectively.

Due to the presence of such fragmented laboratory data, it is difficult to draw conclusions about the trend of laboratory values during hospitalization, although some authors have found a tendency of increased values during hospitalization, in particular in those in critical conditions[9,11,12,68,69]. Whether enzyme elevation is induced directly by the virus or because of the inflammation, congestion, or medications is still not clear. Certainly, many of the drugs used in COVID-19 positive patients turn out to be hepatotoxic such as hydroxychloroquine and antivirals such as ritonavir, lopinavir, and remdesivir[8,66]. The meta-analysis by Wong *et al*[67] and Cai *et al*[66] suggests that liver injury is higher in studies with high usage of lopinavir/ritonavir, despite that their hepatotoxic role is still to be described in patients without pre-existing liver disease, while there was no evidence of a higher risk of liver injury for those treated with antibiotics, nonsteroidal anti-inflammatory drugs, ribavirin, herbal medications, and interferon.

The literature review highlighted the presence of a great discrepancy in the autopsy protocols, with only half of the autopsies performed as complete (full autopsies), while the other half as partial. Macroscopic evaluation of the liver was not frequent, while microscopic assessment was present in almost every case (99%). As expected, congestion and steatosis were the most frequent findings. The congestion can be traced back to the presence in these patients of cardiovascular dysfunction due to the massive inflammation and cytokine storm linked to the infection. The presence of steatosis needs a more complex analysis; lipid accumulation due to SARS-CoV-2 has to be differentiated from pre-existing modifications, typical of patient with metabolic syndrome. COVID-19 lipid accumulation can be explained because of the cytopathic effect of the coronavirus, which induces endoplasmatic stress and lipogenesis[2]. Transcriptomic profiling of COVID-19 patients by Wanner *et al*[5] demonstrated an upregulation of cellular processes involved in lipid/cholesterol synthesis. Furthermore, corticosteroid therapy, widely used in the treatment of COVID-19, is known to be associated with steatosis or glycogenosis[2].

Hepatic necrosis and inflammation can be multifactorial; they can be induced by a cytopathic direct effect of the virus, because of inflammatory storm or hypoxic hepatitis, or may be drug induced. These hepatic changes are the third most frequent finding in liver autopsies of COVID-19 patients[70]. Differentiating the different causes from a pathological point of view is impossible, also in consideration of

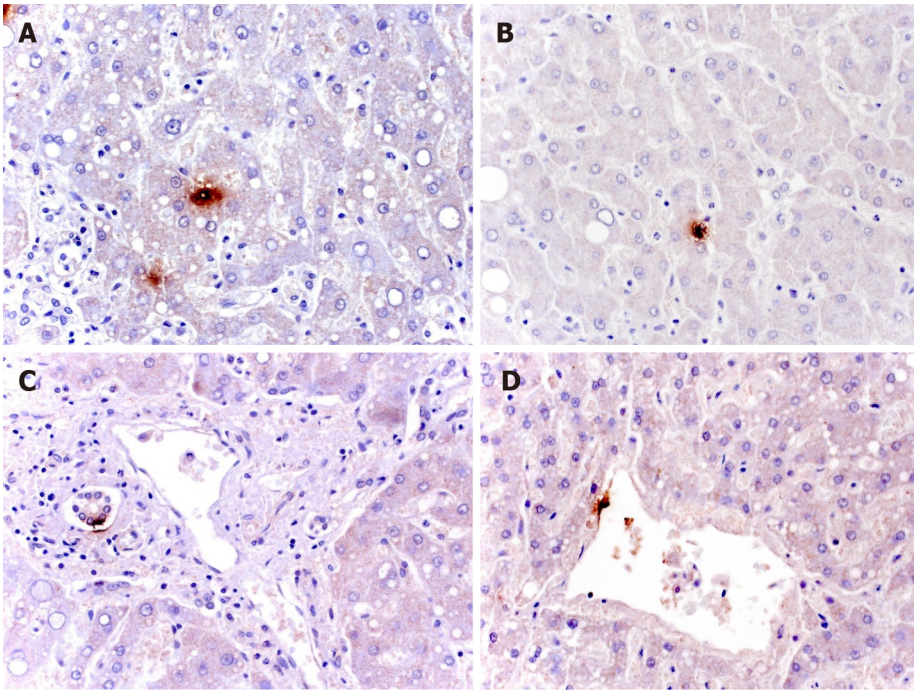

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Figure 2 Immunohistochemical staining for severe acute respiratory syndrome coronavirus 2 in liver tissue. A and B: Spot localization of virus in samples of initial hepatic necrosis and in Kupffer cells; C and D: Spot localization of isolated ductular and endothelial cells (mouse, GeneTex GTX632604, 1A9 clone, 1:100).

the fact that they can overlap one another. In addition, patients with pre-existing liver diseases, such as chronic liver disease, have an increased risk of drug-induced hepatic damage, therefore in those patients the use of hepatotoxic treatments should be weighted. Liver damage in critically ill patients is known and is linked to the so-called hypoxic hepatitis, which is caused by underlying cardiac dysfunction and respiratory failure that decrease the blood flow and oxygenation inducing cellular stress. Moreover, damage could even be mediated by reperfusion, which promotes the production of reactive oxygen species, leading to damage. This process can be highlighted in some cases as a picture of endotheliitis[2,3,11]. Massive inflammation is common in COVID-19 patients and macrophage activation is evidenced by the presence of hemophagocytosis in liver tissue.

Unlike what is reported by Marjot *et al*[4], the frequency of thrombotic phenomena of the hepatic vascular tree is lower, with 12% of cases instead of 29%. As Kleiner[70] noted, death could occur long after the acute phase of liver damage, so the histological changes do not always represent a reliable image of what happened in acute damage, but are the result of damage and reparative modifications. Therefore, to better understand the acute damage, it could be of help to perform a liver biopsy in patients with liver damage. Obviously, it is understood that the execution of such an invasive examination is not a priority in the treatment of these patients, but it could be performed in those cases where the hepatic injury dominates the clinical picture.

Despite the presence of hepatic injury, the presence of SARS-CoV-2 in the liver has been sought infrequently (16% of the cases). Most studies have exploited the RT-PCR to search for the viral genome, but only a few have applied other techniques (IHC, ISH, and transmission electron microscopy) to identify the cells in which the viral proteins were expressed (Figure 2A and B). It is not surprising that by using RT-PCR a greater number of cases resulted positive, because this type of analysis uses a homogenized tissue, which also contains vessels and immunity cells. However, the few available data allow us to confirm the fact that the virus can be found mainly in Kupffer cells, endothelial cells of centrolobulare veins, and cholangiocytes (Figure 2C and D). Note that Wanner *et al*[5] demonstrated that, when comparing the levels of SARS-CoV-2 RNA copies per cell between airway samples and autopsy livers biopsies, the levels of RNA show similar ranges, but with lower median RNA in liver specimens.

CONCLUSION

Postmortem investigations remain the gold standard to investigate the effects of SARS-CoV-2 in different organs and apparatuses. It is well known that the absence of postmortem investigations in the first wave of the pandemic has failed to provide a valuable contribution to the correct management and

treatment of patients. On the other hand, the execution of clinical and forensic autopsies has disclosed several important aspects of the disease, clarifying morphological and virologic features and promoting unexplored therapeutic approaches and new frontiers of research[71-74]. Despite the limited number of performed autopsies worldwide, to date there is no doubt that the liver is a target for the virus, despite minimal viral receptor expression. However, liver damage is not always directly linked to the action of the virus, but can be secondary to inflammation or even simply caused by the therapy administered during hospitalization. Therefore, it is important to monitor patients who use hepatotoxic drugs, to avoid worsening of the liver functions, which can affect the patient's outcome.

ARTICLE HIGHLIGHTS

Research background

Hepatic histologic manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are far to be completely investigated. Many authors demonstrated the presence of angiotensin converting enzyme 2 receptor in the liver as well as transmembrane serine protease 2.

Research motivation

Liver injury was demonstrated in 14%-53% of cases of patients with SARS-CoV-2 infection. In the first wave of the pandemic few autopsies were performed and only few authors can provide a wide casistic. Authors started to study the histologic manifestations of coronavirus disease 2019 (COVID-19) in the lungs, heart, and liver, too.

Research objectives

The objectives of the study were to summarize the biochemical and histological changes in the liver and to promote the leading role of autopsy in the pandemic.

Research methods

Authors provide a systematic review focusing on autopsy studies of COVID-19 deaths and in particular on liver pathology.

Research results

Forty-six articles corresponding to the inclusion criteria were included, with only 994 autopsy cases of COVID-19 patients. Congestion and steatosis were the main histopathological findings, followed by hepatic necrosis, hepatic and portal inflammation, and fibrosis. The most frequent cause of death was respiratory failure, pulmonary thrombosis, and sepsis. Acute liver failure was indicated as the cause of death in two cases.

Research conclusions

The review of the literature highlighted the presence of a great discrepancy in the autopsy protocols, with only half of the autopsies performed as complete (full autopsies), while the other half as partial. Macroscopic and microscopic evaluation of the liver was not always performed or described. Despite the presence of hepatic injury, the presence of SARS-CoV-2 in the liver has been sought infrequently (16% of the cases).

Research perspectives

Much more effort needs to be addressed to completely investigate liver toxicity from COVID-19. Autopsies had a leading role during the pandemic and were important to understand the physiopathology of SARS-CoV-2 infection and should be always considered to improve scientific research.

FOOTNOTES

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REFERENCES

1 **Sun J**, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. *Liver Int* 2020; **40**: 1278-1281 [PMID: 32251539 DOI: 10.1111/liv.14470]
2 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/liv.1473]
3 **Yang RX**, Zheng RD, Fan JG. Etiology and management of liver injury in patients with COVID-19. *World J Gastroenterol* 2020; **26**: 4753-4762 [PMID: 32921955 DOI: 10.3748/wjg.v26.i32.4753]
4 **Marjot T**, Webb GJ, Barritt AS 4th, Moon AM, Stamataki Z, Wong VW, Barnes E. COVID-19 and liver disease: mechanistic and clinical perspectives. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 348-364 [PMID: 33692570 DOI: 10.1038/s41575-021-00426-4]
5 **Wanner N**, Andrieux G, Badia-I-Mompel P, Edler C, Pfefferle S, Lindenmeyer MT, Schmidt-Lauber C, Czogalla J, Wong MN, Okabayashi Y, Braun F, Lütgehetmann M, Meister E, Lu S, Noriega MLM, Günther T, Grundhoff A, Fischer N, Bräuninger H, Lindner D, Westermann D, Haas F, Roedl K, Kluge S, Addo MM, Huber S, Lohse AW, Reiser J, Ondruschka B, Sperhake JP, Saez-Rodriguez J, Boerries M, Hayek SS, Aepfelbacher M, Scaturro P, Puelles VG, Huber TB. Molecular consequences of SARS-CoV-2 liver tropism. *Nat Metab* 2022; **4**: 310-319 [PMID: 35347318 DOI: 10.1038/s42255-022-00552-6]
6 **Pirisi M**, Rigamonti C, D'Alfonso S, Nebuloni M, Fanni D, Gerosa C, Orrù G, Venanzi Rullo E, Pavone P, Faa G, Saba L, Boldorini R. Liver infection and COVID-19: the electron microscopy proof and revision of the literature. *Eur Rev Med Pharmacol Sci* 2021; **25**: 2146-2151 [PMID: 33660834 DOI: 10.26355/eurrev_202102_25120]
7 **Warner FJ**, Rajapaksha H, Shackel N, Herath CB. ACE2: from protection of liver disease to propagation of COVID-19. *Clin Sci (Lond)* 2020; **134**: 3137-3158 [PMID: 33284956 DOI: 10.1042/CS20201268]
8 **Jothimani D**, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: 32553666 DOI: 10.1016/j.jhep.2020.06.006]
9 **Mohammed A**, Paranji N, Chen PH, Niu B. COVID-19 in Chronic Liver Disease and Liver Transplantation: A Clinical Review. *J Clin Gastroenterol* 2021; **55**: 187-194 [PMID: 33394628 DOI: 10.1097/MCG.0000000000001481]
10 **Lizardo-Thiebaud MJ**, Cervantes-Alvarez E, Limon-de la Rosa N, Tejeda-Dominguez F, Palacios-Jimenez M, Méndez-Guerrero O, Delaye-Martinez M, Rodriguez-Alvarez F, Romero-Morales B, Liu WH, Huang CA, Kershenobich D, Navarro-Alvarez N. Direct or Collateral Liver Damage in SARS-CoV-2-Infected Patients. *Semin Liver Dis* 2020; **40**: 321-330 [PMID: 32886936 DOI: 10.1055/s-0040-1715108]
11 **Parker GA**, Picut CA. Liver immunobiology. *Toxicol Pathol* 2005; **33**: 52-62 [PMID: 15805056 DOI: 10.1080/01926230590522365]
12 **Gracia-Ramos AE**, Jaquez-Quintana JO, Contreras-Omaña R, Auron M. Liver dysfunction and SARS-CoV-2 infection. *World J Gastroenterol* 2021; **27**: 3951-3970 [PMID: 34326607 DOI: 10.3748/wjg.v27.i26.3951]
13 **Fiel MI**, El Jamal SM, Paniz-Mondolfi A, Gordon RE, Reidy J, Bandovic J, Advani R, Kilaru S, Pourmand K, Ward S, Thung SN, Schiano T. Findings of Hepatic Severe Acute Respiratory Syndrome Coronavirus-2 Infection. *Cell Mol Gastroenterol Hepatol* 2021; **11**: 763-770 [PMID: 32992052 DOI: 10.1016/j.jcmgh.2020.09.015]
14 **Aguiar D**, Lobrinus JA, Schibler M, Fracasso T, Lardi C. Inside the lungs of COVID-19 disease. *Int J Legal Med* 2020; **134**: 1271-1274 [PMID: 32458044 DOI: 10.1007/s00414-020-02318-9]
15 **Arslan MN**, Büyük Y, Ziyade N, Elgörmüş N, Şirin G, Çoban İ, Gökşen ME, Daş T, Akçay A. COVID-19 autopsies of Istanbul. *Ir J Med Sci* 2022; **191**: 529-541 [PMID: 33755916 DOI: 10.1007/s11845-021-02602-6]
16 **Barton LM**, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 Autopsies, Oklahoma, USA. *Am J Clin Pathol* 2020; **153**: 725-733 [PMID: 32275742 DOI: 10.1093/ajcp/aqaa062]
17 **Beigmohammadi MT**, Jahanbin B, Safaei M, Amoozadeh L, Khoshavi M, Mehrtash V, Jafarzadeh B, Abdollahi A. Pathological Findings of Postmortem Biopsies From Lung, Heart, and Liver of 7 Deceased COVID-19 Patients. *Int J Surg Pathol* 2021; **29**: 135-145 [PMID: 32552178 DOI: 10.1177/1066896920935195]
18 **Bradley BT**, Maioli H, Johnston R, Chaudhry I, Fink SL, Xu H, Najafian B, Deutsch G, Lacy JM, Williams T, Yarid N, Marshall DA. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet* 2020; **396**: 320-332 [PMID: 32682491 DOI: 10.1016/S0140-6736(20)31305-2]
19 **Bryce C**, Grimes Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, Hernandez T, Stock A, Zhao Z, AlRasheed MR, Chen J, Li L, Wang D, Corben A, Haines GK 3rd, Westra WH, Umphlett M, Gordon RE, Reidy J, Petersen B, Salem F, Fiel MI, El Jamal SM, Tsankova NM, Houldsworth J, Mussa Z, Veremis B, Sordillo E, Gitman MR, Nowak M, Brody R, Harpaz N,

Merad M, Gnjatic S, Liu WC, Schotsaert M, Miorin L, Aydillo Gomez TA, Ramos-Lopez I, Garcia-Sastre A, Donnelly R, Seigler P, Keys C, Cameron J, Moultrie I, Washington KL, Treatman J, Sebra R, Jhang J, Firpo A, Lednicky J, Paniz-Mondolfi A, Cordon-Cardo C, Fowkes ME. Pathophysiology of SARS-CoV-2: the Mount Sinai COVID-19 autopsy experience. *Mod Pathol* 2021; **34**: 1456-1467 [PMID: 33795830 DOI: 10.1038/s41379-021-00793-y]

20 **Bösmüller H**, Traxler S, Bitzer M, Häberle H, Raiser W, Nann D, Frauenfeld L, Vogelsberg A, Klingel K, Fend F. The evolution of pulmonary pathology in fatal COVID-19 disease: an autopsy study with clinical correlation. *Virchows Arch* 2020; **477**: 349-357 [PMID: 32607684 DOI: 10.1007/s00428-020-02881-x]

21 **Bugra A**, Das T, Arslan MN, Ziyade N, Buyuk Y. Postmortem pathological changes in extrapulmonary organs in SARS-CoV-2 rt-PCR-positive cases: a single-center experience. *Ir J Med Sci* 2022; **191**: 81-91 [PMID: 33963513 DOI: 10.1007/s11845-021-02638-8]

22 **Chornenkyy Y**, Mejia-Bautista M, Brucal M, Blanke T, Dittmann D, Yeldandi A, Boike JR, Lomasney JW, Nayar R, Jennings LJ, Pezhouh MK. Liver Pathology and SARS-CoV-2 Detection in Formalin-Fixed Tissue of Patients With COVID-19. *Am J Clin Pathol* 2021; **155**: 802-814 [PMID: 33914058 DOI: 10.1093/ajcp/aqab009]

23 **Danics K**, Pesti A, Törő K, Kiss-Dala N, Szlávik J, Lakatos B, Radnai A, Balázs T, Bacskai M, Dobi D, Várkonyi T, Glasz T, Lotz G, Kiss A, Schaff Z, Vályi-Nagy I. A COVID-19-association-dependent categorization of death causes in 100 autopsy cases. *Geroscience* 2021; **43**: 2265-2287 [PMID: 34510338 DOI: 10.1007/s11357-021-00451-w]

24 **Del Nonno F**, Nardacci R, Colombo D, Visco-Comandini U, Cicalini S, Antinori A, Marchioni L, D'Offizi G, Piacentini M, Falasca L. Hepatic Failure in COVID-19: Is Iron Overload the Dangerous Trigger? *Cells* 2021; **10** [PMID: 34064487 DOI: 10.3390/cells10051103]

25 **Edler C**, Schröder AS, Aepfelbacher M, Fitzek A, Heinemann A, Heinrich F, Klein A, Langenwalder F, Lütgehetmann M, Meißner K, Püschel K, Schädler J, Steurer S, Mushumba H, Sperhake JP. Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg, Germany. *Int J Legal Med* 2020; **134**: 1275-1284 [PMID: 32500199 DOI: 10.1007/s00414-020-02317-w]

26 **Elsoukkary SS**, Mostyka M, Dillard A, Berman DR, Ma LX, Chadburn A, Yantiss RK, Jessurun J, Seshan SV, Borczuk AC, Salvatore SP. Autopsy Findings in 32 Patients with COVID-19: A Single-Institution Experience. *Pathobiology* 2021; **88**: 56-68 [PMID: 32942274 DOI: 10.1159/000511325]

27 **Evert K**, Dienemann T, Brochhausen C, Lunz D, Lubnow M, Ritzka M, Keil F, Trummer M, Scheiter A, Salzberger B, Reischl U, Boor P, Gessner A, Jantsch J, Calvisi DF, Evert M, Schmidt B, Simon M. Autopsy findings after long-term treatment of COVID-19 patients with microbiological correlation. *Virchows Arch* 2021; **479**: 97-108 [PMID: 33471172 DOI: 10.1007/s00428-020-03014-0]

28 **Falasca L**, Nardacci R, Colombo D, Lalle E, Di Caro A, Nicastri E, Antinori A, Petrosillo N, Marchioni L, Biava G, D'Offizi G, Palmieri F, Goletti D, Zumla A, Ippolito G, Piacentini M, Del Nonno F. Postmortem Findings in Italian Patients With COVID-19: A Descriptive Full Autopsy Study of Cases With and Without Comorbidities. *J Infect Dis* 2020; **222**: 1807-1815 [PMID: 32914853 DOI: 10.1093/infdis/jiaa578]

29 **Fassan M**, Mescoli C, Sbaraglia M, Guzzardo V, Russo FP, Fabris R, Trevenzoli M, Pelizzaro F, Cattelan AM, Basso C, Navalesi P, Farinati F, Vettor R, Dei Tos AP. Liver histopathology in COVID-19 patients: A mono-Institutional series of liver biopsies and autopsy specimens. *Pathol Res Pract* 2021; **221**: 153451 [PMID: 33932720 DOI: 10.1016/j.prp.2021.153451]

30 **Greuel S**, Ihlow J, Dragomir MP, Streit S, Corman VM, Haberbosch L, Winkler D, Meinhardt J, Aschman T, Schneider J, Trotsyuk I, Kunze CA, Maurer L, Radbruch H, Heppner FL, Horst D, Elezkurtaj S. COVID-19: Autopsy findings in six patients between 26 and 46 years of age. *Int J Infect Dis* 2021; **108**: 274-281 [PMID: 34089883 DOI: 10.1016/j.ijid.2021.05.069]

31 **Grosse C**, Grosse A, Salzer HJF, Dünser MW, Motz R, Langer R. Analysis of cardiopulmonary findings in COVID-19 fatalities: High incidence of pulmonary artery thrombi and acute suppurative bronchopneumonia. *Cardiovasc Pathol* 2020; **49**: 107263 [PMID: 32784110 DOI: 10.1016/j.carpath.2020.107263]

32 **Hanley B**, Naresh KN, Roufosse C, Nicholson AG, Weir J, Cooke GS, Thursz M, Manousou P, Corbett R, Goldin R, Al-Sarraj S, Abdolrasouli A, Swann OC, Baillon L, Penn R, Barclay WS, Viola P, Osborn M. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe* 2020; **1**: e245-e253 [PMID: 32844161 DOI: 10.1016/S2666-5247(20)30115-4]

33 **Hirayama Y**, Daniels NF, Evans S, Clarke D, Purvis S, Oliver C, Woodmansey S, Staniforth J, Soilleux EJ. High Prevalence of Pre-Existing Liver Abnormalities Identified Via Autopsies in COVID-19: Identification of a New Silent Risk Factor? *Diagnostics (Basel)* 2021; **11** [PMID: 34574044 DOI: 10.3390/diagnostics11091703]

34 **Hooper JE**, Padera RF, Dolhnikoff M, da Silva LFF, Duarte-Neto AN, Kapp ME, Lacy JM, Mauad T, Saldiva PHN, Rapkiewicz AV, Wolf DA, Felix JC, Benson P, Shanes E, Gawelek KL, Marshall DA, McDonald MM, Muller W, Priemer DS, Solomon IH, Zak T, Bhattacharjee MB, Fu L, Gilbert AR, Harper HL, Litovsky S, Lomasney J, Mount SL, Reilly S, Sekulic M, Steffensen TS, Threlkeld KJ, Zhao B, Williamson AK. A Postmortem Portrait of the Coronavirus Disease 2019 (COVID-19) Pandemic: A Large Multi-institutional Autopsy Survey Study. *Arch Pathol Lab Med* 2021; **145**: 529-535 [PMID: 33449998 DOI: 10.5858/arpa.2020-0786-SA]

35 **Ihlow J**, Seelhoff A, Corman VM, Gruber AD, Dökel S, Meinhardt J, Radbruch H, Späth-Schwalbe E, Elezkurtaj S, Horst D, Herbst H. COVID-19: a fatal case of acute liver failure associated with SARS-CoV-2 infection in pre-existing liver cirrhosis. *BMC Infect Dis* 2021; **21**: 901 [PMID: 34479499 DOI: 10.1186/s12879-021-06605-7]

36 **Lacy JM**, Brooks EG, Akers J, Armstrong D, Decker L, Gonzalez A, Humphrey W, Mayer R, Miller M, Perez C, Arango JAR, Sathyavagiswaran L, Stroh W, Utley S. COVID-19: Postmortem Diagnostic and Biosafety Considerations. *Am J Forensic Med Pathol* 2020; **41**: 143-151 [PMID: 32379077 DOI: 10.1097/PAF.0000000000000567]

37 **Lagana SM**, Kudose S, Iuga AC, Lee MJ, Fazlollahi L, Remotti HE, Del Portillo A, De Michele S, de Gonzalez AK, Saqi A, Khairallah P, Chong AM, Park H, Uhlemann AC, Lefkowitch JH, Verna EC. Hepatic pathology in patients dying of COVID-19: a series of 40 cases including clinical, histologic, and virologic data. *Mod Pathol* 2020; **33**: 2147-2155 [PMID: 32792598 DOI: 10.1038/s41379-020-00649-x]

38 **Lax SF**, Skok K, Zechner P, Kessler HH, Kaufmann N, Koelblinger C, Vander K, Bargfrieder U, Trauner M. Pulmonary

Arterial Thrombosis in COVID-19 With Fatal Outcome : Results From a Prospective, Single-Center, Clinicopathologic Case Series. *Ann Intern Med* 2020; **173**: 350-361 [PMID: 32422076 DOI: 10.7326/M20-2566]

39 **Malik Y**, Singh K, Yadav S, Vashist YK, Garg A, Kumar S, Sharma G. COVID-19: Asymptomatic Carrier: An Autopsy Case Report. *Int J Appl Basic Med Res* 2021; **11**: 120-124 [PMID: 33912436 DOI: 10.4103/ijabmr.IJABMR_579_20]

40 **Menter T**, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, Frank S, Turek D, Willi N, Pargger H, Bassetti S, Leuppi JD, Cathomas G, Tolnay M, Mertz KD, Tzankov A. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* 2020; **77**: 198-209 [PMID: 32364264 DOI: 10.1111/his.14134]

41 **Nunes MC**, Hale MJ, Mahtab S, Mabena FC, Dludlu N, Baillie VL, Thwala BN, Els T, du Plessis J, Laubscher M, Mckenzie S, Mtshali S, Menezes C, Serafin N, van Blydenstein S, Tsitsi M, Dulisse B, Madhi SA. Clinical characteristics and histopathology of COVID-19 related deaths in South African adults. *PLoS One* 2022; **17**: e0262179 [PMID: 35051205 DOI: 10.1371/journal.pone.0262179]

42 **Oprinca GC**, Muja LA. Postmortem examination of three SARS-CoV-2-positive autopsies including histopathologic and immunohistochemical analysis. *Int J Legal Med* 2021; **135**: 329-339 [PMID: 32851474 DOI: 10.1007/s00414-020-02406-w]

43 **Rapkiewicz AV**, Mai X, Carsons SE, Pittaluga S, Kleiner DE, Berger JS, Thomas S, Adler NM, Charytan DM, Gasmi B, Hochman JS, Reynolds HR. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: A case series. *EClinicalMedicine* 2020; **24**: 100434 [PMID: 32766543 DOI: 10.1016/j.eclinm.2020.100434]

44 **Remmelink M**, De Mendonça R, D'Haene N, De Clercq S, Verocq C, Lebrun L, Lavis P, Racu ML, Trépant AL, Maris C, Rorive S, Goffard JC, De Witte O, Peluso L, Vincent JL, Decaestecker C, Taccone FS, Salmon I. Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care* 2020; **24**: 495 [PMID: 32787909 DOI: 10.1186/s13054-020-03218-5]

45 **Ren L**, Liu Q, Wang R, Chen R, Ao Q, Wang X, Zhang J, Deng F, Feng Y, Wang G, Zhou Y, Li L, Liu L. Clinicopathologic Features of COVID-19: A Case Report and Value of Forensic Autopsy in Studying SARS-CoV-2 Infection. *Am J Forensic Med Pathol* 2021; **42**: 164-169 [PMID: 33464756 DOI: 10.1097/PAF.0000000000000644]

46 **Schmit G**, Lelotte J, Vanhaebost J, Horsmans Y, Van Bockstal M, Baldin P. The Liver in COVID-19-Related Death: Protagonist or Innocent Bystander? *Pathobiology* 2021; **88**: 88-94 [PMID: 33108789 DOI: 10.1159/000512008]

47 **Schweitzer W**, Ruder T, Baumeister R, Bolliger S, Thali M, Meixner E, Ampanozi G. Implications for forensic death investigations from first Swiss post-mortem CT in a case of non-hospital treatment with COVID-19. *Forensic Imaging* 2020; **21**: 200378 [DOI: 10.1016/j.fri.2020.200378]

48 **Shishido-Hara Y**, Furukawa K, Nishio M, Honda K, Tando S, Yaoi T, Kawamoto M, Maehara Y, Nakaya T, Itoh K. An autopsy case of COVID-19 with a sudden death: Clinico-pathological comparison. *Clin Case Rep* 2022; **10**: e5961 [PMID: 35702618 DOI: 10.1002/ccr3.5961]

49 **Sonzogni A**, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, Morotti D, Zerbi P, Carsana L, Rossi R, Lauri E, Pellegrinelli A, Nebuloni M. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. *Liver Int* 2020; **40**: 2110-2116 [PMID: 32654359 DOI: 10.1111/liv.14601]

50 **Suess C**, Hausmann R. Gross and histopathological pulmonary findings in a COVID-19 associated death during self-isolation. *Int J Legal Med* 2020; **134**: 1285-1290 [PMID: 32504146 DOI: 10.1007/s00414-020-02319-8]

51 **Shirazi Tehrani A**, Tabatabaei Mirakabad FS, Abdollahifar MA, Mollazadehghomi S, Darabi S, Forozesh M, Rezaei-Tavirani M, Mahmoudiasl GR, Ahrabi B, Azimzadeh Z, Allah Abbaszadeh H. Severe Acute Respiratory Syndrome Coronavirus 2 Induces Hepatocyte Cell Death, Active Autophagosome Formation and Caspase 3 Up-Regulation in Postmortem Cases: Stereological and Molecular Study. *Tohoku J Exp Med* 2022; **256**: 309-319 [PMID: 35321977 DOI: 10.1620/tjem.2022.J007]

52 **Tian S**, Xiong Y, Liu H, Niu L, Guo J, Liao M, Xiao SY. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod Pathol* 2020; **33**: 1007-1014 [PMID: 32291399 DOI: 10.1038/s41379-020-0536-x]

53 **Varga Z**, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395**: 1417-1418 [PMID: 32325026 DOI: 10.1016/S0140-6736(20)30937-5]

54 **Wang Y**, Liu S, Liu H, Li W, Lin F, Jiang L, Li X, Xu P, Zhang L, Zhao L, Cao Y, Kang J, Yang J, Li L, Liu X, Li Y, Nie R, Mu J, Lu F, Zhao S, Lu J, Zhao J. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol* 2020; **73**: 807-816 [PMID: 32437830 DOI: 10.1016/j.jhep.2020.05.002]

55 **Wang XX**, Shao C, Huang XJ, Sun L, Meng LJ, Liu H, Zhang SJ, Li HJ, Lv FD. Histopathological features of multiorgan percutaneous tissue core biopsy in patients with COVID-19. *J Clin Pathol* 2021; **74**: 522-527 [PMID: 32848014 DOI: 10.1136/jclinpath-2020-206623]

56 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

57 **Yadav J**, Goel G, Purwar S, Saigal S, Tandon A, Joshi A, Patel B, Js S, S M, Singh J, Shankar P, Arora A, Singh S. Clinical, Virological, and Pathological Profile of Patients Who Died of COVID-19: An Autopsy-Based Study From India. *Cureus* 2022; **14**: e23538 [PMID: 35494966 DOI: 10.7759/cureus.23538]

58 **Youd E**, Moore L. COVID-19 autopsy in people who died in community settings: the first series. *J Clin Pathol* 2020; **73**: 840-844 [PMID: 32605920 DOI: 10.1136/jclinpath-2020-206710]

59 **Zhao CL**, Rapkiewicz A, Maghsoodi-Deerwester M, Gupta M, Cao W, Palaia T, Zhou J, Ram B, Vo D, Rafiee B, Hossein-Zadeh Z, Dabiri B, Hanna I. Pathological findings in the postmortem liver of patients with coronavirus disease 2019 (COVID-19). *Hum Pathol* 2021; **109**: 59-68 [PMID: 33307078 DOI: 10.1016/j.humpath.2020.11.015]

60 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]

61 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research

Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

62 **Marjot T**, Moon AM, Cook JA, Abd-Elsalam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: 33035628 DOI: 10.1016/j.jhep.2020.09.024]

63 **Wang X**, Fang X, Cai Z, Wu X, Gao X, Min J, Wang F. Comorbid Chronic Diseases and Acute Organ Injuries Are Strongly Correlated with Disease Severity and Mortality among COVID-19 Patients: A Systemic Review and Meta-Analysis. *Research (Wash D C)* 2020; **2020**: 2402961 [PMID: 32377638 DOI: 10.34133/2020/2402961]

64 **Vila-Corcoles A**, Satue-Gracia E, Vila-Rovira A, de Diego-Cabanes C, Forcadell-Peris MJ, Hospital-Guardiola I, Ochoa-Gondar O, Basora-Gallisa J. COVID19-related and all-cause mortality risk among middle-aged and older adults across the first epidemic wave of SARS-COV-2 infection: a population-based cohort stuJune 2020.dy in Southern Catalonia, Spain, March-. *BMC Public Health* 2021; **21**: 1795 [PMID: 34615512 DOI: 10.1186/s12889-021-11879-2]

65 **Dorjee K**, Kim H, Bonomo E, Dolma R. Prevalence and predictors of death and severe disease in patients hospitalized due to COVID-19: A comprehensive systematic review and meta-analysis of 77 studies and 38,000 patients. *PLoS One* 2020; **15**: e0243191 [PMID: 33284825 DOI: 10.1371/journal.pone.0243191]

66 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

67 **Wong YJ**, Tan M, Zheng Q, Li JW, Kumar R, Fock KM, Teo EK, Ang TL. A systematic review and meta-analysis of the COVID-19 associated liver injury. *Ann Hepatol* 2020; **19**: 627-634 [PMID: 32882393 DOI: 10.1016/j.aohep.2020.08.064]

68 **Wu Y**, Li H, Guo X, Yoshida EM, Mendez-Sanchez N, Levi Sandri GB, Teschke R, Romeiro FG, Shukla A, Qi X. Incidence, risk factors, and prognosis of abnormal liver biochemical tests in COVID-19 patients: a systematic review and meta-analysis. *Hepatol Int* 2020; **14**: 621-637 [PMID: 32710250 DOI: 10.1007/s12072-020-10074-6]

69 **Barnes E**. Infection of liver hepatocytes with SARS-CoV-2. *Nat Metab* 2022; **4**: 301-302 [PMID: 35347317 DOI: 10.1038/s42255-022-00554-4]

70 **Kleiner DE**. Liver Biopsy Shines a Light on COVID-19-Related Liver Injury. *Cell Mol Gastroenterol Hepatol* 2021; **11**: 881-882 [PMID: 33144089 DOI: 10.1016/j.jcmgh.2020.10.003]

71 **D'Errico S**, Zanon M, Montanaro M, Radaelli D, Sessa F, Di Mizio G, Montana A, Corrao S, Salerno M, Pomara C. More than Pneumonia: Distinctive Features of SARS-Cov-2 Infection. From Autopsy Findings to Clinical Implications: A Systematic Review. *Microorganisms* 2020; **8** [PMID: 33114061 DOI: 10.3390/microorganisms8111642]

72 **Macor P**, Durigutto P, Mangogna A, Bussani R, D'Errico S, Zanon M, Pozzi N, Meroni P, Tedesco F. Multi-organ complement deposition in COVID-19 patients. *medRxiv* 2021 [PMID: 33442701 DOI: 10.3390/biomedicines9081003]

73 **Cipolloni L**, Sessa F, Bertozzi G, Baldari B, Cantatore S, Testi R, D'Errico S, Di Mizio G, Asmundo A, Castorina S, Salerno M, Pomara C. Preliminary Post-Mortem COVID-19 Evidence of Endothelial Injury and Factor VIII Hyperexpression. *Diagnostics (Basel)* 2020; **10** [PMID: 32784826 DOI: 10.3390/diagnostics10080575]

74 **Frisoni P**, Neri M, D'Errico S, Alfieri L, Bonuccelli D, Cingolani M, Di Paolo M, Gaudio RM, Lestani M, Marti M, Martelloni M, Moreschi C, Santurro A, Scopetti M, Turriziani O, Zanon M, Scendoni R, Frati P, Fineschi V. Cytokine storm and histopathological findings in 60 cases of COVID-19-related death: from viral load research to immunohistochemical quantification of major players IL-1β, IL-6, IL-15 and TNF-α. *Forensic Sci Med Pathol* 2022; **18**: 4-19 [PMID: 34463916 DOI: 10.1007/s12024-021-00414-9]

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RETRACTION NOTE

Retraction note to: Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial

Qing-Hua Sun, Hong-Yan Wang, Shi-Dong Sun, Xin Zhang, Han Zhang

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Abstract

We have decided to retract the above article for further consideration due to a data labelling error.

Key Words: Retraction note

Core Tip: We have decided to retract the above article due to a data labelling error.

Citation: Sun QH, Wang HY, Sun SD, Zhang X, Zhang H. Retraction note to: Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. *World J Gastroenterol* 2023; 29(1): 221-222
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RETRACTION NOTE

Retraction Note to: Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. *World J Gastroenterol* 2019; 25: 2110-2121 [PMID: 31114137 DOI: 10.3748/wjg.v25.i17.2110]. The online version of the original article can be found at https://www.wjgnet.com/1007-9327/full/v

25/i17/2110.htm.

It has come to my attention that there was a data labelling error in the study[1] we published in the *World Journal of Gastroenterology* four years ago. We discovered that one of our research assistants confused the two drugs by mistake when dispensing them to subjects, meaning that the labels for patients may have been mismatched with the actual treatment they received. Due to this data labelling problem, the validity of the paper and its conclusion have been brought into question even though the methodology of statistical analysis used in the paper was correct. To avoid misleading other readers, I respectfully ask that the article "Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. *World J Gastroenterol* 2019; 25: 2110-2121 [PMID: 31114137 DOI: 10.3748/wjg.v25.i17.2110]" is retracted.

We apologize to the readers and editors of the *World Journal of Gastroenterology* for this error and for any inconvenience caused.

FOOTNOTES

Author contributions: Sun QH, Wang HY, Sun SD, Zhang X, Zhang H wrote this retraction note.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

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REFERENCES

1 **Sun QH**, Wang HY, Sun SD, Zhang X, Zhang H. Beneficial effect of probiotics supplements in reflux esophagitis treated with esomeprazole: A randomized controlled trial. *World J Gastroenterol* 2019; **25**: 2110-2121 [PMID: 31114137 DOI: 10.3748/wjg.v25.i17.2110]

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