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*REVIEW*

# Inflammation, microbiome and colorectal cancer disparity in African-Americans: Are there bugs in the genetics?

Sami Ahmad, Hassan Ashktorab, Hassan Brim, Franck Housseau

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## Abstract

Dysregulated interactions between host inflammation and gut microbiota over the course of life increase the risk of colorectal cancer (CRC). While environmental factors and socio-economic realities of race remain predominant contributors to CRC disparities in African-Americans (AAs), this review focuses on the biological mediators of CRC disparity, namely the under-appreciated influence of inherited ancestral genetic regulation on mucosal innate immunity and its interaction with the microbiome. There remains a poor understanding of mechanisms linking immune-related genetic polymorphisms and microbiome diversity that could influence chronic inflammation and exacerbate CRC disparities in AAs. A better understanding of the relationship between host genetics, bacteria, and CRC pathogenesis will improve the prediction of cancer risk across race/ethnicity groups overall.

**Key Words:** Inflammation; African-American; Population-specific genome wide association studies; Minorities health; Microbiome; Colorectal cancer

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**Core Tip:** Studies largely examine either variations in microbiome composition or host immunity polymorphisms, often using genome-wide association studies comprised of populations mainly of European ancestry. There is, thus, a pressing need for studies that include, recruit, and account for more widely diverse cohorts. Identification of population-associated polymorphisms driving host/microbiome interactions linked to colorectal cancer (CRC) disparity may reveal genes or pathways that could be targeted for patient-specific CRC interception strategies.

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## INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of death amongst cancer patients, an estimated 53000 of whom will die in the United States from CRC in 2021[1]. Strikingly, although total CRC mortality has decreased over the last two decades, particularly in older individuals (age 64 +), CRC incidence has increased in individuals under 50[1]. Recent studies showed early-onset CRC patients were more likely to be African-Americans (AAs), who bear the highest CRC incidence rate between 20-year-old and 44-year-old (7.9/100000) as compared to Caucasian Americans (CAs) (6.7/100000) and Asian-Pacific Islanders (6.3/100000)[2]. AAs commonly display more aggressive types of CRC as well, and are generally diagnosed at more advanced stages of the disease, exhibiting survival rates 7% below those of CAs (58% *vs* 65% 5-year survival)[3]. Such statistics must be interpreted cautiously, since the noted increase of early-onset CRC may result from recently recommended and adopted early colonoscopy (40-45 years old) screening campaigns. Nevertheless, there are multiple proposed influences on CRC disparities in AAs, including differences in health care and treatment access, comorbidities and tumor characteristics[4-8]. Socioeconomic status (SES) also weighs heavily on the late diagnoses and prevention campaign efficacy observed in AA populations[2,6,9]. Undoubtedly the source of CRC disparity is multifactorial, and a layered perspective is imperative to address the alarming rise of early onset CRC, an otherwise preventable disease when detected early.

Herein, we aim to elucidate novel biological factors that may also contribute to the AA disparities in CRC mortality. Specifically, in addition to the genetic influence on CRC pathogenesis, an accumulating body of evidence connects CRC to dysregulated interactions between mucosal innate immunity and the microbiome[10]. Indeed, sustained inflammation promoted by chronic colorectal dysbiosis is an established driver of CRC pathogenesis[11]. Related to this notion is a recent gut microbiome profiling study that found that, in addition to diminished overall species diversity, pro-inflammatory *Fusobacterium nucleatum (F. nucleatum)* and *Enterobacter* species were significantly more abundant in AA CRC patients as compared to a CA cohort[12]. The presence of *F. nucleatum* has also been linked to inflammation-associated microsatellite alterations found more prevalently in AA rectal tumors, a finding linked to worsened CRC prognosis[13-15]. Furthermore, genetic landscape and microbiome composition have been shown to influence the occurrence of proximal colorectal tumors[16], which are more difficult to detect and are diagnosed nearly four times more often in AA than in CA CRC patients[17]. Nearly 80% of sessile serrated polyps are found in the proximal colon, a phenomenon associated with microbial biofilms and *Fusobacteria*, plus the frequency of *BRAF* mutations, CpG island hypermethylation phenotype, and microsatellite instability that increases from the distal to the proximal region[16,18-22]. Altogether, there is emerging research on the genetically tuned relationships between mucosal innate immunity, the microbiome, and disparate CRC development, but a functional understanding of how said relationships impact CRC pathology remains incomplete[23]. Additionally, despite the evidence that mucosal innate immunity and the microbiome are intimately connected, this review highlights how minority health research currently evaluates their contribution to CRC risk in a largely separate fashion [24].

Accordingly, we propose an integrated concept whereby a differential mucosal inflammatory response to gut microbiota, influenced by host genetic ancestry, represents an underappreciated factor affecting population susceptibility to CRC. In support of this concept, a recent study found that the most differentially expressed genes (DEGs) between AA and CA CRC tumors were related to the regulation of inflammatory immunity[25]. More broadly, transcriptional regulation of inflammation was determined the most distinguishing DNA variation between African and European genetic ancestries[26]. Another study demonstrated that African genetic ancestry and level of African admixture (mixture with ancestral African genetic lineage) predicted a stronger inflammatory transcriptional response in macrophages infected with bacterial pathogens[27]. From this framework, one could theorize that in a CRC setting, the same bacterium or a community of bacteria may induce a differentially deleterious

inflammatory response based on a patient's immune-related genetic background. Positive ancestral selection of anti-pathogenic immunity providing survival benefit in endemic areas may, in a modern "westernized life style" context, exacerbate localized inflammation in the tumor micro-environment and, when compounded by SES and environmental factors, accelerate CRC progression into the more aggressive form seen in AA patients, whose self-reported race/ethnicity correlates with an elevated African admixture[28,29] (Figure 1).

Given that SES and a variety of environmental factors associated with CRC pathogenesis and disparities are commonly discussed elsewhere[2,9], we are limiting the scope of the present review to the nascent literature associating CRC first to genetic polymorphisms related to innate immunity, second to those related to the microbiome, and finally explore how they may conjointly contribute to CRC disparities in the AA population. We also emphasize that, by considering admixture and genetic ancestry rather than self-reported race, population-specific risk studies including microbiome genome-wide association studies (GWAS) can more accurately capture human genetic diversity, thereby increasing the likelihood of identifying clinically relevant CRC risk factors associated with African ancestry[30-34]. Furthermore, polymorphisms related to innate immunity as well as the microbiome and contributing to links between CRC risk and African ancestry may have been left undiscovered by the longstanding genetic homogeneity of genomic research cohorts, a problem we discuss in our closing remark. Although technically challenging, expanding GWAS to more diverse "multi-ancestry" cohorts will reveal novel linkages between the microbiome, inflammation, and CRC risk that can build predictive polygenic risk scores adaptable to an equally diverse patient base[35,36]. Crucially, functional evaluation of CRC risk variants associated with African ancestry may offer insights into the trend of aggressive, earlier onset CRC in AA patients, paving the way towards personalized prevention and precision medicine.

## MUCOSAL INFLAMMATION, MICROBIOME, AND CRC

To better appreciate how host genetics may impact CRC risk between populations of different ancestral origins by modulating innate immunity or the microbiome, we will first highlight how mucosal inflammation and the gut microbiome interact to affect CRC pathogenesis. The human gut contains up to $10^{13}$ bacteria that play critical roles in immune, metabolic, cardiovascular, and neurological development [37]. The composition and functions of this bacterial community (microbiota) and its associated genome (microbiome) are highly dynamic and influenced by both environmental factors and host genetic background to maintain immunological and metabolic functionality[38]. Meanwhile, a tightly regulated physical separation between the immune system and commensal bacteria is necessary to limit a chronic inflammatory response to the microbiota[39,40]. The integrity of the intestinal barrier and its epithelium are therefore essential elements of healthy host-microbiota mutualism[40]. To establish a "demilitarized zone" and keep microbes at bay, the epithelium uses different mechanisms including tight junctions between epithelial cells, protective mucus production, and the expression of a complex arsenal of innate receptors that trigger bactericidal mediator secretion[41]. Nevertheless, a permissible level of contact or bacterial penetrance is necessary to facilitate metabolic exchanges and immunity maturation for homeostatic equilibrium between dense microbial flora and the host[39,40].

In the case of a high-fat diet, the cumulative alteration of bacterial metabolites can disrupt this equilibrium, thereby promoting carcinogenic dysbiosis and mucosal inflammation[42]. Diet is, thus, a critical environmental factor when connecting inflammation and the microbiome to CRC risk, especially when considering CRC disparities in AAs compared to Native Africans[43-45]. However, CRC as impacted by the genetic origins of host inflammatory response remains understudied. Inflammatory bowel disease (IBD), a model of perturbed micro-immune crosstalk and a known influencing factor of CRC etiology, can be a useful departure point for this line of inquiry[46]. In fact, multiple GWAS have linked higher risk of IBD, CRC[47-50], and microbiotic dysbiosis to host genetic variations, but surprisingly little is known about how CRC disparities may be compounded by the genetic regulation of host inflammatory response to gut bacteria[51].

There are, however, documented relationships between the genetic regulation of innate inflammatory immunity, the microbiome, and colon carcinogenesis. For example, adenomatous polyposis coli (Apc) $^{Min/+}$ mice knockout for toll-like receptor (TLR) 4 or its signaling adaptor partner myeloid differentiation primary response 88 demonstrated a decreased number of intestinal polyps[52]. Nucleotide-binding oligomerization domain leucine-rich repeat and pyrin domain containing 6 and nucleotide binding oligomerization domain containing protein 2 (NOD2) knockout mice were shown to develop colon tumors following colitis, and fecal microbiota transplantation from these mice into wild type recipients triggered similar tumorigenesis, which interestingly attributed carcinogenic causality to the microbiota [53,54]. In the case of lipocaline-2 knockout mice, *Alistipes spp.* commensals thrived and drove proximal colon tumorigenesis[55]. The nature of host genetic events can therefore drive different microbiome shifts impacting CRC and its anatomical pathogenesis (*i.e.*, distal *vs* proximal).

If inflammation is a mechanism connecting the microbiome and colorectal carcinogenesis, host genetic background, including immune-related single-nucleotide polymorphisms (irSNPs), could differ-

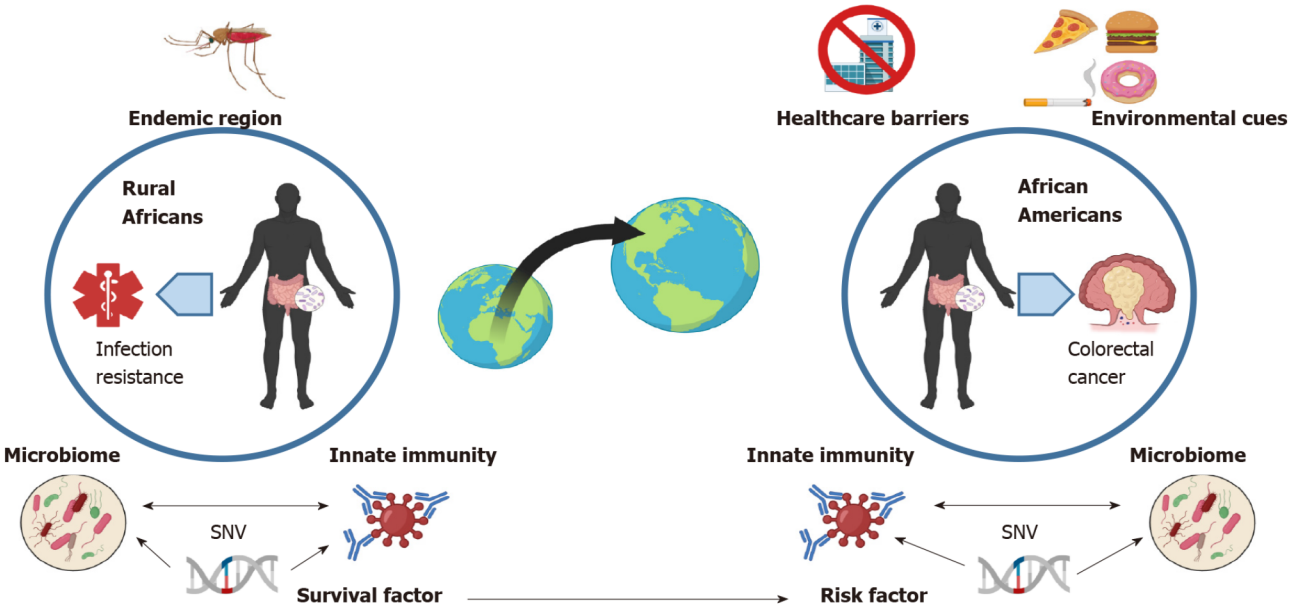


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**Figure 1 Immune-related variant may promote survival to pathogens in ancestral African environment but precipitate cancer in descendent African-Americans.** Pathogens associated with endemic African regions (*e.g.*, malaria) are thought to pressure selection for specific immune-related genetic variants associated with pathogen resistance and survival of Native Africans (left). In the context of westernized diet and lifestyle, this genetic predisposition (represented herein by a single nucleotide variant), when associated with inflammatory regulation and inherited from African ancestors, may lead to altered interactions with bacteria or communities of bacteria of the gut microbiome, thereby precipitating the colon adenoma-carcinoma sequence in African-Americans (right). Higher inflammation associated with lack of exercise, high fat diet, and socio-economic status are thought to be predominant factors driving early colorectal cancer onset in African-Americans *via* their impact on shaping the gut microbiome and its interactions with the host genetic background. SNV: Single nucleotide variant. Created with Biorender.com.

entially regulate such associations based on ancestry. In humans, pattern recognition receptor polymorphisms are associated with both IBD and CRC risk[48]. GWAS have linked genetic loci to increased IBD risk and a variety of risk alleles affect immune response[56], including NOD2 or autophagy-related 16 like 1[57,58]. Such polymorphisms have been associated with microbial dysbiosis and an excessive inflammatory response[59,60]. Namely, Knights *et al*[59] found that among 474 individuals, NOD2 variants were associated with *Enterobacteriaceae* family enrichment, including *Escherichia coli*, a species notably enriched in IBD individuals. Also, Lavoie *et al*[60] described an increase of interleukin (IL)-17-producing CD4$^+$ T (*i.e.*, Th17) cells in the lamina propria of mice engineered to express the polymorphism T300A (rs2241880) in the *Atg16 L1* gene. Although Th17-based colitis was associated with an increase of *Bacteroides ovatus*, T300A did not directly induce the increase of *Bacteroides ovatus* but rather induced the increase of IL23p19, an important cytokine for maintaining the Th17 lineage[61]. Th17 cells and their canonical cytokine IL-17 are critical pro-inflammatory contributors to epithelial homeostasis and mucosal immunity by orchestrating anti-bacterial defense and epithelial repair and regeneration as well as regulating barrier permeability by controlling the expression of occludin proteins[62,63]. When dysregulated in a chronic setting, sustained IL-17 production may promote colon tumorigenesis[64,65]. Several studies have now identified IL-23/Th17 pathway-associated polymorphisms linked to IBD susceptibility and the gut microbiome profile[66-68]. Presumably, genetic regulation of Th17-driven inflammation may impact IBD and ensuing CRC risk *via* the extent or nature of colonic dysbiosis. In sum, these GWAS suggest that by influencing the extent or nature of colonic dysbiosis, genetic regulation of inflammation represents a risk factors for both IBD and CRC. Next, we review how genetic ancestry contributes to this phenomenon, and how it may exacerbate CRC disparity in AAs.

## GEOGRAPHIC AND POPULATION-RELATED INNATE IMMUNITY DISPARITIES THAT MODULATE CRC RISK

The flow of genetic information across time and geography may contribute to current disparities in cancer incidence and progression[69]. Cancer is known to result from an accumulation of somatic genetic and epigenetic alterations that dysregulate the cell cycle but also depends on genetic background and polymorphisms that impact patient risk and predisposition[70,71]. Yet, few GWAS have implicated ancestral genetic variants in cancer predisposition amongst self-identified racial/ethnic

groups[72,73]. Many such polymorphisms regulate gene expression *via* epigenetic or post-translational modification mechanisms, which affect noncoding sequences like microRNA (miRNA) binding sites[74,75]. Yet, the biological and clinical significance of most polymorphisms associated with cancer disparities remains unknown[72]. Mechanistic associations between chronic inflammation and carcinogenesis could link ancestral genetic diversity to cancer, whereby immune-related genetic regulatory variants have the potential to differentially modulate CRC across populations[27].

In fact, a large fraction of population-associated polymorphisms impact gene expression related to inflammation and innate immunity, which, being likely essential for surviving life-threatening infections, evolved under stronger selection pressures than other traits[76,77]. For instance, human genome diversification *via* archaic human genome introgression (*i.e.*, admixture with Neanderthal genome) is a proposed adaptation of ancestral humans to infectious environments following "out of Africa" migration[77,78]. In non-African populations, Neanderthal-introgressed haplotypes reintroduced a splice variant (rs10774671) of the 2'-5'-oligoadenylate synthetase (*OAS*) *1* gene[79]. The *OAS* locus on chromosome 12 encodes three genes, *OAS1*, *OAS2* and *OAS3* that play an important role in virus defense. The elevated frequency of the Neanderthal-derived allele at the *OAS* locus was proposed to be the result of a positive selection in European and East Asian populations. This allele selection has a functional significance since it is associated with the production of a protein variant (OAS1 p46) characterized by higher enzymatic activity and improved resistance to West Nile virus and hepatitis virus C[80]. Therefore, the allele haplotype may provide a survival advantage to infectious agents in the non-African environment and represents an example of variety in baseline inflammation levels that may influence susceptibility to diseases like IBD and CRC in patients with insignificant African ancestry. Selection of genetic variants providing health and survival benefit in endemic areas may represent another means of adaptation and human genome diversification. In a cohort of 158 healthy individuals (distributed as European, Sub-Saharan African, and East-Asian), Barreiro *et al*[81] found that nucleotide diversity of the TLR family was shown to vary between African populations, suggesting pathogen-specific selection pressures. Specifically, the *TLR10/TLR1/TLR6* locus showed signs of recent positive selection amongst non-African populations. Furthermore, of all SNPs in this region, a high frequency *TLR1* single nucleotide variant (SNV) (non-synonymous T1805G variant) found in Europeans was the most significant population differentiator and was associated with a decrease in agonist-mediated nuclear factor-kappa B activation[81]. Although it is unclear if decreased TLR1-mediated immune response confers a selective advantage, it could potentially modulate otherwise harmful inflammatory responses to pathogens[82,83]. This finding suggested that a finely tuned balance between optimal defenses to pathogens and excessive inflammation may have been critical for evolutionary survival[78].

To investigate how ancestral immunity would impact pathogen response, Nédélec *et al*[27] studied interactions between macrophages and live bacteria (*Listeria* and *Salmonella*). Amongst the macrophages, they found that many of the DEGs; (30% of the 11914 genes analyzed) between AAs ($n$ = 77) and CAs ($n$ = 91) were involved in the regulation of the innate immunity. These results built off their previous findings that 9% of macrophage DEGs varied according to ancestry-associated regulation and that increased African ancestry could predict a stronger inflammatory response to infection[27]. Performing quantitative trait locus (QTL) analysis, the authors identified SNVs in 14% of DEGs or using alternative splicing between CA and AA individual-derived macrophages (either non-infected or infected with *Listeria* or *Salmonella*). A large fraction of DEGs were associated with expression QTL only in infected macrophages. In other words, SNVs in a significant fraction of inflammation-related genes were expressed in infected macrophages according to the level of African ancestry. Interestingly, the same authors also found that these DEGs included susceptibility genes previously reported by GWAS for rheumatoid arthritis, systemic sclerosis, or ulcerative colitis, all related to chronic inflammation and conditions with known AA disparity[78]. The interest of such a study, although performed on macrophages *in vitro*, is its illustration of the link between African genetic ancestry and inflammatory response to bacteria, one that could accelerate CRC by aggravating interactions between gut microbiota and the mucosal immune system. Reinforcing this concept is another GWAS that revealed that some of the most differentiating irSNPs between African and European populations were associated with genes regulating nuclear factor-kappa B or chemokine gene clusters[78]. Selected genetic variants may offer protection against infection in endemic regions for native/rural Africans but favor cancer development in descendants bearing the same variants in a western environment, a concept exemplifying the crossroad between host genetics and environmental factors that shapes cancer risk (Figure 1).

Regarding the possibility of a role of associations between irSNP and CRC risk into AA disparity, a recent study by Sanabria-Salas *et al*[33] studied links between pro-inflammatory *IL1B* haplotypes and CRC risk in patients from six Colombian cities. The authors associated the *IL1B* CGTC haplotype with CRC risk exclusively in patients from the coastal regions of Colombia who possessed the highest proportion of admixed African ancestry[33]. The same group has associated *IL1B* irSNPs (four SNPs -3737C/-1464G/-511T/-31C) with African ancestry and elevated cancer risk. The CGTC haplotype was most frequently found and highly expressed in AAs, establishing a functional link between *IL1B* irSNP and CRC risk[84]. Further studies, validating the connection between AA CRC patients and *IL1B* polymorphisms, will be required to confirm the *IL1B* SNP haplotype as a population-associated CRC risk marker; these findings nevertheless showcase a prime example of an exploitable connection

between ancestry-related inflammation and cancer risk disparity.

Next, a cancer genomic meta-analysis using 48 GWAS within the National Cancer Institute GAME-ON Network (64591 cancer and 74467 control patients) across five common cancer sites (ovarian, lung, breast, colorectal, and prostate) found that genetic variants associated with inflammation and innate immune response were relevant to CRC risk, including SH2B adapter protein 3 (*SH2B3*) (rs3184504, $P = 3.32 \times 10^{-5}$), a negative regulator of growth factors and cytokine-induced signaling (Table 1). Unfortunately, irSNP associations with race/ethnicity and geographic distributions were not evaluated[85]. In contrast, Wang *et al*[47] demonstrated the merit of accounting for population diversity in their analysis of associations between innate immunity pathways and CRC risk. In two large studies (discovery and validation cohorts) across five distinct ethnic groups (AA, CA, Japanese-American, Latino, and Native Hawaiian), they found that among more than 600 common variants associated with 37 innate immunity-related genes, a SNV in the second intron of peroxisome proliferator-activated receptor gamma (*PPARG*) (rs9858822) showed a statistically significant association with CRC in the AA population (Table 1). This variant, rare in other non-AA populations, was not previously reported. Importantly, a frequently reported *PPARG* variant rs1801282, inconsistently associated with CRC in other studies[86-88], was not detected in association with CRC in Wang *et al*[47]'s study that carefully controlled for population diversity, supporting the value of multi-ancestry SNP association studies. In another meta-analysis of 308 SNPs performed by Montazeri *et al*[89], 14 SNPs showed credible association with CRC including rs3802842, whose expression was associated with immune infiltration. Curiously, the *PPARG* rs9858822 polymorphism detected by Wang *et al*[47] failed as a positive SNP in this meta-analysis, a finding that warrants additional verification. However, such discrepancies between the multi-ethnic study of Wang *et al*[47] and European study of Montazeri *et al*[89] may highlight the issue of the underrepresented diversity when seeking genetic cancer risk. Population-associated risk may be missed, and alternatively discovered cancer risk may not be relevant for minorities.

Lastly, functional variants in the 3′-untranslated region (UTR) of inflammatory gene miRNA binding sites (miRSNPs) have been associated with CRC risk[75]. Four miRSNPs in the mannose binding lectin 2 (*MBL2*) gene 3′-UTR have been associated with increased CRC risk in the AA population. *MBL2* codes for mannose binding lactose protein, a pattern recognition receptor that binds a wide range of pathogen-expressed sugars, leading to their phagocytosis. Although not assessed in this study, the modulation of the interactions between the mucosal inflammation and the microbiome, *via* MBL2 expression, could be a mechanistic link between African ancestry and higher CRC risk[74]. irSNPs associated with CRC risk are summarized in Table 1.

Ultimately, linking chronic inflammation risk loci to positive selection *via* resistance to past infectious agents and population displacement should be done with caution, as the merits are still debated[78]. Physiological interfacing of the immune system with other biological systems (reproduction and organ development) may also explain positive selection in a manner distinct from past pathogen resistance [78]. Yet, considering the evidence of host innate immunity regulation by population-enriched irSNPS [27], it is reasonable to speculate that the mucosal inflammation associated with commensalism is differentially tuned according to the level of African ancestry and could therefore influence CRC disparities. This view is supported by disparity research in other cancers, such as the finding that *IL10* promoter SNPs enriched in AAs are also potential risk factors for prostate cancer development and progression [90]. Interestingly, one such *IL10* polymorphism (rs1800871) was associated with Proteobacteria load in the gut microbiome (Table 2), but a connection between rs1800871, proteobacteria, and prostate cancer remains to be established[90]. In breast cancer, Jenkins *et al*[91] demonstrated how the ancestral selection of immune variants in the African continent can predispose AA women to ancestry-related differences in tumor immunogenicity. Specifically, the status of a "Duffy-null" polymorphism-regulated atypical chemokine receptor 1 (*ACKR1*) allele linked West African genetic ancestry to tumor immune infiltration. Thus, for breast cancer, duffy antigen receptor for chemokines/ACKR1 polymorphism may serve as a biomarker for precision medicine and immunotherapy in patients bearing significant West African ancestry[91]. This result highlights the potential that ancestry-associated irSNPS have for cancer screening and clinical care when paired with functional analyses and elevating the importance of similar studies for CRC.

## HOST GENETICS AND MICROBIOME INTERACTIONS' CONTRIBUTION TO CRC DISPARITY

While host genetics may impact mucosal inflammation and CRC risk, other factors, including environmental factors such as diet, lifestyle, and antibiotic exposure, undoubtedly influence CRC susceptibility and treatment response by shaping gut microbiome composition[51,92,93]. Notwithstanding such findings, we propose that the predominant reliance of the microbiome on environmental cues in healthy individuals may conceal host genetic contributions (including genetic ancestry and somatic mutations) in disease contexts (*e.g.*, CRC), driving the microbiome response to environmental fluctuations and defining, at least in part, differential susceptibility to cancer in AAs[94-96]. The numerous immune-related genetic variants that delineate chronic disease susceptibility between AAs and CAs (previously

**Table 1 Immune-related single nucleotide polymorphism associated with colorectal cancer**

| Ref. | Size | Analysis | Gene | SNP | Function | Ethnicity | Comment |
|---|---|---|---|---|---|---|---|
| Montazeri *et al*[89], 2020 | 6149 CRC 7337 controls | Meta-analysis | *COLCA1/2*[1] | rs3802842 (11q23.1) | Immune infiltration of LP | Europeans | Confirmed by Lu *et al*[49], 2019[1] |
| | | | *TGFB1*, *SMAD7*, *SMAD7* | rs1800469, rs12953717, rs4464148 | TGFB signaling inhibitor | | |
| Law *et al*[50], 2019 | 34627 CRC 71379 controls | Meta-analysis | *HLA-C* | rs3131043 (6p21.33) | Adaptive immunity | Europeans | |
| | | | *HLA-DRB1/DQA1* | rs9271770 (6q21.33) | Adaptive immunity | | |
| | | | *COLCA1/2* | rs3087967 (11q23.1) | Immune LP leukocytes | | |
| | | | *FUT2* | rs12979278 (19q13.33) | Gut barrier | | |
| Lu *et al*[49], 2019 | | GWAS | *NOD2* | rs2066847 | Innate immunity | East Asians | Confirmed by Montazeri *et al*[89], 2020[2] |
| | | | *GATA3* | rs10795668 (10p14) | T cell transcription factor | | |
| | | | *SMAD7* | rs7229639 | TGFB signaling inhibitor | | |
| | | | *SMAD7* | rs4939827 | TGFB signaling inhibitor | | |
| | | | *COLCA1/2*[2] | rs3802842 (11q23.1) | Immune infiltration of LP | | |
| Sanabria-Salas *et al*[33], 2017 | 391 CRC | GWAS | *IL1B* | CGTC haplotype (2q14) | Inflammation | Columbian Africans | Association with AA admixture |
| Hung *et al*[85], 2015 | 15414 CRC 17688 controls | GWAS | *SH2B3* | rs3184504 (12q24) | Cytokine signaling | Europeans | Confirmed by Schumacher *et al*[135], 2015 |
| Schumacher *et al*[135], 2015 | 18299 CRC, 19656 controls (Europeans), 2098 cases, 6172 controls (Asian 1), 2627 cases, 3797 controls (Asian 2) | Meta-analysis | *SH2B3*[3] | rs3184504 (12q24.12) | Cytokine signaling | Europeans/Asians | Confirmed by Hung *et al*[85], 2015[3] |
| | | | *NOS1* | rs73208120 (12q24.22) | ROS production | | |
| Wang *et al*[47], 2013 | 2535 CRC, 3915 controls (discovery), 2153 CRC, 2630 controls (validation) | GWAS | *PPARG* | rs9858822 | Monocyte activation | Multi-ethnic | High frequency in AA |
| Tsilidis *et al*[136], 2009 | CLUE II cohort, 208 CRC, 381 controls | GWAS | *IL10* | rs1800896, rs1800890, rs3024496, rs3024498 | Increased IL-10 | ND | |

[1]Confirmed by Lu *et al*[49].
[2]Confirmed by Montazeri *et al*[89].
[3]Confirmed by Hung *et al*[85].
AA: African-American; CRC: Colorectal cancer; GWAS: Genome-wide association studies; IL: Interleukin; LP: Lamina propria; ROS: Reactive oxygen species; SNP: Single nucleotide polymorphism; TGF: Transforming growth factor;

ND: Not determined; HLA: Human leukocyte antigen; FUT2: Fucosyltransferase 2; NOD2: Nucleotide binding oligomerization domain containing protein 2; GATA3: GATA binding protein 3; SH2B3: SH2B adapter protein 3; NOS1: Nitric oxide synthase 1; PPARG: Peroxisome proliferator-activated receptor gamma.

discussed) may then contribute to differential inflammatory responses to the microbiome dysbiosis and compound existing CRC disparities (Figure 2).

From this perspective, multiple metagenomic studies of fecal and mucosal samples have already found compositional and metabolomic differences between CRC and healthy patient microbiome[97-100]. Novel meta-analysis approaches combined these metagenome shotgun datasets across heterogeneous populations to explore relationships between the microbiome and CRC; associations were identified at both bacterial strain and gene levels[101-103]. A common core of 29 bacterial species was enriched in CRC cases, and choline metabolism was established as a reproducible biomarker of the CRC-associated microbiome[101,102]. While such findings are correlative and do not suggest causative links, several smaller studies have highlighted the role of specific bacteria[104-106] and microbial dysbiosis in triggering colorectal carcinogenesis in animal models[107]. However, microbiome GWAS (mGWAS) have not yet identified consistent associations between such carcinogenic bacteria and CRC risk[108]. Unfortunately, because population diversity was systematically underrepresented or not annotated in the metagenome datasets, it remains unknown how these results could translate into CRC risk factors or diagnostic biomarkers for specific racial/ethnic groups.

Meanwhile, several taxonomic and metagenomic studies have revealed an intriguing diversity in microbiome composition across racial/ethnic groups, but mechanistic understandings of associations between bacteria or groups of bacteria and race/ethnicity are sparse[34,109-113]. In particular, diversity of the microbiome is highly diet-driven. A study by O'Keefe *et al*[44] showed that a 2-wk food swap between AAs (received high fiber, low fat diet) and rural Africans (received low fiber, high fat diet) produced dramatic changes in mucosal biomarkers and a metabolome switch, illustrated by an increase in saccharolytic fermentation and anti-inflammatory butyrogenesis as well as suppression of secondary bile acid synthesis in AAs. In light of these results, it is critical that mGWAS take diet into consideration as a confounding factor, as its impact will inevitably interfere with the genetic/epigenetic influence on CRC risk[114]. Interestingly though, differences between CAs and AAs with respect to the mean alternate Healthy Eating Index (a measure of diet quality[115]) faded when adjusting for SES, implying that diet cannot entirely account for CRC disparities[116]. Multiple other factors besides diet are known to impact the composition and function of the microbiome, including smoking, alcohol consumption, as well as antibiotic exposure or metabolic condition such as diabetes or obesity, which are coincidentally also risk factors for CRC[117-119]. While these aspects of the microbiome biology have been extensively reviewed[120], we are paying much of our attention herein on the role of the host genetics and the ancestral genetic origin on the microbiome diversity and consequently CRC risk between ethnicities.

Interestingly, using the Healthy Life in an Urban Setting cohort and fecal 16S ribosomal RNA gene sequencing of over 2000 individuals, Deschasaux *et al*[110] found that microbiome diversity between racial/ethnic groups living in the same city was independent of metabolic health and only partially explained by SES, lifestyle, and diet factors. Yet, this finding was not always reproduced in other studies [45,111,121]. Overall, however, there is sufficient evidence to justify additional efforts to clarify if host genetics and population origins are taking part in shaping the microbiome[51,94]. The role of host genetic background has been suggested by mGWAS, which showed that SNPs such as rs4988235,

**Table 2 Interactions of host genetics (metabolic and immune-related single nucleotide polymorphism) with gut bacteria**

| Ref. | Size | Analysis | Gene | SNP | Function | Bacteria | Ethnicity | Comment |
|---|---|---|---|---|---|---|---|---|
| Knights *et al*[59], 2014 | 474 IBD | 16S RNA | *NOD2* | rs5743293, rs104895431, rs104895467, rs2068844, rs2068845, rs5743277, rs5743293 | Innate immunity | *Enterobacteriaceae* | Europeans | IBD |
| Blekhman *et al*[122], 2015 | 93 (HMP) | Metagenomic | *LCT* | rs2304371, rs3754689 | Metabolism | *Bifidobacterium*, *SMB53* (*Clostridiaceae*) | Multi-ethnic | NS |
| | | | *GNA12* | rs1182182 | IBD | | | |
| Goodrich *et al*[123], 2016 | 1126 | Fecal 16S rRNA | *CD36* | rs1360741 | Immune-related | *Blautia* | Europeans | United Kingdom twins |
| Li *et al*[137], 2016 | 10523 IBD, 5726 IBD | Mucosal 16S rRNA | *SLC39A8* | rs13107325 | Immune-related | *Composition* | ND | IBD |
| Bonder *et al*[68], 2016 | 1514 | Metagenomic | *C11orf30-LRRC32* | rs2155219 | IBD | *Coprococcus comes/Proteobacteria* | | AA[1] PCa risk |
| | | | *CCL2* | rs3091315, rs3091316 | Immune-related | *Methonobacteria* | | |
| | | | *DAP2* | rs267939 | Innate immunity | *Bifidobacterium* | | |
| | | | *IL23R* | rs12141575 | Immune-related | *Enterobacteriaceae/E. coli* | | |
| | | | *IL10* | rs1800871[1] | Immune-related | *Proteobacteria/Sutterella* | | |
| | | | *MUC22* | rs3873352 | Barrier defense | | | |
| | | | *NOD1* | rs12669082 | Innate immunity | *Enterobacteriaceae/E.coli* | | |
| | | | | rs41524946 | Innate immunity | *Enteroba cteriaceae/E. coli* | | |
| | | | | rs55689059 | Innate immunity | *Enterobacteriaceae/E. coli* | | |
| | | | | rs55841603 | Innate immunity | *Enterobacteriaceae/E. coli* | | |
| | | | *NOD2* | rs8056611, rs2357792, | Innate immunity | *Enterobacteriaceae/E. coli* | | |
| | | | *CD209* | rs1010046 | Innate immunity | *Bacteroidetes* | | |
| Rühlemann *et al*[138], 2021 | 8956 | 16S rRNA/GWAS | *FUT2* | rs602662 | Barrier defense | *Bacteroides* OTU97_27 | Europeans | |
| | | | *BLVRA* | rs623108 | Innate immunity | *Barnesiella spp*. OTU99_55 | | |

[1]Studies include an analysis according to the population origins.

AA: African-American; GWAS: Genome-wide association studies; HMP: Human Microbiome Project; IBD: Inflammatory bowel disease; SNP: Single nucleotide polymorphism; ND: Not determined; NS: Not significant; PCa: Prostate cancer; rRNA: Ribosomal RNA; *E. coli*: *Escherichia coli*; FUT2: Fucosyltransferase 2; NOD2: Nucleotide binding oligomerization domain containing protein 2; LCT: Lactase; GNA12: Guanine nucleotide-binding protein alpha-12; CCL2: C-C motif chemokine ligand 2; IL: Interleukin; MUC22: Mucin 22; BLVRA: Biliverdin reductase.

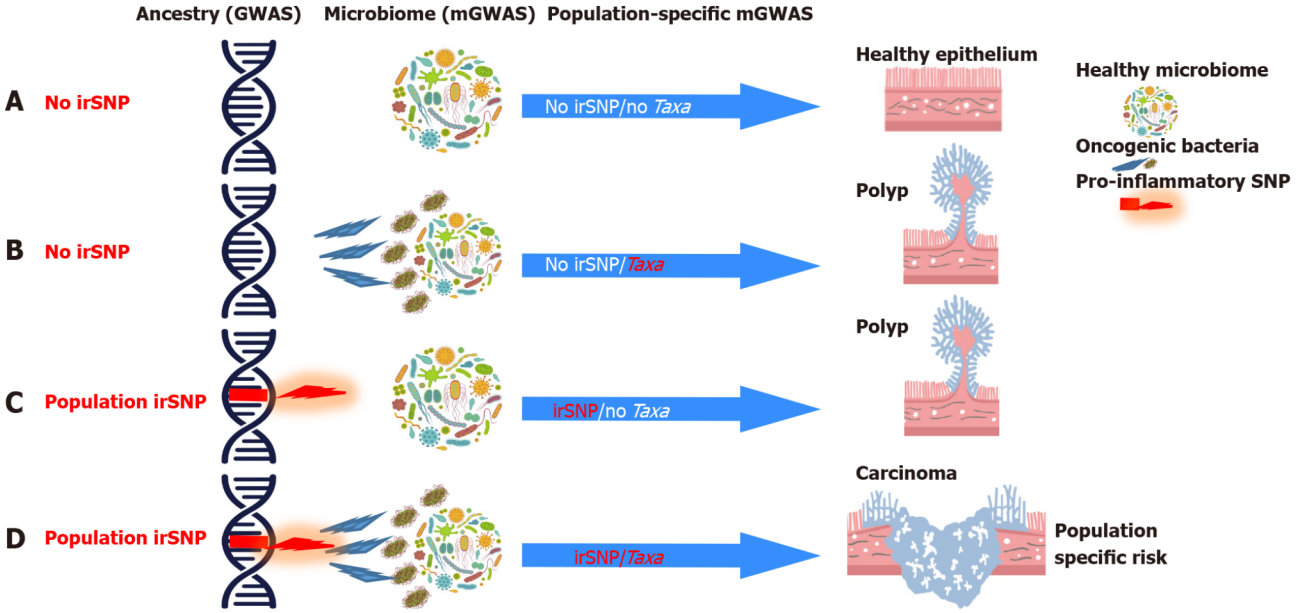


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**Figure 2 Interactions between host genetics and microbiome delineate colorectal cancer risk factors.** Immune-related risk factors of colorectal cancer (CRC) may combine host genetics [including immune-related single-nucleotide polymorphisms (irSNPs)] and microbiome cues. The genetic regulation (irSNP) of bacteria (*Taxa*) recognition by the mucosal immunity may mediate the heterogeneity of CRC risk factors between populations which vary by the representation of irSNPs. A: Absence of irSNPs and pathogenic bacteria (no irSNPs/no *Taxa*) maintain healthy mucosal inflammation and gut barrier; B: Absence of irSNPs with enrichment of pathogenic bacteria (no irSNPs/*Taxa*) may lead to chronic inflammation that slowly promotes polyp formation; C: Presence of irSNP in absence of pathogenic bacteria may also lead to chronic inflammation that slowly promotes polyp formation; D: Combination of irSNP and pathogenic bacteria (dysbiosis) may trigger a smoldering chronic inflammatory response precipitating early progression of the adenoma-carcinoma sequence. Genome-wide association studies (GWAS) (irSNP as CRC risk) and microbiome GWAS (mGWAS) (bacteria as CRC risk) are poised to miss the association of the irSNP with CRC because of the necessity of a combined occurrence of irSNP and specific bacteria enrichment in the microbiota to increase the CRC risk. However, if the irSNP is linked to the genetic ancestry, population-specific microbiome GWAS will likely detect the association of irSNP and the bacteria as a CRC risk in the population carrying this genetic ancestry. CRC: Colorectal cancer; irSNPs: Immune-related single-nucleotide polymorphisms; GWAS: Genome-wide association studies; mGWAS: Microbiome genome-wide association studies.

associated with lactase persistence gene, contributed to microbiome composition[45,68,122,123]. A study performed with 416 twin pairs in the United Kingdom identified 26 "heritable" taxa, also suggesting that specific host genetic variants may participate in microbiome composition (Table 2)[124]. Notably, host genetic variants associated with microbiome composition were found to be enriched in immunity-related pathways[122]. Studies showing associations between metabolic and immune-related polymorphisms and microbiome composition are summarized in Table 2.

Finally, associations between the microbiome and somatic mutations in CRC have been described by Burns *et al*[95], who suggested that genetic determinants of the host and colon tumor mutations alter microbiome structure. The CRC mutanome could therefore help predict the composition and function of CRC-associated microbiomes and the clinical outcome or the response to therapies. To this end, of intrigue is the recent description by Ashktorab *et al*[125] and Brim *et al*[126] of genetic variation in tumor suppressor genes (*APC*), DNA mismatch repair genes, and other driver mutations (*KRAS* and *PIK3C*) in AAs with CRC, some of which were novel and not previously described in other populations. Loss of function mutations in *APC* were correlated with changes in 25 different microbial taxa, including an abundance of *Finegolia* or *Christensenellaceae*. Mutations in the zinc finger protein 717-coding gene were associated with an abundance of *Akkermansia* and *Verrucomicrobiaceae*, both colitis-associated species. Additionally, the same authors have isolated a novel *Streptococcus spp*. VT_162 from colon adenoma and CRC lesions in AAs[126]. Fecal *Streptococcus spp*. VT_162 was also confirmed in an advanced adenoma and CRC Chinese/Hong-Kong cohort[126]. An assessment of this bacterium's relative prevalence in CAs compared to AAs will be necessary. Whether CA genetic background is more restrictive, while AA genetic background is more permissive to this species is not yet established. It may be postulated that, as seen in IBD patients, host genetic background drives the nature of the microbiome and alters the CRC risk posed by procarcinogenic "driver" bacteria[48,51].

Although there is a lack of direct causative links between such bacteria and colon carcinogenesis or growth promotion, this evidence at least signals that CRC disparities in AAs may be related to differential host inflammatory responses to similar bacterial communities (inflammation-driven disparities) and/or the contribution of ancestry-related factors in shaping microbiome diversity (bacteria-driven disparities). The molecular pathological epidemiology (MPE) that aims at uncovering an interactive relationship between environmental features and disease subtypes to understand disease incidence and

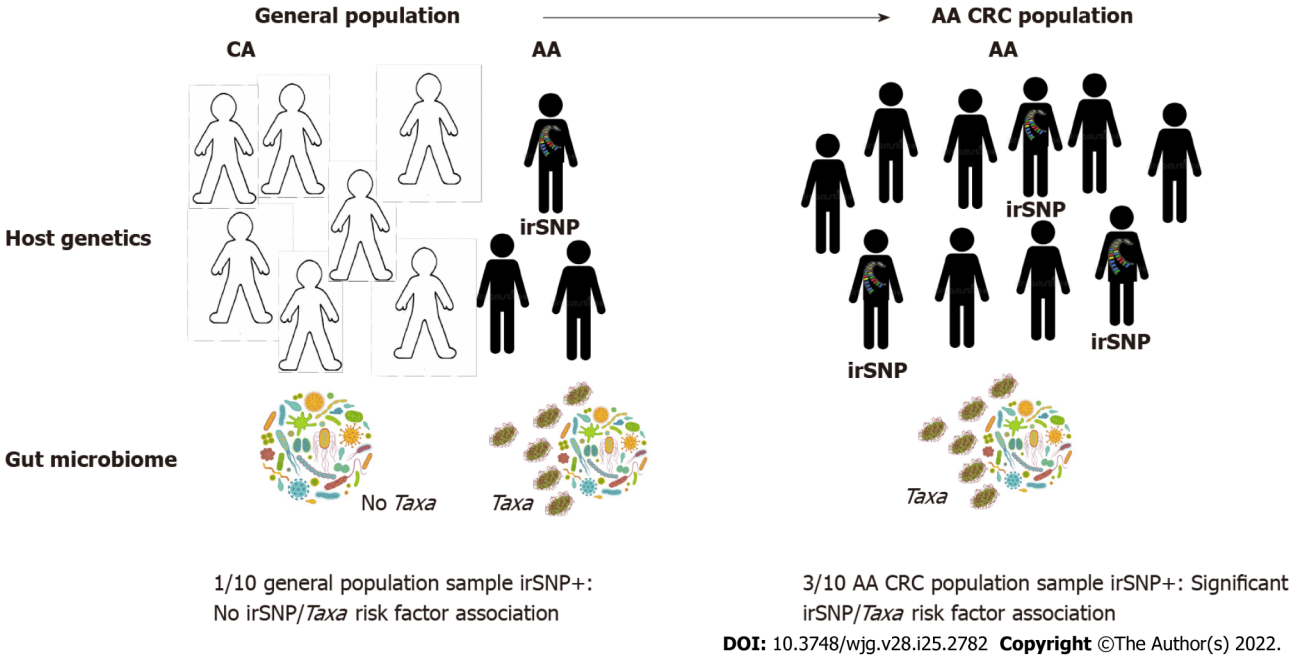


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**Figure 3 Population-specific colorectal cancer microbiome genome-wide association studies to understand colorectal cancer disparities.** Systemic underrepresentation of minorities in microbiome genome-wide association studies (mGWAS) may compromise the identification of microbiome-associated colorectal cancer (CRC) risks in African-Americans (AAs). Left: Associations between AA-enriched immune-related single-nucleotide polymorphisms (irSNPs) (pop-irSNPs) and gut bacteria (*Taxa*) may remain undetected in the global population if pop-irSNP and *Taxa* have too low frequency in the AA population and AAs are underrepresented. Right: If pop-irSNP and pathogenic bacteria are CRC risk factors in AA, they will be enriched in AA CRC patients compared to the general AA population (left), and mGWAS will detect the association between pop-irSNP and *Taxa* in AA CRC cohort. These features justify population-specific CRC mGWAS to detect additional CRC risk resulting from the interaction between ancestral irSNP and bacteria in minorities. CRC: Colorectal cancer; irSNPs: Immune-related single-nucleotide polymorphisms; mGWAS: Microbiome genome-wide association studies; AA: African-American; CA: Caucasian-American.

mortality will provide etiologic and pathogenic insights in CRC disparities[127,128]. However, discoveries made in healthy donors often remain inconsistent from one study to another, perhaps due to multiple confounding environmental factors between heterogeneous cohorts[45,129]. More likely culprits for the inconsistency seen in GWAS that seek to identify the impact of host genetics on microbiome diversity are the homogeneity in sampled cohorts and differences in experimental approaches including stool *vs* mucosa sampling, sequencing approaches, and annotations. Future GWAS must address such issues in order to identify associations between genetic background and the microbiome that can reliably be applied to the question of disparities.

## CONCLUSION

CRC disparity is far from an exclusively biological phenomenon but rather involves a complex interplay of SES, environmental and genetic components that collectively impact CRC risk and prognostics. Although there is a growing understanding of this complexity, studies examining the influence on CRC pathogenesis from ancestry-specific interactions between host genetics and the commensal microbiome are lacking. Such work is nevertheless urgently needed to appropriately mitigate CRC on a population basis and especially to help address alarming new trends, such as early-onset CRC amongst AAs. To facilitate this line of investigation, we reviewed irSNPs identified in CRC mGWAS and proposed that some of such variants (and others yet to be discovered) may alter microbiome composition and/or differential inflammatory responses to bacteria, thereby impacting CRC risk in a manner associated with genetic ancestry. However, testing the functional significance of such variants will require systematic studies that can incorporate the microbiome, mucosal immunity, and host genetics. A recent investigation by DeStefano Shields *et al*[130], although not related to cancer disparity, offers a potential experimental blueprint. Researchers introduced the *BRAF*$^{V600E}$ mutation to a Min$^{Apc\Delta716/+}$ murine model of distal colon polyposis, then colonized *BRAF* mutant and Min$^{Apc\Delta716/+}$ mice with *Enterotoxigenic Bacteroides fragilis*. Distal colon tumorigenesis was observed in Min$^{Apc\Delta716/+}$ mice following colonization, whereas *BRAF*$^{V600E}$ Min$^{Apc\Delta716/+}$ mice developed proximal colon tumors associated with immune signature and microbiome alterations plus sensitivity to anti-programmed death ligand 1[130]. These results suggested that host gene/bacteria interactions may drive CRC risk and pathogenesis, and demonstrate how such interactions can be disentangled mechanistically using experimental models with clinical implications. In the nascent field of precision medicine and multidisciplinary big data integration, the rapidly

evolving MPE represents a successful model of integration of pathology, genomics, microbiome, immunology, epidemiology, and social science[127,128]. MPE will considerably improve precision medicine and prevention allowing, among other things, identifying SNPs that may impact microbiome and inflammation and serve as predictive markers. Genetic ancestry, however, remains to be integrated to this model to precisely address CRC disparities at the molecular level. Elucidating the mechanisms by which ancestry-associated variants impact CRC pathogenesis will be much more challenging, but models such as the one described by Lavoie *et al*[60], who used mice engineered to express the polymorphism T300A (rs2241880) in the *Atg16 L1* gene known to increase IBD risk in humans, represent a promising approach to identifying mechanisms that can lead to personalized interventions applicable to minorities.

Yet, without addressing biases in genomics science, the ability of GWAS to detect variants of significance for AAs (or other minorities in general) will remain stunted[131]. To date, cancer GWAS have examined cohorts predominantly composed of Caucasian individuals, an homogeneity that limits the appreciation of how genetic ancestry impacts cancer risk[101,102]. Critically, the lack of diverse representation in sampling cohorts results in the increased likelihood that, even if identified, cancer-associated variants may be of limited clinical significance for non-Caucasian populations. While commenting on this issue, Davis[131] recently argued that the current state of genomic medicine is inadequately equipped to confront current oncological trends of disparate incidence and mortality in inclusive fashion and advocated for a persistent push towards the prioritization of patient diversity. Such an agenda is not only more harmonious with the principles of ethical human subjects research but is also scientifically meritable, as studies that account for diversity have already revealed novel genomic data that may improve our understanding of cancer etiology[132]. Therefore, for powered GWAS to detect and discern associations between population-enriched irSNPs, the microbiome, and CRC, proper accounting of population diversity and sufficient cohort size (estimated at > 4000 individuals)[45], or even population-specific CRC studies will be essential (illustrated in Figure 3). Encouragingly, recent methodological frameworks for multi-ancestry cohort GWAS have already yielded ancestry-related cancer variant risk factors[133,134]. Moreover, the recent initiative of the National Cancer Institute: Genetic Association and Mechanism in Oncology (GAME-ON; https://epi.grants.cancer.gov), which regrouped genomic data from more than 33 GWAS across five different cancers (CRC, lung, breast, ovary, and oral), is an example of the benefit of data sharing that will help identifying through meta-analysis data cancer risk loci in understudied populations, especially since 40% of the samples are from African, Asian, and Hispanic backgrounds. However, our integrated concept for CRC genomic research proposes that to represent accurately and capture the contribution of irSNP/microbiome interactions to CRC disparities, such diversified host genomic data must be paired with microbiome data.

In sum, population-related irSNP that regulate mucosal inflammation may modify the microbiota and its interaction with colon epithelium. Alternatively, a population-related SNP not involved in immune regulation may also alter the microbiome and trigger procarcinogenic chronic inflammation. Finally, a combination of two SNPs impacting inflammation and the microbiome with minor effects on CRC pathogenesis may trigger a strong procarcinogenic bacteria/inflammation interaction when combined in an at-risk population (Figure 2). By hunting for and characterizing such genetic factors using the emerging genetic admixture and ancestry paradigms, we believe scientists and clinicians will have a precision tool that enables a clearer understanding of the association between CRC with AA populations and the increasing trend of early onset CRC, ultimately to better mitigate CRC outcome disparities.

## FOOTNOTES

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## REFERENCES

1 **Siegel RL**, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 145-164 [PMID: 32133645 DOI: 10.3322/caac.21601]
2 **Carethers JM**, Doubeni CA. Causes of Socioeconomic Disparities in Colorectal Cancer and Intervention Framework and Strategies. *Gastroenterology* 2020; **158**: 354-367 [PMID: 31682851 DOI: 10.1053/j.gastro.2019.10.029]
3 **Carethers JM**. Clinical and Genetic Factors to Inform Reducing Colorectal Cancer Disparitites in African Americans. *Front Oncol* 2018; **8**: 531 [PMID: 30524961 DOI: 10.3389/fonc.2018.00531]
4 **Polite BN**, Sing A, Sargent DJ, Grothey A, Berlin J, Kozloff M, Feng S. Exploring racial differences in outcome and treatment for metastatic colorectal cancer: results from a large prospective observational cohort study (BRiTE). *Cancer* 2012; **118**: 1083-1090 [PMID: 21800287 DOI: 10.1002/cncr.26394]
5 **Sanoff HK**, Sargent DJ, Green EM, McLeod HL, Goldberg RM. Racial differences in advanced colorectal cancer outcomes and pharmacogenetics: a subgroup analysis of a large randomized clinical trial. *J Clin Oncol* 2009; **27**: 4109-4115 [PMID: 19636001 DOI: 10.1200/JCO.2009.21.9527]
6 **Johnston FM**, Yeo HL, Clark C, Stewart JH 4th. Bias Issues in Colorectal Cancer Management: A Review. *Ann Surg Oncol* 2022; **29**: 2166-2173 [PMID: 34142287 DOI: 10.1245/s10434-021-10232-6]
7 **Ciccone G**, Prastaro C, Ivaldi C, Giacometti R, Vineis P. Access to hospital care, clinical stage and survival from colorectal cancer according to socio-economic status. *Ann Oncol* 2000; **11**: 1201-1204 [PMID: 11061620 DOI: 10.1023/a:1008352119907]
8 **Jepson C**, Kessler LG, Portnoy B, Gibbs T. Black-white differences in cancer prevention knowledge and behavior. *Am J Public Health* 1991; **81**: 501-504 [PMID: 2003635 DOI: 10.2105/ajph.81.4.501]
9 **Zavala VA**, Bracci PM, Carethers JM, Carvajal-Carmona L, Coggins NB, Cruz-Correa MR, Davis M, de Smith AJ, Dutil J, Figueiredo JC, Fox R, Graves KD, Gomez SL, Llera A, Neuhausen SL, Newman L, Nguyen T, Palmer JR, Palmer NR, Pérez-Stable EJ, Piawah S, Rodriquez EJ, Sanabria-Salas MC, Schmit SL, Serrano-Gomez SJ, Stern MC, Weitzel J, Yang JJ, Zabaleta J, Ziv E, Fejerman L. Cancer health disparities in racial/ethnic minorities in the United States. *Br J Cancer* 2021; **124**: 315-332 [PMID: 32901135 DOI: 10.1038/s41416-020-01038-6]
10 **Chen J**, Pitmon E, Wang K. Microbiome, inflammation and colorectal cancer. *Semin Immunol* 2017; **32**: 43-53 [PMID: 28982615 DOI: 10.1016/j.smim.2017.09.006]
11 **Dejea C**, Wick E, Sears CL. Bacterial oncogenesis in the colon. *Future Microbiol* 2013; **8**: 445-460 [PMID: 23534358 DOI: 10.2217/fmb.13.17]
12 **Farhana L**, Antaki F, Murshed F, Mahmud H, Judd SL, Nangia-Makker P, Levi E, Yu Y, Majumdar AP. Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans. *World J Gastrointest Pathophysiol* 2018; **9**: 47-58 [PMID: 30283710 DOI: 10.4291/wjgp.v9.i2.47]
13 **Koi M**, Okita Y, Carethers JM. *Fusobacterium nucleatum* Infection in Colorectal Cancer: Linking Inflammation, DNA Mismatch Repair and Genetic and Epigenetic Alterations. *J Anus Rectum Colon* 2018; **2**: 37-46 [PMID: 30116794 DOI: 10.23922/jarc.2017-055]
14 **Devaraj B**, Lee A, Cabrera BL, Miyai K, Luo L, Ramamoorthy S, Keku T, Sandler RS, McGuire KL, Carethers JM. Relationship of EMAST and microsatellite instability among patients with rectal cancer. *J Gastrointest Surg* 2010; **14**: 1521-1528 [PMID: 20844976 DOI: 10.1007/s11605-010-1340-6]
15 **Carethers JM**, Koi M, Tseng-Rogenski SS. EMAST is a Form of Microsatellite Instability That is Initiated by Inflammation and Modulates Colorectal Cancer Progression. *Genes (Basel)* 2015; **6**: 185-205 [PMID: 25836926 DOI: 10.3390/genes6020185]
16 **Dejea CM**, Wick EC, Hechenbleikner EM, White JR, Mark Welch JL, Rossetti BJ, Peterson SN, Snesrud EC, Borisy GG, Lazarev M, Stein E, Vadivelu J, Roslani AC, Malik AA, Wanyiri JW, Goh KL, Thevambiga I, Fu K, Wan F, Llosa N, Housseau F, Romans K, Wu X, McAllister FM, Wu S, Vogelstein B, Kinzler KW, Pardoll DM, Sears CL. Microbiota organization is a distinct feature of proximal colorectal cancers. *Proc Natl Acad Sci U S A* 2014; **111**: 18321-18326 [PMID: 25489084 DOI: 10.1073/pnas.1406199111]
17 **Thornton JG**, Morris AM, Thornton JD, Flowers CR, McCashland TM. Racial variation in colorectal polyp and tumor location. *J Natl Med Assoc* 2007; **99**: 723-728 [PMID: 17668638]
18 **Park CH**, Han DS, Oh YH, Lee AR, Lee YR, Eun CS. Role of Fusobacteria in the serrated pathway of colorectal carcinogenesis. *Sci Rep* 2016; **6**: 25271 [PMID: 27125587 DOI: 10.1038/srep25271]
19 **Yu J**, Chen Y, Fu X, Zhou X, Peng Y, Shi L, Chen T, Wu Y. Invasive Fusobacterium nucleatum may play a role in the carcinogenesis of proximal colon cancer through the serrated neoplasia pathway. *Int J Cancer* 2016; **139**: 1318-1326 [PMID: 27130618 DOI: 10.1002/ijc.30168]
20 **DeDecker L**, Coppedge B, Avelar-Barragan J, Karnes W, Whiteson K. Microbiome distinctions between the CRC carcinogenic pathways. *Gut Microbes* 2021; **13**: 1854641 [PMID: 33446008 DOI: 10.1080/19490976.2020.1854641]
21 **Yang JF**, Tang SJ, Lash RH, Wu R, Yang Q. Anatomic distribution of sessile serrated adenoma/polyp with and without cytologic dysplasia. *Arch Pathol Lab Med* 2015; **139**: 388-393 [PMID: 25724036 DOI: 10.5858/arpa.2013-0523-OA]
22 **Yamauchi M**, Morikawa T, Kuchiba A, Imamura Y, Qian ZR, Nishihara R, Liao X, Waldron L, Hoshida Y, Huttenhower C, Chan AT, Giovannucci E, Fuchs C, Ogino S. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal *versus* distal colorectum. *Gut* 2012; **61**: 847-854 [PMID: 22427238 DOI: 10.1136/gutjnl-2011-300865]
23 **Polite BN**, Adams-Campbell LL, Brawley OW, Bickell N, Carethers JM, Flowers CR, Foti M, Gomez SL, Griggs JJ,

Lathan CS, Li CI, Lichtenfeld JL, McCaskill-Stevens W, Paskett ED. Charting the future of cancer health disparities research: A position statement from the American Association for Cancer Research, the American Cancer Society, the American Society of Clinical Oncology, and the National Cancer Institute. *CA Cancer J Clin* 2017; **67**: 353-361 [PMID: 28738442 DOI: 10.3322/caac.21404]

24 **Zheng D**, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020; **30**: 492-506 [PMID: 32433595 DOI: 10.1038/s41422-020-0332-7]

25 **Jovov B**, Araujo-Perez F, Sigel CS, Stratford JK, McCoy AN, Yeh JJ, Keku T. Differential gene expression between African American and European American colorectal cancer patients. *PLoS One* 2012; **7**: e30168 [PMID: 22276153 DOI: 10.1371/journal.pone.0030168]

26 **Quach H**, Rotival M, Pothlichet J, Loh YE, Dannemann M, Zidane N, Laval G, Patin E, Harmant C, Lopez M, Deschamps M, Naffakh N, Duffy D, Coen A, Leroux-Roels G, Clément F, Boland A, Deleuze JF, Kelso J, Albert ML, Quintana-Murci L. Genetic Adaptation and Neandertal Admixture Shaped the Immune System of Human Populations. *Cell* 2016; **167**: 643-656.e17 [PMID: 27768888 DOI: 10.1016/j.cell.2016.09.024]

27 **Nédélec Y**, Sanz J, Baharian G, Szpiech ZA, Pacis A, Dumaine A, Grenier JC, Freiman A, Sams AJ, Hebert S, Pagé Sabourin A, Luca F, Blekhman R, Hernandez RD, Pique-Regi R, Tung J, Yotova V, Barreiro LB. Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell* 2016; **167**: 657-669.e21 [PMID: 27768889 DOI: 10.1016/j.cell.2016.09.025]

28 **Banda Y**, Kvale MN, Hoffmann TJ, Hesselson SE, Ranatunga D, Tang H, Sabatti C, Croen LA, Dispensa BP, Henderson M, Iribarren C, Jorgenson E, Kushi LH, Ludwig D, Olberg D, Quesenberry CP Jr, Rowell S, Sadler M, Sakoda LC, Sciortino S, Shen L, Smethurst D, Somkin CP, Van Den Eeden SK, Walter L, Whitmer RA, Kwok PY, Schaefer C, Risch N. Characterizing Race/Ethnicity and Genetic Ancestry for 100,000 Subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. *Genetics* 2015; **200**: 1285-1295 [PMID: 26092716 DOI: 10.1534/genetics.115.178616]

29 **Bryc K**, Durand EY, Macpherson JM, Reich D, Mountain JL. The genetic ancestry of African Americans, Latinos, and European Americans across the United States. *Am J Hum Genet* 2015; **96**: 37-53 [PMID: 25529636 DOI: 10.1016/j.ajhg.2014.11.010]

30 **Batai K**, Hooker S, Kittles RA. Leveraging genetic ancestry to study health disparities. *Am J Phys Anthropol* 2021; **175**: 363-375 [PMID: 32935870 DOI: 10.1002/ajpa.24144]

31 **Denny JC**. Chapter 13: Mining electronic health records in the genomics era. *PLoS Comput Biol* 2012; **8**: e1002823 [PMID: 23300414 DOI: 10.1371/journal.pcbi.1002823]

32 **Ruterbusch JJ**, Levin AM, Kittles R, Rybicki BA, Bock CH. Abstract A66: Admixture and fine mapping in African Americans identifies a susceptibility locus for prostate cancer on chromosome 7. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: A66 [DOI: 10.1158/1055-9965.DISP12-A66]

33 **Sanabria-Salas MC**, Hernández-Suárez G, Umaña-Pérez A, Rawlik K, Tenesa A, Serrano-López ML, Sánchez de Gómez M, Rojas MP, Bravo LE, Albis R, Plata JL, Green H, Borgovan T, Li L, Majumdar S, Garai J, Lee E, Ashktorab H, Brim H, Margolin D, Fejerman L, Zabaleta J. IL1B-CGTC haplotype is associated with colorectal cancer in admixed individuals with increased African ancestry. *Sci Rep* 2017; **7**: 41920 [PMID: 28157220 DOI: 10.1038/srep41920]

34 **Nieves Delgado A**, Baedke J. Does the human microbiome tell us something about race? *Humanit Soc Sci Commun* 2021; **8**: 97 [DOI: 10.1057/s41599-021-00772-3]

35 **Gallagher S**, Hughes E, Wagner S, Tshiaba P, Rosenthal E, Roa BB, Kurian AW, Domchek SM, Garber J, Lancaster J, Weitzel JN, Gutin A, Lanchbury JS, Robson M. Association of a Polygenic Risk Score With Breast Cancer Among Women Carriers of High- and Moderate-Risk Breast Cancer Genes. *JAMA Netw Open* 2020; **3**: e208501 [PMID: 32609350 DOI: 10.1001/jamanetworkopen.2020.8501]

36 **Thomas M**, Sakoda LC, Hoffmeister M, Rosenthal EA, Lee JK, van Duijnhoven FJB, Platz EA, Wu AH, Dampier CH, de la Chapelle A, Wolk A, Joshi AD, Burnett-Hartman A, Gsur A, Lindblom A, Castells A, Win AK, Namjou B, Van Guelpen B, Tangen CM, He Q, Li CI, Schafmayer C, Joshu CE, Ulrich CM, Bishop DT, Buchanan DD, Schaid D, Drew DA, Muller DC, Duggan D, Crosslin DR, Albanes D, Giovannucci EL, Larson E, Qu F, Mentch F, Giles GG, Hakonarson H, Hampel H, Stanaway IB, Figueiredo JC, Huyghe JR, Minnier J, Chang-Claude J, Hampe J, Harley JB, Visvanathan K, Curtis KR, Offit K, Li L, Le Marchand L, Vodickova L, Gunter MJ, Jenkins MA, Slattery ML, Lemire M, Woods MO, Song M, Murphy N, Lindor NM, Dikilitas O, Pharoah PDP, Campbell PT, Newcomb PA, Milne RL, MacInnis RJ, Castellví-Bel S, Ogino S, Berndt SI, Bézieau S, Thibodeau SN, Gallinger SJ, Zaidi SH, Harrison TA, Keku TO, Hudson TJ, Vymetalkova V, Moreno V, Martín V, Arndt V, Wei WQ, Chung W, Su YR, Hayes RB, White E, Vodicka P, Casey G, Gruber SB, Schoen RE, Chan AT, Potter JD, Brenner H, Jarvik GP, Corley DA, Peters U, Hsu L. Genome-wide Modeling of Polygenic Risk Score in Colorectal Cancer Risk. *Am J Hum Genet* 2020; **107**: 432-444 [PMID: 32758450 DOI: 10.1016/j.ajhg.2020.07.006]

37 **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]

38 **Sonnenburg ED**, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol* 2019; **17**: 383-390 [PMID: 31089293 DOI: 10.1038/s41579-019-0191-8]

39 **Belkaid Y**, Harrison OJ. Homeostatic Immunity and the Microbiota. *Immunity* 2017; **46**: 562-576 [PMID: 28423337 DOI: 10.1016/j.immuni.2017.04.008]

40 **Hooper LV**, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; **336**: 1268-1273 [PMID: 22674334 DOI: 10.1126/science.1223490]

41 **Johansson ME**, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* 2008; **105**: 15064-15069 [PMID: 18806221 DOI: 10.1073/pnas.0803124105]

42 **Louis P**, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014; **12**: 661-672 [PMID: 25198138 DOI: 10.1038/nrmicro3344]

43 **Ocvirk S**, Wilson AS, Posma JM, Li JV, Koller KR, Day GM, Flanagan CA, Otto JE, Sacco PE, Sacco FD, Sapp FR,

Newton K, Brouard F, DeLany JP, Behnning M, Appolonia CN, Soni D, Bhatti F, Methé B, Fitch A, Morris A, Gaskins HR, Kinross J, Nicholson JK, Thomas TK, O'Keefe SJD. A prospective cohort analysis of gut microbial co-metabolism in Alaska Native and rural African people at high and low risk of colorectal cancer. *Am J Clin Nutr* 2020; **111**: 406-419 [PMID: 31851298 DOI: 10.1093/ajcn/nqz301]

44 **O'Keefe SJ**, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vipperla K, Naidoo V, Mtshali L, Tims S, Puylaert PG, DeLany J, Krasinskas A, Benefiel AC, Kaseb HO, Newton K, Nicholson JK, de Vos WM, Gaskins HR, Zoetendal EG. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun* 2015; **6**: 6342 [PMID: 25919227 DOI: 10.1038/ncomms7342]

45 **Rothschild D**, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018; **555**: 210-215 [PMID: 29489753 DOI: 10.1038/nature25973]

46 **Terzić J**, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology* 2010; **138**: 2101-2114.e5 [PMID: 20420949 DOI: 10.1053/j.gastro.2010.01.058]

47 **Wang H**, Taverna D, Stram DO, Fortini BK, Cheng I, Wilkens LR, Burnett T, Makar KW, Lindor NM, Hopper JL, Gallinger S, Baron JA, Haile R, Kolonel LN, Henderson BE, Newcomb PA, Casey G, Duggan D, Ulrich CM, Le Marchand L. Genetic variation in the inflammation and innate immunity pathways and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 2094-2101 [PMID: 24045924 DOI: 10.1158/1055-9965.EPI-13-0694]

48 **Turpin W**, Goethel A, Bedrani L, Croitoru Mdcm K. Determinants of IBD Heritability: Genes, Bugs, and More. *Inflamm Bowel Dis* 2018; **24**: 1133-1148 [PMID: 29701818 DOI: 10.1093/ibd/izy085]

49 **Lu Y**, Kweon SS, Tanikawa C, Jia WH, Xiang YB, Cai Q, Zeng C, Schmit SL, Shin A, Matsuo K, Jee SH, Kim DH, Kim J, Wen W, Shi J, Guo X, Li B, Wang N, Zhang B, Li X, Shin MH, Li HL, Ren Z, Oh JH, Oze I, Ahn YO, Jung KJ, Conti DV, Schumacher FR, Rennert G, Jenkins MA, Campbell PT, Hoffmeister M, Casey G, Gruber SB, Gao J, Gao YT, Pan ZZ, Kamatani Y, Zeng YX, Shu XO, Long J, Matsuda K, Zheng W. Large-Scale Genome-Wide Association Study of East Asians Identifies Loci Associated With Risk for Colorectal Cancer. *Gastroenterology* 2019; **156**: 1455-1466 [PMID: 30529582 DOI: 10.1053/j.gastro.2018.11.066]

50 **Law PJ**, Timofeeva M, Fernandez-Rozadilla C, Broderick P, Studd J, Fernandez-Tajes J, Farrington S, Svinti V, Palles C, Orlando G, Sud A, Holroyd A, Penegar S, Theodoratou E, Vaughan-Shaw P, Campbell H, Zgaga L, Hayward C, Campbell A, Harris S, Deary IJ, Starr J, Gatcombe L, Pinna M, Briggs S, Martin L, Jaeger E, Sharma-Oates A, East J, Leedham S, Arnold R, Johnstone E, Wang H, Kerr D, Kerr R, Maughan T, Kaplan R, Al-Tassan N, Palin K, Hänninen UA, Cajuso T, Tanskanen T, Kondelin J, Kaasinen E, Sarin AP, Eriksson JG, Rissanen H, Knekt P, Pukkala E, Jousilahti P, Salomaa V, Ripatti S, Palotie A, Renkonen-Sinisalo L, Lepistö A, Böhm J, Mecklin JP, Buchanan DD, Win AK, Hopper J, Jenkins ME, Lindor NM, Newcomb PA, Gallinger S, Duggan D, Casey G, Hoffmann P, Nöthen MM, Jöckel KH, Easton DF, Pharoah PDP, Peto J, Canzian F, Swerdlow A, Eeles RA, Kote-Jarai Z, Muir K, Pashayan N; PRACTICAL consortium, Harkin A, Allan K, McQueen J, Paul J, Iveson T, Saunders M, Butterbach K, Chang-Claude J, Hoffmeister M, Brenner H, Kirac I, Matošević P, Hofer P, Brezina S, Gsur A, Cheadle JP, Aaltonen LA, Tomlinson I, Houlston RS, Dunlop MG. Association analyses identify 31 new risk loci for colorectal cancer susceptibility. *Nat Commun* 2019; **10**: 2154 [PMID: 31089142 DOI: 10.1038/s41467-019-09775-w]

51 **Hall AB**, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. *Nat Rev Genet* 2017; **18**: 690-699 [PMID: 28824167 DOI: 10.1038/nrg.2017.63]

52 **Rakoff-Nahoum S**, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science* 2007; **317**: 124-127 [PMID: 17615359 DOI: 10.1126/science.1140488]

53 **Hu B**, Elinav E, Huber S, Strowig T, Hao L, Hafemann A, Jin C, Wunderlich C, Wunderlich T, Eisenbarth SC, Flavell RA. Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. *Proc Natl Acad Sci U S A* 2013; **110**: 9862-9867 [PMID: 23696660 DOI: 10.1073/pnas.1307575110]

54 **Couturier-Maillard A**, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A, Gaillot O, Schreiber S, Lemoine Y, Ryffel B, Hot D, Nùñez G, Chen G, Rosenstiel P, Chamaillard M. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest* 2013; **123**: 700-711 [PMID: 23281400 DOI: 10.1172/JCI62236]

55 **Moschen AR**, Gerner RR, Wang J, Klepsch V, Adolph TE, Reider SJ, Hackl H, Pfister A, Schilling J, Moser PL, Kempster SL, Swidsinski A, Orth Höller D, Weiss G, Baines JF, Kaser A, Tilg H. Lipocalin 2 Protects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations. *Cell Host Microbe* 2016; **19**: 455-469 [PMID: 27078067 DOI: 10.1016/j.chom.2016.03.007]

56 **Davies JM**, Abreu MT. The innate immune system and inflammatory bowel disease. *Scand J Gastroenterol* 2015; **50**: 24-33 [PMID: 25523553 DOI: 10.3109/00365521.2014.966321]

57 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 Leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603 [PMID: 11385576 DOI: 10.1038/35079107]

58 **Ebbo M**, Crinier A, Vély F, Vivier E. Innate lymphoid cells: major players in inflammatory diseases. *Nat Rev Immunol* 2017; **17**: 665-678 [PMID: 28804130 DOI: 10.1038/nri.2017.86]

59 **Knights D**, Silverberg MS, Weersma RK, Gevers D, Dijkstra G, Huang H, Tyler AD, van Sommeren S, Imhann F, Stempak JM, Vangay P, Al-Ghalith GA, Russell C, Sauk J, Knight J, Daly MJ, Huttenhower C, Xavier RJ. Complex host genetics influence the microbiome in inflammatory bowel disease. *Genome Med* 2014; **6**: 107 [PMID: 25587358 DOI: 10.1186/s13073-014-0107-1]

60 **Lavoie S**, Conway KL, Lassen KG, Jijon HB, Pan H, Chun E, Michaud M, Lang JK, Gallini Comeau CA, Dreyfuss JM, Glickman JN, Vlamakis H, Ananthakrishnan A, Kostic A, Garrett WS, Xavier RJ. The Crohn's disease polymorphism, *ATG16L1* T300A, alters the gut microbiota and enhances the local Th1/Th17 response. *Elife* 2019; **8** [PMID: 30666959

DOI: 10.7554/eLife.39982]

61 **Harrington LE**, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop *via* a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**: 1123-1132 [PMID: 16200070 DOI: 10.1038/ni1254]

62 **Lee JS**, Tato CM, Joyce-Shaikh B, Gulen MF, Cayatte C, Chen Y, Blumenschein WM, Judo M, Ayanoglu G, McClanahan TK, Li X, Cua DJ. Interleukin-23-Independent IL-17 Production Regulates Intestinal Epithelial Permeability. *Immunity* 2015; **43**: 727-738 [PMID: 26431948 DOI: 10.1016/j.immuni.2015.09.003]

63 **Stockinger B**, Veldhoen M. Differentiation and function of Th17 T cells. *Curr Opin Immunol* 2007; **19**: 281-286 [PMID: 17433650 DOI: 10.1016/j.coi.2007.04.005]

64 **Grivennikov SI**, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012; **491**: 254-258 [PMID: 23034650 DOI: 10.1038/nature11465]

65 **Wang K**, Kim MK, Di Caro G, Wong J, Shalapour S, Wan J, Zhang W, Zhong Z, Sanchez-Lopez E, Wu LW, Taniguchi K, Feng Y, Fearon E, Grivennikov SI, Karin M. Interleukin-17 receptor a signaling in transformed enterocytes promotes early colorectal tumorigenesis. *Immunity* 2014; **41**: 1052-1063 [PMID: 25526314 DOI: 10.1016/j.immuni.2014.11.009]

66 **Bank S**, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard J, Ydegaard Turino S, Brodersen JB, Rashid S, Kaiser Rasmussen B, Avlund S, Bastholm Olesen T, Hoffmann HJ, Andersen Nexø B, Sode J, Vogel U, Andersen V. Polymorphisms in the Toll-Like Receptor and the IL-23/IL-17 Pathways Were Associated with Susceptibility to Inflammatory Bowel Disease in a Danish Cohort. *PLoS One* 2015; **10**: e0145302 [PMID: 26698117 DOI: 10.1371/journal.pone.0145302]

67 **Newman WG**, Zhang Q, Liu X, Amos CI, Siminovitch KA. Genetic variants in IL-23R and ATG16L1 independently predispose to increased susceptibility to Crohn's disease in a Canadian population. *J Clin Gastroenterol* 2009; **43**: 444-447 [PMID: 19276991 DOI: 10.1097/MCG.0b013e318168bdf0]

68 **Bonder MJ**, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, Deelen P, Vatanen T, Schirmer M, Smeekens SP, Zhernakova DV, Jankipersadsing SA, Jaeger M, Oosting M, Cenit MC, Masclee AA, Swertz MA, Li Y, Kumar V, Joosten L, Harmsen H, Weersma RK, Franke L, Hofker MH, Xavier RJ, Jonkers D, Netea MG, Wijmenga C, Fu J, Zhernakova A. The effect of host genetics on the gut microbiome. *Nat Genet* 2016; **48**: 1407-1412 [PMID: 27694959 DOI: 10.1038/ng.3663]

69 **Yeyeodu ST**, Kidd LR, Kimbro KS. Protective Innate Immune Variants in Racial/Ethnic Disparities of Breast and Prostate Cancer. *Cancer Immunol Res* 2019; **7**: 1384-1389 [PMID: 31481520 DOI: 10.1158/2326-6066.CIR-18-0564]

70 **Quan B**, Qi X, Yu Z, Jiang Y, Liao M, Wang G, Feng R, Zhang L, Chen Z, Jiang Q, Liu G. Pathway analysis of genome-wide association study and transcriptome data highlights new biological pathways in colorectal cancer. *Mol Genet Genomics* 2015; **290**: 603-610 [PMID: 25362561 DOI: 10.1007/s00438-014-0945-y]

71 **Wallace TA**, Prueitt RL, Yi M, Howe TM, Gillespie JW, Yfantis HG, Stephens RM, Caporaso NE, Loffredo CA, Ambs S. Tumor immunobiological differences in prostate cancer between African-American and European-American men. *Cancer Res* 2008; **68**: 927-936 [PMID: 18245496 DOI: 10.1158/0008-5472.CAN-07-2608]

72 **Özdemir BC**, Dotto GP. Racial Differences in Cancer Susceptibility and Survival: More Than the Color of the Skin? *Trends Cancer* 2017; **3**: 181-197 [PMID: 28718431 DOI: 10.1016/j.trecan.2017.02.002]

73 **Yuan J**, Hu Z, Mahal BA, Zhao SD, Kensler KH, Pi J, Hu X, Zhang Y, Wang Y, Jiang J, Li C, Zhong X, Montone KT, Guan G, Tanyi JL, Fan Y, Xu X, Morgan MA, Long M, Zhang R, Sood AK, Rebbeck TR, Dang CV, Zhang L. Integrated Analysis of Genetic Ancestry and Genomic Alterations across Cancers. *Cancer Cell* 2018; **34**: 549-560.e9 [PMID: 30300578 DOI: 10.1016/j.ccell.2018.08.019]

74 **Zanetti KA**, Haznadar M, Welsh JA, Robles AI, Ryan BM, McClary AC, Bowman ED, Goodman JE, Bernig T, Chanock SJ, Harris CC. 3'-UTR and functional secretor haplotypes in mannose-binding lectin 2 are associated with increased colon cancer risk in African Americans. *Cancer Res* 2012; **72**: 1467-1477 [PMID: 22282660 DOI: 10.1158/0008-5472.CAN-11-3073]

75 **Karimzadeh MR**, Zarin M, Ehtesham N, Khosravi S, Soosanabadi M, Mosallaei M, Pourdavoud P. MicroRNA binding site polymorphism in inflammatory genes associated with colorectal cancer: literature review and bioinformatics analysis. *Cancer Gene Ther* 2020; **27**: 739-753 [PMID: 32203060 DOI: 10.1038/s41417-020-0172-0]

76 **Lazarus R**, Vercelli D, Palmer LJ, Klimecki WJ, Silverman EK, Richter B, Riva A, Ramoni M, Martinez FD, Weiss ST, Kwiatkowski DJ. Single nucleotide polymorphisms in innate immunity genes: abundant variation and potential role in complex human disease. *Immunol Rev* 2002; **190**: 9-25 [PMID: 12493003 DOI: 10.1034/j.1600-065x.2002.19002.x]

77 **Barreiro LB**, Quintana-Murci L. From evolutionary genetics to human immunology: how selection shapes host defence genes. *Nat Rev Genet* 2010; **11**: 17-30 [PMID: 19953080 DOI: 10.1038/nrg2698]

78 **Brinkworth JF**, Barreiro LB. The contribution of natural selection to present-day susceptibility to chronic inflammatory and autoimmune disease. *Curr Opin Immunol* 2014; **31**: 66-78 [PMID: 25458997 DOI: 10.1016/j.coi.2014.09.008]

79 **Sams AJ**, Dumaine A, Nédélec Y, Yotova V, Alfieri C, Tanner JE, Messer PW, Barreiro LB. Adaptively introgressed Neandertal haplotype at the OAS locus functionally impacts innate immune responses in humans. *Genome Biol* 2016; **17**: 246 [PMID: 27899133 DOI: 10.1186/s13059-016-1098-6]

80 **Green R**, Wilkins C, Thomas S, Sekine A, Hendrick DM, Voss K, Ireton RC, Mooney M, Go JT, Choonoo G, Jeng S, de Villena FP, Ferris MT, McWeeney S, Gale M Jr. Oas1b-dependent Immune Transcriptional Profiles of West Nile Virus Infection in the Collaborative Cross. *G3 (Bethesda)* 2017; **7**: 1665-1682 [PMID: 28592649 DOI: 10.1534/g3.117.041624]

81 **Barreiro LB**, Ben-Ali M, Quach H, Laval G, Patin E, Pickrell JK, Bouchier C, Tichit M, Neyrolles O, Gicquel B, Kidd JR, Kidd KK, Alcaïs A, Ragimbeau J, Pellegrini S, Abel L, Casanova JL, Quintana-Murci L. Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. *PLoS Genet* 2009; **5**: e1000562 [PMID: 19609346 DOI: 10.1371/journal.pgen.1000562]

82 **Ness RB**, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol* 2004; **160**: 1033-1038 [PMID: 15561982 DOI: 10.1093/aje/kwh325]

83 **Bidwell J**, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999; **1**: 3-19 [PMID: 11197303 DOI: 10.1038/sj.gene.6363645]

84 **Chen H**, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, Rogus J, Beck JD, Offenbacher S, Cork MJ, Rafie-Kolpin M, Hsieh CM, Kornman KS, Duff GW. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* 2006; **15**: 519-529 [PMID: 16399797 DOI: 10.1093/hmg/ddi469]

85 **Hung RJ**, Ulrich CM, Goode EL, Brhane Y, Muir K, Chan AT, Marchand LL, Schildkraut J, Witte JS, Eeles R, Boffetta P, Spitz MR, Poirier JG, Rider DN, Fridley BL, Chen Z, Haiman C, Schumacher F, Easton DF, Landi MT, Brennan P, Houlston R, Christiani DC, Field JK, Bickeböller H, Risch A, Kote-Jarai Z, Wiklund F, Grönberg H, Chanock S, Berndt SI, Kraft P, Lindström S, Al Olama AA, Song H, Phelan C, Wentzensen N, Peters U, Slattery ML; GECCO, Sellers TA; FOCI, Casey G, Gruber SB; CORECT, Hunter DJ; DRIVE, Amos CI, Henderson B; GAME-ON Network. Cross Cancer Genomic Investigation of Inflammation Pathway for Five Common Cancers: Lung, Ovary, Prostate, Breast, and Colorectal Cancer. *J Natl Cancer Inst* 2015; **107** [PMID: 26319099 DOI: 10.1093/jnci/djv246]

86 **Theodoropoulos G**, Papaconstantinou I, Felekouras E, Nikiteas N, Karakitsos P, Panoussopoulos D, Lazaris ACh, Patsouris E, Bramis J, Gazouli M. Relation between common polymorphisms in genes related to inflammatory response and colorectal cancer. *World J Gastroenterol* 2006; **12**: 5037-5043 [PMID: 16937502 DOI: 10.3748/wjg.v12.i31.5037]

87 **Landi S**, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J, Capella G, Canzian F; Bellvitge Colorectal Cancer Study Group. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* 2003; **63**: 3560-3566 [PMID: 12839942]

88 **Koh WP**, Yuan JM, Van Den Berg D, Ingles SA, Yu MC. Peroxisome proliferator-activated receptor (PPAR) gamma gene polymorphisms and colorectal cancer risk among Chinese in Singapore. *Carcinogenesis* 2006; **27**: 1797-1802 [PMID: 16513680 DOI: 10.1093/carcin/bgl001]

89 **Montazeri Z**, Li X, Nyiraneza C, Ma X, Timofeeva M, Svinti V, Meng X, He Y, Bo Y, Morgan S, Castellví-Bel S, Ruiz-Ponte C, Fernández-Rozadilla C, Carracedo Á, Castells A, Bishop T, Buchanan D, Jenkins MA, Keku TO, Lindblom A, van Duijnhoven FJB, Wu A, Farrington SM, Dunlop MG, Campbell H, Theodoratou E, Zheng W, Little J. Systematic meta-analyses, field synopsis and global assessment of the evidence of genetic association studies in colorectal cancer. *Gut* 2020; **69**: 1460-1471 [PMID: 31818908 DOI: 10.1136/gutjnl-2019-319313]

90 **Abbas M**, Mason T, Ibad A, Khraiwesh M, Apprey V, Kanaan Y, Wilson B, Dunston G, Ricks-Santi L, Brim H. Genetic Polymorphisms in IL-10 Promoter Are Associated With Smoking and Prostate Cancer Risk in African Americans. *Anticancer Res* 2020; **40**: 27-34 [PMID: 31892550 DOI: 10.21873/anticanres.13923]

91 **Jenkins BD**, Martini RN, Hire R, Brown A, Bennett B, Brown I, Howerth EW, Egan M, Hodgson J, Yates C, Kittles R, Chitale D, Ali H, Nathanson D, Nikolinakos P, Newman L, Monteil M, Davis MB. Atypical Chemokine Receptor 1 (*DARC/ACKR1*) in Breast Tumors Is Associated with Survival, Circulating Chemokines, Tumor-Infiltrating Immune Cells, and African Ancestry. *Cancer Epidemiol Biomarkers Prev* 2019; **28**: 690-700 [PMID: 30944146 DOI: 10.1158/1055-9965.EPI-18-0955]

92 **Cho I**, Blaser MJ. The human microbiome: at the interface of health and disease. *Nat Rev Genet* 2012; **13**: 260-270 [PMID: 22411464 DOI: 10.1038/nrg3182]

93 **Gopalakrishnan V**, Helmink BA, Spencer CN, Reuben A, Wargo JA. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell* 2018; **33**: 570-580 [PMID: 29634945 DOI: 10.1016/j.ccell.2018.03.015]

94 **Xu F**, Fu Y, Sun TY, Jiang Z, Miao Z, Shuai M, Gou W, Ling CW, Yang J, Wang J, Chen YM, Zheng JS. The interplay between host genetics and the gut microbiome reveals common and distinct microbiome features for complex human diseases. *Microbiome* 2020; **8**: 145 [PMID: 33032658 DOI: 10.1186/s40168-020-00923-9]

95 **Burns MB**, Montassier E, Abrahante J, Priya S, Niccum DE, Khoruts A, Starr TK, Knights D, Blekhman R. Colorectal cancer mutational profiles correlate with defined microbial communities in the tumor microenvironment. *PLoS Genet* 2018; **14**: e1007376 [PMID: 29924794 DOI: 10.1371/journal.pgen.1007376]

96 **Thyagarajan S**, Zhang Y, Thapa S, Allen MS, Phillips N, Chaudhary P, Kashyap MV, Vishwanatha JK. Comparative analysis of racial differences in breast tumor microbiome. *Sci Rep* 2020; **10**: 14116 [PMID: 32839514 DOI: 10.1038/s41598-020-71102-x]

97 **Yu J**, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, Tang L, Zhao H, Stenvang J, Li Y, Wang X, Xu X, Chen N, Wu WK, Al-Aama J, Nielsen HJ, Kiilerich P, Jensen BA, Yau TO, Lan Z, Jia H, Li J, Xiao L, Lam TY, Ng SC, Cheng AS, Wong VW, Chan FK, Yang H, Madsen L, Datz C, Tilg H, Wang J, Brünner N, Kristiansen K, Arumugam M, Sung JJ. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* 2017; **66**: 70-78 [PMID: 26408641 DOI: 10.1136/gutjnl-2015-309800]

98 **Zeller G**, Tap J, Voigt AY, Sunagawa S, Kultima JR, Costea PI, Amiot A, Böhm J, Brunetti F, Habermann N, Hercog R, Koch M, Luciani A, Mende DR, Schneider MA, Schrotz-King P, Tournigand C, Tran Van Nhieu J, Yamada T, Zimmermann J, Benes V, Kloor M, Ulrich CM, von Knebel Doeberitz M, Sobhani I, Bork P. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol* 2014; **10**: 766 [PMID: 25432777 DOI: 10.15252/msb.20145645]

99 **Baxter NT**, Ruffin MT 4th, Rogers MA, Schloss PD. Microbiota-based model improves the sensitivity of fecal immunochemical test for detecting colonic lesions. *Genome Med* 2016; **8**: 37 [PMID: 27056827 DOI: 10.1186/s13073-016-0290-3]

100 **Zackular JP**, Rogers MA, Ruffin MT 4th, Schloss PD. The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev Res (Phila)* 2014; **7**: 1112-1121 [PMID: 25104642 DOI: 10.1158/1940-6207.CAPR-14-0129]

101 **Thomas AM**, Manghi P, Asnicar F, Pasolli E, Armanini F, Zolfo M, Beghini F, Manara S, Karcher N, Pozzi C, Gandini S, Serrano D, Tarallo S, Francavilla A, Gallo G, Trompetto M, Ferrero G, Mizutani S, Shiroma H, Shiba S, Shibata T, Yachida S, Yamada T, Wirbel J, Schrotz-King P, Ulrich CM, Brenner H, Arumugam M, Bork P, Zeller G, Cordero F,

Dias-Neto E, Setubal JC, Tett A, Pardini B, Rescigno M, Waldron L, Naccarati A, Segata N. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nat Med* 2019; **25**: 667-678 [PMID: 30936548 DOI: 10.1038/s41591-019-0405-7]

102 **Wirbel J**, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, Fleck JS, Voigt AY, Palleja A, Ponnudurai R, Sunagawa S, Coelho LP, Schrotz-King P, Vogtmann E, Habermann N, Niméus E, Thomas AM, Manghi P, Gandini S, Serrano D, Mizutani S, Shiroma H, Shiba S, Shibata T, Yachida S, Yamada T, Waldron L, Naccarati A, Segata N, Sinha R, Ulrich CM, Brenner H, Arumugam M, Bork P, Zeller G. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat Med* 2019; **25**: 679-689 [PMID: 30936547 DOI: 10.1038/s41591-019-0406-6]

103 **Dai Z**, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, Chan FKL, Kristiansen K, Sung JJY, Wong SH, Yu J. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome* 2018; **6**: 70 [PMID: 29642940 DOI: 10.1186/s40168-018-0451-2]

104 **Wu S**, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM, Sears CL. A human colonic commensal promotes colon tumorigenesis *via* activation of T helper type 17 T cell responses. *Nat Med* 2009; **15**: 1016-1022 [PMID: 19701202 DOI: 10.1038/nm.2015]

105 **Arthur JC**, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012; **338**: 120-123 [PMID: 22903521 DOI: 10.1126/science.1224820]

106 **Kostic AD**, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, El-Omar EM, Brenner D, Fuchs CS, Meyerson M, Garrett WS. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; **14**: 207-215 [PMID: 23954159 DOI: 10.1016/j.chom.2013.07.007]

107 **Tomkovich S**, Dejea CM, Winglee K, Drewes JL, Chung L, Housseau F, Pope JL, Gauthier J, Sun X, Mühlbauer M, Liu X, Fathi P, Anders RA, Besharati S, Perez-Chanona E, Yang Y, Ding H, Wu X, Wu S, White JR, Gharaibeh RZ, Fodor AA, Wang H, Pardoll DM, Jobin C, Sears CL. Human colon mucosal biofilms from healthy or colon cancer hosts are carcinogenic. *J Clin Invest* 2019; **129**: 1699-1712 [PMID: 30855275 DOI: 10.1172/JCI124196]

108 **Knippel RJ**, Drewes JL, Sears CL. The Cancer Microbiome: Recent Highlights and Knowledge Gaps. *Cancer Discov* 2021; **11**: 2378-2395 [PMID: 34400408 DOI: 10.1158/2159-8290.CD-21-0324]

109 **Brooks AW**, Priya S, Blekhman R, Bordenstein SR. Gut microbiota diversity across ethnicities in the United States. *PLoS Biol* 2018; **16**: e2006842 [PMID: 30513082 DOI: 10.1371/journal.pbio.2006842]

110 **Deschasaux M**, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, Tremaroli V, Bakker GJ, Attaye I, Pinto-Sietsma SJ, van Raalte DH, Snijder MB, Nicolaou M, Peters R, Zwinderman AH, Bäckhed F, Nieuwdorp M. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med* 2018; **24**: 1526-1531 [PMID: 30150717 DOI: 10.1038/s41591-018-0160-1]

111 **Gupta VK**, Paul S, Dutta C. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Front Microbiol* 2017; **8**: 1162 [PMID: 28690602 DOI: 10.3389/fmicb.2017.01162]

112 **Hester CM**, Jala VR, Langille MG, Umar S, Greiner KA, Haribabu B. Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. *World J Gastroenterol* 2015; **21**: 2759-2769 [PMID: 25759547 DOI: 10.3748/wjg.v21.i9.2759]

113 **Ou J**, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, O'Keefe SJ. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013; **98**: 111-120 [PMID: 23719549 DOI: 10.3945/ajcn.112.056689]

114 **Jung SY**, Papp JC, Sobel EM, Pellegrini M, Yu H, Zhang ZF. Pro-inflammatory cytokine polymorphisms in ONECUT2 and HNF4A and primary colorectal carcinoma: a post genome-wide gene-lifestyle interaction study. *Am J Cancer Res* 2020; **10**: 2955-2976 [PMID: 33042629]

115 **Chiuve SE**, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr* 2012; **142**: 1009-1018 [PMID: 22513989 DOI: 10.3945/jn.111.157222]

116 **Wang DD**, Leung CW, Li Y, Ding EL, Chiuve SE, Hu FB, Willett WC. Trends in dietary quality among adults in the United States, 1999 through 2010. *JAMA Intern Med* 2014; **174**: 1587-1595 [PMID: 25179639 DOI: 10.1001/jamainternmed.2014.3422]

117 **Lu SSM**, Mohammed Z, Häggström C, Myte R, Lindquist E, Gylfe Å, Van Guelpen B, Harlid S. Antibiotics Use and Subsequent Risk of Colorectal Cancer: A Swedish Nationwide Population-Based Study. *J Natl Cancer Inst* 2022; **114**: 38-46 [PMID: 34467395 DOI: 10.1093/jnci/djab125]

118 **Tsong WH**, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. *Br J Cancer* 2007; **96**: 821-827 [PMID: 17311023 DOI: 10.1038/sj.bjc.6603623]

119 **Ali Khan U**, Fallah M, Sundquist K, Sundquist J, Brenner H, Kharazmi E. Risk of colorectal cancer in patients with diabetes mellitus: A Swedish nationwide cohort study. *PLoS Med* 2020; **17**: e1003431 [PMID: 33186354 DOI: 10.1371/journal.pmed.1003431]

120 **Rebersek M**. Gut microbiome and its role in colorectal cancer. *BMC Cancer* 2021; **21**: 1325 [PMID: 34895176 DOI: 10.1186/s12885-021-09054-2]

121 **Scepanovic P**, Hodel F, Mondot S, Partula V, Byrd A, Hammer C, Alanio C, Bergstedt J, Patin E, Touvier M, Lantz O, Albert ML, Duffy D, Quintana-Murci L, Fellay J; Milieu Intérieur Consortium. A comprehensive assessment of demographic, environmental, and host genetic associations with gut microbiome diversity in healthy individuals. *Microbiome* 2019; **7**: 130 [PMID: 31519223 DOI: 10.1186/s40168-019-0747-x]

122 **Blekhman R**, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, Spector TD, Keinan A, Ley RE, Gevers D, Clark AG. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol* 2015; **16**: 191 [PMID: 26374288 DOI: 10.1186/s13059-015-0759-1]

123 **Goodrich JK**, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE.

Genetic Determinants of the Gut Microbiome in UK Twins. *Cell Host Microbe* 2016; **19**: 731-743 [PMID: 27173935 DOI: 10.1016/j.chom.2016.04.017]

124 **Goodrich JK**, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. Human genetics shape the gut microbiome. *Cell* 2014; **159**: 789-799 [PMID: 25417156 DOI: 10.1016/j.cell.2014.09.053]

125 **Ashktorab H**, Azimi H, Varma S, Lee EL, Laiyemo AO, Nickerson ML, Brim H. Driver genes exome sequencing reveals distinct variants in African Americans with colorectal neoplasia. *Oncotarget* 2019; **10**: 2607-2624 [PMID: 31080553 DOI: 10.18632/oncotarget.26721]

126 **Brim H**, Yooseph S, Lee E, Sherif ZA, Abbas M, Laiyemo AO, Varma S, Torralba M, Dowd SE, Nelson KE, Pathmasiri W, Sumner S, de Vos W, Liang Q, Yu J, Zoetendal E, Ashktorab H. A Microbiomic Analysis in African Americans with Colonic Lesions Reveals Streptococcus sp.VT162 as a Marker of Neoplastic Transformation. *Genes (Basel)* 2017; **8** [PMID: 29120399 DOI: 10.3390/genes8110314]

127 **Ogino S**, Nowak JA, Hamada T, Milner DA Jr, Nishihara R. Insights into Pathogenic Interactions Among Environment, Host, and Tumor at the Crossroads of Molecular Pathology and Epidemiology. *Annu Rev Pathol* 2019; **14**: 83-103 [PMID: 30125150 DOI: 10.1146/annurev-pathmechdis-012418-012818]

128 **Nishi A**, Milner DA Jr, Giovannucci EL, Nishihara R, Tan AS, Kawachi I, Ogino S. Integration of molecular pathology, epidemiology and social science for global precision medicine. *Expert Rev Mol Diagn* 2016; **16**: 11-23 [PMID: 26636627 DOI: 10.1586/14737159.2016.1115346]

129 **Turpin W**, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, Guttman DS, Griffiths A, Panaccione R, Otley A, Xu L, Shestopaloff K, Moreno-Hagelsieb G; GEM Project Research Consortium, Paterson AD, Croitoru K. Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat Genet* 2016; **48**: 1413-1417 [PMID: 27694960 DOI: 10.1038/ng.3693]

130 **DeStefano Shields CE**, White JR, Chung L, Wenzel A, Hicks JL, Tam AJ, Chan JL, Dejea CM, Fan H, Michel J, Maiuri AR, Sriramkumar S, Podicheti R, Rusch DB, Wang H, De Marzo AM, Besharati S, Anders RA, Baylin SB, O'Hagan HM, Housseau F, Sears CL. Bacterial-Driven Inflammation and Mutant *BRAF* Expression Combine to Promote Murine Colon Tumorigenesis That Is Sensitive to Immune Checkpoint Therapy. *Cancer Discov* 2021; **11**: 1792-1807 [PMID: 33632774 DOI: 10.1158/2159-8290.CD-20-0770]

131 **Davis MB**. Genomics and Cancer Disparities: The Justice and Power of Inclusion. *Cancer Discov* 2021; **11**: 805-809 [PMID: 33811116 DOI: 10.1158/2159-8290.CD-21-0225]

132 **Sherman RM**, Forman J, Antonescu V, Puiu D, Daya M, Rafaels N, Boorgula MP, Chavan S, Vergara C, Ortega VE, Levin AM, Eng C, Yazdanbakhsh M, Wilson JG, Marrugo J, Lange LA, Williams LK, Watson H, Ware LB, Olopade CO, Olopade O, Oliveira RR, Ober C, Nicolae DL, Meyers DA, Mayorga A, Knight-Madden J, Hartert T, Hansel NN, Foreman MG, Ford JG, Faruque MU, Dunston GM, Caraballo L, Burchard EG, Bleecker ER, Araujo MI, Herrera-Paz EF, Campbell M, Foster C, Taub MA, Beaty TH, Ruczinski I, Mathias RA, Barnes KC, Salzberg SL. Assembly of a pan-genome from deep sequencing of 910 humans of African descent. *Nat Genet* 2019; **51**: 30-35 [PMID: 30455414 DOI: 10.1038/s41588-018-0273-y]

133 **Peterson RE**, Edwards AC, Bacanu SA, Dick DM, Kendler KS, Webb BT. The utility of empirically assigning ancestry groups in cross-population genetic studies of addiction. *Am J Addict* 2017; **26**: 494-501 [PMID: 28714599 DOI: 10.1111/ajad.12586]

134 **Adedokun B**, Du Z, Gao G, Ahearn TU, Lunetta KL, Zirpoli G, Figueroa J, John EM, Bernstein L, Zheng W, Hu JJ, Ziegler RG, Nyante S, Bandera EV, Ingles SA, Press MF, Deming-Halverson SL, Rodriguez-Gil JL, Yao S, Ogundiran TO, Ojengbede O, Blot W, Troester MA, Nathanson KL, Hennis A, Nemesure B, Ambs S, Fiorica PN, Sucheston-Campbell LE, Bensen JT, Kushi LH, Torres-Mejia G, Hu D, Fejerman L, Bolla MK, Dennis J, Dunning AM, Easton DF, Michailidou K, Pharoah PDP, Wang Q, Sandler DP, Taylor JA, O'Brien KM, Kitahara CM, Falusi AG, Babalola C, Yarney J, Awuah B, Addai-Wiafe B; GBHS Study Team, Chanock SJ, Olshan AF, Ambrosone CB, Conti DV, Ziv E, Olopade OI, Garcia-Closas M, Palmer JR, Haiman CA, Huo D. Cross-ancestry GWAS meta-analysis identifies six breast cancer loci in African and European ancestry women. *Nat Commun* 2021; **12**: 4198 [PMID: 34234117 DOI: 10.1038/s41467-021-24327-x]

135 **Schumacher FR**, Schmit SL, Jiao S, Edlund CK, Wang H, Zhang B, Hsu L, Huang SC, Fischer CP, Harju JF, Idos GE, Lejbkowicz F, Manion FJ, McDonnell K, McNeil CE, Melas M, Rennert HS, Shi W, Thomas DC, Van Den Berg DJ, Hutter CM, Aragaki AK, Butterbach K, Caan BJ, Carlson CS, Chanock SJ, Curtis KR, Fuchs CS, Gala M, Giovannucci EL, Gogarten SM, Hayes RB, Henderson B, Hunter DJ, Jackson RD, Kolonel LN, Kooperberg C, Küry S, LaCroix A, Laurie CC, Laurie CA, Lemire M, Levine D, Ma J, Makar KW, Qu C, Taverna D, Ulrich CM, Wu K, Kono S, West DW, Berndt SI, Bezieau S, Brenner H, Campbell PT, Chan AT, Chang-Claude J, Coetzee GA, Conti DV, Duggan D, Figueiredo JC, Fortini BK, Gallinger SJ, Gauderman WJ, Giles G, Green R, Haile R, Harrison TA, Hoffmeister M, Hopper JL, Hudson TJ, Jacobs E, Iwasaki M, Jee SH, Jenkins M, Jia WH, Joshi A, Li L, Lindor NM, Matsuo K, Moreno V, Mukherjee B, Newcomb PA, Potter JD, Raskin L, Rennert G, Rosse S, Severi G, Schoen RE, Seminara D, Shu XO, Slattery ML, Tsugane S, White E, Xiang YB, Zanke BW, Zheng W, Le Marchand L, Casey G, Gruber SB, Peters U. Genome-wide association study of colorectal cancer identifies six new susceptibility loci. *Nat Commun* 2015; **6**: 7138 [PMID: 26151821 DOI: 10.1038/ncomms8138]

136 **Tsilidis KK**, Helzlsouer KJ, Smith MW, Grinberg V, Hoffman-Bolton J, Clipp SL, Visvanathan K, Platz EA. Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. *Cancer Causes Control* 2009; **20**: 1739-1751 [PMID: 19760027 DOI: 10.1007/s10552-009-9427-7]

137 **Li D**, Achkar JP, Haritunians T, Jacobs JP, Hui KY, D'Amato M, Brand S, Radford-Smith G, Halfvarson J, Niess JH, Kugathasan S, Büning C, Schumm LP, Klei L, Ananthakrishnan A, Aumais G, Baidoo L, Dubinsky M, Fiocchi C, Glas J, Milgrom R, Proctor DD, Regueiro M, Simms LA, Stempak JM, Targan SR, Törkvist L, Sharma Y, Devlin B, Borneman J, Hakonarson H, Xavier RJ, Daly M, Brant SR, Rioux JD, Silverberg MS, Cho JH, Braun J, McGovern DP, Duerr RH. A Pleiotropic Missense Variant in SLC39A8 Is Associated With Crohn's Disease and Human Gut Microbiome Composition. *Gastroenterology* 2016; **151**: 724-732 [PMID: 27492617 DOI: 10.1053/j.gastro.2016.06.051]

138 **Rühlemann MC**, Hermes BM, Bang C, Doms S, Moitinho-Silva L, Thingholm LB, Frost F, Degenhardt F, Wittig M, Kässens J, Weiss FU, Peters A, Neuhaus K, Völker U, Völzke H, Homuth G, Weiss S, Grallert H, Laudes M, Lieb W, Haller D, Lerch MM, Baines JF, Franke A. Genome-wide association study in 8,956 German individuals identifies influence of ABO histo-blood groups on gut microbiome. *Nat Genet* 2021; **53**: 147-155 [PMID: 33462482 DOI: 10.1038/s41588-020-00747-1]

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*REVIEW*

# Altered gut microbiota patterns in COVID-19: Markers for inflammation and disease severity

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## Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection leads to a severe respiratory illness and alters the gut microbiota, which dynamically interacts with the human immune system. Microbiota alterations include decreased levels of beneficial bacteria and augmentation of opportunistic pathogens. Here, we describe critical factors affecting the microbiota in coronavirus disease 2019 (COVID-19) patients. These include, such as gut microbiota imbalance and gastrointestinal symptoms, the pattern of altered gut microbiota composition in COVID-19 patients, and crosstalk between the microbiome and the gut-lung axis/gut-brain-lung axis. Moreover, we have illustrated the hypoxia state in COVID-19 associated gut microbiota alteration. The role of ACE2 in the digestive system, and control of its expression using the gut microbiota is discussed, highlighting the interactions between the lungs, the gut, and the brain during COVID-19 infection. Similarly, we address the gut microbiota in elderly or co-morbid patients as well as gut microbiota dysbiosis of in severe COVID-19. Several clinical trials to understand the role of probiotics in COVID-19 patients are listed in this review. Augmented inflammation is one of the major driving forces for COVID-19 symptoms and gut microbiome disruption and is associated with disease severity. However, understanding the role of the gut microbiota in immune modulation during SARS-CoV-2 infection may help improve therapeutic strategies for COVID-19 treatment.

**Key Words:** COVID-19; Inflammation; Gut microbiota; Therapeutic

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**Core Tip:** The gut microbiota of coronavirus disease 2019 (COVID-19) patients is altered compared to that of healthy individuals, with a reduction in the count of beneficial bacteria and an increase in the count of opportunistic fungi. In this review, we elucidate the components governing immune modulation. Additionally, we explore the effect of changes in the microbial ecosystem in COVID-19 patients, with an aim to help develop precise therapeutics and expand our knowledge regarding the pattern of changes in the gut microbiota of COVID-19 patients.

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## INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has stimulated research on several medical conditions and on individual patient variations during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection to unfold underlying disease mechanisms. Scientists have determined the inflammatory response and cellular injury mediated by acute SARS-CoV-2 infection. Moreover, several studies have revealed the involvement of the gastrointestinal (GI) tract and its associated gut microbiome during COVID-19, motivating research in this field. Increasing evidence has surfaced confirming the association of the GI tract and COVID-19, including[1,2] a severe state of gut dysbiosis in COVID-19 patients[3,4]. Similarly, GI symptoms such as vomiting, abdominal pain, and diarrhea have been noted in many COVID-19 patients[5-7]. Moreover, high expression of ACE2 receptor was reported in epithelial cells of the GI tract[8]. SARS-CoV-2 RNA has been identified in rectal and anal swabs, as well as stool specimens[7,9,10]. Finally, liver damage, loss of appetite, and irritable inflammatory diseases have been reported as post-COVID-19 illnesses[11]. These all data strongly indicate a correlation between the GI including the gut microbiome, and COVID-19.

The gut microbiota plays an important role in controlling gut health and acts as a health modulator (Figure 1)[12] aidings in different metabolic activities and extensively impacting health and disease[13,14]. Ongoing research aims to better understand the gut microbiota and provide insights into the mechanistic conditions required to implement normal health functions. The gut microbiota controls specific functions in the host, such as drug and xenobiotic metabolism and nutrient metabolism[15]. Simultaneously, it helps maintain the structural integrity of the gut mucosal barrier, protects against pathogens, and regulates immunomodulation, as well as health and disease conditions[16,17]. Several other studies suggest a possible link between COVID-19 and gut microbiota composition[18,19]. Additionally, an association has been shown between altered gut microbial composition and increased risk factors in COVID-19 patients (Figure 1)[20,21].

Inflammation is a major risk factor in COVID-19 patients[22-24]. During uncontrolled inflammation, abnormal levels of cytokines such as interleukin-1 beta (IL-1β), IL-6, IL-8, IL-10, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) are found in the patients[23,25-27]. Certain abnormal levels of cytokines are substantial related to the severity of COVID-19 and are probably responsible for the "cytokine storm" syndrome manifested during the disease[28-30]. Research has correlated the inflammation during COVID-19 with GI and hepatic manifestations of the disease[31].

Interactions between the gut microbiota and the lungs, known as the gut-lung axis, have sparked interest for gastroenterology studies focusing on COVID-19 as these interactions affect disease severity. Changes in the gut microbiome certainly affect homeostasis and may lead to increased infections[32,33]. Similarly, in addition to the gut, COVID-19 can also have a detrimental effect on the central nervous system (CNS) and the blood-brain barrier (BBB) and disrupt the gut-brain-lung axis. Studies have explored therapeutic options (nicotinic cholinergic agonists and vagus nerve stimulation) to minimize the damage caused to this axis[34].

Research is necessary to understand how the gut microbiome is altered during COVID-19 infection and the factors that influence the microbiome during mild to moderate and severe disease. Studies have been conducted to understand the GI symptoms during COVID-19 and to detect viral shedding using the fecal matter of SARS-CoV-2 patients. The gut microbiota of COVID-19 patients has been mapped to obtain evidence regarding inflammation, disease severity, and therapeutic development.

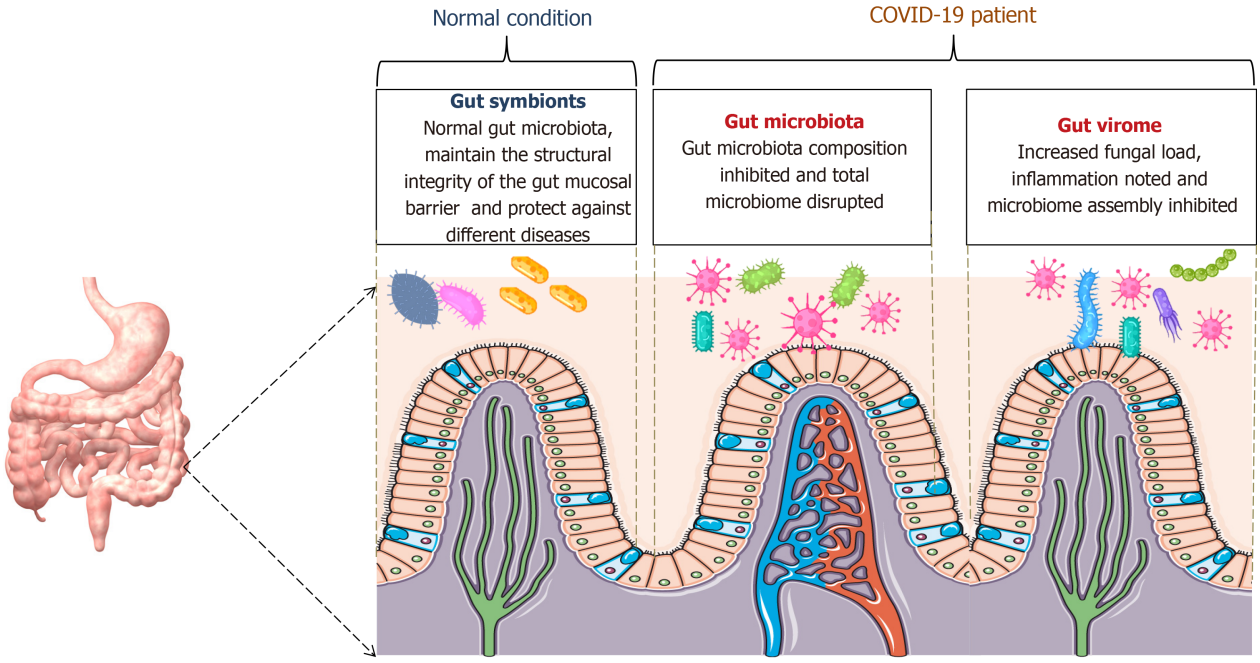


**Figure 1 The schematic diagram shows normal healthy gut and the incidence in gut microbiota and gut virome in coronavirus disease 2019 patients.** COVID-19: Coronavirus disease 2019.

Using these studies, we explore the following critical factors: (1) The gut microbiota imbalance and GI symptoms in COVID-19 patients; (2) fecal viral shedding in COVID-19 patients and restoration of the gut microbiota; (3) the pattern of altered gut microbiota composition in COVID-19 patients; (4) alterations in gut biosynthesis during COVID-19 infection; (5) the role of ACE2 in the digestive system and the gut microbiome; (6) crosstalk between the microbiome and the gut-lung axis during COVID-19 infection; (7) crosstalk between the microbiome and the gut-brain-lung axis during COVID-19 infection; and (8) hypoxia during COVID-19 associated with altered gut microbiota. We also discuss how immune responses and inflammation due to COVID-19 drive the changes in the microbial ecosystem and summarize therapeutic options currently in development.

## GI SYMPTOMS IN COVID-19 PATIENTS

Along with respiratory symptoms and fever, GI symptoms have also been observed in COVID-19 patients (Table 1). A study by Redd *et al*[35] reported abdominal pain (14.5%), nausea (26.4%), diarrhea (33.7%), and vomiting (15.4%) in patients from the United States. Three hundred and eighteen hospitalized COVID-19 patients were evaluated to understand their symptoms. In another study with 204 COVID-19 patients, 50.5% (103 patients) exhibited GI symptoms. Among these 103 patients, 78.6% showed a lack of appetite, 34% had diarrhea, 3.9% vomited, and 1.9% complained of abdominal pain. The authors correlated patients describing GI symptoms with other measurements such as prothrombin time, monocyte count, and liver enzyme levels. Patients with GI symptoms had elevated mean liver enzyme levels, extended prothrombin times, and lower monocyte counts[36]. In a much larger cohort study involving 1099 COVID-19 patients from 552 different hospitals spread to over 30 provinces, only 3.8% of patients experienced diarrhea. The authors concluded that fever and cough are common symptoms, unlike diarrhea, among the COVID-19 patient population[37].

These findings suggest that the virus might be present for a period in the GI tract, which may cause a GI infection (Figure 2). Importantly, fecal viral shedding was noted after clearing SARS-CoV-2 from the respiratory tract, suggesting that the virus can persist for a long time in the GI tract, especially in patients who manifest GI symptoms. During COVID-19 infection, gut microbiota composition is altered, possibly explaining the GI imbalance and manifestations of the different GI symptoms such as abdominal pain, nausea, vomiting, and diarrhea, as described above. This change in the gut microbiota includes reduced levels of commensals microbes and is observed in patient samples even after 30 d of disease remission[38-40]. Additional studies addressed the imbalance of the gut microbiota and its association with different GI-related aspects of COVID-19[41]. The gut microbiota population in COVID-19 patients with low to moderate GI symptoms should also be analyzed. Evaluating these diverse patient populations will enable a thorough description of this phenomenon.

**Table 1 Different gastrointestinal symptoms in coronavirus disease 2019 patients**

| Sl. No. | Total number of human subjects involved in study | Demographics of the study populations | Vomiting | Diarrhea | Nausea | Remarks/study summary | Ref. |
|---|---|---|---|---|---|---|---|
| 1 | 191 | Adults (46-67 years) hospitalised, Chinese peoples, 91 patients having comorbidity | 7 (3.7%) | 9 (4.7%) | 7 (3.7%) | Identification of several risk factors and a detailed clinical course of illness for mortality of COVID-19 patients | [121] |
| 2 | 171 | Minor aged (1 d-15 years, hospitalised, Chinese children, no such comorbidity | 11 (6.4%) | 15 (8.8%) | NA | Report of a spectrum of illness from children infected with SARS-CoV-2 virus | [122] |
| 3 | 1099 | Median age group (35-58 years), hospitalised, Chinese patients without any comorbidity | 55 (5.0%) | 42 (3.8%) | 55 (5.0%) | Identification and definition of clinical characteristics and disease severity of hospitalized COVID-19 patients | [37] |
| 4 | 140 | Adults (25-87 year), hospitalised Chinese patients with high comorbidity | 7 (5.0%) | 18 (12.9%) | 24 (17.3%) | Report on hospitalized patients having COVID-19 with abnormal clinical manifestations (fever, fatigue, gastrointestinal symptoms, allergy) | [123] |
| 5 | 73 | Adults hospitalised Chinese patients, comorbidity reported | NA | 26 (35.6%) | NA | Clinical significance of SARS-CoV-2 by examining viral RNA in feces of COVID-19 patients during hospitalizations | [124] |
| 6 | 52 | Adults (mean age 59.7 year), critically ill ICU- admitted Chinese patients, comorbidity reported | 2 (3.8%) | NA | NA | Retrospective, single-centered, observational study on critically ill, ICU-admitted adult COVID-19 patients | [125] |
| 7 | 138 | Adult (median age 56 years), hospitalised Chinese patients with comorbidities | 5 (3.6%) | 14 (10.1%) | 14 (10.1%) | Clinical characteristics of COVID-19 patients in hospitalized conditions | [126] |
| 8 | 41 | Middle age group (41-58 years) hospitalised Chinese patients with comorbidities | NA | 1 (2.6%) | NA | Epidemiological, laboratory, clinical, and radiological features and treatment with clinical outcomes of hospitalized COVID-19 patients | [46] |
| 9 | 62 | Studied patients (median age 41 years) were hospitalised, Chinese ethnicity and comorbidity reported | NA | 3 (4.8%) | NA | Most common symptoms at onset of illness with clinical data in confirmed COVID-19 patients | [127] |
| 10 | 137 | Studied patients ( mean age 57-55) ware Chinese and hospitalised, comorbidity was also noted | NA | 11 (8%) | NA | Investigation of epidemiological history, clinical characteristics, treatment, and prognosis of COVID-19 patients | [128] |
| 11 | 81 | Chinese patients (mean age was 49.5 years), hospitalised with high comorbidities | 4 (4.9%) | 3 (3.7%) | NA | Report of confirmed COVID-19 patients with chest computer tomography imaging anomalies | [129] |
| 12 | 99 | Hospitalised, Chinese patients (average age of the patients was 55.5 years), comorbidity was reported | 1 (1%) | 2 (2.0%) | 1 (1%) | Inclusive exploration of epidemiology and clinical features of COVID-19 patients | [130] |

NA: Not available; ICU: Intensive care unit; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

## FECAL VIRAL SHEDDING IN COVID-19 PATIENTS

Table 2 lists various cohort studies reporting fecal viral shedding by COVID-19 patients and detecting SARS-CoV-2 RNA in the fecal matter[42,43]. SARS-CoV-2 RNA-positive fecal matter was detected in 66.67% of COVID-19 patients (42 patients) in China[43]. Researchers attempted to evaluate the viral shedding period in stool samples, and noted viral shedding in asymptomatic patients. For example, SARS-CoV-2 RNA was detected from a stool sample of an asymptomatic child 17 d after viral exposure [9].

Certain studies have reported that virus separation from stool samples is difficult. For example, Wölfel *et al*[44] detected viral RNA in stool samples but attempts to isolate the virus were unsuccessful, most likely due to the mild nature of the infection. A viral load below $10^6$ copies per milliliter often hampers viral isolation[36]. The viral load also varies widely from one sample to another, including stool, serum, and respiratory samples[44-46]. However, understanding the correlation between the altered gut microbiota and the viral load in patient samples is essential for advancing therapeutic strategies centered around restoring the microbiota.

Additionally, efforts should focus on determining the possible correlation between fecal viral shedding and altered gut microbiota composition at different stages the infection, *i.e.*, mild to moderate

**Table 2 Fecal viral shedding in coronavirus disease 2019 patients**

| Sl. No. | Total number of human subjects in study | Demographics of the study populations | Gastrointestinal symptoms | Confirmed cases of fecal shedding | Remarks/study summary | Ref. |
|---|---|---|---|---|---|---|
| 1 | 205 | Patients (mean age of 44 years) were hospitalised, Chinese without any comorbidities | No symptoms | 44 | Evidence-based study for gastrointestinal infection of SARS-CoV-2 virus and its possible fecal-oral transmission route in humans | [131] |
| 2 | 73 | Different age group (10 mo to 78 years old), hospitalised Chinese patients without report any comorbidities | Gastrointestinal bleeding, diarrhea | 39 | Description of epidemiological and clinical characteristics of COVID-19 patients | [124] |
| 3 | 10 | Chinese patients have aged 19-40 years, hospitalised and no such comorbidity was reported | Hemoptysis, diarrhea, cough | 8 | Report of median aged COVID-19 confirmed patients in ICU | [127] |
| 4 | 14 | Patients (18-87 years) were hospitalized, Chinese individuals without any comorbidities | No symptoms | 5 | Retrospective analysis of laboratory-confirmed COVID-19 cases in hospitalized conditions | [132] |
| 5 | 66 | Chinese patients (median age of 44 years) were hospitalised, comorbidity was not reported | No symptoms | 11 | Viral RNA detection was performed from throat swabs, stool, urine, and serum samples in different clinical conditions in COVID-19 patients | [133] |
| 6 | 18 | Adults patients (median age, 47 years) from Singapore were hospitalised and comorbidities was noted | No symptoms | 4 | COVID-19 patient case series using clinical, laboratory, and radiological data | [134] |
| 7 | 74 | Studied paients belonged from China and were hospitalised with comorbidities | No symptoms | 41 | Analysis of respiratory and fecal samples to determine clinical symptoms and medical treatments of COVID-19 patients | [135] |
| 8 | 9 | Adults Chinese patients were hospitalised without any comorbidities | Diarrhea and urinary irritation | 2 | Detection of SARS-CoV-2 RNA in urine and blood samples, and anal, oropharyngeal swabs of confirmed COVID-19 patients | [136] |

ICU: Intensive care unit; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

or severe COVID-19.

## ALTERED GUT MICROBIOTA COMPOSITION IN COVID-19 PATIENTS

SARS-CoV-2 infections have led to changes in the ecology of the gut microbiota in patients (compared to that seen in controls). These changes are influenced by the immune responses elicited during COVID-19 (Table 3). Different studies have revealed the growth of unusual microorganisms and depletion of common gut microbes (bacterial, viral, and fungal populations) in COVID-19 patients (Figure 3).

To understand the severity of disease in COVID-19 patients, the gut microbiota composition of 100 COVID-19 patients was analyzed in two hospital cohorts. Stool samples were collected from 27 of the 100 patients. The gut microbiome compositions were characterized using total DNA extracted from stool samples. The authors demonstrated that the number of gut commensals and Bifidobacteria was low and correlated with several factors of disease severity, such as high concentrations of inflammatory cytokines and C-reactive protein (CRP). These data suggests that the composition of the microbiota is associated with disease severity[38]. Another study carried out RNA and DNA profiling by sequencing of the virome using fecal matter from COVID-19 patients. The fecal matter of 98 COVID-19 patients was analyzed to understand COVID-19 severity and its association with the gut virome. The study showed that COVID-19 severity is inversely correlated with gut viruses, and older patients are more prone to severe COVID-19 outcomes[47]. Alterations in fungal microbiomes during COVID-19 have also been investigated. Analysis of the fecal mycobiome using the deep shotgun method showed heterogeneous microbial profiles, with enrichment of fungal genera such as Aspergillus and Candida. Two species of *Aspergillus* (*Aspergillus flavus* and *Aspergillus niger*) were identified in fecal samples after clearance of SARS-CoV-2 from nasopharyngeal samples[48]. Additionally, there is evidence of abundant symbionts among COVID-19 patients including *Clostridium ramosum*, *Coprobacillus*, and *Clostridium hathewayi*,

**Table 3 Analysis of gut microbiota in coronavirus disease 2019 patients in different cohorts**

| Sl. No. | Cohort composition | No of Patients | Demographics of the study populations | Country | Significant gut microbiota found | Study conclusion | Reference |
|---|---|---|---|---|---|---|---|
| 1 | A pilot study with 15 healthy individuals (controls) and 15 patients with COVID-19 | 15 | Study performed with hospitalised patients (median age 55 years), Chinese ethnicity and comorbidities were reported | Hong Kong | Abundance of *Clostridium hathewayi, Clostridium ramosum, Coprobacillus,* which are correlated with COVID-19 severity | Change in the fecal microbiome of COVID-19 patients during hospitalization, compared to healthy individuals (controls) | [48] |
| 2 | The two-hospitals cohort, serial stool samples collected from 27 COVID-19 patients among 100 | 27 | Adults hospitalised Chinese patients, comorbidities were noted | Hong Kong | *Faecalibacterium prausnitzii, Eubacteriumrectale* and bifidobacteria | Gut microbiome involved in COVID-19 severity | [38] |
| 3 | United States cohort (majority African American) | 50 | Studied patients (mean age 62.3 years) were hospitalised with comorbidities, American ethnicity | United States | Some of the significant genera (*Corynebacterium Peptoniphilus, Campylobacter, etc.*) | No significant associations found between the composition microbiome and disease severity from COVID-19 patient gut microbiota | [50] |
| 4 | The study used 53 COVID-19 patients and 76 healthy individuals. 81 fecal samples collected during hospitalization | 53 | Adults Chinase hospitalised patients, no such comorbidities were noted | China | Elevated gut microbes such as *Rothia mucilaginosa, Granulicatella* spp, *etc.* | COVID-19 infection linked with change of the microbiome in COVID-19 patients | [137] |
| 5 | 15 patients Cohort | 15 | Study performed adults hospitalised patients with comorbidities, Chinese ethnicity | Hong Kong | Elevated bacterial species *Collinsella tanakaei, Collinsella aerofaciens, Morganella morganii, Streptococcus infantis* | The study found fecal viral (SARS-CoV-2) activity | [54] |
| 6 | Two-hospital cohort with a total of 100 patients. Stool samples collected from 27 patients | 27 | Hospitalised adults patients were from China, comorbidities were noted | Hong Kong | Several gut microbiota such as *Faecalibacterium prausnitzii, Eubacterium rectale,* and bifidobacteria | Gut microbiota associated disease severity and inflammation in COVID-19 patients | [38] |
| 7 | 98 COVID-19 patients (3 asymptomatic, 34 moderate, 53 mild, 3 critical, 5 severe), serial fecal samples collected from 37 COVID-19 patients | 37 | Adults (mean age 37 years) patients, hospitalised condition from Chinese ethnicity, comorbidities were reported | Hong Kong | A total of 10 virus species in fecal matter (9 DNA virus species and 1 RNA virus, pepper chlorotic spot virus) | Analysis of gut virome (RNA and DNA virome) in COVID-19 patients | [47] |
| 8 | Study of fecal samples from 30 COVID-19 patients | 30 | Patients (mean age 46 years) were hospitalised from Chinese groups, comorbidities were noted | Hong Kong | Increased proportions of fungal pathogens ( *Candida albicans, Candida auris, Aspergillus flavus, Aspergillus niger*) in fecal samples | Analysis of fecal fungal microbiome of COVID-19 patients | [48] |

COVID-19: Coronavirus disease 2019.

which directly correlated with disease severity. Conversely, *Faecalibacterium prausnitzii,* which was also abundant among the patients, was inversely correlated with disease severity[49].

Similarly, in a study by Yeoh *et al*[38], stool samples from 27 patients were correlated with blood markers and inflammatory cytokines. The study concluded that the scale of COVID-19 severity might be associated with the gut microbiome and linked it to COVID-19 inflammation[46]. In another study containing a greater number of African Americans, enriched genera (*Campylobacter, Corynebacterium,* and *Peptoniphilus)* were mapped in the COVID-19 patient population, the gut microbial composition was markedly different between positive and negative samples. However, the study did not identify any considerable association between COVID-19 severity and microbiome composition[50].

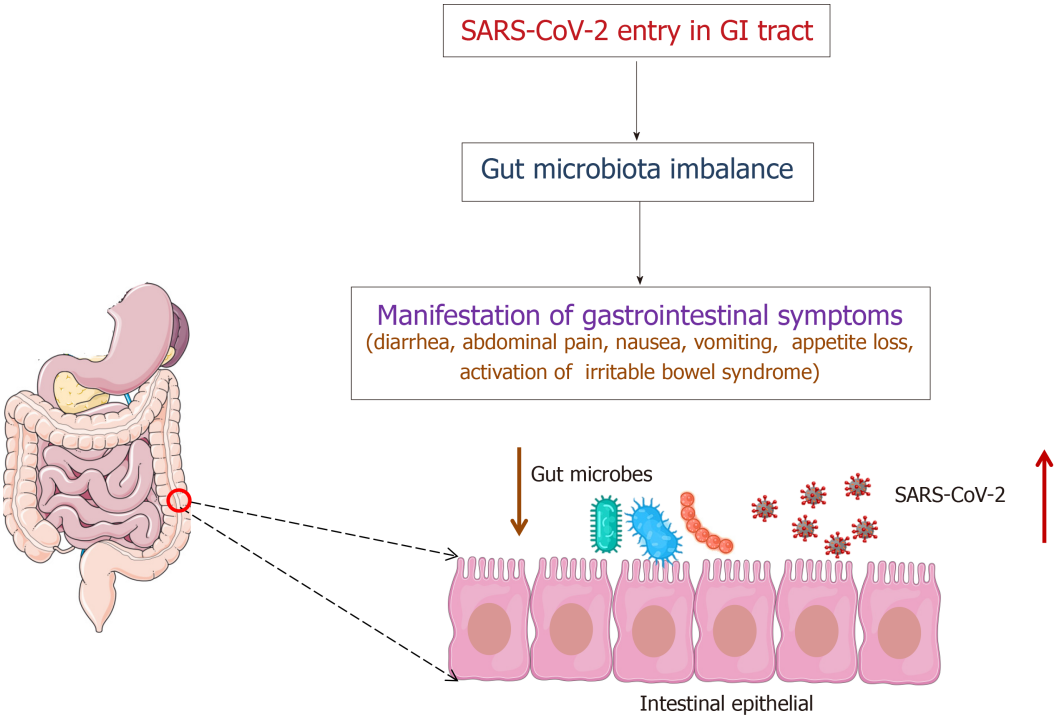


**Figure 2 The schematic diagram illustrates the severe acute respiratory syndrome coronavirus 2 entry in the body, causes of gut microbiota imbalance which assists in manifesting the gastrointestinal symptoms in coronavirus disease 2019 patients.** GI: Gastrointestinal; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

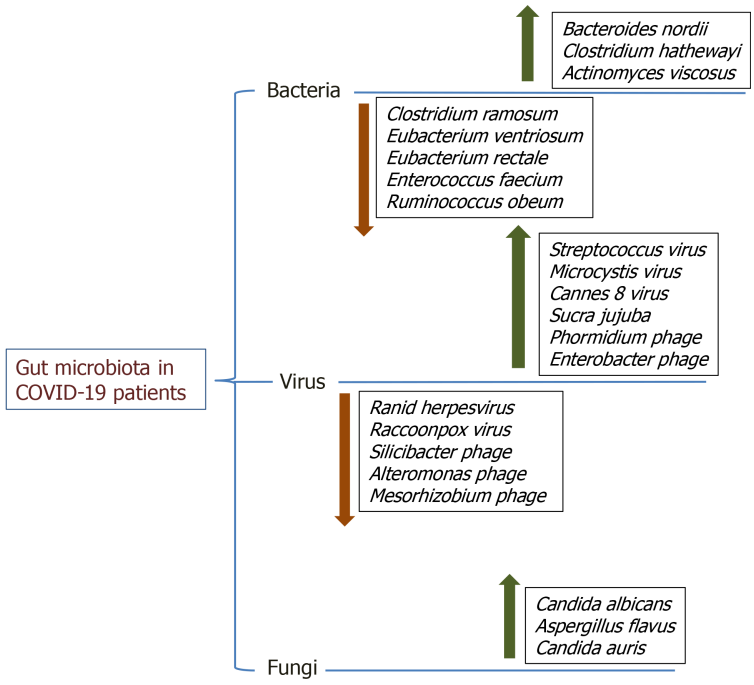


**Figure 3 The diagram illustrates increased or decreased gut microbiota in coronavirus disease 2019 patients, including bacterial, viral, and fungal populations.** COVID-19: Coronavirus disease 2019.

Certain studies even noted a reduction in fiber-utilizing bacteria such as *Prevotella*, *Bacteroides plebius*, and *Faecalibacterium prausnitzii* (*F. prausnitzii*), and a low Firmicute/Bacteroidetes ratio[51]. Poor outcomes were noted in special populations, such as hypertensive, diabetic, and elderly patients[52,53]. Research is still underway to ascertain the different types of gut microbial populations (pro-inflammatory, opportunistic, beneficial, or anti-inflammatory) present depending on COVID-19 severity (Figure 4).

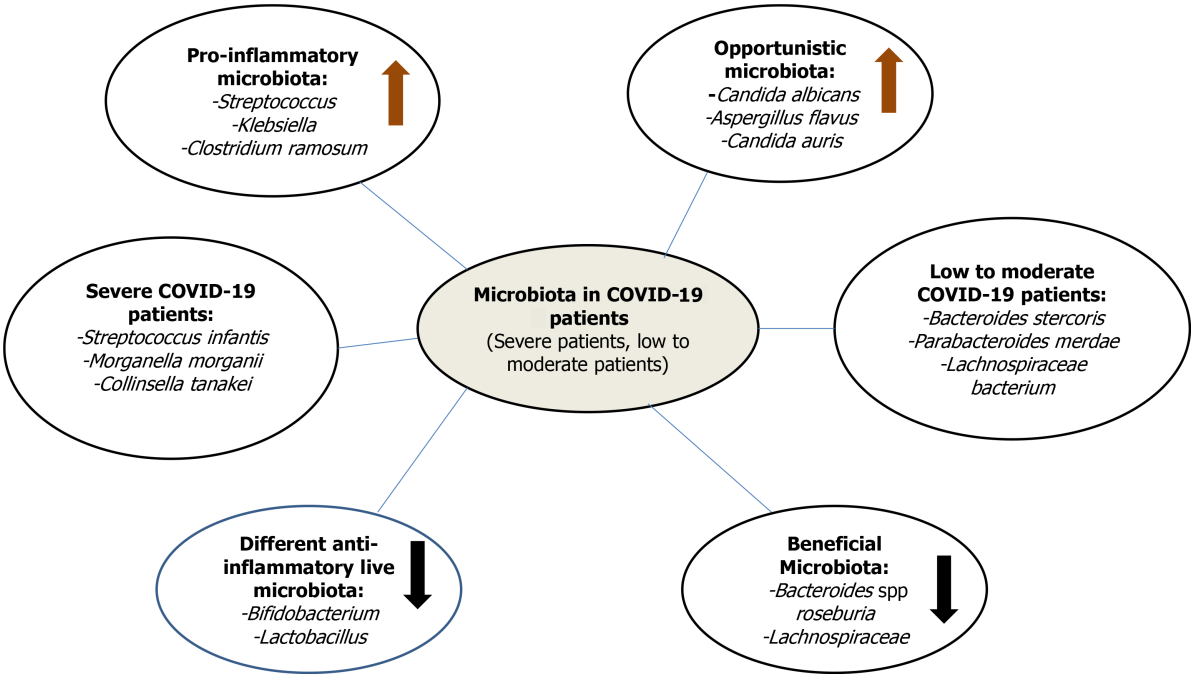


**Figure 4 The diagram illustrates different types of mapped gut microbiota in coronavirus disease 2019 patients.** Pro-inflammatory microbiota, opportunistic microbiota, the microbiome in severe coronavirus disease 2019 (COVID-19) patients, and the microbiome in low to moderate COVID-19 patients, anti-inflammatory microbiota, and beneficial microbiota. COVID-19: Coronavirus disease 2019.

These studies help us understand how gut microbiota composition affects patients with moderate to severe COVID-19 and how gut microbiota diversity might alter immunity in COVID-19 patients.

## ALTERATIONS IN THE BIOSYNTHESIS OF BIOLOGICAL COMPOUNDS IN THE GUT DURING COVID-19 INFECTION

Other than compositional changes in gut microbiota, functional changes during SARS-CoV-2 infection were observed in some patients. The gut microbiota aids in different biosynthetic pathways, such as amino acid biosynthesis, carbohydrate metabolism, nucleotide de novo biosynthesis, and glycolysis. This might be due to the abundance of bacterial components such as *Collinsella tanakaei, Streptococcus infantis, Morganella morganii,* and *Collinsella aerofaciens*, *etc.* Apart from these microbes, many short-chain fatty acid (SCFA) synthesis bacteria, such as *Lachnospiraceae bacteria*, *Bacteroides stercoris*, *Alistipes onderdonkii,* and *Parabacteroides merdae* were present in COVID-19 samples with mild symptoms and in non-COVID-19 samples[54]. In a study using non-human primate models, β diversity analysis and 16S rRNA gene profiling were carried out to understand the gut microbiota composition during SARS-CoV-2 infection. The study revealed substantial changes in the gut microbiota composition and metabolism and a reduction in the concentration of SCFAs as well as a difference in the concentrations of bile acids. The study also found alterations in tryptophan metabolites during SARS-CoV-2 infection in the animal models[55].

Shotgun metagenomic sequencing using fecal samples has also been performed to profile the gut microbiome in SARS-CoV-2 infected patients. Researchers observed prolonged impairment of L-isoleucine biosynthesis and SCFAs due to alterations in the gut microbiome of patients with COVID-19 [56].

## ROLE OF ACE2 IN THE DIGESTIVE SYSTEM AND THE GUT MICROBIOME

The ACE2 (angiotensin-converting enzyme 2) receptor acts as a binding site by which SARS-CoV-2 enters host cells[57,58]. A higher expression of ACE2 in the cell favors SARS-CoV-2 infection. Despite this, ACE2 deficiency can play a vital role in SARS-CoV-2 infection[59]. Increased ACE2 expression is found in the epithelial cells of the respiratory tract (nasal mucosa, nasopharynx, and lungs), in different parts of the intestine, and in different types of epithelial cells, including nasal, corneal, and intestinal epithelial cells in humans[60]. In addition, this protein is expressed in different parts of the digestive system, such as the small intestine, stomach, colon, and liver[61]. However, ACE2 expression is

controlled by distinct microbial communities in several body tissues. Mouse model studies suggest an association between certain microbial communities and overexpression of ACE2. This overexpression may prevent detrimental changes in hypoxia-induced gut pathophysiology and pulmonary pathophysiology[62]. ACE2 expression is controlled in the GI and respiratory tract[63]. Additionally, it can also be controlled by some bacterial species from important phyla. Downregulation of ACE2 expression was associated with the Bacteroidetes phylum. Among all species of this phylum, *Bacteroides dorei* has been shown to inhibit ACE2 expression in the colon, whereas the Firmicutes phylum plays a variable role in its modulation[20,49,64]. These findings are supported by other studies describing the modulation of ACE2 expression in the gut by the microbiota[65,66].

## GUT-LUNG AXIS CROSSTALK DURING COVID-19 INFECTION

Several reports indicate that manipulation of the gut microbiota may be used to treat pulmonary diseases[67]. Therefore, the gut-lung axis crosstalk can help to elucidate these respiratory and digestive system interactions (Figure 5). Dysbiosis occurs when there are detrimental changes in the microbial composition of the gut or respiratory tract. It often leads to altered immune responses and the development of diseases, such as COVID-19. Nonetheless, of gut dysbiosis can be manipulated for treatment purposes[32,67-69]. Studies suggest that SARS-CoV-2 from the lungs travels to the gut *via* the lymphatic system leading to disrupted gut permeability[70,71]. Furthermore, the extent of dysbiosis is associated with COVID-19 severity[4,72]. Therefore, understanding the crosstalk between the microbiome and the gut-lung axis during COVID-19 infection may provide therapeutic approaches.

## GUT-BRAIN-LUNG AXIS CROSSTALK DURING COVID-19 INFECTION

Like the gut-lung axis, crosstalk between the microbiome and the gut-brain axis has been recognized and remains the topic[73-75]. Several studies have illustrated the role of the microbiome-gut-brain axis in different neurological disorders[76,77].

The interaction between the brain and the gut (also called the gut-brain axis) is bidirectional, with several pathways involved, including bacterial metabolites, neuroanatomical communications, neurotransmitters, and hormones[78]. The vagus nerve is primarily involved in such communication, and these molecules (neurotransmitters/hormones) are produced in the GI tract. During communication between neurotransmitters and hormones, they might interact with the receptors on the vagus nerve, relaying information to the brain[78-81]. Many hormones can cross the BBB and affect the CNS directly. Additionally, neuroendocrine pathways which operate *via* the hypothalamic-pituitary-adrenal (HPA) axis associated with stress also affect the BBB. The stress-HPA axis is associated with the release of glucocorticoids such as cortisol from the adrenal cortex. Cortisol, is associated with augmented intestinal permeability and GI motility, affecting the gut microbiota[78,82-84]. The stress-HPA axis may also lead to inflammation and bacteria-derived impaired metabolite production, especially SCFAs[78,84]. Therefore, a thorough understanding of the gut-brain axis can help the development of therapeutic approaches *via* modulation of the gut microbial composition.

The gut microbiota might play a distinct role in controlling the host immune system, and research is underway to uncover more in this field[85,86]. The involvement of the lungs (gut-brain-lung axis) occurs when inflammation and neurodegeneration in the brain stem due to COVID-19 prevent cranial nerve signaling, disrupting anti-inflammatory pathways and normal respiratory and GI functions. Recently, the lungs have been associated in the crosstalk among the microbiota-gut-brain axis components, and this axis was also noted during COVID-19 (Figure 6)[34,78]. Moreover, in COVID-19 patients, alterations in the gut microbiota have been shown to reduce live microbes (Bifidobacterium and Lactobacillus) during intestinal microbial dysbiosis[87].

The microbial translocation to the gut and its subsequent damage may play a vital role in inferior clinical outcomes for the disease. The gut-brain-lung axis during COVID-19 infection can also offer clues indicate viable directions for therapeutic development[34].

## HYPOXIA IN COVID-19 AND GUT MICROBIOTA

Abnormal cytokine release (cytokine storms) and inflammatory responses may be associated with hypoxia during severe COVID-19. Viral replication in the lungs leads to a cytokine storm, destroying normal lung function and causing hypoxemia, *i.e.*, low oxygen levels in the blood. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that regulates cellular functions such as cell proliferation and angiogenesis. In hypoxic conditions, HIF-1α binds to the hypoxemic response element and induces the production of cytokines such as IL-6 and TNF-α, leading to hypoxia[88]. There are other collective causes of hypoxia, including pulmonary infiltration and thrombosis. The COVID-19 virus induces

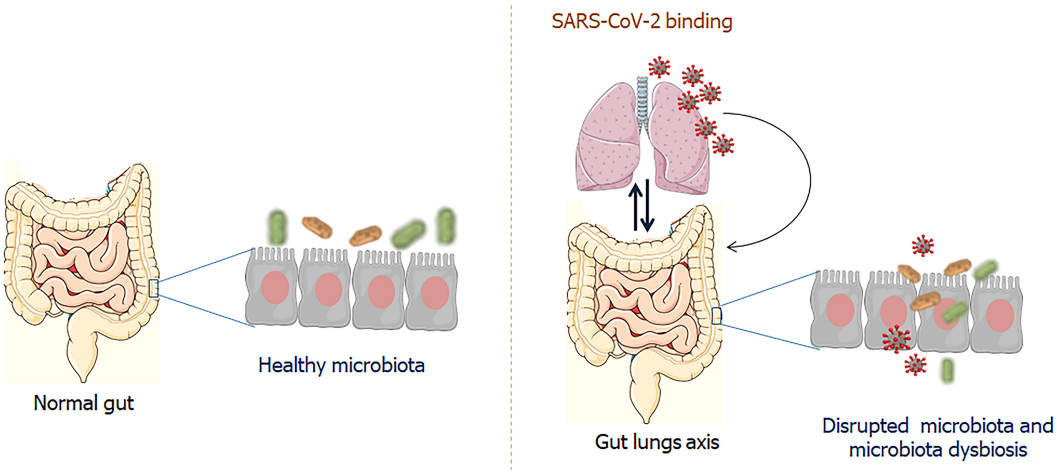


**Figure 5 The diagram points out the normal gut and its microbial association. The figure also illustrates the crosstalk between the microbiome and gut-lung axis.** SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

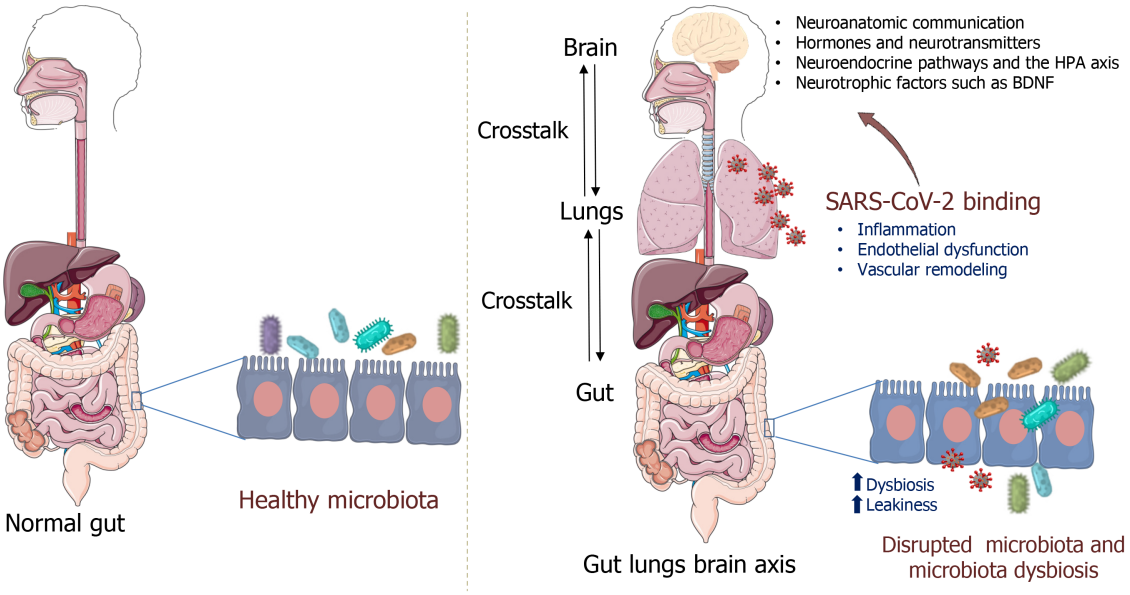


**Figure 6 The diagram describes the normal gut and its microbial association. The figure also illustrates the crosstalk between the microbiome and gut-brain-lung axis.** BDNF: Brain-derived neurotrophic factor; HPA: Hypothalamic-pituitary-adrenal; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

pneumonia that causes atelectasis (collapsing of air sacs), leading to low oxygen levels in the body[89]. Additionally, COVID-19 leads to mitochondrial damage, production of reactive oxygen species, and subsequently HIF-1α, further promoting viral infections and inflammation[90].

As part of its normal metabolic functions, the gut microbiota produces neurotropic metabolites, neurotransmitters, peptides, and SCFA, whose levels are disrupted due to COVID-19. SCFA such as butyrate confer neuroprotection. Modulation of gut microbes (responsible for such metabolite production) by SARS-CoV-2 alters hypoxia-sensing, negatively impacting the CNS[91]. Therefore, an association between gut microbiota and hypoxia in COVID-19 patients can be speculated, and may be linked to the CNS (Figure 7).

## ALTERATION OF GUT MICROBIOTA IN COVID-19: EVIDENCE FOR INFLAMMATION OR DIEASE, SEVERITY?

Under normal conditions, colonization of the normal microbiota in the gut causes resistance to pathogen [92,93]. Much of the normal gut microbiota belongs to Clostridia., which produces butyric acid. This SCFA is produced during dietary fiber fermentation along with acetic acid and propionic acid, which

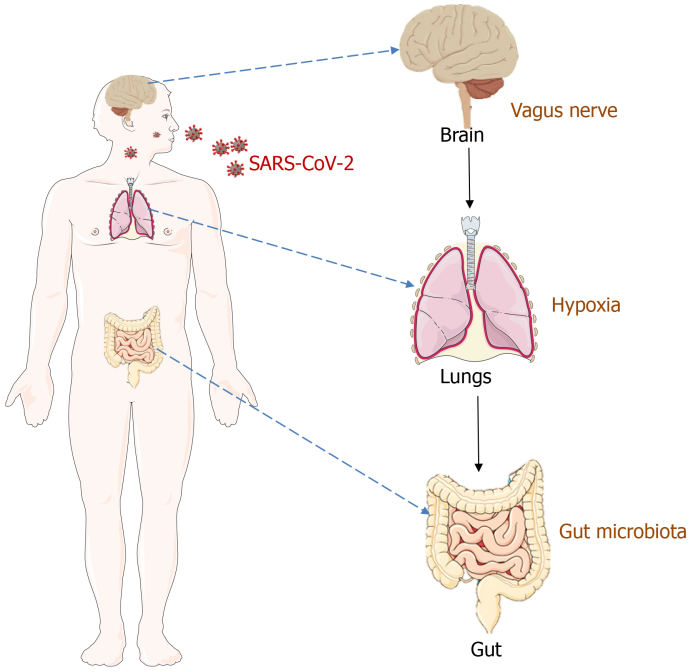


**Figure 7 The figure illustrates an association between gut microbiota and hypoxia in coronavirus disease 2019 patients, and it is connected with central nervous system.** SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

play a critical role in gut health (Figure 8A)[94,95]. Butyric acid helps in maintain the integrity of the gut barrier by providing a vital energy resource for colonocytes. This SCFA also hinders histone deacetylase activity and inhibits the activation of the nuclear factor (NF)-κB signaling pathway activation. This phenomenon may activate the G protein-coupled receptor pair (GPR41 /GPR43). These events help exert an anti-inflammatory response in normal gut health and stimulate regulatory T cells (Treg cells) [96-100]. Treg cells play a central role in suppressing inflammatory responses[97,101]. However, in COVID-19 patients, typical microbiota dysbiosis causes an imbalance in all these events.

There is a distinct connection between dysbiosis of the gut microbiota and hyper-inflammatory responses, especially cytokine release, in some COVID-19 patients[102] (Figure 8). Researchers noted that gut microbiota composition is related to the COVID-19 severity of and observed an association between altered cytokine levels and gut microbiota composition[38]. Cytokines/inflammatory factors, such as IL-1β, IL-6, and TNF-α, are usually associated with inflammation during disease[103]. In the case of severe COVID-19, the levels of certain cytokines, such as IL-6, IL-10, TNF-α, and IFN-are raised abnormally, and in some cases, cytokine storms are observed (Figure 8B)[23]. In pilot study, the quality of gut microbial composition was associated with the severity COVID-19 in 15 patients at the time of hospitalization in Hong Kong. The study showed an abundance of microbes such as *Clostridium hathewayi*, *Clostridium ramosum*, and *Coprobacillus* in COVID-19 patients. Moreover, an anti-inflammatory bacteria, *Faecalibacterium prausnitzii*, was be inversely correlated with disease severity[49].

Nonetheless, more detailed studies are needed to understand the impaired gut health during COVID-19, especially in extreme forms of the disease. Another study confirmed microbiota dysbiosis in COVID-19 patients. This study found differential bacterial populations with a decrease in *F. prausnitzii* and *Clostridium* spp and an association of IL-21 in mild to severe COVID-19 patients[51].

A gut microbiota richness analysis in COVID-19 patients was conducted over through a six-month evaluation using 16S rDNA sequencing. This study showed that, patients with decreased post-convalescence richness in bacterial microbiota had high disease severity with increased CRP. Additionally, the authors observed increased incidence of intensive care unit admissions with worse pulmonary functions in these patients[104]. The study suggested an association between the hyper-inflammatory response in COVID-19 and gut dysbiosis. However, a greater number of studies testing patients well after recovery are required to fully illustrate gut dysbiosis, associated factors, and the hyper-inflammatory response during COVID-19.

## GUT MICROBIOTA IN ELDERLY OR CO-MORBID COVID-19 PATIENTS

Researchers have attempted to understand the role of the gut microbiota in elderly or co-morbid COVID-19 patients. A recent study evaluated the association of the gut microbiota and its modulation in COVID-19 patients. In this study, the cohort comprised approximately 200 severe COVID-19 patients

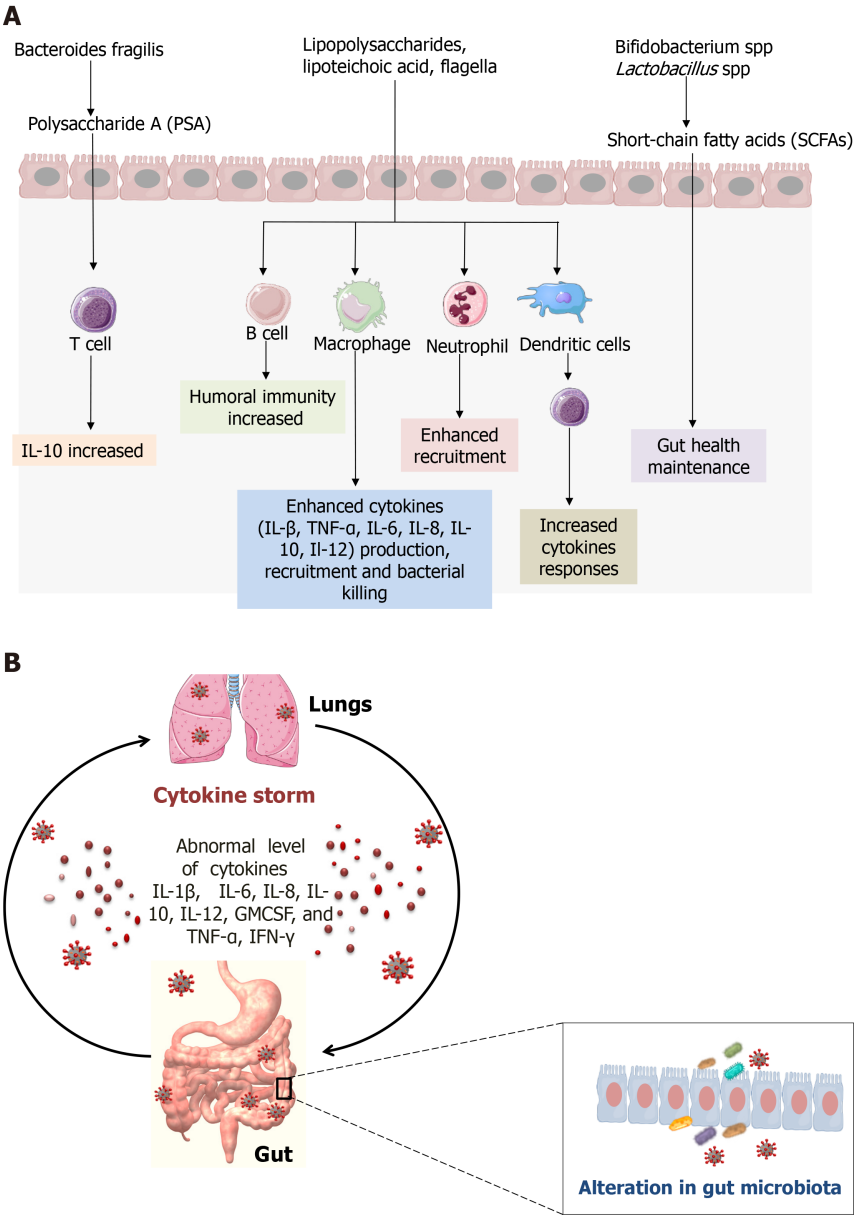


**Figure 8 The figure illustrates normal gut microbiota and immunological consequences, and coronavirus disease 2019 related altered gut microbiota associated inflammation.** A: Normal gut microbiota and immunological consequences for healthy gut; B: Coronavirus disease 2019 (COVID-19) related altered gut microbiota associated inflammation. The inflammatory condition in COVID-19 patients causes the abnormal release of different cytokines, such as interleukin-1 beta (IL-1β), IL-6, IL-8, IL-10, IL-12, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-alpha, and interferon-gamma. PSA: Polysaccharide A; SCFA: Short-chain fatty acid; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; GMCSF: Granulocyte-macrophage colony-stimulating factor; TNF-α: Tumor necrosis factor-alpha; IFN-γ: Interferon-gamma.

hospitalized with pneumonia. Researchers considered elderly patients (age 62 years to 64 years) and their comorbidity. Patients in this study received two types of treatments: one group was treated with only the best available therapy (BAT), and the other group was treated with oral bacteriotherapy and BAT. Researchers found a decline in mortality and decreased progress in severe disease. Finally, researchers concluded that oral bacteriotherapy might be helpful in the management of hospitalized COVID-19 patients[105]. Similarly, Rao *et al*[106] noted that people with the comorbidities are more prone to COVID-19-related complications. In this case, immune system deregulation and deaths were also noted. However, researchers used-glucan to enhance the immune system in COVID-19 patients. This glucan was used to augment the activity of macrophages, natural killer cells, and IL-8, implicating that it might enhance the defense mechanisms to combat the virus[106].

Recently, Liu *et al*[1] evaluated the role of the gut microbiota composition and its association with the post-acute COVID-19 syndrome (PACS). In this study, researchers considered the comorbidities and dietary patterns during patient selection compare gut microbiota compositions. However, no considerable differences were observed in age, comorbidities, gender, antibiotics, or antiviral drug use between patients with PACS or without PACS[1].

Therefore, in cases of elderly or co-morbid COVID-19 patients, the gut microbiota might play an important role in immune system deregulation, although further studies are required to validate the findings.

## GUT MICROBIOTA BASED ON ANTIBIOTIC USAGE IN COVID-19 PATIENTS

In COVID-19 patients, the use of antibiotics is relatively common. The frequently used antibiotics in COVID-19 patients are Azithromycin, Amoxicillin Clavulanate, Cephalosporin, Tetracycline[49,107], *etc*. The composition of the gut microbiota is hampered in COVID-19 patients due to the usage of antibiotics, occasionally causing antibiotic-associated diarrhea (AAD)[108]. Antibiotics usages in COVID-19 patients were increased the number of opportunistic pathogens compared with that detected in an untreated control group. Zuo *et al*[49] reported that the gut of COVID-19 patients, using antibiotics contains opportunistic bacterial pathogens such as *Bacteroides nordii*, *Actinomyces viscosus*, and *Clostridium hathewayi*. Additional studies also reported this phenomenon[22,109]. An increase of opportunistic bacterial pathogens causes dysbiosis of the gut. Rafiqul Islam *et al*[110] also noted that the abundance of opportunistic pathogens in COVID-19 patients in Bangladesh could cause dysbiosis, with 46 genera of opportunistic bacteria being identified patient GI samples. However, a study demonstrated that particular strains of probiotics may be useful for AAD[111]. Scientists have shown that the administration of oral probiotics can recover gut health and have antiviral effects[112,53]. For probiotic strain identification, Mak *et al*[113] highlight the need for effective research to easily recognize the probiotic strains of therapeutic use. In this case, the probiotics should be specific for COVID-19, and help reduce the susceptibility to COVID-19 preventing severe COVID-19 disease.

## GUT MICROBIOTA DYSBIOSIS DURING COVID-19 AND USE OF PROBIOTICS

Scientists identified an association between the gut microbiota dysbiosis and the severity of COVID-19. Magalhães *et al*[52] noted that gut microbiota dysbiosis causes poor outcomes in elderly COVID-19 patients with hypertension and diabetes. Additionally, co-morbid elderly COVID-19 patients were prone to increased inflammatory situations due to the dysbiosis. The elevated amount of bacterial products in the gut might translocate into the blood due to the increased permeability across the intestinal epithelium. Bacterial toxin products, such as lipopolysaccharides (LPS), may accumulate in blood, aggravating TLR4 and subsequent downstream signaling. This could contribute to the "cytokine storm", and result in complications in elderly COVID-19 patients[54]. Researchers also found a different route of activation of toll-like receptor (TLR4/TLR5) in COVID-19 patients[114-116]. Hung *et al*[53] also reported that gut microbiota dysbiosis increases COVID-19 severity in the elderly. However, the use of probiotics is a novel way to reduce COVID-19 severity in elderly populations.

## THERAPEUTIC IMPLICATIONS AND CLINICAL TRIALS TO UNDERSTAND THE ROLE OF THE GUT MICROBIOTA DURING COVID-19

A careful analysis of the microbiome-gut-lung axis during COVID-19 infection can direct research towards therapeutic options for restoring gut health. As an altered gut microbiota is strongly associated with COVID-19 and its severity, supplementation of bacterial metabolites or commensals and prebiotics to enrich the microbial ecosystem is a path toward effective therapeutic options.

However, very few studies have explored this. A randomized clinical trial with 300 registered participants assessed the effectiveness of combination therapy using *Lactobacillus plantarum* (*L. plantarum* ) CECT 7484, *L. plantarum* CECT 30292, *Pediococcus acidilactici* (*P. acidilactici*) CECT 7483, and *L. plantarum* CECT 7485, in adult COVID-19 patients (ClinicalTrials.gov; Clinical trial no. NCT04517422). Nonetheless, a deficiency of well-established data calls for more studies of this nature[41]. An open-label, randomized clinical trial with 350 participants conducted by Kaleido Biosciences sought to determine the effectiveness of a novel glycan molecule (KB109) in patients with mild to moderate COVID-19 (ClinicalTrials.gov; Clinical trial no. NCT04414124)[117]. The synthetic glycan molecule reduced the number of acute care visits by COVID-19 patients. Additionally, disease resolution in patients with comorbidities was improved, compared to that in patients relying solely on supportive self-care.

A similar study attempted to evaluate the glycan molecule's effectiveness (KB109) associated with gut microbiota function in COVID-19 patients. The same organization conducted the clinical study, an open-label, randomized clinical trial in 49 participants in the United States (ClinicalTrials.gov; Clinical trial no. NCT04486482)[118]. There were no conclusive results; however, more studies are likely to be conducted in this sense. A complete list of the clinical trials initiated to understand the role of the gut microbiota in COVID-19 and its therapeutic implications are shown in Table 4.

**Table 4 List of clinical trials initiated to understand the role of gut microbiota in coronavirus disease 2019 and its therapeutic implications**

| Sl. No. | Objective of clinical trials | Clinical trials No. | Description of clinical trials | Remarks |
|---|---|---|---|---|
| 1 | Evaluate the combination of probiotics (*P. acidilactici* and *L. plantarum*) to reduce the viral load of moderate or severe COVID-19 patients | NCT04517422 | It was a randomized controlled trial, 300 participants, treatment by dietary supplement (probiotics) | Observational study of adult and older adult, trial completed |
| 2 | To explore the natural history of mild-to-moderate COVID-19 illness and safety of a novel glycan (KB109) and self-supportive care | NCT04414124 | It was a randomized, prospective, open-label, parallel-group controlled clinical study of 350 participants | Observational study of adults (both male and female), trial completed |
| 3 | Investigate the physiologic effects of the novel glycan (KB109) on patients with COVID-19 illness on gut microbiota structure and function in the outpatient | NCT04486482 | It was a randomized, open-label clinical study of 49 participants | Observational study of adults patients with mild-to-moderate COVID-19 infections, trial completed |
| 4 | Evaluate the clinical contribution of the gut microbiota and its diversity on the COVID-19 disease severity and the viral load | NCT05107245 | It was case-control, diagnostic study of 143 participants | Observational study on the diagnostic evaluation of the human intestinal microbiota, trial completed |
| 5 | Studied the effects of *Lactobacillus coryniformis* K8 intake on the prevalence and severity of COVID-19 in health professional | NCT04366180 | A randomized, interventional study of 314 participants | Investigation of probiotic effects to healthcare personnel exposed to COVID-19 infection |
| 6 | Investigate to exploring the role of nutritional support by probiotics to COVID-19 outpatients (adult) | NCT04907877 | Randomized, evidence based study of 300 participants | Used of probiotics as dietary supplement that enhance specific immune response of patients having COVID-19 respiratory infection |
| 7 | Use of dietary supplement (Omni-Biotic® 10 AAD) can decrease the intestinal inflammation and improves dysbiosis for COVID-19 patients | NCT04420676 | It was a randomized Interventional study of 30 participants | This study performed as double blind, placebo-controlled study |
| 8 | Evaluate the probiotics efficacy to decrease the COVID-19 infection symptoms and duration of COVID-19 positive patients | NCT04621071 | The double-blind, randomized, controlled trial of 17 participants | This study performed to explored the effects of dietary supplement: Probiotics (2 strains $10 \times 10^9$ UFC), trial completed |
| 9 | Impact analysis of probiotic strain *L. reuteri* DSM 17938 for specific Abs response against SARS-CoV-2 infection | NCT04734886 | It was control, randomized trial of 161 participants | To assess the upon and after COVID-19 infection in healthy adults, trial completed |
| 10 | To evaluate the primary efficacy of live microbials (probiotics) for boosting up the immunity of SARS-CoV-2 infected persons (unvaccinated) | NCT04847349 | It was double-blind, randomized, controlled trial of 54 participants | Efficacy analysis of dietary supplement (combination of live microbials) as anti COVID-19 infection, trial completed |
| 11 | Evaluate the follow -up of Symprove (probiotic) to COVID-19 positive patients | NCT04877704 | The randomized clinical trial was performed with 60 patients | Observational study to supervision of hospitalized COVID-19 patients |
| 12 | Study was performed to evaluate the possible effect of a probiotic mixtures in the improvement of COVID-19 infection symptoms | NCT04390477 | It was randomized case control, clinical trial of 41 participants | Observational study of dietary supplement: Probiotic to COVID-19 patients |
| 13 | The probiotic ( Omni-Biotic Pro Vi 5) use for investigate the side effect of post-COVID syndrome | NCT04813718 | It was a randomized trial of 20 participants | It was a therapeutic target study of probiotic for treatment of acute COVID-19 and prevention of post COVID infections |
| 14 | To evaluate the effect of a probiotic strain on the occurrence and severity of COVID-19 in hospitalised elderly population | NCT04756466 | Randomized control trial of 201 participants | It was observational study, probiotic sued for improve the immune response of elderly patients |
| 15 | This study assesses the beneficial effects of the nutritional supplementation (ABBC1) to individuals taken the COVID-10 vaccine | NCT04798677 | It was a double-blinded, placebo-controlled, randomized clinical study of 90 participants | Used as knowing the microbiome modulating properties, observational study |
| 16 | To investigate the consequence of *Ligilactobacillus salivarius* MP101 to hospitalised elderly individuals | NCT04922918 | Non-randomised study of 25 participants | Observational study of aged patients having highly affected by COVID-19 |
| 17 | Study was performed to explored the effect of the probiotic *Lactobacillus rhamnosus* GG | NCT04399252 | It was a randomized double-blind, placebo-controlled trail of 182 participants | Observational study of individuals microbiome of household contacts exposed to COVID-19 |
| 18 | Treatment approaches by probiotics to human gut microbiome and growing the anti-inflammatory response for COVID-19 infected patients | NCT04854941 | It was a randomized controlled open-label study of 200 participants | The optimizing treatment approaches based observational study, trial completed |
| 19 | To evaluate the capability of the novel | NCT04666116 | Randomized, single blind clinical | Used of dietary supplementation with |

| | | | | |
|---|---|---|---|---|
| | nutritional supplement (probiotics and other vitamins) to COVID-19 infected and hospitalised patients | | trial of 96 participants | probiotics aims to reduce the viral load |
| 20 | Using of probiotics for COVID 19 transmission reduction to health care professionals | NCT04462627 | It was a non-randomized trial of 500 participants | Analysis and reduction of COVID-19 viral load to health care professionals |

*P. acidilactici*: *Pediococcus acidilactici*; *L. plantarum*: *Lactobacillus plantarum*; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

As the pandemic persists, it is critical to assess the effect of next-generation probiotics, prebiotics, synbiotics, and increased fiber intake on changes in gut microbiota composition in patients with mild to moderate and severe COVID-19.

## FUTURE PERSPECTIVE

In several cases, complex pathophysiological and immunological responses are reported in the host due to SARS-CoV-2 infection. However, very little is known regarding the changes in gut virome in the COVID-19 patients, and this should be explored in future studies should explore it further. Moreover, the possible role of the gut microbiota in COVID-19 should be explored in future research. Likewise, population-based cohorts should be generated to illustrate the function of the altered gut microbiota during COVID-19 in different populations. This will enable the design of diagnostics and therapeutics for COVID-19 in different population types. Simultaneously, population-specific changes need to be described as this can help resolve severe conditions in COVID-19 patients. In the future, researchers should attempt to understand population-specific gut microbiota alteration during COVID-19 to design therapeutic interventions as required. Moreover, research could focus on the population specific changes in the immune response generated against the two altered gut microbiota during COVID-19.

## CONCLUSION

Presently, abundant research has described the marked changes in the gut microbiomes of COVID-19 patients. Therefore, an apparent association exsists between the overall health of the gut microbiome and the progression of COVID-19[119]. Furthermore, the altered gut microbiota has been shown to persist in patients even after several days of recovery from COVID-19.

However, poor outcome were observed in elderly or co-morbid patients[97,120]. Recently, several studies discussed the factors associated with the modified gut microbiota in COVID-19 patients manifesting GI symptoms. According to some reports, increased inflammation may lead to a leaky gut, which enables the translocation of bacterial metabolites and toxins into the systemic circulation[97,120]. This might cause further complications to the severe COVID-19 patients.

In this review, we have illustrated various GI aspects of COVID-19 patients including the gut microbiota imbalance and GI symptoms, the patterns of altered gut microbiota composition, the crosstalk between the microbiome and the gut-lung axis, the crosstalk between the microbiome and the gut-brain-lung axis, as well as hypoxia associated with altered gut microbiota. We also highlighted the association between the gut microbiota and elderly or co-morbid COVID-19 patients, as well as that of gut microbiota dysbiosis and COVID-19 severity. Additionally, we explored the correlation between, probiotics usage and the gut microbiota based on antibiotic usage in COVID-19 patients. Therefore, our review will provide a distinct outline for researchers working in the field. Also, it will provide valuable insight into the role of gut microbiomes in COVID-19 patients.

Currently, therapeutics are in development to combat COVID-19. In addition to antiviral therapeutics, probiotics might be effective for improving gut health through the gut-lung axis. Recently, several clinical trials have been initiated to understand the role of probiotics in COVID-19 patients. The ongoing clinical trials will elucidate the role of probiotic therapeutics or for COVID-19 patients, and offer new alternatives in COVID-19 treatment.

## FOOTNOTES

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## REFERENCES

1 **Liu Q**, Mak JWY, Su Q, Yeoh YK, Lui GC, Ng SSS, Zhang F, Li AYL, Lu W, Hui DS, Chan PK, Chan FKL, Ng SC. Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut* 2022; **71**: 544-552 [PMID: 35082169 DOI: 10.1136/gutjnl-2021-325989]
2 **Troisi J**, Venutolo G, Pujolassos Tanyà M, Delli Carri M, Landolfi A, Fasano A. COVID-19 and the gastrointestinal tract: Source of infection or merely a target of the inflammatory process following SARS-CoV-2 infection? *World J Gastroenterol* 2021; **27**: 1406-1418 [PMID: 33911464 DOI: 10.3748/wjg.v27.i14.1406]
3 **Al Kassaa I**, El Omari S, Abbas N, Papon N, Drider D, Kassem II, Osman M. High association of COVID-19 severity with poor gut health score in Lebanese patients. *PLoS One* 2021; **16**: e0258913 [PMID: 34673813 DOI: 10.1371/journal.pone.0258913]
4 **Donati Zeppa S**, Agostini D, Piccoli G, Stocchi V, Sestili P. Gut Microbiota Status in COVID-19: An Unrecognized Player? *Front Cell Infect Microbiol* 2020; **10**: 576551 [PMID: 33324572 DOI: 10.3389/fcimb.2020.576551]
5 **Gu J**, Han B, Wang J. COVID-19: Gastrointestinal Manifestations and Potential Fecal-Oral Transmission. *Gastroenterology* 2020; **158**: 1518-1519 [PMID: 32142785 DOI: 10.1053/j.gastro.2020.02.054]
6 **Ramachandran P**, Onukogu I, Ghanta S, Gajendran M, Perisetti A, Goyal H, Aggarwal A. Gastrointestinal Symptoms and Outcomes in Hospitalized Coronavirus Disease 2019 Patients. *Dig Dis* 2020; **38**: 373-379 [PMID: 32599601 DOI: 10.1159/000509774]
7 **Han C**, Duan C, Zhang S, Spiegel B, Shi H, Wang W, Zhang L, Lin R, Liu J, Ding Z, Hou X. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. *Am J Gastroenterol* 2020; **115**: 916-923 [PMID: 32301761 DOI: 10.14309/ajg.0000000000000664]
8 **Ng SC**, Tilg H. COVID-19 and the gastrointestinal tract: more than meets the eye. *Gut* 2020; **69**: 973-974 [PMID: 32273292 DOI: 10.1136/gutjnl-2020-321195]
9 **Tang A**, Tong ZD, Wang HL, Dai YX, Li KF, Liu JN, Wu WJ, Yuan C, Yu ML, Li P, Yan JB. Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China. *Emerg Infect Dis* 2020; **26**: 1337-1339 [PMID: 32150527 DOI: 10.3201/eid2606.200301]
10 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**: 744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]
11 **Mehandru S**, Merad M. Pathological sequelae of long-haul COVID. *Nat Immunol* 2022; **23**: 194-202 [PMID: 35105985 DOI: 10.1038/s41590-021-01104-y]
12 **Holmes E**, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* 2012; **16**: 559-564 [PMID: 23140640 DOI: 10.1016/j.cmet.2012.10.007]
13 **Nicholson JK**, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012; **336**: 1262-1267 [PMID: 22674330 DOI: 10.1126/science.1223813]
14 **Joyce SA**, Gahan CG. The gut microbiota and the metabolic health of the host. *Curr Opin Gastroenterol* 2014; **30**: 120-127 [PMID: 24468803 DOI: 10.1097/MOG.0000000000000039]
15 **Jandhyala SM**, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015; **21**: 8787-8803 [PMID: 26269668 DOI: 10.3748/wjg.v21.i29.8787]
16 **Corfield AP**. The Interaction of the Gut Microbiota with the Mucus Barrier in Health and Disease in Human. *Microorganisms* 2018; **6** [PMID: 30072673 DOI: 10.3390/microorganisms6030078]
17 **Tang WH**, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. *Circ Res* 2017; **120**: 1183-1196 [PMID: 28360349 DOI: 10.1161/CIRCRESAHA.117.309715]
18 **Dhar D**, Mohanty A. Gut microbiota and Covid-19-possible link and implications. *Virus Res* 2020; **285**: 198018 [PMID: 32430279 DOI: 10.1016/j.virusres.2020.198018]

19 **Zuo T**, Wu X, Wen W, Lan P. Gut Microbiome Alterations in COVID-19. *Genomics Proteomics Bioinformatics* 2021 [PMID: 34560321 DOI: 10.1016/j.gpb.2021.09.004]

20 **Chen J**, Hall S, Vitetta L. Altered gut microbial metabolites could mediate the effects of risk factors in Covid-19. *Rev Med Virol* 2021; **31**: 1-13 [PMID: 34546607 DOI: 10.1002/rmv.2211]

21 **Yamamoto S**, Saito M, Tamura A, Prawisuda D, Mizutani T, Yotsuyanagi H. The human microbiome and COVID-19: A systematic review. *PLoS One* 2021; **16**: e0253293 [PMID: 34161373 DOI: 10.1371/journal.pone.0253293]

22 **Chiappetta S**, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. *Int J Obes (Lond)* 2020; **44**: 1790-1792 [PMID: 32409680 DOI: 10.1038/s41366-020-0597-4]

23 **Del Valle DM**, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Marron TU, Xie H, Patel M, Tuballes K, Van Oekelen O, Rahman A, Kovatch P, Aberg JA, Schadt E, Jagannath S, Mazumdar M, Charney AW, Firpo-Betancourt A, Mendu DR, Jhang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnjatic S. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med* 2020; **26**: 1636-1643 [PMID: 32839624 DOI: 10.1038/s41591-020-1051-9]

24 **Chen L**, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2018; **9**: 7204-7218 [PMID: 29467962 DOI: 10.18632/oncotarget.23208]

25 **Tang Y**, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. *Front Immunol* 2020; **11**: 1708 [PMID: 32754163 DOI: 10.3389/fimmu.2020.01708]

26 **Costela-Ruiz VJ**, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev* 2020; **54**: 62-75 [PMID: 32513566 DOI: 10.1016/j.cytogfr.2020.06.001]

27 **García LF**. Immune Response, Inflammation, and the Clinical Spectrum of COVID-19. *Front Immunol* 2020; **11**: 1441 [PMID: 32612615 DOI: 10.3389/fimmu.2020.01441]

28 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]

29 **Sinha P**, Matthay MA, Calfee CS. Is a "Cytokine Storm" Relevant to COVID-19? *JAMA Intern Med* 2020; **180**: 1152-1154 [PMID: 32602883 DOI: 10.1001/jamainternmed.2020.3313]

30 **Ragab D**, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. *Front Immunol* 2020; **11**: 1446 [PMID: 32612617 DOI: 10.3389/fimmu.2020.01446]

31 **Perisetti A**, Gajendran M, Mann R, Elhanafi S, Goyal H. COVID-19 extrapulmonary illness -special gastrointestinal and hepatic considerations. *Dis Mon* 2020; **66**: 101064 [PMID: 32807535 DOI: 10.1016/j.disamonth.2020.101064]

32 **de Oliveira GLV**, Oliveira CNS, Pinzan CF, de Salis LVV, Cardoso CRB. Microbiota Modulation of the Gut-Lung Axis in COVID-19. *Front Immunol* 2021; **12**: 635471 [PMID: 33717181 DOI: 10.3389/fimmu.2021.635471]

33 **Zhou D**, Wang Q, Liu H. Coronavirus disease 2019 and the gut-lung axis. *Int J Infect Dis* 2021; **113**: 300-307 [PMID: 34517046 DOI: 10.1016/j.ijid.2021.09.013]

34 **Johnson SD**, Olwenyi OA, Bhyravbhatla N, Thurman M, Pandey K, Klug EA, Johnston M, Dyavar SR, Acharya A, Podany AT, Fletcher CV, Mohan M, Singh K, Byrareddy SN. Therapeutic implications of SARS-CoV-2 dysregulation of the gut-brain-lung axis. *World J Gastroenterol* 2021; **27**: 4763-4783 [PMID: 34447225 DOI: 10.3748/wjg.v27.i29.4763]

35 **Redd WD**, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Thompson CC, Shen L, Chan WW. Prevalence and Characteristics of Gastrointestinal Symptoms in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 Infection in the United States: A Multicenter Cohort Study. *Gastroenterology* 2020; **159**: 765-767.e2 [PMID: 32333911 DOI: 10.1053/j.gastro.2020.04.045]

36 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

37 **Guan W-j**, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, Liu L, Shan H, Lei C-l, Hui DS. Clinical characteristics of coronavirus disease 2019 in China. *New Engl J of Med* 2020; **382** (18): 1708-1720

38 **Yeoh YK**, Zuo T, Lui GC, Zhang F, Liu Q, Li AY, Chung AC, Cheung CP, Tso EY, Fung KS, Chan V, Ling L, Joynt G, Hui DS, Chow KM, Ng SSS, Li TC, Ng RW, Yip TC, Wong GL, Chan FK, Wong CK, Chan PK, Ng SC. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut* 2021; **70**: 698-706 [PMID: 33431578 DOI: 10.1136/gutjnl-2020-323020]

39 **Shi J**, Sun J, Hu Y. Enteric involvement of SARS-CoV-2: Implications for the COVID-19 management, transmission, and infection control. *Virulence* 2020; **11**: 941-944 [PMID: 32715925 DOI: 10.1080/21505594.2020.1794410]

40 **Villapol S**. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; **226**: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]

41 **Kaźmierczak-Siedlecka K**, Vitale E, Makarewicz W. COVID-19 -gastrointestinal and gut microbiota-related aspects. *Eur Rev Med Pharmacol Sci* 2020; **24**: 10853-10859 [PMID: 33155247 DOI: 10.26355/eurrev_202010_23448]

42 **Zhang T**, Cui X, Zhao X, Wang J, Zheng J, Zheng G, Guo W, Cai C, He S, Xu Y. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. *J Med Virol* 2020; **92**: 909-914 [PMID: 32222992 DOI: 10.1002/jmv.25795]

43 **Chen Y**, Chen L, Deng Q, Zhang G, Wu K, Ni L, Yang Y, Liu B, Wang W, Wei C, Yang J, Ye G, Cheng Z. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *J Med Virol* 2020; **92**: 833-840 [PMID: 32243607 DOI: 10.1002/jmv.25825]

44 **Wölfel R**, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; **581**: 465-469 [PMID: 32235945 DOI: 10.1038/s41586-020-2196-x]

45 **Kevadiya BD**, Machhi J, Herskovitz J, Oleynikov MD, Blomberg WR, Bajwa N, Soni D, Das S, Hasan M, Patel M,

Senan AM, Gorantla S, McMillan J, Edagwa B, Eisenberg R, Gurumurthy CB, Reid SPM, Punyadeera C, Chang L, Gendelman HE. Diagnostics for SARS-CoV-2 infections. *Nat Mater* 2021; **20**: 593-605 [PMID: 33589798 DOI: 10.1038/s41563-020-00906-z]

46 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

47 **Zuo T**, Liu Q, Zhang F, Yeoh YK, Wan Y, Zhan H, Lui GCY, Chen Z, Li AYL, Cheung CP, Chen N, Lv W, Ng RWY, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Temporal landscape of human gut RNA and DNA virome in SARS-CoV-2 infection and severity. *Microbiome* 2021; **9**: 91 [PMID: 33853691 DOI: 10.1186/s40168-021-01008-x]

48 **Zuo T**, Zhan H, Zhang F, Liu Q, Tso EYK, Lui GCY, Chen N, Li A, Lu W, Chan FKL, Chan PKS, Ng SC. Alterations in Fecal Fungal Microbiome of Patients With COVID-19 During Time of Hospitalization until Discharge. *Gastroenterology* 2020; **159**: 1302-1310.e5 [PMID: 32598884 DOI: 10.1053/j.gastro.2020.06.048]

49 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955.e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]

50 **Newsome RC**, Gauthier J, Hernandez MC, Abraham GE, Robinson TO, Williams HB, Sloan M, Owings A, Laird H, Christian T, Pride Y, Wilson KJ, Hasan M, Parker A, Senitko M, Glover SC, Gharaibeh RZ, Jobin C. The gut microbiome of COVID-19 recovered patients returns to uninfected status in a minority-dominated United States cohort. *Gut Microbes* 2021; **13**: 1-15 [PMID: 34100340 DOI: 10.1080/19490976.2021.1926840]

51 **Khan M**, Mathew BJ, Gupta P, Garg G, Khadanga S, Vyas AK, Singh AK. Gut Dysbiosis and IL-21 Response in Patients with Severe COVID-19. *Microorganisms* 2021; **9** [PMID: 34199203 DOI: 10.3390/microorganisms9061292]

52 **Magalhães NS**, Savino W, Silva PMR, Martins MA, Carvalho VF. Gut Microbiota Dysbiosis Is a Crucial Player for the Poor Outcomes for COVID-19 in Elderly, Diabetic and Hypertensive Patients. *Front Med (Lausanne)* 2021; **8**: 644751 [PMID: 34458281 DOI: 10.3389/fmed.2021.644751]

53 **Hung YP**, Lee CC, Lee JC, Tsai PJ, Ko WC. Gut Dysbiosis during COVID-19 and Potential Effect of Probiotics. *Microorganisms* 2021; **9** [PMID: 34442684 DOI: 10.3390/microorganisms9081605]

54 **Zuo T**, Liu Q, Zhang F, Lui GC, Tso EY, Yeoh YK, Chen Z, Boon SS, Chan FK, Chan PK, Ng SC. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. *Gut* 2021; **70**: 276-284 [PMID: 32690600 DOI: 10.1136/gutjnl-2020-322294]

55 **Sokol H**, Contreras V, Maisonnasse P, Desmons A, Delache B, Sencio V, Machelart A, Brisebarre A, Humbert L, Deryuter L, Gauliard E, Heumel S, Rainteau D, Dereuddre-Bosquet N, Menu E, Ho Tsong Fang R, Lamaziere A, Brot L, Wahl C, Oeuvray C, Rolhion N, Van Der Werf S, Ferreira S, Le Grand R, Trottein F. SARS-CoV-2 infection in nonhuman primates alters the composition and functional activity of the gut microbiota. *Gut Microbes* 2021; **13**: 1-19 [PMID: 33685349 DOI: 10.1080/19490976.2021.1893113]

56 **Zhang F**, Wan Y, Zuo T, Yeoh YK, Liu Q, Zhang L, Zhan H, Lu W, Xu W, Lui GCY, Li AYL, Cheung CP, Wong CK, Chan PKS, Chan FKL, Ng SC. Prolonged Impairment of Short-Chain Fatty Acid and L-Isoleucine Biosynthesis in Gut Microbiome in Patients With COVID-19. *Gastroenterology* 2022; **162**: 548-561.e4 [PMID: 34687739 DOI: 10.1053/j.gastro.2021.10.013]

57 **Shang J**, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020; **581**: 221-224 [PMID: 32225175 DOI: 10.1038/s41586-020-2179-y]

58 **Chakraborty C**, Sharma AR, Mallick B, Bhattacharya M, Sharma G, Lee SS. Evaluation of molecular interaction, physicochemical parameters and conserved pattern of SARS-CoV-2 Spike RBD and hACE2: in silico and molecular dynamics approach. *Eur Rev Med Pharmacol Sci* 2021; **25**: 1708-1723 [PMID: 33629340 DOI: 10.26355/eurrev_202102_24881]

59 **Verdecchia P**, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med* 2020; **76**: 14-20 [PMID: 32336612 DOI: 10.1016/j.ejim.2020.04.037]

60 **Sungnak W**, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JL; HCA Lung Biological Network. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* 2020; **26**: 681-687 [PMID: 32327758 DOI: 10.1038/s41591-020-0868-6]

61 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]

62 **Sharma RK**, Oliveira AC, Yang T, Karas MM, Li J, Lobaton GO, Aquino VP, Robles-Vera I, de Kloet AD, Krause EG, Bryant AJ, Verma A, Li Q, Richards EM, Raizada MK. Gut Pathology and Its Rescue by ACE2 (Angiotensin-Converting Enzyme 2) in Hypoxia-Induced Pulmonary Hypertension. *Hypertension* 2020; **76**: 206-216 [PMID: 32418496 DOI: 10.1161/HYPERTENSIONAHA.120.14931]

63 **Koester ST**, Li N, Lachance DM, Morella NM, Dey N. Variability in digestive and respiratory tract Ace2 expression is associated with the microbiome. *PLoS One* 2021; **16**: e0248730 [PMID: 33725024 DOI: 10.1371/journal.pone.0248730]

64 **Geva-Zatorsky N**, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, Yanortsang TB, Yang L, Jupp R, Mathis D, Benoist C, Kasper DL. Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell* 2017; **168**: 928-943.e11 [PMID: 28215708 DOI: 10.1016/j.cell.2017.01.022]

65 **Penninger JM**, Grant MB, Sung JJY. The Role of Angiotensin Converting Enzyme 2 in Modulating Gut Microbiota, Intestinal Inflammation, and Coronavirus Infection. *Gastroenterology* 2021; **160**: 39-46 [PMID: 33130103 DOI: 10.1053/j.gastro.2020.07.067]

66 **Viana SD**, Nunes S, Reis F. ACE2 imbalance as a key player for the poor outcomes in COVID-19 patients with age-

related comorbidities -Role of gut microbiota dysbiosis. *Ageing Res Rev* 2020; **62**: 101123 [PMID: 32683039 DOI: 10.1016/j.arr.2020.101123]

67 **Budden KF**, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, Hansbro PM. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol* 2017; **15**: 55-63 [PMID: 27694885 DOI: 10.1038/nrmicro.2016.142]

68 **Dumas A**, Bernard L, Poquet Y, Lugo-Villarino G, Neyrolles O. The role of the lung microbiota and the gut-lung axis in respiratory infectious diseases. *Cell Microbiol* 2018; **20**: e12966 [PMID: 30329198 DOI: 10.1111/cmi.12966]

69 **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]

70 **S A**, K G, A AM. Intermodulation of gut-lung axis microbiome and the implications of biotics to combat COVID-19. *J Biomol Struct Dyn* 2021; 1-17 [PMID: 34699326 DOI: 10.1080/07391102.2021.1994875]

71 **Aktas B**, Aslim B. Gut-lung axis and dysbiosis in COVID-19. *Turk J Biol* 2020; **44**: 265-272 [PMID: 32595361 DOI: 10.3906/biy-2005-102]

72 **Hernández-Terán A**, Mejía-Nepomuceno F, Herrera MT, Barreto O, García E, Castillejos M, Boukadida C, Matias-Florentino M, Rincón-Rubio A, Avila-Rios S, Mújica-Sánchez M, Serna-Muñoz R, Becerril-Vargas E, Guadarrama-Pérez C, Ahumada-Topete VH, Rodríguez-Llamazares S, Martínez-Orozco JA, Salas-Hernández J, Pérez-Padilla R, Vázquez-Pérez JA. Dysbiosis and structural disruption of the respiratory microbiota in COVID-19 patients with severe and fatal outcomes. *Sci Rep* 2021; **11**: 21297 [PMID: 34716394 DOI: 10.1038/s41598-021-00851-0]

73 **Zhu X**, Han Y, Du J, Liu R, Jin K, Yi W. Microbiota-gut-brain axis and the central nervous system. *Oncotarget* 2017; **8**: 53829-53838 [PMID: 28881854 DOI: 10.18632/oncotarget.17754]

74 **Morais LH**, Schreiber HL 4th, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. *Nat Rev Microbiol* 2021; **19**: 241-255 [PMID: 33093662 DOI: 10.1038/s41579-020-00460-0]

75 **Mayer EA**. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* 2011; **12**: 453-466 [PMID: 21750565 DOI: 10.1038/nrn3071]

76 **Taché Y**, Saavedra JM. Introduction to the Special Issue "The Brain-Gut Axis". *Cell Mol Neurobiol* 2022; **42**: 311-313 [PMID: 34652580 DOI: 10.1007/s10571-021-01155-7]

77 **Dogra N**, Mani RJ, Katare DP. The Gut-Brain Axis: Two Ways Signaling in Parkinson's Disease. *Cell Mol Neurobiol* 2022; **42**: 315-332 [PMID: 33649989 DOI: 10.1007/s10571-021-01066-7]

78 **Manosso LM**, Arent CO, Borba LA, Ceretta LB, Quevedo J, Réus GZ. Microbiota-Gut-Brain Communication in the SARS-CoV-2 Infection. *Cells* 2021; **10** [PMID: 34440767 DOI: 10.3390/cells10081993]

79 **Strandwitz P**. Neurotransmitter modulation by the gut microbiota. *Brain Res* 2018; **1693**: 128-133 [PMID: 29903615 DOI: 10.1016/j.brainres.2018.03.015]

80 **O'Donnell MP**, Fox BW, Chao PH, Schroeder FC, Sengupta P. A neurotransmitter produced by gut bacteria modulates host sensory behaviour. *Nature* 2020; **583**: 415-420 [PMID: 32555456 DOI: 10.1038/s41586-020-2395-5]

81 **Mittal R**, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, Grati M, Mittal J, Yan D, Eshraghi AA, Deo SK, Daunert S, Liu XZ. Neurotransmitters: The Critical Modulators Regulating Gut-Brain Axis. *J Cell Physiol* 2017; **232**: 2359-2372 [PMID: 27512962 DOI: 10.1002/jcp.25518]

82 **Konturek PC**, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *J Physiol Pharmacol* 2011; **62**: 591-599 [PMID: 22314561]

83 **Vodička M**, Ergang P, Hrnčíř T, Mikulecká A, Kvapilová P, Vagnerová K, Šestáková B, Fajstová A, Hermanová P, Hudcovic T, Kozáková H, Pácha J. Microbiota affects the expression of genes involved in HPA axis regulation and local metabolism of glucocorticoids in chronic psychosocial stress. *Brain Behav Immun* 2018; **73**: 615-624 [PMID: 29990567 DOI: 10.1016/j.bbi.2018.07.007]

84 **Ojeda J**, Ávila A, Vidal PM. Gut Microbiota Interaction with the Central Nervous System throughout Life. *J Clin Med* 2021; **10** [PMID: 33801153 DOI: 10.3390/jcm10061299]

85 **Zheng D**, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020; **30**: 492-506 [PMID: 32433595 DOI: 10.1038/s41422-020-0332-7]

86 **Cerf-Bensussan N**, Gaboriau-Routhiau V. The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol* 2010; **10**: 735-744 [PMID: 20865020 DOI: 10.1038/nri2850]

87 **Xu K**, Cai H, Shen Y, Ni Q, Chen Y, Hu S, Li J, Wang H, Yu L, Huang H, Qiu Y, Wei G, Fang Q, Zhou J, Sheng J, Liang T, Li L. [Management of COVID-19: the Zhejiang experience]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2020; **49**: 147-157 [PMID: 32391658 DOI: 10.3785/j.issn.1008-9292.2020.02.02]

88 **Jahani M**, Dokaneheifard S, Mansouri K. Hypoxia: A key feature of COVID-19 launching activation of HIF-1 and cytokine storm. *J Inflamm (Lond)* 2020; **17**: 33 [PMID: 33139969 DOI: 10.1186/s12950-020-00263-3]

89 **Rahman A**, Tabassum T, Araf Y, Al Nahid A, Ullah MA, Hosen MJ. Silent hypoxia in COVID-19: pathomechanism and possible management strategy. *Mol Biol Rep* 2021; **48**: 3863-3869 [PMID: 33891272 DOI: 10.1007/s11033-021-06358-1]

90 **Tian M**, Liu W, Li X, Zhao P, Shereen MA, Zhu C, Huang S, Liu S, Yu X, Yue M, Pan P, Wang W, Li Y, Chen X, Wu K, Luo Z, Zhang Q, Wu J. HIF-1α promotes SARS-CoV-2 infection and aggravates inflammatory responses to COVID-19. *Signal Transduct Target Ther* 2021; **6**: 308 [PMID: 34408131 DOI: 10.1038/s41392-021-00726-w]

91 **Gopal AB**, Chakraborty S, Padhan PK, Barik A, Dixit P, Chakraborty D, Poirah I, Samal S, Sarkar A, Bhattacharyya A. Silent hypoxia in COVID-19: a gut microbiota connection. *Curr Opin Physiol* 2021; **23**: 100456 [PMID: 34250324 DOI: 10.1016/j.cophys.2021.06.010]

92 **Belkaid Y**, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014; **157**: 121-141 [PMID: 24679531 DOI: 10.1016/j.cell.2014.03.011]

93 **von Rosenvinge EC**, O'May GA, Macfarlane S, Macfarlane GT, Shirtliff ME. Microbial biofilms and gastrointestinal diseases. *Pathog Dis* 2013; **67**: 25-38 [PMID: 23620117 DOI: 10.1111/2049-632X.12020]

94 **Ríos-Covián D**, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilán CG, Salazar N. Intestinal Short

Chain Fatty Acids and their Link with Diet and Human Health. *Front Microbiol* 2016; **7**: 185 [PMID: 26925050 DOI: 10.3389/fmicb.2016.00185]

95 **Parada Venegas D**, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, Harmsen HJM, Faber KN, Hermoso MA. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol* 2019; **10**: 277 [PMID: 30915065 DOI: 10.3389/fimmu.2019.00277]

96 **Odenwald MA**, Turner JR. The intestinal epithelial barrier: a therapeutic target? *Nat Rev Gastroenterol Hepatol* 2017; **14**: 9-21 [PMID: 27848962 DOI: 10.1038/nrgastro.2016.169]

97 **Kim HS**. Do an Altered Gut Microbiota and an Associated Leaky Gut Affect COVID-19 Severity? *mBio* 2021; **12** [PMID: 33436436 DOI: 10.1128/mBio.03022-20]

98 **Inan MS**, Rasoulpour RJ, Yin L, Hubbard AK, Rosenberg DW, Giardina C. The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. *Gastroenterology* 2000; **118**: 724-734 [PMID: 10734024 DOI: 10.1016/s0016-5085(00)70142-9]

99 **Maslowski KM**, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; **461**: 1282-1286 [PMID: 19865172 DOI: 10.1038/nature08530]

100 **Samuel BS**, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U S A* 2008; **105**: 16767-16772 [PMID: 18931303 DOI: 10.1073/pnas.0808567105]

101 **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]

102 **Chhibber-Goel J**, Gopinathan S, Sharma A. Interplay between severities of COVID-19 and the gut microbiome: implications of bacterial co-infections? *Gut Pathog* 2021; **13**: 14 [PMID: 33632296 DOI: 10.1186/s13099-021-00407-7]

103 **Kany S**, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *Int J Mol Sci* 2019; **20** [PMID: 31795299 DOI: 10.3390/ijms20236008]

104 **Chen Y**, Gu S, Chen Y, Lu H, Shi D, Guo J, Wu WR, Yang Y, Li Y, Xu KJ, Ding C, Luo R, Huang C, Yu L, Xu M, Yi P, Liu J, Tao JJ, Zhang H, Lv L, Wang B, Sheng J, Li L. Six-month follow-up of gut microbiota richness in patients with COVID-19. *Gut* 2022; **71**: 222-225 [PMID: 33833065 DOI: 10.1136/gutjnl-2021-324090]

105 **Ceccarelli G**, Borrazzo C, Pinacchio C, Santinelli L, Innocenti GP, Cavallari EN, Celani L, Marazzato M, Alessandri F, Ruberto F, Pugliese F, Venditti M, Mastroianni CM, d'Ettorre G. Oral Bacteriotherapy in Patients With COVID-19: A Retrospective Cohort Study. *Front Nutr* 2020; **7**: 613928 [PMID: 33505983 DOI: 10.3389/fnut.2020.613928]

106 **Rao KS**, Suryaprakash V, Senthilkumar R, Preethy S, Katoh S, Ikewaki N, Abraham SJK. Role of Immune Dysregulation in Increased Mortality Among a Specific Subset of COVID-19 Patients and Immune-Enhancement Strategies for Combatting Through Nutritional Supplements. *Front Immunol* 2020; **11**: 1548 [PMID: 32733487 DOI: 10.3389/fimmu.2020.01548]

107 **Al-Hadidi SH**, Alhussain H, Abdel Hadi H, Johar A, Yassine HM, Al Thani AA, Eltai NO. The Spectrum of Antibiotic Prescribing During COVID-19 Pandemic: A Systematic Literature Review. *Microb Drug Resist* 2021; **27**: 1705-1725 [PMID: 34077290 DOI: 10.1089/mdr.2020.0619]

108 **Ramirez J**, Guarner F, Bustos Fernandez L, Maruy A, Sdepanian VL, Cohen H. Antibiotics as Major Disruptors of Gut Microbiota. *Front Cell Infect Microbiol* 2020; **10**: 572912 [PMID: 33330122 DOI: 10.3389/fcimb.2020.572912]

109 **Chattopadhyay I**, Shankar EM. SARS-CoV-2-Indigenous Microbiota Nexus: Does Gut Microbiota Contribute to Inflammation and Disease Severity in COVID-19? *Front Cell Infect Microbiol* 2021; **11**: 590874 [PMID: 33791231 DOI: 10.3389/fcimb.2021.590874]

110 **Rafiqul Islam SM**, Foysal MJ, Hoque MN, Mehedi HMH, Rob MA, Salauddin A, Tanzina AY, Biswas S, Noyon SH, Siddiki AMAMZ, Tay A, Mannan A. Dysbiosis of Oral and Gut Microbiomes in SARS-CoV-2 Infected Patients in Bangladesh: Elucidating the Role of Opportunistic Gut Microbes. *Front Med (Lausanne)* 2022; **9**: 821777 [PMID: 35237631 DOI: 10.3389/fmed.2022.821777]

111 **Baud D**, Dimopoulou Agri V, Gibson GR, Reid G, Giannoni E. Using Probiotics to Flatten the Curve of Coronavirus Disease COVID-2019 Pandemic. *Front Public Health* 2020; **8**: 186 [PMID: 32574290 DOI: 10.3389/fpubh.2020.00186]

112 **Xia Y**, Cao J, Wang M, Lu M, Chen G, Gao F, Liu Z, Zhang D, Ke X, Yi M. Effects of Lactococcus lactis subsp. lactis JCM5805 on colonization dynamics of gut microbiota and regulation of immunity in early ontogenetic stages of tilapia. *Fish Shellfish Immunol* 2019; **86**: 53-63 [PMID: 30428393 DOI: 10.1016/j.fsi.2018.11.022]

113 **Mak JW**, Chan FK, Ng SC. Probiotics and COVID-19-Authors' reply. *Lancet Gastroenterol Hepatol* 2020; **5** (8): 722-723

114 **Bhattacharya M**, Sharma AR, Mallick B, Sharma G, Lee SS, Chakraborty C. Immunoinformatics approach to understand molecular interaction between multi-epitopic regions of SARS-CoV-2 spike-protein with TLR4/MD-2 complex. *Infect Genet Evol* 2020; **85**: 104587 [PMID: 33039603 DOI: 10.1016/j.meegid.2020.104587]

115 **Petruk G**, Puthia M, Petrlova J, Samsudin F, Strömdahl AC, Cerps S, Uller L, Kjellström S, Bond PJ, Schmidtchen AA. SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity. *J Mol Cell Biol* 2020; **12**: 916-932 [PMID: 33295606 DOI: 10.1093/jmcb/mjaa067]

116 **Chakraborty C**, Sharma AR, Bhattacharya M, Sharma G, Lee SS, Agoramoorthy G. Consider TLR5 for new therapeutic development against COVID-19. *J Med Virol* 2020; **92**: 2314-2315 [PMID: 32391920 DOI: 10.1002/jmv.25997]

117 **Haran JP**, Pinero JC, Zheng Y, Palma NA, Wingertzahn M. Virtualized clinical studies to assess the natural history and impact of gut microbiome modulation in non-hospitalized patients with mild to moderate COVID-19 a randomized, open-label, prospective study with a parallel group study evaluating the physiologic effects of KB109 on gut microbiota structure and function: a structured summary of a study protocol for a randomized controlled study. *Trials* 2021; **22**: 245 [PMID: 33810796 DOI: 10.1186/s13063-021-05157-0]

118 **van der Lelie D**, Taghavi S. COVID-19 and the Gut Microbiome: More than a Gut Feeling. *mSystems* 2020; **5** [PMID: 32694127 DOI: 10.1128/mSystems.00453-20]

119 **Buffie CG**, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol* 2013; **13**: 790-801 [PMID: 24096337 DOI: 10.1038/nri3535]

120 **Vignesh R**, Swathirajan CR, Tun ZH, Rameshkumar MR, Solomon SS, Balakrishnan P. Could Perturbation of Gut Microbiota Possibly Exacerbate the Severity of COVID-19 *via* Cytokine Storm? *Front Immunol* 2020; **11**: 607734 [PMID: 33569053 DOI: 10.3389/fimmu.2020.607734]

121 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

122 **Lu X**, Zhang L, Du H, Zhang J, Li YY, Qu J, Zhang W, Wang Y, Bao S, Li Y. SARS-CoV-2 infection in children. *New Engl J of Med* 2020; **382** (17): 1663-1665

123 **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: 32077115 DOI: 10.1111/all.14238]

124 **Xiao F**, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-1833.e3 [PMID: 32142773 DOI: 10.1053/j.gastro.2020.02.055]

125 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

126 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020; **323**: 1061-1069 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

127 **Xu XW**, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ* 2020; **368**: m606 [PMID: 32075786 DOI: 10.1136/bmj.m606]

128 **Liu K**, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl)* 2020; **133**: 1025-1031 [PMID: 32044814 DOI: 10.1097/CM9.0000000000000744]

129 **Shi H**, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis* 2020; **20**: 425-434 [PMID: 32105637 DOI: 10.1016/S1473-3099(20)30086-4]

130 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

131 **Wang W**, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA* 2020; **323**: 1843-1844 [PMID: 32159775 DOI: 10.1001/jama.2020.3786]

132 **Zhang J**, Wang S, Xue Y. Fecal specimen diagnosis 2019 novel coronavirus-infected pneumonia. *J Med Virol* 2020; **92**: 680-682 [PMID: 32124995 DOI: 10.1002/jmv.25742]

133 **Ling Y**, Xu SB, Lin YX, Tian D, Zhu ZQ, Dai FH, Wu F, Song ZG, Huang W, Chen J, Hu BJ, Wang S, Mao EQ, Zhu L, Zhang WH, Lu HZ. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J (Engl)* 2020; **133**: 1039-1043 [PMID: 32118639 DOI: 10.1097/CM9.0000000000000774]

134 **Young BE**, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng OT, Marimuthu K, Ang LW, Mak TM, Lau SK, Anderson DE, Chan KS, Tan TY, Ng TY, Cui L, Said Z, Kurupatham L, Chen MI, Chan M, Vasoo S, Wang LF, Tan BH, Lin RTP, Lee VJM, Leo YS, Lye DC; Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA* 2020; **323**: 1488-1494 [PMID: 32125362 DOI: 10.1001/jama.2020.3204]

135 **Wu Y**, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; **5**: 434-435 [PMID: 32199469 DOI: 10.1016/S2468-1253(20)30083-2]

136 **Peng L**, Liu J, Xu W, Luo Q, Chen D, Lei Z, Huang Z, Li X, Deng K, Lin B, Gao Z. SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. *J Med Virol* 2020; **92**: 1676-1680 [PMID: 32330305 DOI: 10.1002/jmv.25936]

137 **Wu Y**, Cheng X, Jiang G, Tang H, Ming S, Tang L, Lu J, Guo C, Shan H, Huang X. Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in COVID-19 patients during hospitalization. *NPJ Biofilms Microbiomes* 2021; **7**: 61 [PMID: 34294722 DOI: 10.1038/s41522-021-00232-5]

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*REVIEW*

# Long noncoding RNAs in hepatitis B virus replication and oncogenesis

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## Abstract

Several diverse long noncoding RNAs (lncRNAs) have been identified to be involved in hepatitis B virus (HBV) replication and oncogenesis, especially those dysregulated in HBV-related hepatocellular carcinoma (HCC). Most of these dysregulated lncRNAs are modulated by the HBV X protein. The regulatory mechanisms of some lncRNAs in HBV replication and oncogenesis have been characterized. Genetic polymorphisms of several lncRNAs affecting HBV replication or oncogenesis have also been studied. The prognosis of HCC remains poor. It is important to identify novel tumor markers for early diagnosis and find more therapeutic targets for effective treatments of HCC. Some dysregulated lncRNAs in HBV-related HCC may become biomarkers for early diagnosis and/or the therapeutic targets of HCC. This mini-review summarizes these findings briefly, focusing on recent developments.

**Key Words:** Hepatitis B virus; Hepatocellular carcinoma; Long noncoding RNAs; Hepatitis B virus X protein; Biomarker

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**Core Tip:** The prognosis of hepatocellular carcinoma (HCC) remains poor. It is important to identify novel tumor markers for early diagnosis and find more therapeutic targets for effective treatments of HCC. Several diverse long noncoding RNAs (lncRNAs) have been identified to be involved in hepatitis B virus (HBV)-related HCC. A better understanding of the molecular mechanisms underlying lncRNA-mediated hepatocarcinogenesis may help for their use in early diagnosis and to identify appropriate targets for the prevention and treatment of HBV-related HCC.

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## INTRODUCTION

Majority of the human genome (70%–90%) is actively transcribed. Only approximately 2% of these transcribed RNAs translate into proteins, while more than 90% of them are noncoding RNAs (ncRNAs). Based on size, ncRNAs are classified into small ncRNAs [less than 200 nucleotides (nt)] and long noncoding RNAs (lncRNAs, more than 200 nt). Several kinds of small ncRNAs have been characterized, including transfer-, ribo-, small nucleolar-, piwi -and miRNAs. It is well known that miRNAs (generally 22–25 nt) can regulate gene expression by suppressing protein translation or by mRNA degradation through interacting with their target mRNA sequences, usually at the 3′-untranslated regions (3′UTR). Various miRNAs have been found to be dysregulated and play important roles in hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC) progression[1].

LncRNAs are mostly transcribed by RNA polymerase II, sometimes polyadenylated and located in nuclear and/or cytosolic fractions. LncRNAs are divided into intergenic, intronic, bidirectional, sense, and antisense lncRNAs based on their genomic location and strand orientation. These lncRNAs may function as signals, decoys, guides, or scaffolds to regulate the expression of their target genes through interacting with their partner molecules[2]. In this way, lncRNAs can modulate gene expression at different levels, including epigenetic silencing, transcriptional control, post-transcriptional regulation, and protein stability modulation[2]. Thus, they are involved in many biological processes such as cell proliferation, invasion and metastasis, autophagy, and apoptosis. Indeed, dysregulation of lncRNAs has now been implicated in numerous human diseases, especially cancers, *e.g.*, HCC[2].

Liver cancer was the sixth most common cancer and the third leading cause of cancer death in the world in 2020 (https://gco.iarc.fr/today/home). HCC represents approximately 90% of all cases of primary liver cancer. The leading cause of HCC is persistent HBV infection, which occurs in more than half of the HCC cases[3]. The management of HBV-related HCC has improved in the past decade. However, the outcome of HCC is still poor. Understanding more regarding the underlying mechanism of HBV-related HCC is required for improving the prevention, diagnosis, and treatment of HCC. The roles of lncRNAs in HBV-related HCC have received much more attention lately.

In this mini-review, we briefly outline the involvement of lncRNAs in HBV replication. We summarize the findings of dysregulated lncRNAs in HBV-related diseases, especially HCC, and discuss their roles and the potential clinical applications as diagnostic or therapeutic targets for HCC.

## LNCRNAS IN HBV REPLICATION

HBV, belonging to the *Hepadnaviridae* family, is a small enveloped DNA virus. Ten HBV genotypes (A–J), which are based on eight percent or more sequence divergence across the viral genome, have been identified[4]. Even with the genotype differences, HBVs share the same pattern of replication cycle in the cells[5]. HBV infection begins with the attachment of virions to the surface proteoglycans on hepatocytes, followed by high affinity binding with more specific receptors (*e.g.*, sodium taurocholate cotransporting polypeptide; NTCP)[6]. After entry into the cell, the viral compact, partially double-stranded genome (*i.e.*, relaxed circular DNA) will form the stable covalently closed circular DNA (cccDNA) by viral and host factors[7]. Then, the viral RNAs [including sub-genomic mRNAs and pre-genomic RNA (pgRNA)] are transcribed from cccDNA using the cellular transcriptional machinery. The pgRNAs and the viral polymerase proteins (HBpAg) will be encapsidated by HBV core antigen (HBcAg) proteins. The newly formed nucleocapsids containing partially double-stranded HBV DNA made from pgRNA by HBpAg then either re-enter the nucleus to produce more cccDNA or release as mature virions following the assembly with HBV surface proteins (HBsAg)[5].

The 3.2 kilobases (kb) HBV genome contains four open reading frames (ORFs): Surface (S), precore (pre-C)/core (C), polymerase (P), and X. The S ORF encodes three viral envelope proteins initiated from different start codons: Large, middle, and small surface antigens (HBsAg). HBV e antigen and core antigen (HBcAg) are encoded in the pre-C/C ORF[5]. The P ORF encodes viral polymerase (HBpAg) possessing reverse transcriptase, RNase H, and DNA polymerase activities. HBV X protein (HBxAg, HBx) derived from X ORF can transactivate the expression of both cellular and viral genes required for viral replication[5].

Nearly 257 million people worldwide have been infected with HBV, resulting in 887000 people dying of liver cirrhosis or HCC annually. Therefore, identification of new therapeutic targets against HBV is urgently needed. LncRNAs could be potential targets as a growing number of them have been found to play a role in HBV replication[8] (Figure 1).

### *LncRNAs facilitating HBV replication*

LncRNA deleted in lymphocytic leukemia 2 (DLEU2): HBx was found to bind the DLEU2 promoter to enhance its transcription. Nuclear DLEU2 could directly bind to HBx or the histone methyltransferase enhancer of zeste homolog 2 (EZH2). The interaction of HBx and DLEU2 on the viral cccDNA displaces EZH2 from the viral chromatin to boost viral transcription and replication[9].

HOX transcript antisense RNA (HOTAIR): HOTAIR was significantly upregulated in HBV-infected cells. HOTAIR promoted HBV transcription and replication by elevating the activities of HBV promoters *via* modulation of the levels of cccDNA-bound SP1[10].

LncRNA proliferating cell nuclear antigen (PCNA) pseudogene 1 (PCNAP1): The expression levels of PCNAP1 and PCNA were significantly elevated in the livers of HBV cccDNA-positive HCC patients. PCNA could interact with HBV cccDNA in a HBc-dependent manner. PCNAP1 enhanced PCNA through sponging miR-154, which targets the 3'UTR of PCNA mRNA. Moreover, PCNAP1 or PCNA enhanced HBV replication significantly both *in vitro* and *in vivo*. Thus, lncRNA PCNAP1 enhances HBV replication through the miR-154/PCNA/HBV cccDNA axis[11].

LncRNA highly upregulated in liver cancer (HULC): The HULC gene is located on chromosome 6p24.3 and contains two exons and one intron. HULC is an lncRNA of around 500 nt mainly localized in the cytoplasm. HULC was found to elevate HBx, which coactivated STAT3 to stimulate the miR-539 promoter. Elevated miR-539, which targets the 3'UTR of APOBEC3B mRNA, downregulated APOBEC3B and promoted HBV replication. Thus, HULC activates HBV through the HBx/STAT3/miR-539/APOBEC3B axis[12]. Another study demonstrated that lncRNA HULC enhanced HBV replication through the HAT1/HULC/HBc complex responsible for the accumulation on cccDNA minichromosome[13]. Moreover, the HULC genetic variant rs7763881 is associated with HBV infection[14].

LncRNA zinc ribbon domain containing 1-antisense RNA 1 (ZNRD1-AS1): A ZNRD1, cloned from the human leukocyte antigen region, should play an important role in immune response against HBV infection. ZNRD1-AS1 is an important regulator of ZNRD1. The variant allele of ZNRD1-AS1 (rs3757328) was reported to be associated with HBV clearance[15].

LncRNA HBx-long interspersed nuclear element 1 (HBx-LINE1): HBx-LINE1 suppresses miR-122 [16], a miRNA that has been demonstrated to inhibit HBV replication by directly targeting the HBV pgRNA sequence[17]. Thus, by depleting miR-122, HBx-LINE1 enhances HBV replication.

LncRNA AP000253: AP000253 was found to promote HBV transcription and replication in hepatoma cells[18]. However, the AP000253 expression in liver tissues and the molecular mechanism of its involvement in HBV infection are not clear yet.

### *LncRNAs suppressing HBV replication*

LncRNA HOXA transcript at the distal tip (HOTTIP): HOTTIP is a 3764 nt transcript mapped to the HOXA locus. HOTTIP was found to be induced by HBV in vitro. Further studies demonstrated that HBpAg could bind to and stabilize cAMP-responsive element-binding protein 1 (CREB1) mRNA to facilitate its translation. Then, the CREB1 protein would bind to the regulatory element of HOTTIP to promote its expression. HOTTIP significantly suppresses HBV replication through its downstream factor HOXA13, which was found to bind to HBV Enh I/Xp to reduce the production of pgRNA as well as HBV replication. Thus, HBpAg attenuates HBV replication *via* activation of the CREB1/HOTTIP/HOXA13 axis. In this way, lncRNA HOTTIP could restrain HBV replication and contribute to viral persistent infection[19].

### *Other lncRNAs involved in HBV replication*

H11, a novel inhibitor of La protein, suppressed HBV replication by blocking the interaction between La protein and HBV RNA. Further studies have shown that 61 lncRNAs were upregulated and 74 lncRNAs were downregulated in an H11 treatment group when compared with the control group[20]. These lncRNAs should affect HBV replication though further verification is required.

The importance of lncRNAs in HBV replication has started to emerge. However, many unidentified lncRNAs critical for HBV replication should exist, such as those regulated by HBx (mentioned in the following sections). HBx is required for transcription from the viral cccDNA minichromosome. Thus, HBx modulates HBV replication. The lncRNAs affected by HBx may modulate HBV replication. The

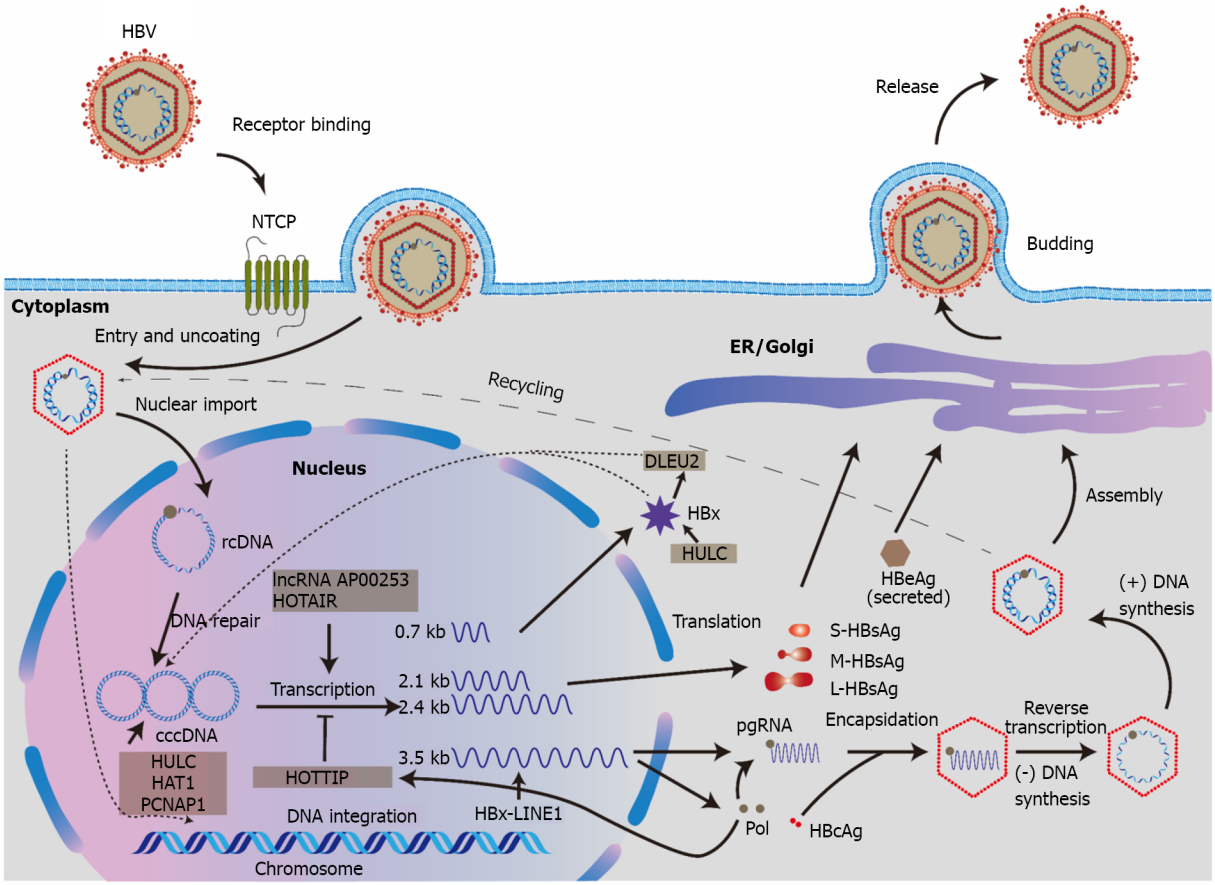


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**Figure 1 Long noncoding RNAs involved in the lifecycle of the hepatitis B virus.** Highly upregulated in liver cancer, HAT1, PCNAP1, and DLEU2 could enhance hepatitis B virus (HBV) replication[9,11,13]. LncRNA AP00253[18] and HOX transcript antisense RNA[10] promote, while HOXA transcript at the distal tip[19] inhibits, HBV transcription. HBV X protein-long interspersed nuclear elements 1 promotes HBV replication by suppressing miR-122, a miRNA directly targeting the HBV pgRNA sequence[16]. HULC: Highly upregulated in liver cancer; HBV: Hepatitis B virus; HBx: HBV X protein; LINE: Long interspersed nuclear elements; HOTTIP: HOXA transcript at the distal tip; HOTAIR: HOX transcript antisense RNA.

roles of these lncRNAs in HBV replication require further investigation.

## LNCRNAS IN HBV-RELATED IMMUNE RESPONSES

The roles of lncRNAs in the host immune system during HBV infection have just started to emerge. LncRNA ENST00000519726 (lncRNA-HEIM) was highly expressed in monocytes and was further upregulated upon HBV infection. Elevated lncRNA-HEIM expression was remarkably correlated with the TGF- signaling pathway. Furthermore, altering the endogenous lncRNA-HEIM amount in monocytes significantly affected TGF- production[21].

LncRNA FTX downregulates the expression of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, and nuclear factor kB (NF-kB) but upregulates the expression of Tim-3. This result demonstrated the effect of FTX on the expression of inflammatory cytokines through FTX-miR-545-TIM3 axis[22].

High lncRNA-CD160 expression level can inhibit interferon (IFN)-γ and TNF-α secretion in CD8+ T cells and decrease the immune response of CD8+ T cells. LncRNA-CD160 can interact with histone-modification enzyme gene histone deacetylase 11 (HDAC-11) to form a complex on the promoters of IFN-γ and TNF-α to inhibit their expression. Thus, lncRNA-CD160 acts as an immune suppressor. Indeed, knockdown of lncRNA-CD160 can block HBV infection[23].

Lnc-DC is a specific group of lncRNAs in dendritic cells. Lnc-DC could be activated by HBV infection. In addition, lnc-DC is important in regulating the growth, apoptosis, and immune response of dendritic cells mediated by TLR9/STAT3 signaling. Moreover, the regulation of lnc-DC controlled the immune response by reduction in secreted TNF-α, IL-6, IL-12, and IFN-γ, while increasing the IL-1β concentration in dendritic cells[24].

LncRNA#32 could positively regulate IFN-stimulated gene expression by interacting with activating transcription factor 2. Indeed, depletion of lncRNA#32 resulted in a significant increase in the replication of several viruses, including HBV. Thus, lncRNA#32 plays a role in host antiviral responses

[25].

The role of lncRNAs in the HBV-related immune responses remains unclear. There should be many unidentified lncRNAs important for HBV-related immune responses, such as those involved in HBV-related HCC.

## LNCRNAS IN HBV-RELATED CHRONIC DISEASES

Progression of liver disease from chronic HBV infection to HCC may include several stages[26], such as fibrosis and cirrhosis (Figure 2). The roles of lncRNAs in these HBV-related chronic diseases are largely unknown.

Transforming growth factor-β (TGF-β) plays an important role in various pathogenic processes, from inflammation, fibrosis, and cirrhosis to cancer. LncRNA-ATB, which is activated by TGF-β, is a key regulator of the TGF-β signaling pathway. The plasma levels of lncRNA-ATB in HBV-related cirrhosis patients were significantly higher than those in healthy controls[27].

Serum lincRNA-p21 levels in CHB patients, those with hepatitis B cirrhosis, and HBV-related HCC, were higher than those in the control subjects. LincRNA-p21 level was negatively correlated with levels of HBV DNA, alanine aminotransferase, and aspartate aminotransferase in patients with liver diseases. Thus, serum lincRNA-p21 may serve as a potential biomarker for liver cell damage in patients with hepatitis virus infection, hepatitis B cirrhosis, and HBV-related HCC[28].

Compared with that in healthy controls, HBV carriers, and CHB patients, the expression of lnc-TCL6 was obviously upregulated in Child–Pugh A patients with liver cirrhosis. Thus, lnc-TCL6 was identified as a sensitive biomarker for early diagnosis of liver cirrhosis (Child–Pugh A)[29].

LncRNA metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) expression in CHB group was significantly upregulated compared to the control group. Moreover, the thioredoxin interacting protein (TXNIP) was also significantly upregulated in the CHB group. Further studies indicate that the MALAT1/hsa-miR-20b-5p/TXNIP axis may mediate CHB-induced inflammatory damage in chronic HBV infection complicated with non-alcoholic fatty liver disease[30].

LncRNA-maternally expressed gene-3 (MEG3) was reported to be significantly downregulated in human HCC cell lines possibly due to the MEG3 promoter being hyper-methylated. Further studies indicated that the serum level of lncRNA-MEG3 was lower in CHB patients, which is negatively correlated to the liver fibrotic degree. In vitro experiments verified those results. Thus, lncRNA-MEG3 may serve as a diagnostic biomarker for CHB[31].

LncRNA growth arrest specific transcript 5 (GAS5) is significantly downregulated in CHB patients due to its promoter methylation. Compared with the sera of healthy controls, lower GAS5 levels were detected in the sera of CHB patients. Thus, lncRNA GAS5 is also reported as a biomarker for liver fibrosis in CHB patients[32].

CHB patients might progress to acute-on-chronic liver failure (ACLF) with a high fatality rate. Four lncRNAs (RP11-25K21.6, THRB, RAB27A, and GNPTAB) were found to be differentially expressed between the ACLF and the control groups. Aberrant lncRNAs might be used to develop novel diagnostic biomarkers and/or therapeutic targets for ACLF[33].

The role of lncRNAs in the HBV-related chronic diseases is not clear yet. Those lncRNAs involved in HBV-related HCC (mentioned in the following section) may also play roles in HBV-related chronic diseases. This requires further investigation.

## LNCRNAS DYSREGULATED IN HBV-RELATED HCC

HCC was the sixth most common cancer in world in 2020. Risk factors of HCC include external stimuli such as HBV or hepatitis C virus infection, intake of aflatoxin B1, alcohol consumption, smoking, and host factors such as age, gender, genetics, and comorbidities. Globally, approximately 2 billion people have been infected with HBV. Among them, more than 350 million people are chronic HBV carriers. Chronic HBV infection has been implicated in HCC development. In fact, persistent HBV infection occurs in more than half of HCC, particularly in developing countries. Comparing with other solid malignancies, HCC is characterized by its highly invasive and metastatic potential. Indeed, HCC is the third leading cause of cancer death in world. This is in part due to the fact that patients may not exhibit symptoms at early stages of HCC[34]. Therefore, comprehensive approaches are warranted to identify novel tumor markers and find more effective therapeutic targets to improve the diagnosis and treatment of HCC[8]. In recent years the idea has emerged that regulatory ncRNAs, such as miRNAs and lncRNAs, should play regulatory roles in cancers such as HCC[1]. Specifically, various lncRNAs were reported to regulate the expression of tumor suppressor genes or oncogenes involved in cancer development[2].

HCC caused by HBV infection is related to different HBV genotypes, the mutation status of viral genomes, integration of viral DNAs, and the dysregulation of signaling pathways affected by HBV. However, the detailed mechanisms of HBV-related HCC remain to be determined. The development of

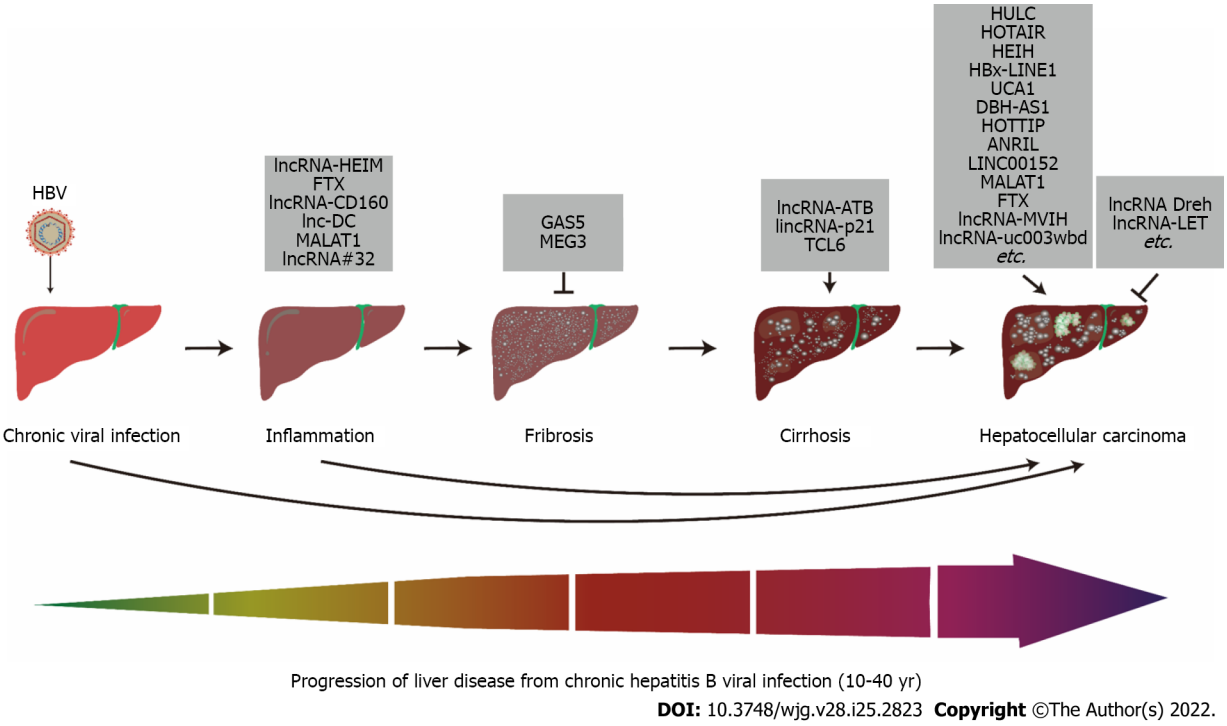


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**Figure 2 Various long noncoding RNAs are involved in the progression of liver diseases caused by the hepatitis B virus[26].** HULC: Highly upregulated in liver cancer; HBV: Hepatitis B virus; HBx: HBV X protein; LINE: Long interspersed nuclear elements; HOTTIP: HOXA transcript at the distal tip; HOTAIR: HOX transcript antisense RNA; GAS5: Growth arrest specific transcript 5; MEG3: Maternally expressed gene-3; MALAT1: Metastasis associated in lung adenocarcinoma transcript 1.

the majority of HBV-related HCCs is associated with: (1) Chronic inflammation; (2) Insertion of viral sequences into the cellular chromosomes; (3) Transactivation of growth regulatory genes by HBx; and (4) Altered versions of the preS/S envelope proteins[35]. If the host immune system fails to clear HBV, the infection will become chronic. Then, HBV DNA may integrate into the cellular chromosome. Indeed, integrated viral DNA is found in 85%–90% of HBV-related HCCs. Insertion of the HBV DNA occurs preferentially at certain sites in the host genome, including repetitive elements such as LINEs and Alu repeats[36]. The HBx gene is the smallest ORF encoding a 154-amino acid regulatory protein. HBx is reported to contribute to the pathogenesis of HCC by trans-modulating many growth regulatory genes and activating various signaling pathways, including p53, NF-kB, and Wnt signaling. Diverse lncRNAs involved in the events of viral immune responses, viral integration, and HBx regulatory activities should play roles in HBV-related HCC[37]. Studies on lncRNAs which are differentially expressed in HBV-HCC tissue samples may elucidate oncogenic pathways and identify novel diagnostic and therapeutic targets[8]. When compared to normal and/or non-HBV HCC samples, hundreds of dysregulated lncRNAs in HBV-related HCC tissues have been detected[38-40]. It is also reported that HBx could alter the expression of 2002 lncRNA[41]. In this mini-review, the better characterized lncRNAs in HBV-related HCC will be discussed.

## UPREGULATED LNCRNAS IN HBV-RELATED HCC

### *HULC*

HULC was the first lncRNA reported to be specifically upregulated in HCC through microarray analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). By interacting with CREB, HBx up-regulates the HULC expression in hepatoma cell lines and HBV-related HCC tissues. HULC downregulates the expression of p18, a tumor suppressor gene close to HULC, and thus promotes the proliferation of hepatoma cells[42]. Moreover, HULC can act as a molecular sponge for miR-107. By sponging miR-107, HULC upregulates E2F1 and then activates SPHK1 transcription in hepatoma cell lines and HBV-related HCC tissues. Thus, HULC promotes tumor angiogenesis through miR-107/E2F1/SPHK1 signaling[43]. Altogether, these studies indicate that HULC serves as an oncogene important for HBV-related HCC. HULC is also demonstrated to act as an endogenous 'sponge' for various other miRNAs (*e.g.*, miR-372, miR-186, miR-488, miR-200a-5p, miR-6825-5p, miR-6845-5p, and miR-6886-3p) in hepatoma cell lines and HCC tissues[44]. Those findings provide new insights into the mechanism of HULC in the HCC development (Table 1).

**Table 1 Long noncoding RNAs up-regulated in hepatitis B virus-related hepatocellular carcinoma**

| LncRNA name | Regulatory mechanism | Biological function |
|---|---|---|
| HULC[43] | Sequesters microRNAs and decreases p18 expression | Promotes proliferation of hepatoma cells and tumor angiogenesis *in vitro* and *in vivo* |
| HOTAIR[45,47] | Suppresses miRNA-218 expression and inactivation of P14 and P16 signaling | Promotes migration and invasion of HCC cells |
| HEIH[48] | Binds to EZH2 and represses EZH2 targets | Promotes cell proliferation and tumor growth, modulates cell cycle |
| HBx-LINE1[16,49] | Sequesters miR-122 and activates Wnt signaling pathway | Promotes cell motility through EMT |
| UCA1[53,54] | Through the HBx/UCA1/EZH2/p27 axis in nucleus; sequesters miR-216b and miR-203 in cytoplasm | Promotes growth, metastasis, and EMT of HCC cell lines |
| DBH-AS1[56] | Activates the ERK/p38/JNK MAPK signaling pathway | Inhibits serum starvation-induced apoptosis of HCC cells, promotes tumor growth, proliferation, and cell-cycle progression of HCC cells |
| HOTTIP[57] | Upregulates the expression of HOXA genes (*e.g.*, HOXA 10, 11, and 13) | Inhibits proliferation and migration of HCC cells *in vitro* and reduces tumorigenesis and pulmonary metastasis *in vivo* |
| ANRIL[59,60] | Represses the KLF2 transcription through binding to PRC2; serves as a molecular sponge for miR-122-5p | Inhibits apoptosis of HCC cells *in vitro* and promotes proliferation, invasion, and migration of HCC cells *in vitro* |
| LINC00152[61] | Inhibits the expression of E-cadherin; activates the mTOR pathway | Promotes the proliferation and EMT of HCC cell lines and tumorigenesis |
| MALAT1[63,66] | Upregulates LTBP3 transcription | Promotes migration and invasion of HCC cells *in vitro* and tumor growth *in vivo* |
| Ftx[22] | Production of miR-545/374a | Promotes tumorigenesis and tumor progression |
| MVIH[68] | Activates angiogenesis through reducing the secretion of PGK1 | Promotes tumor growth and metastasis |
| Unigene56159[69] | Sequesters miR-140-5p | Promotes EMT, migration, and invasion of hepatoma cells |
| LINC01232[70] | Downregulation of miR-708-5p | Promotes tumor progression *in vitro* |
| SAMD12-AS1[71] | Inhibition of p53 | Promotes cell proliferation and inhibits apoptosis |
| lnc-HUR1[72] | Inhibition of p53 | Promotes cell proliferation and tumorigenesis |
| n335586[73] | n335586/miR-924/CKMT1A axis | Contributes to cell migration and invasion |
| XIST[74] | XIST/miR-192/TRIM25 axis | Enhances the proliferation and migration of HCC cells |
| SNHG5[81] | SNHG5/miR-26a-5p/GSK3β signaling pathway | Promotes HCC progression |
| WEE2-AS1[76] | HBx/WEE2-AS1/FERMT3 pathway | Promotes HCC proliferation and invasion |
| MAFG-AS1[77] | HBx/ MAFG-AS1/MAFG | Promotes HCC proliferation and migration |
| lncRNA-ATB[78] | Induces autophagy | Promotes cell invasion and migration |
| TRERNA1[79] | TRERNA1/miR-22-3p/NRAS axis | Promotes cell proliferation in HCC |
| lncRNA IHS[80] | Regulates the ERK- and AKT/GSK-3β signaling pathways | Promotes tumor proliferation and metastasis in HCC |
| SNHG20[81] | Negatively regulated PTEN protein level | Facilitates the proliferation of HCC cells |
| AX800134[82] | Not known | Antiapoptosis |

LncRNAs: Long noncoding RNAs; HCC: Hepatocellular carcinoma; EMT: Epithelial-to-mesenchymal transition; HULC: Highly upregulated in liver cancer; HBV: Hepatitis B virus; HBx: HBV X protein; LINE: Long interspersed nuclear elements; HOTTIP: HOXA transcript at the distal tip; HOTAIR: HOX transcript antisense RNA; GAS5: Growth arrest specific transcript 5; MEG3: Maternally expressed gene-3; MALAT1: Metastasis associated in lung adenocarcinoma transcript 1; PGK1: Phosphoglycerate kinase 1.

### *HOTAIR*

The *HOTAIR* gene is on chromosome 12. LncRNA HOTAIR is a 2158 nt transcript derived from the *HOTAIR* gene. HOTAIR is involved in the occurrence of HBV-related HCC. HBV replication and, in particular, HBx production, stimulates expression of HOTAIR and Plk1. The combination of Plk1 and HOTAIR are involved in epigenetic reprogramming associated with oncogenic transformation[45]. *In vitro* studies have demonstrated that HOTAIR has sequence-specific effects and interacts with various chromatin modifying proteins, *e.g.*, Polycomb repressive complex 2 (PRC2)[46]. Another study showed

that HOTAIR might mediate hepatocarcinogenesis by down-regulating miR-218 and in-activating P14 and P16 signaling[47]. These findings suggest that lncRNA HOTAIR should play an important role in hepatocarcinogenesis (Table 1).

### *High expression in HCC*

The *high expression in HCC* (*HEIH*) gene is on chromosome 5. LncRNA HEIH is a polyadenylated, approximately 1600 nt in length, lncRNA, whose expression is strongly linked to HBV-associated HCC. It is located both in the nucleus and cytoplasm. HEIH could promote cell proliferation by upregulating PCNA and decreasing the expression of p16, p21, and p27 in cells. Moreover, HEIH could promote tumor growth in nude mice. Mechanistically, HEIH is physiologically associated with EZH2, the catalytic subunit of the PRC2. The association of HEIH with EZH2 is needed to repress the EZH2 target genes[48]. These studies demonstrate that HEIH contributes to HBV-related HCC through the participation of epigenetic silencing (Table 1).

### *HBx-LINE1*

Almost all HBV-related HCC tumors (85%–90%) have at least one HBV insertion site in the host genome. Thus, integration of HBV DNA should be important for the HCC development. LINEs appear to be a favored site for HBV DNA integration. To investigate the effect of HBV integration on genome disruption, HBx-LINE1, a viral-human chimeric fusion transcript derived from viral sequences containing HBx and cellular LINEs, was found functioning as an lncRNA. HBx-LINE1 was detected in 23.3% of HBV-related HCC patients[49,50]. HBx-LINE1, on chromosome 8p11.21, was transcribed from the HBx promoter. HBx-LINE1 contains six miR-122-binding sites and serves as a molecular sponge to sequester cellular miR-122. By downregulating miR-122, HBx-LINE1 activates the β-catenin signaling pathway, and in turn enhances HCC cell proliferation, invasion, and migration[16]. However, these findings were not consistent with those from other studies[51]. More studies with a larger sample size should help further clarify the role of HBx-LINE1 in the HBV-related HCC (Table 1).

### *Urothelial carcinoma associated 1*

The *urothelial carcinoma associated 1* (*UCA1*) gene is approximately 7.3 kb in length on chromosome 19p13.12 and contains three exons. It has three transcriptional isoforms. UCA1, around 1400 nt in length, is the most abundant isoform of the *UCA1* gene[52]. UCA1 was originally identified in the bladder cancer cell line and also played an important role in HBV-related HCC. HBx could upregulate UCA1 expression[37,53]. UCA1, an lncRNA detected in both the cytoplasm and nucleus[53], has diverse functions. In nucleus, UCA1 recruits EZH2 to the p27 promoter, reduces the p27 expression, and enhances CDK2. Thus, UCA1 could promote the growth of hepatic and hepatoma cells through the HBx/UCA1/EZH2/p27 axis[53]. In cytoplasm, UCA1 serves as a molecular sponge for miR-216b and miR-203. Through sequestering miR-216b, UCA1 could promote the growth and metastasis of HCC cell lines by up-regulating the expression of fibroblast growth factor receptor 1 and activating the extracellular signal-regulated kinase signaling pathway[54]. In addition, sequestering miR-203 facilitates epithelial-to-mesenchymal transition (EMT) in HCC cells through Snail2 upregulation[55]. Thus, UCA1 could promote cell cycle progression, enhance cell proliferation, and decrease cell apoptosis in HBx-related HCC[37] (Table 1).

### *DBH-AS1*

DBH-AS1 is an lncRNA of approximate 2 kb located on chromosome 9q34. HBx can upregulate the expression of DBH-AS1, which promotes HCC cell proliferation[37]. DBH-AS1 activates the ERK/p38/JNK MAPK signaling pathway. Once activated, ERK/p38/JNK MAPK can upregulate cyclin-dependent kinase 6 (CDK6), CCND1, and CCNE1 (members of cyclins) and downregulate p16, p21, and p27 (inhibitors of CDK). Thus, DBH-AS1 could induce the G1/S and G2/M transitions and promote cell proliferation. DBH-AS1 was also shown to protect HCC cells from serum starvation-induced apoptosis. These results suggested that DBH-AS1 acts as an oncogene[56]. However, a recent study showed contradicting results regarding the expression pattern of DBH-AS1[57]. To elucidate the exact role of DBH-AS1 in HBV-related HCC, more research is required (Table 1).

### *HOTTIP*

LncRNA HOTTIP, a 3764 nt transcript, is located on chromosome 7p15.2 and encodes from a genomic region in the 5′tip of the HOXA locus. LncRNA-HOTTIP was significantly overexpressed in tumor tissues compared to adjacent non-tumor tissues of the HCC patients. Patients with high HOTTIP expression were associated with increased metastasis formation and decreased overall survival. HOTTIP has also been detected to be upregulated significantly in HBV-related HCC patients[57]. Elevated HOTTIP expression could enhance cell proliferation and migration and contribute to metastasis of HCC partly by upregulating its neighboring *HOXA* genes (*e.g.*, HOXA 10, 11 and 13), which are associated with various cancer types. These findings suggest that HOTTIP acts as an oncogene in HBV-related HCC (Table 1).

### *Antisense ncRNA in the INK4 locus*

Antisense ncRNA in the INK4 locus (ANRIL), a 3800 nt lncRNA, is in the *INK4BARF-INK4A* gene cluster[58]. ANRIL is upregulated in several cancers including HBV-related HCC[57]. The ANRIL expression was associated with tumor size, histological grade, and overall survival in HCC patients[59]. These findings indicate that ANRIL plays a role in the HCC development, particularly HBV-related HCC. Knockdown of ANRIL expression in HCC cells *in vitro* could induce apoptosis and reduce the proliferation, invasion, and migration of these HCC cells[60]. Furthermore, inhibition of ANRIL led to slower tumor growth *in vivo*[59,60]. ANRIL represses the KLF2 transcription through binding with PRC2[59]. ANRIL also serves as a molecular sponge for miR-122-5p, whose overexpression significantly repressed the proliferation, migration, and invasion of HCC cells[60]. These findings indicate that ANRIL is also an oncogene in HBV-related HCC (Table 1).

### *LINC00152*

LINC00152, an lncRNA of 828 nt, is mapped to chromosome 2p11.2 and contains four exons. It is mainly localized in the nucleus of HCC cells. The LINC00152 expression is associated with tumor size, HBV infection, and HBx amount[61]. Elevated LINC00152 expression also results in decreased overall survival[61]. LINC00152 is up-regulated by HBx protein and enhances proliferation and EMT of HCC cell lines *in vitro* and tumorigenesis *in vivo*[61]. LINC00152 has been shown to activate the mTOR pathway, which is a classic dysregulated pathway involved in the pathogenesis of HCC. In addition, LINC00152 promotes EMT by reducing the binding of EZH2 to the E-cadherin promoter and suppressing E-cadherin expression in HCC cell lines[61]. Ablation of E-cadherin will lose cell–cell contacts, resulting in EMT. These studies suggest that LINC00152 contributes to HBV-related HCC (Table 1).

### *Metastasis-associated lung adenocarcinoma transcript 1*

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (also known as nuclear-enriched abundant transcript 2), an lncRNA of approximate 8000 nt in length, is mainly localized in the nucleus [62]. MALAT1 expression is elevated by HBx in HCC tissues and cell lines[63]. Sp1 and Sp3, also found upregulated, would bind to the proximal promoter region and enhance the transcription of MALAT1 [64]. MALAT1 affects alternative splicing and gene expression[62,65]. MALAT1 could promote tumor growth and metastasis by upregulating latent-transforming growth factor beta-binding protein 3 (LTBP3) expression. These results suggest that MALAT1 mediates the oncogenic effect of HBx through enhancing the LTBP3 expression, which promotes early metastatic events[63]. Moreover, a higher MALAT1 expression correlates to HCC recurrence after liver transplantation[66]. Additionally, knockdown of MALAT1 has been shown to reduce cell viability, motility, and invasiveness and increase sensitivity to apoptosis in HepG2 cells[66]. Therefore, MALAT1 is also an oncogene contributing to the risk of HBV-related HCC (Table 1).

### *Ftx*

LncRNA Ftx is transcribed within the X-inactivation center[67]. Ftx encodes four miRNA clusters in its introns, among them, miR-545/374a located in intron b of Ftx has been implicated in HBV-associated HCC[22]. Expression of miR-374a and miR-545 was significantly higher in tumor tissues of HBV-related HCC. Further investigations suggested that miR-545/374a may contribute to poor prognosis by enhancing tumor invasion. This was supported by the observation that the proliferation of malignant cells was significantly suppressed in the presence of miR-545/374a inhibitors. On the other hand, overexpression of the miRNAs resulted in increased proliferation. miR-545/374a also enhanced migration and invasion abilities of HCC cells. Moreover, clinical samples positive for HBV DNA showed an increase in miR-545/374a. The upregulation of miR545 was also found to be HBx-dependent. Three targets for miR-545/374a were identified: Estrogen-related receptor alpha, estrogen-related receptor gamma (ESRRG), and arginine and glutamate rich. Experimental evidence demonstrated that miR-545 could downregulate ESRRG expression. Additionally, miR-374a had been shown to activate Wnt signaling pathway. In conclusion, lncRNA Ftx involves HBV-related HCC by serving as miRNAs precursor (Table 1).

### *Microvascular invasion in HCC*

Microvascular invasion in HCC (MVIH) is situated within the intron of the *ribosomal protein S24* gene. The MVIH expression was significantly upregulated in HBV-related HCC. Highly expressed MVIH was associated with frequent microvascular invasion and decreased overall survival[68]. Further investigations found that MVIH could promote tumor growth and metastasis by enhancing angiogenesis through reducing the secretion of phosphoglycerate kinase 1 suppressing angiogenesis[68] (Table 1).

### *Unigene56159*

Unigene56159, an lncRNA of 2653 nt, is in the second intron of ROBO1. Unigene56159 was elevated in HBV-related HCC and HBV-producing cell line. Further investigations have found that Unigene56159, induced by HBV, could promote the EMT, migration, and invasion of hepatoma cells through seques-

tering miR-140-5p and up-regulating the Slug expression[69].

In addition to the abovementioned lncRNAs, the expression of LINC01232[70], SAMD12-AS1[71], lncHUR1[72], n335586[73], XIST[74], and SNHG5[75] was found to be increased in HBV-related HCC. Moreover, the expression of WEE2-AS1[76], MAFG-AS1[77], lncRNA-ATB[78], TRERNA1[79], lncRNA-IHS[80], SNHG20[81], and AX800134[82] was upregulated by HBx.

Those upregulated lncRNAs by HBV and/or HBx may be involved in HCC progress through diverse mechanisms to enhance HCC cell proliferation, invasion, and metastasis, and/or prevent the death of HCC cells (*e.g.*, apoptosis)[83] (Table 1).

## DOWNREGULATED LNCRNAS IN HBV RELATED HCC

### *Downregulated expression by HBx*

Downregulated expression by HBx (Dreh), an lncRNA of approximate 700 nt in length, is mapped to chromosome 5. The Dreh expression was downregulated by HBx in HCC cell lines. Dreh is also significantly downregulated in HBV-related HCC tissues. Suppression of DREH facilitates proliferation of hepatoma cells *in vitro* and also tumor growth *in vivo*[84]. Lower Dreh expression is associated with the recurrence-free survival and overall survival of HCC patients[84]. Playing as a tumor suppressor in the development of HBV-related HCC, Dreh suppressed cell proliferation and cell migration *in vitro* and *in vivo*. By binding to the intermediate filament protein vimentin, lncRNA Dreh inhibits its expression and alters its filament structure to repress tumor cell migration. Therefore, HBx inhibits Dreh expression and in turn facilitates HCC (Table 2).

### *Low expression in tumor*

LncRNA-low expression in tumor (LET) is an lncRNA identified to be decreased in HBV-related HCC. LncRNA-LET and nuclear factor 90 (NF90) are associated with each other. LncRNA-LET could downregulate NF90. NF90 has been implicated in the stabilization of many factors [*e.g.*, hypoxia induced factor 1-α (HIF-1α)] related to tumor growth and metastasis[85,86]. The LET/NF90/HIF-1α axis may be critical for HCC invasion in hypoxic environments. Under hypoxia conditions, induced HDAC-3 suppressed LET expression, which increased the expression of NF90 and HIF-1α, and, hence, enhanced the invasiveness of HCC and contributed to HCC progression[85].

In addition to the abovementioned lncRNAs, the expression of uc.306[87], UPAT[88], SEMA6A-AS1 [89], BANCR[90], and miR143HG[91] was found to be decreased in HBV-related HCC. Moreover, the expression of lncRNA-6195[92], LINC01352[93], and F11-AS1[94] was suppressed by HBx. These downregulated lncRNAs by HBV and/or HBx serve as tumor repressors and suppress HCC cell proliferation (Table 2).

## OTHER LNCRNAS IN HBV-RELATED HCC

### *H19*

The *H19* gene is located adjacent to the *insulin-like growth factor 2* gene on chromosome 11p15.5. This gene produces a 2.3 kb lncRNA, which is exclusively expressed from the maternal allele. Reports of H19 expression in HCC are controversial. Recently, lncRNA H19 was found to be upregulated in CHB patients[95]. On the other hand, lncRNA H19 could suppress the growth of hepatoblastoma cells by promoting their apoptosis[96].

Most of these dysregulated lncRNAs are modulated by HBx, it would help to know whether altered versions of the preS/S envelope proteins would modulate the expression of lncRNAs or not. The progress of research on the role of lncRNAs in HBV-related HCC is impressive. However, the functions of a large proportion of lncRNAs dysregulated in HBV-related HCC remain elusive. Future investigation on the function of lncRNAs in HBV-related HCC will shed the light on understanding the detailed mechanisms regarding the development of HBV-induced HCC. Understanding more regarding the molecular mechanisms underlying lncRNA-mediated oncogenesis should help for their use in diagnosis and to identify appropriate targets for prevention and treatment of HBV-related HCC.

## GENETIC POLYMORPHISMS OF LNCRNAS IN HBV REPLICATION AND ONCOGENESIS

A number of dysregulated lncRNAs have been identified in HBV replication and oncogenesis[2,37]. Genetic polymorphisms affecting the expression (changes in the enhancer/promoter region) or activity (differences in the coding region) of these lncRNAs may also affect HBV replication and oncogenesis. Association studies are used to identify genetic variations of lncRNAs involved in HBV replication and oncogenesis[2].

**Table 2 Long noncoding RNAs downregulated in hepatitis B virus-related hepatocellular carcinoma**

| LncRNA name | Regulatory mechanism | Biological function |
|---|---|---|
| Dreh[84] | Inhibits the vimentin expression and alters its structure | Inhibits proliferation of HCC cells *in vitro* and tumor growth *in vivo* |
| LET[85] | LET/NF90/HIF1-α axis | Inhibits HCC invasion |
| uc.306[87] | May participate in the Wnt pathway | Not known |
| UPAT[88] | Promotes ZEB1 degradation | Suppresses cellular migration, invasion, and EMT processes |
| SEMA6A-AS1[89] | Not known | Not known |
| BANCR[90] | Not known | Not known |
| miR143HG[91] | Suppresses the MAPK and Wnt signaling pathways | Inhibits tumor multiplication and metastasis |
| LncRNA-6195[92] | Represses the enzymatic activity of ENO1 | Represses the growth of HCC |
| LINC01352[93] | LINC01352/miR-135b/APC axis | Facilitates HCC progression |
| F11-AS1[94] | lncRNA F11-AS1/miR-211-5p/NR1l3 axis | Inhibits HCC growth and metastasis |

LncRNAs: Long noncoding RNAs; HCC: Hepatocellular carcinoma; EMT: Epithelial-to-mesenchymal transition; HIF-1α: Hypoxia induced factor 1-α.

## GENETIC POLYMORPHISMS IN THE ENHANCER/PROMOTER OF LNCRNAS

The intronic enhancer of HOTAIR had significantly higher HOTAIR levels in the rs920778 TT genotype than in the CC genotype. By upregulating HOTAIR, the rs920778 TT genotype promotes the development of HBVrelated HCC and increases proliferation of HCC cells[97].

LncRNA GAS5 (growth arrest special 5) has been found to be downregulated in HCC patients[98]. A five-base-pair Ins/Del polymorphism (rs145204276) in the promoter region of GAS5 could affect the GAS5 expression. The deletion allele of rs145204276 increases the risk of HBV-related HCC significantly, though the detailed mechanism is unclear[99].

The sequence of RERT-lncRNA overlaps with those of the Ras-related GTP-binding protein 4b and prolyl hydroxylase 1 (EGLN2). An Ins/Del polymorphism (rs10680577) in the distal promoter of RERT-lncRNA may alter the structure of RERT-lncRNA and regulate the expression of RERT-lncRNA and EGLN2. A 4-bp deletion allele of rs10680577 in RERT-lncRNA increases expression of RERT-lncRNA and EGLN2 and promotes the occurrence of HBV-related HCC[100].

## GENETIC POLYMORPHISMS IN THE CODING REGION OF LNCRNAS

A polymorphism in the coding region of KCNQ1-overlapping transcript 1 (KCNQ1OT1), *i.e.* rs35622507, may alter the structure of KCNQ1OT1 and modulate the expression of KCNQ1OT1 and cyclin-dependent kinase inhibitor 1C (CDKN1C). The homozygous 10-10 genotype with significantly lower expression of KCNQ1OT1 and higher expression of CDKN1C was shown to increase the risk of HBV-related HCC[101].

LINC01149 variant rs2844512 was identified to facilitate HBV spontaneous recovery but increase the risk of HCC. Further studies indicated this variant created a binding site for miR-128-3p and upregulated MICA expression by serving as a miRNA sponge[102].

## OTHER GENETIC POLYMORPHISMS IN LNCRNAS

The variant allele of ZNRD1-AS1 (*i.e.*, rs3757328) is reported to associate with HBV clearance[15].

The SNPs in glutamate-ammonia ligase overlapping with LINC00272 were associated with increased risk of HBV-related HCC[103].

Caspase recruitment domain family, member 8 (CARD8) can coordinate innate and adaptive immune responses and may participate in HBV-related HCC. Several SNPs (rs7248320) within a new identified lncRNA AC008392.1, which is located in the upstream region of CARD8 in the long arm of the 9-chromosome, affect the CARD8 expression and may serve as a susceptibility marker for HBV-related HCC[104].

Three regulatory SNPs (rs3757328, rs6940552 and rs9261204) in the ZNRD1-AS1 and the HBV genotype significantly affected HCC susceptibility. The results indicate that ZNRD1-AS1 accompanied by HBV genotypes may influence HCC risk[15].

Tumor necrosis factor receptor superfamily member 10 (TNFRSF10) including TNFRSF10A and TNFRSF10B is a death domain-containing receptor for the apoptotic ligand TNFSF10. In the upstream region of TNFRSF10A and downstream region of TNFRSF10B, lncRNAs RP11-1149O23.3 and RP11-459E5.1 were identified, respectively. The two lncRNAs are possibly involved in the regulation of TNFRSF10A and TNFRSF10B. Several SNPs (*i.e.*, rs79037040-T and rs2055822-A) in RP11-1149O23.3 and RP11-459E5.1 may affect the expression of TNFRSF10A and TNFRSF10B and may be susceptibility markers for HCC and chronic HBV infection[105].

Paired-box family member PAX8 encodes a transcription factor that may participate in the prognosis of HCC. Several SNPs (rs4848320 and rs1110839) within AC016683.6 lncRNA may affect the PAX8 expression and affect the prognosis of HBV-related HCC[106].

The association of lnc-RP11-150O12.3 variants (rs2275959, rs1008547, and rs11776545) with HBV-related HCC risk and progression has been reported[107].

LncRNAs HULC and MALAT1 have been upregulated in HBV-related HCC. Genetic variants of lncRNA HULC (rs7763881) and lncRNA MALAT1 (rs619586) are associated with the decreased susceptibility to HCC[108].

These studies suggest that genetic polymorphisms in lncRNAs should affect the development and outcome of HBV-related HCC. This may help to identify appropriate targets for prevention and treatment of HCC in the age of personalized medicine.

## REGULATORY MECHANISMS OF LNCRNAS IN HBV REPLICATION AND ONCOGENESIS

LncRNAs can function as signals, decoys, guides, or scaffolds through interacting with their partner molecules and regulate gene expression at different levels. Up to now, at least seven mechanisms have been found to affect HBV replication and oncogenesis by dysregulated lncRNAs: Epigenetic silencing, transcriptional control, splicing regulation, molecular sponge, production of miRNAs, protein stability modulation, and production of a small polypeptide (Figure 3)[2,109,110].

Epigenetic silencing: Polycomb group (PcG) proteins are epigenetic regulators of transcription. Through multiprotein complexes PRCs, PcGs could modify histones (and other proteins) and silence target genes. LncRNAs may decrease the expression of their target genes through epigenetic silencing either by altering the expression level of PcGs or by interacting with PcGs directly and then silencing their target genes. HOTAIR enhances the degradation of suppressor of Zeste 12 homolog, a key subunit of PRC2, through Plk1[45]. Several lncRNAs interact with EZH2, a component of PRC2 to modulate (repress in most cases) the expression of their target genes, *e.g.*, HEIH[48], UCA1[53], HOTAIR[47], LINC00152[61], and PVT1[111]. ANRIL can interact with PRC2[112].

Transcriptional control: LncRNAs can activate the transcription of their target genes in cis or in trans. HOTTIP could promote the expression of its neighboring homeobox A genes (*HOAX* genes) in HCC [57]. LINC00152 binds to the promoters of epithelial cell adhesion molecule and IL-23 to activate their transcription in HCC patients[113]. MALAT1 could up-regulate the expression of LTBP3[63].

Splicing regulation: LncRNAs could affect the expression of their target genes through alternative splicing regulation. MALAT1, a nuclear lncRNA, could interact with the SR proteins (serine/arginine-rich family of nuclear phosphoproteins) and change the cellular levels of the phosphorylated forms of SR proteins to modulate alternative splicing in HCC[62].

Molecular sponge: LncRNAs could exert their effects by acting as molecular sponges for miRNAs, *i.e.*, competitive endogenous RNA (ceRNA)[114]. Through molecular sponges, lncRNAs sequester miRNAs and then de-repress the expression of the miRNA target genes. Several lncRNAs, such as Unigene56159 [69], HULC[115], HBx-LINE1[16], UCA1[55], ANRIL[60], LINC01149 variant[102], LINC01352[93], F11-AS1[94], LINC01232[70], n335586[73], XIST[74], SNHG5[75], SSTR5-AS1[116], and TRERNA1[79] are involved in the development of HBV-related HCC by sequestering miRNAs.

Production of miRNAs: LncRNAs could produce miRNAs to regulate their target genes by serving as precursors of miRNAs. The Ftx transcript encodes two clusters of miRNAs (*i.e.*, miR-374a and miR-545) in its introns in HBV-related HCC[117].

Protein stability modulation: LncRNAs could stabilize their protein targets by direct interactions. LncRNA-Dreh could bind vimentin, a type III intermediate filament, and stabilize its filament structure [84]. LncRNAs could also stabilize their protein targets by reduced degradation of these proteins indirectly. By enhancing ubiquitin-specific peptidase 22, HULC suppresses ubiquitin-mediated degradation of cyclooxygenase-2 and silent information regulator 1 in HCC[118].

Production of a small polypeptide: A putative lncRNA HBVPTPAP could encode a small polypeptide to induce the apoptosis of HCC cells[110].

Through these different mechanisms, lncRNAs can regulate gene expression. As more lncRNAs are identified, more novel mechanisms will be revealed.

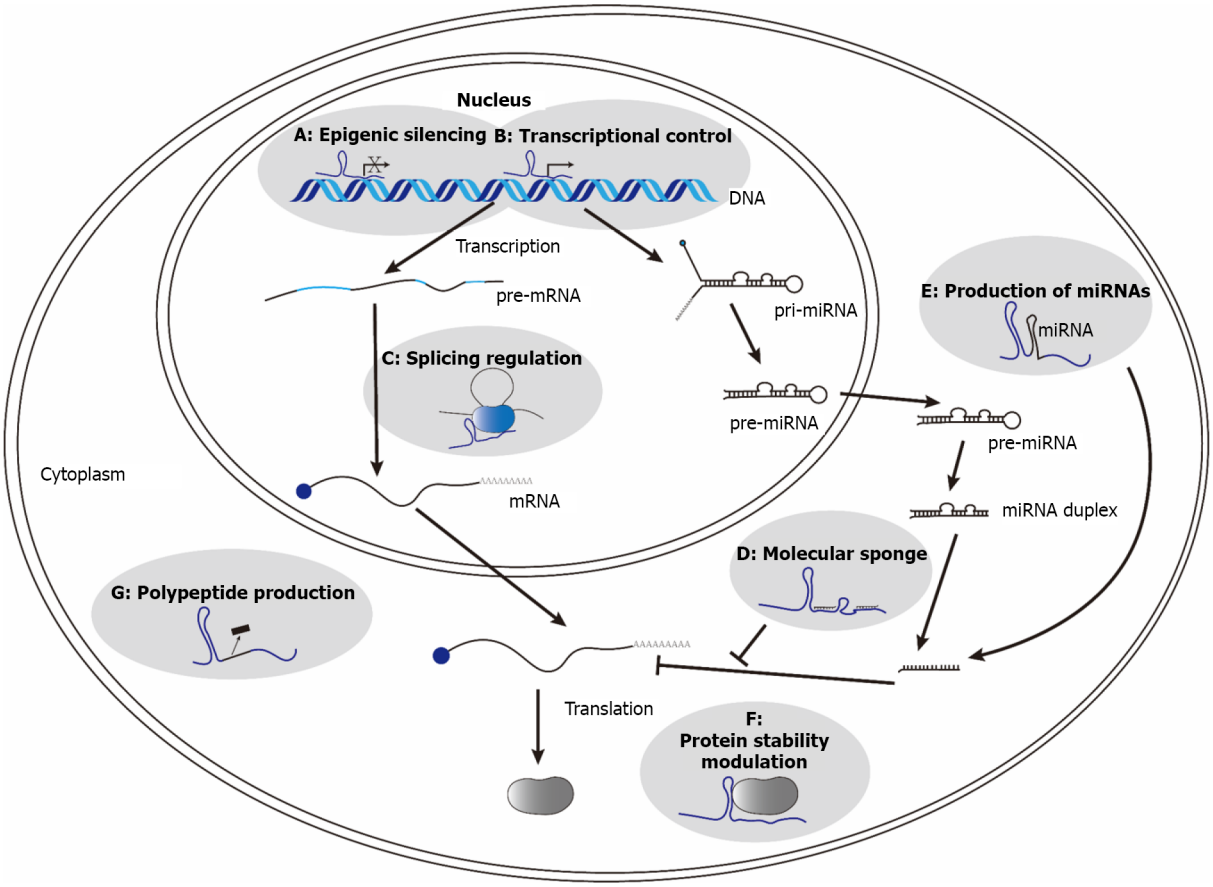


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**Figure 3 Regulatory mechanisms of various long noncoding RNAs in hepatitis B virus replication and oncogenesis[2].** A: HOX transcript antisense RNA (HOTAIR), high expression in hepatocellular carcinoma, urothelial carcinoma associated 1 (UCA1), HOTAIR, LINC00152, PVT1, and antisense noncoding RNA in the INK4 locus (ANRIL) could regulate gene expression through epigenetic silencing; B: HOXA transcript at the distal tip, LINC00152, and metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) could increase the expression of their target genes through transcriptional control; C: MALAT1 could modulate alternative splicing; D: Unigene56159, highly upregulated in liver cancer (HULC), hepatitis B virus (HBV) X protein-long interspersed nuclear elements 1, UCA1, ANRIL, LINC01149 variant, LINC01352, F11-AS1, LINC01232, n335586, XIST, SNHG5, SSTR5-AS1, and TRERNA1 regulate the gene expression through molecular sponging to sequester miRNAs; E: Long noncoding RNAs (lncRNAs) Ftx could produce miRNAs to regulate their target genes; F: LncRNA-Dreh and HULC could modulate protein stability; G: LncRNA HBVPTPAP could encode a small polypeptide to exert its function.

## LNCRNAS AS POTENTIAL BIOMARKERS FOR DIAGNOSIS AND THERAPY OF HBV-RELATED HCC

It is suggested that HOTAIR may potentially be a novel HBV diagnostic biomarker based on its ability to facilitate HBV transcription and replication. This may not be practical because the commercially available diagnostic kits for HBV infection already have high specificities and sensitivities. On the other hand, the specificity and sensitivity of currently available tumor markers for HBV-related HCC (*e.g.*, α-fetoprotein) are not sufficient. This results in the poor prognosis of HBV-related HCC. This has encouraged researchers to search for novel potential biomarkers of HCC. Various miRNAs have been accepted to be biomarkers for diagnosis and therapy for HCC[119]. As an increasing number of lncRNAs, as well as the genetic polymorphisms in lncRNAs, have been found to contribute in the development of HBV-related HCC, researchers have tried to study whether these lncRNAs could be used as novel biomarkers for HBV-related HCC[120]. To be tumor markers, lncRNAs have the advantage over proteins because they can be amplified easily, *e.g.*, by RT-PCR. A number of lncRNAs dysregulated in HBV-related HCC have been suggested to be potential markers for HCCs. If these lncRNAs can be secreted to biological fluids, *e.g.*, blood, they can be easily detected. Therefore, those secreted lncRNAs are suggested to be potential markers for HBV-related HCC, such as HULC[121], IGF2AS[122], Linc00152[121], lncRNA-uc003wbd[123], lncRNA-AF085935[123], uc001ncr[124], AX800134[124], and UCA1[53-55]. However, most of these circulating lncRNAs aberrantly expressed in HBV-related HCC are also dysregulated in other tumors. It is better to identify a circulating lncRNA specific for HBV-related HCC diagnosis[125]. Alternatively, detection of several lncRNAs together could be an option[126-130].

Gene therapies such as RNA interference may become a common treatment for HCC in the future. In this way, lncRNAs can be the new therapeutic targets for HBV-related HCC. It is reported that metformin can suppress the HULC expression and block the progression of HBV-related HCC[131]. Thus, HULC could be a potential therapeutic target for RNA interference in HBV-related HCC treatment. Other lncRNAs upregulated in HBV-related HCC are also reported to be potential therapeutic targets[8] , such as MVIH[68] and HBx-LINE1[16]. On the other hand, an effective way to deliver lncRNA MEG3 RNA using MS2 virus-like-particles to inhibit HCC cells has been developed [132]. Other lncRNAs downregulated in HBV-related HCC could use a similar approach to treat HBV-related HCC, such as DREH[84] and LET[85]. Other advances in gene and epigenetic editing will enable correcting the aberrations caused by the dysregulated lncRNAs in HBV-related HCC in the future.

## CONCLUSION

The important roles of lncRNAs in HBV replication and oncogenesis have started to emerge in recent years. Growing evidence indicates that some lncRNAs are dysregulated in HBV-infected cells and/or HBV-related HCC. Functional characterization of these lncRNAs has led to a more comprehensive understanding of HBV replication and oncogenesis. These lncRNAs have diverse functions, which are now beginning to be revealed. Although the progress regarding the role of lncRNAs in HBV-related HCC has been impressive, their roles in HBV replication, host immune responses, and HBV-related chronic diseases need further characterization. Even in HBV-related HCC, only a small part of lncRNAs has been well characterized, and a large portion of lncRNAs remains to be further explored. Thus, more efforts are required to understand the detailed mechanisms of these lncRNAs in the progression of HCC. The growing studies on the roles of lncRNAs in hepatocarcinogenesis could result in designing LncRNAs as potential biomarkers for diagnosis and therapy of HBV-related HCC.

Several technologies such as chromatin immunoprecipitation (ChIP), RNA sequencing, ChIP sequencing, tiled RNA expression arrays, cap analysis of gene expression, and serial analysis of gene expression have been used to detect lncRNAs. Only recently, different types of post-transcriptional chemical modifications of RNAs have been detected and characterized through sequencing-based, transcriptome-wide studies, *e.g.*, pseudouridine (Ψ), N6–methyladenosine and 5-methylcytosine. These modifications have been shown to affect the fate of RNA, including lncRNAs[133]. Further details about the roles of these chemical modifications of lncRNAs in HBV replication and oncogenesis are expected in the near future.

## FOOTNOTES

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## REFERENCES

1 **Han TS**, Hur K, Cho HS, Ban HS. Epigenetic Associations between lncRNA/circRNA and miRNA in Hepatocellular Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 32937886 DOI: 10.3390/cancers12092622]
2 **Zhang H**, Chen X, Zhang J, Wang X, Chen H, Liu L, Liu S. Long noncoding RNAs in HBVrelated hepatocellular carcinoma (Review). *Int J Oncol* 2020; **56**: 18-32 [PMID: 31746420 DOI: 10.3892/ijo.2019.4909]
3 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

4 **Tong S**, Revill P. Overview of hepatitis B viral replication and genetic variability. *J Hepatol* 2016; **64**: S4-S16 [PMID: 27084035 DOI: 10.1016/j.jhep.2016.01.027]

5 **Tsukuda S**, Watashi K. Hepatitis B virus biology and life cycle. *Antiviral Res* 2020; **182**: 104925 [PMID: 32866519 DOI: 10.1016/j.antiviral.2020.104925]

6 **Herrscher C**, Roingeard P, Blanchard E. Hepatitis B Virus Entry into Cells. *Cells* 2020; **9** [PMID: 32570893 DOI: 10.3390/cells9061486]

7 **Wei L**, Ploss A. Hepatitis B virus cccDNA is formed through distinct repair processes of each strand. *Nat Commun* 2021; **12**: 1591 [PMID: 33707452 DOI: 10.1038/s41467-021-21850-9]

8 **Qiu L**, Tang Q, Li G, Chen K. Long non-coding RNAs as biomarkers and therapeutic targets: Recent insights into hepatocellular carcinoma. *Life Sci* 2017; **191**: 273-282 [PMID: 28987633 DOI: 10.1016/j.lfs.2017.10.007]

9 **Salerno D**, Chiodo L, Alfano V, Floriot O, Cottone G, Paturel A, Pallocca M, Plissonnier ML, Jeddari S, Belloni L, Zeisel M, Levrero M, Guerrieri F. Hepatitis B protein HBx binds the DLEU2 LncRNA to sustain cccDNA and host cancer-related gene transcription. *Gut* 2020; **69**: 2016-2024 [PMID: 32114505 DOI: 10.1136/gutjnl-2019-319637]

10 **Ren F**, Ren JH, Song CL, Tan M, Yu HB, Zhou YJ, Qin YP, Cheng ST, Zhang Y, Huang AL, Chen J, Yang X. LncRNA HOTAIR modulates hepatitis B virus transcription and replication by enhancing SP1 transcription factor. *Clin Sci (Lond)* 2020; **134**: 3007-3022 [PMID: 33103728 DOI: 10.1042/CS20200970]

11 **Feng J**, Yang G, Liu Y, Gao Y, Zhao M, Bu Y, Yuan H, Yuan Y, Yun H, Sun M, Gao H, Zhang S, Liu Z, Yin M, Song X, Miao Z, Lin Z, Zhang X. LncRNA PCNAP1 modulates hepatitis B virus replication and enhances tumor growth of liver cancer. *Theranostics* 2019; **9**: 5227-5245 [PMID: 31410212 DOI: 10.7150/thno.34273]

12 **Liu Y**, Feng J, Sun M, Yang G, Yuan H, Wang Y, Bu Y, Zhao M, Zhang S, Zhang X. Long non-coding RNA HULC activates HBV by modulating HBx/STAT3/miR-539/APOBEC3B signaling in HBV-related hepatocellular carcinoma. *Cancer Lett* 2019; **454**: 158-170 [PMID: 30981758 DOI: 10.1016/j.canlet.2019.04.008]

13 **Yang G**, Feng J, Liu Y, Zhao M, Yuan Y, Yuan H, Yun H, Sun M, Bu Y, Liu L, Liu Z, Niu JQ, Yin M, Song X, Miao Z, Lin Z, Zhang X. HAT1 signaling confers to assembly and epigenetic regulation of HBV cccDNA minichromosome. *Theranostics* 2019; **9**: 7345-7358 [PMID: 31695772 DOI: 10.7150/thno.37173]

14 **Shahen SM**, Elshenawy SZ, Mohamed SE, Talaat RM. Genetic polymorphisms in the miR-372 (rs12983273) and LncRNA HULC (rs7763881) genes and susceptibility to Hepatitis B virus (HBV) infection. *Mol Biol Rep* 2021; **48**: 7901-7906 [PMID: 34677712 DOI: 10.1007/s11033-021-06818-8]

15 **Wen J**, Liu Y, Liu J, Liu L, Song C, Han J, Zhu L, Wang C, Chen J, Zhai X, Shen H, Hu Z. Expression quantitative trait loci in long non-coding RNA ZNRD1-AS1 influence both HBV infection and hepatocellular carcinoma development. *Mol Carcinog* 2015; **54**: 1275-1282 [PMID: 25110835 DOI: 10.1002/mc.22200]

16 **Liang HW**, Wang N, Wang Y, Wang F, Fu Z, Yan X, Zhu H, Diao W, Ding Y, Chen X, Zhang CY, Zen K. Hepatitis B virus-human chimeric transcript HBx-LINE1 promotes hepatic injury *via* sequestering cellular microRNA-122. *J Hepatol* 2016; **64**: 278-291 [PMID: 26409216 DOI: 10.1016/j.jhep.2015.09.013]

17 **Li C**, Wang Y, Wang S, Wu B, Hao J, Fan H, Ju Y, Ding Y, Chen L, Chu X, Liu W, Ye X, Meng S. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol* 2013; **87**: 2193-2205 [PMID: 23221562 DOI: 10.1128/JVI.02831-12]

18 **Hao Q**, Wang Z, Wang Q, Chen B, Qian H, Liu X, Cao H, Xia W, Jiang J, Lu Z. Identification and characterization of lncRNA AP000253 in occult hepatitis B virus infection. *Virol J* 2021; **18**: 125 [PMID: 34112188 DOI: 10.1186/s12985-021-01596-y]

19 **Zhao X**, Fan H, Chen X, Zhao X, Wang X, Feng Y, Liu M, Li S, Tang H. Hepatitis B Virus DNA Polymerase Restrains Viral Replication Through the CREB1/HOXA Distal Transcript Antisense RNA Homeobox A13 Axis. *Hepatology* 2021; **73**: 503-519 [PMID: 32314410 DOI: 10.1002/hep.31284]

20 **Pan J**, Tong S, Tang J. LncRNA expression profiles in HBV-transformed human hepatocellular carcinoma cells treated with a novel inhibitor of human La protein. *J Viral Hepat* 2018; **25**: 391-400 [PMID: 29091324 DOI: 10.1111/jvh.12821]

21 **Yao J**, Lin C, Jiang J, Zhang X, Li F, Liu T, Diao H. lncRNA-HEIM Facilitated Liver Fibrosis by Up-Regulating TGF-$\beta$ Expression in Long-Term Outcome of Chronic Hepatitis B. *Front Immunol* 2021; **12**: 666370 [PMID: 34168644 DOI: 10.3389/fimmu.2021.666370]

22 **Liu X**, Li C, Zhu J, Li W, Zhu Q. Dysregulation of FTX/miR-545 signaling pathway downregulates Tim-3 and is responsible for the abnormal activation of macrophage in cirrhosis. *J Cell Biochem* 2018 [PMID: 30304545 DOI: 10.1002/jcb.27562]

23 **Wu J**, Niu Q, Yuan J, Xu X, Cao L. lncRNA-CD160 decreases the immunity of $CD8^+$ T cells through epigenetic mechanisms in hepatitis B virus infection. *Oncol Lett* 2020; **20**: 235-247 [PMID: 32565950 DOI: 10.3892/ol.2020.11534]

24 **Zhuang L**, Tian J, Zhang X, Wang H, Huang C. Lnc-DC regulates cellular turnover and the HBV-induced immune response by TLR9/STAT3 signaling in dendritic cells. *Cell Mol Biol Lett* 2018; **23**: 43 [PMID: 30202418 DOI: 10.1186/s11658-018-0108-y]

25 **Nishitsuji H**, Ujino S, Yoshio S, Sugiyama M, Mizokami M, Kanto T, Shimotohno K. Long noncoding RNA #32 contributes to antiviral responses by controlling interferon-stimulated gene expression. *Proc Natl Acad Sci U S A* 2016; **113**: 10388-10393 [PMID: 27582466 DOI: 10.1073/pnas.1525022113]

26 **D'souza S**, Lau KC, Coffin CS, Patel TR. Molecular mechanisms of viral hepatitis induced hepatocellular carcinoma. *World J Gastroenterol* 2020; **26**: 5759-5783 [PMID: 33132633 DOI: 10.3748/wjg.v26.i38.5759]

27 **Shoraka S**, Mohebbi SR, Hosseini SM, Asadzadeh Aghdaei H, Zali MR. Identification of plasma lncRNA-ATB levels in hepatitis B virus-related cirrhosis and non-cirrhotic chronic hepatitis B patients. *Virus Res* 2021; **303**: 198503 [PMID: 34331990 DOI: 10.1016/j.virusres.2021.198503]

28 **Wang S**, Jiang J, Zhang C, Zhang X, Wang C. Serum lincRNA-p21 expression in primary liver diseases and liver metastatic diseases. *Pathol Res Pract* 2019; **215**: 779-783 [PMID: 30704781 DOI: 10.1016/j.prp.2019.01.014]

29 **Li LJ**, Wu XY, Tan SW, Xie ZJ, Pan XM, Pan SW, Bai WR, Li HJ, Liu HL, Jiang J, Wu B. Lnc-TCL6 is a potential biomarker for early diagnosis and grade in liver-cirrhosis patients. *Gastroenterol Rep (Oxf)* 2019; **7**: 434-443 [PMID: 31857905 DOI: 10.1093/gastro/goz050]

30 **Li JZ**, Ye LH, Wang DH, Zhang HC, Li TY, Liu ZQ, Dai EH, Li MR. The identify role and molecular mechanism of the MALAT1/hsa-mir-20b-5p/TXNIP axis in liver inflammation caused by CHB in patients with chronic HBV infection complicated with NAFLD. *Virus Res* 2021; **298**: 198405 [PMID: 33775752 DOI: 10.1016/j.virusres.2021.198405]

31 **Chen MJ**, Wang XG, Sun ZX, Liu XC. Diagnostic value of LncRNA-MEG3 as a serum biomarker in patients with hepatitis B complicated with liver fibrosis. *Eur Rev Med Pharmacol Sci* 2019; **23**: 4360-4367 [PMID: 31173310 DOI: 10.26355/eurrev_201905_17943]

32 **Guo Y**, Li C, Zhang R, Zhan Y, Yu J, Tu J, Zheng J. Epigenetically-regulated serum GAS5 as a potential biomarker for patients with chronic hepatitis B virus infection. *Cancer Biomark* 2021; **32**: 137-146 [PMID: 34092613 DOI: 10.3233/CBM-203169]

33 **Fu X**, Cheng D, Ouyang Y, Li Y, Li R, Peng S, Fu L. Integrated Analysis of Long Noncoding RNA Expression Profiles in Acute-on-Chronic Liver Failure. *Biomed Res Int* 2021; **2021**: 5387856 [PMID: 34104647 DOI: 10.1155/2021/5387856]

34 **Kulik L**, El-Serag HB. Epidemiology and Management of Hepatocellular Carcinoma. *Gastroenterology* 2019; **156**: 477-491.e1 [PMID: 30367835 DOI: 10.1053/j.gastro.2018.08.065]

35 **Chaturvedi VK**, Singh A, Dubey SK, Hetta HF, John J, Singh MP. Molecular mechanistic insight of hepatitis B virus mediated hepatocellular carcinoma. *Microb Pathog* 2019; **128**: 184-194 [PMID: 30611768 DOI: 10.1016/j.micpath.2019.01.004]

36 **Bousali M**, Papatheodoridis G, Paraskevis D, Karamitros T. Hepatitis B Virus DNA Integration, Chronic Infections and Hepatocellular Carcinoma. *Microorganisms* 2021; **9** [PMID: 34442866 DOI: 10.3390/microorganisms9081787]

37 **Zhang B**, Han S, Feng B, Chu X, Chen L, Wang R. Hepatitis B virus X protein-mediated non-coding RNA aberrations in the development of human hepatocellular carcinoma. *Exp Mol Med* 2017; **49**: e293 [PMID: 28186085 DOI: 10.1038/emm.2016.177]

38 **Pan YF**, Qin T, Feng L, Yu ZJ. Expression profile of altered long non-coding RNAs in patients with HBV-associated hepatocellular carcinoma. *J Huazhong Univ Sci Technolog Med Sci* 2013; **33**: 96-101 [PMID: 23392715 DOI: 10.1007/s11596-013-1078-y]

39 **Yu TT**, Xu XM, Hu Y, Deng JJ, Ge W, Han NN, Zhang MX. Long noncoding RNAs in hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2015; **21**: 7208-7217 [PMID: 26109807 DOI: 10.3748/wjg.v21.i23.7208]

40 **Fang ZX**, Niu JJ, Liu PG, Lin Y. Genome-wide long noncoding RNA and mRNA expression profiles demonstrate associations between exposure to inorganic elements and the risk of developing hepatocellular carcinoma. *BMC Med Genomics* 2021; **14**: 85 [PMID: 33736645 DOI: 10.1186/s12920-021-00927-w]

41 **Jin Y**, Wu D, Yang W, Weng M, Li Y, Wang X, Zhang X, Jin X, Wang T. Hepatitis B virus x protein induces epithelial-mesenchymal transition of hepatocellular carcinoma cells by regulating long non-coding RNA. *Virol J* 2017; **14**: 238 [PMID: 29258558 DOI: 10.1186/s12985-017-0903-5]

42 **Du Y**, Kong G, You X, Zhang S, Zhang T, Gao Y, Ye L, Zhang X. Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation *via* down-regulating p18. *J Biol Chem* 2012; **287**: 26302-26311 [PMID: 22685290 DOI: 10.1074/jbc.M112.342113]

43 **Lu Z**, Xiao Z, Liu F, Cui M, Li W, Yang Z, Li J, Ye L, Zhang X. Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). *Oncotarget* 2016; **7**: 241-254 [PMID: 26540633 DOI: 10.18632/oncotarget.6280]

44 **Yu X**, Zheng H, Chan MT, Wu WK. HULC: an oncogenic long non-coding RNA in human cancer. *J Cell Mol Med* 2017; **21**: 410-417 [PMID: 27781386 DOI: 10.1111/jcmm.12956]

45 **Zhang H**, Diab A, Fan H, Mani SK, Hullinger R, Merle P, Andrisani O. PLK1 and HOTAIR Accelerate Proteasomal Degradation of SUZ12 and ZNF198 during Hepatitis B Virus-Induced Liver Carcinogenesis. *Cancer Res* 2015; **75**: 2363-2374 [PMID: 25855382 DOI: 10.1158/0008-5472.CAN-14-2928]

46 **Balas MM**, Hartwick EW, Barrington C, Roberts JT, Wu SK, Bettcher R, Griffin AM, Kieft JS, Johnson AM. Establishing RNA-RNA interactions remodels lncRNA structure and promotes PRC2 activity. *Sci Adv* 2021; **7** [PMID: 33853770 DOI: 10.1126/sciadv.abc9191]

47 **Fu WM**, Zhu X, Wang WM, Lu YF, Hu BG, Wang H, Liang WC, Wang SS, Ko CH, Waye MM, Kung HF, Li G, Zhang JF. Hotair mediates hepatocarcinogenesis through suppressing miRNA-218 expression and activating P14 and P16 signaling. *J Hepatol* 2015; **63**: 886-895 [PMID: 26024833 DOI: 10.1016/j.jhep.2015.05.016]

48 **He Y**, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J. Long noncoding RNAs: Novel insights into hepatocelluar carcinoma. *Cancer Lett* 2014; **344**: 20-27 [PMID: 24183851 DOI: 10.1016/j.canlet.2013.10.021]

49 **Lau CC**, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KF, Chan TF, Wong N. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell* 2014; **25**: 335-349 [PMID: 24582836 DOI: 10.1016/j.ccr.2014.01.030]

50 Hepatitis B viral insertion generates an oncogenic long noncoding RNA. *Cancer Discov* 2014; **4**: OF13 [PMID: 24795017 DOI: 10.1158/2159-8290.CD-RW2014-058]

51 **Trung NT**, Hai LT, Giang DP, Hoan PQ, Binh MT, Hoan NX, Toan NL, Meyer CG, Velavan TP, Bang MH, Song LH. No expression of HBV-human chimeric fusion transcript (HBx-LINE1) among Vietnamese patients with HBV-associated hepatocellular carcinoma. *Ann Hepatol* 2019; **18**: 404-405 [PMID: 31036498 DOI: 10.1016/j.aohep.2019.02.002]

52 **Xue M**, Chen W, Li X. Urothelial cancer associated 1: a long noncoding RNA with a crucial role in cancer. *J Cancer Res Clin Oncol* 2016; **142**: 1407-1419 [PMID: 26341664 DOI: 10.1007/s00432-015-2042-y]

53 **Hu JJ**, Song W, Zhang SD, Shen XH, Qiu XM, Wu HZ, Gong PH, Lu S, Zhao ZJ, He ML, Fan H. HBx-upregulated lncRNA UCA1 promotes cell growth and tumorigenesis by recruiting EZH2 and repressing p27Kip1/CDK2 signaling. *Sci Rep* 2016; **6**: 23521 [PMID: 27009634 DOI: 10.1038/srep23521]

54 **Wang F**, Ying HQ, He BS, Pan YQ, Deng QW, Sun HL, Chen J, Liu X, Wang SK. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. *Oncotarget* 2015; **6**: 7899-7917 [PMID: 25760077 DOI: 10.18632/oncotarget.3219]

55 **Xiao JN**, Yan TH, Yu RM, Gao Y, Zeng WL, Lu SW, Que HX, Liu ZP, Jiang JH. Long non-coding RNA UCA1

regulates the expression of Snail2 by miR-203 to promote hepatocellular carcinoma progression. *J Cancer Res Clin Oncol* 2017; **143**: 981-990 [PMID: 28271214 DOI: 10.1007/s00432-017-2370-1]

56 **Bao J**, Chen X, Hou Y, Kang G, Li Q, Xu Y. LncRNA DBH-AS1 facilitates the tumorigenesis of hepatocellular carcinoma by targeting miR-138 *via* FAK/Src/ERK pathway. *Biomed Pharmacother* 2018; **107**: 824-833 [PMID: 30142544 DOI: 10.1016/j.biopha.2018.08.079]

57 **Zhang Q**, Matsuura K, Kleiner DE, Zamboni F, Alter HJ, Farci P. Analysis of long noncoding RNA expression in hepatocellular carcinoma of different viral etiology. *J Transl Med* 2016; **14**: 328 [PMID: 27894309 DOI: 10.1186/s12967-016-1085-4]

58 **Pasmant E**, Laurendeau I, Héron D, Vidaud M, Vidaud D, Bièche I. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. *Cancer Res* 2007; **67**: 3963-3969 [PMID: 17440112 DOI: 10.1158/0008-5472.CAN-06-2004]

59 **Huang MD**, Chen WM, Qi FZ, Xia R, Sun M, Xu TP, Yin L, Zhang EB, De W, Shu YQ. Long non-coding RNA ANRIL is upregulated in hepatocellular carcinoma and regulates cell apoptosis by epigenetic silencing of KLF2. *J Hematol Oncol* 2015; **8**: 50 [PMID: 25966845 DOI: 10.1186/s13045-015-0146-0]

60 **Ma J**, Li T, Han X, Yuan H. Knockdown of LncRNA ANRIL suppresses cell proliferation, metastasis, and invasion *via* regulating miR-122-5p expression in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2018; **144**: 205-214 [PMID: 29127494 DOI: 10.1007/s00432-017-2543-y]

61 **Deng X**, Zhao XF, Liang XQ, Chen R, Pan YF, Liang J. Linc00152 promotes cancer progression in hepatitis B virus-associated hepatocellular carcinoma. *Biomed Pharmacother* 2017; **90**: 100-108 [PMID: 28343069 DOI: 10.1016/j.biopha.2017.03.031]

62 **Tripathi V**, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, Blencowe BJ, Prasanth SG, Prasanth KV. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 2010; **39**: 925-938 [PMID: 20797886 DOI: 10.1016/j.molcel.2010.08.011]

63 **Hou Z**, Xu X, Fu X, Tao S, Zhou J, Liu S, Tan D. HBx-related long non-coding RNA MALAT1 promotes cell metastasis *via* up-regulating LTBP3 in hepatocellular carcinoma. *Am J Cancer Res* 2017; **7**: 845-856 [PMID: 28469957]

64 **Huang Z**, Huang L, Shen S, Li J, Lu H, Mo W, Dang Y, Luo D, Chen G, Feng Z. Sp1 cooperates with Sp3 to upregulate MALAT1 expression in human hepatocellular carcinoma. *Oncol Rep* 2015; **34**: 2403-2412 [PMID: 26352013 DOI: 10.3892/or.2015.4259]

65 **Miyagawa R**, Tano K, Mizuno R, Nakamura Y, Ijiri K, Rakwal R, Shibato J, Masuo Y, Mayeda A, Hirose T, Akimitsu N. Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles. *RNA* 2012; **18**: 738-751 [PMID: 22355166 DOI: 10.1261/rna.028639.111]

66 **Lai MC**, Yang Z, Zhou L, Zhu QQ, Xie HY, Zhang F, Wu LM, Chen LM, Zheng SS. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. *Med Oncol* 2012; **29**: 1810-1816 [PMID: 21678027 DOI: 10.1007/s12032-011-0004-z]

67 **Romito A**, Rougeulle C. Origin and evolution of the long non-coding genes in the X-inactivation center. *Biochimie* 2011; **93**: 1935-1942 [PMID: 21820484 DOI: 10.1016/j.biochi.2011.07.009]

68 **Yuan SX**, Yang F, Yang Y, Tao QF, Zhang J, Huang G, Wang RY, Yang S, Huo XS, Zhang L, Wang F, Sun SH, Zhou WP. Long noncoding RNA associated with microvascular invasion in hepatocellular carcinoma promotes angiogenesis and serves as a predictor for hepatocellular carcinoma patients' poor recurrence-free survival after hepatectomy. *Hepatology* 2012; **56**: 2231-2241 [PMID: 22706893 DOI: 10.1002/hep.25895]

69 **Lv J**, Fan HX, Zhao XP, Lv P, Fan JY, Zhang Y, Liu M, Tang H. Long non-coding RNA Unigene56159 promotes epithelial-mesenchymal transition by acting as a ceRNA of miR-140-5p in hepatocellular carcinoma cells. *Cancer Lett* 2016; **382**: 166-175 [PMID: 27597739 DOI: 10.1016/j.canlet.2016.08.029]

70 **Guo L**, Gao S, Sun W, Wang Y, Zhao J. Elevated LINC01232 is associated with poor prognosis and HBV infection in hepatocellular carcinoma patients and contributes to tumor progression in vitro. *Clin Res Hepatol Gastroenterol* 2022; **46**: 101813 [PMID: 34583064 DOI: 10.1016/j.clinre.2021.101813]

71 **Liu Q**, Liu N, Shangguan Q, Zhang F, Chai W, Tong X, Zhao X, Li Z, Qi D, Ye X. LncRNA SAMD12-AS1 promotes cell proliferation and inhibits apoptosis by interacting with NPM1. *Sci Rep* 2019; **9**: 11593 [PMID: 31406141 DOI: 10.1038/s41598-019-48116-1]

72 **Liu N**, Liu Q, Yang X, Zhang F, Li X, Ma Y, Guan F, Zhao X, Li Z, Zhang L, Ye X. Hepatitis B Virus-Upregulated LNC-HUR1 Promotes Cell Proliferation and Tumorigenesis by Blocking p53 Activity. *Hepatology* 2018; **68**: 2130-2144 [PMID: 29790592 DOI: 10.1002/hep.30098]

73 **Fan H**, Lv P, Mu T, Zhao X, Liu Y, Feng Y, Lv J, Liu M, Tang H. LncRNA n335586/miR-924/CKMT1A axis contributes to cell migration and invasion in hepatocellular carcinoma cells. *Cancer Lett* 2018; **429**: 89-99 [DOI: 10.1016/j.canlet.2018.05.010]

74 **Wang J**, Yin G, Bian H, Yang J, Zhou P, Yan K, Liu C, Chen P, Zhu J, Li Z, Xue T. LncRNA XIST upregulates TRIM25 *via* negatively regulating miR-192 in hepatitis B virus-related hepatocellular carcinoma. *Mol Med* 2021; **27**: 41 [PMID: 33858324 DOI: 10.1186/s10020-021-00278-3]

75 **Li Y**, Guo D, Zhao Y, Ren M, Lu G, Wang Y, Zhang J, Mi C, He S, Lu X. Long non-coding RNA SNHG5 promotes human hepatocellular carcinoma progression by regulating miR-26a-5p/GSK3β signal pathway. *Cell Death Dis* 2018; **9**: 888 [PMID: 30166525 DOI: 10.1038/s41419-018-0882-5]

76 **Hu Z**, Huang P, Yan Y, Zhou Z, Wang J, Wu G. Hepatitis B virus X protein related lncRNA WEE2-AS1 promotes hepatocellular carcinoma proliferation and invasion. *Biochem Biophys Res Commun* 2019; **508**: 79-86 [PMID: 30471857 DOI: 10.1016/j.bbrc.2018.11.091]

77 **Zhang F**, Li Y, Gan L, Tong X, Qi D, Wang Q, Ye X. HBx-upregulated MAFG-AS1 promotes cell proliferation and migration of hepatoma cells by enhancing MAFG expression and stabilizing nonmuscle myosin IIA. *FASEB J* 2021; **35**: e21529 [PMID: 33813778 DOI: 10.1096/fj.202002374R]

78 **Zhang Y**, Li J, Wang S, Yang F, Zhou Y, Liu Y, Zhu W, Shi X. HBxassociated long noncoding RNA activated by TGFβ

promotes cell invasion and migration by inducing autophagy in primary liver cancer. *Int J Oncol* 2020; **56**: 337-347 [PMID: 31746419 DOI: 10.3892/ijo.2019.4908]

79 **Song W**, Zheng C, Liu M, Xu Y, Qian Y, Zhang Z, Su H, Li X, Wu H, Gong P, Li Y, Fan H. TRERNA1 upregulation mediated by HBx promotes sorafenib resistance and cell proliferation in HCC *via* targeting NRAS by sponging miR-22-3p. *Mol Ther* 2021; **29**: 2601-2616 [PMID: 33839325 DOI: 10.1016/j.ymthe.2021.04.011]

80 **Chen Z**, Yu W, Zhou Q, Zhang J, Jiang H, Hao D, Wang J, Zhou Z, He C, Xiao Z. A Novel lncRNA IHS Promotes Tumor Proliferation and Metastasis in HCC by Regulating the ERK- and AKT/GSK-3β-Signaling Pathways. *Mol Ther Nucleic Acids* 2019; **16**: 707-720 [PMID: 31128422 DOI: 10.1016/j.omtn.2019.04.021]

81 **Tu W**, Yang Y, Song Y, Zhu W. Hepatitis B virus x protein accelerated the proliferation of hepatocellular carcinoma cell through lncRNA SNHG20/PTEN pathway. *J Biochem* 2019; **165**: 423-431 [PMID: 30690477 DOI: 10.1093/jb/mvy120]

82 **Zuo K**, Kong L, Xue D, Yang Y, Xie L. The expression and role of lncRNA AX800134 in hepatitis B virus-related hepatocellular carcinoma. *Virus Genes* 2018; **54**: 475-483 [PMID: 29789998 DOI: 10.1007/s11262-018-1564-1]

83 **Qiu L**, Wang T, Xu X, Wu Y, Tang Q, Chen K. Long Non-Coding RNAs in Hepatitis B Virus-Related Hepatocellular Carcinoma: Regulation, Functions, and Underlying Mechanisms. *Int J Mol Sci* 2017; **18** [PMID: 29168767 DOI: 10.3390/ijms18122505]

84 **Lv D**, Wang Y, Zhang Y, Cui P, Xu Y. Downregulated long non-coding RNA DREH promotes cell proliferation in hepatitis B virus-associated hepatocellular carcinoma. *Oncol Lett* 2017; **14**: 2025-2032 [PMID: 28789433 DOI: 10.3892/ol.2017.6436]

85 **Yang F**, Huo XS, Yuan SX, Zhang L, Zhou WP, Wang F, Sun SH. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. *Mol Cell* 2013; **49**: 1083-1096 [PMID: 23395002 DOI: 10.1016/j.molcel.2013.01.010]

86 **Castella S**, Bernard R, Corno M, Fradin A, Larcher JC. Ilf3 and NF90 functions in RNA biology. *Wiley Interdiscip Rev RNA* 2015; **6**: 243-256 [PMID: 25327818 DOI: 10.1002/wrna.1270]

87 **Luo HL**, Chen J, Luo T, Wu FX, Liu JJ, Wang HF, Chen M, Li LQ, Li H. Downregulation of Macrophage-Derived T-UCR uc.306 Associates with Poor Prognosis in Hepatocellular Carcinoma. *Cell Physiol Biochem* 2017; **42**: 1526-1539 [PMID: 28723685 DOI: 10.1159/000479269]

88 **Xiang L**, Huang X, Wang S, Ou H, Chen Z, Hu Z, Huang Y, Li X, Yuan Y, Yang D. Deficiency of pseudogene UPAT leads to hepatocellular carcinoma progression and forms a positive feedback loop with ZEB1. *Cancer Sci* 2020; **111**: 4102-4117 [PMID: 32808348 DOI: 10.1111/cas.14620]

89 **Yu S**, Li N, Wang J, Fu Y, Huang Y, Yi P, Chen R, Tang D, Hu X, Fan X. Correlation of Long Noncoding RNA SEMA6A-AS1 Expression with Clinical Outcome in HBV-Related Hepatocellular Carcinoma. *Clin Ther* 2020; **42**: 439-447 [PMID: 32070484 DOI: 10.1016/j.clinthera.2020.01.012]

90 **Zhao NN**, Wang C, Lai CC, Cheng SJ, Yan J, Hong ZX, Yu LX, Zhu ZY, Zhang PR, Wang ZH, Wang XL, Zhang SG, Yang PH. Downregulation of BRAF-activated non-protein coding RNA in patients with hepatitis B virus-associated hepatocellular carcinoma. *Oncol Lett* 2018; **15**: 7794-7798 [PMID: 29725471 DOI: 10.3892/ol.2018.8327]

91 **Lin X**, Xiaoqin H, Jiayu C, Li F, Yue L, Ximing X. Long non-coding RNA miR143HG predicts good prognosis and inhibits tumor multiplication and metastasis by suppressing mitogen-activated protein kinase and Wnt signaling pathways in hepatocellular carcinoma. *Hepatol Res* 2019; **49**: 902-918 [PMID: 30945380 DOI: 10.1111/hepr.13344]

92 **Yu S**, Li N, Huang Z, Chen R, Yi P, Kang R, Tang D, Hu X, Fan X. A novel lncRNA, TCONS_00006195, represses hepatocellular carcinoma progression by inhibiting enzymatic activity of ENO1. *Cell Death Dis* 2018; **9**: 1184 [PMID: 30518748 DOI: 10.1038/s41419-018-1231-4]

93 **Huang P**, Xu Q, Yan Y, Lu Y, Hu Z, Ou B, Zhang H, Mao K, Zhang J, Wang J, Xiao Z. HBx/ERα complex-mediated LINC01352 downregulation promotes HBV-related hepatocellular carcinoma *via* the miR-135b-APC axis. *Oncogene* 2020; **39**: 3774-3789 [PMID: 32157216 DOI: 10.1038/s41388-020-1254-z]

94 **Deng Y**, Wei Z, Huang M, Xu G, Wei W, Peng B, Nong S, Qin H. Long non-coding RNA F11-AS1 inhibits HBV-related hepatocellular carcinoma progression by regulating NR1I3 *via* binding to microRNA-211-5p. *J Cell Mol Med* 2020; **24**: 1848-1865 [PMID: 31880390 DOI: 10.1111/jcmm.14881]

95 **Liu Y**, Xu L, Lu B, Zhao M, Li L, Sun W, Qiu Z, Zhang B. LncRNA H19/microRNA-675/PPARα axis regulates liver cell injury and energy metabolism remodelling induced by hepatitis B X protein *via* Akt/mTOR signalling. *Mol Immunol* 2019; **116**: 18-28 [PMID: 31574452 DOI: 10.1016/j.molimm.2019.09.006]

96 **Ge L**, Zhang X, Hu S, Song Y, Kong J, Zhang B, Yang X. H19 suppresses the growth of hepatoblastoma cells by promoting their apoptosis *via* the signaling pathways of miR-675/FADD and miR-138/PTK2. *J Cell Biochem* 2019; **120**: 5218-5231 [PMID: 30367502 DOI: 10.1002/jcb.27797]

97 **Li H**, Tang XM, Liu Y, Li W, Chen Q, Pan Y. Association of Functional Genetic Variants of HOTAIR with Hepatocellular Carcinoma (HCC) Susceptibility in a Chinese Population. *Cell Physiol Biochem* 2017; **44**: 447-454 [PMID: 29141248 DOI: 10.1159/000485011]

98 **Chang L**, Li C, Lan T, Wu L, Yuan Y, Liu Q, Liu Z. Decreased expression of long non-coding RNA GAS5 indicates a poor prognosis and promotes cell proliferation and invasion in hepatocellular carcinoma by regulating vimentin. *Mol Med Rep* 2016; **13**: 1541-1550 [PMID: 26707238 DOI: 10.3892/mmr.2015.4716]

99 **Tao R**, Hu S, Wang S, Zhou X, Zhang Q, Wang C, Zhao X, Zhou W, Zhang S, Li C, Zhao H, He Y, Zhu S, Xu J, Jiang Y, Li L, Gao Y. Association between indel polymorphism in the promoter region of lncRNA GAS5 and the risk of hepatocellular carcinoma. *Carcinogenesis* 2015; **36**: 1136-1143 [PMID: 26163879 DOI: 10.1093/carcin/bgv099]

100 **Zhu Z**, Gao X, He Y, Zhao H, Yu Q, Jiang D, Zhang P, Ma X, Huang H, Dong D, Wan J, Gu Z, Jiang X, Yu L, Gao Y. An insertion/deletion polymorphism within RERT-lncRNA modulates hepatocellular carcinoma risk. *Cancer Res* 2012; **72**: 6163-6172 [PMID: 23026137 DOI: 10.1158/0008-5472.CAN-12-0010]

101 **Wan J**, Huang M, Zhao H, Wang C, Zhao X, Jiang X, Bian S, He Y, Gao Y. A novel tetranucleotide repeat polymorphism within KCNQ1OT1 confers risk for hepatocellular carcinoma. *DNA Cell Biol* 2013; **32**: 628-634 [PMID: 23984860 DOI: 10.1089/dna.2013.2118]

102 **Zhong R**, Tian J, Fu M, Ma S, Liu L, Li J, Shen N, Ke J, Yang Y, Gong Y, Zhu Y, Wang Y, Gong J, Chang J, Lei P,

Cheng X, Huang K, Shen G, Miao X. LINC01149 variant modulates MICA expression that facilitates hepatitis B virus spontaneous recovery but increases hepatocellular carcinoma risk. *Oncogene* 2020; **39**: 1944-1956 [PMID: 31754211 DOI: 10.1038/s41388-019-1117-7]

103 **Lin YY**, Yu MW, Lin SM, Lee SD, Chen CL, Chen DS, Chen PJ. Genome-wide association analysis identifies a GLUL haplotype for familial hepatitis B virus-related hepatocellular carcinoma. *Cancer* 2017; **123**: 3966-3976 [PMID: 28662289 DOI: 10.1002/cncr.30851]

104 **Yin J**, Wen J, Hang D, Han J, Jiang J, Song C, Liu Y, Liu J, Liu L, Zhu L, Chen J, Zhai X, Xie S, Hu Z, Shen H, Dai M, Li N. Expression Quantitative Trait Loci for CARD8 Contributes to Risk of Two Infection-Related Cancers--Hepatocellular Carcinoma and Cervical Cancer. *PLoS One* 2015; **10**: e0132352 [PMID: 26147888 DOI: 10.1371/journal.pone.0132352]

105 **Wen J**, Song C, Liu J, Chen J, Zhai X, Hu Z. Expression quantitative trait loci for TNFRSF10 influence both HBV infection and hepatocellular carcinoma development. *J Med Virol* 2016; **88**: 474-480 [PMID: 26297860 DOI: 10.1002/jmv.24363]

106 **Ma S**, Yang J, Song C, Ge Z, Zhou J, Zhang G, Hu Z. Expression quantitative trait loci for PAX8 contributes to the prognosis of hepatocellular carcinoma. *PLoS One* 2017; **12**: e0173700 [PMID: 28339471 DOI: 10.1371/journal.pone.0173700]

107 **Liu Q**, Liu G, Lin Z, Tian N, Lin X, Tan J, Huang B, Ji X, Pi L, Yu X, Liu L, Gao Y. The association of lncRNA SNPs and SNPs-environment interactions based on GWAS with HBV-related HCC risk and progression. *Mol Genet Genomic Med* 2021; **9**: e1585 [PMID: 33432784 DOI: 10.1002/mgg3.1585]

108 **Huang X**, Zhang W, Shao Z. Association between long non-coding RNA polymorphisms and cancer risk: a meta-analysis. *Biosci Rep* 2018; **38** [PMID: 29802154 DOI: 10.1042/BSR20180365]

109 **Bhat SA**, Ahmad SM, Mumtaz PT, Malik AA, Dar MA, Urwat U, Shah RA, Ganai NA. Long non-coding RNAs: Mechanism of action and functional utility. *Noncoding RNA Res* 2016; **1**: 43-50 [PMID: 30159410 DOI: 10.1016/j.ncrna.2016.11.002]

110 **Lun YZ**, Pan ZP, Liu SA, Sun J, Han M, Liu B, Dong W, Pan LH, Cheng J. The peptide encoded by a novel putative lncRNA HBVPTPAP inducing the apoptosis of hepatocellular carcinoma cells by modulating JAK/STAT signaling pathways. *Virus Res* 2020; **287**: 198104 [PMID: 32755630 DOI: 10.1016/j.virusres.2020.198104]

111 **Jiang B**, Yang B, Wang Q, Zheng X, Guo Y, Lu W. lncRNA PVT1 promotes hepatitis B viruspositive liver cancer progression by disturbing histone methylation on the cMyc promoter. *Oncol Rep* 2020; **43**: 718-726 [PMID: 31894346 DOI: 10.3892/or.2019.7444]

112 **Kotake Y**, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, Xiong Y. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene* 2011; **30**: 1956-1962 [PMID: 21151178 DOI: 10.1038/onc.2010.568]

113 **Chen T**, Pei J, Wang J, Luo R, Liu L, Wang L, Jia H. HBx-related long non-coding RNA 01152 promotes cell proliferation and survival by IL-23 in hepatocellular carcinoma. *Biomed Pharmacother* 2019; **115**: 108877 [PMID: 31054511 DOI: 10.1016/j.biopha.2019.108877]

114 **Salmena L**, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011; **146**: 353-358 [PMID: 21802130 DOI: 10.1016/j.cell.2011.07.014]

115 **Wang Y**, Chen F, Zhao M, Yang Z, Li J, Zhang S, Zhang W, Ye L, Zhang X. The long noncoding RNA HULC promotes liver cancer by increasing the expression of the HMGA2 oncogene *via* sequestration of the microRNA-186. *J Biol Chem* 2017; **292**: 15395-15407 [PMID: 28765279 DOI: 10.1074/jbc.M117.783738]

116 **Xu J**, Zhang J, Shan F, Wen J, Wang Y. SSTR5AS1 functions as a ceRNA to regulate CA2 by sponging miR15b5p for the development and prognosis of HBVrelated hepatocellular carcinoma. *Mol Med Rep* 2019; **20**: 5021-5031 [PMID: 31638225 DOI: 10.3892/mmr.2019.10736]

117 **Lin Y**, Shen Y, Chen J, Hu C, Zhou Z, Yuan C. The Function of LncRNA FTX in Several Common Cancers. *Curr Pharm Des* 2021; **27**: 2381-2386 [PMID: 33121404 DOI: 10.2174/1381612826666201029164036]

118 **Xiong H**, Li B, He J, Zeng Y, Zhang Y, He F. lncRNA HULC promotes the growth of hepatocellular carcinoma cells *via* stabilizing COX-2 protein. *Biochem Biophys Res Commun* 2017; **490**: 693-699 [PMID: 28634076 DOI: 10.1016/j.bbrc.2017.06.103]

119 **Nagy Á**, Lánczky A, Menyhárt O, Győrffy B. Author Correction: Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Sci Rep* 2018; **8**: 11515 [PMID: 30046141 DOI: 10.1038/s41598-018-29514-3]

120 **Li H**, Zhao X, Li C, Sheng C, Bai Z. Integrated analysis of lncRNA-associated ceRNA network reveals potential biomarkers for the prognosis of hepatitis B virus-related hepatocellular carcinoma. *Cancer Manag Res* 2019; **11**: 877-897 [PMID: 30697079 DOI: 10.2147/CMAR.S186561]

121 **Li J**, Wang X, Tang J, Jiang R, Zhang W, Ji J, Sun B. HULC and Linc00152 Act as Novel Biomarkers in Predicting Diagnosis of Hepatocellular Carcinoma. *Cell Physiol Biochem* 2015; **37**: 687-696 [PMID: 26356260 DOI: 10.1159/000430387]

122 **Qi J**, Li J, Sun J, Li C. Long Non-Coding RNA IGF2AS in Serum may be a Biomarker for Diagnosis of Hepatitis B Virus-Related Hepatocellular Carcinoma. *Clin Lab* 2019; **65** [PMID: 31850718 DOI: 10.7754/Clin.Lab.2019.190442]

123 **Lu J**, Xie F, Geng L, Shen W, Sui C, Yang J. Investigation of serum lncRNA-uc003wbd and lncRNA-AF085935 expression profile in patients with hepatocellular carcinoma and HBV. *Tumour Biol* 2015; **36**: 3231-3236 [PMID: 25501706 DOI: 10.1007/s13277-014-2951-4]

124 **Wang K**, Guo WX, Li N, Gao CF, Shi J, Tang YF, Shen F, Wu MC, Liu SR, Cheng SQ. Serum LncRNAs Profiles Serve as Novel Potential Biomarkers for the Diagnosis of HBV-Positive Hepatocellular Carcinoma. *PLoS One* 2015; **10**: e0144934 [PMID: 26674525 DOI: 10.1371/journal.pone.0144934]

125 **Xie H**, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. *Biomed Res Int* 2013; **2013**: 136106 [PMID: 23762823 DOI: 10.1155/2013/136106]

126 **Huang ZL**, Li W, Chen QF, Wu PH, Shen LJ. Eight key long non-coding RNAs predict hepatitis virus positive

hepatocellular carcinoma as prognostic targets. *World J Gastrointest Oncol* 2019; **11**: 983-997 [PMID: 31798779 DOI: 10.4251/wjgo.v11.i11.983]

127 **Xia Q**, Shu Z, Ye T, Zhang M. Identification and Analysis of the Blood lncRNA Signature for Liver Cirrhosis and Hepatocellular Carcinoma. *Front Genet* 2020; **11**: 595699 [PMID: 33365048 DOI: 10.3389/fgene.2020.595699]

128 **Liu H**, Zhao P, Jin X, Zhao Y, Chen Y, Yan T, Wang J, Wu L, Sun Y. A 9lncRNA risk score system for predicting the prognosis of patients with hepatitis B viruspositive hepatocellular carcinoma. *Mol Med Rep* 2019; **20**: 573-583 [PMID: 31115573 DOI: 10.3892/mmr.2019.10262]

129 **Bai Z**, Li H, Li C, Sheng C, Zhao X. Integrated analysis identifies a long non-coding RNAs-messenger RNAs signature for prediction of prognosis in hepatitis B virus-hepatocellular carcinoma patients. *Medicine (Baltimore)* 2020; **99**: e21503 [PMID: 33019382 DOI: 10.1097/MD.0000000000021503]

130 **Zhao X**, Bai Z, Li C, Sheng C, Li H. Identification of a Novel Eight-lncRNA Prognostic Signature for HBV-HCC and Analysis of Their Functions Based on Coexpression and ceRNA Networks. *Biomed Res Int* 2020; **2020**: 8765461 [PMID: 32382578 DOI: 10.1155/2020/8765461]

131 **Jiang Z**, Liu H. Metformin inhibits tumorigenesis in HBV-induced hepatocellular carcinoma by suppressing HULC overexpression caused by HBX. *J Cell Biochem* 2018; **119**: 4482-4495 [PMID: 29231260 DOI: 10.1002/jcb.26555]

132 **Chang L**, Wang G, Jia T, Zhang L, Li Y, Han Y, Zhang K, Lin G, Zhang R, Li J, Wang L. Armored long non-coding RNA MEG3 targeting EGFR based on recombinant MS2 bacteriophage virus-like particles against hepatocellular carcinoma. *Oncotarget* 2016; **7**: 23988-24004 [PMID: 26992211 DOI: 10.18632/oncotarget.8115]

133 **Jacob R**, Zander S, Gutschner T. The Dark Side of the Epitranscriptome: Chemical Modifications in Long Non-Coding RNAs. *Int J Mol Sci* 2017; **18** [PMID: 29125541 DOI: 10.3390/ijms18112387]

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*MINIREVIEWS*

# Characteristics of inflammatory bowel diseases in patients with concurrent immune-mediated inflammatory diseases

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## Abstract

Patients with inflammatory bowel disease (IBD) are more likely to have concurrent immune-mediated inflammatory diseases (IMIDs) than those without IBD. IMIDs have been observed to alter the phenotype and outcomes of IBD in recent studies. Several studies have found that IBD patients with concurrent IMIDs may have more extensive or severe disease phenotypes, and are considered to be at increased risk of requiring biologics and IBD-related surgeries, suggesting that having multiple IMIDs is a poor prognostic factor for IBD. Furthermore, IBD patients with primary sclerosing cholangitis and Takayasu arteritis are reported to have unique endoscopic phenotypes, suggesting concurrent IMIDs can influence IBD phenotype with specific intestinal inflammatory distributions. In this review, we discuss the pathogenesis, disease phenotypes, and clinical outcomes in IBD patients with concomitant IMIDs.

**Key Words:** Inflammatory bowel disease; Phenotypes; Outcomes; Concurrent immune-mediated inflammatory diseases

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**Core Tip:** Patients with inflammatory bowel disease (IBD) are more likely to acquire other immune-mediated inflammatory diseases (IMIDs). IBD patients with concurrent IMIDs were more likely to require biologics and IBD-related surgeries than non-IBD patients due to extensive disease phenotypes according to recent studies. As a result, when treating IBD patients, we must be aware of the concurrence of other IMIDs and understand its pathogenesis to select biologic and small molecule therapies that treat multiple concomitant diseases.

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## INTRODUCTION

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic relapsing intestinal inflammatory disorder[1]. IBD is a complex disease that is linked to both hereditary and environmental variables, although the etiology is unknown[2-4]. Previous genome-wide association studies (GWASs) identified 99 loci associated with UC and revealed a minimum of 28 shared associations between UC and CD[5]. GWASs have revealed 71 unique loci related to CD and significant genetic overlap with loci related to other immune-mediated inflammatory diseases (IMIDs)[6].

In the pathophysiology of intestinal inflammation in IBD, antigen-presenting cells (APCs) and effector T cells, such as T helper (Th) 1, Th2, and Th17 cells play a pathogenic role[4]. T-cell-mediated immune responses are triggered and modulated by APCs, such as dendritic cells and macrophages. Interleukin (IL)-12 activates Th1 cells that produce interferon-γ, tumor necrosis factor (TNF)-α, and IL-12 [7] (Figure 1). TNF-α is released from activated macrophages in response to interferon, leading to the differentiation of stromal cells into myofibroblasts and the synthesis of matrix metalloproteinases that destroy tissue[8]. While aberrant Th1 responses are thought to involve in CD intestinal inflammation, Th2 cell-mediated inflammation is suggested to be fundamental to the pathogenesis of UC[8]. Th17 cells are characterized by a distinct subset of $CD4^+$ T cells promoting the expression of IL-17 and maintained by IL-23[9,10] (Figure 1). Th17 cells and their cytokines play important roles in the inflammatory pathways of both UC and CD[11]. Innate lymphoid cells (ILCs), which do not exhibit antigen receptors to distinguish T cells from B cells, contribute to the pathogenesis in IBD as well, particularly in CD[12]. ILCs are divided into three groups based on the cytokine profiles of helper T-cell subsets, with ILC1, ILC2, and ILC3 serving as the counterparts of Th1, Th2, and Th17 cells, respectively[12].

Given that IBD patients are more likely to have additional IMIDs than nonIBD patients in various studies[13-15], multiple immune pathways may be shared between IBD and other IMIDs. Among recent cross-sectional research of members of a Northern California managed care organization, 17% of IBD patients had at least one IMID diagnosis, whereas the proportion of concurrent IMIDs was 10% in nonIBD individuals[13]. While another study found no significant differences in CD phenotype between individuals with and without IMID, UC patients with another IMID were more likely to have a pancolitis phenotype than those who did not. Furthermore, IBD patients with another IMID had a higher percentage of patients who needed TNF inhibitors or surgery than those without[14]. Of note, previous research found that IBD patients with primary sclerosing cholangitis (PSC) or Takayasu arteritis (TAK) have distinct endoscopic IBD phenotypes[16,17]. IBD can affect the phenotypes and disease courses of concurrent IMIDs as well, according to a recent meta-analysis[18]. All these studies reveal that the presence of other IMIDs in IBD patients can influence disease phenotypes and outcomes and vice versa.

We evaluate the pathogenesis, disease phenotypes, and clinical outcomes in IBD patients with concurrent IMIDs in this review, which includes IMIDs with a high prevalence among patients with IBD and IMIDs that alter IBD phenotypes and outcomes.

## IMIDS WITH HIGH PREVALENCE AMONG IBD

Asthma, psoriasis, and rheumatoid arthritis (RA) were the most common IMIDs among IBD patients, according to the data from Northern California managed care organizations[13]. Another study based on prospective registry data found that 21% of IBD patients also had another IMID. Asthma (9.9%), psoriasis (6.1%), and RA (2.0%) were consistently reported to coexist with IBD[14].

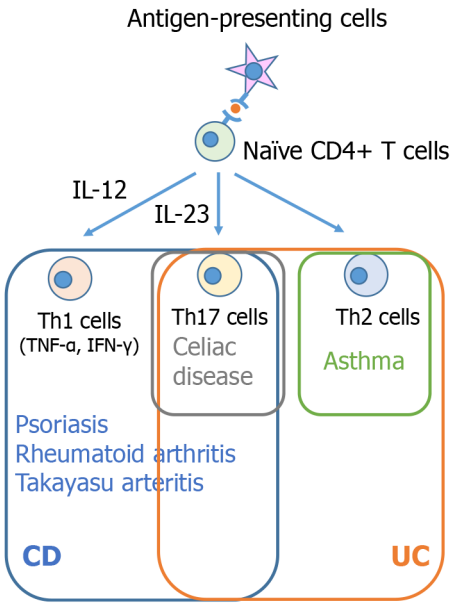


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**Figure 1 The potential common immunological mechanisms shared between inflammatory bowel diseases and other immune-mediated inflammatory diseases.** IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; IL: Interleukin; Th17 cells: IL-17 producing T helper cells; Th1 cells: T helper 1 cells; Th2 cells: T helper 2 cells; CD: Crohn's disease; UC: Ulcerative colitis.

### *Asthma and IBD*

Asthma is a prevalent chronic airway disease characterized by respiratory symptoms such as coughing and shortness of breath[19]. Asthma is a Th2 cell-mediated disease. Dendritic cells capture inhaled allergens and present them to naïve $CD4^+$ T cells, resulting in the polarization of Th2 cells. These cells produce IL-4, IL-5, and IL-13, promoting eosinophil migration to the airway, bronchial hyperresponsiveness, mucus overproduction, and immunoglobulin E synthesis[19]. ILC2 also plays a role in the pathogenesis of asthma. ILC2 is activated by IL-25 and IL-33 and can produce Th2 cell-associated cytokines, causing airway hypersensitivity and tissue remodeling[19]. Th2 cell-mediated inflammatory pathways are also implicated in IBD patients, particularly UC patients (Figure 1 and Table 1). Macrophages or dendritic cells acquire luminal antigens in the intestine and present them to naïve $CD4^+$ T cells, boosting their differentiation into Th2 cells in the immunological pathways involved in UC[20]. Given the high transcript levels of IL-17A that are detected in both of CD and UC, Th17 cells are also linked to the development of UC[11]. Although ILC1 and ILC3 are considered to be involved in the pathogenesis of CD, it is unclear if ILC2 contributes to the development of UC[12,21].

### *Psoriasis and IBD*

Psoriasis is an immune-mediated skin disorder that affects the skin and/or joints[22] resulting in pruritic and painful skin plaques. Psoriasis is caused by the activation of plasmacytoid dendritic cells, which release various proinflammatory cytokines including TNF, interferon-γ, IL-12, and IL-23. IL-12 aids in the development of Th1 cells from naïve T cells, while IL-23 aids in the maintenance of IL-17-producing Th17 cells. Th17 cells generate IL-17A, IL-17F, and IL-22, while activated Th1 cells release interferon-γ and TNF-α, causing keratinocyte hyperproliferation and other psoriasis clinical features [23]. Similarly, Th1 and Th17 cells are involved in the major immunological mechanism of IBD, particularly CD[24]. ILC3, the counterpart of Th17 cells, is an important source of IL-17 in both psoriasis and CD[21]. Indeed, a systematic review and meta-analysis found that psoriasis patients had an increased risk of IBD, particularly CD[25]. A multicenter retrospective study found that 61% of IBD patients who started ustekinumab, an antagonist of the p40 subunit of IL-12 and IL-23, for concomitant active psoriasis achieved clinical remission of IBD[26]. Although TNF inhibitors are effective in the treatment of both psoriasis and IBD, they can also cause psoriasis[27]. TNF-α inhibition is speculated to cause uncontrolled production of interferon-γ by plasmacytoid dendritic cells, resulting in the recruitment of Th1 or Th17 cells into the dermis, which produces proinflammatory cytokines, such as IL-12 and IL-23 [28]. Thus, these data support the notion that the immune mechanism involving Th1 cells, Th17 cells, and ILC3 may be the common pathway between psoriasis and IBD (Figure 1 and Table 1).

### *RA and IBD*

RA is a chronic inflammatory disease that affects the cartilage and bone[29] resulting in painful and disfiguring joint deformities. Dendritic cells or macrophages uptake antigens and present them to T cells in the pathogenesis of RA, resulting in Th1 or Th17 cell-mediated diseases in the synovial tissue.

**Table 1 Characteristic and clinical outcomes in inflammatory bowel disease patients with concurrent immune-mediated inflammatory diseases**

| IMIDs with high prevalence among patients with IBD | Possible shared immune cells contributing to pathogenesis | IBD phenotypes | IBD outcomes | Possible shared therapies |
|---|---|---|---|---|
| Asthma | Th2 cells | Indefinite | Indefinite | Indefinite |
| Psoriasis | Th1 cells, Th17 cells, and ILC3 | Indefinite | Indefinite | Ustekinumab; TNF inhibitors |
| Rheumatoid arthritis | Th1 cells, Th17 cells, ILC1, and ILC3 | Indefinite | Indefinite | Tofacitinib; TNF inhibitors |
| **IMIDs affecting phenotypes or outcomes of IBD** | | | | |
| PSC | Indefinite (leaky gut theory) | Severe right-sided colitis, rectal sparing, and backwash ileitis | PSC-IBD patients have fewer or no symptoms, and are less likely to require immunosuppressants, hospitalization, and surgery than IBD patients alone | Indefinite |
| Celiac disease | Th17 cells | CD patients with celiac disease are less likely to have ileocolonic involvement than CD patients alone | CD patients with celiac disease are less likely to require TNF inhibitors or azathioprine than CD patients alone. UC patients with celiac disease have an increased risk of colectomy | Indefinite |
| Takayasu arteritis | Th1 cells and Th17 cells | Discontinuous aphthous erosions/ulcers or focal mucosal inflammation | Indefinite | Tofacitinib; Ustekinumab; TNF inhibitors |

IBD: Inflammatory bowel disease; PSC: Primary sclerosing cholangitis; IMIDs: Immune-mediated inflammatory diseases; TNF: Tumor necrosis factor; Th17 cells: Interleukin-17 producing T helper cells; Th1 cells: T helper 1 cells; Th2 cells: T helper 2 cells; CD: Crohn's disease; UC: Ulcerative colitis; ILC: Innate lymphoid cell.

Subsequently, the stimulated T cells activate macrophages, B cells, fibroblasts, and osteoclasts. TNF, IL-1, and IL-6 are secreted by the activated macrophages. Inflammatory mediators including matrix metalloproteinases are then produced by fibroblast-like synoviocytes, resulting in cartilage destruction. The activation of osteoclasts induces bone erosions as well[30]. A lymph node activation in RA patients is associated with an imbalance of ILCs, and ILC1 and ILC3 within the lymph node microenvironment can cause inflammation[21]. As a result, RA may have similar immune mechanisms with a CD rather than UC (Figure 1 and Table 1).

Ustekinumab was found to be ineffective in clinical trials of RA patients, whereas TNF, IL-6, and Janus kinase (JAK) inhibitors such as tofacitinib significantly improved RA disease activity when compared to placebo[31]. As a result, among medications approved for RA and IBD, TNF and JAK inhibitors may be effective for both IMIDs. Given that JAKs bind to cytokine receptors and transmit various extracellular cytokine signals such as IL-6, IL-12, IL-23, and interferons, JAK inhibitors are therefore expected to be effective for various IMIDs[28,32] (Table 1).

## IMIDS AFFECTING IBD PHENOTYPES AND OUTCOMES

Several recent population-based studies and meta-analyses have shown that the presence of IMIDs may influence the disease course of IBD. According to a nationwide study of the Danish population, 22.5% of IBD patients had at least one concurrent IMID. This study found that risk factors such as older age and methotrexate use were associated with an increased risk of concurrent IMIDs, whereas male gender, a high socioeconomic status, and infliximab use were associated with a reduced risk of concurrent IMIDs. The impact of at least one concurrent IMID on the disease course of IBD was also investigated in this study. The occurrence of IMIDs increased the risk of surgery in CD patients but not in UC patients in the subgroup who developed IMIDs after the onset of IBD. Both UC and CD patients who had concurrent IMIDs were more likely to require biologics than those who did not[33]. IBD patients with concurrent IMIDs had an increased risk of extensive colitis/pancolitis and IBD-related surgeries compared with those without IMIDs, according to a recent systematic review and meta-analysis involving 16064 IBD patients with concurrent IMIDs and 3451414 IBD patients without IMIDs. Furthermore, those with concurrent IMIDs were at an increased risk of requiring immunomodulators and biologics[34]. Overall, these findings suggest that the coexistence of IMIDs is associated with severe IBD phenotypes.

Another recent systematic review and meta-analysis found that CD patients with concurrent IMIDs were more likely to have upper gastrointestinal involvement and nonstricturing and nonpenetrating phenotypes than those without IMIDs. Given that this meta-analysis suggests that CD patients with concurrent IMIDs may have a milder disease course, which contradicts the findings of the previous meta-analysis, more research is needed to better understand CD patients' behaviors if they develop other IMIDs and require medication[35].

While it is unclear how different IMIDs affect IBD phenotypes and outcomes, previous research has consistently suggested that IMIDs such as PSC or TAK can influence the endoscopic findings of IBD to specific phenotypes[16,17,36,37]. On the other hand, recent studies have shown that there is an association between celiac disease and IBD and that IBD patients with celiac disease can have distinct phenotypic characteristics and outcomes[38,39].

### PSC and IBD

PSC is a chronic and progressive cholestatic disease that affects the intrahepatic and extrahepatic bile ducts[40]. PSC was found to be present in 2.2% of IBD patients. In one study, PSC was found to be present in 2.5% of UC patients and 0.96% of CD patients, respectively[41]. Patients with "extensive UC" had a higher prevalence of PSC than those with left-sided colitis. PSC was more common in CD patients with ileocolonic or colonic involvement than in those with ileal involvement[35,41]. PSC-IBD patients have a predilection for more severe right-sided colitis according to several studies[42,43]. Furthermore, patients with IBD who have PSC frequently develop a distinct IBD phenotype characterized by "rectal sparing" and "backwash ileitis"[16]. According to a systematic review of 11406 PSC-IBD patients, the pooled rate of rectal sparing and backwash ileitis was 9.9% and 12.3%, respectively[36]. Based on this systematic review, a meta-analysis found that the pooled event rate of pancolitis and rectal sparing was significantly higher in PSC-IBD patients than in IBD patients without PSC, but the rate of backwash ileitis was not statistically significant between the two groups[43].

Although PSC-IBD patients frequently develop pancolonic involvement with significant right-sided colitis, several studies have shown that their disease course was mild[43]. Previous studies found that PSC-IBD patients had fewer or no symptoms[44] and were less likely to require immunosuppressants such as steroids, thiopurines, hospitalization[45], and surgery than IBD patients without PSC[46]. In addition, patients with PSC-CD had a higher risk of nonstricturing and nonpenetrating behavior than those without PSC, as well as being less likely to undergo CD-related surgeries, according to a recent meta-analysis[35]. All of these findings suggest that PSC-IBD patients may have better IBD outcomes, suggesting that PSC-IBD is a distinct disease phenotype from IBD phenotypes in patients with other IMIDs (Table 1).

The cause of the disparity between endoscopic disease activity and clinical outcomes in patients with PSC-IBD is unknown. In a retrospective study, immunosuppressant use and colonic or ileal surgical resection were found to be less common in PSC-IBD patients than in IBD patients without PSC. However, this study discovered that a higher percentage of PSC-IBD patients used 5-aminosalicylates, suggesting that PSC-IBD patients are more likely to use 5-aminosalicylates to lower their risk of colorectal cancer or dysplasia and that their use can be associated with mild disease activity[46-48]. Meanwhile, a case-controlled study found that PSC-UC patients had a milder disease course than those with UC alone, even though the number of patients taking 5-aminosalicylates or sulfasalazine was the same[49]. Hence, more research is needed to understand if differences in the pathogenesis or medical therapies between PSC-IBD patients and patients with IBD alone can explain the unique disease phenotype of PSC-IBD.

Many studies have been carried out to better understand the mechanisms of PSC-IBD, and it has been discovered that there is less genetic overlap between PSC and IBD[50]. Thus, environmental factors such as the gut microbiome have been highlighted. According to the "leaky gut" theory, IBD-related mucosal injury allows the colonic bacteria to cross the gut barrier and into the liver, resulting in the development of PSC[43]. Although the exact mechanism is unknown, PSC-IBD patients were more likely to develop colorectal cancer than IBD patients without PSC[43], so UC guidelines recommend colorectal cancer screening at the time of PSC diagnosis[51].

### Celiac disease and IBD

Celiac disease is a gluten-induced disease that frequently coexists in patients with IMIDs[52]. Patients with IBD have a higher risk of celiac disease compared to controls according to a systematic review and meta-analysis (risk ratio 3.96; 95% confidence interval: 2.23-7.02). Patients with celiac disease have a significantly increased risk of IBD compared to controls (risk ratio 9.88; 95% confidence interval: 4.03-24.2), suggesting the shared pathogenesis between both diseases[38]. A case–control study based on a national registry of pediatric IBD patients found that CD patients with celiac disease were less likely to have ileocolonic involvement and require medical therapies such as TNF inhibitors or azathioprine than CD patients alone. While disease distribution and medical treatments were not significantly different between UC patients with and without celiac disease, UC patients with celiac disease had a higher risk of colectomy[39], suggesting that CD patients with celiac disease may have better outcomes, whereas the concurrence of celiac disease can potentially worsen UC outcomes (Table 1).

Some genetic, environmental, and immunological factors are shared by celiac disease and IBD. GWASs revealed that celiac disease and CD share genetic risk loci including PTPN2, IL18RAP, TAGAP, and PUS10[53]. Both diseases have been linked to microbial factors[54,55]. Celiac disease is a $CD4^+$ T-cell-dependent disease associated with chronic intestinal inflammation, according to the immunological mechanism[56]. APCs present gliadin peptide to $CD4^+$ T cells, which then produce proinflammatory cytokines such as interferon-γ and IL-21, increasing intraepithelial lymphocytes (IELs)[56]. The IELs such as $CD8^+$ T cells carrying αβ T-cell receptor (TCR) or $CD4^-CD8^-$ γδ $TCR^+$ T cells cause cytolysis or apoptosis in intestinal epithelial cells[57,58]. Patients with celiac disease have mucosal Th17 cells, and an increased level of IL-17A expression is associated with villous atrophy, a hallmark pathologic feature of celiac disease[59,60]. Th17 cells produce IL-17, interferon-γ, and IL-21, which play a role in celiac disease pathogenesis[61]. In terms of ILCs, it remains unclear how these cells are associated with the disease initiation and progression[62]. These findings support that Th17 cells may be involved in both IBD and celiac disease (Figure 1 and Table 1).

### *TAK and IBD*

TAK is a type of chronic large-vessel vasculitis that primarily affects the aorta, and its major branches, as well as pulmonary arteries[63]. The prevalence of UC and CD among TAK patients was reported to be 6.4% (30/470) and 9.0% (4/44), respectively[64,65]. Endoscopic features of IBD in TAK patients were found to be atypical for UC or CD in a previous retrospective study[17]. This study, which included 142 Japanese TAK patients, identified 13 TAK-IBD patients (9.2%), and their endoscopic findings at the initial time of IBD diagnosis were assessed. As a result, 7/8 (87.5%) of TAK patients had "discontinuous aphthous erosions/ulcers or focal mucosal inflammation" with only one patient having continuous inflammation typical of UC[17], suggesting that TAK-IBD patients have a distinct endoscopic IBD phenotype. This study also discovered that patients with TAK-IBD were more likely to have the HLA haplotype, which has a susceptible effect on UC, when compared with TAK patients without IBD, implying that TAK-IBD may have a similar immune mechanism to UC. A recent case study describing the evolution of colonic inflammation in a TAK-IBD patient during 10-year observation found that colonic inflammation was initially discontinuous and more severe in the proximal colon than in the remaining colon[37], supporting the presence of a distinct IBD phenotype in TAK-IBD patients (Table 1).

Th1 and Th17 cells are predominant in the pathophysiology of TAK just as they are in the pathogenesis of CD[66] (Figure 1 and Table 1). TAK patients' aorta expresses higher levels of IL-6, IL-12, IL-17, and interferon-γ[67], and a single nucleotide polymorphism encoding a common component of IL-12 and IL-23 is associated with the development of TAK[64]. In a previous study, ustekinumab was shown to reduce inflammatory markers and the dose of steroid needed in the treatment of TAK patients [68]. Many case studies have described TAK-IBD patients' therapeutic approaches to date. Tofacitinib, a JAK inhibitor that was recently approved for UC[69], was found to be effective for both inflammatory conditions[70,71], implying that it can inhibit multiple inflammatory signals involved in TAK and IBD, suggesting that JAK pathways could be a promising therapeutic target. To better understand the prognosis of IBD in such patients with TAK, further studies are needed.

Figures 2 and 3 show representative endoscopic findings of PSC-IBD and TAK-IBD, respectively.

## CONCLUSION

Recent research has revealed that IBD and other IMIDs share overlapping genetic and immunological etiologies. Indeed, IBD patients frequently develop concurrent IMIDs, and these patients have a higher risk of severe IBD phenotypes, implying that the presence of IMIDs is a poor prognostic factor for IBD. As a result, when treating IBD patients, we should be aware of the presence of other IMIDs, as biologics and small molecule therapies may be able to treat multiple disease states simultaneously. Multidisciplinary care with rheumatologists or dermatologists is also important when deciding the therapeutic strategy for IBD patients with concurrent IMIDs.

Furthermore, patients with IMIDs such as PSC or TAK can have distinct endoscopic phenotypes of IBD, suggesting that the immune mechanisms of these IMIDs may affect how intestinal inflammation is distributed. Even though IBD patients with concurrent IMIDs are more likely to require biologics or surgery, the disease course of PSC-IBD patients is not often associated with poor IBD outcomes, although these patients are more likely to develop pancolonic inflammation. Further research involving various concurrent IMIDs is needed in light of these findings to better understand how each IMID and its medication may affect the phenotype and natural history of IBD.

In the future, clinicians and researchers will face a challenge in identifying therapeutic targets to improve patient outcomes. Previous studies have shown that Th1 and Th17 cells (rather than Th2 cells) are more likely to be involved in the concurrence of other IMIDs in IBD patients, implying that these effector T cells and their cytokines could be promising therapeutic targets. Future research is needed to gain further insights into the shared immune mechanisms involved in the pathophysiology of IBD and other IMIDs to develop appropriate therapeutic targets to treat both diseases.

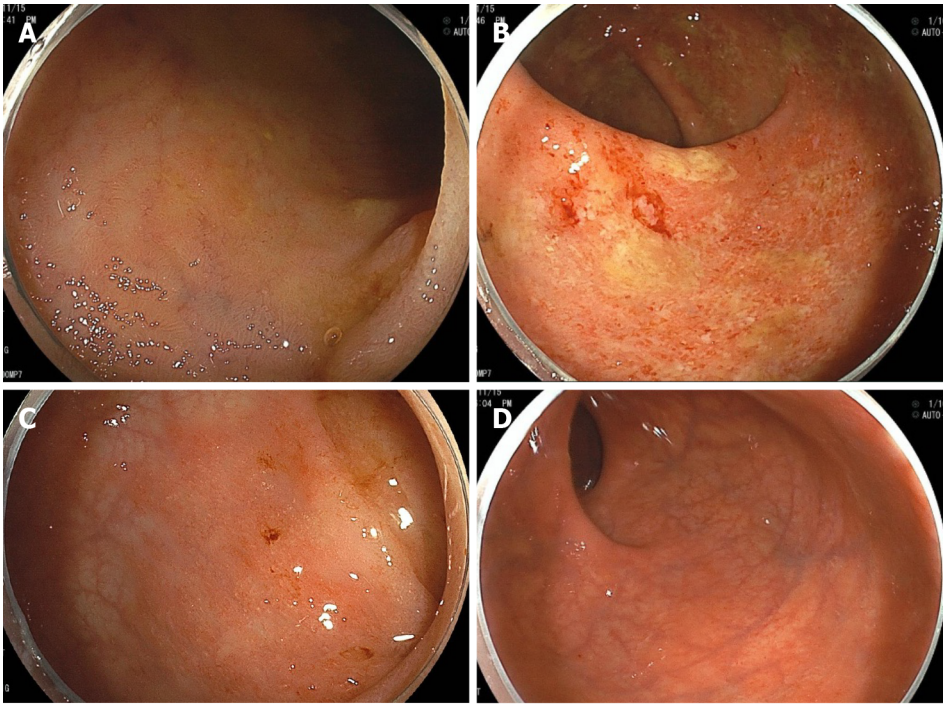


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**Figure 2 Colonoscopy showing representative endoscopic findings of a patient with primary sclerosing cholangitis and ulcerative colitis.** Right-sided predominant colitis and rectal sparing. A: Terminal ileum with normal mucosa; B: Ascending colon, diffuse inflammation with erosions, loss of vascular pattern and friability; C: Sigmoid colon, with mild erythematous inflammation and decreased vascular pattern; D: Rectum, with normal mucosa.

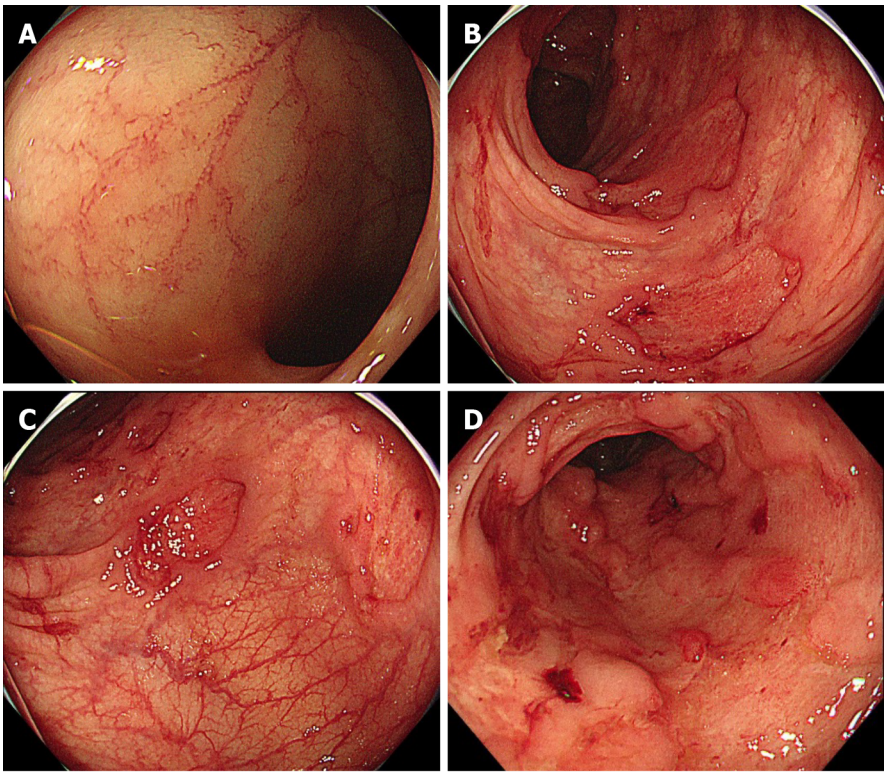


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**Figure 3 Colonoscopy showing representative endoscopic findings of a patient with Takayasu arteritis and inflammatory bowel disease.** A: Terminal ileum with normal mucosa; B and C: Sigmoid and rectosigmoid colon, respectively, with discontinuous round ulcers; D: Lower rectum, with deep and large longitudinal ulcers.

## FOOTNOTES

**Author contributions:** Akiyama S designed the research; Akiyama S and Fukuda S performed the research and analyzed the data; Akiyama S, Fukuda S, Steinberg JM, Suzuki H, and Tsuchiya K wrote the paper.

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## REFERENCES

1 **Rosen MJ**, Dhawan A, Saeed SA. Inflammatory Bowel Disease in Children and Adolescents. *JAMA Pediatr* 2015; **169**: 1053-1060 [PMID: 26414706 DOI: 10.1001/jamapediatrics.2015.1982]
2 **Ananthakrishnan AN**, Bernstein CN, Iliopoulos D, Macpherson A, Neurath MF, Ali RAR, Vavricka SR, Fiocchi C. Environmental triggers in IBD: a review of progress and evidence. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 39-49 [PMID: 29018271 DOI: 10.1038/nrgastro.2017.136]
3 **Younis N**, Zarif R, Mahfouz R. Inflammatory bowel disease: between genetics and microbiota. *Mol Biol Rep* 2020; **47**: 3053-3063 [PMID: 32086718 DOI: 10.1007/s11033-020-05318-5]
4 **Khor B**, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; **474**: 307-317 [PMID: 21677747 DOI: 10.1038/nature10209]
5 **Anderson CA**, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel JF, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RS, Floyd JA, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot JP, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra HJ, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; **43**: 246-252 [PMID: 21297633 DOI: 10.1038/ng.764]
6 **Franke A**, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010; **42**: 1118-1125 [PMID: 21102463 DOI: 10.1038/ng.717]
7 **Monteleone G**, Biancone L, Marasco R, Morrone G, Marasco O, Luzza F, Pallone F. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 1997; **112**: 1169-1178 [PMID: 9098000 DOI: 10.1016/s0016-5085(97)70128-8]
8 **Geremia A**, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun Rev* 2014; **13**: 3-10 [PMID: 23774107 DOI: 10.1016/j.autrev.2013.06.004]
9 **Akiyama S**, Sakuraba A. Distinct roles of interleukin-17 and T helper 17 cells among autoimmune diseases. *J Transl Autoimmun* 2021; **4**: 100104 [PMID: 34179741 DOI: 10.1016/j.jtauto.2021.100104]

10 **Yen D**, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation *via* IL-17 and IL-6. *J Clin Invest* 2006; **116**: 1310-1316 [PMID: 16670770 DOI: 10.1172/jci21404]

11 **Kobayashi T**, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R, Kitazume MT, Nakazawa A, Sugita A, Koganei K, Isobe K, Hibi T. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. *Gut* 2008; **57**: 1682-1689 [PMID: 18653729 DOI: 10.1136/gut.2007.135053]

12 **Panda SK**, Colonna M. Innate Lymphoid Cells in Mucosal Immunity. *Front Immunol* 2019; **10**: 861 [PMID: 31134050 DOI: 10.3389/fimmu.2019.00861]

13 **Weng X**, Liu L, Barcellos LF, Allison JE, Herrinton LJ. Clustering of inflammatory bowel disease with immune mediated diseases among members of a northern california-managed care organization. *Am J Gastroenterol* 2007; **102**: 1429-1435 [PMID: 17437504 DOI: 10.1111/j.1572-0241.2007.01215.x]

14 **Conway G**, Velonias G, Andrews E, Garber JJ, Yajnik V, Ananthakrishnan AN. The impact of co-existing immune-mediated diseases on phenotype and outcomes in inflammatory bowel diseases. *Aliment Pharmacol Ther* 2017; **45**: 814-823 [PMID: 28105709 DOI: 10.1111/apt.13940]

15 **Halling ML**, Kjeldsen J, Knudsen T, Nielsen J, Hansen LK. Patients with inflammatory bowel disease have increased risk of autoimmune and inflammatory diseases. *World J Gastroenterol* 2017; **23**: 6137-6146 [PMID: 28970729 DOI: 10.3748/wjg.v23.i33.6137]

16 **Loftus EV Jr**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

17 **Akiyama S**, Fujii T, Matsuoka K, Yusuke E, Negi M, Takenaka K, Nagahori M, Ohtsuka K, Isobe M, Watanabe M. Endoscopic features and genetic background of inflammatory bowel disease complicated with Takayasu arteritis. *J Gastroenterol Hepatol* 2017; **32**: 1011-1017 [PMID: 28449344 DOI: 10.1111/jgh.13640]

18 **Attauabi M**, Wewer MD, Bendtsen F, Seidelin JB, Burisch J. Inflammatory Bowel Diseases Affect the Phenotype and Disease Course of Coexisting Immune-Mediated Inflammatory Diseases: A Systematic Review With Meta-Analysis. *Inflamm Bowel Dis* 2022 [PMID: 35134921 DOI: 10.1093/ibd/izac003]

19 **Lambrecht BN**, Hammad H, Fahy JV. The Cytokines of Asthma. *Immunity* 2019; **50**: 975-991 [PMID: 30995510 DOI: 10.1016/j.immuni.2019.03.018]

20 **Ordás I**, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**: 1606-1619 [PMID: 22914296 DOI: 10.1016/S0140-6736(12)60150-0]

21 **Mohammadi H**, Sharafkandi N, Hemmatzadeh M, Azizi G, Karimi M, Jadidi-Niaragh F, Baradaran B, Babaloo Z. The role of innate lymphoid cells in health and disease. *J Cell Physiol* 2018; **233**: 4512-4529 [PMID: 29058773 DOI: 10.1002/jcp.26250]

22 **Hoegler KM**, John AM, Handler MZ, Schwartz RA. Generalized pustular psoriasis: a review and update on treatment. *J Eur Acad Dermatol Venereol* 2018; **32**: 1645-1651 [PMID: 29573491 DOI: 10.1111/jdv.14949]

23 **Korman NJ**. Management of psoriasis as a systemic disease: what is the evidence? *Br J Dermatol* 2020; **182**: 840-848 [PMID: 31225638 DOI: 10.1111/bjd.18245]

24 **Torres J**, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet* 2017; **389**: 1741-1755 [PMID: 27914655 DOI: 10.1016/S0140-6736(16)31711-1]

25 **Fu Y**, Lee CH, Chi CC. Association of Psoriasis With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *JAMA Dermatol* 2018; **154**: 1417-1423 [PMID: 30422277 DOI: 10.1001/jamadermatol.2018.3631]

26 **Pugliese D**, Daperno M, Fiorino G, Savarino E, Mosso E, Biancone L, Testa A, Sarpi L, Cappello M, Bodini G, Caprioli F, Festa S, Laino G, Maconi G, Mazzuoli S, Mocci G, Sartini A, D'Amore A, Alivernini S, Gremese E, Armuzzi A. Real-life effectiveness of ustekinumab in inflammatory bowel disease patients with concomitant psoriasis or psoriatic arthritis: An IG-IBD study. *Dig Liver Dis* 2019; **51**: 972-977 [PMID: 30992173 DOI: 10.1016/j.dld.2019.03.007]

27 **Pugliese D**, Guidi L, Ferraro PM, Marzo M, Felice C, Celleno L, Landi R, Andrisani G, Pizzolante F, De Vitis I, Papa A, Rapaccini GL, Armuzzi A. Paradoxical psoriasis in a large cohort of patients with inflammatory bowel disease receiving treatment with anti-TNF alpha: 5-year follow-up study. *Aliment Pharmacol Ther* 2015; **42**: 880-888 [PMID: 26235565 DOI: 10.1111/apt.13352]

28 **Akiyama S**, Lin A, Traboulsi C, Rubin DT. Treatment of Crohn's Disease and Concomitant Alopecia Areata With Tofacitinib. *ACG Case Rep J* 2021; **8**: e00690 [PMID: 34840997 DOI: 10.14309/crj.0000000000000690]

29 **McInnes IB**, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011; **365**: 2205-2219 [PMID: 22150039 DOI: 10.1056/NEJMra1004965]

30 **Smolen JS**, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. *Lancet* 2007; **370**: 1861-1874 [PMID: 17570481 DOI: 10.1016/S0140-6736(07)60784-3]

31 **Kerschbaumer A**, Sepriano A, Smolen JS, van der Heijde D, Dougados M, van Vollenhoven R, McInnes IB, Bijlsma JWJ, Burmester GR, de Wit M, Falzon L, Landewé R. Efficacy of pharmacological treatment in rheumatoid arthritis: a systematic literature research informing the 2019 update of the EULAR recommendations for management of rheumatoid arthritis. *Ann Rheum Dis* 2020; **79**: 744-759 [PMID: 32033937 DOI: 10.1136/annrheumdis-2019-216656]

32 **Winthrop KL**. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat Rev Rheumatol* 2017; **13**: 234-243 [PMID: 28250461 DOI: 10.1038/nrrheum.2017.23]

33 **Burisch J**, Jess T, Egeberg A. Incidence of Immune-Mediated Inflammatory Diseases Among Patients With Inflammatory Bowel Diseases in Denmark. *Clin Gastroenterol Hepatol* 2019; **17**: 2704-2712.e3 [PMID: 30936024 DOI: 10.1016/j.cgh.2019.03.040]

34 **Attauabi M**, Zhao M, Bendtsen F, Burisch J. Systematic Review with Meta-analysis: The Impact of Co-occurring Immune-mediated Inflammatory Diseases on the Disease Course of Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2021; **27**: 927-939 [PMID: 32628745 DOI: 10.1093/ibd/izaa167]

35 **Attauabi M**, Zhao M, Bendtsen F, Burisch J. Systematic review and meta-analysis: the impact of co-occurring immune-mediated inflammatory diseases on the disease localization and behavior of Crohn's disease. *Therap Adv Gastroenterol*

2021; **14**: 17562848211004839 [PMID: 34234844 DOI: 10.1177/17562848211004839]
36 **de Vries AB**, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol* 2015; **21**: 1956-1971 [PMID: 25684965 DOI: 10.3748/wjg.v21.i6.1956]
37 **Wada A**, Higashiyama M, Hirata D, Ito S, Tanemoto R, Nishii S, Mizoguchi A, Inaba K, Sugihara N, Hanawa Y, Horiuchi K, Akita Y, Narimatsu K, Komoto S, Tomita K, Hokari R. Changes in Colonic Inflammation Related with Takayasu Arteritis during a 10-year Observation Period. *Intern Med* 2022; **61**: 475-480 [PMID: 34393161 DOI: 10.2169/internalmedicine.7287-21]
38 **Pinto-Sanchez MI**, Seiler CL, Santesso N, Alaedini A, Semrad C, Lee AR, Bercik P, Lebwohl B, Leffler DA, Kelly CP, Moayyedi P, Green PH, Verdu EF. Association Between Inflammatory Bowel Diseases and Celiac Disease: A Systematic Review and Meta-Analysis. *Gastroenterology* 2020; **159**: 884-903.e31 [PMID: 32416141 DOI: 10.1053/j.gastro.2020.05.016]
39 **Bramuzzo M**, Lionetti P, Miele E, Romano C, Arrigo S, Cardile S, Di Nardo G, Illiceto MT, Pastore M, Felici E, Fuoti M, Banzato C, Citrano M, Congia M, Norsa L, Pozzi E, Zuin G, Agrusti A, Bianconi M, Grieco C, Giudici F, Aloi M, Alvisi P. Phenotype and Natural History of Children With Coexistent Inflammatory Bowel Disease and Celiac Disease. *Inflamm Bowel Dis* 2021; **27**: 1881-1888 [PMID: 33452803 DOI: 10.1093/ibd/izaa360]
40 **Beheshti-Maal A**, Tamimi A, Iravani S, Memarnejadian A, Sorouri M, Aghdaei HA, Zali MR, Hossein Khannazer N, Vosough M. PSC associated inflammatory bowel disease: a distinct entity. *Expert Rev Gastroenterol Hepatol* 2022; **16**: 129-139 [PMID: 35078376 DOI: 10.1080/17474124.2022.2031979]
41 **Barberio B**, Massimi D, Cazzagon N, Zingone F, Ford AC, Savarino EV. Prevalence of Primary Sclerosing Cholangitis in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Gastroenterology* 2021; **161**: 1865-1877 [PMID: 34425093 DOI: 10.1053/j.gastro.2021.08.032]
42 **Krugliak Cleveland N**, Rubin DT, Hart J, Weber CR, Meckel K, Tran AL, Aelvoet AS, Pan I, Gonsalves A, Gaetano JN, Williams KM, Wroblewski K, Jabri B, Pekow J. Patients With Ulcerative Colitis and Primary Sclerosing Cholangitis Frequently Have Subclinical Inflammation in the Proximal Colon. *Clin Gastroenterol Hepatol* 2018; **16**: 68-74 [PMID: 28756053 DOI: 10.1016/j.cgh.2017.07.023]
43 **Ricciuto A**, Kamath BM, Griffiths AM. The IBD and PSC Phenotypes of PSC-IBD. *Curr Gastroenterol Rep* 2018; **20**: 16 [PMID: 29594739 DOI: 10.1007/s11894-018-0620-2]
44 **Palmela C**, Peerani F, Castaneda D, Torres J, Itzkowitz SH. Inflammatory Bowel Disease and Primary Sclerosing Cholangitis: A Review of the Phenotype and Associated Specific Features. *Gut Liver* 2018; **12**: 17-29 [PMID: 28376583 DOI: 10.5009/gnl16510]
45 **Moayyeri A**, Daryani NE, Bahrami H, Haghpanah B, Nayyer-Habibi A, Sadatsafavi M. Clinical course of ulcerative colitis in patients with and without primary sclerosing cholangitis. *J Gastroenterol Hepatol* 2005; **20**: 366-370 [PMID: 15740478 DOI: 10.1111/j.1440-1746.2005.03727.x]
46 **Sokol H**, Cosnes J, Chazouilleres O, Beaugerie L, Tiret E, Poupon R, Seksik P. Disease activity and cancer risk in inflammatory bowel disease associated with primary sclerosing cholangitis. *World J Gastroenterol* 2008; **14**: 3497-3503 [PMID: 18567077 DOI: 10.3748/wjg.14.3497]
47 **van Staa TP**, Card T, Logan RF, Leufkens HG. 5-Aminosalicylate use and colorectal cancer risk in inflammatory bowel disease: a large epidemiological study. *Gut* 2005; **54**: 1573-1578 [PMID: 15994215 DOI: 10.1136/gut.2005.070896]
48 **Eaden J**, Abrams K, Ekbom A, Jackson E, Mayberry J. Colorectal cancer prevention in ulcerative colitis: a case-control study. *Aliment Pharmacol Ther* 2000; **14**: 145-153 [PMID: 10651654 DOI: 10.1046/j.1365-2036.2000.00698.x]
49 **Lundqvist K**, Broomé U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. *Dis Colon Rectum* 1997; **40**: 451-456 [PMID: 9106695 DOI: 10.1007/BF02258391]
50 **Ellinghaus D**, Folseraas T, Holm K, Ellinghaus E, Melum E, Balschun T, Laerdahl JK, Shiryaev A, Gotthardt DN, Weismüller TJ, Schramm C, Wittig M, Bergquist A, Björnsson E, Marschall HU, Vatn M, Teufel A, Rust C, Gieger C, Wichmann HE, Runz H, Sterneck M, Rupp C, Braun F, Weersma RK, Wijmenga C, Ponsioen CY, Mathew CG, Rutgeerts P, Vermeire S, Schrumpf E, Hov JR, Manns MP, Boberg KM, Schreiber S, Franke A, Karlsen TH. Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. *Hepatology* 2013; **58**: 1074-1083 [PMID: 22821403 DOI: 10.1002/hep.25977]
51 **Rubin DT**, Ananthakrishnan AN, Siegel CA, Sauer BG, Long MD. ACG Clinical Guideline: Ulcerative Colitis in Adults. *Am J Gastroenterol* 2019; **114**: 384-413 [PMID: 30840605 DOI: 10.14309/ajg.0000000000000152]
52 **Lundin KE**, Wijmenga C. Coeliac disease and autoimmune disease-genetic overlap and screening. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 507-515 [PMID: 26303674 DOI: 10.1038/nrgastro.2015.136]
53 **Festen EA**, Goyette P, Green T, Boucher G, Beauchamp C, Trynka G, Dubois PC, Lagacé C, Stokkers PC, Hommes DW, Barisani D, Palmieri O, Annese V, van Heel DA, Weersma RK, Daly MJ, Wijmenga C, Rioux JD. A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. *PLoS Genet* 2011; **7**: e1001283 [PMID: 21298027 DOI: 10.1371/journal.pgen.1001283]
54 **Caminero A**, Meisel M, Jabri B, Verdu EF. Mechanisms by which gut microorganisms influence food sensitivities. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 7-18 [PMID: 30214038 DOI: 10.1038/s41575-018-0064-z]
55 **Harris KG**, Chang EB. The intestinal microbiota in the pathogenesis of inflammatory bowel diseases: new insights into complex disease. *Clin Sci (Lond)* 2018; **132**: 2013-2028 [PMID: 30232239 DOI: 10.1042/CS20171110]
56 **Hisamatsu T**, Erben U, Kühl AA. The Role of T-Cell Subsets in Chronic Inflammation in Celiac Disease and Inflammatory Bowel Disease Patients: More Common Mechanisms or More Differences? *Inflamm Intest Dis* 2016; **1**: 52-62 [PMID: 29922658 DOI: 10.1159/000445133]
57 **Sollid LM**. Intraepithelial lymphocytes in celiac disease: license to kill revealed. *Immunity* 2004; **21**: 303-304 [PMID: 15357941 DOI: 10.1016/j.immuni.2004.08.002]
58 **Meresse B**, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* 2004; **21**: 357-366 [PMID: 15357947 DOI:

10.1016/j.immuni.2004.06.020]
59 **Fernández S**, Molina IJ, Romero P, González R, Peña J, Sánchez F, Reynoso FR, Pérez-Navero JL, Estevez O, Ortega C, Santamaría M. Characterization of gliadin-specific Th17 cells from the mucosa of celiac disease patients. *Am J Gastroenterol* 2011; **106**: 528-538 [PMID: 21206487 DOI: 10.1038/ajg.2010.465]
60 **Lahdenperä AI**, Fälth-Magnusson K, Högberg L, Ludvigsson J, Vaarala O. Expression pattern of T-helper 17 cell signaling pathway and mucosal inflammation in celiac disease. *Scand J Gastroenterol* 2014; **49**: 145-156 [PMID: 24325470 DOI: 10.3109/00365521.2013.863966]
61 **Monteleone I**, Sarra M, Del Vecchio Blanco G, Paoluzi OA, Franzè E, Fina D, Fabrizi A, MacDonald TT, Pallone F, Monteleone G. Characterization of IL-17A-producing cells in celiac disease mucosa. *J Immunol* 2010; **184**: 2211-2218 [PMID: 20061410 DOI: 10.4049/jimmunol.0901919]
62 **Yu X**, Vargas J, Green PHR, Bhagat G. Innate Lymphoid Cells and Celiac Disease: Current Perspective. *Cell Mol Gastroenterol Hepatol* 2021; **11**: 803-814 [PMID: 33309944 DOI: 10.1016/j.jcmgh.2020.12.002]
63 **Alibaz-Oner F**, Direskeneli H. Update on Takayasu's arteritis. *Presse Med* 2015; **44**: e259-e265 [PMID: 26033561 DOI: 10.1016/j.lpm.2015.01.015]
64 **Terao C**, Matsumura T, Yoshifuji H, Kirino Y, Maejima Y, Nakaoka Y, Takahashi M, Amiya E, Tamura N, Nakajima T, Origuchi T, Horita T, Matsukura M, Kochi Y, Ogimoto A, Yamamoto M, Takahashi H, Nakayamada S, Saito K, Wada Y, Narita I, Kawaguchi Y, Yamanaka H, Ohmura K, Atsumi T, Tanemoto K, Miyata T, Kuwana M, Komuro I, Tabara Y, Ueda A, Isobe M, Mimori T, Matsuda F. Takayasu arteritis and ulcerative colitis: high rate of co-occurrence and genetic overlap. *Arthritis Rheumatol* 2015; **67**: 2226-2232 [PMID: 25931203 DOI: 10.1002/art.39157]
65 **Reny JL**, Paul JF, Lefèbvre C, Champion K, Emmerich J, Blétry O, Piette JC, Fiessinger JN. Association of Takayasu's arteritis and Crohn's disease. Results of a study on 44 Takayasu patients and review of the literature. *Ann Med Interne (Paris)* 2003; **154**: 85-90 [PMID: 12746644 DOI: 10.1016/s0248-8663(97)80232-9]
66 **Saadoun D**, Garrido M, Comarmond C, Desbois AC, Domont F, Savey L, Terrier B, Geri G, Rosenzwajg M, Klatzmann D, Fourret P, Cluzel P, Chiche L, Gaudric J, Koskas F, Cacoub P. Th1 and Th17 cytokines drive inflammation in Takayasu arteritis. *Arthritis Rheumatol* 2015; **67**: 1353-1360 [PMID: 25604824 DOI: 10.1002/art.39037]
67 **Savioli B**, Abdulahad WH, Brouwer E, Kallenberg CGM, de Souza AWS. Are cytokines and chemokines suitable biomarkers for Takayasu arteritis? *Autoimmun Rev* 2017; **16**: 1071-1078 [PMID: 28778711 DOI: 10.1016/j.autrev.2017.07.023]
68 **Gon Y**, Yoshifuji H, Nakajima T, Murakami K, Nakashima R, Ohmura K, Mimori T, Terao C. Long-term outcomes of refractory Takayasu arteritis patients treated with biologics including ustekinumab. *Mod Rheumatol* 2021; **31**: 678-683 [PMID: 32700985 DOI: 10.1080/14397595.2020.1800560]
69 **Sandborn WJ**, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, Friedman G, Lawendy N, Yu D, Woodworth D, Mukherjee A, Zhang H, Healey P, Panés J; OCTAVE Induction 1, OCTAVE Induction 2, and OCTAVE Sustain Investigators. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2017; **376**: 1723-1736 [PMID: 28467869 DOI: 10.1056/NEJMoa1606910]
70 **Kuwabara S**, Tanimura S, Matsumoto S, Nakamura H, Horita T. Successful remission with tofacitinib in a patient with refractory Takayasu arteritis complicated by ulcerative colitis. *Ann Rheum Dis* 2020; **79**: 1125-1126 [PMID: 32213500 DOI: 10.1136/annrheumdis-2019-216606]
71 **Sato S**, Matsumoto H, Temmoku J, Fujita Y, Matsuoka N, Furuya M, Gunji N, Fujiwara T, Asano T, Onizawa M, Kobayashi H, Watanabe H, Ohira H, Migita K. A case of Takayasu arteritis complicated by refractory ulcerative colitis successfully treated with tofacitinib. *Rheumatology (Oxford)* 2020; **59**: 1773-1775 [PMID: 31776552 DOI: 10.1093/rheumatology/kez580]

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*MINIREVIEWS*

# Correlation of molecular alterations with pathological features in hepatocellular carcinoma: Literature review and experience of an Italian center

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## Abstract

Hepatocellular carcinoma (HCC) represents the primary carcinoma of the liver and the fourth leading cause of cancer-related deaths. The World Health Organization estimates an increase in cases in the coming years. The risk factors of HCC are multiple, and the incidence in different countries is closely related to the different risk factors to which the population is exposed. The molecular mechanisms that drive HCC tumorigenesis are extremely complex, but understanding this multistep process is essential for the identification of diagnostic, prognostic, and therapeutic markers. The development of multigenic next-generation sequencing panels through the parallel analysis of multiple markers can provide a landscape of the genomic status of the tumor. Considering the literature and our preliminary data based on 36 HCCs, the most frequently altered genes in HCCs are *TERT*, *CTNNB1*, and *TP53*. Over the years, many groups have attempted to classify HCCs on a molecular basis, but a univocal classification has never been achieved. Nevertheless, statistically significant correlations have been found in HCCs between the molecular signature and morphologic features, and this leads us to think that it would be desirable to integrate the approach between anatomic pathology and molecular laboratories.

**Key Words:** Hepatocarcinoma; Mutation; Next-generation sequencing; Review; *TP53*; *CTNNB1*; *TERT*

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**Core Tip:** The molecular mechanisms that drive hepatocellular carcinoma tumorigenesis are extremely complex, and a univocal classification based on molecular features has not been defined. In the age of precision medicine, the study of hepatocellular carcinoma mutations is still a field worth investigating. Based on this, we wanted to analyze the possible correlations between molecular alterations and pathological features. Considering both the literature data and our personal experience, about 80% of hepatocellular carcinomas harbor mutations in at least one of the following genes, *TERT*, *TP53*, or *CTNNB1*, with different biological and clinical implications.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the world-leading cancers, representing approximately 80% of the primary carcinomas of the liver[1] and the fourth most common cause of cancer-related deaths. The World Health Organization (WHO) estimated more than 905500 new HCC cases in 2020 worldwide [2], and based on its projection an increase of 58% is evaluated by 2040 with a total of 1400000 new cases and 1000000 deaths in 2030[2].

The etiological factors for HCC development are: (1) Infections, including hepatitis B virus (HBV) and hepatitis C virus (HCV), with or without coinfection of hepatitis delta virus; (2) Lifestyle risk factors and behaviors, such as alcohol addiction and smoking; (3) Environment, such as dietary toxins (*e.g.*, aflatoxins, or aristolochic acid); (4) Underlying diseases, such as obesity, type 2 diabetes, nonalcoholic liver steatohepatitis/nonalcoholic fatty liver disease; and (5) Genetics, some single nucleotide polymorphisms are identified to be associated with HCC risk at different stages, from predisposition to risk factors to the severity of the chronic liver disease and its evolution to cirrhosis or to the malignant transformation and tumor progression[3,4]. For example, a single nucleotide polymorphism correlated with higher infection risk (MDM2 Promoter SNP309, MDM2 G-309T, rs2279744) has been associated with HCC patients with chronic hepatitis C[5].

The incidence of HCC in different countries varies considering the different risk factors mentioned above. In Eastern Asian countries and most African countries, the incidence of HCC is mostly due to aflatoxin exposure and HBV infection, except for Northern Africa where HCV infection is prevalent[6, 7]. In traditional Chinese herbal medicines, practiced particularly in China, Vietnam, and Southeast Asia, plants containing aristolochic acid are commonly used. In this area, next-generation sequencing studies underlined that a fraction of HCCs harbored high rates of mutations matching a distinctive mutational signature of aristolochic acid exposure[8-10]. Moving to Western countries, the incidence of HCC is usually associated with HCV infection, dietary habits, and related metabolic diseases, such as nonalcoholic liver steatohepatitis and nonalcoholic fatty liver disease. In this area, the low incidence of HCC due to HBV/HCV infections can be explained considering the use of the vaccine for HBV and anti-viral treatments against HCV in contrast with the increased incidence of metabolic syndrome[11].

All the aforementioned risk factors lead to liver disease (cirrhosis or chronic inflammation) that causes an accumulation of genomic alterations driving HCC. In general, HCC arises during the progression of pre-existing chronic hepatitis, and in the vast majority (80%) of patients, HCC occurs in the setting of cirrhosis[12]. The development of HCC is a process characterized by a specific sequence of lesions, from regenerative nodules in cirrhosis, low-grade dysplastic nodules and high-grade dysplastic nodules to early and progressed HCC[13,14].

The molecular mechanisms driving HCC tumorigenesis are extremely complex. Understanding this multistep process, with underlying genetic alteration, is essential for prevention, diagnostic, prognostic, and therapeutic purposes. Considering the future perspective, a better knowledge of molecular mechanisms involved in HCC tumorigenesis would help for a correct classification of HCC, for improving patient outcomes, and to develop new therapeutic targets. The advent of NGS technologies may help in the comprehensive study of genetic alteration and the different pathways involved in the initiation and progression of HCC. In fact, the development of NGS multi-gene panels allows the parallel analysis of multiple markers giving a broad view of the genomic situation[15,16]. To date, this

molecular landscape is crucial for therapeutic decision-making in other solid tumors[15]. The Cancer Genome Atlas Research Network investigated a total of 559 cases of HCC[17]. This study found that *TERT, TP53*, and *CTNNB1* are the most frequently altered genes in HCCs; 77% of HCCs showed a mutation in at least one of these three genes[17]. Correlation data between HCC molecular signatures and etiological agents are shown in Table 1.

Bearing in mind all this evidence, the present review will discuss the main molecular mutations in HCC, with particular emphasis on the influence that these alterations have on HCC morphology and biological aggressiveness.

## MOLECULAR ALTERATIONS IN HCC

### *TERT*

During cycles of genomic replication, the linear organization of chromosomes brings with it the problem of erosion of the 5'-terminus due to non-reproduction of the RNA primer binding site. Indeed, this erosion does not happen thanks to telomerase, constituted by telomerase reverse transcriptase (encoded by the *TERT* gene) and RNA template (encoded by the *TERC* gene). The telomerase complex adds nucleotides onto telomeres, preventing them from shortening. Telomeres are short tandem repeats of DNA (TTAGGG) coated by a protein complex known as Shelterin to protect the end of the chromosome where telomeres are located. Telomere synthesis is a controlled process activated in stem cells but deactivated in most somatic cells due to epigenetic silencing during the differentiation process. In the mature hepatocytes, the telomerase is not expressed[18,19]. The shortening of the telomeres exposes chromosomes to damage resulting in cellular senescence and is thought to be responsible for a sequence of events that drive to cancer[20].

Reactivation of *TERT* expression has been observed in several cancers (*e.g.*, melanomas, gliomas, poorly differentiated bladder cancer, anaplastic thyroid carcinomas, basal cell, squamous cell carcinomas) leading to a restoration of the telomerase activity[19] (Figure 1). This event avoids cellular senescence and leads cancer cells to acquire replicative immortality, a crucial feature in the progression of the neoplasm rather than in the transformation of the cells into malignant ones[21-24]. This upregulation of *TERT* in cancer can occur through several mechanisms, which are generally mutually exclusive: (1) Gene amplification, found in ovarian cancer, adrenocortical carcinoma, lung adenocarcinoma, and esophageal carcinoma[25]; (2) Gene rearrangements, found in high-risk neuroblastoma[26]; and (3) Gene mutations in hot-spot regions of the promoter region, found in melanomas, thyroid tumors, gliomas[19].

Alterations in the gene promoter region are the most common and most frequently detected, in particular C>T transition at chr5:1295228 (−124 or C228T) or chr5:1295250 (−146 or C250T). The C228T and C250T TERT mutations separately create an identical 11-base sequence that acts as a novel E-twenty-sis transcription factor binding site, causing *TERT* overexpression[21].

***TERT* and HCC:** Telomere length and telomere expression play a key role in the pathogenesis of HCC. Several studies have found telomere shortening in cirrhotic tissue, independently of the etiology of the liver disease (*e.g.*, alcohol abuse or viral hepatitis), suggesting that this event might represent a hallmark of liver senescence and chronic hepatitis[27-29]. In contrast to cirrhotic tissue, in 44%-59% of the HCC a reactivation of the *TERT* gene is observed[30]. Cellular senescence found in cirrhotic tissue followed by *TERT* reactivation is one of the mechanisms that may explain the development and progression of HCC in cirrhosis. In particular, with the accumulation of gene alterations senescence can induce neoplastic transformation, whereas subsequent telomerase activation can lead to a neoplastic progression (Figure 1).

Reactivation of TERT can also be caused by HBV infection (Figure 1). HBV is an enveloped virus with partially double-stranded DNA with the capacity to integrate its own genome into that of the host, leading to the deregulation of the gene involved. *TERT* promoter is the most frequent site of integration (38.5%) in HBV-related cancers, and the viral integration leads to *TERT* overexpression[31,32]. Intriguingly, *TERT* mutations have never been described in hepatocellular adenoma (HCA) in contrast to *CTNNB1* (see "*CTNNB1* and HCC" paragraph)[13,32-34].

*TERT* mutations in HCC have been statistically correlated with: (1) Age over 65 years ($P$ = 0.018), HCV infection more than HBV ($P$ = 0.048), and intratumoral morphological heterogeneity ($P$ = 0.0001) [35,36]. In a study performed on 97 HCCs by Kwa *et al*[35], the histological patterns in the tumor areas were classified into four groups: early, well, moderate, and poor. In particular, regarding the morphological aspect in *TERT* mutated HCC they observed two or more histological patterns as opposed to *TERT* wild-type HCCs, which showed only a single dominant pattern[35]; (2) Alcohol consumption[32, 37]. Schulze *et al*[32] performed a study on 243 surgically resected HCCs, and 60% of the alcohol-related HCCs had a mutation in the *TERT* gene promoter; (3) *CTNNB1* mutations. Several studies have shown the association between *CTNNB1* and *TERT*[38-40]. This correlation was demonstrated for the first time in a mice model in which it was observed that β-catenin binds the *TERT* promoter and participates in the control of its expression[40]; and (4) Poor prognosis, ($P$ = 0.041). A study by Oh *et al*[41] on telomere length in HCC showed that telomere elongation was a poor prognostic factor, as it decreased overall

**Table 1 Recurrent gene mutations in hepatocellular carcinoma related to risk factors**

| Frequency mutation | | Etiological factor | | |
|---|---|---|---|---|
| | | HBV | HCV | Non-viral |
| Driver gene | *TERT* | 50% | 61% | 65% |
| | *CTNNB1* | 15% | 30% | 39% |
| | *TP53* | 10%-65 % | 24% | 16% |

HBV: Hepatitis B virus; HCV: Hepatitis C virus.

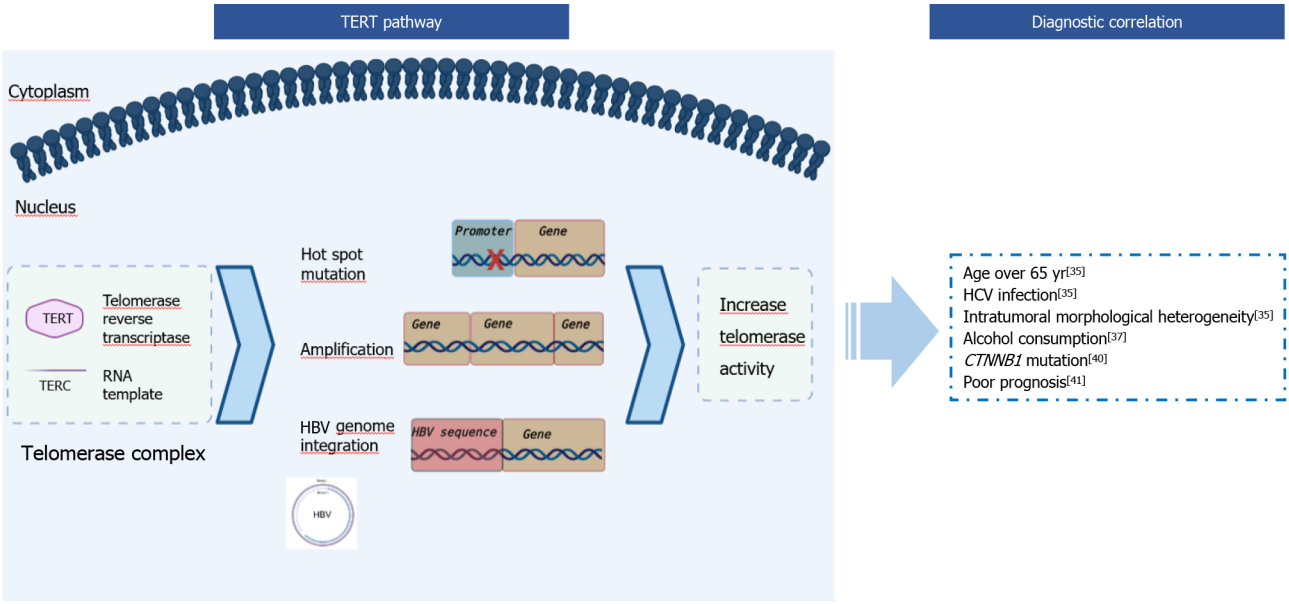


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**Figure 1 Main cellular pathways and clinical implications of *TERT* mutations in hepatocellular carcinoma.** Created with BioRender.com. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

survival ($P$ = 0.044). Moreover, in the case of high telomerase activity the prognosis was unfavorable ($P$ = 0.009)[41] (Figure 1).

### *CTNNB1*

The *CTNNB1* gene encodes β-catenin, a protein that performs several cellular functions. When interacting with the cadherin protein complex, β-catenin is important for the stabilization of the cytoskeleton and intracellular adhesions, but it also plays a role as a transcription factor in the canonical Wnt/β-catenin pathway. This pathway is involved in embryonic development, cellular homeostasis, and several diseases[42]. The cytoplasmic concentration of β-catenin is tightly controlled through its ubiquitination and proteasomal degradation. The phosphorylation required for this degradation mechanism is performed by glycogen synthase kinase 3 alpha and beta through the action of axin and the protein adenomatous polyposis coli (APC)[43,44]. In the cytoplasmic membrane, there are receptors for the Wnt molecules, called frizzled. The ligand-receptor complex triggers a cascade of cytoplasmic reactions, leading to the activation of the disheveled protein. This protein binds axin, preventing the bond between axin and glycogen synthase kinase 3[45]. This mechanism inhibits the proteasomal degradation of β-catenin. Given that *CTNNB1* continues to be transcribed, the β-catenin cytoplasmic concentration increases.

Once all the β-catenin cytoplasmic binding sites are saturated, β-catenin protein is translocated into the nucleus. Here β-catenin interacts with many transcriptional factors, in particular with T-cell factor/lymphoid enhancing factor to promote the transcription of target genes, such as *c-Myc*, *CyclinD-1*, and *Jun* (Figure 2). Most of these gene targets encode for oncoproteins, leading to the activation of oncogenic mechanisms (*e.g.*, uncontrolled growth or escape from apoptosis)[46]. For this reason, β-catenin is a molecule that may be involved in carcinogenesis and tumor progression of several cancers: HCC, lung cancer, brain and cerebellum cancer, breast cancer, colon cancer, leukemia, and others[47-49].

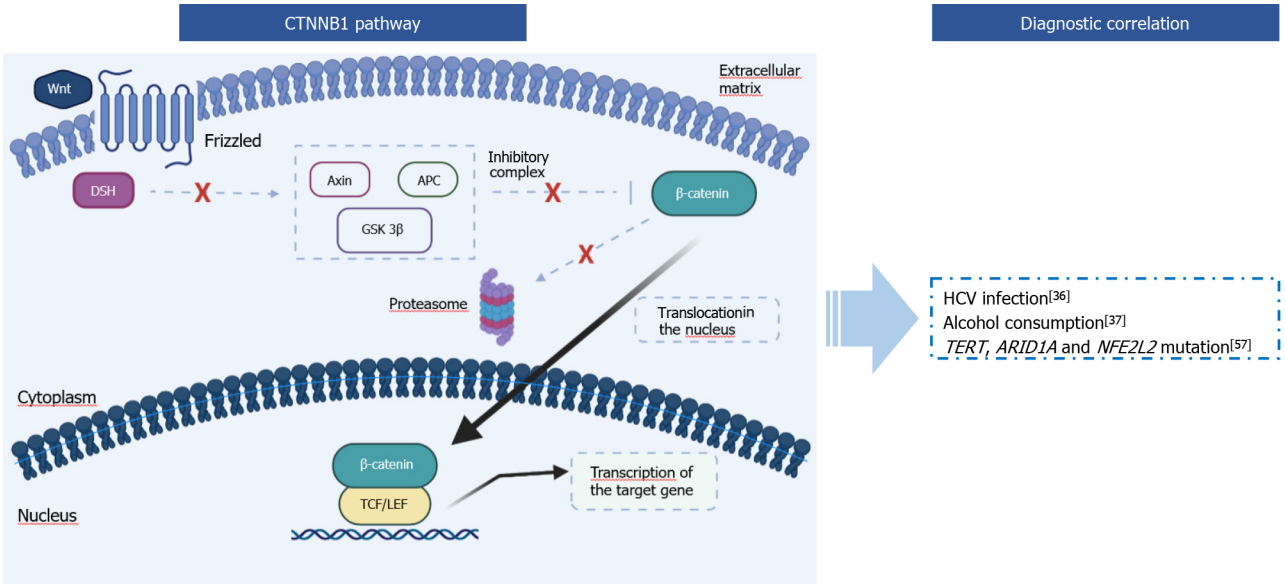


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**Figure 2 Main cellular pathways and clinical implications of *CTNNB1* mutations in hepatocellular carcinoma.** Created with BioRender.com. APC: Adenomatous polyposis coli; DSH: Disheveled; GSK: Glycogen synthase kinase; HCV: Hepatitis C virus; LEF: Lymphoid enhancing factor; TCF: T-cell factor.

The Wnt pathway can also be activated by transforming growth factor-β. Dysregulation of its signaling pathway is associated with an invasive phenotype and plays a central role in inflammation, fibrogenesis, and immunomodulation in the HCC microenvironment[50,51].

Activating mutations found in the *CTNNB1* gene are generally substitutions or in-frame deletions in hotspot regions that encode for the part of the protein that acts as a domain for the *APC/AXIN1/* glycogen synthase kinase 3B complex. Thus, β-catenin is not degraded by the proteosome and then uncontrollably activates the transcription of oncogenes[52].

**CTNNB1 and HCC:** In HCCs, *CTNNB1* mutations are among the most encountered genetic alterations, with a frequency of 20%-40%[32]. Regarding therapy, *CTNNB1* mutations induce resistance to immune checkpoint inhibitors (anti-PD-1/PD-L1 inhibitors and anti-CTLA4)[53]. Another important aspect concerns HCA. According to the literature, 5%-10% of HCA are subject to malignant transformation, but the most recent WHO guidelines considered CTNNB1-mutated HCAs as a specific subtype, with a higher risk for malignant transformation that could lead to the development of HCC. In HCA *CTNNB1* mutations are identified in 11%-43%[54-56].

*CTNNB1* mutations are not the only alterations found in HCCs, regarding Wnt/β-catenin pathway. In fact, mutations in Axin and APC in HCCs have been detected in 6%-15% and 2%-4%, respectively[57]. Generally, mutations in *CTNNB1*, Axin, and *APC* are mutually exclusive[57].

HCCs with mutated *CTNNB1* are statistically correlated to: (1) HCV infection[36]. For example, in a study on 22 HCV-related HCCs, an association between HCV infection and activation of the Wnt signaling pathway caused by the β-catenin mutation was found in 41% of cases[36], while according to the WHO, 30% of HCCs caused by HCV harbors a mutation in the *CTNNB1* gene; (2) Alcohol consumption. Schulze *et al*[37] studied 243 surgically resected HCCs, and 37% of the alcohol-related HCCs harbored a mutation in the *CTNNB1* gene; (3) *TERT* mutation[38,39]. Correlations between *CTNNB1* and *TERT* have been described in the "*TERT* and HCC" paragraph; and (4) *ARID1A* mutations ($P$ = 0.05) and *NFE2L2* mutation ($P$ = 0.015) associations with *CTNNB1* were demonstrated in a study performed on 125 HCCs[57] (Figure 2).

### *TP53*

The *TP53* gene encodes for the p53 protein, which owes its name to its molecular mass (53 kDa). p53 is called "the guardian of the genome" because it is an oncosuppressor that regulates the cell cycle, apoptosis, and genomic stability by preventing genomic mutations. The p53 pathway is crucial in cellular mechanisms as it interacts with other signal transduction pathways (*e.g.*, retinoblastoma pathway, Wnt-β-catenin, cyclin-cdk). Plenty of positive and negative autoregulatory feedback mechanisms act on p53 functions[58,59]. The activation of p53 occurs in response to many different stressors, both intrinsic and extrinsic to the cell (*e.g.*, gamma or UV radiation, oxidative stress, osmotic shock) that put faithful duplication of genetic material at risk[60]. The key event for p53 activation is the phosphorylation of the N-terminal domain by protein kinases. This event leads to the accumulation of p53 in the stressed cells through an increase in the half-life of the protein and an increase in efficiency as a transcription factor. After this activation, p53 initiates a program that blocks the cell cycle, leads the

cell to senescence, and then to apoptosis[61] (Figure 3).

In unstressed cells, cytoplasmic levels of p53 are kept in check through its degradation. The Mdm2 protein binds p53, transports it from the nucleus to the cytosol, and acts as a ubiquitin ligase so that ubiquitin binds to p53 leading to proteasome degradation[62]. If the *TP53* gene is altered, the p53 protein cannot function properly, driving tumorigenesis and tumor progression. As early as 1990, *TP53* was defined as the most frequently mutated gene in human cancers. *TP53* mutations remain among the most frequent and most significant in more common human cancers, although the frequency of mutations is highly variable depending on the type of cancer: from 90% in the ovary, 50%-80% in the lung up to less than 5% in the cervix[63]. Individuals affected by Li Fraumeni syndrome carry a mutated allele of *TP53*, and this syndrome predisposes to the development of several types of cancers[64].

***TP53* and HCC:** Approximately 15%-40% of HCCs carry mutations in the *TP53* gene, with a higher frequency in advanced tumors[65]. Intriguingly, a specific *TP53* mutation is significantly associated with dietary intake of aflatoxin B1, a mycotoxin produced by *Aspergillus* fungi. Exposure to aflatoxin B1 induces the transversion G → T at the *TP53* codon 249, leading to the p.R249S (c.747G>T) substitution. This mutation could then be considered a mutational signature of exposure to aflatoxin B1 in HCC[66-68].

HCCs with mutated *TP53* are statistically correlated to: (1) HBV infection and aflatoxin B1 exposure [68,69]. Lunn *et al*[69] conducted a population-based study on 110 HCCs. The relative risk (RR) that they obtained for HBV infection (RR=17.0), aflatoxin B1 exposure (RR=17.4), and the two risk agents together (RR=67.7) confirmed the correlation between these agents and HCC development. Exposure to aflatoxin B1 induces the p.R249S substitution in the *TP53* gene, and HBV infection causes integration of the viral genome into that of the host, promoting mutations in genes crucial for cellular regulation, such as *TP53*. For these reasons HBV infection and aflatoxin B1 promote a high rate of mutagenesis in HCC[68,69]; and (2) *TP53* alterations were usually exclusive from *CTNNB1* mutations ($P$ = 0.0001) but not from *AXIN1* and *APC*[57] (Figure 3).

## MOLECULAR HCC SUBTYPE CLASSIFICATION

Over the years, many groups have tried to classify HCCs according to a molecular basis, but a univocal classification has been never reached.

Kabashima *et al*[70] grouped different classifications, starting from that of Shimada *et al*[71], and grouped HCCs into three molecular subtypes (MS1, MS2, MS3). The groups identified by the different studies are only fairly overlapping with each other. These classifications were based on clinical, molecular, and immunological features[70,71].

Regarding gene alterations, some correlations were found between *TP53* and *CTNNB1*. *TP53*-mutated HCCs were classified into the MS1 group, which correlated with unfavorable prognosis, viral infection, high serum alpha-fetoprotein levels, vascular invasion and proliferation, extensive mitotic activity resulting in chromosomal instability, and stem cell-like properties.

*CTNNB1*-mutated HCCs were placed into the MS2 group, correlating with aberrant activation of Wnt/ β-Catenin pathway, which could explain the high rate of methylation in CpG islands present in this group, as the constitutively active β-catenin protein recruits a DNMT1 methyltransferase. Another feature associated with the MS2 group is the immunosuppressive phenotype. Moreover, this class is considered non-proliferative/less progressive than others.

The MS3 group is not associated with molecular signatures, but only with metabolic disease-associated tumors[51,72-74].

The *TERT* gene is rarely found in these classifications. However, it should be considered that the most frequent mutations in *TERT* fall in a promoter region usually not covered by the exome sequencing studies. The Cancer Genome Atlas 2017 classification detected *TERT* mutations in HCC and included HCC-TERT samples in iCluster2 and iCluster3[17]. In 2019, further classification was drafted by Yang *et al*[75] that divides HCCs into 3 groups (C1, C2, C3), overlapping with a previous study performed by Hoshida *et al*[51]: C1→S3, C2→S1, C3→S2.

## MOLECULAR HCC SUBTYPES AND PATHOLOGICAL FEATURES

In 2017, Calderaro *et al*[76] discriminated HCCs based on the presence of *TP53* or *CTNNB1* mutations, considering that these are two genes that mutate in a mutually exclusive manner and together comprise 57% of HCCs. In this study, CTNNB1-mutated HCCs were described as larger than the CTNNB1-wildtype HCCs but characterized by a lower tumor grade, with microtrabecular and pseudoglandular patterns of growth, without inflammatory infiltrates and with the presence of cholestasis[76]. Conversely, TP53-mutated HCCs were described as poorly differentiated tumors, with large multinucleated and pleomorphic cells, solid pattern of growth, frequent vascular invasion, and angiogenesis[76].

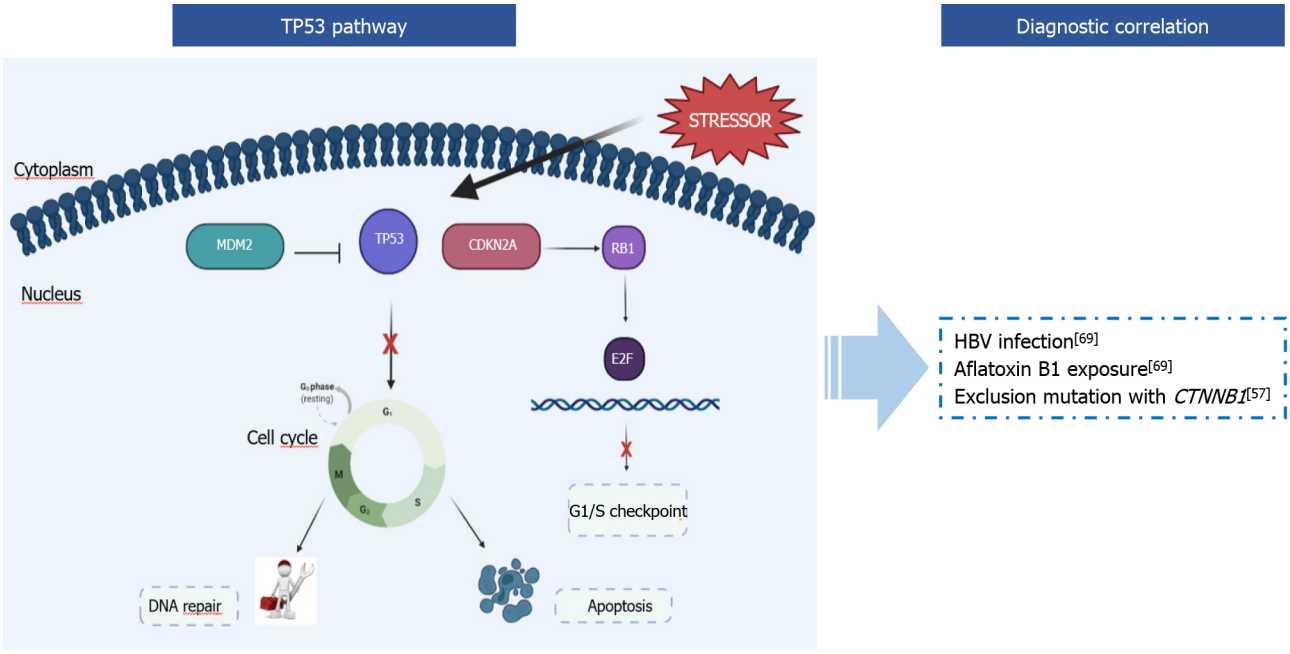


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**Figure 3 Main cellular pathways and clinical implications of *TP53* mutations in hepatocellular carcinoma.** Created with BioRender.com. HBV: Hepatitis B virus.

Selected subtypes of HCCs recognized by the most recent WHO Classification of Tumors were described to have specific molecular alterations (Figures 4 and 5)[14]: (1) Up to 63% of steatohepatitic HCCs were associated with nonalcoholic fatty liver disease. This type of HCC is characterized by the following histologic features: macrovesicular steatosis, Mallory-Denk bodies, ballooning of tumoral hepatocytes, inflammation, and trabecular or pericellular fibrosis. With regard to key molecular features, steatohepatitic HCCs were significantly associated with a lower frequency of *CTNNB1* mutations, higher rate of mutations in *TERT* and *TP53*, IL-6/JAK/STAT activation, high level of C-reactive protein, and serum amyloid A positive at immunohistochemistry[14,77-80]; (2) Clear cell HCCs are considered a well-differentiated type, characterized by a cytoplasmic clearing, due to accumulation of glycogen, lipopolysaccharides, mucopolysaccharides, or cytoplasmic vesicles. *IDH* mutations were identified in 25% of clear cell HCCs, and these alterations were significantly associated with a worse prognosis. Moreover, *IDH* mutations are also found in intrahepatic cholangiocarcinoma, a tumor with a significantly worse prognosis than HCC[14,80-83]; (3) Macrotrabecular massive HCCs are frequently larger than 50 mm with vascular invasion, correlated high alpha-fetoprotein serum levels, high expression of angiopoietin 2, and vascular endothelial growth factor A. At the histological level, this subgroup is characterized by massive trabeculae surrounded by vascular spaces and coated by immature endothelial cells. On the molecular side, *TP53* mutations and *FGF19* amplifications have been detected. Macrotrabecular massive HCC is an aggressive phenotype associated with a worse prognosis [14,80,84,85]; and (5) Scirrhous HCCs develop in the non-cirrhotic liver, and they are characterized by hyaline stroma, intratumoral fibrosis with thin trabecular pattern growth (due to this characteristic is easily confusable radiologically to cholangiocarcinoma), or the lymphoepithelioma-like subtype, consisting of dense intratumor lymphocytic infiltration. Scirrhous HCCs may exhibit *TSC1*/*TSC2* mutations and transforming growth factor-β signaling activation. Regarding prognosis, scirrhous HCCs are an aggressive subgroup, often with invasion of the portal vein, but as far as long-term follow-up is concerned, the prognosis is similar or sometimes better to conventional HCCs[76,86,87].

## EXPERIENCE FROM OUR CENTER

Our preliminary results focused on 36 prospectively enrolled patients, all resected for HCC and selected for NGS by means of a laboratory-developed multi-gene panel Gene-Studio S5 sequencer, which comprises specific target regions including *TERT*[15]. We detected single mutations in the *TERT* promoter in 7 (19.4%) cases, in *TP53* in 4 (11.1%) cases, and in *CTNNB1* in 2 (5.6%) cases. *TERT* and *CTNNB1* coexistent mutations were observed in 8 (22.2%) cases, while *TERT* and *TP53* were in 8 (22.2%) cases. In 7 (19.4%) cases no mutations in these three genes were detected (Figure 6).

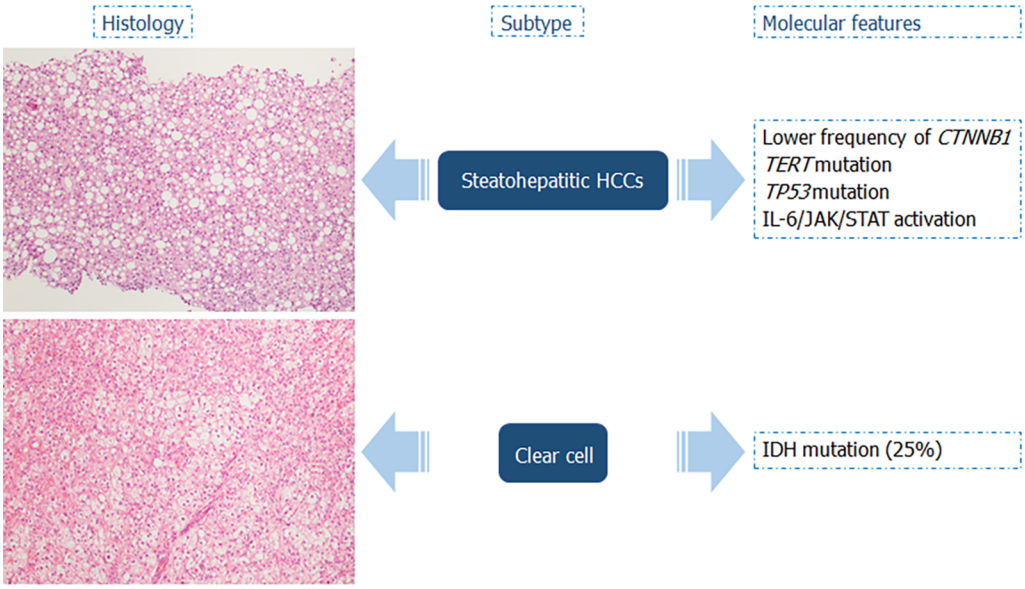


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**Figure 4 Steatohepatitic hepatocellular carcinomas and clear cell carcinoma and their molecular features.** Hematoxylin-eosin stain ( 10 magnification). HCC: Hepatocellular carcinoma.

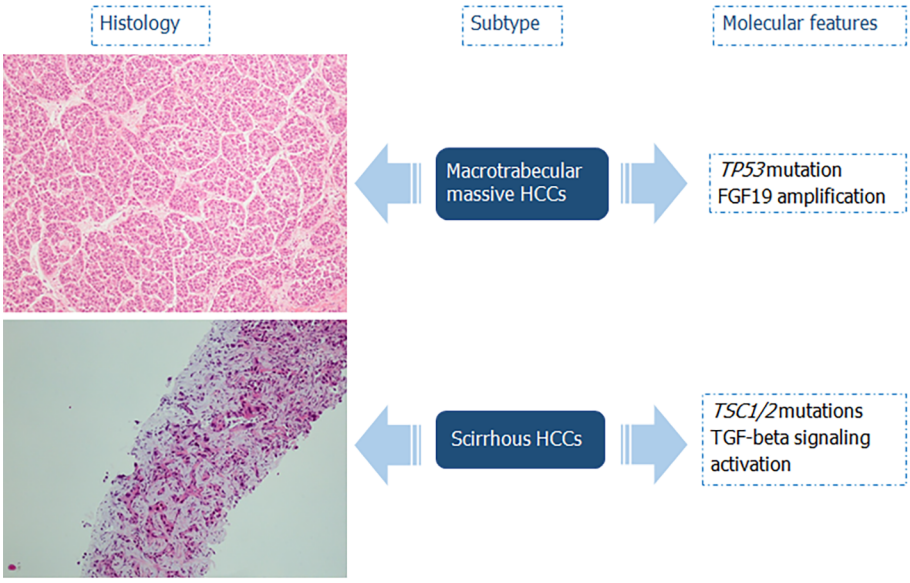


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**Figure 5 Macrotrabecular massive hepatocellular carcinomas and scirrhous hepatocellular carcinoma and their molecular features.** Hematoxylin-eosin stain ( 10 magnification). HCC: Hepatocellular carcinoma; TGF: Transforming growth factor.

In line with a previous study by Calderaro *et al*[76], we observed a trend of *TERT*-mutated HCC towards a macrotrabecular or solid architecture. Moreover, the presence of *TERT* promoter mutations in combination with *TP53* mutation correlated with high-grade HCC ($P = 0.011$; Figure 7).

Interestingly, no correlations were found between mutations and tumor dimensions. This evidence leads us to hypothesize that the presence of *TERT* promoter mutations, alone or in combination with *TP53* alteration, correlates with a morphological progression in HCC, in terms of a higher tumor grade and an architecture more related to aggressive behavior (solid, macrotrabecular) but not of a dimensional evolution.

Most of the HCC in non-cirrhotic livers of our series showed no mutations or harbored only a *CTNNB1* mutation ($P = 0.031$), as a countercheck of the correlation between tumor progression and mutations. The validation of these results on a larger series as well as with post-surgical follow-up might indicate that small HCC may have an aggressive behavior from a molecular and morphological point of view, despite their dimensions.

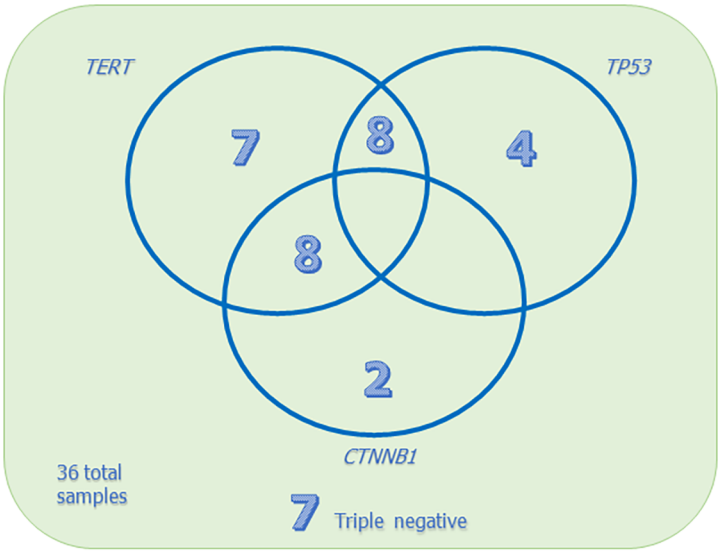


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**Figure 6 Representation of our data based on the number of samples grouped according to their mutational status.**

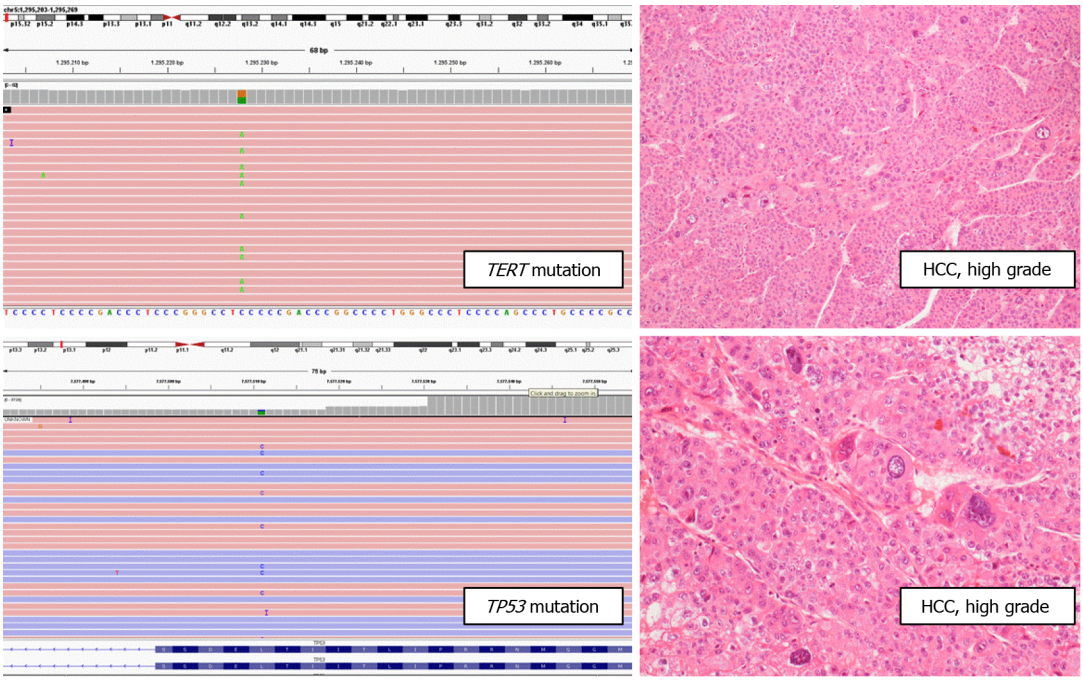


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**Figure 7 A case from our series of high-grade (Edmondson's 4) hepatocellular carcinoma, with tumor giant cells and macrotrabecular architecture.** Left boxes: coexistent *TERT* and *TP53* mutations detected by next-generation sequencing analysis; Right boxes: Hematoxylin-eosin stain ( 10 and 20 magnification). HCC: Hepatocellular carcinoma.

## CONCLUSION

The molecular signature of a tumor is becoming increasingly important in the approach of patients with different types of cancers, on diagnostic, prognostic, and predictive grounds. In the age of precision medicine, the study of HCC mutations is still a field that is worth investigating. Considering both the literature data and our personal experience, about 80% of HCCs harbor mutations in at least one gene among *TERT*, *TP53*, or *CTNNB1*, with different biological and clinical implications.

In the near future, a deeper analysis of these three genes is surely desirable since a molecular characterization of HCC would open up the possibility of personalized therapies, as has happened for other cancers (*e.g.*, lung adenocarcinomas, melanomas, gastrointestinal stromal tumors, colorectal adenocarcinomas). Moreover, the evidence of a tight correlation between the mutational profile and the HCC morphology is likely to imply an increasing integrative approach between anatomic pathology and

molecular laboratories.

## FOOTNOTES

**Author contributions:** Vasuri F and de Biase D contributed equally to this paper; Maloberti T and Vasuri F designed the research study; Maloberti T, De Leo A, Sanza V, Gruppioni E, Altimari A, Riefolo M, Visani M, and Malvi D performed the research; Maloberti T and Vasuri F analyzed the data and wrote the manuscript; D'Errico A, Tallini G, and de Biase D supervised the manuscript preparation; All authors have read and approved the final manuscript.

**Conflict-of-interest statement:** Dario de Biase has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim and Eli Lilly, unrelated to the current work.

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## REFERENCES

1 **Harris PS**, Hansen RM, Gray ME, Massoud OI, McGuire BM, Shoreibah MG. Hepatocellular carcinoma surveillance: An evidence-based approach. *World J Gastroenterol* 2019; **25**: 1550-1559 [PMID: 30983815 DOI: 10.3748/wjg.v25.i13.1550]
2 **World Health Organization**. Cancer Today. Available from: https://gco.iarc.fr/today/home
3 **Nahon P**, Zucman-Rossi J. Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. *J Hepatol* 2012; **57**: 663-674 [PMID: 22609306 DOI: 10.1016/j.jhep.2012.02.035]
4 **Bidkhori G**, Benfeitas R, Klevstig M, Zhang C, Nielsen J, Uhlen M, Boren J, Mardinoglu A. Metabolic network-based stratification of hepatocellular carcinoma reveals three distinct tumor subtypes. *Proc Natl Acad Sci U S A* 2018; **115**: E11874-E11883 [PMID: 30482855 DOI: 10.1073/pnas.1807305115]
5 **Dharel N**, Kato N, Muroyama R, Moriyama M, Shao RX, Kawabe T, Omata M. MDM2 promoter SNP309 is associated with the risk of hepatocellular carcinoma in patients with chronic hepatitis C. *Clin Cancer Res* 2006; **12**: 4867-4871 [PMID: 16914573 DOI: 10.1158/1078-0432.CCR-06-0111]
6 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: 25752327 DOI: 10.1111/liv.12818]
7 **Wild CP**, Miller JD, Groopman JD. Mycotoxin Control in Low- And Middle-Income Countries. In: Wild CP, Miller JD, Groopman JD, editors. Mycotoxin Control in Low- and Middle-Income Countries. Lyon (FR), 2015
8 **Ng AWT**, Poon SL, Huang MN, Lim JQ, Boot A, Yu W, Suzuki Y, Thangaraju S, Ng CCY, Tan P, Pang ST, Huang HY, Yu MC, Lee PH, Hsieh SY, Chang AY, Teh BT, Rozen SG. Aristolochic acids and their derivatives are widely implicated in liver cancers in Taiwan and throughout Asia. *Sci Transl Med* 2017; **9** [PMID: 29046434 DOI: 10.1126/scitranslmed.aan6446]
9 **Arlt VM**, Stiborova M, Schmeiser HH. Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. *Mutagenesis* 2002; **17**: 265-277 [PMID: 12110620 DOI: 10.1093/mutage/17.4.265]
10 **Yang JD**, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 589-604 [PMID: 31439937 DOI: 10.1038/s41575-019-0186-y]
11 **Baecker A**, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. *Eur J Cancer Prev* 2018; **27**: 205-212 [PMID: 29489473 DOI: 10.1097/CEJ.0000000000000428]
12 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
13 **Zucman-Rossi J**, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 1226-1239.e4 [PMID: 26099527 DOI: 10.1053/j.gastro.2015.05.061]
14 **Tumours WCo**. Digestive System Tumours. 5th Edition. Lyon: IARC, 2019
15 **de Biase D**, Acquaviva G, Visani M, Sanza V, Argento CM, De Leo A, Maloberti T, Pession A, Tallini G. Molecular Diagnostic of Solid Tumor Using a Next Generation Sequencing Custom-Designed Multi-Gene Panel. *Diagnostics (Basel)*

2020; **10** [PMID: 32340363 DOI: 10.3390/diagnostics10040250]
16 **Malvi D**, de Biase D, Fittipaldi S, Grillini M, Visani M, Pession A, D'Errico A, Vasuri F. Immunomorphology and molecular biology of mixed primary liver cancers: is Nestin a marker of intermediate-cell carcinoma? *Histopathology* 2020; **76**: 265-274 [PMID: 31374137 DOI: 10.1111/his.13966]
17 **Cancer Genome Atlas Research Network**. ; Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]
18 **Nandakumar J**, Cech TR. Finding the end: recruitment of telomerase to telomeres. *Nat Rev Mol Cell Biol* 2013; **14**: 69-82 [PMID: 23299958 DOI: 10.1038/nrm3505]
19 **Heidenreich B**, Kumar R. TERT promoter mutations in telomere biology. *Mutat Res Rev Mutat Res* 2017; **771**: 15-31 [PMID: 28342451 DOI: 10.1016/j.mrrev.2016.11.002]
20 **Mason PJ**, Perdigones N. Telomere biology and translational research. *Transl Res* 2013; **162**: 333-342 [PMID: 24070997 DOI: 10.1016/j.trsl.2013.08.009]
21 **Colebatch AJ**, Dobrovic A, Cooper WA. *TERT* gene: its function and dysregulation in cancer. *J Clin Pathol* 2019; **72**: 281-284 [PMID: 30696697 DOI: 10.1136/jclinpath-2018-205653]
22 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
23 **Dratwa M**, Wysoczańska B, Łacina P, Kubik T, Bogunia-Kubik K. TERT-Regulation and Roles in Cancer Formation. *Front Immunol* 2020; **11**: 589929 [PMID: 33329574 DOI: 10.3389/fimmu.2020.589929]
24 **Esopi D**, Graham MK, Brosnan-Cashman JA, Meyers J, Vaghasia A, Gupta A, Kumar B, Haffner MC, Heaphy CM, De Marzo AM, Meeker AK, Nelson WG, Wheelan SJ, Yegnasubramanian S. Pervasive promoter hypermethylation of silenced TERT alleles in human cancers. *Cell Oncol (Dordr)* 2020; **43**: 847-861 [PMID: 32468444 DOI: 10.1007/s13402-020-00531-7]
25 **Barthel FP**, Wei W, Tang M, Martinez-Ledesma E, Hu X, Amin SB, Akdemir KC, Seth S, Song X, Wang Q, Lichtenberg T, Hu J, Zhang J, Zheng S, Verhaak RG. Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat Genet* 2017; **49**: 349-357 [PMID: 28135248 DOI: 10.1038/ng.3781]
26 **Peifer M**, Hertwig F, Roels F, Dreidax D, Gartlgruber M, Menon R, Krämer A, Roncaioli JL, Sand F, Heuckmann JM, Ikram F, Schmidt R, Ackermann S, Engesser A, Kahlert Y, Vogel W, Altmüller J, Nürnberg P, Thierry-Mieg J, Thierry-Mieg D, Mariappan A, Heynck S, Mariotti E, Henrich KO, Gloeckner C, Bosco G, Leuschner I, Schweiger MR, Savelyeva L, Watkins SC, Shao C, Bell E, Höfer T, Achter V, Lang U, Theissen J, Volland R, Saadati M, Eggert A, de Wilde B, Berthold F, Peng Z, Zhao C, Shi L, Ortmann M, Büttner R, Perner S, Hero B, Schramm A, Schulte JH, Herrmann C, O'Sullivan RJ, Westermann F, Thomas RK, Fischer M. Telomerase activation by genomic rearrangements in high-risk neuroblastoma. *Nature* 2015; **526**: 700-704 [PMID: 26466568 DOI: 10.1038/nature14980]
27 **Kitada T**, Seki S, Kawakita N, Kuroki T, Monna T. Telomere shortening in chronic liver diseases. *Biochem Biophys Res Commun* 1995; **211**: 33-39 [PMID: 7779103 DOI: 10.1006/bbrc.1995.1774]
28 **Wiemann SU**, Satyanarayana A, Tsahuridu M, Tillmann HL, Zender L, Klempnauer J, Flemming P, Franco S, Blasco MA, Manns MP, Rudolph KL. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. *FASEB J* 2002; **16**: 935-942 [PMID: 12087054 DOI: 10.1096/fj.01-0977com]
29 **Carulli L**, Anzivino C. Telomere and telomerase in chronic liver disease and hepatocarcinoma. *World J Gastroenterol* 2014; **20**: 6287-6292 [PMID: 24876749 DOI: 10.3748/wjg.v20.i20.6287]
30 **Quaas A**, Oldopp T, Tharun L, Klingenfeld C, Krech T, Sauter G, Grob TJ. Frequency of TERT promoter mutations in primary tumors of the liver. *Virchows Arch* 2014; **465**: 673-677 [PMID: 25267585 DOI: 10.1007/s00428-014-1658-7]
31 **Jang JW**, Kim HS, Kim JS, Lee SK, Han JW, Sung PS, Bae SH, Choi JY, Yoon SK, Han DJ, Kim TM, Roberts LR. Distinct Patterns of HBV Integration and *TERT* Alterations between in Tumor and Non-Tumor Tissue in Patients with Hepatocellular Carcinoma. *Int J Mol Sci* 2021; **22** [PMID: 34209079 DOI: 10.3390/ijms22137056]
32 **Schulze K**, Nault JC, Villanueva A. Genetic profiling of hepatocellular carcinoma using next-generation sequencing. *J Hepatol* 2016; **65**: 1031-1042 [PMID: 27262756 DOI: 10.1016/j.jhep.2016.05.035]
33 **Lee SE**, Chang SH, Kim WY, Lim SD, Kim WS, Hwang TS, Han HS. Frequent somatic TERT promoter mutations and CTNNB1 mutations in hepatocellular carcinoma. *Oncotarget* 2016; **7**: 69267-69275 [PMID: 27661004 DOI: 10.18632/oncotarget.12121]
34 **Nault JC**, Zucman-Rossi J. TERT promoter mutations in primary liver tumors. *Clin Res Hepatol Gastroenterol* 2016; **40**: 9-14 [PMID: 26336998 DOI: 10.1016/j.clinre.2015.07.006]
35 **Kwa WT**, Effendi K, Yamazaki K, Kubota N, Hatano M, Ueno A, Masugi Y, Sakamoto M. Telomerase reverse transcriptase (TERT) promoter mutation correlated with intratumoral heterogeneity in hepatocellular carcinoma. *Pathol Int* 2020; **70**: 624-632 [PMID: 32559017 DOI: 10.1111/pin.12974]
36 **Huang H**, Fujii H, Sankila A, Mahler-Araujo BM, Matsuda M, Cathomas G, Ohgaki H. Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. *Am J Pathol* 1999; **155**: 1795-1801 [PMID: 10595907 DOI: 10.1016/s0002-9440(10)65496-x]
37 **Schulze K**, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505-511 [PMID: 25822088 DOI: 10.1038/ng.3252]
38 **Nault JC**, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, Laurent A, Cherqui D, Balabaud C, Zucman-Rossi J. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* 2013; **4**: 2218 [PMID: 23887712 DOI: 10.1038/ncomms3218]
39 **Pinyol R**, Tovar V, Llovet JM. TERT promoter mutations: gatekeeper and driver of hepatocellular carcinoma. *J Hepatol* 2014; **61**: 685-687 [PMID: 24859456 DOI: 10.1016/j.jhep.2014.05.028]
40 **Hoffmeyer K**, Raggioli A, Rudloff S, Anton R, Hierholzer A, Del Valle I, Hein K, Vogt R, Kemler R. Wnt/β-catenin

signaling regulates telomerase in stem cells and cancer cells. *Science* 2012; **336**: 1549-1554 [PMID: 22723415 DOI: 10.1126/science.1218370]

41 **Oh BK**, Kim H, Park YN, Yoo JE, Choi J, Kim KS, Lee JJ, Park C. High telomerase activity and long telomeres in advanced hepatocellular carcinomas with poor prognosis. *Lab Invest* 2008; **88**: 144-152 [PMID: 18158557 DOI: 10.1038/Labinvest.3700710]

42 **Shang S**, Hua F, Hu ZW. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget* 2017; **8**: 33972-33989 [PMID: 28430641 DOI: 10.18632/oncotarget.15687]

43 **Xing Y**, Clements WK, Kimelman D, Xu W. Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. *Genes Dev* 2003; **17**: 2753-2764 [PMID: 14600025 DOI: 10.1101/gad.1142603]

44 **Liu J**, Xing Y, Hinds TR, Zheng J, Xu W. The third 20 amino acid repeat is the tightest binding site of APC for beta-catenin. *J Mol Biol* 2006; **360**: 133-144 [PMID: 16753179 DOI: 10.1016/j.jmb.2006.04.064]

45 **Fiedler M**, Mendoza-Topaz C, Rutherford TJ, Mieszczanek J, Bienz M. Dishevelled interacts with the DIX domain polymerization interface of Axin to interfere with its function in down-regulating β-catenin. *Proc Natl Acad Sci U S A* 2011; **108**: 1937-1942 [PMID: 21245303 DOI: 10.1073/pnas.1017063108]

46 **Nishida N**, Goel A. Genetic and epigenetic signatures in human hepatocellular carcinoma: a systematic review. *Curr Genomics* 2011; **12**: 130-137 [PMID: 21966251 DOI: 10.2174/138920211795564359]

47 **Kobayashi M**, Honma T, Matsuda Y, Suzuki Y, Narisawa R, Ajioka Y, Asakura H. Nuclear translocation of beta-catenin in colorectal cancer. *Br J Cancer* 2000; **82**: 1689-1693 [PMID: 10817505 DOI: 10.1054/bjoc.1999.1112]

48 **Khramtsov AI**, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. *Am J Pathol* 2010; **176**: 2911-2920 [PMID: 20395444 DOI: 10.2353/ajpath.2010.091125]

49 **Gekas C**, D'Altri T, Aligué R, González J, Espinosa L, Bigas A. β-Catenin is required for T-cell leukemia initiation and MYC transcription downstream of Notch1. *Leukemia* 2016; **30**: 2002-2010 [PMID: 27125305 DOI: 10.1038/leu.2016.106]

50 **Chen J**, Gingold JA, Su X. Immunomodulatory TGF-β Signaling in Hepatocellular Carcinoma. *Trends Mol Med* 2019; **25**: 1010-1023 [PMID: 31353124 DOI: 10.1016/j.molmed.2019.06.007]

51 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]

52 **de La Coste A**, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, Fabre M, Chelly J, Beldjord C, Kahn A, Perret C. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 1998; **95**: 8847-8851 [PMID: 9671767 DOI: 10.1073/pnas.95.15.8847]

53 **Pinyol R**, Sia D, Llovet JM. Immune Exclusion-Wnt/CTNNB1 Class Predicts Resistance to Immunotherapies in HCC. *Clin Cancer Res* 2019; **25**: 2021-2023 [PMID: 30617138 DOI: 10.1158/1078-0432.CCR-18-3778]

54 **Nault JC**, Couchy G, Balabaud C, Morcrette G, Caruso S, Blanc JF, Bacq Y, Calderaro J, Paradis V, Ramos J, Scoazec JY, Gnemmi V, Sturm N, Guettier C, Fabre M, Savier E, Chiche L, Labrune P, Selves J, Wendum D, Pilati C, Laurent A, De Muret A, Le Bail B, Rebouissou S, Imbeaud S; GENTHEP Investigators, Bioulac-Sage P, Letouzé E, Zucman-Rossi J. Molecular Classification of Hepatocellular Adenoma Associates With Risk Factors, Bleeding, and Malignant Transformation. *Gastroenterology* 2017; **152**: 880-894.e6 [PMID: 27939373 DOI: 10.1053/j.gastro.2016.11.042]

55 **Shen XY**, Hu XG, Kim YB, Kim MN, Hong SY, Kim BW, Wang HJ. Molecular classification of hepatocellular adenoma: A single-center experience. *Ann Hepatobiliary Pancreat Surg* 2019; **23**: 109-114 [PMID: 31225410 DOI: 10.14701/ahbps.2019.23.2.109]

56 **Rebouissou S**, Franconi A, Calderaro J, Letouzé E, Imbeaud S, Pilati C, Nault JC, Couchy G, Laurent A, Balabaud C, Bioulac-Sage P, Zucman-Rossi J. Genotype-phenotype correlation of CTNNB1 mutations reveals different ß-catenin activity associated with liver tumor progression. *Hepatology* 2016; **64**: 2047-2061 [PMID: 27177928 DOI: 10.1002/hep.28638]

57 **Guichard C**, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 694-698 [PMID: 22561517 DOI: 10.1038/ng.2256]

58 **Harris SL**, Levine AJ. The p53 pathway: positive and negative feedback loops. *Oncogene* 2005; **24**: 2899-2908 [PMID: 15838523 DOI: 10.1038/sj.onc.1208615]

59 **Toufektchan E**, Toledo F. The Guardian of the Genome Revisited: p53 Downregulates Genes Required for Telomere Maintenance, DNA Repair, and Centromere Structure. *Cancers (Basel)* 2018; **10** [PMID: 29734785 DOI: 10.3390/cancers10050135]

60 **Overholtzer M**, Rao PH, Favis R, Lu XY, Elowitz MB, Barany F, Ladanyi M, Gorlick R, Levine AJ. The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability. *Proc Natl Acad Sci U S A* 2003; **100**: 11547-11552 [PMID: 12972634 DOI: 10.1073/pnas.1934852100]

61 **Jin S**, Levine AJ. The p53 functional circuit. *J Cell Sci* 2001; **114**: 4139-4140 [PMID: 11739646]

62 **Purvis JE**, Karhohs KW, Mock C, Batchelor E, Loewer A, Lahav G. p53 dynamics control cell fate. *Science* 2012; **336**: 1440-1444 [PMID: 22700930 DOI: 10.1126/science.1218351]

63 **Leroy B**, Anderson M, Soussi T. TP53 mutations in human cancer: database reassessment and prospects for the next decade. *Hum Mutat* 2014; **35**: 672-688 [PMID: 24665023 DOI: 10.1002/humu.22552]

64 **Gargallo P**, Yáñez Y, Segura V, Juan A, Torres B, Balaguer J, Oltra S, Castel V, Cañete A. Li-Fraumeni syndrome heterogeneity. *Clin Transl Oncol* 2020; **22**: 978-988 [PMID: 31691207 DOI: 10.1007/s12094-019-02236-2]

65 **Lombardo D**, Saitta C, Giosa D, Di Tocco FC, Musolino C, Caminiti G, Chines V, Franzè MS, Alibrandi A, Navarra G, Raimondo G, Pollicino T. Frequency of somatic mutations in *TERT* promoter, *TP53* and *CTNNB1* genes in patients with hepatocellular carcinoma from Southern Italy. *Oncol Lett* 2020; **19**: 2368-2374 [PMID: 32194736 DOI: 10.3892/ol.2020.11332]

66 **Bressac B**, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350**: 429-431 [PMID: 1672732 DOI: 10.1038/350429a0]

67 **Unsal H**, Yakicier C, Marçais C, Kew M, Volkmann M, Zentgraf H, Isselbacher KJ, Ozturk M. Genetic heterogeneity of hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 1994; **91**: 822-826 [PMID: 8290606 DOI: 10.1073/pnas.91.2.822]

68 **Gouas D**, Shi H, Hainaut P. The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy. *Cancer Lett* 2009; **286**: 29-37 [PMID: 19376640 DOI: 10.1016/j.canlet.2009.02.057]

69 **Lunn RM**, Zhang YJ, Wang LY, Chen CJ, Lee PH, Lee CS, Tsai WY, Santella RM. p53 mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. *Cancer Res* 1997; **57**: 3471-3477 [PMID: 9270015]

70 **Kabashima A**, Shimada S, Shimokawa M, Akiyama Y, Tanabe M, Tanaka S. Molecular and immunological paradigms of hepatocellular carcinoma: Special reference to therapeutic approaches. *J Hepatobiliary Pancreat Sci* 2021; **28**: 62-75 [PMID: 33259135 DOI: 10.1002/jhbp.874]

71 **Shimada S**, Mogushi K, Akiyama Y, Furuyama T, Watanabe S, Ogura T, Ogawa K, Ono H, Mitsunori Y, Ban D, Kudo A, Arii S, Tanabe M, Wands JR, Tanaka S. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. *EBioMedicine* 2019; **40**: 457-470 [PMID: 30598371 DOI: 10.1016/j.ebiom.2018.12.058]

72 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676 [PMID: 15349906 DOI: 10.1002/hep.20375]

73 **Boyault S**, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45**: 42-52 [PMID: 17187432 DOI: 10.1002/hep.21467]

74 **Chiang DY**, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, Llovet JM. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 6779-6788 [PMID: 18701503 DOI: 10.1158/0008-5472.CAN-08-0742]

75 **Yang C**, Huang X, Liu Z, Qin W, Wang C. Metabolism-associated molecular classification of hepatocellular carcinoma. *Mol Oncol* 2020; **14**: 896-913 [PMID: 31955511 DOI: 10.1002/1878-0261.12639]

76 **Calderaro J**, Couchy G, Imbeaud S, Amaddeo G, Letouzé E, Blanc JF, Laurent C, Hajji Y, Azoulay D, Bioulac-Sage P, Nault JC, Zucman-Rossi J. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* 2017; **67**: 727-738 [PMID: 28532995 DOI: 10.1016/j.jhep.2017.05.014]

77 **Salomao M**, Yu WM, Brown RS Jr, Emond JC, Lefkowitch JH. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. *Am J Surg Pathol* 2010; **34**: 1630-1636 [PMID: 20975341 DOI: 10.1097/PAS.0b013e3181f31caa]

78 **Ando S**, Shibahara J, Hayashi A, Fukayama M. β-catenin alteration is rare in hepatocellular carcinoma with steatohepatitic features: immunohistochemical and mutational study. *Virchows Arch* 2015; **467**: 535-542 [PMID: 26311355 DOI: 10.1007/s00428-015-1836-2]

79 **Taniai M**, Hashimoto E, Tobari M, Kodama K, Tokushige K, Yamamoto M, Takayama T, Sugitani M, Sano K, Kondo F, Fukusato T. Clinicopathological investigation of steatohepatitic hepatocellular carcinoma: A multicenter study using immunohistochemical analysis of adenoma-related markers. *Hepatol Res* 2018; **48**: 947-955 [PMID: 30058778 DOI: 10.1111/hepr.13203]

80 **El Jabbour T**, Lagana SM, Lee H. Update on hepatocellular carcinoma: Pathologists' review. *World J Gastroenterol* 2019; **25**: 1653-1665 [PMID: 31011252 DOI: 10.3748/wjg.v25.i14.1653]

81 **Lee WS**, Lee KW, Heo JS, Kim SJ, Choi SH, Kim YI, Joh JW. Comparison of combined hepatocellular and cholangiocarcinoma with hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *Surg Today* 2006; **36**: 892-897 [PMID: 16998683 DOI: 10.1007/s00595-006-3276-8]

82 **Lee JH**, Shin DH, Park WY, Shin N, Kim A, Lee HJ, Kim YK, Choi KU, Kim JY, Yang YI, Lee CH, Sol MY. IDH1 R132C mutation is detected in clear cell hepatocellular carcinoma by pyrosequencing. *World J Surg Oncol* 2017; **15**: 82 [PMID: 28403884 DOI: 10.1186/s12957-017-1144-1]

83 **Bannasch P**, Ribback S, Su Q, Mayer D. Clear cell hepatocellular carcinoma: origin, metabolic traits and fate of glycogenotic clear and ground glass cells. *Hepatobiliary Pancreat Dis Int* 2017; **16**: 570-594 [PMID: 29291777 DOI: 10.1016/S1499-3872(17)60071-7]

84 **Vasuri F**, Fittipaldi S, Giunchi F, Monica M, Ravaioli M, Degiovanni A, Bonora S, Golfieri R, Bolondi L, Grigioni WF, Pasquinelli G, D'Errico-Grigioni A. Facing the enigma of the vascular network in hepatocellular carcinomas in cirrhotic and non-cirrhotic livers. *J Clin Pathol* 2016; **69**: 102-108 [PMID: 26243063 DOI: 10.1136/jclinpath-2015-203028]

85 **Ziol M**, Poté N, Amaddeo G, Laurent A, Nault JC, Oberti F, Costentin C, Michalak S, Bouattour M, Francoz C, Pageaux GP, Ramos J, Decaens T, Luciani A, Guiu B, Vilgrain V, Aubé C, Derman J, Charpy C, Zucman-Rossi J, Barget N, Seror O, Ganne-Carrié N, Paradis V, Calderaro J. Macrotrabecular-massive hepatocellular carcinoma: A distinctive histological subtype with clinical relevance. *Hepatology* 2018; **68**: 103-112 [PMID: 29281854 DOI: 10.1002/hep.29762]

86 **Matsuura S**, Aishima S, Taguchi K, Asayama Y, Terashi T, Honda H, Tsuneyoshi M. 'Scirrhous' type hepatocellular carcinomas: a special reference to expression of cytokeratin 7 and hepatocyte paraffin 1. *Histopathology* 2005; **47**: 382-390 [PMID: 16178893 DOI: 10.1111/j.1365-2559.2005.02230.x]

87 **Li W**, Gomez E, Zhang Z. Immunohistochemical expression of stromal cell-derived factor-1 (SDF-1) and CXCR4 ligand receptor system in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2007; **26**: 527-533 [PMID: 18365549]

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*MINIREVIEWS*

# Micelles as potential drug delivery systems for colorectal cancer treatment

Zaynab Fatfat, Maamoun Fatfat, Hala Gali-Muhtasib

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## Abstract

Despite the significant progress in cancer therapy, colorectal cancer (CRC) remains one of the most fatal malignancies worldwide. Chemotherapy is currently the mainstay therapeutic modality adopted for CRC treatment. However, the long-term effectiveness of chemotherapeutic drugs has been hampered by their low bioavailability, non-selective tumor targeting mechanisms, non-specific biodistribution associated with low drug concentrations at the tumor site and undesirable side effects. Over the last decade, there has been increasing interest in using nanotechnology-based drug delivery systems to circumvent these limitations. Various nanoparticles have been developed for delivering chemotherapeutic drugs among which polymeric micelles are attractive candidates. Polymeric micelles are biocompatible nanocarriers that can bypass the biological barriers and preferentially accumulate in tumors *via* the enhanced permeability and retention effect. They can be easily engineered with stimuli-responsive and tumor targeting moieties to further ensure their selective uptake by cancer cells and controlled drug release at the desirable tumor site. They have been shown to effectively improve the pharmacokinetic properties of chemotherapeutic drugs and enhance their safety profile and anticancer efficacy in different types of cancer. Given that combination therapy is the new strategy implemented in cancer therapy, polymeric micelles are suitable for multidrug delivery and allow drugs to act concurrently at the action site to achieve synergistic therapeutic outcomes. They also allow the delivery of anticancer genetic material along with chemotherapy drugs offering a novel approach for CRC therapy. Here, we highlight the properties of polymeric micelles that make them promising drug delivery systems for CRC treatment. We also review their application in CRC chemotherapy and gene therapy as well as in combination cancer chemotherapy.

**Key Words:** Polymeric micelles; Drug delivery; Colorectal cancer; Chemotherapy; Gene

therapy; Combination cancer therapy

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**Core Tip:** Polymeric micelle-based drug delivery has demonstrated promising therapeutic outcomes against colorectal cancer. These safe nanocarriers exhibit high encapsulation efficiency of chemotherapeutic drugs, improve their water solubility and enhance the stability of nucleic acid-based therapeutics. They also accumulate preferentially at colorectal cancer sites, increase the anticancer effect of the delivered therapeutics and reduce their side effects. Incorporating stimuli-responsive and tumor targeting moieties to their structure further enhances their targeting and therapeutic efficacy. This platform also provides an opportunity to simultaneously deliver different chemotherapeutic drugs or nucleic acid-based therapeutics with chemotherapeutic drugs to the colorectal tumor to achieve an enhanced anticancer response.

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## INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent cancer and the second leading cause of cancer-related deaths in men and women globally with an estimated 1.9 million new cases and 0.9 million deaths in 2020[1]. These numbers are predicted to reach 3.1 million and 1.6 million, respectively, within the next two decades[2]. The majority of CRC patients present with localized tumors, and nearly 20%-30% of CRC patients have unresectable metastasis[3]. While the 5-year survival rate for localized CRCs is nearly 90%, it is reduced to about 12% for distant metastatic CRCs[4].

Surgery is the primary treatment for patients in early stages of CRC, whereas preoperative or postoperative radiotherapy and chemotherapy are added for patients in advanced stages[5]. Numerous chemotherapeutic drugs, approved by the Food and Drug Administration, have been used for CRC treatment including 5-fluorouracil (5-FU), irinotecan (IRI), oxaliplatin (OXA) and capecitabine[3]. However, their clinical efficacy has been constrained by the adverse side effects and drug resistance that causes cancer relapse and treatment failure[6-8]. To address these drawbacks, combination therapy has been used as an alternative therapeutic approach to cancer monotherapy. Combining therapeutic agents with different modes of action demonstrated great potential in achieving synergistic therapeutic outcomes with reduced side effects in addition to overcoming drug resistance[9]. Several combination chemotherapeutic regimens such as FOLFIRI (folinic acid, 5-FU and IRI), XELOX/CAPOX (OXA and capecitabine) and FOLFOX (folinic acid, 5-FU and OXA) are widely used in clinical treatment for CRC [10]. However, and despite the continued efforts devoted to advance CRC chemotherapy, the prognosis of this disease is still unsatisfactory, particularly for metastatic CRC patients[11].

The main concerns limiting the success of single agent or combination chemotherapy are poor solubility, short half-life, rapid metabolism of the anticancer drugs, lack of selectivity for the tumor sites, non-specific biodistribution and subsequent failure to achieve effective therapeutic concentrations at the action sites[12,13]. Over the past decades, nanotechnology-based drug delivery systems have emerged as a modern approach to overcome these limitations and improve drug performance. They enhance the pharmacokinetic proprieties of the drugs, ensure their delivery to the tumor, prevent toxicity to healthy tissues, increase drug concentration at the tumor site and therefore potentiate their anticancer activity while mitigating their associated side effects[14]. In combination therapy, they can effectively carry several drugs with dissimilar pharmacokinetic profiles and maintain the optimized synergistic drug ratio until they reach the target cancer cells[15]. Additionally, nanosized drug delivery systems opened up new opportunities for the delivery of novel therapeutics along with chemotherapeutic agents, such as genetic anticancer agents, which are considered to be innovative approaches in CRC management [15].

Among the broad range of nanocarriers, polymeric micelles have received particular attention for therapeutic applications in cancer. Many anticancer agents are polycyclic compounds with poor aqueous solubility[16], and polymeric micelles have proven to be powerful vehicles for loading and delivering hydrophobic drugs[17]. Besides being biocompatible and biodegradable, polymeric micelles provide distinct advantages including small size, low-cost, ease of preparation, high drug encapsulation capacity and structural stability[18-20]. They can be easily functionalized with stimuli-responsive and tumor targeting moieties to achieve site-specific delivery and controlled drug release[21]. They also allow the integration of multiple functions other than drug delivery including gene delivery and

imaging[21]. To date, two polymeric micelles, Genexol-PM loading paclitaxel (PTX) and Nanoxel loading docetaxel (DTX), have been approved in Korea and India, respectively, for the treatment of different types of cancer including breast and non-small cell lung cancers[22]. As these nanocarriers have shown therapeutic potential for these solid tumors, they could serve as drug delivery platforms to alleviate the current limitations of CRC chemotherapy and promote the introduction of gene therapy in the management of this disease. Here, we review the composition and characteristics of polymeric micelles in addition to the achievements in micelle-based drug delivery for CRC chemotherapy and gene therapy. Published studies included in this minireview were identified through searching PubMed and Google scholar using different permutations of these keywords "colorectal cancer" or "colon cancer," "chemotherapy" or "gene therapy," "combination" and "micelle." Clinical trials were identified through searching https://clinicaltrials.gov using two keywords "micelle" and "cancer".

## COMPOSITION AND CHARACTERISTICS OF POLYMERIC MICELLES

Polymeric micelles are formed by self-assembly of amphiphilic di-block, tri-block or grafted copolymers in aqueous solutions[23]. Micelles have a two-phase structure including an inner core composed of hydrophobic blocks surrounded by a corona of hydrophilic blocks[24,25]. The widely used core-forming polymers include polyesters, polyethers or polyamino acids, while the most used hydrophilic corona-forming polymer is polyethylene glycol (PEG)[26]. Alternatively, other hydrophilic polymers could be used including chitosan, dextran and hyaluronic acid (HA)[27]. The hydrophilic corona confers steric stability to the micelle, decreases its interactions with serum components and prevents its recognition and early elimination by the reticuloendothelial system, thus enhancing its retention in the systemic circulation[28,29]. The hydrophobic core forms a loading site for lipophilic drugs. It was reported that encapsulating drugs in micelles enhanced their water solubility by 10- to 500-fold[30]. Non-polar drugs can be loaded in micelles by physical entrapment or chemical conjugation[30]. Polymeric micelles used in gene delivery are known as micelleplexes and have cationic properties, which allow the association of nucleic acids *via* electrostatic interactions. The hydrophilic blocks of these micelles are usually composed of polycations including polyethyleneimine (PEI), poly (2-dimethylaminoethyl methacrylate) and polyamino acids[31].

The concentration of copolymers that is needed to form micelles is known as the critical micelle concentration. The critical micelle concentration should be low enough to prevent the dissociation of the micelles upon dilution in the bloodstream[32]. The size of polymeric micelles typically ranges from 10 to 100 nm[33]. Given that nanoparticles that are less than 10 nm can easily undergo renal clearance and those larger than 100 nm can be rapidly eliminated by the liver and spleen, the suitable size of micelles limits their rapid clearance from the circulation[34]. In addition, the nanosize of micelles allows them to passively accumulate in tumor tissues by the enhanced permeability and retention effect[27]. This passive targeting is due to the aberrant and leaky vascular architecture coupled with defective lymphatic drainage that uniquely characterize solid tumors[35]. Being unable to pass through the normal blood vessel walls, the micelles extravasate through the leaky tumor blood vessels, accumulate preferentially at the tumor site and consequently promote the retention of the delivered drug at the tumor tissue[36].

The polymeric micelles deliver the drug payload to the cancer cells by two mechanisms. The therapeutic agents can enter the cancer cell as free drugs after their release from the micelles or be internalized as drugs encapsulated within the micelles to be released within the cell[37]. Chemically conjugated drugs are released by bulk degradation or surface erosion of the micelles, while the physically entrapped drugs are mainly released by diffusion[38]. Intact drug-loaded micelles are internalized *via* cell- dependent pinocytosis, which includes macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis and clathrin- and caveolae-independent endocytosis[39-42] Internalized micelles have been shown to localize in acidic organelles, namely endosomes or lysosomes[40,43]. The loaded drugs were suggested to be released from the micelles in these organelles[40,43] and to subsequently exert their therapeutic effect at the action site[40] (Figure 1).

Active targeting can be used to complement passive targeting for enhanced tumor selectivity, improved cellular uptake and reduced off-target effects[21,23]. The surface of the micelles can be modified with ligands that can specifically recognize and bind to receptors or proteins overexpressed on the tumor cells but that are less expressed on normal cells[44]. Different types of ligands have been conjugated to micelles to ensure their active targeting to cancer cells including antibodies and antibody fragments, proteins including transferrin, peptides such as iRGD, nucleic acid-based ligands such as the AS1411 aptamer and small molecules including folic acid (FA) and HA[45].

Another interesting approach for active targeting is to achieve adequate drug release at the tumor site by designing stimuli-responsive micelles taking advantage of the unique features of the tumor microenvironment. The tumor tissues are characterized by a low pH, upregulation of specific enzymes and elevated levels of glutathione compared with the normal tissues[34]. Stimuli-sensitive polymeric micelles are developed by introducing pH-sensitive linkers in their structures, such as hydrazone bonds or pH-sensitive ionizable functional groups, enzyme-sensitive moieties or redox-sensitive linkers

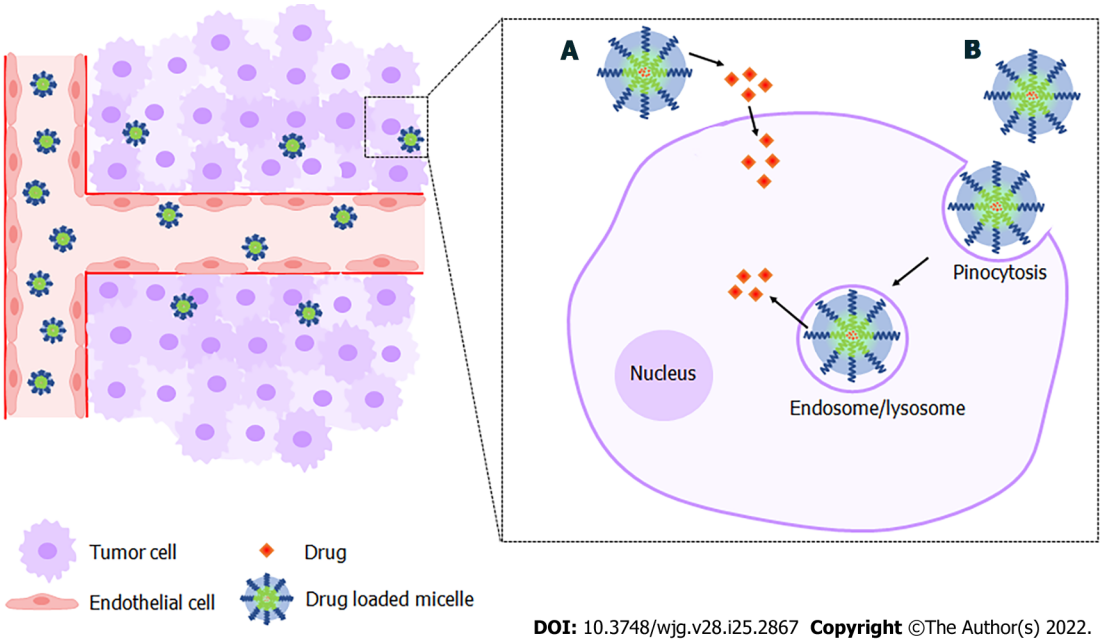


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**Figure 1 Schematic illustration of the passive targeting of drug formulated micelles to the tumor tissue by enhanced permeability and retention effect and the proposed mechanisms of drug release in the cancer cell.** A: The drug can enter the cancer cell in its free form after its release from the micelle; B: The drug can be internalized within the micelle after which it is released in the cancer cell.

including disulfide bonds[34]. When the micelles reach the tumor, the linker degrades in response to the internal stimulus leading to the disassembly of micelles and subsequent drug release[34]. In addition to internal stimuli, polymeric micelles responsive to external stimuli such as magnetic fields, ultrasound and light are designed to achieve a temporal and spatial controlled release of the drug in the desired tumor tissue[34]. After releasing the drug, micelles can dissociate into monomers that are excreted by the kidneys, therefore avoiding any long-term adverse effects[46]. All these properties make polymeric micelles an attractive platform for drug delivery against CRC.

## APPLICATION OF POLYMERIC MICELLES IN CRC TREATMENT

The properties and the cellular and molecular mechanisms of action of polymeric micelles used for drug delivery in CRC treatment are presented in Table 1.

### *Chemotherapy*

Numerous studies have demonstrated the potential of polymeric micelles to ensure the targeted delivery of chemotherapeutic drugs to colorectal tumor sites and enhance their therapeutic effectiveness.

IRI is a topoisomerase I inhibitor that is converted by enzymatic activation in the liver and tumors to 7-ethyl-10-hydroxy-camptothecin (SN-38). The latter is about 100-1000 times more potent than IRI against cancer cells[47]. However, only 2%-5% of the administered dose of IRI is converted to SN-38 in the clinic[48]. Although SN-38 demonstrated effective anticancer activity, its direct use as a free drug in the clinic was limited by its hydrophobicity[49]. To circumvent this problem, Koizumi *et al*[50] conjugated SN-38 to PEG-poly(glutamic acid) copolymer with a phenyl ester bond to develop SN-38-loaded polymeric micelles (NK012). The micelle formulation of SN-38 demonstrated extended blood circulation compared with free IRI in mice bearing CRC xenografts. It also prolonged and improved the distribution of SN-38 in the tumor and produced stronger antitumor activities compared to free IRI. To test the correlation between the efficacy of micellar drugs and tumor hypervascularity and hyperpermeability, the effect of NK012 and free IRI was tested in a vascular endothelial growth factor (VEGF)–secreting tumor model namely SBC-3/VEGF. Interestingly, NK012 was found to improve the accumulation and the antitumor activity of SN-38 in SBC-3/VEGF tumors compared to SBC-3/Neo tumors. This was due to the high vascular density and permeability induced by VEGF. However, treatment with free IRI neither significantly increased SN-38 concentration in SBC-3/VEGF tumors nor effectively suppressed tumor growth[50].

Doxorubicin (Dox) is an anthracycline antibiotic that is commonly used to treat several types of solid cancer. However, its clinical use is hampered by dose-dependent cardiotoxicity due to its nonspecific biodistribution[51]. Recently, Brunato *et al*[52] developed methoxyPEG (mPEG)-polyamino acid based

**Table 1 Overview of micelles used for drug delivery in colorectal cancer treatment, their characteristics and cellular and molecular mechanisms of action**

| Block copolymer | CMC | Size in nm | Zeta potential in mV | Entrapment efficiency in % | Therapeutic agent | Cell line or animal model | Cellular and molecular mechanisms of action of micelles loaded with the therapeutic agents | Ref. |
|---|---|---|---|---|---|---|---|---|
| PEG-poly (glutamic acid) | N/A | 20 | N/A | N/A | SN-38 | WiDR, SW480, Lovo and HT-29 human colon cancer cells; Female BALB/c nude mice subcutaneously injected with HT-29 cells | | Koizumi *et al* [50] |
| mPEG5kDa-b-[(Dox-hydGlu)$_6$-r-Leu$_{10}$] | 4.6 ± 0.2 μmol/L | 29.2 ± 1.1 | 3.61 ± 0.28 | N/A | Doxorubucin | CT26 murine colorectal cancer cells; BALB/c mice subcutaneously injected with CT26 cells | (1) Do not cause hemolysis; (2) Do not induce a significant increase of the levels of blood markers for organ toxicity AST, BUN and CPK; and (3) Induce a slight increase of ALT and LDH | Brunato *et al* [52] |
| PEG-poly (L-lactate-co-hexamethylene-co-adipate) (PEG-PLLHA) and FA-PEG-poly (hexamethylene adipate-co-hexamethylene 2-hydroxyl succinate) | 3.65 μg/mL | 215.6 ± 3.1 | −2.4 ± 0.2 | 82.1 ± 0.6 | Docetaxel | CT-26 cells; Female BALB/c mice subcutaneously injected with CT-26 cells | (1) Induce a more severe tumor necrosis compared to their non-targeted counterparts; (2) Do not cause hemolysis or erythrocyte agglutination; (3) Do not induce histological damage to the major organs of the treated mice; (4) Induce a slight increase of BUN levels; and (5) Do not affect the concentrations of ALT, AST, ALP, and CRE | Su *et al*[54] |
| PEG-poly (D,L lactate-co-hexamethylene-co-adipate) (PEG-PDLLHA) and FA-PEG-poly (hexamethylene adipate-co-hexamethylene 2-hydroxyl succinate) | 3.50 μg/mL | 245.5 ± 4.3 | −2.8 ± 0.1 | 79.9 ± 1.0 | | | | |
| D-α-tocopherol succinate (TOS)-conjugated-hyaluronic acid | N/A | 95.5 ± 13.7 | N/A | 90 | Paclitaxel | CT26 mouse colon carcinoma cells; NIH-3T3 mouse embryo fibroblasts; HT29 and Lovo human colorectal adenocarcinoma cells; BALB/c mouse subcutaneously injected with CT26 cells | (1) Induce early and late apoptosis in HT29 and Lovo cancer cells *in vitro*; and (2) Induce apoptosis and decrease tumor cell proliferation *in vivo* | Zhu *et al*[58] |
| mPEG-PCL and DOTAP | N/A | 144.8 | 46.4 | N/A | Bcl-xl siRNA and Mcl1 siRNA | C26 cells; BALB/c mice inoculated with C26 cells | | Lu *et al*[59] |
| mPEG-PCL and DOTAP | N/A | 46.4 ± 3.7 | 44.1 ± 1.5 | N/A | Plasmid pVAX1-mIL22BP expressing murine IL-22BP | C26 *Mus musculus* colon carcinoma cells; 293t human embryonic kidney cells; BALB/c mice intraperitoneally injected with C26 cells | (1) Induce apoptosis *in vitro*; (2) Decrease the microvessel density characterized by CD31 positive staining; and (3) Induce lymphocyte infiltration in tumor microenvironment as indicated by the detection of CD8+ | Men *et al*[60] |

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| | | | | | | | and CD4+ cells in the tumor tissues | |
| mPEG-PCL and DOTAP | N/A | 46 ± 5.6 | 41.8 ± 0.5 | N/A | Plasmid pcDNA-Survivin-T34A expressing Survivin-T34A | C-26 murine colon adenocarcinoma cells; BALB/c mice intraperitoneally injected with C-26 cells | | Duan *et al*[61] |
| PEI-deoxycholic acid | N/A | 88.4 ± 16 | N/A | N/A | XIAP siRNA and paclitaxel | HCT-116 human colorectal cancer cells; Male BALB/c nu/nu mice subcutaneously injected with HCT-116 cells | | Jang *et al*[63] |
| PEI-poly (DL-lactic acid) | 0.1167 mg/mL | 235 ± 25 | −22 | 100 | Survivin shRNA and camptothecin | C26 and CHO cells; Female BALB/c mice subcutaneously inoculated with C26 cells | (1) Induce a more pronounced apoptosis *in vitro* compared with their non-targeted counterparts; and (2) Have a lower accumulation in vital organs *in vivo* compared with their non-targeted counterparts | Sanati *et al*[64] |
| PDMA-b-PCL and mPEG-PCL | N/A | 222.1 | 21.1 | N/A | SN-38, USPIO and VEGF siRNA | LS174T human colon adenocarcinoma cells; Female BALB/c athymic nu+/nu+ mice subcutaneously injected with LS174T cells | | Lee *et al*[66] |
| PEI-poly (D,L lactide) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-mPEG | N/A | 171.25 ± 4.70 | 15.12 ± 0.36 | 81.25 ± 3.12 | miRNA-34a and irinotecan | CT-26 murine colon adenocarcinoma cells; Female BALB/c mice injected with CT-26 cells | (1) Upregulate miR-34a and reduce the expression of Bcl-2 and the phosphorylation level of mTOR; (2) Negligible hemolytic activity; and (3) Do not significantly alter the levels of ALP, ALT, ALB, AST, CK, LDH, BUN and CRE | Li *et al*[67] |
| PEG-lysyl-(α-fluorenylmethyl-oxycarbonyl-ε-Cbz-lysine)$_2$ | 2.6 μmol/L | 25.4 ± 0.8 | 0.519 ± 0.730 | N/A | Doxorubicin and dasatinib | HCT-116 human colon cancer cells | | Zhang *et al*[69] |
| Poly {(*N*-methyl-diethenamine sebacate)-*co*-[(cholesteryl oxocarbonylamido ethyl) methyl bis (ethylene) ammonium bromide] sebacate} | N/A | 230 | 70 | N/A | Doxorubicin and TRAIL | SW480 human colorectal adenocarcinoma epithelial cells; WI38 human lung fibroblasts | Induce caspase-dependent apoptosis | Lee *et al*[70] |
| Cholesteryl-modified single strand DNA (Chl–ssDNA) and its complementary sequence | 249 pmol/L | 371.3 ± 3.1 | -7.07 ± 2.3 | 84.9 ± 5.21 | Doxorubicin and KLA peptide | C57/BL6 mice injected with C26 cells | | Charbgoo *et al*[71] |
| FA-dextran-paclitaxel | 3.1 μg/mL | 76 ± 2 | -11.2 ± 0.8 | N/A | Adjudin and paclitaxel | HCT-8 and HCT-8/PTX cells; Mouse subcutaneously injected with HCT-8/PTX cells | (1) Reduce mitochondrial membrane potential and the levels of ATP; and (2) Do not cause hemolysis | Chen *et al*[72] |
| Poly-lactic-co-glycolic acid grafted branched PEI | 1.32 ± 0.003 mg/mL | 137.98 ± 2.13 | 12.3 ± 0.2 | 70.38 ± 2.34 | 5-fluorouracil and methotrexate | HCT 116 colon cancer cells | | Ashwanikumar *et al*[73] |
| mPEG-PCL | 56 | 167.5 | -0.11 | 68.8 | Doxifluridine | HT-29 human | | Sawdon *et al* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | mg/L | | | | and doxorubicin | colorectal adenocarcinoma cells | [74] |
| | | 267.5 | 1.01 | 86.3 | Doxifluridine and SN-38 | | |
| Chitosan-PCL | 40 mg/mL | 163.7 | 38.8 | N/A | Doxifluridine and SN-38 | HT-29 human colorectal adenocarcinoma cells | Wang *et al*[75] |

CMC: Critical micelle concentration; mPEG: Methoxypolyethylene glycol; Dox: Doxorubicin; hydGlu: Acid-γ-hydrazide; Leu: Leucine; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen; CPK: Creatine phosphokinase; CK: Creatine kinase; LDH: Lactate dehydrogenase; FA: Folic acid; ALP: Alkaline phosphatase; CRE: Creatinine; DOTAP: N-[1-(2, 3-dioleoyloxy) propyl]-N, N, N-trimethylammonium methyl sulfate; PEI: Polyethyleneimine; PCL: Poly(ε-caprolactone); ALB: Albumin; siRNA: Small interfering RNA; XIAP: X-linked inhibitor of apoptosis; shRNA: Short hairpin RNA; VEGF: Vascular endothelial growth factor; USPIO: Ultra-small superparamagnetic iron oxide nanoparticles; miR-34a: microRNA-34a; TRAIL: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand; IL-22BP: Interleukin-22 binding protein; mTOR: Mammalian target of rapamycin; SN-38: 7-ethyl-10-hydroxy-camptothecin; N/A: Not available; PEG: Polyethylene glycol; TOS: D-α-tocopherol succinate; PTX: Paclitaxel.

micelles for the delivery and controlled release of Dox in CRC. The hydrophobic block of the copolymer included six glutamic acid-γ-hydrazide and ten leucines. Dox was conjugated to glutamic acid through pH-sensitive hydrazone bond to trigger its release in the acidic lysosomal microenvironment. The release of Dox was faster at pH 5.5, which mimics the lysosomal environment. In addition, the intracellular delivery of Dox by the micelles and its trafficking through the lysosomal compartment of CRC cells, where the acidic environment can induce the cleavage of the hydrazone bond, were confirmed by confocal microscopy. Therefore, encapsulating Dox in the formulated micelles may prevent its undesired release in the bloodstream. Interestingly, Dox-loaded micelles had good tolerability, produced a higher antitumor effect compared to the free drug and had limited toxicity in CRC bearing mice[52].

DTX is a microtubule inhibitor that is approved to treat numerous types of cancer. Due to its low water solubility, a mixture of Tween 80 and ethanol is required to solubilize DTX. Yet, Tween 80 used in the available marketed formulation of DTX Taxotere® was associated with adverse effects[53]. Recently, FA-targeted PEG-polyester micelles were formulated for the targeted delivery of DTX against colon cancer. The targeted formulation resulted in an accelerated internalization of the micelles by FA receptor-positive colon cancer cells *in vitro* and in a higher and preferential accumulation of the micelles in colon tumors *in vivo* compared to the non-targeted formulation. Besides being biocompatible and safe, the FA-targeted DTX-loaded micelles exhibited a remarkable 97% tumor-inhibiting efficiency, which was higher than that of non-targeted DTX-loaded micelles (85%) and free DTX (31%) in mice bearing colon tumor xenografts[54].

PTX is a microtubule stabilizing agent commonly used against a variety of cancers including CRC [55]. Because of its low aqueous solubility, Taxol®, the commercial formulation of PTX, uses a nonionic surfactant namely Cremophor® EL[56]. However, Cremophor® EL has been associated with serious side effects such as hypersensitivity, nephrotoxicity and neurotoxicity[57]. Zhu *et al*[58] developed smart micelles for the targeted delivery and controlled release of PTX in colon cancer. Micelles are made from the self-assembly of D-α-tocopherol succinate (TOS) conjugated to HA *via* a redox responsive disulfide bond. Their findings demonstrated that these micelles could specifically accumulate in orthotopic colon tumors and metastatic tumor cells rather than in normal intestinal tissue through binding of HA to CD44 receptors overexpressed on primary and metastatic colon tumor cells. They could also selectively release PTX in orthotopic colon tumors and metastatic tumor cells where the high levels of glutathione break disulfide bonds thus causing drug release. PTX-encapsulated micelles induced the highest survival rate (100%) in mice bearing orthotopic colon tumors compared to untreated group (60%) and free PTX (Taxol) treated group (40%). They also suppressed primary colon tumors and metastatic sites in the intestine and decreased metastasis to other organs and to the peritoneum[58].

### Gene therapy

Polymeric micelles were also reported to successfully deliver anticancer genetic material to CRC. Cationic hybrid micelles composed of amphiphilic cationic lipid N-[1-(2,3-dioleoyloxy) propyl]-N, N, N-trimethylammonium methyl sulfate (DOTAP) and mPEG-poly (ε-caprolactone) (PCL) have been used to deliver genes or small interfering RNA (siRNA) for the treatment of colon cancer in preclinical studies. The cationic hydrophilic head of DOTAP ensures the adsorption of the anionic nucleic acid onto the surface of the micelles *via* electrostatic interaction. These hybrid micelles demonstrated an effective and safe delivery of siRNA targeting colon cancer cells. The micelle-based delivery of siRNA caused inhibition of the two anti-apoptotic genes *Bcl-xl* and *Mcl1*, which consequently induced apoptosis in colon cancer cells *in vitro* and suppressed the growth of colon tumors *in vivo*[59]. In another report, these hybrid micelles were used for the delivery of interleukin-22 binding protein (*IL-22BP*) gene, which is known to play a role in blocking the IL-22/IL-22R1 signal axis involved in tumor growth and

metastasis. The micelle-delivered *IL-22BP* gene was efficiently expressed in colon tumors *in vivo* leading to the inhibition of the growth of abdominal cavity metastases. Its anticancer mechanism involved blockage of IL-22, induction of apoptosis and anticancer immune response in addition to inhibition of angiogenesis[60]. In a study conducted by Duan *et al*[61], these hybrid micelles were found to efficiently deliver the classic suicide gene survivin-T34A that acts as a competitor in the survivin pathway involved in apoptosis inhibition[62]. The survivin-T34A gene-loaded micelles inhibited the proliferation of colon cancer cells *in vitro* and the growth of abdominal metastatic colon cancer and malignant ascites *in vivo* by inducing apoptosis[61]. Importantly, in all these studies, this micellar carrier did not induce any pathological changes in the vital organs of the mice with colon cancer.

### Combination of chemotherapy with gene therapy

The micellar dual delivery of chemotherapeutic drugs with agents targeted against specific genes have been shown to produce synergistic therapeutic responses against CRC. Cationic micelles based on PEI-deoxycholic acid conjugates have been used for the co-delivery of PTX and siRNA targeting the X-linked inhibitor of apoptosis gene to CRC. The micellar co-delivery of siRNA targeting X-linked inhibitor of apoptosis gene and PTX effectively decreased the expression of X-linked inhibitor of apoptosis at the mRNA and protein levels *in vitro* and *in vivo*. In addition, it was more potent than PTX-loaded micelles as it caused a greater reduction in cell viability of CRC cells and inhibited the growth of colorectal tumors *in vivo*[63]. In another study, Sanati *et al*[64] synthesized cationic micelles for the targeted dual delivery of camptothecin (CPN), a topoisomerase inhibitor, and short hairpin RNA targeting survivin to colon cancer. These micelles containing PEI-poly (D,L-lactic acid) copolymers were coated with polycarboxylic acid dextran and were subsequently modified with AS1411 aptamer that has high affinity for the nucleolin receptors expressed on the surface of colon cancer cells. Cell surface nucleolin expression is known to be more elevated in cancer cells than in normal cells[65]. The conjugation of AS1411 aptamer on the micelles improved their cellular internalization through nucleolin-mediated endocytosis *in vitro* and enhanced their accumulation in colon tumors *in vivo* in comparison with their non-targeted counterparts. While the non-targeted micellar formulation of each of CPN and short hairpin RNA targeting survivin exhibited 3% and 5% tumor inhibitory effect *in vivo*, the targeted and non-targeted formulations of the combination of CPN and short hairpin RNA targeting survivin exerted 93% and 87% tumor inhibitory effects, respectively. It is important to note that the free CPN did not have any anticancer effects *in vivo,* and its micellar formulation prevented the development of its associated systemic toxicity[64]. Moreover, Lee *et al*[66] designed theranostic micelles from cationic PDMA-b-PCL and mPEG-PCL polymers for the combined delivery of SN-38, siRNA targeting human VEGF in addition to a magnetic resonance imaging contrast agent namely ultra-small superparamagnetic iron oxide nanoparticles. The SN-38/ultra-small superparamagnetic iron oxide nanoparticles/siRNA-loaded mixed micelles were targeted passively to colorectal tumor regions *in vivo.* They induced a synergistic tumor growth suppression and allowed the tracking of anticancer effects by magnetic resonance imaging during the treatment[66]. Additionally, hybrid micelles co-self-assembled from PEI-poly (D,L lactide), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-mPEG were used to deliver IRI and tumor suppressive microRNA-34a (miR-34a) to colon cancer. The micellar co-delivery of the two therapeutic agents showed higher efficacy in inducing apoptosis and inhibiting migration of colon cancer cells *in vitro* compared with free IRI and the single agent formulation. The micellar IRI and miR-34a combination also accumulated in colon tumors *in vivo* and showed superior tumor growth inhibition with low acute systemic toxicity compared with free IRI and the micellar formulation of individual agents[67].

### Combination cancer chemotherapy

For dual combination therapy, the encapsulation of drugs in a micellar system could be performed in two ways (Figure 2). In the first method, both drugs are physically loaded in the micelles by hydrophobic interactions. Alternatively, one hydrophobic drug is conjugated to the amphiphilic copolymer, which self-assembles and forms prodrug micelles to subsequently encapsulate the second hydrophobic drug in its core[68].

The chemotherapeutic drug Dox and the tyrosine kinase inhibitor dasatinib were effectively co-encapsulated in the inner hydrophobic core of micelles formed by PEG-peptidic conjugate namely PEG-lysyl-(α-fluorenylmethyloxycarbonyl-ε-Cbz-lysine)$_2$. The dual drug-loaded micelles exhibited greater inhibition of colon cancer cell proliferation compared with the micellar formulation or the free form of individual drugs[69]. In another study, Dox was co-delivered with the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in cationic micelles to CRC. The micelles were prepared with poly {(*N*-methyldietheneamine sebacate)-*co*-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate. Dox was encapsulated in the micelles, whereas TRAIL was complexed onto their surface. The internalization of these micelles by CRC cells was mediated by the death receptors expressed on the cell surface. Compared to the formulation of single agents, Dox and TRAIL-loaded micelles enhanced the inhibition of CRC cell viability, the increase of sub-G1 population and the reduction of long-term survival and clonogenicity of cancer cells after the treatment. Interestingly, these remarkable anticancer effects were reported in both TRAIL sensitive and resistant CRC cells. In addition, the cytotoxic effect of the co-loaded micelles was selectively higher against cancer cells than

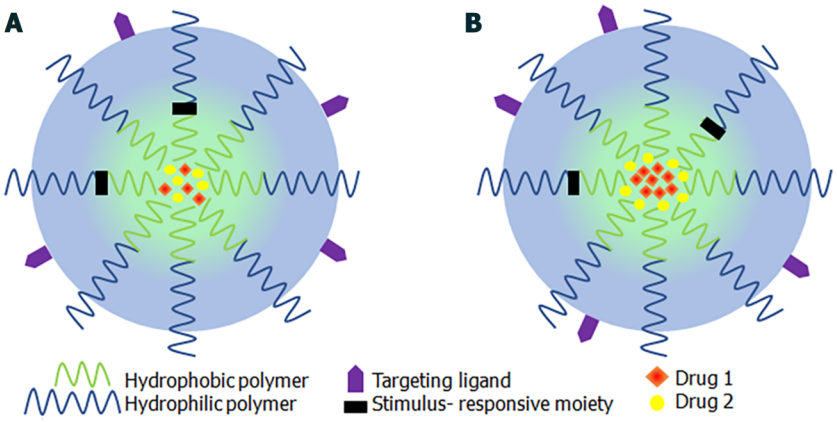


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**Figure 2 Polymeric micelles for dual drug delivery.** A: Both drugs are physically loaded in the core of the micelle; B: One hydrophobic drug is conjugated to the amphiphilic copolymer forming the micelle and the second is encapsulated in its core.

normal cells. This was suggested to be due to the relative disparities in expression levels of decoy and death receptors between normal and cancer cells[70]. A third study reported the combined delivery of Dox and the proapoptotic cationic peptide KLA using DNA micelles. These micelles were made using cholesteryl-modified single strand DNA and its complementary sequence to which the KLA peptide was conjugated. The cationic peptides enhanced the stability of the micelles by protecting them from digestion by nucleases. Since oligonucleotides cannot cross cell membranes, DNA micelles were conjugated to a mucine1 aptamer to target mucine1 receptors overexpressed on the surface of cancer cells. These micelles were found to specifically bind to the mucine1 receptors overexpressing cells and to accumulate within them. The co-delivery of KLA and Dox using the targeted micelles potentiated the inhibition of colon tumor growth *in vivo* compared to free Dox in addition to attenuating its toxic effects [71]. A recent study reported the formulation of a folate receptor-targeted and redox-responsive micellar system for the co-delivery of PTX and adjudin (ADD), a mitochondrial inhibitor, to reverse multidrug resistance in colon cancer. In this drug delivery system, PTX was conjugated to dextran *via* a disulfide bond to form the PTX prodrug, and FA was conjugated to the side chain of dextran-PTX. The resulting PTX prodrug micelles were further used to encapsulate ADD in its hydrophobic core. *In vitro* studies showed that conjugation of FA to the surface of these micelles enhanced their internalization by PTX resistant CRC cells overexpressing the FA receptor. They also demonstrated that ADD inhibited PTX efflux, which consequently improved intracellular accumulation of PTX and therefore its cytotoxic activity. In addition, PTX and ADD-loaded micelles exhibited a prolonged circulation in the blood compared with free PTX *in vivo*. They also increased the concentrations of drugs in resistant colon tumors compared to their non-targeted counterparts and free PTX. Importantly, they demonstrated a strong tumor growth inhibition owing to the active tumor-targeting, the co-delivery of two therapeutic drugs and glutathione-responsive drug release[72]. In another study, micelles engineered with poly-lactic-co-glycolic acid grafted branched PEI were used to co-deliver 5-FU and methotrexate (MTX), which is an antagonist of FA and is known to have anticancer effects against different types of neoplasm. MTX was conjugated to the copolymer forming the prodrug micelles, which was further used to encapsulate 5-FU. In addition to its therapeutic potential, MTX has structural similarity with FA and can therefore act as an FA receptor targeting agent. The conjugation of MTX to the micelles improved their internalization by colon cancer cells compared to the non-conjugated micelles. The combined drug-loaded micelles were more effective in attenuating the viability of colon cancer cells compared to free 5-FU or the micellar MTX[73]. Doxifluridine, a prodrug of 5-FU, has been commonly used in cancer treatment to circumvent the toxicity of 5-FU. Doxifluridine is converted into toxic 5-FU by endogenous thymidine phosphorylase in cancer cells[74]. In a study conducted by Wang *et al*[75], doxifluridine was conjugated to PCL to form a hydrophobic segment that was grafted with hydrophilic chitosan. The produced polymeric prodrugs self-assembled into micelles used for SN-38 encapsulation. The dually loaded micelles were found to reduce the viability of colon cancer cells *in vitro* to a greater extent than doxifluridine-loaded micelles. In another study, doxifluridine-PCL hydrophobic segment was grafted with mPEG to form prodrug micelles serving as carriers to further encapsulate Dox or SN-38. The micellar co-delivery of doxifluridine with Dox or SN-38 exhibited superior inhibition of CRC cell viability with respect to doxifluridine-loaded micelles[74].

### *Polymeric micelles in clinical trials*

Despite their promising potential in preclinical studies against colon cancer, few polymeric micelles have reached the clinical setting. A phase I clinical trial of NK012 was conducted in Japan with a total of 24 patients with solid tumors including 12 patients with CRC. NK012 was found to be well tolerated at

doses up to 28 mg/m$^2$ when administered every 3 wk. None of the CRC patients had a partial response. However, 5 CRC patients with a history of treatment with OXA and IRI had stable disease; 4 of them received the NK012 regimen for six cycles of treatment or more[76]. Subsequently, the efficacy and safety of NK012 was evaluated in a phase II clinical trial at an initial dose of 28 mg/m$^2$ in 58 Japanese patients with unresectable metastatic CRC who had been treated with OXA-based chemotherapy. Administration of NK012 resulted in a response rate similar to that of free IRI. In addition, febrile neutropenia and grade ≥ 3 neutropenia were reported in these patients suggesting that an initial dose of 28 mg/m$^2$ of NK012 may be too high. Further studies should be conducted to determine the optimal dose of NK012 and improve its efficacy and safety[77]. In another study, a phase I clinical trial of NK105, PTX-loaded micelles, was conducted with 19 patients with advanced solid tumors including 1 patient with colon cancer. NK105 was well tolerated, and the recommended phase II dose was determined to be 150 mg/m$^2$ every 3 wk. The colon cancer patient was reported to have a stable disease for longer than 4 weeks at the time of the completion of the study[78]. Two additional clinical trials on polymeric micelles in CRC were launched but their results have not been reported yet. The first is a phase I clinical trial that aims to determine the maximum tolerated dose/recommended phase II dose of the combination of NK012 with 5-FU and leucovorin in patients with CRC (NCT01238939)[79]. The second is a phase II clinical trial that aims to investigate the safety and the diagnostic performance of ONM-100, a polymer micelle covalently conjugated to an intraoperative fluorescence imaging agent indocyanine green, for the detection of cancer in patients with solid tumors including CRC patients undergoing routine surgery (NCT03735680)[80].

## CONCLUSION

Polymeric micelles are potential candidates for delivery of chemotherapeutic drugs and therapeutic genetic material in single or combination treatment against CRC. Polymeric micelles exhibit a safe profile, help in solubilizing hydrophobic chemotherapeutic drugs and achieve prolonged and stabilized drug circulation in the blood. They also promote the passive accumulation of drugs at CRC sites, potentiate their anticancer activity and reduce their unwanted side effects. Conjugating polymeric micelles with stimuli-responsive and tumor targeting moieties further increases their internalization by cancer cells, thus achieving selective drug release at colorectal tumor sites and enhancing drug anticancer effects. In addition, polymeric micelles ensure an effective and safe delivery of genetic material to modulate the expression of apoptotic players in CRC. Co-delivering genetic anticancer agents with chemotherapeutic drugs using micellar systems is known to augment the therapeutic outcome of the latter and therefore represent a promising combination strategy for combating CRC.

This nanocarrier system also allows the incorporation of imaging agents with therapeutics and offers great potential to achieve tumor diagnosis, in addition to tracking the delivery and release of the therapeutic agent and evaluating its therapeutic performance. Although most of the studies evaluating polymeric micelle-mediated combination chemotherapy in CRC were performed *in vitro*, they yielded interesting results. The delivery of drug combinations using polymeric micelles could further intensify their inhibitory effects against CRC and help overcome drug resistance. However, further pharmacokinetic and pharmacodynamic studies are needed prior to the clinical translation of drug-loaded polymeric micelles. Few polymeric micelles have been used in the clinical setting for CRC treatment and diagnosis with promising outcomes, thus their application merits further investigation.

## FOOTNOTES

**Author contributions:** Fatfat Z and Fatfat M reviewed the literature and drafted the manuscript; Gali-Muhtasib H initiated the idea and revised the manuscript; All authors have read and approved the final manuscript.

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## REFERENCES

1 **Xi Y**, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 2021; **14**: 101174 [PMID: 34243011 DOI: 10.1016/j.tranon.2021.101174]
2 **World Health Organization**. International Agency for Research on Cancer: GLOBOCAN: Cancer tomorrow, 2022. Available from:https://gco.iarc.fr/tomorrow/en/dataviz/isotype?group_cancers=1&multiple_cancers=1&cancers=8_9_10&single_unit=50000 [DOI: 10.1002/food.19770210141]
3 **Matos AI**, Carreira B, Peres C, Moura LIF, Conniot J, Fourniols T, Scomparin A, Martínez-Barriocanal Á, Arango D, Conde JP, Préat V, Satchi-Fainaro R, Florindo HF. Nanotechnology is an important strategy for combinational innovative chemo-immunotherapies against colorectal cancer. *J Control Release* 2019; **307**: 108-138 [PMID: 31226355 DOI: 10.1016/j.jconrel.2019.06.017]
4 **Naeem M**, Awan UA, Subhan F, Cao J, Hlaing SP, Lee J, Im E, Jung Y, Yoo JW. Advances in colon-targeted nano-drug delivery systems: challenges and solutions. *Arch Pharm Res* 2020; **43**: 153-169 [PMID: 31989477 DOI: 10.1007/s12272-020-01219-0]
5 **Lei X**, He Q, Li Z, Zou Q, Xu P, Yu H, Ding Y, Zhu W. Cancer stem cells in colorectal cancer and the association with chemotherapy resistance. *Med Oncol* 2021; **38**: 43 [PMID: 33738588 DOI: 10.1007/s12032-021-01488-9]
6 **Rejhová A**, Opattová A, Čumová A, Slíva D, Vodička P. Natural compounds and combination therapy in colorectal cancer treatment. *Eur J Med Chem* 2018; **144**: 582-594 [PMID: 29289883 DOI: 10.1016/j.ejmech.2017.12.039]
7 **Hammond WA**, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: a review. *Ther Adv Med Oncol* 2016; **8**: 57-84 [PMID: 26753006 DOI: 10.1177/1758834015614530]
8 **Huang JR**, Lee MH, Li WS, Wu HC. Liposomal Irinotecan for Treatment of Colorectal Cancer in a Preclinical Model. *Cancers (Basel)* 2019; **11** [PMID: 30818855 DOI: 10.3390/cancers11030281]
9 **Bayat Mokhtari R**, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, Yeger H. Combination therapy in combating cancer. *Oncotarget* 2017; **8**: 38022-38043 [PMID: 28410237 DOI: 10.18632/oncotarget.16723]
10 **Moradi-Marjaneh R**, Paseban M, Moradi Marjaneh M. Hsp70 inhibitors: Implications for the treatment of colorectal cancer. *IUBMB Life* 2019; **71**: 1834-1845 [PMID: 31441584 DOI: 10.1002/iub.2157]
11 **Ginghină O**, Hudiță A, Zaharia C, Tsatsakis A, Mezhuev Y, Costache M, Gălățeanu B. Current Landscape in Organic Nanosized Materials Advances for Improved Management of Colorectal Cancer Patients. *Materials (Basel)* 2021; **14** [PMID: 34066710 DOI: 10.3390/ma14092440]
12 **Entezar-Almahdi E**, Mohammadi-Samani S, Tayebi L, Farjadian F. Recent Advances in Designing 5-Fluorouracil Delivery Systems: A Stepping Stone in the Safe Treatment of Colorectal Cancer. *Int J Nanomedicine* 2020; **15**: 5445-5458 [PMID: 32801699 DOI: 10.2147/IJN.S257700]
13 **Arshad U**, Sutton PA, Ashford MB, Treacher KE, Liptrott NJ, Rannard SP, Goldring CE, Owen A. Critical considerations for targeting colorectal liver metastases with nanotechnology. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2020; **12**: e1588 [PMID: 31566913 DOI: 10.1002/wnan.1588]
14 **Cabeza L**, Perazzoli G, Mesas C, Jiménez-Luna C, Prados J, Rama AR, Melguizo C. Nanoparticles in Colorectal Cancer Therapy: Latest In Vivo Assays, Clinical Trials, and Patents. *AAPS PharmSciTech* 2020; **21**: 178 [PMID: 32591920 DOI: 10.1208/s12249-020-01731-y]
15 **Gurunathan S**, Kang MH, Qasim M, Kim JH. Nanoparticle-Mediated Combination Therapy: Two-in-One Approach for Cancer. *Int J Mol Sci* 2018; **19** [PMID: 30347840 DOI: 10.3390/ijms19103264]
16 **Majumder N**, G Das N, Das SK. Polymeric micelles for anticancer drug delivery. *Ther Deliv* 2020; **11**: 613-635 [PMID: 32933425 DOI: 10.4155/tde-2020-0008]
17 **Deshmukh AS**, Chauhan PN, Noolvi MN, Chaturvedi K, Ganguly K, Shukla SS, Nadagouda MN, Aminabhavi TM. Polymeric micelles: Basic research to clinical practice. *Int J Pharm* 2017; **532**: 249-268 [PMID: 28882486 DOI: 10.1016/j.ijpharm.2017.09.005]
18 **Jhaveri AM**, Torchilin VP. Multifunctional polymeric micelles for delivery of drugs and siRNA. *Front Pharmacol* 2014; **5**: 77 [PMID: 24795633 DOI: 10.3389/fphar.2014.00077]
19 **Bholakant R**, Dong B, Zhou X, Huang X, Zhao C, Huang D, Zhong Y, Qian H, Chen W, Feijen J. Multi-functional polymeric micelles for chemotherapy-based combined cancer therapy. *J Mater Chem B* 2021; **9**: 8718-8738 [PMID: 34635905 DOI: 10.1039/d1tb01771c]
20 **Tawfik SM**, Azizov S, Elmasry MR, Sharipov M, Lee YI. Recent Advances in Nanomicelles Delivery Systems. *Nanomaterials (Basel)* 2020; **11** [PMID: 33396938 DOI: 10.3390/nano11010070]
21 **Raval N**, Maheshwari R, Shukla H, Kalia K, Torchilin VP, Tekade RK. Multifunctional polymeric micellar nanomedicine in the diagnosis and treatment of cancer. *Mater Sci Eng C Mater Biol Appl* 2021; **126**: 112186 [PMID: 34082985 DOI: 10.1016/j.msec.2021.112186]
22 **Mi P**, Miyata K, Kataoka K, Cabral H. Clinical translation of Self-Assembled cancer nanomedicines. *Adv Therap* 2021; **4**: 2000159 [DOI: 10.1002/adtp.202170001]
23 **Oerlemans C**, Bult W, Bos M, Storm G, Nijsen JF, Hennink WE. Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. *Pharm Res* 2010; **27**: 2569-2589 [PMID: 20725771 DOI: 10.1007/s11095-010-0233-4]
24 **Yokoyama M**. Polymeric micelles as drug carriers: their lights and shadows. *J Drug Target* 2014; **22**: 576-583 [PMID: 25012065 DOI: 10.3109/1061186X.2014.934688]
25 **Torchilin VP**. Targeted polymeric micelles for delivery of poorly soluble drugs. *Cell Mol Life Sci* 2004; **61**: 2549-2559 [PMID: 15526161 DOI: 10.1007/s00018-004-4153-5]

26 **Morshed RA**, Cheng Y, Auffinger B, Wegscheid ML, Lesniak MS. The potential of polymeric micelles in the context of glioblastoma therapy. *Front Pharmacol* 2013; **4**: 157 [PMID: 24416018 DOI: 10.3389/fphar.2013.00157]

27 **Ghosh B**, Biswas S. Polymeric micelles in cancer therapy: State of the art. *J Control Release* 2021; **332**: 127-147 [PMID: 33609621 DOI: 10.1016/j.jconrel.2021.02.016]

28 **Yousefpour Marzbali M**, Yari Khosroushahi A. Polymeric micelles as mighty nanocarriers for cancer gene therapy: a review. *Cancer Chemother Pharmacol* 2017; **79**: 637-649 [PMID: 28314988 DOI: 10.1007/s00280-017-3273-1]

29 **Almeida M**, Magalhães M, Veiga F, Figueiras A. Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer. *J Polym Res* 2017; **25**: 1-14 [DOI: 10.1007/s10965-017-1426-x]

30 **Hanafy NAN**, El-Kemary M, Leporatti S. Micelles Structure Development as a Strategy to Improve Smart Cancer Therapy. *Cancers (Basel)* 2018; **10** [PMID: 30037052 DOI: 10.3390/cancers10070238]

31 **Pereira-Silva M**, Alvarez-Lorenzo C, Concheiro A, Santos AC, Veiga F, Figueiras A. Nanomedicine in osteosarcoma therapy: Micelleplexes for delivery of nucleic acids and drugs toward osteosarcoma-targeted therapies. *Eur J Pharm Biopharm* 2020; **148**: 88-106 [PMID: 31958514 DOI: 10.1016/j.ejpb.2019.10.013]

32 **Glavas L**, Olsén P, Odelius K, Albertsson AC. Achieving micelle control through core crystallinity. *Biomacromolecules* 2013; **14**: 4150-4156 [PMID: 24066701 DOI: 10.1021/bm401312j]

33 **Felber AE**, Dufresne MH, Leroux JC. pH-sensitive vesicles, polymeric micelles, and nanospheres prepared with polycarboxylates. *Adv Drug Deliv Rev* 2012; **64**: 979-992 [PMID: 21996056 DOI: 10.1016/j.addr.2011.09.006]

34 **Zhou Q**, Zhang L, Yang T, Wu H. Stimuli-responsive polymeric micelles for drug delivery and cancer therapy. *Int J Nanomedicine* 2018; **13**: 2921-2942 [PMID: 29849457 DOI: 10.2147/IJN.S158696]

35 **Kalyane D**, Raval N, Maheshwari R, Tambe V, Kalia K, Tekade RK. Employment of enhanced permeability and retention effect (EPR): Nanoparticle-based precision tools for targeting of therapeutic and diagnostic agent in cancer. *Mater Sci Eng C Mater Biol Appl* 2019; **98**: 1252-1276 [PMID: 30813007 DOI: 10.1016/j.msec.2019.01.066]

36 **Tan C**, Wang Y, Fan W. Exploring polymeric micelles for improved delivery of anticancer agents: recent developments in preclinical studies. *Pharmaceutics* 2013; **5**: 201-219 [PMID: 24300405 DOI: 10.3390/pharmaceutics5010201]

37 **Jarak I**, Varela CL, Tavares da Silva E, Roleira FFM, Veiga F, Figueiras A. Pluronic-based nanovehicles: Recent advances in anticancer therapeutic applications. *Eur J Med Chem* 2020; **206**: 112526 [PMID: 32971442 DOI: 10.1016/j.ejmech.2020.112526]

38 **Kedar U**, Phutane P, Shidhaye S, Kadam V. Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine* 2010; **6**: 714-729 [PMID: 20542144 DOI: 10.1016/j.nano.2010.05.005]

39 **Zhao Z**, Wang Y, Han J, Wang K, Yang D, Yang Y, Du Q, Song Y, Yin X. Self-assembled micelles of amphiphilic poly(L-phenylalanine)-b-poly(L-serine) polypeptides for tumor-targeted delivery. *Int J Nanomedicine* 2014; **9**: 5849-5862 [PMID: 25540585 DOI: 10.2147/IJN.S73111]

40 **Zhang C**, Jin S, Li S, Xue X, Liu J, Huang Y, Jiang Y, Chen WQ, Zou G, Liang XJ. Imaging intracellular anticancer drug delivery by self-assembly micelles with aggregation-induced emission (AIE micelles). *ACS Appl Mater Interfaces* 2014; **6**: 5212-5220 [PMID: 24606837 DOI: 10.1021/am5005267]

41 **Fang XB**, Zhang JM, Xie X, Liu D, He CW, Wan JB, Chen MW. pH-sensitive micelles based on acid-labile pluronic F68-curcumin conjugates for improved tumor intracellular drug delivery. *Int J Pharm* 2016; **502**: 28-37 [PMID: 26784981 DOI: 10.1016/j.ijpharm.2016.01.029]

42 **Qiu L**, Shan X, Long M, Ahmed KS, Zhao L, Mao J, Zhang H, Sun C, You C, Lv G, Chen J. Elucidation of cellular uptake and intracellular trafficking of heparosan polysaccharide-based micelles in various cancer cells. *Int J Biol Macromol* 2019; **130**: 755-764 [PMID: 30851320 DOI: 10.1016/j.ijbiomac.2019.02.133]

43 **Sakai-Kato K**, Un K, Nanjo K, Nishiyama N, Kusuhara H, Kataoka K, Kawanishi T, Goda Y, Okuda H. Elucidating the molecular mechanism for the intracellular trafficking and fate of block copolymer micelles and their components. *Biomaterials* 2014; **35**: 1347-1358 [PMID: 24290812 DOI: 10.1016/j.biomaterials.2013.11.027]

44 **Yu G**, Ning Q, Mo Z, Tang S. Intelligent polymeric micelles for multidrug co-delivery and cancer therapy. *Artif Cells Nanomed Biotechnol* 2019; **47**: 1476-1487 [PMID: 31070063 DOI: 10.1080/21691401.2019.1601104]

45 **Amjad MW**, Kesharwani P, Mohd Amin MCI, Iyer AK. Recent advances in the design, development, and targeting mechanisms of polymeric micelles for delivery of siRNA in cancer therapy. *Prog Polym Sci* 2017; **64**: 154-181 [DOI: 10.1016/j.progpolymsci.2016.09.008]

46 **Cabral H**, Kataoka K. Progress of drug-loaded polymeric micelles into clinical studies. *J Control Release* 2014; **190**: 465-476 [PMID: 24993430 DOI: 10.1016/j.jconrel.2014.06.042]

47 **Xu G**, Shi C, Guo D, Wang L, Ling Y, Han X, Luo J. Functional-segregated coumarin-containing telodendrimer nanocarriers for efficient delivery of SN-38 for colon cancer treatment. *Acta Biomater* 2015; **21**: 85-98 [PMID: 25910639 DOI: 10.1016/j.actbio.2015.04.021]

48 **Senter PD**, Beam KS, Mixan B, Wahl AF. Identification and activities of human carboxylesterases for the activation of CPT-11, a clinically approved anticancer drug. *Bioconjug Chem* 2001; **12**: 1074-1080 [PMID: 11716702 DOI: 10.1021/bc0155420]

49 **Carie A**, Rios-Doria J, Costich T, Burke B, Slama R, Skaff H, Sill K. IT-141, a Polymer Micelle Encapsulating SN-38, Induces Tumor Regression in Multiple Colorectal Cancer Models. *J Drug Deliv* 2011; **2011**: 869027 [PMID: 22187652 DOI: 10.1155/2011/869027]

50 **Koizumi F**, Kitagawa M, Negishi T, Onda T, Matsumoto S, Hamaguchi T, Matsumura Y. Novel SN-38-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors. *Cancer Res* 2006; **66**: 10048-10056 [PMID: 17047068 DOI: 10.1158/0008-5472.CAN-06-1605]

51 **Kim TH**, Mount CW, Gombotz WR, Pun SH. The delivery of doxorubicin to 3-D multicellular spheroids and tumors in a murine xenograft model using tumor-penetrating triblock polymeric micelles. *Biomaterials* 2010; **31**: 7386-7397 [PMID: 20598741 DOI: 10.1016/j.biomaterials.2010.06.004]

52 **Brunato S**, Mastrotto F, Bellato F, Bastiancich C, Travanut A, Garofalo M, Mantovani G, Alexander C, Preat V, Salmaso S, Caliceti P. PEG-polyaminoacid based micelles for controlled release of doxorubicin: Rational design, safety and efficacy study. *J Control Release* 2021; **335**: 21-37 [PMID: 33989691 DOI: 10.1016/j.jconrel.2021.05.010]

53 **Repp L**, Unterberger CJ, Ye Z, Feltenberger JB, Swanson SM, Marker PC, Kwon GS. Oligo(Lactic Acid)$_8$-Docetaxel Prodrug-Loaded PEG-*b*-PLA Micelles for Prostate Cancer. *Nanomaterials (Basel)* 2021; **11** [PMID: 34685195 DOI: 10.3390/nano11102745]

54 **Su M**, Xie J, Zeng Q, Shu M, Liu J, Jiang Z. Enzymatic synthesis of PEGylated lactide-diester-diol copolyesters for highly efficient targeted anticancer drug delivery. *Mater Sci Eng C Mater Biol Appl* 2020; **115**: 111125 [PMID: 32600724 DOI: 10.1016/j.msec.2020.111125]

55 **Zhu L**, Chen L. Progress in research on paclitaxel and tumor immunotherapy. *Cell Mol Biol Lett* 2019; **24**: 40 [PMID: 31223315 DOI: 10.1186/s11658-019-0164-y]

56 **Repp L**, Rasoulianboroujeni M, Lee HJ, Kwon GS. Acyl and oligo(lactic acid) prodrugs for PEG-b-PLA and PEG-b-PCL nano-assemblies for injection. *J Control Release* 2021; **330**: 1004-1015 [PMID: 33166607 DOI: 10.1016/j.jconrel.2020.11.008]

57 **Yang T**, Cui FD, Choi MK, Cho JW, Chung SJ, Shim CK, Kim DD. Enhanced solubility and stability of PEGylated liposomal paclitaxel: in vitro and in vivo evaluation. *Int J Pharm* 2007; **338**: 317-326 [PMID: 17368984 DOI: 10.1016/j.ijpharm.2007.02.011]

58 **Zhu C**, Zhang H, Li W, Luo L, Guo X, Wang Z, Kong F, Li Q, Yang J, Du Y, You J. Suppress orthotopic colon cancer and its metastasis through exact targeting and highly selective drug release by a smart nanomicelle. *Biomaterials* 2018; **161**: 144-153 [PMID: 29421551 DOI: 10.1016/j.biomaterials.2018.01.043]

59 **Lu Y**, Zhong L, Jiang Z, Pan H, Zhang Y, Zhu G, Bai L, Tong R, Shi J, Duan X. Cationic micelle-based siRNA delivery for efficient colon cancer gene therapy. *Nanoscale Res Lett* 2019; **14**: 193 [PMID: 31165329 DOI: 10.1186/s11671-019-2985-z]

60 **Men K**, Huang R, Zhang X, Zhang R, Zhang Y, Peng Y, Tong R, Yang L, Wei Y, Duan X. Delivery of interleukin-22 binding protein (IL-22BP) gene by cationic micelle for colon cancer gene therapy. *RSC Adv* 2018; **8**: 16537-16548 [PMID: 35540501 DOI: 10.1039/c8ra02580k]

61 **Duan X**, Wang P, Men K, Gao X, Huang M, Gou M, Chen L, Qian Z, Wei Y. Treating colon cancer with a suicide gene delivered by self-assembled cationic MPEG-PCL micelles. *Nanoscale* 2012; **4**: 2400-2407 [PMID: 22388488 DOI: 10.1039/c2nr30079f]

62 **Martínez-García D**, Manero-Rupérez N, Quesada R, Korrodi-Gregório L, Soto-Cerrato V. Therapeutic strategies involving survivin inhibition in cancer. *Med Res Rev* 2019; **39**: 887-909 [PMID: 30421440 DOI: 10.1002/med.21547]

63 **Jang YL**, Yun UJ, Lee MS, Kim MG, Son S, Lee K, Chae SY, Lim DW, Kim HT, Kim SH, Jeong JH. Cell-penetrating peptide mimicking polymer-based combined delivery of paclitaxel and siRNA for enhanced tumor growth suppression. *Int J Pharm* 2012; **434**: 488-493 [PMID: 22613208 DOI: 10.1016/j.ijpharm.2012.04.083]

64 **Sanati S**, Taghavi S, Abnous K, Taghdisi SM, Babaei M, Ramezani M, Alibolandi M. Fabrication of anionic dextran-coated micelles for aptamer targeted delivery of camptothecin and survivin-shRNA to colon adenocarcinoma. *Gene Ther* 2022; **29**: 55-68 [PMID: 33633357 DOI: 10.1038/s41434-021-00234-0]

65 **Berger CM**, Gaume X, Bouvet P. The roles of nucleolin subcellular localization in cancer. *Biochimie* 2015; **113**: 78-85 [PMID: 25866190 DOI: 10.1016/j.biochi.2015.03.023]

66 **Lee SY**, Yang CY, Peng CL, Wei MF, Chen KC, Yao CJ, Shieh MJ. A theranostic micelleplex co-delivering SN-38 and VEGF siRNA for colorectal cancer therapy. *Biomaterials* 2016; **86**: 92-105 [PMID: 26896610 DOI: 10.1016/j.biomaterials.2016.01.068]

67 **Li Y**, Jia F, Deng X, Wang X, Lu J, Shao L, Cui X, Pan Z, Wu Y. Combinatorial miRNA-34a replenishment and irinotecan delivery *via* auto-fluorescent polymeric hybrid micelles for synchronous colorectal cancer theranostics. *Biomater Sci* 2020; **8**: 7132-7144 [PMID: 33150879 DOI: 10.1039/d0bm01579b]

68 **Yang T**, Lan Y, Cao M, Ma X, Cao A, Sun Y, Yang J, Li L, Liu Y. Glycyrrhetinic acid-conjugated polymeric prodrug micelles co-delivered with doxorubicin as combination therapy treatment for liver cancer. *Colloids Surf B Biointerfaces* 2019; **175**: 106-115 [PMID: 30529816 DOI: 10.1016/j.colsurfb.2018.11.082]

69 **Zhang P**, Li J, Ghazwani M, Zhao W, Huang Y, Zhang X, Venkataramanan R, Li S. Effective co-delivery of doxorubicin and dasatinib using a PEG-Fmoc nanocarrier for combination cancer chemotherapy. *Biomaterials* 2015; **67**: 104-114 [PMID: 26210177 DOI: 10.1016/j.biomaterials.2015.07.027]

70 **Lee AL**, Dhillon SH, Wang Y, Pervaiz S, Fan W, Yang YY. Synergistic anti-cancer effects *via* co-delivery of TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) and doxorubicin using micellar nanoparticles. *Mol Biosyst* 2011; **7**: 1512-1522 [PMID: 21350763 DOI: 10.1039/c0mb00266f]

71 **Charbgoo F**, Alibolandi M, Taghdisi SM, Abnous K, Soltani F, Ramezani M. MUC1 aptamer-targeted DNA micelles for dual tumor therapy using doxorubicin and KLA peptide. *Nanomedicine* 2018; **14**: 685-697 [PMID: 29317345 DOI: 10.1016/j.nano.2017.12.010]

72 **Chen D**, Ge S, Zuo L, Wang S, Liu M, Li S. Adjudin-loaded redox-sensitive paclitaxel-prodrug micelles for overcoming multidrug resistance with efficient targeted Colon cancer therapy. *Drug Deliv* 2020; **27**: 1094-1105 [PMID: 32706289 DOI: 10.1080/10717544.2020.1797245]

73 **Ashwanikumar N**, Kumar NA, Nair SA, Kumar GSV. Dual drug delivery of 5-fluorouracil (5-FU) and methotrexate (MTX) through random copolymeric nanomicelles of PLGA and polyethylenimine demonstrating enhanced cell uptake and cytotoxicity. *Colloids Surf B Biointerfaces* 2014; **122**: 520-528 [PMID: 25108479 DOI: 10.1016/j.colsurfb.2014.07.024]

74 **Sawdon AJ**, Zhang J, Wang X, Peng CA. Enhanced Anticancer Activity of 5'-DFUR-PCL-MPEG Polymeric Prodrug Micelles Encapsulating Chemotherapeutic Drugs. *Nanomaterials (Basel)* 2018; **8** [PMID: 30551585 DOI: 10.3390/nano8121041]

75 **Wang J**, Peng CA. Anticancer effectiveness of polymeric drug nanocarriers on colorectal cancer cells. *Annu Int Conf IEEE Eng Med Biol Soc* 2011; **2011**: 3249-3252 [PMID: 22255032 DOI: 10.1109/IEMBS.2011.6090883]

76 **Hamaguchi T**, Doi T, Eguchi-Nakajima T, Kato K, Yamada Y, Shimada Y, Fuse N, Ohtsu A, Matsumoto S, Takanashi M, Matsumura Y. Phase I study of NK012, a novel SN-38-incorporating micellar nanoparticle, in adult patients with solid tumors. *Clin Cancer Res* 2010; **16**: 5058-5066 [PMID: 20943763 DOI: 10.1158/1078-0432.CCR-10-0387]

77 **Hamaguchi T**, Tsuji A, Yamaguchi K, Takeda K, Uetake H, Esaki T, Amagai K, Sakai D, Baba H, Kimura M, Matsumura

Y, Tsukamoto T. A phase II study of NK012, a polymeric micelle formulation of SN-38, in unresectable, metastatic or recurrent colorectal cancer patients. *Cancer Chemother Pharmacol* 2018; **82**: 1021-1029 [PMID: 30284603 DOI: 10.1007/s00280-018-3693-6]

78 **Hamaguchi T**, Kato K, Yasui H, Morizane C, Ikeda M, Ueno H, Muro K, Yamada Y, Okusaka T, Shirao K, Shimada Y, Nakahama H, Matsumura Y. A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. *Br J Cancer* 2007; **97**: 170-176 [PMID: 17595665 DOI: 10.1038/sj.bjc.6603855]

79 **Kayaku N**. Study of NK012 and 5-FU/LV in Solid Tumors Followed by Dose Expansion in Colorectal Cancer. [accessed 2022 Mar 21]. In: ClinicalTrials.gov [Internet]. Nashville (TN): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT01238939 ClinicalTrials.gov Identifier: NCT01238939

80 **OncoNano Medicine**. A Study to Evaluate ONM-100, an Intraoperative Fluorescence Imaging Agent for the Detection of Cancer. [accessed 2022 Mar 21]. In: ClinicalTrials.gov [Internet]. Philadelphia (PA): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03735680 ClinicalTrials.gov Identifier: NCT03735680

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*MINIREVIEWS*

# Incretin based therapy and pancreatic cancer: Realising the reality

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## Abstract

Incretin-based therapies like glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors help maintain the glycaemic control in patients with type 2 diabetes mellitus with additional systemic benefits and little risk of hypoglycaemia. These medications are associated with low-grade chronic pancreatitis in animal models inconsistently. The incidence of acute pancreatitis was also reported in some human studies. This inflammation provides fertile ground for developing pancreatic carcinoma (PC). Although the data from clinical trials and population-based studies have established safety regarding PC, the pathophysiological possibility that low-grade chronic pancreatitis leads to PC remains. We review the existing literature and describe the relationship between incretin-based therapies and PC.

**Key Words:** Diabetes mellitus; Dipeptidyl peptidase-4 inhibitor; Glucagon-like peptide-1 receptor agonist; Incretin; Pancreatitis; Pancreatic Cancer

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**Core Tip:** Incretin-based therapies are increasingly being used to treat patients with type 2 diabetes mellitus . The early literature regarding pancreatic safety of incretin-based therapies was discordant. However, the follow-up data of various randomised trials have consistently shown that these medications are safe.

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## INTRODUCTION

Incretin-based therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists (RAs) and dipeptidyl peptidase-4 inhibitors (DPP-4Is) are being increasingly used to treat patients with type 2 diabetes mellitus (T2DM). GLP-1 RAs mimic the action of endogenous GLP-1, whereas DPP-4Is prevent the degradation of GLP-1. These medications can control blood glucose levels without an increased risk of hypoglycemic episodes or weight gain. The initial clinical trials for GLP-1 RA and DPP-4I were conducted in 2003 and 2004, respectively[1,2]. The United States Food and Drug Administration (FDA) issued an alert regarding the potential risk of acute pancreatitis in 2007[3]. DPP-4Is have been widely used owing to the oral administration and low risk of hypoglycemia. However, the use of GLP-1 RA was limited until 2015. But the data from cardiovascular outcome trials (CVOTs) of GLP-1 RAs changed the scenario. The significant reduction in cardiovascular deaths with GLP-1 RAs such as liraglutide and semaglutide tilted the benefit-risk relationship. These therapies have become the next treatment choice after metformin in patients with or at high risk of atherosclerotic vascular disease[4]. Newer generation incretin-based therapies [coagonists of GLP-1 with gastric inhibitory peptide (GIP) or glucagon] are in the pipeline. They are presumed to have higher efficacy than GLP-1 RAs and DPP-4Is. Various incretin-based medications categorised according to their mechanism of action are shown in Table 1.

Incretin-based therapies are speculated to induce overstimulation of the GLP-1 receptor, leading to pancreatitis and pancreatic carcinoma (PC). However, the risk of both PC and pancreatitis is increased in DM compared to non-diabetic counterparts[5]. For PC, inflammation is relevant both as a risk factor and an effect of cancer. Patients with chronic pancreatitis carry a higher risk of developing PC. The initial postmarketing surveillance studies for incretin-based therapies revealed an increase in pancreatic adverse events[6]. Further observational studies of pancreatic adverse events showed conflicting results. Post hoc analyses of pertinent randomized controlled trials (RCTs) failed to distinguish pancreatic adverse events between controls and patients on incretin-based therapies[7]. However, the theoretical plausibility of PC in patients receiving incretin-based therapies still remains valid. It is worth reviewing the status of the incretin-based therapies now as an increasing number of therapies based on incretin are becoming available. The treating clinician must be aware of the benefit-risk ratio (RR) when prescribing incretin-based therapy.

## SEARCH STRATEGY

Two authors (VS, AR) conducted the initial search in PubMed and Reference Citation Analysis ( https://www.referencecitationanalysis.com/) database for relevant articles. The references of these articles were searched for additional relevant studies. The keywords used in the search were: 'Incretin'; 'pancreatic cancer'; glucagon-like peptide-1 receptor agonist'; 'dipeptidyl peptidase-4 inhibitor'; incretin and pancreatic cancer; 'GLP1-RA and pancreatic cancer'; 'DPP-4I and pancreatic cancer'. Only English language publications were included. SK, DN, and JS selected the appropriate articles to be included.

## PATHOPHYSIOLOGY

The critical pathophysiologic mechanisms for developing PC in patients using incretin-based therapies are chronic low-grade inflammation and proliferative changes. A flowchart outlining the pathophysiology of pancreatic cancer is shown in Figure 1.

### *Role of inflammation*

Even without any therapy, both PC and pancreatitis incidence is increased in T2DM[8,9]. Long term treatment of T2DM patients with DPP-4Is/GLP-1 RAs may exacerbate the pre-existing chronic inflammation. Any additional mutations in the background of inflammation can tilt the balance toward the

**Table 1 Various incretin-based therapies**

| Class of drugs | Medications |
|---|---|
| GLP-1RA (oral/subcutaneous) | Subcutaneous-Exenatide, Albiglutide, Lixisenatide, Liraglutide, Semaglutide. Oral-Semaglutide |
| DPP-4I (oral) | Saxagliptin, Vildaglipitn, Sitagliptin, Aloglipitn, Linagliptin, Teneligliptin |
| Newer drugs/drugs in development | Tirzepatide (GLP1 + GIP co-agnoist) |
| | Cotadutide (GLP1 + glucagon co-agonist) |
| | Teduglutide (GLP-2 RA) |
| | Triple agnoists (GLP1 + Glucagon + GIP agnoists) |

GLP-1RA: Glucagon like peptide receptor-1 receptor agonist; DPP-4I: Dipeptidyl peptidase-4 inhibitor; GIP: Gastric inhibitory peptide; GLP-2RA: Glucagon like peptide receptor-2 receptor agonist.

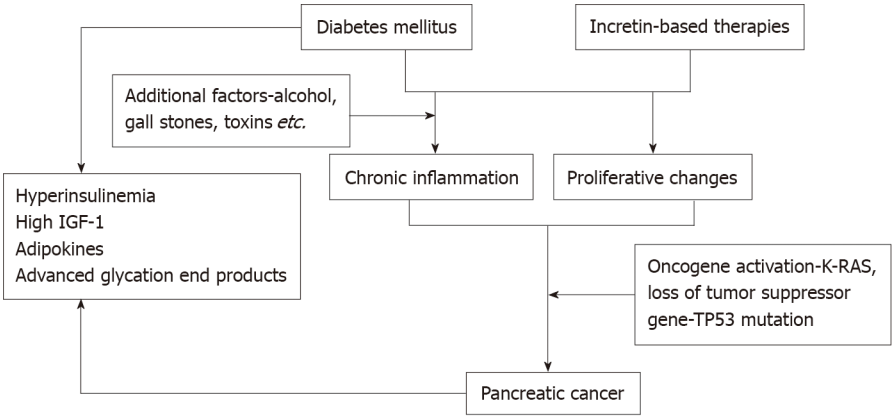


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**Figure 1 Flowchart explaining the mechanism of development of pancreatic cancer.** IGF-1: Insulin-like growth factor-1; K-RAS: Kirsten rat sarcoma virus gene.

progression of neoplasia. Various researchers conducted animal studies to substantiate this hypothesis using rodents with mutant Kirsten rat sarcoma virus gene (K-RAS), leading to constitutive activation. The K-RAS gene is mutated in > 90% of human PC cases[10]. KC mice model has a single activating mutation (G12D) in the K-RAS gene. Prenatal expression of mutant K-RAS in all exocrine lineages of KC mice resulted in histological changes suggestive of pancreatitis, implying a cooperative relationship between K-RAS activation and inflammation. Treatment of KC mice with the pancreatitis-inducing agent caerulein (a cholecystokinin analog) dramatically accelerates the progression to pancreatic ductal adenocarcinoma (PDAC) within a few weeks[11]. Further animal experiments have shown that K-RAS mutation alone cannot reach the expected theoretical levels of activity required for the progression of carcinogenesis. Nevertheless, agents which cause inflammation can hyperstimulate K-RAS by modulating its activity above the putative threshold necessary for carcinogenesis. The constitutive activation of nuclear factor kappa B pathway in addition to K-RAS in acinar cells significantly accelerates carcinogenesis, whereas cyclooxygenase inhibition is associated with a reduced risk of PDAC. These findings highlight the importance of inflammation in the progression of PDAC[12].

Incretin-based medications induced pancreatitis in animal experiments[13-15]. However, most PDAC cases develop without clinically apparent acute or chronic pancreatitis[16]. This observation can be explained by low-grade subclinical inflammation being sufficient to promote carcinogenesis in the presence of additional drivers of carcinogenesis. On the other hand, it can also be a consequence of the earliest events in carcinogenesis.

### *Role of proliferative changes*

In addition to low-grade asymptomatic chronic pancreatitis, incretin-based medications induce proliferative changes in the islets. Premalignant changes that precede the onset of PDAC are known as pancreatic intraepithelial neoplasia (PanIN) lesions. They herald the start of PDAC. PanINs can be found in up to 50% of the middle-aged population. However, only a few progress to PDAC[17]. As progression of PanINs to PDAC occurs *via* the accumulation of additional somatic mutations, any factor that increases cellular replication in PanINs is likely to increase the likelihood of PDAC. Both PanINs and PDAC express the human GLP-1 receptor. Acinar and duct cells proliferate in response to incretin-

based medications in the normal pancreas with an increase in pancreatic weight[18]. In the setting of chronic pancreatitis, as shown in animal models treated with incretin-based therapies, this proliferation may be sufficient to initiate carcinogenesis event sequences. Treatment with exenatide in KC mice resulted in the formation and growth of dysplastic PanIN lesions in addition to pancreatitis.

### Histological changes following incretin-based therapies

The controversy about pancreatic adverse events due to incretin-based therapies was amplified by a publication by Butler *et al*[19]. The authors examined age-matched organ donor samples obtained from the Network for Pancreatic Organ Donation (nPOD). They included a total of 34 subjects with T2DM treated with incretin-based therapies ($n$ = 8) or non-incretin-based therapies ($n$ = 12) and nondiabetic control subjects ($n$ = 14). The incretin group had an increase in pancreatic mass by 40% with increased exocrine cell proliferation and dysplasia. The authors also noticed alpha and beta-cell hyperplasia along with glucagon-expressing microadenomas in incretin-treated subjects. Islet cell costaining for insulin and glucagon was higher in DM subjects than non-DM control subjects and increased further with incretin therapy. The authors concluded that incretin therapy in humans results in the expansion of both exocrine and endocrine pancreatic compartments and that there is a potential for evolution into neuroendocrine tumors. However, this study was met with sharp criticism owing to methodological flaws. Harja *et al*[20], in a more detailed analysis of the nPOD database, found that the baseline characteristics were not comparable between the incretin group and the other two groups. Pancreatic weight data were missing for half of the subjects. The increase in PanINs can be explained by the nearly 20-year age difference between the incretin group and the other two groups. Bonner-Weir *et al*[21] and Kahn[22] also re-analyzed the data from nPOD and reached similar conclusions.

Despite the flaws in the study methodology, this study caught the attention of the FDA and the European Medicines Agency (EMA). Both agencies reviewed all available animal and human data for incretin-based therapies. Microscopic examinations from animal studies did not reveal pancreatic lesions or pancreatitis. Even at doses greater than human clinical exposure to incretin-based therapies, there were no tumours in rodents for up to 2 years (lifespan of rodents). The FDA studied the effect of exenatide in Zucker diabetic fatty rat, a chemical-induced pancreatitis model, and C57 black 6 (C57BL/6) high-fat diet mouse model. There was no identifiable pancreatic pathology in the pancreatitis mouse model and Zucker diabetic fatty rat model. After three months of exenatide in the C57BL/6 high-fat diet mouse, a minimal-to-moderate worsening of background findings was noted. Based on the available data, they could not draw any conclusions about the risk of pancreatic adverse events in patients using incretin-based therapies[23].

Ueberberg *et al*[24] conducted a study on cadaveric pancreata, similar to Butler *et al*[19]. They obtained pancreatic tissue during surgery from 13 diabetic patients (7 in the incretin group and 6 in the non-incretin group), 11 non-diabetic controls, and nine brain-dead organ donors. There were no differences between groups in the alpha cell area, beta and alpha cell replication, acinar, and ductal cell replication. Coexpression of insulin and glucagon has not been demonstrated. PanIN lesions were more common in the diabetic group, although the prevalence was low. Considering the small sample size and large interindividual variability, the authors advised caution on coming to any conclusion from such studies.

Chadwick *et al*[25] attempted to determine the background incidence of spontaneous pancreatic lesions in different rat strains fed a standard or high-fat diet over four months. They found that the pancreatic lesions previously thought to be due to incretins are common baseline findings. These lesions can be seen without any drug treatment. These lesions were independent of diet or glycaemic status. The authors concluded that we need to be cautious when interpreting patients' pancreatic findings on incretin-based therapies. Aston-Mourney *et al*[26] tried to characterize the amyloidogenic potential of sitagliptin in rodents. Human islet amyloid polypeptide transgenic mice, untreated nontransgenic mice, and those on treatment with sitagliptin, metformin, or the combination were followed up for one year. There was no increase in amyloid formation or ductal proliferation. However, there was an improvement in β-cell secretion, suggesting endocrine protective effects without associated toxicity to the exocrine compartment of the pancreas.

### Effect of incretin-based therapies on cancer cells

The effect of incretin-based therapies on PC cells has also been studied *in vitro*. Lu *et al*[27] found that liraglutide in combination with metformin has synergistic anti-tumor effects *in vitro*. Yan *et al*[28] examined the effect of exendin-4 on a xenograft tumor model. Exendin-4 suppressed the PC cell proliferation by attenuating the function of pancreatic stellate cells (PSC) and suppressing extracellular matrix deposition. Zhao *et al*[29] examined the effect of liraglutide on PC cell line (PANC-1) cocultured with and without PSCs and found that liraglutide significantly reduced the migration and invasion of the PANC-1 cells. The authors hypothesized that the effect is probably mediated by calcium and calcium-binding proteins. In another study, Zhao *et al*[30] examined the effects of liraglutide on the chemosensitivity of PC cells to gemcitabine in PANC-1 and gemcitabine resistant cell lines (PANC-GR). Liraglutide inhibited proliferation and promoted apoptosis of the PANC-GR cells in a dose-dependent manner. It also increased GLP-1 receptor and protein kinase-A expression in the PANC-GR cells. In rodent studies, liraglutide treatment was observed to increase the chemosensitivity of PC cells to

gemcitabine.

### DPP4 and cancer

DPP-4 cleaves many other polypeptides, such as chemokines, neuropeptides in addition to GLP-1. DPP-4, also known as cluster differentiation marker 26, plays a vital role in inflammation by modulating the inactivation of cytokines and chemokines. DPP-4 is expressed in various malignancies. The overexpression of DPP-4 exerts an antitumour effect predominantly through immunomodulation[31]. DPP-4 inhibition has been shown to improve antitumor immune response by preserving the function of a chemokine C-X-C motif ligand and through interleukin 33 (IL-33) mediated tumour control[32,33]. The effects of DPP-4 on cancer cells appear to be heterogeneous, depending on tumor types, stages, microenvironment, and host condition. In breast cancer and small cell lung cancers, decreased expression of DPP-4 is associated with more aggressiveness of the tumour. However, increased DPP-4 expression levels have been associated with poor prognosis in patients with PC[31].

## INCRETIN-BASED THERAPIES AND PC-HUMAN STUDIES

### Data from observational studies

Early case reports of pancreatitis following exenatide and sitagliptin led to the issue of FDA alert in 2007. These reports led to many database analyses to look for the prevalence of pancreas-related adverse events. Elashoff *et al*[6] raised concern regarding PDAC by studying the FDA adverse events reporting system (FDA AERS) during 2004-09. This study resulted in widespread coverage in media regarding the risk of PC. Elashoff *et al*[6] examined FDA AERS database for adverse events associated with exenatide and sitagliptin in this study. The odds ratio for reported pancreatitis was 6-fold higher for sitagliptin or exenatide users than other therapies. PC was also more frequent (approximately 3-fold) among patients who took sitagliptin or exenatide. However, we must understand that this was a retrospective, record-based study. The likelihood of reporting events is higher with new drugs than with the patients on older drugs. A series of observational studies followed this study and showed discordant results. Most of the observational studies were retrospective and record-based. Both the FDA and the EMA reviewed their clinical safety databases, and the pooled data did not reveal any compelling evidence of an increased risk of pancreatitis or PC.

The latest observational study from Montvida *et al*[34] used Centricity Electronic Medical Records from the United States. The authors assessed the time to pancreatic events for incretin-based and nonincretin therapies (sulfonylurea, thiazolidinedione, and insulin). This study surprisingly found that the group treated with insulin had higher pancreatitis events with a short time to event as compared to incretin-based therapies. For PC, the time to event rate was not significantly different between the groups. Among the older studies, few studies showed an increased risk of pancreatitis and PC, but most studies could not find any association between pancreatic adverse events and incretin-based therapies [34-38]. Although the sample size was adequate for these studies, we must keep in mind the unaccounted confounders. The baseline characteristics and the comparator drugs varied among different studies. A summary of these studies is compiled in Table 2.

### Data from randomised controlled trials

Data from RCTs have conventionally been considered superior to data from observational studies. There have been many follow-up studies of the patients recruited for CVOT of the incretin-based therapies. Among the individual CVOT data, there was a non-significant trend for or against the development of PC for different incretin-based therapies. Many researchers also assessed the PC risk with incretin-based therapies using meta-analyses and systematic reviews. Alves *et al*[39] evaluated the association of liraglutide or exenatide with PC in a meta-analysis that included 25 longitudinal studies in 2012. Neither GLP-1 RAs nor DPP-4Is were associated with increased risk for PC.

The first meta-analysis evaluating PC association with all types of incretin-based therapies was published by Chen *et al*[40] in 2016. This meta-analysis included 24 RCTs enrolling 47904 patients. This meta-analysis also could not identify any increased risk of PC either as monotherapy (RR = 0.62, 95%CI: 0.38–1.01) or combination therapy (RR = 0.92, 95%CI: 0.45–1.90). This result was followed by a series of meta-analyses with a similar conclusion[41-43]. The latest meta-analysis is from Abd El Aziz *et al*[43], which evaluated 11 CVOTs studying 55921 patients. In the individual study data, lixisenatide and semaglutide were associated with nominally reduced RR, whereas liraglutide was associated with a slightly elevated RR. However, none of these RRs were statistically significant. In this meta-analysis, neither GLP-1 RAs nor DPP-4Is were associated with a significant increase or decrease in risk of PC [RR for PC- for GLP-1 RA 0.99 (95%CI: 0.9-1.08); DPP-4I 0.92 (95%CI: 0.83-1.01)]. The summary of the data from various meta-analyses is compiled in Table 3.

### Newer incretin-based therapies

Tirzepatide is a dual agonist at GLP-1 and GIP receptors. It was formulated as a fatty acid-modified

**Table 2 Important observational studies which evaluated the relationship between incretin-based therapies and pancreatic carcinoma**

| Ref. | Study design | Population | Findings |
|---|---|---|---|
| Elashoff *et al* [6], 2011 | Retrospective study (Control drugs-rosiglitazone, glinides, glipizide), 2004-09 | Database-FDA AERS. Patients of T2DM on exenatide and sitagliptin. *n* = 1541 events (exenatide). *n* = 322 (sitagliptin). *n* = 691 (controls) | PC was more common among patients who took sitagliptin (2.7-fold) or exenatide (2.9-fold) as compared with other therapies |
| Montvida *et al*[34], 2019 | Retrospective record-based study. 2005 onwards. Follow-up duration 2.27-4.3 yr | Centricity electronic medical record, United States. DPP-4i *n* = 50095. GLP-1 RA *n* = 12654. SU *n* = 110747. TZD *n* = 17597. Insulin *n* = 34805 | Compared with DPP-4i, the GLP-1 RA group developed PC 3 yr later (95%CI: 0.84-5.16). No other significant differences were observed between groups |
| Nagel *et al* [35], 2016 | Retrospective study (Control drugs-rosiglitazone, glinides, glipizide), 1968-2013 | Database-FDA AERS. Patients of T2DM on sitagliptin, saxagliptin, linagliptin, and alogliptin. *n* = 156 PC patients | EB05 was 10.3 for sitagliptin, 7.1 for saxagliptin, 4.9 for linagliptin, and 1.4 for alogliptin, compared with all other agents |
| Azoulay *et al* [36], 2016 | Nested case control analysis (control drug- sulfonylureas), 2007-2014. Follow-up 1.3-2.8 yr | Database-CNODES (Canada, United States, United Kingdom). *n* = 972384 | Compared with SUs, incretin-based drugs were not associated with an increased risk of PC-pooled aHR 1.02 (95%CI: 0.84-1.23) |
| Tseng *et al* [37], 2017 | Retrospective population-based cohort study, 1997-2010. Follow up-till occurrence of adverse pancreatic event | Database-The Taiwan National Health Insurance Research Database. *n* = 13171 incretin. *n* = 13171 non-incretins | PC occurred in 6 (0.05%) and 10 (0.08%) patients in the incretin and non- incretin cohort, respectively |
| Boniol *et al* [38], 2018 | Retrospective cohort study, 2008-2013. Follow-up 1.8-2.3 yr | Public health insurance databases of Belgium, Lombardy (Italy). *n* = 33292 incretin. *n* = 525733 control | The aHR for PC was 2.14 (95%CI: 1.71–2.67) for incretin group compared with control |

FDA AERS: Food and drug administration adverse event reporting system; T2DM: Type 2 diabetes mellitus; PC: Pancreatic carcinoma; EB05: Empirical Bayesian fifty centile; aHR: Adjusted hazard ratio; CI: Confidence interval; CNODES: Canadian network for observational drug effect studies; SU: Sulfonylurea; OHA: Oral hypoglycemic agent; RR: Risk ratio; TZD: Thiazolidinedione.

**Table 3 Important systematic reviews and meta-analyses which evaluated the relationship between incretin-based therapies and pancreatic cancer**

| Ref. | Description | Findings |
|---|---|---|
| Alves *et al* [39], 2012 | All studies (25 RCT/longitudinal observational) assessing the estimate of pancreatitis/PC in patients with T2DM using exenatide or liraglutide | For PC risk, the OR of exenatide was 0.86 (95%CI: 0.29-2.60) and liraglutide was 1.35 (95%CI: 0.70-2.59) |
| Chen *et al* [40], 2016 | All RCTs reporting PC with use of incretin-based therapies compared with placebo or non-incretin anti-diabetic drugs in patients with T2DM | Overall, no increased risk of PC was detected in association with incretin-based treatment (RR = 0.7, 95%CI: 0.37–1.05). The incidence of PC was even lower among incretin-based groups than controls (RR = 0.50, 95%CI: 0.29–0.87) in trials with duration more than 104 wk |
| Zhang *et al* [41], 2017 | 6 prospective randomized controlled trials (EXAMINE, SAVOR-TIMI 53, TECOS, ELIXA, LEADER and SUSTAIN-6)-3 trials for DPP-4is and 3 trials for GLP-1 RAs | Incretin-based agents did not significantly affect PC-OR: 0.71 (95%CI: 0.45–1.11) |
| Pinto *et al* [42], 2019 | 12 RCTs with GLP-1 RAs as an intervention, from database inception till 2017 | GLP-1 RAs did not increase the risk for pancreatic cancer when compared to other treatments-OR: 1.06 (95%CI: 0.67-1.67) |
| Abd El Aziz *et al* [43], 2020 | Meta-analysis of cases of acute pancreatitis and PC as well as any malignant neoplasm reported in 11 CVOTs with GLP-1 RAs and DPP-4i | Neither GLP-1 RAs nor DPP-4is were associated with a significantly elevated or reduced risk of PC. For GLP-1 RA OR was 1.14 (95%CI: 0.77-1.7) and for DPP4i OR was 0.94 (95%CI: 0.52-1.68) |

PC: Pancreatic cancer; RCT: Randomised controlled trial; T2DM: Type 2 diabetes mellitus; OR: Odds ratio; RR: Risk ratio; CVOT: Cardiovascular outcome trial; GLP-1 RA: Glucagon like peptide-1 receptor agonists; DPP-4i: Dipeptidyl Peptidase-4 inhibitors; OHA: Oral hypoglycemic agent.

peptide based on the native GIP sequence. Tirzepatide demonstrated a more significant reduction in glycosylated hemoglobin, superior weight loss, and comparable adverse effects compared to semaglutide in a phase 3 trial. Pancreatitis was observed in four patients in the tirzepatide arm and three patients in the semaglutide arm during the study duration[44]. Cotadutide is a balanced GLP-1 and glucagon receptor dual agonist. Phase 2 clinical trials for cotadutide were published recently. The efficacy of cotadutide also appears to be better than that of semaglutide, although no head-to-head comparison is available. No case of pancreatitis has been reported in the phase 2a study of cotadutide, although the frequency of nausea and vomiting was increased in the cotadutide arm[45]. We must therefore wait until we have sufficient data on these new drugs. It is premature to make comment on the pancreatic safety of these drugs at present.

## CONCLUSION

We have collected extensive data on the safety of incretin-based therapies over the past two decades. It seems that incretin-based therapies do not increase the risk for PC. Instead, new pre-clinical experimental data have shown beneficial effects on cancer cell lines that require further evaluation. The uncertain risks of PC appear to be smaller compared to the beneficial pleiotropic effects of incretin-based therapies. However, with newer incretin-based therapies, we should keep the theoretical possibility of PC in mind and be cautious until we obtain sufficient data.

## FOOTNOTES

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## REFERENCES

1 **Keating GM**. Exenatide. *Drugs* 2005; **65**: 1681-92; discussion 1693 [PMID: 16060703 DOI: 10.2165/00003495-200565120-00008]
2 **Ahrén B**, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 2078-2084 [PMID: 15126524 DOI: 10.1210/jc.2003-031907]
3 **Butler PC**, Elashoff M, Elashoff R, Gale EA. A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe? *Diabetes Care* 2013; **36**: 2118-2125 [PMID: 23645885 DOI: 10.2337/dc12-2713]
4 **American Diabetes Association**. 9. Pharmacologic Approaches to Glycemic Treatment: *Standards of Medical Care in Diabetes-2021*. *Diabetes Care* 2021; **44**: S111-S124 [PMID: 33298420 DOI: 10.2337/dc21-S009]
5 **Roy A**, Sahoo J, Kamalanathan S, Naik D, Mohan P, Kalayarasan R. Diabetes and pancreatic cancer: Exploring the two-way traffic. *World J Gastroenterol* 2021; **27**: 4939-4962 [PMID: 34497428 DOI: 10.3748/wjg.v27.i30.4939]
6 **Elashoff M**, Matveyenko AV, Gier B, Elashoff R, Butler PC. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology* 2011; **141**: 150-156 [PMID: 21334333 DOI: 10.1053/j.gastro.2011.02.018]
7 **Nauck MA**. The rollercoaster history of using physiological and pharmacological properties of incretin hormones to develop diabetes medications with a convincing benefit-risk relationship. *Metabolism* 2020; **103**: 154031 [PMID: 31785258 DOI: 10.1016/j.metabol.2019.154031]
8 **Méndez-Bailón M**, de Miguel Yanes JM, Jiménez-García R, Hernández-Barrera V, Pérez-Farinós N, López-de-Andrés A. National trends in incidence and outcomes of acute pancreatitis among type 2 diabetics and non-diabetics in Spain (2001-2011). *Pancreatology* 2015; **15**: 64-70 [PMID: 25500341 DOI: 10.1016/j.pan.2014.11.004]
9 **Koo DH**, Han KD, Park CY. The Incremental Risk of Pancreatic Cancer According to Fasting Glucose Levels: Nationwide Population-Based Cohort Study. *J Clin Endocrinol Metab* 2019; **104**: 4594-4599 [PMID: 31498870 DOI: 10.1210/jc.2019-00033]
10 **di Magliano MP**, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 2013; **144**: 1220-1229 [PMID: 23622131 DOI: 10.1053/j.gastro.2013.01.071]
11 **Carrière C**, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. *Biochem Biophys Res Commun* 2009; **382**: 561-565 [PMID: 19292977 DOI: 10.1016/j.bbrc.2009.03.068]
12 **Zambirinis CP**, Pushalkar S, Saxena D, Miller G. Pancreatic cancer, inflammation, and microbiome. *Cancer J* 2014; **20**:

195-202 [PMID: 24855007 DOI: 10.1097/PPO.0000000000000045]
13 **Rouse R**, Zhang L, Shea K, Zhou H, Xu L, Stewart S, Rosenzweig B, Zhang J. Extended exenatide administration enhances lipid metabolism and exacerbates pancreatic injury in mice on a high fat, high carbohydrate diet. *PLoS One* 2014; **9**: e109477 [PMID: 25291183 DOI: 10.1371/journal.pone.0109477]
14 **Bonner-Weir S**, Sullivan BA, Weir GC. Human Islet Morphology Revisited: Human and Rodent Islets Are Not So Different After All. *J Histochem Cytochem* 2015; **63**: 604-612 [PMID: 25604813 DOI: 10.1369/0022155415570969]
15 **Vrang N**, Jelsing J, Simonsen L, Jensen AE, Thorup I, Søeborg H, Knudsen LB. The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis. *Am J Physiol Endocrinol Metab* 2012; **303**: E253-E264 [PMID: 22589391 DOI: 10.1152/ajpendo.00182.2012]
16 **Ilic M**, Ilic I. Epidemiology of pancreatic cancer. *World J Gastroenterol* 2016; **22**: 9694-9705 [PMID: 27956793 DOI: 10.3748/wjg.v22.i44.9694]
17 **Sipos B**, Frank S, Gress T, Hahn S, Klöppel G. Pancreatic intraepithelial neoplasia revisited and updated. *Pancreatology* 2009; **9**: 45-54 [PMID: 19077454 DOI: 10.1159/000178874]
18 **Perfetti R**, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000; **141**: 4600-4605 [PMID: 11108273 DOI: 10.1210/endo.141.12.7806]
19 **Butler AE**, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* 2013; **62**: 2595-2604 [PMID: 23524641 DOI: 10.2337/db12-1686]
20 **Harja E**, Lord J, Skyler JS. An analysis of characteristics of subjects examined for incretin effects on pancreatic pathology. *Diabetes Technol Ther* 2013; **15**: 609-618 [PMID: 23927624 DOI: 10.1089/dia.2013.0177]
21 **Bonner-Weir S**, In't Veld PA, Weir GC. Reanalysis of study of pancreatic effects of incretin therapy: methodological deficiencies. *Diabetes Obes Metab***16**: 661-666 [PMID: 24400596 DOI: 10.1111/dom.12257]
22 **Kahn SE**. Incretin therapy and islet pathology: a time for caution. *Diabetes* 2013; **62**: 2178-2180 [PMID: 23596147 DOI: 10.2337/db13-0520]
23 **Egan AG**, Blind E, Dunder K, de Graeff PA, Hummer BT, Bourcier T, Rosebraugh C. Pancreatic safety of incretin-based drugs--FDA and EMA assessment. *N Engl J Med* 2014; **370**: 794-797 [PMID: 24571751 DOI: 10.1056/NEJMp1314078]
24 **Ueberberg S**, Jütte H, Uhl W, Schmidt W, Nauck M, Montanya E, Tannapfel A, Meier J. Histological changes in endocrine and exocrine pancreatic tissue from patients exposed to incretin-based therapies. *Diabetes Obes Metab* 2016; **18**: 1253-1262 [PMID: 27545110 DOI: 10.1111/dom.12766]
25 **Chadwick KD**, Fletcher AM, Parrula MC, Bonner-Weir S, Mangipudy RS, Janovitz E, Graziano MJ, Roy D, Reilly TP. Occurrence of spontaneous pancreatic lesions in normal and diabetic rats: a potential confounding factor in the nonclinical assessment of GLP-1-based therapies. *Diabetes* 2014; **63**: 1303-1314 [PMID: 24222349 DOI: 10.2337/db13-1268]
26 **Aston-Mourney K**, Subramanian SL, Zraika S, Samarasekera T, Meier DT, Goldstein LC, Hull RL. One year of sitagliptin treatment protects against islet amyloid-associated β-cell loss and does not induce pancreatitis or pancreatic neoplasia in mice. *Am J Physiol Endocrinol Metab* 2013; **305**: E475-E484 [PMID: 23736544 DOI: 10.1152/ajpendo.00025.2013]
27 **Lu R**, Yang J, Wei R, Ke J, Tian Q, Yu F, Liu J, Zhang J, Hong T. Synergistic anti-tumor effects of liraglutide with metformin on pancreatic cancer cells. *PLoS One* 2018; **13**: e0198938 [PMID: 29897998 DOI: 10.1371/journal.pone.0198938]
28 **Yan M**, Shen M, Xu L, Huang J, He G, An M, Li X, Gao Z, Meng X. Inactivation of Pancreatic Stellate Cells by Exendin-4 Inhibits the Migration and Invasion of Pancreatic Cancer Cells. *Onco Targets Ther* 2020; **13**: 9455-9463 [PMID: 33061431 DOI: 10.2147/OTT.S259853]
29 **Zhao H**, Jiang X, Duan L, Yang L, Wang W, Ren Z. Liraglutide suppresses the metastasis of PANC-1 co-cultured with pancreatic stellate cells through modulating intracellular calcium content. *Endocr J* 2019; **66**: 1053-1062 [PMID: 31474673 DOI: 10.1507/endocrj.EJ19-0215]
30 **Zhao HJ**, Jiang X, Hu LJ, Yang L, Deng LD, Wang YP, Ren ZP. Activation of GLP-1 receptor enhances the chemosensitivity of pancreatic cancer cells. *J Mol Endocrinol* 2020; **64**: 103-113 [PMID: 31855560 DOI: 10.1530/JME-19-0186]
31 **Kawakita E**, Koya D, Kanasaki K. CD26/DPP-4: Type 2 Diabetes Drug Target with Potential Influence on Cancer Biology. *Cancers (Basel)* 2021; **13** [PMID: 34063285 DOI: 10.3390/cancers13092191]
32 **Hollande C**, Boussier J, Ziai J, Nozawa T, Bondet V, Phung W, Lu B, Duffy D, Paradis V, Mallet V, Eberl G, Sandoval W, Schartner JM, Pol S, Barreira da Silva R, Albert ML. Inhibition of the dipeptidyl peptidase DPP4 (CD26) reveals IL-33-dependent eosinophil-mediated control of tumor growth. *Nat Immunol* 2019; **20**: 257-264 [PMID: 30778250 DOI: 10.1038/s41590-019-0321-5]
33 **Barreira da Silva R**, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. *Nat Immunol* 2015; **16**: 850-858 [PMID: 26075911 DOI: 10.1038/ni.3201]
34 **Montvida O**, Green JB, Atherton J, Paul SK. Treatment with incretins does not increase the risk of pancreatic diseases compared to older anti-hyperglycaemic drugs, when added to metformin: real world evidence in people with Type 2 diabetes. *Diabet Med* 2019; **36**: 491-498 [PMID: 30306620 DOI: 10.1111/dme.13835]
35 **Nagel AK**, Ahmed-Sarwar N, Werner PM, Cipriano GC, Van Manen RP, Brown JE. Dipeptidyl Peptidase-4 Inhibitor-Associated Pancreatic Carcinoma: A Review of the FAERS Database. *Ann Pharmacother* 2016; **50**: 27-31 [PMID: 26497885 DOI: 10.1177/1060028015610123]
36 **Azoulay L**, Filion KB, Platt RW, Dahl M, Dormuth CR, Clemens KK, Durand M, Juurlink DN, Targownik LE, Turin TC, Paterson JM, Ernst P; Canadian Network for Observational Drug Effect Studies Investigators. Incretin based drugs and the risk of pancreatic cancer: international multicentre cohort study. *BMJ* 2016; **352**: i581 [PMID: 26888382 DOI: 10.1136/bmj.i581]
37 **Tseng CM**, Liao WC, Chang CY, Lee CT, Tseng CH, Hsu YC, Lin JT. Incretin-based pharmacotherapy and risk of adverse

pancreatic events in the ethnic Chinese with diabetes mellitus: A population-based study in Taiwan. *Pancreatology* 2017; **17**: 76-82 [PMID: 27743712 DOI: 10.1016/j.pan.2016.10.003]

38 **Boniol M**, Franchi M, Bota M, Leclercq A, Guillaume J, van Damme N, Corrao G, Autier P, Boyle P. Incretin-Based Therapies and the Short-term Risk of Pancreatic Cancer: Results From Two Retrospective Cohort Studies. *Diabetes Care* 2018; **41**: 286-292 [PMID: 29146599 DOI: 10.2337/dc17-0280]

39 **Alves C**, Batel-Marques F, Macedo AF. A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. *Diabetes Res Clin Pract* 2012; **98**: 271-284 [PMID: 23010561 DOI: 10.1016/j.diabres.2012.09.008]

40 **Chen H**, Zhou X, Chen T, Liu B, Jin W, Gu H, Hong T, Zhang G. Incretin-Based Therapy and Risk of Pancreatic Cancer in Patients with Type 2 Diabetes Mellitus: A Meta-analysis of Randomized Controlled Trials. *Diabetes Ther* 2016; **7**: 725-742 [PMID: 27655330 DOI: 10.1007/s13300-016-0198-3]

41 **Zhang Z**, Chen X, Lu P, Zhang J, Xu Y, He W, Li M, Zhang S, Jia J, Shao S, Xie J, Yang Y, Yu X. Incretin-based agents in type 2 diabetic patients at cardiovascular risk: compare the effect of GLP-1 agonists and DPP-4 inhibitors on cardiovascular and pancreatic outcomes. *Cardiovasc Diabetol* 2017; **16**: 31 [PMID: 28249585 DOI: 10.1186/s12933-017-0512-z]

42 **Pinto LC**, Falcetta MR, Rados DV, Leitão CB, Gross JL. Glucagon-like peptide-1 receptor agonists and pancreatic cancer: a meta-analysis with trial sequential analysis. *Sci Rep* 2019; **9**: 2375 [PMID: 30787365 DOI: 10.1038/s41598-019-38956-2]

43 **Abd El Aziz M**, Cahyadi O, Meier JJ, Schmidt WE, Nauck MA. Incretin-based glucose-lowering medications and the risk of acute pancreatitis and malignancies: a meta-analysis based on cardiovascular outcomes trials. *Diabetes Obes Metab* 2020; **22**: 699-704 [PMID: 31750601 DOI: 10.1111/dom.13924]

44 **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]

45 **Parker VER**, Robertson D, Wang T, Hornigold DC, Petrone M, Cooper AT, Posch MG, Heise T, Plum-Moerschel L, Schlichthaar H, Klaus B, Ambery PD, Meier JJ, Hirshberg B. Efficacy, Safety, and Mechanistic Insights of Cotadutide, a Dual Receptor Glucagon-Like Peptide-1 and Glucagon Agonist. *J Clin Endocrinol Metab* 2020; **105** [PMID: 31608926 DOI: 10.1210/clinem/dgz047]

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MINIREVIEWS

# Non-alcoholic fatty liver disease and the impact of genetic, epigenetic and environmental factors in the offspring

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## Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide and is strongly associated with metabolic deregulation. More recently, a significant impact of parental NAFLD in the offspring was demonstrated and has been widely discussed. However, pathogenetic pathways implicated in the inheritance by the offspring and relatives are still under debate. Probably, multiple mechanisms are involved as well as in NAFLD pathogenesis itself. Among the multifactorial involved mechanisms, genetic, epigenetic and environmental backgrounds are strongly related to NAFLD development in the offspring. Thus, based on recent evidence from the available literature concerning genetic, epigenetic and environmental disease modifiers, this review aimed to discuss the relationship between parental NAFLD and its impact on the offspring.

**Key Words:** Steatosis; Genetic; Epigenetic; Environmental; Offspring; Non-alcoholic fatty liver disease

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**Core Tip:** Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disease and familial clustering has been described, although there is still some debate about this association. Among the factors that contribute to the disease in the offspring of NAFLD patients, genetic, epigenetic and environmental factors are the most plausible ones. In this review we describe the main genetic, environmental and epigenetic factors linked to NAFLD and the studies investigating the relation of NAFLD in parents and its offspring. Although there are many experimental studies in animals, there is still much to be elucidated regarding studies and interventions in human beings.

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## INTRODUCTION

Although non-alcoholic fatty liver disease (NAFLD) is being replaced by metabolic dysfunction-associated fatty liver disease[1], studies concerning genetic and epigenetic factors in this new scenario are still scarce. This way, we will still adopt the nomenclature NAFLD when discussing the studies in this review.

NAFLD affects about 25% to 45% of the world's western population[2]. The spectrum of the disease includes simple steatosis, steatohepatitis with or without fibrosis, leading to cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC)[3]. NAFLD is currently the third indication for liver transplantation worldwide, and it will potentially be the leading indication in 2030[4].

Many cofactors have been recognized and related to NAFLD's high prevalence and severity. Metabolic syndrome, obesity and type 2 diabetes mellitus (T2DM) are the most relevant factors associated with progression from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) and fibrosis. Patients with T2DM have a higher prevalence of NAFLD, with a high prevalence of NASH and advanced fibrosis[5]. In a bidirectional relation, NAFLD also increases up to 5.5 times the risk of future development of T2DM and could be considered an early predictor of the disease[6]. Ethnicity also influences NAFLD prevalence, with Hispanics presenting a higher prevalence than Caucasians and African Americans, independently of metabolic factors. The genetic and environmental basis could be responsible for these findings in diverse ethnic groups[7]. Accordingly, the observation of NASH and cirrhosis familial clusters suggests a substantial hereditary influence on NAFLD progression [8]. Data from diverse epidemiological, familial aggregation and twin-cohorts studies, with a well-designed methodology, suggest that hepatic steatosis is highly heritable[9-12]. Some of these studies used magnetic resonance elastography to assess liver fibrosis or serum aminotransferase levels to infer hepatic steatosis. They demonstrated a high prevalence of NAFLD in family members of children with NAFLD, monozygotic and dizygotic twins, and first-degree family members of T2DM patients[11,13]. So far, the risk of hepatic steatosis and more severe disease in family members and children of patients with NAFLD is not fully understood, as well as the pathogenetic pathways involved in this process.

Genome-wide association studies have demonstrated the association of single nucleotide polymorphisms (SNP) with NAFLD. Patatin-like phospholipase-domain-containing 3 (rs738409 C>G encoding for PNPLA3 I148M), also known as adiponutrin gene, is located at chromosome 22 and was the first SNP described[14]. Although this is the most robust variant linked to NAFLD, additional genetic variants have been identified subsequently: Transmembrane 6 superfamily member 2 (TM6SF2) [15], glucokinase regulator (GCKR)[16], membrane-bound O-acyltransferase domain-containing 7 (MBOAT7)[17] and hydroxysteroid 17 β-dehydrogenase (HSD17B13)[18], among others. These variants have been associated with multiple pleiotropic effects, including a protective effect for NAFLD as seems to occur with the HSD17B13 polymorphism[18]. The different phenotypes resulting from these genes might partially explain the heritable component and metabolic profile of NAFLD patients and their offspring[9].

Although our understanding of genetic influence has exponentially increased in the past few years, it cannot thoroughly explain the high prevalence of NAFLD in family members of patients with the disease. Experimental studies have investigated different pathways related to NAFLD development in the offspring[9,19,20]. In this context, environmental and epigenetic mechanisms play an essential role in the occurrence and progression of NAFLD. Epigenetic factors involve mechanisms that affect and regulate gene expression without changes in DNA sequences[21]. Therefore, gene expression and cell phenotype related to NAFLD might depend on the genetic information encoded by DNA sequences and epigenetic factors[22]. Figure 1 shows the multifactorial mechanisms implicated in the offspring's NAFLD development. This review aims to discuss the impact of genetic, epigenetic and environment-related variables associated with NAFLD in the offspring.

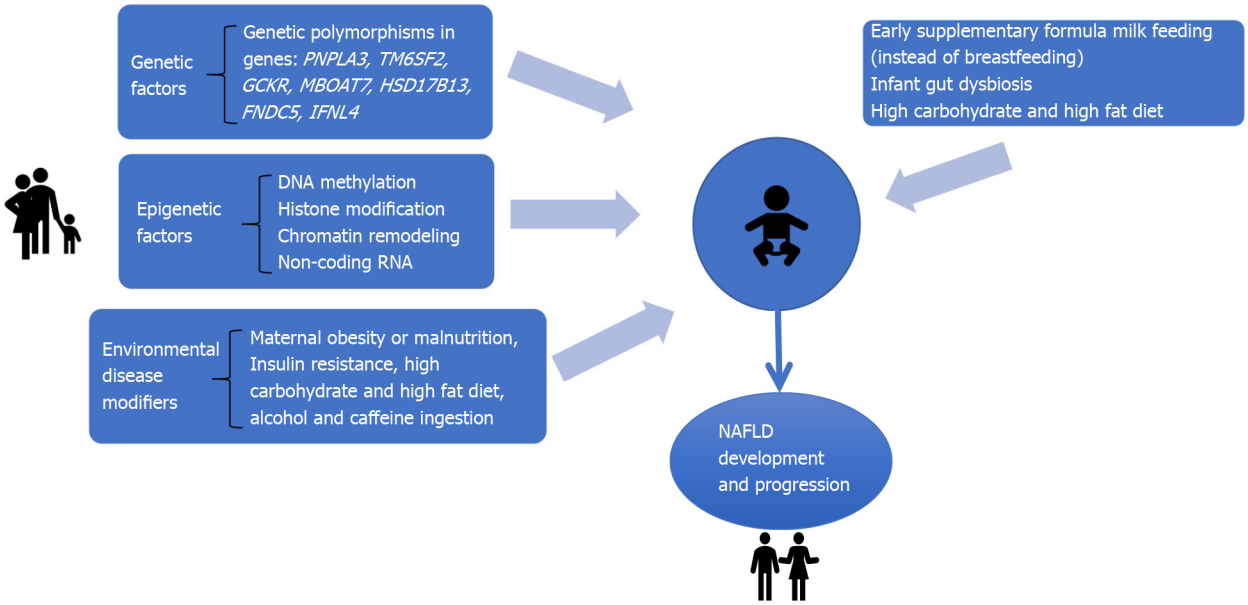


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**Figure 1 The interplay among genetic, epigenetic and environmental factors in pre and postnatal periods that impact the development of non-alcoholic fatty liver disease in the offspring.** NAFLD: Non-alcoholic fatty liver disease.

## HERITAGE AND GENETIC FACTORS

### *Studies in familial clusters and twin cohorts*

Several studies have shown a solid familial clustering of NAFLD, particularly in coexisting metabolic traits[23-25]. Familial combined hyperlipidemia is the most frequent genetic dyslipidemia with a high risk of premature atherothrombotic cardiovascular disease. To assess whether liver steatosis is involved in the pathogenetic pathway of familial combined hyperlipidemia, Brouwers *et al*[23] studied family members with steatosis and twenty spouses. Fatty liver diagnosed by ultrasound was significantly more prevalent in familial combined hyperlipidemia probands (40%) and relatives (35%) compared with their spouses[23]. Moreover, the authors evaluated the correlations between indicators of fatty liver with plasma lipid levels. Liver steatosis and alanine aminotransferase levels correlated with triglyceride levels in all familial combined hyperlipidemia family members[23].

In the multigenerational Framingham Heart Study, a community-based study, individuals with at least one parent presenting hepatic steatosis had two-fold increased odds of having liver steatosis themselves than those without a parental history. More participants without metabolic diseases had liver steatosis if they had at least one parent with liver steatosis than those without any parent with steatosis. On the other hand, there was no difference in the prevalence of steatosis in patients with high cardiometabolic risk among participants with or without a parental history of liver steatosis. Based on these findings, this study suggested that a family history of liver steatosis was a significant risk factor for liver steatosis, but only in metabolically healthy participants[26]. This study goes against the previous one, which showed a higher prevalence of steatosis in those with familial hyperlipidemia. There was no investigation if the genetic aspects of those patients with familial hyperlipidemia could have influenced the higher prevalence of steatosis.

Schwimmer *et al*[11] evaluated 33 overweight children with biopsy-proven NAFLD and 11 overweight children without; NAFLD was significantly more observed in siblings and parents of the NAFLD children group. The correlation of liver fat fraction to body mass index (BMI) was more substantial in overweight children with NAFLD than without NAFLD, showing that there is likely an interaction between BMI and genetic factors on steatosis severity in families of children with NAFLD [11].

Similar to steatosis, hepatic fibrosis in NAFLD is also a heritable trait. Familial aggregation studies revealed a marked coexistence of advanced fibrosis or NAFLD cirrhosis among index patients and their first-degree relatives[24,25]. A cross-sectional analysis demonstrated that first-degree relatives of probands with NAFLD cirrhosis present a 12 times higher risk of advanced fibrosis compared with the relatives of non-NAFLD controls[25]. Interestingly, in another recent cross-sectional study of a prospective cohort comprising 156 twins and their families, the same authors identified a metabolite (3-4-hydroxyphenyl lactate) related to the abundance of several gut microbiota species in individuals with advanced fibrosis. Then, in their conclusions, they propose a link between genetics and microbiota composition concerning NAFLD heritability[27].

The potential genetic link of NAFLD regarding steatosis and fibrosis inheritance triggered the development of studies in twins to evaluate if both steatosis and fibrosis had a significant shared gene. The first study on twins regarding NAFLD inheritance included 60 monozygotic and dizygotic twins [13]. Both liver steatosis and fibrosis were non-invasively quantified by magnetic resonance imaging. The presence of hepatic steatosis by proton-density fat fraction magnetic resonance imaging (MRI) and fibrosis by magnetic resonance elastography correlated between monozygotic twins but not between dizygotic twins, providing evidence that both hepatic steatosis and fibrosis might be heritable traits as well[13].

Following the same rationale, Cui *et al*[10] investigated a prospective cohort of community-dwelling monozygotic and dizygotic twin pairs living in Southern California, using non-invasive proton-density fat fraction MRI and magnetic resonance elastography to assess steatosis and fibrosis. They investigated if individuals prone to genetic susceptibilities to steatosis and fibrosis also had genetic susceptibilities to metabolic variables such as arterial hypertension, dyslipidemia, insulin resistance and diabetes mellitus. The authors have shown that hepatic steatosis and fibrosis have statistically and clinically significant shared genetic determination and metabolic traits such as high-density lipoprotein, triglycerides, insulin resistance, and glycosylated hemoglobin[10]. In another study, the same cohort of twins was evaluated regarding the metabolites of the gut microbiome and its effect on steatosis and liver fibrosis compared to a biopsy-proven NAFLD cohort. This proof of concept study provided a link between the gut-microbiome and 3-lactate that shared gene-effect with hepatic steatosis and fibrosis[27]. Hence, the heritage of NAFLD might relate to multiple factors like a genetic inheritance that could directly affect steatosis and fibrosis and heritable traits of the gut microbiome inherited, or even be influenced by a shared lifestyle in the probands and its parents.

### *Genetic polymorphisms*

Genetic polymorphisms are involved in NAFLD expression regarding its relation with liver steatosis, advanced stages of fibrosis, and even a possible protective effect for disease progression[9,18,28]. However, studies evaluating their impact on the offspring of patients with NAFLD are scarce.

As previously described, PNPLA3 rs738409 C>G variant is associated with hepatic steatosis and severity of NAFLD, progression to cirrhosis and HCC, resulting in a worse prognosis[14]. PNPLA3 encodes a triacylglycerol lipase, and this variant promotes hepatic triglyceride accumulation by restricting substrate access to the catalytic dyad, thus inhibiting triglyceride hydrolysis in the cell[14].

TM6SF2 function is related to regulating cholesterol synthesis and secretion of lipoproteins. Individuals who carry the SNP rs58542926 C>T, which encodes the E167K amino acidic substitution, have a higher risk of NAFLD and histological disease severity. However, there is still a strong debate if it has a protective effect on coronary artery disease. A large study with 60801 patients with coronary artery disease compared to 123504 healthy individuals described a protective effect of the T variant of TM6SF2 on this disease and found an equivalent, although modest effect for the G variant of PNPLA3, that was more intense in the recessive model (genotype GG). At last, an exome study including more than 300000 individuals showed that both TM6SF2 and PNPLA3 polymorphisms induce a protective effect on coronary artery disease and an increased risk of NAFLD[29]. So far, there is no study regarding the evaluation of the impact of TM6SF2 in the offspring of NAFLD patients.

In young adolescents, the rs1260326 C>T variant in GCKR was significantly associated with de novo lipogenesis in those with TT genotype. Another variant in GCKR, the rs780094 A>G, was also associated with NAFLD in a meta-analysis involving 2091 cases and 3003 controls[30].

MBOAT7 was first studied in alcohol abusers and was related to a higher risk of cirrhosis. It encodes a protein involved in the re-acylation of phospholipids as a component of the phospholipid-remodeling pathway, known as the land cycle. Subsequently, the rs641738 C>T variant in this gene was associated with increased hepatic fat, more severe liver damage and fibrosis in NAFLD individuals of European descent; moreover, it has been demonstrated that the T allele may predispose to HCC in patients without cirrhosis[31].

Recently, three polymorphisms have been identified as protective against advanced stages of NAFLD. Results from exome-sequence data from 46455 individuals have shown an association of rs72613567:TA in HSD17B13, a variant with an adenine insertion, with lower levels of aminotransferases and reduced risk of chronic liver disease, including NASH[18]. Pirola *et al*[32] demonstrated the effect of this variant on a Hispanic population submitted to liver biopsy, investigating its association with histological parameters of NAFLD. They identified a lower risk of ballooning degeneration, lobular inflammation and liver fibrosis, mediated by reduced enzyme activity in converting retinol to retinoic acid, suggesting a protective effect in inflammation and fibrosis[32]. Di Sessa *et al*[33] evaluated 685 obese children (mean age 10.56 ± 2.94 years) and demonstrated that carriers of the HSD17B13 A allele had a lower percentage of liver steatosis on ultrasound imaging and lower serum aminotransferases levels[33].

Petta *et al*[34] evaluated the role of irisin, a myokine encoded by the fibronectin type III domain-containing protein 5 gene (FNDC5), in NAFLD patients. The variant rs3480 A>G was not associated with the severity of steatosis and NASH but was correlated with a lower prevalence of clinically significant fibrosis (F2-F4), showing a protective effect against fibrosis. They also found that irisin is expressed in human activated hepatic stellate cells, promoting profibrogenic actions and collagen

synthesis. Thus, the FNDC5 genotype might affect hepatic fibrogenesis by modulating irisin secretion [34].

The genetic polymorphisms associated with NAFLD, their functions and effects are summarized in Table 1.

Concerning NAFLD and family inheritance, the PNPLA3 polymorphism was the only one studied. Overweight and obese children with NAFLD confirmed by histology were evaluated regarding the role of lifetime exposures in association with a genetic predisposition, parental obesity, economic income, programming during fetal life, being breastfed or not, and later biomarkers of dietary habits and lifestyle, correlating with fibrosis. In this study, 75% of the children had fibrosis, independently associated with PNPLA3-GG genotype, parental obesity, not being breastfed, vitamin D levels (< 20 mg/dL) and fructose consumption. Notably, a high socioeconomic maternal occupation was related to less severe fibrosis[35]. These findings reinforce the multifactorial impact of NAFLD inheritance. Recently, Jain *et al*[36] studied 51 patients with NAFLD and their parents compared to 50 individuals without NAFLD and their parents as a control group. They observed that parents of the NAFLD group had a higher frequency of GG genotype when compared to parents of those without NAFLD (15% *vs* 5%)[36]. In this study, no other factors except for PNPLA3 polymorphism were evaluated.

## ENVIRONMENTAL AND EPIGENETIC FACTORS: EVIDENCE IN EXPERIMENTAL AND CLINICAL STUDIES

In addition to the genetic information encoded by DNA sequences, epigenetic modifications increase or inhibit the expression of specific genes and affect chromatin structure without modifying nucleotide sequence. Epigenetics implies inheritable changes in the expression of genes, but they can also be acquired and may occur in response to environmental factors, such as nutrition, contributing to disease risk and severity[37]. These alterations can be transferred to the next generation and, in this way, may modify metabolic and NAFLD risk in the offspring. As epigenetic changes can be inheritable and modulated by environmental stimuli, they are considered reversible and could offer new individualized prevention and therapy[37]. So far, the impact of maternal and/or paternal risk factors on the clinical phenotypes of the offspring and the underlying epigenetic mechanisms has not been fully elucidated [37].

Epigenetic phenomena include four regulatory mechanisms: Modification in DNA methylation, covalent histone modification, chromatin remodeling, and RNA-based mechanisms, such as non-coding RNA. DNA methylation is the most studied[22,38].

### *Experimental studies*

Some experimental studies, most of them in mice, tried to elucidate the mechanisms involved in the inheritance of NAFLD and the external factors that could modulate NAFLD development in the offspring through epigenetic factors. It has been shown that many factors during pregnancy may activate lipogenic and inflammatory pathways leading to NAFLD in the progeny[19]. Many authors have studied the impact of breastfeeding, maternal obesity and diet before or during pregnancy in animal models.

Oben *et al*[19] demonstrated that maternal obesity before and throughout pregnancy and lactation could be linked to dysmetabolism in the offspring of female mice. Offspring of obese dams showed a dysmetabolic pattern related to insulin resistance and NAFLD phenotype. Moreover, the offspring of lean dams fed by obese dams presented increased body weight and higher insulin levels and cytokines such as leptin, interleukin-6 and tumor necrosis factor-alpha. Raised levels of leptin were also observed in the breast milk of obese mice compared to lean ones. They proposed that a modified pathway over hypothalamic appetite nuclei signaling by maternal breast milk and neonatal adipose tissue-derived leptin in the early postnatal period was the mechanism behind these findings.

Considering the hypothesis that diet during and after pregnancy might also be involved in NAFLD in the post-weaning period, Pruis *et al*[39] observed that a maternal western-type diet during pregnancy could stimulate metabolic programming or phenotype induction, leading to NAFLD development.

Another study[40] suggested that modifying the diet during pregnancy could benefit the offspring by preventing a disrupted liver lipid profile[61]. When pregnant mice were fed either with a high fat-slow digestive diet or a rapid digestive diet, the offspring of the high fat-rapid digestive diet showed an abnormal liver lipid profile. However, it was not observed in their counterparts born from high fat-slow digestive diet fed-mice.

The relationship between obesity in pregnancy and circadian cycle deregulation might affect metabolic pathways related to NAFLD in adults. Mouralidarane *et al*[20] suggested that, in addition to an obesogenic post-weaning diet, obesity in the mother might lead to NAFLD by disrupting the liver's canonical metabolic rhythmicity gene expression. It implicates the role of abnormal circadian rhythm in the genesis of NAFLD, and alterations in this system during critical developmental periods might be responsible for the onset of the disease later in adulthood.

**Table 1 Genetic polymorphisms involved in non-alcoholic fatty liver disease occurrence and related phenotypes**

| Gene | Variant (s) | Function | Phenotype |
|---|---|---|---|
| *PNPLA3* | rs738409 C>G | Triglyceride hydrolysis | ↑ NAFLD, NASH, fibrosis, HCC |
| *TM6SF2* | rs58542926 C>T | Lipoproteins secretion | ↑ NAFLD, NASH, fibrosis |
| *GCKR* | rs1260326 C>Trs780094 A>G | De novo lipogenesis regulation | ↑ NAFLD, NASH, fibrosis |
| *MBOAT7* | rs641738 C>T | Phospholipid metabolism | ↑ NAFLD and fibrosis |
| *HSD17B13* | rs72613567:TA | Conversion of retinol to retinoic acid | ↓ NASH and fibrosis |
| *FNDC5* | rs3480 A>G | Hepatic fibrogenesis | ↓ fibrosis |

rs: Reference SNP; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma.

Another issue that might be considered regarding further development of NAFLD after birth is ethanol exposure[41]. Shen *et al*[41] developed a rat model of intrauterine growth retardation by prenatal ethanol exposure. These models were fed with normal and high-fat diets. Enhanced liver expression of the insulin growth factor-1 pathway, gluconeogenesis, lipid synthesis and diminished expression of lipid output were accompanied in prenatal ethanol exposure female offspring fed with a high-fat diet.

Oliveira *et al*[42] studied Wistar rats fed with a standard diet and a high-fat diet. Rats born from mice fed with a standard diet were not affected by changes in liver morphology, as did the offspring of high-fat-fed rats. Therefore, the study concluded that fructose intake during adolescence hastens NAFLD onset and reveals a differentiated hepatic response to metabolic insult, depending on the maternal diet. Notwithstanding, Nicolás-Toledo *et al*[43] showed that sucrose intake in adulthood increases fat content only in female rat offspring of dams fed with a low-protein diet during pregnancy, reinforcing the influence of maternal diet in the offspring[43]. Of note, regarding specific epigenetic mechanisms, Suter *et al*[44] have described that epigenetic changes to histones may act as a molecular memory of intrauterine exposure, rendering the risk of adult disease. The genome-wide epigenetic modifications in the fetal liver of susceptible offspring were analyzed, concluding that a maternal high-fat diet is associated with functional alterations to fetal hepatic histones, some of which may persist up to five weeks of age[44].

Another study by Wei *et al*[45] connected NAFLD with epigenetic methylation of specific genes in fathers that can be transmitted from gametes to embryos across generations. They have shown that even paternal diet patterns and prediabetes increase the risk of diabetes in the offspring through gametic epigenetic alterations such as different methylation of genes in the sperm of prediabetic fathers.

All these experimental studies in animal models have revealed that maternal obesity and parental diet during pregnancy or lactation may significantly influence NAFLD and lipid dysmetabolism in the offspring, either by environmental factors or through epigenetic factors, some yet to be better specified, mainly concerning environmental factors. Hence, cofactors as alcohol and fructose intake, among others not yet identified, may activate lipogenic and inflammatory pathways that can lead to NAFLD in the offspring.

### *Clinical studies*

**Studies in mothers and newborns:** Animal studies confirmed that disruptions during early development could lead to increased susceptibility to metabolic dysfunctions later in life. Likewise, human data support that metabolic dysfunction and its contribution to NAFLD can be closely related to genetic and environmental predisposing factors. However, the precise mechanisms that link changes in pre and postnatal environments with NAFLD development risk in adolescence and adulthood remain poorly understood. These mechanisms involve shifts in lipid metabolism, mitochondrial dysfunction, altered gut microbiota, macrophage programming and activation of epigenetic changes.

In prior studies, it was demonstrated that low birth weight babies exhibit an altered postnatal metabolism after developing an adaptative response to a suboptimal fetal environment[46,47]. Although the mechanism is not entirely understood, exposure to excessive and deficient nutrition during the prenatal period may induce a nutritional mismatch between metabolic efficiency and energy expenditure, increasing the risk of future cardiometabolic diseases. If confirmed, an early and straightforward nutritional intervention might prevent the further development of metabolic diseases in adulthood.

Modi *et al*[48] evaluated 105 healthy mother-neonate pairs. They measured neonatal adipose tissue content by whole-body MRI and intrahepatic lipid content by a proton magnetic resonance spectroscopy. They have demonstrated that infant adiposity, particularly abdominal adipose tissue and intra hepatocellular lipid correlated with increased maternal BMI. Recently, Bedogni *et al*[49] studied the prevalence and risk factors associated with bright liver in 391 1-year-old toddlers born from healthy

mothers. The PNPLA3 I148M variant and maternal weight gain during pregnancy were related to the presence of bright liver in the ultrasonography[49]. Thus, interestingly, the authors suggested a potential gene-environment interchange between PNPLA3 and maternal environmental factors contributing to the risk of fatty liver disease at this earlier age, reinforcing the multifactorial inheritance of NAFLD.

In a large study, Ayonrinde *et al*[50] investigated the relation of maternal factors and infant nutrition with the future development of NAFLD in adolescents aged 17 years. They concluded that average pre-gestational BMI, breastfeeding for at least six months and avoiding early supplementary formula milk feeding reduce the risk of NAFLD diagnosis by liver ultrasound[50]. Additionally, more extended maintenance of breastfeeding resulted in multiple benefits on maternal metabolism and a lower risk of NAFLD in mid-life[51-53].

The Healthy Start study examined a cohort of 951 mothers from different ethnicities[54]. Similar to others, they found that maternal BMI was correlated to increased neonatal adiposity. It has also been demonstrated that increased maternal insulin resistance and fasting glucose levels contribute to this association. Excessive insulin resistance during pregnancy activates placental inflammatory pathways and affects the fetus indirectly by increasing placental nutrient transfer capacity[55].

Still regarding insulin-glucose metabolism, elevated blood glucose and insulin concentrations exacerbate de novo lipogenesis, resulting in increased intrahepatic lipids. Additionally, reduced glucose and pyruvate consumption in parallel with increased triglyceride concentrations and excess fatty acids incompletely oxidized can impair mitochondrial function and gene expression, limiting mitochondrial biogenesis and leading to NAFLD[55].

Peroxisome proliferator-activated receptor γcoactivator 1 (PGC1) gene is a transcriptional coactivator that participates in mitochondrial biogenesis and function and hypermethylation PGC1 promoter was associated with decreased mitochondrial DNA content and insulin resistance in NAFLD patients[56]. In a cross-sectional analysis, Gemma *et al*[57] noticed a positive correlation between maternal BMI and methylation of the PGC1 gene in the umbilical cord of their babies[57]. Based on their findings, the authors speculated that PGC1 might be a promising candidate gene involved in metabolic programming by epigenetic regulation[57]. DNA methylation in regulatory regions of different genes participates in NAFLD development and progression. Other epigenetic mechanisms affecting NAFLD pathogenesis include histone modification and microRNA (miRNA)-mediated processes. Notably, circulating miRNAs have been associated with the presence and heritability of NAFLD in a population study in 40 pairs of twins. Serum miR-331-3p and miR-30c were identified among the 21 miRs that differed between NAFLD and non-NAFLD individuals. These miRNAs are highly inheritable and correlate with each other suggesting a common pathway related to NAFLD[58].

Although shreds of evidence support that high pre-pregnancy BMI in the mothers may lead to significant modifications in the infant gut microbiome[59], few studies link maternal obesity and infant dysbiosis with NAFLD risk in later life. The neonatal gut microbiome can be essential for later homeostasis, and disruption of this early process may increase the risk of future metabolic diseases[60]. Emerging data provides evidence that the gut-liver axis is a fundamental element in the onset and progression of NAFLD. Gut microbiota dysbiosis may contribute to NAFLD by increasing concentrations of bacteria-derived endotoxins, pro-inflammatory cytokines, amino-acid metabolites, short-chain fatty acids and bile acids, all of which might exert effects that promote macrophage programming and activation, favoring liver injury[61].

## CONCLUSION

The interplay among multiple genetic, epigenetic and environmental factors determine an individual's susceptibility to NAFLD. Current evidence points to genetic polymorphisms as pleiotropic tools that lead to diverse traits and phenotypes, including typical metabolic profiles in parents and their offspring. Importantly, epigenetic markers can also be transferred to successors by transgenerational epigenetic inheritance. Current studies in mothers and their offspring, although still small, show a direct effect of these factors and their related outcome, NAFLD. Future studies may clarify what interventions are essential for preventing this complex disease in the perinatal or postnatal period to reach the better liver and metabolic-related outcomes in the upcoming adult population.

## FOOTNOTES

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## REFERENCES

1 **Kawaguchi T**, Tsutsumi T, Nakano D, Torimura T. MAFLD: Renovation of clinical practice and disease awareness of fatty liver. *Hepatol Res* 2021 [PMID: 34472683 DOI: 10.1111/hepr.13706]
2 **Bellentani S**, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; **8** Suppl 1: S4-S8 [PMID: 19381118]
3 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152]
4 **Shaker M**, Tabbaa A, Albeldawi M, Alkhouri N. Liver transplantation for nonalcoholic fatty liver disease: new challenges and new opportunities. *World J Gastroenterol* 2014; **20**: 5320-5330 [PMID: 24833862 DOI: 10.3748/wjg.v20.i18.5320]
5 **Leite NC**, Villela-Nogueira CA, Pannain VL, Bottino AC, Rezende GF, Cardoso CR, Salles GF. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. *Liver Int* 2011; **31**: 700-706 [PMID: 21457442 DOI: 10.1111/j.1478-3231.2011.02482.x]
6 **Shibata M**, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. *Diabetes Care* 2007; **30**: 2940-2944 [PMID: 17666460 DOI: 10.2337/dc07-0792]
7 **Wagenknecht LE**, Scherzinger AL, Stamm ER, Hanley AJ, Norris JM, Chen YD, Bryer-Ash M, Haffner SM, Rotter JI. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. *Obesity (Silver Spring)* 2009; **17**: 1240-1246 [PMID: 19584882 DOI: 10.1038/oby.2009.4]
8 **Adibi A**, Janghorbani M, Shayganfar S, Amini M. First-degree relatives of patients with type 2 diabetes mellitus and risk of non-alcoholic Fatty liver disease. *Rev Diabet Stud* 2007; **4**: 236-241 [PMID: 18338077 DOI: 10.1900/RDS.2007.4.236]
9 **Eslam M**, George J. Genetic contributions to NAFLD: leveraging shared genetics to uncover systems biology. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 40-52 [PMID: 31641249 DOI: 10.1038/s41575-019-0212-0]
10 **Cui J**, Chen CH, Lo MT, Schork N, Bettencourt R, Gonzalez MP, Bhatt A, Hooker J, Shaffer K, Nelson KE, Long MT, Brenner DA, Sirlin CB, Loomba R; For The Genetics Of Nafld In Twins Consortium. Shared genetic effects between hepatic steatosis and fibrosis: A prospective twin study. *Hepatology* 2016; **64**: 1547-1558 [PMID: 27315352 DOI: 10.1002/hep.28674]
11 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592 [PMID: 19208353 DOI: 10.1053/j.gastro.2009.01.050]
12 **Dickson I**. NAFLD: Increased familial risk of fibrosis in NAFLD. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 450 [PMID: 28676712 DOI: 10.1038/nrgastro.2017.95]
13 **Loomba R**, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, Nguyen P, Hernandez C, Richards L, Salotti J, Lin S, Seki E, Nelson KE, Sirlin CB, Brenner D; Genetics of NAFLD in Twins Consortium. Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study. *Gastroenterology* 2015; **149**: 1784-1793 [PMID: 26299412 DOI: 10.1053/j.gastro.2015.08.011]
14 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
15 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]
16 **Yang J**, Trépo E, Nahon P, Cao Q, Moreno C, Letouzé E, Imbeaud S, Bayard Q, Gustot T, Deviere J, Bioulac-Sage P, Calderaro J, Ganne-Carrié N, Laurent A, Blanc JF, Guyot E, Sutton A, Ziol M, Zucman-Rossi J, Nault JC. A 17-Beta-Hydroxysteroid Dehydrogenase 13 Variant Protects From Hepatocellular Carcinoma Development in Alcoholic Liver Disease. *Hepatology* 2019; **70**: 231-240 [PMID: 30908678 DOI: 10.1002/hep.30623]
17 **Basyte-Bacevice V**, Skieceviciene J, Valantiene I, Sumskiene J, Petrenkiene V, Kondrackiene J, Petrauskas D, Lammert F, Kupcinskas J. *TM6SF2* and *MBOAT7* Gene Variants in Liver Fibrosis and Cirrhosis. *Int J Mol Sci* 2019; **20** [PMID: 30875804 DOI: 10.3390/ijms20061277]
18 **Abul-Husn NS**, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, Liu Y, Kozlitina J, Stender S, Wood GC, Stepanchick AN, Still MD, McCarthy S, O'Dushlaine C, Packer JS, Balasubramanian S, Gosalia N, Esopi D, Kim SY, Mukherjee S,

Lopez AE, Fuller ED, Penn J, Chu X, Luo JZ, Mirshahi UL, Carey DJ, Still CD, Feldman MD, Small A, Damrauer SM, Rader DJ, Zambrowicz B, Olson W, Murphy AJ, Borecki IB, Shuldiner AR, Reid JG, Overton JD, Yancopoulos GD, Hobbs HH, Cohen JC, Gottesman O, Teslovich TM, Baras A, Mirshahi T, Gromada J, Dewey FE. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *N Engl J Med* 2018; **378**: 1096-1106 [PMID: 29562163 DOI: 10.1056/NEJMoa1712191]

19 **Oben JA**, Mouralidarane A, Samuelsson AM, Matthews PJ, Morgan ML, McKee C, Soeda J, Fernandez-Twinn DS, Martin-Gronert MS, Ozanne SE, Sigala B, Novelli M, Poston L, Taylor PD. Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *J Hepatol* 2010; **52**: 913-920 [PMID: 20413174 DOI: 10.1016/j.jhep.2009.12.042]

20 **Mouralidarane A**, Soeda J, Sugden D, Bocianowska A, Carter R, Ray S, Saraswati R, Cordero P, Novelli M, Fusai G, Vinciguerra M, Poston L, Taylor PD, Oben JA. Maternal obesity programs offspring non-alcoholic fatty liver disease through disruption of 24-h rhythms in mice. *Int J Obes (Lond)* 2015; **39**: 1339-1348 [PMID: 25971926 DOI: 10.1038/ijo.2015.85]

21 **Meaney MJ**. Epigenetics and the biological definition of gene x environment interactions. *Child Dev* 2010; **81**: 41-79 [PMID: 20331654 DOI: 10.1111/j.1467-8624.2009.01381.x]

22 **Chen L**, Huang W, Wang L, Zhang Z, Zhang F, Zheng S, Kong D. The effects of epigenetic modification on the occurrence and progression of liver diseases and the involved mechanism. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 259-270 [PMID: 32124651 DOI: 10.1080/17474124.2020.1736042]

23 **Brouwers MC**, Cantor RM, Kono N, Yoon JL, van der Kallen CJ, Bilderbeek-Beckers MA, van Greevenbroek MM, Lusis AJ, de Bruin TW. Heritability and genetic loci of fatty liver in familial combined hyperlipidemia. *J Lipid Res* 2006; **47**: 2799-2807 [PMID: 16971732 DOI: 10.1194/jlr.M600312-JLR200]

24 **Struben VM**, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000; **108**: 9-13 [PMID: 11059435 DOI: 10.1016/s0002-9343(99)00315-0]

25 **Caussy C**, Soni M, Cui J, Bettencourt R, Schork N, Chen CH, Ikhwan MA, Bassirian S, Cepin S, Gonzalez MP, Mendler M, Kono Y, Vodkin I, Mekeel K, Haldorson J, Hemming A, Andrews B, Salotti J, Richards L, Brenner DA, Sirlin CB, Loomba R; Familial NAFLD Cirrhosis Research Consortium. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. *J Clin Invest* 2017; **127**: 2697-2704 [PMID: 28628033 DOI: 10.1172/JCI93465]

26 **Long MT**, Gurary EB, Massaro JM, Ma J, Hoffmann U, Chung RT, Benjamin EJ, Loomba R. Parental non-alcoholic fatty liver disease increases risk of non-alcoholic fatty liver disease in offspring. *Liver Int* 2019; **39**: 740-747 [PMID: 30179294 DOI: 10.1111/liv.13956]

27 **Caussy C**, Hsu C, Lo MT, Liu A, Bettencourt R, Ajmera VH, Bassirian S, Hooker J, Sy E, Richards L, Schork N, Schnabl B, Brenner DA, Sirlin CB, Chen CH, Loomba R; Genetics of NAFLD in Twins Consortium. Link between gut-microbiome derived metabolite and shared gene-effects with hepatic steatosis and fibrosis in NAFLD. *Hepatology* 2018; **68**: 918-932 [PMID: 29572891 DOI: 10.1002/hep.29892]

28 **Anstee QM**, Day CP. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on PNPLA3 and TM6SF2. *Semin Liver Dis* 2015; **35**: 270-290 [PMID: 26378644 DOI: 10.1055/s-0035-1562947]

29 **Simons N**, Isaacs A, Koek GH, Kuč S, Schaper NC, Brouwers MCGJ. PNPLA3, TM6SF2, and MBOAT7 Genotypes and Coronary Artery Disease. *Gastroenterology* 2017; **152**: 912-913 [PMID: 28157516 DOI: 10.1053/j.gastro.2016.12.020]

30 **Zain SM**, Mohamed Z, Mohamed R. Common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: a meta-analysis. *J Gastroenterol Hepatol* 2015; **30**: 21-27 [PMID: 25167786 DOI: 10.1111/jgh.12714]

31 **Donati B**, Dongiovanni P, Romeo S, Meroni M, McCain M, Miele L, Petta S, Maier S, Rosso C, De Luca L, Vanni E, Grimaudo S, Romagnoli R, Colli F, Ferri F, Mancina RM, Iruzubieta P, Craxi A, Fracanzani AL, Grieco A, Corradini SG, Aghemo A, Colombo M, Soardo G, Bugianesi E, Reeves H, Anstee QM, Fargion S, Valenti L. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci Rep* 2017; **7**: 4492 [PMID: 28674415 DOI: 10.1038/s41598-017-04991-0]

32 **Pirola CJ**, Garaycoechea M, Flichman D, Arrese M, San Martino J, Gazzi C, Castaño GO, Sookoian S. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. *J Lipid Res* 2019; **60**: 176-185 [PMID: 30323112 DOI: 10.1194/jlr.P089953]

33 **Di Sessa A**, Umano GR, Cirillo G, Passaro AP, Verde V, Cozzolino D, Guarino S, Marzuillo P, Miraglia Del Giudice E. Pediatric non-alcoholic fatty liver disease and kidney function: Effect of *HSD17B13* variant. *World J Gastroenterol* 2020; **26**: 5474-5483 [PMID: 33024398 DOI: 10.3748/wjg.v26.i36.5474]

34 **Petta S**, Valenti L, Svegliati-Baroni G, Ruscica M, Pipitone RM, Dongiovanni P, Rychlicki C, Ferri N, Cammà C, Fracanzani AL, Pierantonelli I, Di Marco V, Meroni M, Giordano D, Grimaudo S, Maggioni M, Cabibi D, Fargion S, Craxì A. Fibronectin Type III Domain-Containing Protein 5 rs3480 A>G Polymorphism, Irisin, and Liver Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *J Clin Endocrinol Metab* 2017; **102**: 2660-2669 [PMID: 28472477 DOI: 10.1210/jc.2017-00056]

35 **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]

36 **Jain S**, Thanage R, Panchal F, Rathi PM, Munshi R, Udgirkar SS, Contractor QQ, Chandnani SJ, Sujit NP, Debnath P, Singh A. Screening of Family Members of Nonalcoholic Fatty Liver Disease Patients can Detect Undiagnosed Nonalcoholic Fatty Liver Disease Among Them: Is There a Genetic Link? *J Clin Exp Hepatol* 2021; **11**: 466-474 [PMID: 34276153 DOI: 10.1016/j.jceh.2020.10.003]

37 **Jonas W**, Schürmann A. Genetic and epigenetic factors determining NAFLD risk. *Mol Metab* 2021; **50**: 101111 [PMID: 33160101 DOI: 10.1016/j.molmet.2020.101111]

38 **Botello-Manilla AE**, Chávez-Tapia NC, Uribe M, Nuño-Lámbarri N. Genetics and epigenetics purpose in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 733-748 [PMID: 32552211 DOI: 10.1080/17474124.2020.1780915]

39 **Pruis MG**, Lendvai A, Bloks VW, Zwier MV, Baller JF, de Bruin A, Groen AK, Plösch T. Maternal western diet primes non-alcoholic fatty liver disease in adult mouse offspring. *Acta Physiol (Oxf)* 2014; **210**: 215-227 [PMID: 24224789 DOI: 10.1111/apha.12197]

40 **Salto R**, Manzano M, Girón MD, Cano A, Castro A, Vílchez JD, Cabrera E, López-Pedrosa JM. A Slow-Digesting Carbohydrate Diet during Rat Pregnancy Protects Offspring from Non-Alcoholic Fatty Liver Disease Risk through the Modulation of the Carbohydrate-Response Element and Sterol Regulatory Element Binding Proteins. *Nutrients* 2019; **11** [PMID: 31013988 DOI: 10.3390/nu11040844]

41 **Shen L**, Liu Z, Gong J, Zhang L, Wang L, Magdalou J, Chen L, Wang H. Prenatal ethanol exposure programs an increased susceptibility of non-alcoholic fatty liver disease in female adult offspring rats. *Toxicol Appl Pharmacol* 2014; **274**: 263-273 [PMID: 24275070 DOI: 10.1016/j.taap.2013.11.009]

42 **Oliveira LS**, Caetano B, Miranda RA, Souza AFP, Cordeiro A, Woyames J, Andrade CBV, Atella GC, Takiya CM, Fortunato RS, Trevenzoli IH, Souza LL, Pazos-Moura CC. Differentiated Hepatic Response to Fructose Intake during Adolescence Reveals the Increased Susceptibility to Non-Alcoholic Fatty Liver Disease of Maternal High-Fat Diet Male Rat Offspring. *Mol Nutr Food Res* 2020; **64**: e1900838 [PMID: 31916388 DOI: 10.1002/mnfr.201900838]

43 **Nicolás-Toledo L**, Cervantes-Rodríguez M, Cuevas-Romero E, Corona-Quintanilla DL, Pérez-Sánchez E, Zambrano E, Castelán F, Rodríguez-Antolín J. Hitting a triple in the non-alcoholic fatty liver disease field: sucrose intake in adulthood increases fat content in the female but not in the male rat offspring of dams fed a gestational low-protein diet. *J Dev Orig Health Dis* 2018; **9**: 151-159 [PMID: 29249214 DOI: 10.1017/S204017441700099X]

44 **Suter MA**, Ma J, Vuguin PM, Hartil K, Fiallo A, Harris RA, Charron MJ, Aagaard KM. In utero exposure to a maternal high-fat diet alters the epigenetic histone code in a murine model. *Am J Obstet Gynecol* 2014; **210**: 463.e1-463.e11 [PMID: 24793723 DOI: 10.1016/j.ajog.2014.01.045]

45 **Wei Y**, Yang CR, Wei YP, Zhao ZA, Hou Y, Schatten H, Sun QY. Paternally induced transgenerational inheritance of susceptibility to diabetes in mammals. *Proc Natl Acad Sci U S A* 2014; **111**: 1873-1878 [PMID: 24449870 DOI: 10.1073/pnas.1321195111]

46 **Barker DJ**, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993; **36**: 62-67 [PMID: 8436255 DOI: 10.1007/BF00399095]

47 **Sarr O**, Yang K, Regnault TR. In utero programming of later adiposity: the role of fetal growth restriction. *J Pregnancy* 2012; **2012**: 134758 [PMID: 23251802 DOI: 10.1155/2012/134758]

48 **Modi N**, Murgasova D, Ruager-Martin R, Thomas EL, Hyde MJ, Gale C, Santhakumaran S, Doré CJ, Alavi A, Bell JD. The influence of maternal body mass index on infant adiposity and hepatic lipid content. *Pediatr Res* 2011; **70**: 287-291 [PMID: 21629154 DOI: 10.1203/PDR.0b013e318225f9b1]

49 **Bedogni G**, De Matteis G, Fabrizi M, Alisi A, Crudele A, Pizzolante F, Signore F, Dallapiccola B, Nobili V, Manco M. Association of Bright Liver With the PNPLA3 I148M Gene Variant in 1-Year-Old Toddlers. *J Clin Endocrinol Metab* 2019; **104**: 2163-2170 [PMID: 30649436 DOI: 10.1210/jc.2018-01998]

50 **Ayonrinde OT**, Oddy WH, Adams LA, Mori TA, Beilin LJ, de Klerk N, Olynyk JK. Infant nutrition and maternal obesity influence the risk of non-alcoholic fatty liver disease in adolescents. *J Hepatol* 2017; **67**: 568-576 [PMID: 28619255 DOI: 10.1016/j.jhep.2017.03.029]

51 **Gunderson EP**, Jacobs DR Jr, Chiang V, Lewis CE, Feng J, Quesenberry CP Jr, Sidney S. Duration of lactation and incidence of the metabolic syndrome in women of reproductive age according to gestational diabetes mellitus status: a 20-Year prospective study in CARDIA (Coronary Artery Risk Development in Young Adults). *Diabetes* 2010; **59**: 495-504 [PMID: 19959762 DOI: 10.2337/db09-1197]

52 **Stuebe AM**, Rich-Edwards JW, Willett WC, Manson JE, Michels KB. Duration of lactation and incidence of type 2 diabetes. *JAMA* 2005; **294**: 2601-2610 [PMID: 16304074 DOI: 10.1001/jama.294.20.2601]

53 **Ajmera V**, Liu A, Bettencourt R, Dhar D, Richards L, Loomba R. The impact of genetic risk on liver fibrosis in non-alcoholic fatty liver disease as assessed by magnetic resonance elastography. *Aliment Pharmacol Ther* 2021; **54**: 68-77 [PMID: 33975381 DOI: 10.1111/apt.16392]

54 **Shapiro AL**, Schmiege SJ, Brinton JT, Glueck D, Crume TL, Friedman JE, Dabelea D. Testing the fuel-mediated hypothesis: maternal insulin resistance and glucose mediate the association between maternal and neonatal adiposity, the Healthy Start study. *Diabetologia* 2015; **58**: 937-941 [PMID: 25628236 DOI: 10.1007/s00125-015-3505-z]

55 **Dimasuay KG**, Boeuf P, Powell TL, Jansson T. Placental Responses to Changes in the Maternal Environment Determine Fetal Growth. *Front Physiol* 2016; **7**: 12 [PMID: 26858656 DOI: 10.3389/fphys.2016.00012]

56 **Puigserver P**, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003; **24**: 78-90 [PMID: 12588810 DOI: 10.1210/er.2002-0012]

57 **Gemma C**, Sookoian S, Alvariñas J, García SI, Quintana L, Kanevsky D, González CD, Pirola CJ. Maternal pregestational BMI is associated with methylation of the PPARGC1A promoter in newborns. *Obesity (Silver Spring)* 2009; **17**: 1032-1039 [PMID: 19148128 DOI: 10.1038/oby.2008.605]

58 **Zarrinpar A**, Gupta S, Maurya MR, Subramaniam S, Loomba R. Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study. *Gut* 2016; **65**: 1546-1554 [PMID: 26002934 DOI: 10.1136/gutjnl-2015-309456]

59 **Galley JD**, Bailey M, Kamp Dush C, Schoppe-Sullivan S, Christian LM. Maternal obesity is associated with alterations in the gut microbiome in toddlers. *PLoS One* 2014; **9**: e113026 [PMID: 25409177 DOI: 10.1371/journal.pone.0113026]

60 **Soderborg TK**, Clark SE, Mulligan CE, Janssen RC, Babcock L, Ir D, Young B, Krebs N, Lemas DJ, Johnson LK, Weir T, Lenz LL, Frank DN, Hernandez TL, Kuhn KA, D'Alessandro A, Barbour LA, El Kasmi KC, Friedman JE. The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. *Nat Commun* 2018; **9**: 4462 [PMID: 30367045 DOI: 10.1038/s41467-018-06929-0]

61 **Wesolowski SR**, Kasmi KC, Jonscher KR, Friedman JE. Developmental origins of NAFLD: a womb with a clue. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 81-96 [PMID: 27780972 DOI: 10.1038/nrgastro.2016.160]

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*MINIREVIEWS*

# Role of transcribed ultraconserved regions in gastric cancer and therapeutic perspectives

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## Abstract

Gastric cancer (GC) is the fourth leading cause of cancer-related death. The occurrence and development of GC is a complex process involving multiple biological mechanisms. Although traditional regulation modulates molecular functions related to the occurrence and development of GC, the comprehensive mechanisms remain unclear. Ultraconserved region (UCR) refers to a genome sequence that is completely conserved in the homologous regions of the human, rat and mouse genomes, with 100% identity, without any insertions or deletions, and often located in fragile sites and tumour-related genes. The transcribed UCR (T-UCR) is transcribed from the UCR and is a new type of long noncoding RNA. Recent studies have found that the expression level of T-UCRs changes during the occurrence and development of GC, revealing a new mechanism underlying GC. Therefore, this article aims to review the relevant research on T-UCRs in GC, as well as the function of T-UCRs and their regulatory role in the occurrence and development of GC, to provide new strategies for GC diagnosis and treatment.

**Key Words:** Transcribed ultraconserved region; Gastric cancer; Development; Function; Therapeutic perspectives

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**Core Tip:** Transcribed ultraconserved region (T-UCR) is abnormally expressed in gastric cancer (GC) cells and tumors. It has been found that a variety of T-UCR affects downstream genes and related pathways, and plays a regulatory role in the proliferation, migration and invasion of GC. However, there are few relevant reviews, and this paper aims to review the related studies of T-UCR in GC. And the function of T-UCR and its regulatory role in the occurrence and development of GC, thus providing a new strategy for the diagnosis and treatment of GC.

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## INTRODUCTION

Gastric cancer (GC) is one of the most common malignant tumours in the world. Although the incidence of GC has decreased, GC ranks fourth in cancer-related deaths due to its high lethality[1,2]. Due to the limited specific biomarkers of GC, patients usually have advanced GC when they are diagnosed. The tumourigenesis and development of GC is a multistep process involving countless signal transduction pathways and gene regulation. Among them, oncogenic and tumour suppressor factors, such as the transcribed ultraconserved region (T-UCR), play a key role[3,4].

In the human genome, 93% of genes can be transcribed to produce RNA, but only 2% are translated to protein. This type of RNA that lacks the potential for translation into protein is called noncoding RNA[5-9]. According to the number of bases it contains, noncoding RNA is divided into long noncoding RNAs (lncRNAs, > 200 bp) and small noncoding RNAs (< 200 bp, including rRNA, miRNA, snRNA, snoRNA, siRNA and pi-RNA)[10-13]. T-UCRs are lncRNAs, transcribed from UCRs. Recent studies have found that the expression level of T-UCRs is altered and abnormally expressed in human GC[14-17], which reveals a new mechanism for the occurrence and development of GC. Therefore, this article aims to review the relevant research on T-UCRs in GC, to further understand the specific mechanism of T-UCRs in GC cells, which will facilitate preventive measures against GC, early diagnoses, and new treatments.

## ULTRACONSERVED REGION AND T-UCR

In 2004, Bejerano *et al*[18] compared the genomes of humans, mice, and rats. They found highly conserved DNA sequences, which were subsequently renamed ultraconserved regions (UCRs)[19]. These regions were absolutely conserved in the three species (100% identical, no insertions or deletions) and were often located in fragile sites and genomic regions of tumour-related genes. UCRs are highly conserved in the evolutionary process, due to the long-distance enhancers and ultraconserved exons that originated from the short interspersed repetitive element retroposon family 400 million years ago. At present, such extreme conservation is still active in the Indonesian "coelacanth"[20].

UCRs represent a small part of the human genome, forming a subset of conserved sequences in intragenic and intergenic regions. UCRs are functional but do not encode proteins[21]. The length of UCRs is between 200 and 799 bp. To date, humans have found at least 481 UCR regions[18,22]. It is known that a large part of these regions can actively transcribe RNA, some of them overlap with known protein-coding sequences, and more than half are predicted to not contain any protein-coding sequences [23]. Based on their overlap with known protein-coding genes, these 481 ultraconserved elements were initially divided into three categories: Nonexons, exons and possibly exons[18,19]. However, based on the positional relationship between UCRs and genes, Mestdagh *et al*[24] reclassified them into 5 categories: Exon containing (4.2%), exonic (5%), partly exonic (5%), intergenic (38.7%) and intronic (42.6%) (Figure 1). The sense/antisense strand of each region produces two transcripts, resulting in a total of 962 possible transcripts: One corresponding to the sense genome sequence (named "+") and the other corresponding to the antisense sequence (named "+ A")[25].

T-UCRs are the transcripts of UCRs[26]. Due to their high degree of conservation, T-UCRs may have fundamental functional importance for the ontogeny and phylogeny of mammals and other vertebrates. Recent studies have shown that a T-UCR acts as a regulator in a variety of pathways (such as pri-miRNA processing, transcription regulation, translation and chromatin modification)[27-29]. It is speculated that T-UCRs may be candidate genes for cancer susceptibility because the transcription level of some UCRs is dysregulated in cancer[30,31].

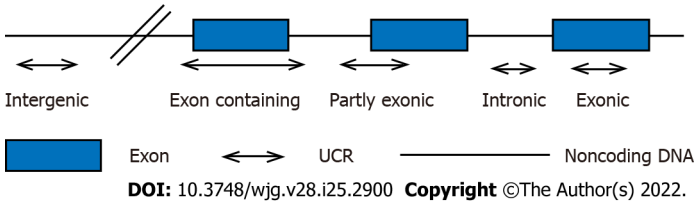


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**Figure 1 Ultraconserved region classification based on the relationship between ultraconserved region and gene position.** UCR: Ultraconserved region.

## T-UCRS AND CANCER

Although transcribed T-UCRs are a type of lncRNA, transcribed by UCRs, they are completely different from lncRNA. T-UCRs are absolutely conserved, while lncRNAs are the least conserved among noncoding RNA[32]. The amazing evolutionary retention of T-UCRs strongly suggests their profound biological role in various physiological responses. As a type of evolutionarily conserved ncRNA, T-UCRs are regarded as essential for life by acting as an antisense transcription inhibitors for nearby protein coding RNA and other ncRNA. T-UCRs are thought to be involved in RNA processing or transcriptional regulation[18]. It has previously been demonstrated that T-UCRs act as regulators of gene expression[20,33]. In recent decades, increasing evidence has shown that T-UCRs are involved in carcinogenesis[23,30,31,34]. Recent studies have identified changes in T-UCR expression patterns associated with specific tumour phenotypes, such as hepatocellular carcinoma[35-37], pancreatic cancer [38,39], bladder cancer[40,41], colorectal cancer[22,26,42,43], prostate cancer[16,25,44,45], cervical cancer [46], neuroblastoma[24], breast cancer[47], lung cancer[22,48-50] and leukaemia[51], indicating a mechanism by which T-UCRs are involved in cancer development. Related studies in GC have also described changes in the expression pattern of T-UCRs[3,4,14-16], indicating that T-UCRs also play a regulatory role in the occurrence and development of GC.

## T-UCRS IN GC

Uc.160+ is transcribed from the UCR on the 5q14.1 chromosome band. Honma *et al*[15] used real-time fluorescent quantitative polymerase chain reaction and in situ hybridization to detect Uc.160+. Compared with nontumour tissues, Uc.160+ expression is downregulated in GC and adenoma tissues. To further understand the biological mechanism of Uc.160+ in GC, the Uc.160+ overexpression vector was used to transfect GC cell lines MKN-1 and MKN-45, and then Western blotting was performed to detect the involvement of mitogens. Compared with the control group, the expression of phosphorylated-Akt and phosphorylated-ERK in the cell line transfected with the Uc.160+ overexpression vector decreased, while the expression of PTEN increased. These results suggest that Uc.160+ exclusively repressed the phosphorylation of Akt by regulating PTEN expression. In addition, Pang *et al* [17] found through a series of phenotypic experiments that the proliferation and activity of GC cells were inhibited after Uc.160 was overexpressed, and cell apoptosis was enhanced, which further proved the inhibitory effect of Uc.160 on GC. In conclusion, UC.160 plays an inhibitory role in the occurrence and development of GC.

Sakamoto *et al*[14] analysed the expression and distribution of Uc.63+ by using qRT-PCR and in situ hybridization, and they found that all GC tissues showed high Uc.63+ expression compared with normal tissues. The expression of Uc.63+ was also elevated in GC cell lines. After overexpression of Uc.63+ was induced by transfection of Uc.63+ expression vector, GC cell proliferation was significantly enhanced. After the expression of Uc.63+ was inhibited by siRNA, the proliferation of GC cells was inhibited. These results supported the possibility that Uc.63+ had a carcinogenic effect in GC. Additionally, they found that Uc.63+ had no effect on the survival rate of cancer patients, but Uc.63+ overexpression was associated with advanced cancer and GC classification. Uc.63+ was preferentially overexpressed in diffuse GC, but not in intestinal GC. In conclusion, Uc.63+ plays a key role in the classification and progression of GC.

Goto *et al*[16] found that UC.416+A is overexpressed in GC compared with normal tissues by in situ hybridization. The growth of GC cells was significantly inhibited after Uc.416+A was downregulated by siRNA. These results suggest that UC.416+A, as an oncogene, plays an important role in promoting the proliferation of GC cells. Global genetic analysis using Affymetrix GeneChips showed that the most upregulated gene was IGFBP1 (insulin-like growth factor binding protein 1) when Uc.416+A was overexpressed. Compared with the corresponding nontumour gastric mucosa, IGFBP6 was significantly downregulated in GC tissue, indicating that Uc.416+A might promote the proliferation of GC cells by inhibiting IGFBP6.

**Table 1 Transcribed ultraconserved region in gastric cancer**

| T-UCR name | Location | Orientation | Expression in gastric cancer | Biological or molecular functions in gastric cancer | Ref. |
|---|---|---|---|---|---|
| Uc.63+ | Chr.2 | Sense | Downregulated | Associated with the classification and progression of gastric cancer | [14] |
| Uc.118 | Chr.3 | Antisense | Downregulated | - | [16] |
| Uc.158 | Chr.5 | Antisense | Downregulated | - | [16] |
| uc.160 | Chr.5 | Sense | Downregulated | Inhibits cell proliferation and promotes apoptosis in AGS and SGC-7901 cell lines, promoting gastric cancer tumourigenesis | [15, 17] |
| Uc.241 | - | - | Downregulated | - | [16] |
| Uc.244 | Chr.8 | Sense | Down/upregulated | - | [16, 23] |
| Uc.249 | Chr.9 | Sense | Down/upregulated | - | [16, 23] |
| Uc.252 | Chr.9 | Antisense | Down/upregulated | - | [16, 23] |
| Uc.261 | Chr.9 | Sense | Down/upregulated | - | [16, 23] |
| Uc.282 | Chr.9 | Sense | Down/upregulated | - | [16, 23] |
| Uc.283 | - | - | - | - | [16] |
| Uc.346 | Chr.12 | - | Downregulated | - | [23] |
| Uc.359 | Chr.14 | Antisense | Down/upregulated | - | [16, 23] |
| Uc.416 | - | Antisense | Upregulated | Promotes proliferation in MKN-74 cell line, promoting gastric cancer tumourigenesis | [16] |

T-UCR: Transcribed ultraconserved region.

In addition, the expression levels of Uc.118, Uc.158, Uc.241 and Uc.346 were found to be significantly downregulated in GC; Uc.244, Uc.249, Uc.252, Uc.261, Uc.282, Uc.283 and Uc.359 Levels were undetermined (Table 1).

## REGULATORY MECHANISM OF T-UCRS IN GC

The regulatory mechanism of T-UCRs in tumourigenesis and the development of cancer is largely unclear, but current studies have found that T-UCR are involved in three different regulatory mechanisms in tumours: CpG island methylation[52], interaction with miRNAs[53-55] and direct binding to the target mRNAs[27,43]. In GC related research, it was only found that T-UCRs participate in the occurrence and development of GC through the first two pathways (Figure 2).

### *CpG island methylation*

The field of epigenetics describes the transmission of information through heritable changes in phenotypes that do not involve changes in DNA sequences during cell division[56]. CpG island methylation, histone modification and chromatin delivery structure are the potential mechanisms of epigenetic transmission, and CpG island methylation is a key component of the changes in gene expression associated with human cancer, especially the expression of GC related genes[57-60]. GC is the most susceptible of all cancers to epigenetic changes without any changes in DNA[59,61-63]. CpG islands are DNA fragments of at least 0.5 kb, that are rich in G:C and CpG content, and are present in approximately 70% of human gene promoters. In other words, CpG islands are tandem repeats of cytosine (C) and guanidine (G), where p is the phosphoric acid between C and G. In brief, the methylation of CpG occurs under the action of methylase whereby the hydrogen on the cytosine (C) 5 carbon atom is replaced by methyl (CH3)[60,64-66]. Demethylation is the opposite process. It has been found that more than 50% of human genes are regulated by promoters including CpG islands[67]. In normal cells, except for genes with inactive X chromosomes or genes related to imprinted genes, promoter CpG islands are usually unmethylated[68]. Although the aetiology is still unclear, promoter CpG island methylation may be related to cancer development and ageing[69]. It is a common feature of

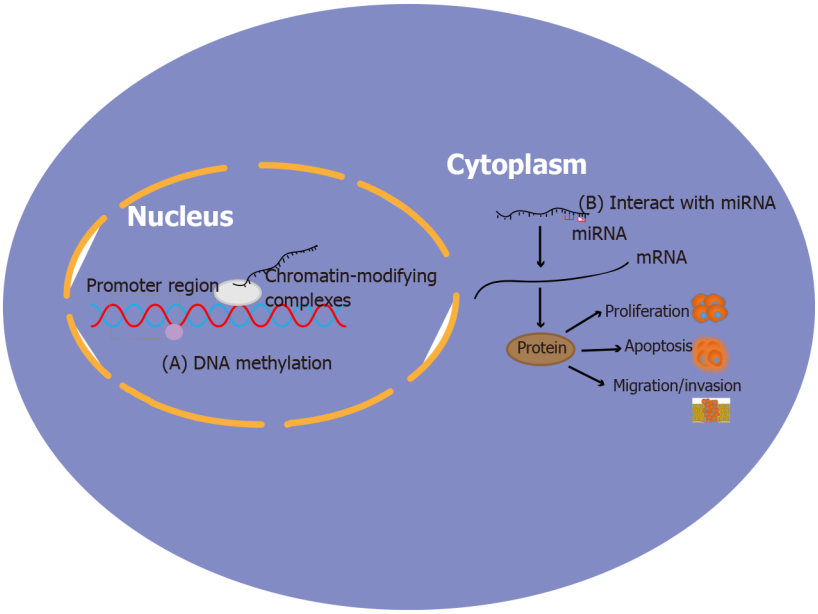


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**Figure 2 The mechanism by which transcribed ultraconserved region affects gastric cancer.** A: DNA methylation; B: Interaction with miRNAs.

human cancers that low expression of tumour-suppressive ncRNA causes CpG island methylation and then affects epigenetic silencing. Promoter CpG island methylation has been found in almost all human cancer tissue types, and it is an important mechanism for the inactivation of tumour suppressor genes and tumour-related genes[70]. GC is the most common human cancer caused by methylation of the promoter CpG island. Interestingly, T-UCRs undergo DNA methylation-related silencing in cancer cells, and promoter CpG island methylation is now considered to be important for inactivating tumour suppressor genes or tumour-related genes. Previous studies have shown that T-UCR regulation in the occurrence and development of GC is closely related to the methylation of CpG islands of the host gene promoter[16].

Goto *et al*[16] discovered that there are CpG islands approximately 500bp upstream of the Uc.158+A transcription gene. Bisulfite genomic sequencing of GC cell lines and GC tissue samples showed specific DNA methylation of GC, which contained UCRs. The luciferase vector of the CpG island upstream of the Uc.158+A transcription gene proved that when the upstream sequence of the Uc.158+A transcription gene was methylated, reporter activity was significantly inhibited. Thus, this suggests that Uc.158+A expression is silenced by DNA methylation in the promoter region upstream of its transcription gene.

### *Interaction with miRNAs*

Previous studies have shown that miRNAs affect the regulation of gene expression at both the transcriptional and posttranscriptional levels[71-73]. MiRNAs are also almost completely conserved[74,75]. Do T-UCRs interact with miRNAs to regulate the occurrence and development of cancer? In 2007, Calin and his collaborators demonstrated for the first time that certain T-UCRs are altered due to the direct regulation of high-level miRNAs in chronic lymphocytic leukaemia, and the expression of each T-UCR is negatively correlated with the corresponding microRNA level[19]. Recent studies have found that a T-UCR interacts with miR-596 and synergistically promotes the development of bladder cancer[28]. Terreri *et al*[55] found that the formation of T-UCR::miRNA pairs may have different effects, either targeting T-UCR or forming sponges that capture miRNAs. They also reported that the interaction between miRNAs and T-UCRs can act as a network to regulate the availability of certain lncRNAs in bladder urothelial carcinoma cells. These results indicated that miRNAs are involved in the process of T-UCR-mediated tumour regulation. In GC, we found that T-UCRs represent the possible targets of miRNAs[14-16], and these interactions may have biological and prognostic significance for cancer patients.

Goto *et al*[16] determined that the expression of miR-153 in GC cell lines with higher expression of Uc.416+A was significantly reduced, suggesting an inverse correlation between Uc.416+A and miR-153. The direct interaction between Uc.416+A and miR-153 was confirmed by a luciferase activity assay. This result showed that the overexpression of Uc.416+A is related to the downregulation of miR-153 in GC. Overexpression of UC.416+A caused changes in downstream related genes. The expression of insulin-like growth factor binding protein 6 (IGFBP6) was upregulated, and alcohol dehydrogenase 1C (ADH1C), homeobox B5 (HOXB5) and homeobox B6 (HOXB6) levels were downregulated. The expression of IGFBP6, HOXB5 and HOXB6 in GC and adjacent tissues showed statistically significant differences, indicating that the overexpression of UC.416+A is regulated by miR-153, which then affects

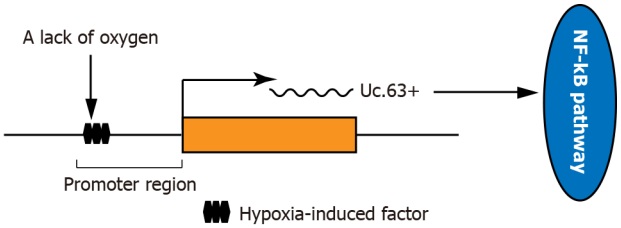


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**Figure 3 The mechanism of Uc.63 + in gastric cancer.**

the gene changes of downstream coding proteins and plays a promoting role in the growth of GC.

### *CpG island methylation and miRNAs play a role together*

Honma *et al*[15] found that the low expression of Uc.160+ in GC was caused by methylation of its upstream promoter region, and the low expression of Uc.160+ caused a decrease in PTEN expression, an increase in phosphatase activity and activation of the MAPK pathway. In addition, Pang *et al*[17]'s study found that UC.160 was also regulated by miR-155, which then regulated PTEN and affected the MAPK pathway. In other words, the low expression of UC.160 in GC is regulated by miR-155 and affected by methylation in the upstream promoter region of its transcriptional genes.

### *Other factors affecting T-UCR transcription*

Sakamoto *et al*[14] found that the expression of UC.63+ is regulated by hypoxia. The promoter region located upstream of the Uc.63+ transcription gene is considered to have hypoxia-induced binding sites. Hypoxia induces overexpression of UC.63+, and the overexpression of UC.63+ upregulates its downstream target P65 and activates the NF-KB signalling pathway to promote the occurrence of GC (Figure 3).

## CONCLUSION

In GC, T-UCRs change at the transcriptional level, and the abnormal expression of T-UCRs can lead to the occurrence and development of GC. Compared with normal cells, T-UCRs in GC cells have a unique expression profile, which indicates that changes in T-UCRs are involved in this malignant process. This study may provide new ideas and directions for GC diagnosis and prognosis. Compared with coding RNA, T-UCRs have incomparable advantages. Because T-UCRs do not code for proteins, they are relatively less regulated and more accurate. In addition, some T-UCR expression levels are completely different in different stages of cancer, and can also be used as one of the criteria for judging prognosis.

Despite the fact that this new type of dysregulated molecule seems to be useful in future clinical applications, further research is needed before it can be used as a valuable clinical biomarker for GC. First, although approximately 98% of the entire genome contains nonprotein coding genes, the proportion of T-UCRs is currently unclear. Second, current screening methods for T-UCRs (RT-qPCR, T-UCR chip) are useful, but simpler and more practical techniques will help in the identification and screening of disease-related T-UCRs. Third, it has been proven that T-UCRs are involved in the occurrence and development of GC, but their biological mechanism has not been fully elucidated. In addition, more powerful strategies are needed to clarify the regulatory role of T-UCR by constructing an interaction network, or to evaluate its function in typical signalling pathways. Since the knowledge of T-UCR is still in its infancy-especially when compared to other ncRNAs, further research is needed to convincingly incorporate these ncRNAs into the growing field of cancer therapeutics.

At present, there are few studies on T-UCR therapy, so there is still much room to explore treatment measures aimed at T-UCRs. Perhaps we can achieve a treatment purpose by changing the expression of T-UCRs. In the reference cell experiment, restoring the downregulated levels of T-UCRs or suppressing the overexpressed levels of T-UCRs *via* overexpression vectors or small interfering RNA methods can reverse the tumour phenotype. In clinical treatment, we can use the negative regulation between T-UCRs and miRNAs to inhibit the expression of T-UCRs, and methylation inhibitors can be used to restore the expression of T-UCRs with downregulated levels in tumours, thereby delaying or even reversing tumour progression.

Although these hypotheses have not been confirmed, we believe that as research on T-UCRs continues, the insight gained will definitely provide a novel strategy for the diagnosis, prognosis and treatment of tumours.

## FOOTNOTES

**Author contributions:** Guo XB and Gao SS conceived of the presented idea and researched the background of the study; Zhang ZZ and Wang XB prepared the figures and tables; Ma Y and Yin GQ wrote the manuscript; all the authors read and approved the final manuscript.

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## REFERENCES

1 **Thrift AP**, El-Serag HB. Burden of Gastric Cancer. *Clin Gastroenterol Hepatol* 2020; **18**: 534-542 [PMID: 31362118 DOI: 10.1016/j.cgh.2019.07.045]
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 **Ghafouri-Fard S**, Taheri M. Long non-coding RNA signature in gastric cancer. *Exp Mol Pathol* 2020; **113**: 104365 [PMID: 31899194 DOI: 10.1016/j.yexmp.2019.104365]
4 **Yu H**, Rong L. Emerging role of long non-coding RNA in the development of gastric cancer. *World J Gastrointest Oncol* 2018; **10**: 260-270 [PMID: 30254721 DOI: 10.4251/wjgo.v10.i9.260]
5 **ENCODE Project Consortium**. . An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012; **489**: 57-74 [PMID: 22955616 DOI: 10.1038/nature11247]
6 **Oe S**, Kimura T, Yamada H. Regulatory non-coding RNAs in nervous system development and disease. *Front Biosci (Landmark Ed)* 2019; **24**: 1203-1240 [PMID: 31136976 DOI: 10.2741/4776]
7 **Tsagakis I**, Douka K, Birds I, Aspden JL. Long non-coding RNAs in development and disease: conservation to mechanisms. *J Pathol* 2020; **250**: 480-495 [PMID: 32100288 DOI: 10.1002/path.5405]
8 **Rederstorff M**, Hüttenhofer A. Small non-coding RNAs in disease development and host-pathogen interactions. *Curr Opin Mol Ther* 2010; **12**: 684-694 [PMID: 21154160 DOI: 10.2174/1566524107939377741]
9 **Dey BK**, Mueller AC, Dutta A. Long non-coding RNAs as emerging regulators of differentiation, development, and disease. *Transcription* 2014; **5**: e944014 [PMID: 25483404 DOI: 10.4161/21541272.2014.944014]
10 **Beermann J**, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. *Physiol Rev* 2016; **96**: 1297-1325 [PMID: 27535639 DOI: 10.1152/physrev.00041.2015]
11 **Zhang X**, Hamblin MH, Yin KJ. The long noncoding RNA Malat1: Its physiological and pathophysiological functions. *RNA Biol* 2017; **14**: 1705-1714 [PMID: 28837398 DOI: 10.1080/15476286.2017.1358347]
12 **Ransohoff JD**, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol* 2018; **19**: 143-157 [PMID: 29138516 DOI: 10.1038/nrm.2017.104]
13 **Romano G**, Veneziano D, Acunzo M, Croce CM. Small non-coding RNA and cancer. *Carcinogenesis* 2017; **38**: 485-491 [PMID: 28449079 DOI: 10.1093/carcin/bgx026]
14 **Sakamoto N**, Sekino Y, Fukada K, Pham QT, Honma R, Taniyama D, Ukai S, Takashima T, Hattori T, Naka K, Tanabe K, Ohdan H, Yasui W. Uc.63+ contributes to gastric cancer progression through regulation of NF-kB signaling. *Gastric Cancer* 2020; **23**: 863-873 [PMID: 32323025 DOI: 10.1007/s10120-020-01070-8]
15 **Honma R**, Goto K, Sakamoto N, Sekino Y, Sentani K, Oue N, Yasui W. Expression and function of Uc.160+, a transcribed ultraconserved region, in gastric cancer. *Gastric Cancer* 2017; **20**: 960-969 [PMID: 28382457 DOI: 10.1007/s10120-017-0714-9]
16 **Goto K**, Ishikawa S, Honma R, Tanimoto K, Sakamoto N, Sentani K, Oue N, Teishima J, Matsubara A, Yasui W. The transcribed-ultraconserved regions in prostate and gastric cancer: DNA hypermethylation and microRNA-associated regulation. *Oncogene* 2016; **35**: 3598-3606 [PMID: 26640143 DOI: 10.1038/onc.2015.445]
17 **Pang L**, Li Q, Zhang Y, Deng B, Wu F, Wang J, Wu K, Ding Y, Yu D. Transcribed ultraconserved noncoding RNA uc.160 acts as a negative regulator in gastric cancer. *Am J Transl Res* 2018; **10**: 2822-2833 [PMID: 30323869]
18 **Bejerano G**, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, Haussler D. Ultraconserved elements in the human

genome. *Science* 2004; **304**: 1321-1325 [PMID: 15131266 DOI: 10.1126/science.1098119]

19 **Calin GA**, Liu CG, Ferracin M, Hyslop T, Spizzo R, Sevignani C, Fabbri M, Cimmino A, Lee EJ, Wojcik SE, Shimizu M, Tili E, Rossi S, Taccioli C, Pichiorri F, Liu X, Zupo S, Herlea V, Gramantieri L, Lanza G, Alder H, Rassenti L, Volinia S, Schmittgen TD, Kipps TJ, Negrini M, Croce CM. Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. *Cancer Cell* 2007; **12**: 215-229 [PMID: 17785203 DOI: 10.1016/j.ccr.2007.07.027]

20 **Bejerano G**, Lowe CB, Ahituv N, King B, Siepel A, Salama SR, Rubin EM, Kent WJ, Haussler D. A distal enhancer and an ultraconserved exon are derived from a novel retroposon. *Nature* 2006; **441**: 87-90 [PMID: 16625209 DOI: 10.1038/nature04696]

21 **Qian XX**, Peng JC, Xu AT, Zhao D, Qiao YQ, Wang TR, Shen J, Ran ZH. Noncoding Transcribed Ultraconserved Region (T-UCR) uc.261 Participates in Intestinal Mucosa Barrier Damage in Crohn's Disease. *Inflamm Bowel Dis* 2016; **22**: 2840-2852 [PMID: 27846191 DOI: 10.1097/MIB.0000000000000945]

22 **Zhang Y**, Wang S, Qian W, Ji D, Wang Q, Zhang Z, Ji B, Fu Z, Sun Y. uc.338 targets p21 and cyclin D1 *via* PI3K/AKT pathway activation to promote cell proliferation in colorectal cancer. *Oncol Rep* 2018; **40**: 1119-1128 [PMID: 29901203 DOI: 10.3892/or.2018.6480]

23 **Pereira Zambalde E**, Mathias C, Rodrigues AC, de Souza Fonseca Ribeiro EM, Fiori Gradia D, Calin GA, Carvalho de Oliveira J. Highlighting transcribed ultraconserved regions in human diseases. *Wiley Interdiscip Rev RNA* 2020; **11**: e1567 [PMID: 31489780 DOI: 10.1002/wrna.1567]

24 **Mestdagh P**, Fredlund E, Pattyn F, Rihani A, Van Maerken T, Vermeulen J, Kumps C, Menten B, De Preter K, Schramm A, Schulte J, Noguera R, Schleiermacher G, Janoueix-Lerosey I, Laureys G, Powel R, Nittner D, Marine JC, Ringnér M, Speleman F, Vandesompele J. An integrative genomics screen uncovers ncRNA T-UCR functions in neuroblastoma tumours. *Oncogene* 2010; **29**: 3583-3592 [PMID: 20383195 DOI: 10.1038/onc.2010.106]

25 **Hudson RS**, Yi M, Volfovsky N, Prueitt RL, Esposito D, Volinia S, Liu CG, Schetter AJ, Van Roosbroeck K, Stephens RM, Calin GA, Croce CM, Ambs S. Transcription signatures encoded by ultraconserved genomic regions in human prostate cancer. *Mol Cancer* 2013; **12**: 13 [PMID: 23409773 DOI: 10.1186/1476-4598-12-13]

26 **Sana J**, Hankeova S, Svoboda M, Kiss I, Vyzula R, Slaby O. Expression levels of transcribed ultraconserved regions uc.73 and uc.388 are altered in colorectal cancer. *Oncology* 2012; **82**: 114-118 [PMID: 22328099 DOI: 10.1159/000336479]

27 **Liz J**, Portela A, Soler M, Gómez A, Ling H, Michlewski G, Calin GA, Guil S, Esteller M. Regulation of pri-miRNA processing by a long noncoding RNA transcribed from an ultraconserved region. *Mol Cell* 2014; **55**: 138-147 [PMID: 24910097 DOI: 10.1016/j.molcel.2014.05.005]

28 **Ding L**, Gong C, Zhao J, Liu X, Li T, Rao S, Wang S, Liu Y, Peng S, Xiao W, Xiong C, Wang R, Liang S, Xu H. Noncoding transcribed ultraconserved region (T-UCR) UC.48+ is a novel regulator of high-fat diet induced myocardial ischemia/reperfusion injury. *J Cell Physiol* 2019; **234**: 9849-9861 [PMID: 30417395 DOI: 10.1002/jcp.27674]

29 **Terracciano D**, Terreri S, de Nigris F, Costa V, Calin GA, Cimmino A. The role of a new class of long noncoding RNAs transcribed from ultraconserved regions in cancer. *Biochim Biophys Acta Rev Cancer* 2017; **1868**: 449-455 [PMID: 28916343 DOI: 10.1016/j.bbcan.2017.09.001]

30 **Fabris L**, Calin GA. Understanding the Genomic Ultraconservations: T-UCRs and Cancer. *Int Rev Cell Mol Biol* 2017; **333**: 159-172 [PMID: 28729024 DOI: 10.1016/bs.ircmb.2017.04.004]

31 **Peng JC**, Shen J, Ran ZH. Transcribed ultraconserved region in human cancers. *RNA Biol* 2013; **10**: 1771-1777 [PMID: 24384562 DOI: 10.4161/rna.26995]

32 **Johnsson P**, Lipovich L, Grandér D, Morris KV. Evolutionary conservation of long non-coding RNAs; sequence, structure, function. *Biochim Biophys Acta* 2014; **1840**: 1063-1071 [PMID: 24184936 DOI: 10.1016/j.bbagen.2013.10.035]

33 **Katzman S**, Kern AD, Bejerano G, Fewell G, Fulton L, Wilson RK, Salama SR, Haussler D. Human genome ultraconserved elements are ultraselected. *Science* 2007; **317**: 915 [PMID: 17702936 DOI: 10.1126/science.1142430]

34 **Scaruffi P**. The transcribed-ultraconserved regions: a novel class of long noncoding RNAs involved in cancer susceptibility. *ScientificWorldJournal* 2011; **11**: 340-352 [PMID: 21298224 DOI: 10.1100/tsw.2011.35]

35 **Luo HL**, Chen J, Luo T, Wu FX, Liu JJ, Wang HF, Chen M, Li LQ, Li H. Downregulation of Macrophage-Derived T-UCR uc.306 Associates with Poor Prognosis in Hepatocellular Carcinoma. *Cell Physiol Biochem* 2017; **42**: 1526-1539 [PMID: 28723685 DOI: 10.1159/000479269]

36 **Carotenuto P**, Fassan M, Pandolfo R, Lampis A, Vicentini C, Cascione L, Paulus-Hock V, Boulter L, Guest R, Quagliata L, Hahne JC, Ridgway R, Jamieson T, Athineos D, Veronese A, Visone R, Murgia C, Ferrari G, Guzzardo V, Evans TRJ, MacLeod M, Feng GJ, Dale T, Negrini M, Forbes SJ, Terracciano L, Scarpa A, Patel T, Valeri N, Workman P, Sansom O, Braconi C. Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers. *Gut* 2017; **66**: 1268-1277 [PMID: 27618837 DOI: 10.1136/gutjnl-2016-312278]

37 **Kogure T**, Yan IK, Lin WL, Patel T. Extracellular Vesicle-Mediated Transfer of a Novel Long Noncoding RNA TUC339: A Mechanism of Intercellular Signaling in Human Hepatocellular Cancer. *Genes Cancer* 2013; **4**: 261-272 [PMID: 24167654 DOI: 10.1177/1947601913499020]

38 **Jiang J**, Azevedo-Pouly AC, Redis RS, Lee EJ, Gusev Y, Allard D, Sutaria DS, Badawi M, Elgamal OA, Lerner MR, Brackett DJ, Calin GA, Schmittgen TD. Globally increased ultraconserved noncoding RNA expression in pancreatic adenocarcinoma. *Oncotarget* 2016; **7**: 53165-53177 [PMID: 27363020 DOI: 10.18632/oncotarget.10242]

39 **Liu C**, Wang J, Yuan X, Qian W, Zhang B, Shi M, Xie J, Shen B, Xu H, Hou Z, Chen H. Long noncoding RNA uc.345 promotes tumorigenesis of pancreatic cancer by upregulation of hnRNPL expression. *Oncotarget* 2016; **7**: 71556-71566 [PMID: 27689400 DOI: 10.18632/oncotarget.12253]

40 **Terreri S**, Mancinelli S, Ferro M, Vitale MC, Perdonà S, Castaldo L, Gigantino V, Mercadante V, De Cecio R, Aquino G, Montella M, Angelini C, Del Prete E, Aprile M, Ciaramella A, Liguori GL, Costa V, Calin GA, La Civita E, Terracciano D, Febbraio F, Cimmino A. Subcellular Localization of uc.8+ as a Prognostic Biomarker in Bladder Cancer Tissue. *Cancers (Basel)* 2021; **13** [PMID: 33567603 DOI: 10.3390/cancers13040681]

41 **Sekino Y**, Sakamoto N, Ishikawa A, Honma R, Shigematsu Y, Hayashi T, Sentani K, Oue N, Teishima J, Matsubara A, Yasui W. Transcribed ultraconserved region Uc.63+ promotes resistance to cisplatin through regulation of androgen

receptor signaling in bladder cancer. *Oncol Rep* 2019; **41**: 3111-3118 [PMID: 30864720 DOI: 10.3892/or.2019.7039]

42 **Zheng Z**, Hong D, Zhang X, Chang Y, Sun N, Lin Z, Li H, Huang S, Zhang R, Xie Q, Huang H, Jin H. uc.77-Downregulation Promotes Colorectal Cancer Cell Proliferation by Inhibiting FBXW8-Mediated CDK4 Protein Degradation. *Front Oncol* 2021; **11**: 673223 [PMID: 34094975 DOI: 10.3389/fonc.2021.673223]

43 **Wang C**, Wang Z, Zhou J, Liu S, Wu C, Huang C, Ding Y. TUC.338 promotes invasion and metastasis in colorectal cancer. *Int J Cancer* 2017; **140**: 1457-1464 [PMID: 27914101 DOI: 10.1002/ijc.30542]

44 **Sekino Y**, Sakamoto N, Goto K, Honma R, Shigematsu Y, Sentani K, Oue N, Teishima J, Matsubara A, Yasui W. Transcribed ultraconserved region Uc.63+ promotes resistance to docetaxel through regulation of androgen receptor signaling in prostate cancer. *Oncotarget* 2017; **8**: 94259-94270 [PMID: 29212226 DOI: 10.18632/oncotarget.21688]

45 **Bao BY**, Lin VC, Yu CC, Yin HL, Chang TY, Lu TL, Lee HZ, Pao JB, Huang CY, Huang SP. Genetic variants in ultraconserved regions associate with prostate cancer recurrence and survival. *Sci Rep* 2016; **6**: 22124 [PMID: 26902966 DOI: 10.1038/srep22124]

46 **Li Q**, Li X, Wang C. Uc.206 regulates cell proliferation and apoptosis by targeting P53 in cervical cancer cells. *Neoplasma* 2016; **63**: 411-418 [PMID: 26925787 DOI: 10.4149/310_151017N538]

47 **Pereira Zambalde E**, Bayraktar R, Schultz Jucoski T, Ivan C, Rodrigues AC, Mathias C, Knutsen E, Silveira de Lima R, Fiori Gradia D, de Souza Fonseca Ribeiro EM, Hannash S, Adrian Calin G, Carvalhode Oliveira J. A novel lncRNA derived from an ultraconserved region: lnc-*uc.147*, a potential biomarker in luminal A breast cancer. *RNA Biol* 2021; **18**: 416-429 [PMID: 34387142 DOI: 10.1080/15476286.2021.1952757]

48 **Liu X**, Zhou X, Deng CJ, Zhao Y, Shen J, Wang Y, Zhang YL. Comprehensive analyses of T-UCR expression profiles and exploration of the efficacy of uc.63- and uc.280+ as biomarkers for lung cancer in Xuanwei, China. *Pathol Res Pract* 2020; **216**: 152978 [PMID: 32360249 DOI: 10.1016/j.prp.2020.152978]

49 **Gao X**, Gao X, Li C, Zhang Y, Gao L. Knockdown of Long Noncoding RNA uc.338 by siRNA Inhibits Cellular Migration and Invasion in Human Lung Cancer Cells. *Oncol Res* 2016; **24**: 337-343 [PMID: 27712590 DOI: 10.3727/096504016X14666990347671]

50 **Tian Y**, Feng Y. Up-regulation of long noncoding RNA uc.338 predicts poor survival in non-small cell lung cancer. *Cancer Biomark* 2018; **22**: 781-785 [PMID: 29843223 DOI: 10.3233/CBM-181331]

51 **das Chagas PF**, de Sousa GR, Kodama MH, de Biagi Junior CAO, Yunes JA, Brandalise SR, Calin GA, Tone LG, Scrideli CA, de Oliveira JC. Ultraconserved long non-coding RNA uc.112 is highly expressed in childhood T versus B-cell acute lymphoblastic leukemia. *Hematol Transfus Cell Ther* 2021; **43**: 28-34 [PMID: 32014474 DOI: 10.1016/j.htct.2019.12.003]

52 **Lujambio A**, Portela A, Liz J, Melo SA, Rossi S, Spizzo R, Croce CM, Calin GA, Esteller M. CpG island hypermethylation-associated silencing of non-coding RNAs transcribed from ultraconserved regions in human cancer. *Oncogene* 2010; **29**: 6390-6401 [PMID: 20802525 DOI: 10.1038/onc.2010.361]

53 **Xiao L**, Wu J, Wang JY, Chung HK, Kalakonda S, Rao JN, Gorospe M. Long Noncoding RNA uc.173 Promotes Renewal of the Intestinal Mucosa by Inducing Degradation of MicroRNA 195. *Gastroenterology* 2018; **154**: 599-611 [PMID: 29042220 DOI: 10.1053/j.gastro.2017.10.009]

54 **Wang JY**, Cui YH, Xiao L, Chung HK, Zhang Y, Rao JN, Gorospe M, Wang JY. Regulation of Intestinal Epithelial Barrier Function by Long Noncoding RNA *uc.173* through Interaction with MicroRNA 29b. *Mol Cell Biol* 2018; **38** [PMID: 29632078 DOI: 10.1128/MCB.00010-18]

55 **Terreri S**, Durso M, Colonna V, Romanelli A, Terracciano D, Ferro M, Perdonà S, Castaldo L, Febbraio F, de Nigris F, Cimmino A. New Cross-Talk Layer between Ultraconserved Non-Coding RNAs, MicroRNAs and Polycomb Protein YY1 in Bladder Cancer. *Genes (Basel)* 2016; **7** [PMID: 27983635 DOI: 10.3390/genes7120127]

56 **Bhol CS**, Panigrahi DP, Praharaj PP, Mahapatra KK, Patra S, Mishra SR, Behera BP, Bhutia SK. Epigenetic modifications of autophagy in cancer and cancer therapeutics. *Semin Cancer Biol* 2020; **66**: 22-33 [PMID: 31158463 DOI: 10.1016/j.semcancer.2019.05.020]

57 **Patel TN**, Roy S, Ravi R. Gastric cancer and related epigenetic alterations. *Ecancermedicalscience* 2017; **11**: 714 [PMID: 28144288 DOI: 10.3332/ecancer.2017.714]

58 **Choi SJ**, Jung SW, Huh S, Chung YS, Cho H, Kang H. Alteration of DNA Methylation in Gastric Cancer with Chemotherapy. *J Microbiol Biotechnol* 2017; **27**: 1367-1378 [PMID: 28621113 DOI: 10.4014/jmb.1704.04035]

59 **Song Y**, Tu J, Cheng Y, Zhou F, Liu P, Zhou S, Gu Y, Sun Y. HHIP Overexpression Suppresses Human Gastric Cancer Progression and Metastasis by Reducing Its CpG Island Methylation. *Front Oncol* 2020; **10**: 1667 [PMID: 33415068 DOI: 10.3389/fonc.2020.01667]

60 **Issa JP**. CpG-island methylation in aging and cancer. *Curr Top Microbiol Immunol* 2000; **249**: 101-118 [PMID: 10802941 DOI: 10.1007/978-3-642-59696-4_7]

61 **Shigeyasu K**, Nagasaka T, Mori Y, Yokomichi N, Kawai T, Fuji T, Kimura K, Umeda Y, Kagawa S, Goel A, Fujiwara T. Clinical Significance of MLH1 Methylation and CpG Island Methylator Phenotype as Prognostic Markers in Patients with Gastric Cancer. *PLoS One* 2015; **10**: e0130409 [PMID: 26121593 DOI: 10.1371/journal.pone.0130409]

62 **Tedaldi G**, Molinari C, São José C, Barbosa-Matos R, André A, Danesi R, Arcangeli V, Ravegnani M, Saragoni L, Morgagni P, Rebuzzi F, Canale M, Pignatta S, Ferracci E, Martinelli G, Ranzani GN, Oliveira C, Calistri D, Ulivi P. Genetic and Epigenetic Alterations of *CDH1* Regulatory Regions in Hereditary and Sporadic Gastric Cancer. *Pharmaceuticals (Basel)* 2021; **14** [PMID: 34066170 DOI: 10.3390/ph14050457]

63 **Loh M**, Liem N, Vaithilingam A, Lim PL, Sapari NS, Elahi E, Mok ZY, Cheng CL, Yan B, Pang B, Salto-Tellez M, Yong WP, Iacopetta B, Soong R. DNA methylation subgroups and the CpG island methylator phenotype in gastric cancer: a comprehensive profiling approach. *BMC Gastroenterol* 2014; **14**: 55 [PMID: 24674026 DOI: 10.1186/1471-230X-14-55]

64 **Curtin K**, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. *Patholog Res Int* 2011; **2011**: 902674 [PMID: 21559209 DOI: 10.4061/2011/902674]

65 **Draht MX**, Riedl RR, Niessen H, Carvalho B, Meijer GA, Herman JG, van Engeland M, Melotte V, Smits KM. Promoter CpG island methylation markers in colorectal cancer: the road ahead. *Epigenomics* 2012; **4**: 179-194 [PMID: 22449189 DOI: 10.2217/epi.12.9]

66 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; **59**: 5438-5442 [PMID: 10554013]

67 **Nazemalhosseini Mojarad E**, Kuppen PJ, Aghdaei HA, Zali MR. The CpG island methylator phenotype (CIMP) in colorectal cancer. *Gastroenterol Hepatol Bed Bench* 2013; **6**: 120-128 [PMID: 24834258]

68 **De Carvalho DD**, You JS, Jones PA. DNA methylation and cellular reprogramming. *Trends Cell Biol* 2010; **20**: 609-617 [PMID: 20810283 DOI: 10.1016/j.tcb.2010.08.003]

69 **Ehrlich M**. DNA hypermethylation in disease: mechanisms and clinical relevance. *Epigenetics* 2019; **14**: 1141-1163 [PMID: 31284823 DOI: 10.1080/15592294.2019.1638701]

70 **Malta TM**, de Souza CF, Sabedot TS, Silva TC, Mosella MS, Kalkanis SN, Snyder J, Castro AVB, Noushmehr H. Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications. *Neuro Oncol* 2018; **20**: 608-620 [PMID: 29036500 DOI: 10.1093/neuonc/nox183]

71 **Kang BW**, Chau I. Current status and future potential of predictive biomarkers for immune checkpoint inhibitors in gastric cancer. *ESMO Open* 2020; **5** [PMID: 32817133 DOI: 10.1136/esmoopen-2020-000791]

72 **Gu H**, Zhong Y, Liu J, Shen Q, Wei R, Zhu H, Zhang X, Xia X, Yao M, Ni M. The Role of miR-4256/HOXC8 Signaling Axis in the Gastric Cancer Progression: Evidence From lncRNA-miRNA-mRNA Network Analysis. *Front Oncol* 2021; **11**: 793678 [PMID: 35111675 DOI: 10.3389/fonc.2021.793678]

73 **Liu J**, Wang H, Liao X. MicroRNA-223-5p targets long non-coding RNA TP73 antisense RNA1 to promote the invasion of gastric cancer. *Hum Cell* 2020; **33**: 676-682 [PMID: 32248369 DOI: 10.1007/s13577-020-00349-3]

74 **Mohr AM**, Mott JL. Overview of microRNA biology. *Semin Liver Dis* 2015; **35**: 3-11 [PMID: 25632930 DOI: 10.1055/s-0034-1397344]

75 **Andrés-León E**, Cases I, Alonso S, Rojas AM. Novel miRNA-mRNA interactions conserved in essential cancer pathways. *Sci Rep* 2017; **7**: 46101 [PMID: 28387377 DOI: 10.1038/srep46101]

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MINIREVIEWS

# Multiple roles for cholinergic signaling in pancreatic diseases

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## Abstract

Cholinergic nerves are widely distributed throughout the human body and participate in various physiological activities, including sensory, motor, and visceral activities, through cholinergic signaling. Cholinergic signaling plays an important role in pancreatic exocrine secretion. A large number of studies have found that cholinergic signaling overstimulates pancreatic acinar cells through muscarinic receptors, participates in the onset of pancreatic diseases such as acute pancreatitis and chronic pancreatitis, and can also inhibit the progression of pancreatic cancer. However, cholinergic signaling plays a role in reducing pain and inflammation through nicotinic receptors, but enhances the proliferation and invasion of pancreatic tumor cells. This review focuses on the progression of cholinergic signaling and pancreatic diseases in recent years and reveals the role of cholinergic signaling in pancreatic diseases.

**Key Words:** Acetylcholine; Muscarinic receptors; Nicotinic receptors; Pancreatic exocrine; Pancreatitis; Pancreatic cancer

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**Core Tip:** The pancreas is a nerve-rich organ that lies behind the peritoneum and is surrounded by many nerve plexuses. Studies have found that cholinergic signaling is involved in the physiological function of the pancreas and the pathological process of pancreatic diseases due to its action on different receptors. Perhaps starting with cholinergic receptors could uncover potential therapeutic targets for pancreatic diseases.

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## INTRODUCTION

Acetylcholine is a neurotransmitter released by all cholinergic neurons that plays an important role in the peripheral and central nervous systems. The vagus nerve is the longest nerve in the human body and provides innervation to most organs, especially the organs of the digestive system. Cholinergic signaling released by vagus nerve activation mainly acts on cholinergic receptors. According to pharmacological properties, cholinergic receptors are divided into muscarinic receptors (M receptors) and nicotinic receptors (N receptors). Among them, M receptors are widely distributed in smooth muscles and glands and participate in the secretion of glands. M receptors are divided into 5 subtypes of M1-M5, among which M1, M3, and M5 receptors are coupled to Gq protein and M2 and M4 receptors are coupled to Gi protein[1,2]. Each of these five subtypes of M receptors has a unique distribution pattern and is expressed in many areas of the central nervous system and peripheral tissues[1,3-5]. N receptors are ligand-gated ion channels, a pentamer made up of 5 identical homologous subunits (including α1-10, β1-β5, γ, δ, and ε) and retain the potential to be activated by the appropriate agonist[6-8].

The pancreas is an important digestive organ of the human body and plays an important role in the digestion and absorption of food. Pancreatic diseases include acute pancreatitis (AP), chronic pancreatitis (CP), and pancreatic cancer (PCa). Recently, the incidence of pancreatic disease has been increasing year by year, posing a serious threat to human life and health[9-11]. Recent studies have found that cholinergic neuromodulation is involved in the occurrence and development of pancreatic diseases[12-14]. This article reviews the relationship between the three main types of pancreatic diseases (AP, CP, PCa) and cholinergic signaling.

## CHOLINERGIC SIGNALING AND PANCREATIC EXOCRINE FUNCTION

The exocrine system of the pancreas includes three different stages, namely, the cephalic stage, the stomach stage, and the intestinal stage[15]. The total amount of daily exocrine activity of the human pancreas is 1-2 L, and approximately 20% occurs in the cephalic stage, which is under the control of the vagus nerve[16-18]. Studies on humans and animals have shown that the cholinergic system regulates pancreatic exocrine secretion through the vagus nerve reflex. These reflexes originate in the dorsal motor nucleus of the vagus nerve in the medulla oblongata. The cranial preganglionic nerve fibers exit the vagus nerve and terminate at the intrapancreatic ganglia to form synapses through cholinergic preganglionic fibers[19,20].

The intrapancreatic ganglion are the integration center of pancreatic exocrine secretion, and terminal axons from these ganglia innervate approximately every acinar artery. Although preganglionic neurotransmission is mediated by acetylcholine through nicotinic and muscarinic receptors, postpancreatic innervation can be mediated by a variety of neurotransmitters, including acetylcholine, which acts on muscarinic receptors in pancreatic acinar cells[21,22]. Cholinergic agonists produce a pancreatic secretory response similar to that of cephalic stimulation, whereas cholinergic antagonists or resection of the vagus nerve can block the cephalic response. These results show that the acetylcholine released by the efferents of the vagus nerve is the primary mechanism by which sensory input leads to the regulation of pancreatic exocrine secretion[23-26]. The cholinergic system also plays an important role in the gastric and intestinal stages of pancreatic secretion regulation[27,28], which are regulated by nerves and bodily fluids. In the intestinal phase, exocrine secretion of the pancreas is mainly regulated by cholecystokinin (CCK) and other gastrointestinal hormones. However, the cholinergic system also plays a role in the secretion of human pancreatic enzymes stimulated by CCK[21,29]. The postprandial physiological dose of CCK mainly acts on the afferent pathway of the vagus nerve in the gastric and duodenal mucosa and stimulates pancreatic exocrine secretion through the cholinergic efferent nerve [19].

Studies have found that human pancreatic acinar cells preferentially express the M3 receptor[30] and are highly expressed in acinar cells[31]. Acetylcholine acts on the M3 receptor and couples to the Gq protein to activate phospholipase C and promote the release of intracellular calcium ions by initiating the phosphatidyl C-inositol triphosphate cascade and promoting the secretion of pancreatic acinar cells [2,32,33]. According to drug blocking studies, the M3 receptor is a muscarinic receptor that stimulates exocrine secretion form the pancreas[34-36]. However, some studies have shown that the M1 receptor can also control pancreatic exocrine secretion[37,38]. The inhibitory effect of drugs blocking the M1 receptor on the secretion of amylase in isolated pancreatic acinar cells was significantly greater than that of drugs blocking the M3 receptor[39]. Carbachol-induced amylase secretion was significantly impaired in the isolated acinars with M1 and M3 muscarinic receptor single knockout (KO) mice, and amylase secretion was eliminated in the acinar preparation of M1 and M3 muscarinic receptor double KO mice. Therefore, it is proposed that cholinergic signaling stimulates the secretion of pancreatic amylase and is mediated by the combination of M1 and M3 receptors[40].

## CHOLINERGIC SIGNALING AND AP

AP is a common pancreatic disease in clinical practice. AP has a rapid onset and progress, and high morbidity and mortality worldwide[11]. There are many causes of AP, such as biliary, hypertriglyceridemia, and chronic alcohol consumption, among which biliary is the most common factor[41]. However, the specific mechanism underlying the cause of pancreatitis is not clear. When the human body is exposed to cholinergic agonists, such as scorpion stings or organophosphorus pesticide poisoning, some patients will develop AP. This is direct evidence that cholinergic signaling stimulation is associated with the occurrence of human AP[42-45]. Organophosphorus pesticides can inhibit cholinesterase activity and cause a large amount of acetylcholine to accumulate in nerve endings. Similarly, scorpion toxin is a neurotoxic protein that can cause AP by activating the nerve pathway that releases acetylcholine[46,47].

Pancreatic duct ligation in the rat model is often used to simulate clinical AP caused by gallstone obstruction[48], but the severity of experimental pancreatitis is low except when used in the possum model. However, some researchers have found in a pancreatic duct ligation rat model that cholinergic signaling stimulation can aggravate AP inflammation, suggesting that cholinergic signaling may be involved in the pathogenesis of AP caused by cholangiopancreatic duct obstruction[49]. This may be due to an increase in M3 receptor expression induced by rat pancreatic duct ligation, which amplifies the overstimulation of cholinergic signaling on acinar cells and aggravates the intracellular stress response[50]. Before the first clinical onset of acute alcoholic pancreatitis, patients usually have a history of alcohol abuse for many years. Chronic alcohol intake can affect the exocrine regulation of the pancreas by interfering with the cholinergic and trypsin pathways[51]. Long-term use of ethanol feeding can significantly reduce pancreatic acetylcholinesterase activity in rats, while the expression level of pancreatic cholinergic M receptors has not changed, which increases the level and duration of acetylcholine in the pancreas and leads to excessive cholinergic signaling stimulation and damage to pancreatic acinar cells[52,53]. Ethanol-treated pancreatic acinar cells can aggravate the pancreatic injury response caused by the cholinergic agonist carbachol, and its effect may be mediated by protein kinase C downstream signaling of cholinergic receptors[54]. The cholinergic receptor antagonist atropine can improve pancreatitis induced by the combination of alcohol and cerulein suggesting that the cholinergic signaling pathway is involved in the pathogenesis of pancreatitis[52]. Therefore, acetylcholine may play a key role in the pathogenesis of acute alcoholic pancreatitis[12].

The M3 receptor is highly expressed in human pancreatic acinar cells[31]. Wan *et al*[55] used chemical genetic technology, in which designer receptors are exclusively activated by designer drugs, to express mutant M3 receptors in mouse acinar cells, causing them to lose their response to acetylcholine but can be activated by the specific drug clozapine-N-oxide (CNO). CNO can induce AP in mutant M3 receptor mice and cause more extensive acinar cell necrosis and inflammation. In addition, the use of M3 receptor antagonists can improve the severity of AP induced by cerulein in wild-type mice (Figure 1)[55].

These results indicate that the activation of the M3 receptor by cholinergic nerve terminals releasing acetylcholine may be one of the pathogeneses of AP. Muscarinic receptor agonists stimulate the activation of trypsinogen and nuclear factor-kappaB, which are two key signaling pathways in the pathogenesis of pancreatitis[56]. Reducing inflammation has always been a major goal in the treatment of AP, and many anti-inflammatory drugs have shown beneficial responses in experimental pancreatitis[57]. However, due to the lack of an in-depth understanding of its pathogenesis, there is currently no effective prevention and treatment strategy[58]. Through in-depth research on the cholinergic signaling pathway, it may be possible to block cholinergic signaling early to treat AP, especially in the prevention of pancreatitis after endoscopic retrograde cholangiopancreatography.

## CHOLINERGIC SIGNALING AND CP

CP is characterized by chronic inflammation and fibrosis of the pancreas caused by multiple factors. The incidence and prevalence of CP are increasing each year, but there is no current specific treatment[10]. The most common causes of CP are excessive drinking, smoking or genetic mutations[59]. Alcohol is considered to be one of the main risk factors for CP. A total of 40%-70% of CP patients become sick due to excessive alcohol consumption[10]. At the same time, excessive alcohol consumption increases the risk of PCa in individuals[60].

Recurrent episodes of AP have been associated with the progression of CP, which is more common in patients with alcoholism. It has been reported that a certain extent of chronic pancreatic damage was already present at the time of AP episodes[41]. The dose-response relationship between alcohol consumption for AP and CP is linear in males[61]. Alcohol-induced pancreatitis may be caused by the alcohol-induced increased viscosity of pancreatic secretions, which blocks the pancreatic duct, and by premature activation of trypsinogen in acinar cells[62].

The hypertonicity of intrapancreatic cholinergic neurons caused by chronic alcoholism may be involved in the pathogenesis of CP[13]. In CP patients, ethanol can cause excessive sensitivity of the

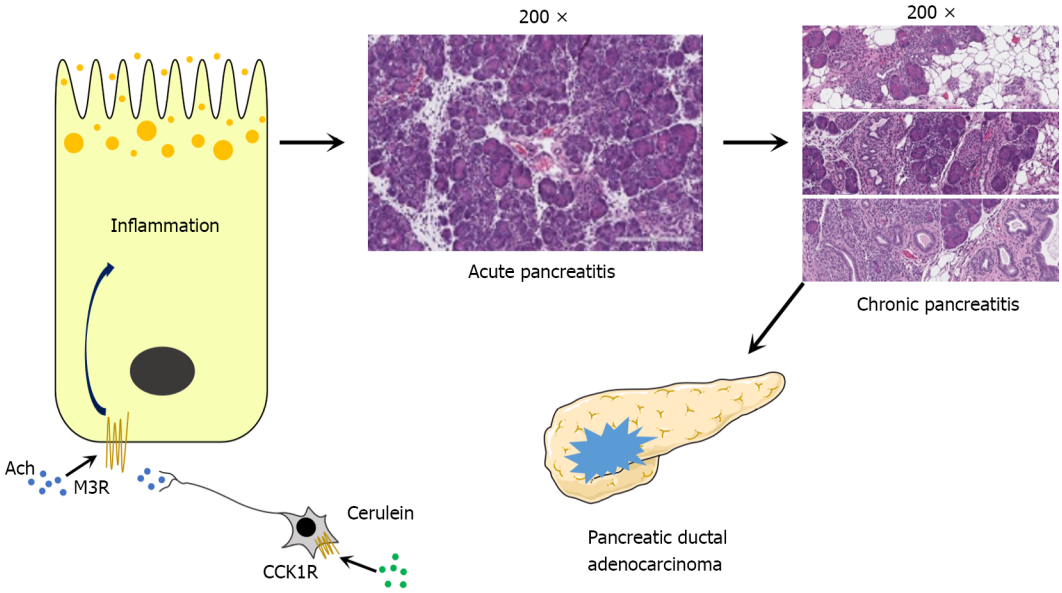


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**Figure 1 M3 receptor activation on pancreatic acinar cells causes acute and chronic pancreatitis.** Ach: Acetylcholine; CCK: Cholecystokinin.

pancreatic parasympathetic nerve pathways after a meal[63]. This may be due to chronic alcoholism interrupting the autonomic nerve suppression reflex, leading to the decentralization of the intermediate autonomic nerve (located in the gastric antrum and duodenum) and the intrapancreatic ganglia. Due to an increase in the activity of these autonomic nerves, cholinergic signaling in the pancreas increases, resulting in excessive protein secretion and obstruction of pancreatic juice flow[64,65]. Histopathological analysis of pancreatic tissue samples from patients with CP (including alcoholic pancreatitis) demonstrated that the density of cholinergic fibers in the pancreas of patients with CP was slightly increased compared with normal pancreatic tissue samples[66]. Therefore, the impaired interaction between cholinergic signaling and their receptors on pancreatic acinar cells may be a mechanism of the pathogenesis of alcoholic pancreatitis.

Compared with AP, few CP models use injury mechanisms that may be related to the pathogenesis of human diseases, and the clinical relevance of the pathogenesis of most CP models is unclear[48]. However, Wan *et al*[55] used a mutant M3 receptor mouse-induced CP model and observed typical CP features, such as extensive chronic inflammation, fibrosis, adipose tissue infiltration, and pancreatic atrophy (Figure 1)[55]. The use of this M3 receptor model may increase our understanding of the pathophysiological process of human CP and identify specific treatments.

Long-term and recurrent pain is a common characteristic of CP[10,67]. A large number of studies have shown that cholinergic nerves have a significant analgesic effect on chronic neuropathic pain, inflammatory pain, and visceral pain[68-70]. Choline transporter (CHT1) is considered to be the rate-limiting step of neuronal acetylcholine synthesis and is essential for the effective recovery of acetylcholine[71,72]. CHT1 is upregulated in CP-induced pain models. CHT1 specific inhibitor, hemicholinium-3, can significantly enhance CP-induced hyperalgesia and reduce the amount of acetylcholine in the dorsal root ganglia of the pancreas in a dose-dependent manner[73]. Further research found that acetylcholine reduces pain and inflammation through α7 nicotinic acetylcholine receptors (α7 nAChRs)[70,74-77]. The activation of α7 nAChRs enhances the autophagic flux of acinar cells mediated by the transcription factor EB pathway and promotes lysosomal degradation to inhibit acinar cell damage, thereby protecting experimental pancreatitis. It has been suggested that cholinergic signaling activation of endogenous α7 nAChRs in the pancreas may be an endogenous protective mechanism in the process of pancreatitis[78]. Therefore, the use of α7 nAChRs to develop analgesic and anti-inflammatory drugs will be a promising target, especially for the treatment of CP.

## CHOLINERGIC SIGNALING AND PCa

PCa is a gastrointestinal tumor with a poor prognosis, and more than 90% of PCa are pancreatic ductal adenocarcinoma (PDAC). According to reports, the 5-year survival rate for PCa patients in the United States from 2009 to 2015 was only 9%[79]. With the continuous increase in morbidity and mortality, PCa is expected to become the second leading cause of cancer-related deaths by 2030[80].

CP has been identified as a risk factor for PCa[81,82]. A meta-analysis found that CP increases the risk of PCa, and five years after the diagnosis of CP the risk of PCa increased nearly eightfold[83]. Pain is a characteristic feature of CP and PCa. Studies have found that increased nerve fiber density and hypertrophy are typical features of CP and PCa, and this pathological change appears to enhance and produce pancreatic neuropathic pain[84]. In vitro, myenteric plexus and dorsal root ganglia neurons were isolated from neonatal rats with CP and PCa, and they exhibited strong neurite outgrowth, more complex branching patterns, and somatic hypertrophy. They findings suggest that the intrapancreatic microenvironment in CP and PCa appears to be a key factor in the generation of pancreatic neuropathy and neural plasticity[85].

In-depth research on the tumor microenvironment found that perineural invasion is an important feature of PCa, which can lead to local tumor recurrence and poor prognosis[86]. As the degree of invasion increases, the survival rate of PCa patients is significantly reduced[87]. However, cholinergic signaling appears to play a tumor-suppressing role in PCa[14,88]. Regarding the relationship between heart rate variability (HRV) as an indicator of vagus nerve activity and the overall survival of patients with advanced PCa, it was found that higher vagus nerve activity represented by a higher initial HRV was significantly associated with a lower risk of death from PCa and was not affected by confounding factors such as age and cancer treatment[89]. The activation of M receptors by cholinergic signals can inhibit the progression of pancreatic tumors. Inhibition of the downstream EGFR/MAPK and PI3K/AKT signaling pathways of PCa cells through the M1 receptor signaling pathway inhibits tumor stem cells, $CD11b^+$ cells, tumor necrosis factor-α levels, and liver metastasis[14].

However, M3 receptor expression seems to be a biomarker for the poor prognosis of PDAC. Compared with patients with low M3 expression, patients with high M3 expression have a worse prognosis and shorter survival time, but the study did not find a statistically significant relationship between M3 receptor expression and peripheral nerve infiltration in PCa[90]. The presence of nonneuronal acetylcholine secreted by fibroblasts and pancreatic stellate cells in the microenvironment of pancreatic tumors activates the M3 receptor, leading to further tumor progression[91].

Oxidative stress and inflammatory signaling contribute to the development of pancreatitis. Español *et al*[92] reported that long-term inflammatory stimulation by LPS plus interferon-γ (IFN-γ) could induce muscarinic receptor expression which mainly involved the M3 and M5 receptors. It could also upregulate the expression of NOS and COX-2 to enhance the effect of carbachol on NO and $PGE_2$ production[92]. Transgenic overexpression of COX-2 in the pancreas induces CP and the formation of preinvasive ductal tumors[93,94]. Muscarinic receptors could serve as promising therapeutic targets for pancreatic inflammation and could prevent the transformation of CP to PCa.

The increase in acetylcholine levels by nAChRs can inhibit histone deacetylase 1-mediated CCL5 chemokines. This could weaken the ability of PDAC cells to recruit $CD8^+$ T cells and directly inhibit the production of IFN-γ by $CD8^+$ T cells. This effect is conducive to Th2 differentiation and could thereby promot tumor growth[86]. Smoking is one of the main causes of PCa[95], and approximately 21% of PCa deaths are attributed to smoking[96]. Studies have shown that nicotine (nAChR agonist) can increase the proliferation activity and self-renewal ability of PCa stem cells by activating the sonic hedgehog signaling pathway[97]. α7 nAChRs have also been shown to upregulate mucin-4 by coactivating the JAK2/STAT3 downstream signaling cascade *via* the MEK/ERK1/2 pathway, thereby increasing the migration and invasion capabilities of PCa cells[98]. Because the pancreas is widely innervated by many different neurons, the relationship between PCa and the nervous system is complicated[99]. Cholinergic signaling produces different effects based on the different receptor mechanisms involved and may become a potential therapeutic target for PCa in the future.

## CONCLUSION

Cholinergic signaling participates in the physiological function of the pancreas and the pathological process of pancreatic diseases. Although the mechanism by which cholinergic signaling regulates pancreatic diseases is still unclear, an increasing number of studies have shown that cholinergic signaling plays a key role in the occurrence and development of pancreatic diseases. There are many animal models of pancreatitis that can be used to help us study the pathogenesis and pathophysiological process of pancreatitis. Among them, rodent models are most commonly used to study acute and CP, but experimental pancreatitis is not necessarily the most relevant to human diseases[48]. Due to the lack of an in-depth understanding of its pathogenesis, there is no effective prevention and treatment strategy at present. Activation of the M3 receptor may be one of the causes of pancreatitis since muscarinic receptors are widely expressed in various glands and smooth muscles throughout the body. The mutant M3 receptor model provides an alternative method for the study of pancreatitis[55]. The pancreas is an organ rich in nervous tissue and lies behind the peritoneum and is surrounded by many nerve plexus. Cholinergic signaling has different effects due to its action on different receptors, which leads to a complicated pathogenesis of pancreatic diseases such as AP, CP, and pancreatic tumors (Table 1). Reducing or enhancing the signal downstream of the receptor may provide a potential therapeutic target in the future.

**Table 1 Role of different cholinergic receptors in pancreatic diseases**

| Pancreatic diseases | Receptor type | Relevant mechanism | Effect | Ref. |
|---|---|---|---|---|
| Acute pancreatitis | M3 | Receptor overexpression | Acinar cell hypersecretion | [49,50,52,55] |
| Chronic pancreatitis | M3 | Cholinergic signaling increases | Acinar cell hypersecretion | [13,64-66] |
| | M3/M5 | Receptor overexpression | Induce fibroblast proliferation | [92] |
| | α7 | Enhances the autophagic flux of acinar cells | Inhibit acinar cell damage | [70,74-77] |
| Pancreatic cancer | M1 | Inhibition of the EGFR/MAPK and PI3K/AKT signaling pathways | Inhibit the progression of pancreatic tumors | [14] |
| | M3 | Receptor overexpression | Induction of preinvasive ductal tumor formation | [90,92-94] |
| | α7 | Activating the JAK2/STAT3 signaling pathway | Increasing the migration and invasion capabilities of tumor cells | [86,97,98] |

## FOOTNOTES

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## REFERENCES

1 **Caulfield MP**, Birdsall NJ. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol Rev* 1998; **50**: 279-290 [PMID: 9647869]
2 **Qin K**, Dong C, Wu G, Lambert NA. Inactive-state preassembly of G(q)-coupled receptors and G(q) heterotrimers. *Nat Chem Biol* 2011; **7**: 740-747 [PMID: 21873996 DOI: 10.1038/nchembio.642]
3 **Wess J**. Molecular biology of muscarinic acetylcholine receptors. *Crit Rev Neurobiol* 1996; **10**: 69-99 [PMID: 8853955 DOI: 10.1615/critrevneurobiol.v10.i1.40]
4 **Volpicelli LA**, Levey AI. Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. *Prog Brain Res* 2004; **145**: 59-66 [PMID: 14650906 DOI: 10.1016/S0079-6123(03)45003-6]
5 **Abrams P**, Andersson KE, Buccafusco JJ, Chapple C, de Groat WC, Fryer AD, Kay G, Laties A, Nathanson NM, Pasricha PJ, Wein AJ. Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *Br J Pharmacol* 2006; **148**: 565-578 [PMID: 16751797 DOI: 10.1038/sj.bjp.0706780]
6 **Wang J**, Lindstrom J. Orthosteric and allosteric potentiation of heteromeric neuronal nicotinic acetylcholine receptors. *Br J Pharmacol* 2018; **175**: 1805-1821 [PMID: 28199738 DOI: 10.1111/bph.13745]
7 **Wonnacott S**, Bermudez I, Millar NS, Tzartos SJ. Nicotinic acetylcholine receptors. *Br J Pharmacol* 2018; **175**: 1785-1788 [PMID: 29878346 DOI: 10.1111/bph.14209]
8 **Hurst R**, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: from basic science to therapeutics. *Pharmacol Ther* 2013; **137**: 22-54 [PMID: 22925690 DOI: 10.1016/j.pharmthera.2012.08.012]
9 **Mizrahi JD**, Surana R, Valle JW, Shroff RT. Pancreatic cancer. *Lancet* 2020; **395**: 2008-2020 [PMID: 32593337 DOI: 10.1016/S0140-6736(20)30974-0]
10 **Beyer G**, Habtezion A, Werner J, Lerch MM, Mayerle J. Chronic pancreatitis. *Lancet* 2020; **396**: 499-512 [PMID:

32798493 DOI: 10.1016/S0140-6736(20)31318-0]
11 **Petrov MS**, Yadav D. Global epidemiology and holistic prevention of pancreatitis. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 175-184 [PMID: 30482911 DOI: 10.1038/s41575-018-0087-5]
12 **Grönroos JM**, Kaila T, Aho HJ, Nevalainen TJ. Decrease in the number of muscarinic receptors in rat pancreas after chronic alcohol intake. *Pharmacol Toxicol* 1989; **64**: 356-359 [PMID: 2748543 DOI: 10.1111/j.1600-0773.1989.tb00664.x]
13 **Tiscornia OM**, Dreiling DA. Physiopathogenic hypothesis of alcoholic pancreatitis: supranormal ecbolic stimulation of the "pancreon" units secondary to the loss of the negative component of pancreas innervation. *Pancreas* 1987; **2**: 604-612 [PMID: 3313381]
14 **Renz BW**, Tanaka T, Sunagawa M, Takahashi R, Jiang Z, Macchini M, Dantes Z, Valenti G, White RA, Middelhoff MA, Ilmer M, Oberstein PE, Angele MK, Deng H, Hayakawa Y, Westphalen CB, Werner J, Remotti H, Reichert M, Tailor YH, Nagar K, Friedman RA, Iuga AC, Olive KP, Wang TC. Cholinergic Signaling *via* Muscarinic Receptors Directly and Indirectly Suppresses Pancreatic Tumorigenesis and Cancer Stemness. *Cancer Discov* 2018; **8**: 1458-1473 [PMID: 30185628 DOI: 10.1158/2159-8290.CD-18-0046]
15 **Konturek SJ**, Pepera J, Zabielski K, Konturek PC, Pawlik T, Szlachcic A, Hahn EG. Brain-gut axis in pancreatic secretion and appetite control. *J Physiol Pharmacol* 2003; **54**: 293-317 [PMID: 14566070]
16 **Anagnostides A**, Chadwick VS, Selden AC, Maton PN. Sham feeding and pancreatic secretion. Evidence for direct vagal stimulation of enzyme output. *Gastroenterology* 1984; **87**: 109-114 [PMID: 6724252]
17 **Konturek SJ**, Zabielski R, Konturek JW, Czarnecki J. Neuroendocrinology of the pancreas; role of brain-gut axis in pancreatic secretion. *Eur J Pharmacol* 2003; **481**: 1-14 [PMID: 14637169 DOI: 10.1016/j.ejphar.2003.08.042]
18 **Defilippi C**, Solomon TE, Valenzuela JE. Pancreatic secretory response to sham feeding in humans. *Digestion* 1982; **23**: 217-223 [PMID: 6183160 DOI: 10.1159/000198753]
19 **Owyang C**. Physiological mechanisms of cholecystokinin action on pancreatic secretion. *Am J Physiol* 1996; **271**: G1-G7 [PMID: 8760100 DOI: 10.1152/ajpgi.1996.271.1.G1]
20 **Owyang C**, Logsdon CD. New insights into neurohormonal regulation of pancreatic secretion. *Gastroenterology* 2004; **127**: 957-969 [PMID: 15362050 DOI: 10.1053/j.gastro.2004.05.002]
21 **Niebergall-Roth E**, Singer MV. Central and peripheral neural control of pancreatic exocrine secretion. *J Physiol Pharmacol* 2001; **52**: 523-538 [PMID: 11787756]
22 **Singer MV**, Niebergall-Roth E. Secretion from acinar cells of the exocrine pancreas: role of enteropancreatic reflexes and cholecystokinin. *Cell Biol Int* 2009; **33**: 1-9 [PMID: 18948215 DOI: 10.1016/j.cellbi.2008.09.008]
23 **Becker S**, Niebel W, Singer MV. Nervous control of gastric and pancreatic secretory response to 2-deoxy-D-glucose in the dog. *Digestion* 1988; **39**: 187-196 [PMID: 3209002 DOI: 10.1159/000199624]
24 **Holtmann G**, Singer MV, Kriebel R, Stäcker KH, Goebell H. Differential effects of acute mental stress on interdigestive secretion of gastric acid, pancreatic enzymes, and gastroduodenal motility. *Dig Dis Sci* 1989; **34**: 1701-1707 [PMID: 2582983 DOI: 10.1007/BF01540047]
25 **Chariot J**, de la Tour J, Anglade P, Rozé C. Cholinergic mechanisms in the pancreas after extrinsic denervation in the rat. *Am J Physiol* 1987; **252**: G755-G761 [PMID: 3591943 DOI: 10.1152/ajpgi.1987.252.6.G755]
26 **Park HS**, Lee YL, Kwon HY, Chey WY, Park HJ. Significant cholinergic role in secretin-stimulated exocrine secretion in isolated rat pancreas. *Am J Physiol* 1998; **274**: G413-G418 [PMID: 9486197 DOI: 10.1152/ajpgi.1998.274.2.G413]
27 **You CH**, Rominger JM, Chey WY. Effects of atropine on the action and release of secretin in humans. *Am J Physiol* 1982; **242**: G608-G611 [PMID: 7091334 DOI: 10.1152/ajpgi.1982.242.6.G608]
28 **Blair EL**, Brown JC, Harper AA, Scratcherd T. A gastric phase of pancreatic secretion. *J Physiol* 1966; **184**: 812-824 [PMID: 5912208 DOI: 10.1113/jphysiol.1966.sp007949]
29 **Gabryelewicz A**, Kulesza E, Konturek SJ. Comparison of loxiglumide, a cholecystokinin receptor antagonist, and atropine on hormonal and meal-stimulated pancreatic secretion in man. *Scand J Gastroenterol* 1990; **25**: 731-738 [PMID: 2396088 DOI: 10.3109/00365529008997600]
30 **Lugea A**, Waldron RT, Mareninova OA, Shalbueva N, Deng N, Su HY, Thomas DD, Jones EK, Messenger SW, Yang J, Hu C, Gukovsky I, Liu Z, Groblewski GE, Gukovskaya AS, Gorelick FS, Pandol SJ. Human Pancreatic Acinar Cells: Proteomic Characterization, Physiologic Responses, and Organellar Disorders in ex Vivo Pancreatitis. *Am J Pathol* 2017; **187**: 2726-2743 [PMID: 28935577 DOI: 10.1016/j.ajpath.2017.08.017]
31 **Ji B**, Bi Y, Simeone D, Mortensen RM, Logsdon CD. Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. *Gastroenterology* 2001; **121**: 1380-1390 [PMID: 11729117 DOI: 10.1053/gast.2001.29557]
32 **Futatsugi A**, Nakamura T, Yamada MK, Ebisui E, Nakamura K, Uchida K, Kitaguchi T, Takahashi-Iwanaga H, Noda T, Aruga J, Mikoshiba K. IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism. *Science* 2005; **309**: 2232-2234 [PMID: 16195467 DOI: 10.1126/science.1114110]
33 **Wakui M**, Osipchuk YV, Petersen OH. Receptor-activated cytoplasmic Ca2+ spiking mediated by inositol trisphosphate is due to Ca2(+)-induced Ca2+ release. *Cell* 1990; **63**: 1025-1032 [PMID: 1701691 DOI: 10.1016/0092-8674(90)90505-9]
34 **Louie DS**, Owyang C. Muscarinic receptor subtypes on rat pancreatic acini: secretion and binding studies. *Am J Physiol* 1986; **251**: G275-G279 [PMID: 2426974 DOI: 10.1152/ajpgi.1986.251.2.G275]
35 **Iwatsuki K**, Horiuchi A, Yonekura H, Homma N, Haruta K, Chiba S. Subtypes of muscarinic receptors in pancreatic exocrine secretion in anesthetized dog. *Pancreas* 1989; **4**: 339-345 [PMID: 2734277 DOI: 10.1097/00006676-198906000-00011]
36 **van Zwam AJ**, Willems PH, Rodrigues de Miranda JF, de Pont JJ, van Ginneken CA. Binding characteristics of the muscarinic receptor subtype in rabbit pancreas. *J Recept Res* 1990; **10**: 119-135 [PMID: 2262932 DOI: 10.3109/10799899009064661]
37 **Singer MV**, Teyssen S, Küppers U. Influence of the M1-receptor antagonists telenzepine and pirenzepine on pancreatic secretory response to intraduodenal tryptophan in dogs. *Digestion* 1991; **48**: 34-42 [PMID: 1868967 DOI: 10.1159/000200661]
38 **Teyssen S**, Niebergall E, Chari ST, Singer MV. Comparison of two dose-response techniques to study the pancreatic

secretory response to intraduodenal tryptophan in the absence and presence of the M1-receptor antagonist telenzepine. *Pancreas* 1995; **10**: 368-373 [PMID: 7792293 DOI: 10.1097/00006676-199505000-00008]
39 **Schmid SW**, Modlin IM, Tang LH, Stoch A, Rhee S, Nathanson MH, Scheele GA, Gorelick FS. Telenzepine-sensitive muscarinic receptors on rat pancreatic acinar cells. *Am J Physiol* 1998; **274**: G734-G741 [PMID: 9575856 DOI: 10.1152/ajpgi.1998.274.4.G734]
40 **Gautam D**, Han SJ, Heard TS, Cui Y, Miller G, Bloodworth L, Wess J. Cholinergic stimulation of amylase secretion from pancreatic acinar cells studied with muscarinic acetylcholine receptor mutant mice. *J Pharmacol Exp Ther* 2005; **313**: 995-1002 [PMID: 15764735 DOI: 10.1124/jpet.105.084855]
41 **Lankisch PG**, Apte M, Banks PA. Acute pancreatitis. *Lancet* 2015; **386**: 85-96 [PMID: 25616312 DOI: 10.1016/S0140-6736(14)60649-8]
42 **Bartholomew C**. Acute scorpion pancreatitis in Trinidad. *Br Med J* 1970; **1**: 666-668 [PMID: 5443968 DOI: 10.1136/bmj.1.5697.666]
43 **Singh S**, Bhardwaj U, Verma SK, Bhalla A, Gill K. Hyperamylasemia and acute pancreatitis following anticholinesterase poisoning. *Hum Exp Toxicol* 2007; **26**: 467-471 [PMID: 17698941 DOI: 10.1177/0960327107076814]
44 **Roeyen G**, Chapelle T, Jorens P, de Beeck BO, Ysebaert D. Necrotizing pancreatitis due to poisoning with organophosphate pesticides. *Acta Gastroenterol Belg* 2008; **71**: 27-29 [PMID: 18396746]
45 **Marsh WH**, Vukov GA, Conradi EC. Acute pancreatitis after cutaneous exposure to an organophosphate insecticide. *Am J Gastroenterol* 1988; **83**: 1158-1160 [PMID: 3421227]
46 **Becerril B**, Marangoni S, Possani LD. Toxins and genes isolated from scorpions of the genus Tityus. *Toxicon* 1997; **35**: 821-835 [PMID: 9241777 DOI: 10.1016/s0041-0101(96)00198-5]
47 **Fletcher PL Jr**, Fletcher MD, Possani LD. Characteristics of pancreatic exocrine secretion produced by venom from the Brazilian scorpion, Tityus serrulatus. *Eur J Cell Biol* 1992; **58**: 259-270 [PMID: 1385124]
48 **Lerch MM**, Gorelick FS. Models of acute and chronic pancreatitis. *Gastroenterology* 2013; **144**: 1180-1193 [PMID: 23622127 DOI: 10.1053/j.gastro.2012.12.043]
49 **Samuel I**, Chaudhary A, Fisher RA, Joehl RJ. Exacerbation of acute pancreatitis by combined cholinergic stimulation and duct obstruction. *Am J Surg* 2005; **190**: 721-724 [PMID: 16226947 DOI: 10.1016/j.amjsurg.2005.07.010]
50 **Samuel I**, Zaheer S, Fisher RA, Zaheer A. Cholinergic receptor induction and JNK activation in acute pancreatitis. *Am J Surg* 2003; **186**: 569-574 [PMID: 14599627 DOI: 10.1016/j.amjsurg.2003.07.016]
51 **Grönroos JM**, Aho HJ, Nevalainen TJ. Cholinergic hypothesis of alcoholic pancreatitis. *Dig Dis* 1992; **10**: 38-45 [PMID: 1551245 DOI: 10.1159/000171342]
52 **Lugea A**, Gong J, Nguyen J, Nieto J, French SW, Pandol SJ. Cholinergic mediation of alcohol-induced experimental pancreatitis. *Alcohol Clin Exp Res* 2010; **34**: 1768-1781 [PMID: 20626730 DOI: 10.1111/j.1530-0277.2010.01264.x]
53 **Grönroos JM**, Kaila T, Hietaranta AJ. Alcohol, pancreatic muscarinic receptors and acute pancreatitis. *Exp Toxicol Pathol* 1994; **45**: 503-505 [PMID: 8054828 DOI: 10.1016/S0940-2993(11)80513-0]
54 **Satoh A**, Gukovskaya AS, Reeve JR Jr, Shimosegawa T, Pandol SJ. Ethanol sensitizes NF-kappaB activation in pancreatic acinar cells through effects on protein kinase C-epsilon. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G432-G438 [PMID: 16574982 DOI: 10.1152/ajpgi.00579.2005]
55 **Wan J**, Wang J, Wagner LE 2nd, Wang OH, Gui F, Chen J, Zhu X, Haddock AN, Edenfield BH, Haight B, Mukhopadhyay D, Wang Y, Yule DI, Bi Y, Ji B. Pancreas-specific CHRM3 activation causes pancreatitis in mice. *JCI Insight* 2021; **6** [PMID: 34314386 DOI: 10.1172/jci.insight.132585]
56 **Zhan X**, Wang F, Bi Y, Ji B. Animal models of gastrointestinal and liver diseases. Animal models of acute and chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G343-G355 [PMID: 27418683 DOI: 10.1152/ajpgi.00372.2015]
57 **Sah RP**, Saluja A. Molecular mechanisms of pancreatic injury. *Curr Opin Gastroenterol* 2011; **27**: 444-451 [PMID: 21844752 DOI: 10.1097/MOG.0b013e328349e346]
58 **Kambhampati S**, Park W, Habtezion A. Pharmacologic therapy for acute pancreatitis. *World J Gastroenterol* 2014; **20**: 16868-16880 [PMID: 25493000 DOI: 10.3748/wjg.v20.i45.16868]
59 **Singh VK**, Yadav D, Garg PK. Diagnosis and Management of Chronic Pancreatitis: A Review. *JAMA* 2019; **322**: 2422-2434 [PMID: 31860051 DOI: 10.1001/jama.2019.19411]
60 **Wang YT**, Gou YW, Jin WW, Xiao M, Fang HY. Association between alcohol intake and the risk of pancreatic cancer: a dose-response meta-analysis of cohort studies. *BMC Cancer* 2016; **16**: 212 [PMID: 26968702 DOI: 10.1186/s12885-016-2241-1]
61 **Samokhvalov AV**, Rehm J, Roerecke M. Alcohol Consumption as a Risk Factor for Acute and Chronic Pancreatitis: A Systematic Review and a Series of Meta-analyses. *EBioMedicine* 2015; **2**: 1996-2002 [PMID: 26844279 DOI: 10.1016/j.ebiom.2015.11.023]
62 **Klochkov A**, Kudaravalli P, Lim Y, Sun Y. Alcoholic Pancreatitis. StatPearls. Treasure Island (FL), 2021
63 **Hirano H**, Shimosegawa T, Meguro T, Shiga N, Koizumi M, Toyota T. Effects of ethanol on meal-stimulated secretion of pancreatic polypeptide and cholecystokinin: comparison of healthy volunteers, heavy drinkers, and patients with chronic pancreatitis. *J Gastroenterol* 1996; **31**: 86-93 [PMID: 8808434 DOI: 10.1007/BF01211192]
64 **Gregg JA**, Sharma MM. Endoscopic measurement of pancreatic juice secretory flow rates and pancreatic secretory pressures after secretin administration in human controls and in patients with acute relapsing pancreatitis, chronic pancreatitis, and pancreatic cancer. *Am J Surg* 1978; **136**: 569-574 [PMID: 707739 DOI: 10.1016/0002-9610(78)90312-4]
65 **Renner IG**, Rinderknecht H, Valenzuela JE, Douglas AP. Studies of pure pancreatic secretions in chronic alcoholic subjects without pancreatic insufficiency. *Scand J Gastroenterol* 1980; **15**: 241-244 [PMID: 7384747 DOI: 10.3109/00365528009181462]
66 **Ceyhan GO**, Demir IE, Rauch U, Bergmann F, Müller MW, Büchler MW, Friess H, Schäfer KH. Pancreatic neuropathy results in "neural remodeling" and altered pancreatic innervation in chronic pancreatitis and pancreatic cancer. *Am J Gastroenterol* 2009; **104**: 2555-2565 [PMID: 19568227 DOI: 10.1038/ajg.2009.380]
67 **Di Sebastiano P**, di Mola FF, Bockman DE, Friess H, Büchler MW. Chronic pancreatitis: the perspective of pain

generation by neuroimmune interaction. *Gut* 2003; **52**: 907-911 [PMID: 12740353 DOI: 10.1136/gut.52.6.907]
68 **Zhang XL**, Albers KM, Gold MS. Inflammation-induced increase in nicotinic acetylcholine receptor current in cutaneous nociceptive DRG neurons from the adult rat. *Neuroscience* 2015; **284**: 483-499 [PMID: 25453771 DOI: 10.1016/j.neuroscience.2014.10.018]
69 **Joshi SK**, Mikusa JP, Weaver B, Honore P. Morphine and ABT-594 (a nicotinic acetylcholine agonist) exert centrally mediated antinociception in the rat cyclophosphamide cystitis model of visceral pain. *J Pain* 2008; **9**: 146-156 [PMID: 18088559 DOI: 10.1016/j.jpain.2007.09.004]
70 **Freitas K**, Ghosh S, Ivy Carroll F, Lichtman AH, Imad Damaj M. Effects of α7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models. *Neuropharmacology* 2013; **65**: 156-164 [PMID: 23079470 DOI: 10.1016/j.neuropharm.2012.08.022]
71 **Okuda T**, Haga T. High-affinity choline transporter. *Neurochem Res* 2003; **28**: 483-488 [PMID: 12675135 DOI: 10.1023/a:1022809003997]
72 **Yamamura HI**, Snyder SH. Choline: high-affinity uptake by rat brain synaptosomes. *Science* 1972; **178**: 626-628 [PMID: 5086398 DOI: 10.1126/science.178.4061.626]
73 **Luo D**, Chen L, Yu B. Inhibition of the high affinity choline transporter enhances hyperalgesia in a rat model of chronic pancreatitis. *Biochem Biophys Res Commun* 2017; **488**: 204-210 [PMID: 28483526 DOI: 10.1016/j.bbrc.2017.05.036]
74 **van Westerloo DJ**, Giebelen IA, Florquin S, Bruno MJ, Larosa GJ, Ulloa L, Tracey KJ, van der Poll T. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. *Gastroenterology* 2006; **130**: 1822-1830 [PMID: 16697744 DOI: 10.1053/j.gastro.2006.02.022]
75 **Schneider L**, Jabrailova B, Soliman H, Hofer S, Strobel O, Hackert T, Büchler MW, Werner J. Pharmacological cholinergic stimulation as a therapeutic tool in experimental necrotizing pancreatitis. *Pancreas* 2014; **43**: 41-46 [PMID: 24212240 DOI: 10.1097/MPA.0b013e3182a85c21]
76 **Damaj MI**, Meyer EM, Martin BR. The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. *Neuropharmacology* 2000; **39**: 2785-2791 [PMID: 11044748 DOI: 10.1016/s0028-3908(00)00139-8]
77 **Rowley TJ**, McKinstry A, Greenidge E, Smith W, Flood P. Antinociceptive and anti-inflammatory effects of choline in a mouse model of postoperative pain. *Br J Anaesth* 2010; **105**: 201-207 [PMID: 20511332 DOI: 10.1093/bja/aeq113]
78 **Li B**, Wu J, Bao J, Han X, Shen S, Ye X, Dai J, Wu Z, Niu M, He Y, Ni J, Wen L, Wang X, Hu G. Activation of α7nACh receptor protects against acute pancreatitis through enhancing TFEB-regulated autophagy. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165971 [PMID: 32950676 DOI: 10.1016/j.bbadis.2020.165971]
79 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 7-30 [PMID: 31912902 DOI: 10.3322/caac.21590]
80 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]
81 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]
82 **Malka D**, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, Lévy P, Ruszniewski P. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut* 2002; **51**: 849-852 [PMID: 12427788 DOI: 10.1136/gut.51.6.849]
83 **Kirkegård J**, Mortensen FV, Cronin-Fenton D. Chronic Pancreatitis and Pancreatic Cancer Risk: A Systematic Review and Meta-analysis. *Am J Gastroenterol* 2017; **112**: 1366-1372 [PMID: 28762376 DOI: 10.1038/ajg.2017.218]
84 **Ceyhan GO**, Bergmann F, Kadihasanoglu M, Altintas B, Demir IE, Hinz U, Müller MW, Giese T, Büchler MW, Giese NA, Friess H. Pancreatic neuropathy and neuropathic pain--a comprehensive pathomorphological study of 546 cases. *Gastroenterology* 2009; **136**: 177-186.e1 [PMID: 18992743 DOI: 10.1053/j.gastro.2008.09.029]
85 **Demir IE**, Ceyhan GO, Rauch U, Altintas B, Klotz M, Müller MW, Büchler MW, Friess H, Schäfer KH. The microenvironment in chronic pancreatitis and pancreatic cancer induces neuronal plasticity. *Neurogastroenterol Motil* 2010; **22**: 480-490, e112 [PMID: 19912545 DOI: 10.1111/j.1365-2982.2009.01428.x]
86 **Yang MW**, Tao LY, Jiang YS, Yang JY, Huo YM, Liu DJ, Li J, Fu XL, He R, Lin C, Liu W, Zhang JF, Hua R, Li Q, Jiang SH, Hu LP, Tian GA, Zhang XX, Niu N, Lu P, Shi J, Xiao GG, Wang LW, Xue J, Zhang ZG, Sun YW. Perineural Invasion Reprograms the Immune Microenvironment through Cholinergic Signaling in Pancreatic Ductal Adenocarcinoma. *Cancer Res* 2020; **80**: 1991-2003 [PMID: 32098780 DOI: 10.1158/0008-5472.CAN-19-2689]
87 **Liebl F**, Demir IE, Mayer K, Schuster T, D'Haese JG, Becker K, Langer R, Bergmann F, Wang K, Rosenberg R, Novotny AR, Feith M, Reim D, Friess H, Ceyhan GO. The impact of neural invasion severity in gastrointestinal malignancies: a clinicopathological study. *Ann Surg* 2014; **260**: 900-7; discussion 907 [PMID: 25379860 DOI: 10.1097/SLA.0000000000000968]
88 **Kamiya A**, Hiyama T, Fujimura A, Yoshikawa S. Sympathetic and parasympathetic innervation in cancer: therapeutic implications. *Clin Auton Res* 2021; **31**: 165-178 [PMID: 32926324 DOI: 10.1007/s10286-020-00724-y]
89 **De Couck M**, Maréchal R, Moorthamers S, Van Laethem JL, Gidron Y. Vagal nerve activity predicts overall survival in metastatic pancreatic cancer, mediated by inflammation. *Cancer Epidemiol* 2016; **40**: 47-51 [PMID: 26618335 DOI: 10.1016/j.canep.2015.11.007]
90 **Zhang L**, Xiu D, Zhan J, He X, Guo L, Wang J, Tao M, Fu W, Zhang H. High expression of muscarinic acetylcholine receptor 3 predicts poor prognosis in patients with pancreatic ductal adenocarcinoma. *Onco Targets Ther* 2016; **9**: 6719-6726 [PMID: 27826198 DOI: 10.2147/OTT.S111382]
91 **Pfitzinger PL**, Fangmann L, Wang K, Demir E, Gürlevik E, Fleischmann-Mundt B, Brooks J, D'Haese JG, Teller S, Hecker A, Jesinghaus M, Jäger C, Ren L, Istvanffy R, Kühnel F, Friess H, Ceyhan GO, Demir IE. Indirect cholinergic activation slows down pancreatic cancer growth and tumor-associated inflammation. *J Exp Clin Cancer Res* 2020; **39**: 289 [PMID: 33357230 DOI: 10.1186/s13046-020-01796-4]
92 **Español AJ**, Maddaleno MO, Lombardi MG, Cella M, Martínez Pulido P, Sales ME. Treatment with LPS plus INF-γ induces the expression and function of muscarinic acetylcholine receptors, modulating NIH3T3 cell proliferation:

participation of NOS and COX. *Br J Pharmacol* 2014; **171**: 5154-5167 [PMID: 24990429 DOI: 10.1111/bph.12834]

93 **Colby JK**, Klein RD, McArthur MJ, Conti CJ, Kiguchi K, Kawamoto T, Riggs PK, Pavone AI, Sawicki J, Fischer SM. Progressive metaplastic and dysplastic changes in mouse pancreas induced by cyclooxygenase-2 overexpression. *Neoplasia* 2008; **10**: 782-796 [PMID: 18670639 DOI: 10.1593/neo.08330]

94 **Müller-Decker K**, Fürstenberger G, Annan N, Kucher D, Pohl-Arnold A, Steinbauer B, Esposito I, Chiblak S, Friess H, Schirmacher P, Berger I. Preinvasive duct-derived neoplasms in pancreas of keratin 5-promoter cyclooxygenase-2 transgenic mice. *Gastroenterology* 2006; **130**: 2165-2178 [PMID: 16762637 DOI: 10.1053/j.gastro.2006.03.053]

95 **Vincent A**, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378**: 607-620 [PMID: 21620466 DOI: 10.1016/S0140-6736(10)62307-0]

96 **GBD 2017 Pancreatic Cancer Collaborators**. . The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019; **4**: 934-947 [PMID: 31648972 DOI: 10.1016/S2468-1253(19)30347-4]

97 **Al-Wadei MH**, Banerjee J, Al-Wadei HA, Schuller HM. Nicotine induces self-renewal of pancreatic cancer stem cells *via* neurotransmitter-driven activation of sonic hedgehog signalling. *Eur J Cancer* 2016; **52**: 188-196 [PMID: 26689865 DOI: 10.1016/j.ejca.2015.10.003]

98 **Momi N**, Ponnusamy MP, Kaur S, Rachagani S, Kunigal SS, Chellappan S, Ouellette MM, Batra SK. Nicotine/cigarette smoke promotes metastasis of pancreatic cancer through α7nAChR-mediated MUC4 upregulation. *Oncogene* 2013; **32**: 1384-1395 [PMID: 22614008 DOI: 10.1038/onc.2012.163]

99 **Hutchings C**, Phillips JA, Djamgoz MBA. Nerve input to tumours: Pathophysiological consequences of a dynamic relationship. *Biochim Biophys Acta Rev Cancer* 2020; **1874**: 188411 [PMID: 32828885 DOI: 10.1016/j.bbcan.2020.188411]

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ORIGINAL ARTICLE

Basic Study

# Fecal gene detection based on next generation sequencing for colorectal cancer diagnosis

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## Abstract

### *BACKGROUND*

Colorectal cancer (CRC) is one of the most common malignancies worldwide. Given its insidious onset, the condition often already progresses to advanced stage when symptoms occur. Thus, early diagnosis is of great significance for timely clinical intervention, efficacy enhancement, and prognostic improvement. Featuring high throughput, fastness, and rich information, next generation sequencing (NGS) can greatly shorten the detection time, which is a widely used detection technique at present.

### *AIM*

To screen specific genes or gene combinations in fecal DNA that are suitable for diagnosis and prognostic prediction of CRC, and to establish a technological

platform for CRC screening, diagnosis, and efficacy monitoring through fecal DNA detection.

***METHODS***

NGS was used to sequence the stool DNA of patients with CRC, which were then compared with the genetic testing results of the stool samples of normal controls and patients with benign intestinal disease, as well as the tumor tissues of CRC patients. Specific genes or gene combinations in fecal DNA suitable for diagnosis and prognostic prediction of CRC were screened, and their significances in diagnosing CRC and predicting patients' prognosis were comprehensively evaluated.

***RESULTS***

High mutation frequencies of *TP53*, *APC,* and *KRAS* were detected in the stools and tumor tissues of CRC patients prior to surgery. Contrastively, no pathogenic mutations of the above three genes were noted in the postoperative stools, the normal controls, or the benign intestinal disease group. This indicates that tumor-specific DNA was detectable in the preoperative stools of CRC patients. The preoperative fecal expression of tumor-associated genes can reflect the gene mutations in tumor tissues to some extent. Compared to the postoperative stools and the stools in the two control groups, the pathogenic mutation frequencies of *TP53* and *KRAS* were significantly higher for the preoperative stools ($\chi^2 = 7.328$, $P < 0.05$; $\chi^2 = 4.219$, $P < 0.05$), suggesting that fecal *TP53* and *KRAS* genes can be used for CRC screening, diagnosis, and prognostic prediction. No significant difference in the pathogenic mutation frequency of the *APC* gene was found from the postoperative stools or the two control groups ($\chi^2 = 0.878$, $P > 0.05$), so further analysis with larger sample size is required. Among CRC patients, the pathogenic mutation sites of *TP53* occurred in 16 of 27 preoperative stools, with a true positive rate of 59.26%, while the pathogenic mutation sites of *KRAS* occurred in 10 stools, with a true positive rate of 37.04%. The sensitivity and negative predictive values of the combined genetic testing of *TP53* and *KRAS* were 66.67% (18/27) and 68.97%, respectively, both of which were higher than those of *TP53* or *KRAS* mutation detection alone, suggesting that the combined genetic testing can improve the CRC detection rate. The mutation sites *TP53* exon 4 A84G and *EGFR* exon 20 I821T (mutation start and stop positions were both 7579436 for the former, while 55249164 for the latter) were found in the preoperative stools and tumor tissues. These "undetected" mutation sites may be new types of mutations occurring during the CRC carcinogenesis and progression, which needs to be confirmed through further research. Some mutations of "unknown clinical significance" were found in such genes as *TP53*, *PTEN*, *KRAS*, *BRAF*, *AKT1*, and *PIK3CA*, whose clinical values is worthy of further exploration.

***CONCLUSION***

NGS-based fecal genetic testing can be used as a complementary technique for the CRC diagnosis. Fecal *TP53* and *KRAS* can be used as specific genes for the screening, diagnosis, prognostic prediction, and recurrence monitoring of CRC. Moreover, the combined testing of *TP53* and *KRAS* genes can improve the CRC detection rate.

**Key Words:** Colorectal cancer; Feces; Next generation sequencing; Diagnosis; Gene

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**Core Tip:** Colorectal cancer (CRC) is characterized by high morbidity and mortality, as well as low early diagnosis rate. The development of current gold standard for clinical diagnosis of CRC is restricted due to its invasiveness. The purpose of this study is to explore the potential value of fecal gene detection based on next generation sequencing in the diagnosis of CRC, to screen specific genes or gene combinations suitable for CRC diagnosis and prognosis prediction in fecal DNA, and to establish a technical platform for fecal DNA detection for CRC screening, diagnosis, and efficacy monitoring.

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## INTRODUCTION

Colorectal cancer (CRC), as a health issue of great concern worldwide, poses a serious threat to human health. According to the latest data released by the International Agency for Research on Cancer, there were approximately 19.3 million new cancer cases and nearly 10 million cancer deaths globally in 2020. Among them, new CRC cases amounted to about 1.93 million (10%), ranking third, while CRC deaths amounted to about 0.94 million (9.4%), ranking second. For female patients, there were approximately 860000 new cases of CRC (9.4%), second only to breast cancer, and nearly 870000 CRC deaths (9.5%), ranking third. As for male population, the numbers of new cases and deaths both ranked third for CRC, which were 930000 (10.6%) and 50000 (9.3%), respectively[1].

In recent years, the morbidity and mortality of CRC have been on the rise in China, and the onset age of patients has gradually become younger. Early detection of CRC is difficult due to the insidious onset, long progressive course, and unobvious symptoms and signs of patients at the onset stage. As the tumor grows and gradually compresses the intestinal cavity, such symptoms as altered bowel habit and abnormal stool color or traits may occur. At this time, the condition often already progresses to advanced stage. Studies have shown that the 5-year survival of patients with early CRC can reach 90%, while is only about 12.5% for advanced stage patients[2]. Thus, early diagnosis is conducive to early intervention and treatment of CRC, which is of great significance for improving the efficacy and prognosis[3,4]. In response, the American Cancer Society recommended the CRC screening since the age of 45 for adults in 2018, which is 5 years earlier than the previously recommended age[5].

The occurrence and progression are a multi-factor, multi-stage complex process[6-8]. It is generally believed that CRC is caused by the mutations and malignant proliferation of colorectal mucosal epithelial cells. Other studies have shown that CRC can develop from inflammatory bowel disease (IBD). IBD is mainly characterized by intestinal mucosal barrier dysfunction, because the changes of cytokines aggravate tissue damage and eventually lead to tumorigenesis. It is reported that patients with ulcerative colitis have a higher risk of developing CRC than the general population[9]. In addition, microRNA and tumor microenvironment also have potential pathogenic effects on CRC[10,11]. Currently, the clinically recognized gold standard for CRC detection remains endoscopy combined with histopathological analysis. Given the heterogeneity of tumor tissues and the invasiveness of endoscopy, the same patient cannot be sampled multiple times, so that the genetic testing results cannot fully display the mutation spectrum of oncogenic genes[12]. According to the Chinese *Expert Consensus on Early Diagnosis and Screening Strategies for Colorectal Tumors* published in October 2018, fecal detection is a promising method for early diagnosis[13]. Its theoretical basis is that the tumor cells in the intestinal cavity of CRC patients adhere less to the base and can be continuously exfoliated from the colorectal mucosa. Studies have shown that tumor-associated DNA can stably exist in the stools, which has high specificity for the detection of CRC and is the most suitable biomarker in fecal detection[14-16]. By extracting the DNA of shed tumor cells in stools and analyzing their mutations, the traits of intestinal tumor cells can be reflected. Fecal DNA detection, as an emerging, noninvasive technique for screening CRC, has attracted great attention in recent years, which has the advantages of noninvasiveness, convenient access to materials, good patient compliance, and continuous dynamic monitoring. It is in line with the concept and conforms to the trend of "precision medicine", which is an exceptionally promising detection method.

With the continuous development of molecular biotechnology, next generation sequencing (NGS) has emerged to gradually become a hot research topic worldwide. It is also known as high-throughput sequencing, which operates on the following principles: Four different deoxynucleotide triphosphates (dNTPs) are labeled with different fluorescent colors. Conforming to the complementary base pairing rule, the bound base releases the corresponding fluorescence by the action of DNA polymerase every time a dNTP is added. Finally, the base is identified according to the fluorescent signal. Compared to the first-generation Sanger sequencing, NGS is a set of technologies that completely change the standard concept of nucleic acid sequencing. Capable of detecting millions of DNA molecules simultaneously, it achieves concurrent synthesis and sequencing, which can greatly shorten the detection time, and has the advantages of high throughput, high efficiency, and rich information[17,18]. NGS can more comprehensively display the full spectrum of tumor-associated genes, which can reflect the changes in tumor-specific genes, expand the understanding of tumor-specific genes, and improve the predictability of targeted therapy, suggesting its significance in achieving personalized tumor treatment and developing new therapeutic strategies[19-21]. Currently, NGS has been widely applied in the research of various solid tumors, including CRC. Kraus and his colleagues performed NGS analysis on the tissue paraffin sections and blood samples of 152 CRC patients based on 18 CRC-related genes. Their results showed that NGS had a high detection rate and could effectively identify the high-risk patients with hereditary CRC[22]. In a study by Li *et al*[23], the NGS (Ion Torrent PGM platform) technology was applied to analyze 22 tumor-associated mutation hotspots in 526 CRC patients, finding that *TP53* and *KRAS* mutations were the most common in tumor tissues, and confirming that NGS was a highly sensitive detection technique.

This study performed NGS-based genetic testing of 50 tumor-associated mutation hotspots in the stools of CRC patients, and compared them with the genetic testing results of corresponding tumor tissues, with an aim to find the fecal genes or gene combinations with high specificity and sensitivity,

and to establish a technological platform for CRC screening, diagnosis, and efficacy monitoring through fecal DNA detection. The findings herein will provide support and basis for personalized diagnosis and treatment of CRC.

## MATERIALS AND METHODS

### Subjects

Fifty tumor tissues, 27 preoperative stools, and 19 postoperative stools (stools formed over 7 d postoperatively) collected from patients with CRC were included in an experimental group. Twenty stool samples collected from healthy subjects were included in a normal control group. Seventeen stool samples of patients with benign intestinal polyps confirmed by digestive endoscopy were collected, as well as three stool samples from patients with ulcerative colitis, totaling 20 samples, were included in a benign control group. Postoperative pathological reports of polyp patients revealed inflammatory or adenomatous polyps, none of which had progressed to the CRC stage.

This study was approved by the Ethics Committee of Taizhou People's Hospital in Jiangsu, and the patients provided signed informed consent for sample collection.

### Main reagents

Fecal DNA sample preservation tubes, tissue nucleic acid preservation tubes, fecal DNA extraction kit (immunomagnetic bead method), universal columnar genomic nucleic acid extraction kit, fast DNA library preparation kit were provided by Cowin Bio., Jiangsu, while high-throughput kit (MGISEQ-2000RS) was provided by MGI, Shenzhen.

### Samples sources

The subjects of this project are patients who were diagnosed with CRC for the first time and underwent surgery at Taizhou People's Hospital from January 2019 to January 2021. A total of 54 tumor tissue samples, 45 preoperative stool samples, and 41 postoperative stool samples were collected. The normal control group consisted of healthy physical examination takers without malignancies (including CRC), intestinal polyps, or IBD, from whom 20 stool samples were collected. The benign control group comprised 20 patients who were diagnosed with intestinal polyps or IBD by digestive endoscopy, and admitted to the Department of Gastroenterology, Taizhou People's Hospital for polypectomy or anti-inflammatory treatment. Twenty formed stool samples were collected from patients who had not taken laxatives preoperatively or from patients before receiving anti-inflammatory therapies. The above samples were tested by NGS, unreliable results were filtered during analysis of genetic testing results based on biological information, and poor quality samples were eliminated (heterogeneous tumor tissues, atypical fecal specimens, and little or degraded DNA in stools). Finally, effectively detected samples included 50 tumor tissues, 27 preoperative stools, 19 postoperative stools, 20 stools in the normal control group, and 20 stools in the benign control group.

### Sample collection and processing

**Stool samples:** Patients were instructed to use the fecal DNA preservation tube correctly (tubes contained protective solution to prevent the degradation of nucleic acid, while steel beads played a mixing role). They were asked to take samples immediately after natural defecation. Using sampling spoon located on the tube cap, typical formed stool about the size of soybean was excavated while avoiding spillover of protective solution and steel beads in the tube. After tightening the tube cap, the patients were asked to shake the tubes for approximately 30 s for uniform mixing. The samples were stored in a -80 °C refrigerator for subsequent testing.

**Tissue samples:** The freshly isolated tumor tissues were quickly cut into pieces (< 0.5 cm in thickness) using sterile tissue scissors, and then the tissue pieces were soaked in the protective solution of preservation tubes (if the volume was excessively large, the protective solution would not easily penetrate the tissues, which could not protect the nucleic acid from degradation). After tightening the tube caps, the samples were stored in a 4–8 °C refrigerator for subsequent testing.

### DNA extraction from stool samples

The DNA in stool samples should be extracted strictly in accordance with the kit instructions (immunomagnetic bead method). Initially, the samples were homogenized, and then the precipitates (fecal particles) were removed, and the supernatants were retained for subsequent use. Different reagents were added to the corresponding wells of deep 96-well plates as *per* the instruction procedures, and then the deep well plates and magnetic sleeves containing the reagents were placed at the corresponding positions of nucleic acid extractor, followed by running of the stool extraction program. Under high salinity condition, DNA can bind to the surfaces of silica-coated Magbeads. After rinsing, high-purity DNA was eluted and stored in Buffer GE. The DNA-containing Buffer GE was transferred to an EP tube and stored under refrigeration for subsequent testing.

Table 1 details the reaction system. Relevant reagents were added to the corresponding wells of deep 96-well plates (wells 1–6 corresponded to one sample, wells 7–12 corresponded to another sample, and so forth).

The deep 96-well plates and corresponding magnetic sleeves were placed into the corresponding positions of an automatic nucleic acid extractor, and then the fecal DNA extraction program was initiated. After running for about 20 min, the program was paused, and each 220 μL of pre-prepared mixture (Magbeads PN 20 μL, isopropanol 200 μL) was added to the first well of each sample, followed by continuation of the program. The product in the last well of each sample was transferred to the centrifuge tube and stored at low temperature for subsequent testing.

### DNA extraction from tissue samples

DNA was extracted from tissue samples strictly following the instructions of universal columnar genomic nucleic acid extraction kit. Initially, fresh tumor tissues were ground to small pieces and added with lysis buffer and proteinase K for complete digestion and lysis. Then, RNase was added to remove RNA. Since DNA was insoluble in absolute ethanol, the samples added with absolute ethanol were centrifuged to adsorb the DNA on the column. The residual impurities dissolved in absolute ethanol were discarded along with the liquid waste in the collection tube. Next, the collection tube was replaced with a new one, and the centrifugation was repeated several times. The DNA-containing adsorption column was placed at room temperature, and let stand to dry the absolute ethanol. The elution buffer was suspended, added to the adsorption column with a new collection tube, and centrifuged, and then the DNA-containing eluate in the collection tube was collected and refrigerated for subsequent testing.

The nucleic acid extraction was carried out in Cowin Bio., Jiangsu.

### Library construction and NGS detection

In accordance with the instructions of fast DNA library preparation kit, the hotspot mutation panels of 50 tumor-associated genes designed for specific genomic regions or targets were added initially based on the multiplex polymerase chain reaction (PCR) library construction technology, and then multiplex PCR enrichment was performed on specific gene region fragments. Next, the obtained target gene fragments were subjected to end repair, barcode adapter ligation, and PCR amplification (Eastwin Scientific Equipment, Suzhou) to prepare a library. Afterwards, the library was quantified with Qubit 3.0 fluorescence quantifier (Thermo Fisher Scientific, Shanghai), and the fragment size of the library was analyzed with the Bioptic Qsep 100 automatic nucleic acid analyzer (Bioptic Inc., Jiangsu) to evaluate the library quality. Finally, a high-throughput sequencer (MGI, Shenzhen) was used for sequencing, and the data splitting, denoising, and comparison were accomplished in the Linux system to obtain the gene mutation results of various samples.

The library construction and NGS were carried out in Cowin Bio., Jiangsu.

### Statistical analysis

Data were analyzed using SPSS 26.0 software. The gene mutation frequencies are expressed as the numbers of cases or percentages. Inter-group differences were examined by $\chi^2$ and Fisher exact tests. $P < 0.05$ was considered statistically significant.

## RESULTS

### Clinical data analysis of CRC patients

Among the CRC patients corresponding to 50 tumor tissue samples, 24 were female (48.00%) and 26 were male (52.00%). The age span was large (35–84 years), and the median age was 59.5 years. According to the histopathological results of tumor tissues, there were 29 cases of rectal cancer (58.00%), 11 cases of left colon cancer + sigmoid colon cancer (22.00%), and 10 cases of right colon cancer (20.00%). Twelve cases (24.00%) were well-differentiated + well-to-moderately differentiated, 25 (50.00%) were moderately differentiated, and 12 (24.00%) were moderately-to-poorly differentiated + poorly differentiated (the degree of differentiation was not determined in 1 of 50 colorectal cancer tumor tissues). Among 19 cases (38.00%) of lymph node metastasis, only 1 had distant metastasis (metastasis to the liver). Regarding pathological staging (according to eighth edition of AJCC TNM staging system), 33 cases (66.00%) were at stages I + II, and 17 (34.00%) were at stages III + IV (Table 2).

### Gene mutations in various samples

As is clear from the NGS testing results of CRC specimens in Table 3, genes like *TP53*, *APC*, *KRAS*, *PTEN*, *MET*, *HRAS*, and *PDGFRA* had high incidences of mutations in tumor tissues and preoperative stools, while in postoperative stools, the mutation frequencies of *TP53*, *HRAS*, and *PDGFRA* were high. Despite high mutation frequencies of *HRAS* and *PDGFRA* in the three groups of samples, their mutations were mostly same sense mutations, meaning that there were only base mutations, without changes in amino acids, which had no clinical significance.

**Table 1 The reaction system**

| Position | Reagent |
|---|---|
| Wells 1 and 7 | Proteinase K: 20 μL |
| | Lysate: 200 μL |
| Wells 2 and 8 | Buffer KCL: 750 μL |
| Wells 3 and 9 | Buffer GW1: 750 μL |
| Wells 4 and 10 | Buffer GW2: 750 μL |
| Wells 5 and 11 | Buffer MW3: 750 μL |
| Wells 6 and 12 | Buffer GE: 100 μL |

**Table 2 Clinical data analysis of colorectal cancer patients**

| Clinical feature | Stratification | Number of people (%) |
|---|---|---|
| Age (years old) | ≥ 60 | 30 (60.00) |
| | < 60 | 20 (40.00) |
| Sex | Male | 26 (52.00) |
| | Female | 24 (48.00) |
| Tumor location | Sigmoid colon + left hemicolon | 11 (22.00) |
| | Right hemicolon | 10 (20.00) |
| | Rectum | 29 (58.00) |
| Tumor size (cm) | ≥ 6 | 12 (24.00) |
| | < 6 | 38 (76.00) |
| Degree of tumor differentiation | High + medium high | 12 (24.00) |
| | Moderately | 25 (50.00) |
| | Low medium + low | 12 (24.00) |
| TNM classification | I + II | 33 (66.00) |
| | III + IV | 17 (34.00) |

As shown in Table 4, the *PDGFRA*, *HRAS*, and *KIT* mutations occurred in only 3 of 20 stool samples in the normal control group. In the benign control group, only 4 stool samples had *PDGFRA*, *HRAS*, *KIT*, or *STK11* mutations.

Since every gene has numerous mutation sites, different site mutations have different meanings. We classified and summarized the results of gene sequencing, and queried them online (https://www.ncbi.nlm.nih.gov/clinvar/). After combining the start and stop positions of each gene mutation site, various gene mutation sites were classified into four types depending on their pathogenicity: "Pathogenic", "benign", "unknown clinical significance", and "undetected in the system" (hereinafter referred to as "undetected"). "Benign" gene mutation sites imply a large number of mutations that can occur multiple times in the normal population, albeit less than 1% incidences. Mutation sites of "unknown clinical significance" indicate that such mutations are neither pathogenic nor benign, on whom the experimental conclusions in the literature reports are inconsistent. There is insufficient evidence to clearly classify them, and further exploration is required. "Undetected" mutation sites are identified by comprehensively checking multiple items such as the exons, the types of amino acid mutations, and the start and end positions of mutations. In the case of non-conformity of any item, the corresponding mutation site is regarded as "undetected". In Table 3, the mutations of the *PTEN* gene in tumor tissues were quite frequent, all of which were mutation sites of "unknown clinical significance". Among the 27 cases of preoperative stools, 1 had pathogenic mutation. Given the small case number, the possible cause of tumor heterogeneity was not ruled out. The *MET* gene exhibited high mutation frequencies in both tumor tissues and preoperative stools, all of which were "benign" mutations upon query. The four *MET* mutations in the postoperative stools were all "undetected" mutations. In Table 4, the mutation sites of *KIT* and *STK11* in the benign control group were benign mutation sites, while the rest were all same sense mutations and had no clinical significance.

**Table 3 Summary of gene mutations in colorectal cancer patients**

| Gene | Tumor tissue | | Preoperative feces | | Postoperative stools | |
|---|---|---|---|---|---|---|
| | Total number of mutations | Mutation frequency | Total number of mutations | Mutation frequency | Total number of mutations | Mutation frequency |
| *TP53* | 40 | 62.00% (31/50) | 27 | 59.26% (16/27) | 7 | 31.58% (6/19) |
| *APC* | 15 | 24.00% (12/50) | 3 | 11.11% (3/27) | 0 | 0 |
| *KRAS* | 30 | 58.00% (29/50) | 11 | 37.04% (10/27) | 0 | 0 |
| *PIK3CA* | 13 | 22.00% (11/50) | 1 | 3.70% (1/27) | 2 | 10.53% (2/19) |
| *FBXW7* | 5 | 10.00% (5/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *GNAS* | 2 | 4.00% (2/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *PTEN* | 11 | 22.00% (11/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *ABL1* | 0 | 0 | 1 | 3.70% (1/27) | 0 | 0 |
| *PDGFRA* | 12 | 24.00% (12/50) | 8 | 29.63% (8/27) | 6 | 31.58% (6/19) |
| *ATM* | 1 | 2.00% (1/50) | 0 | 0 | 0 | 0 |
| *SMAD4* | 1 | 2.00% (1/50) | 0 | 0 | 0 | 0 |
| *BRAF* | 2 | 4.00% (2/50) | 0 | 0 | 2 | 10.53% (2/19) |
| *PTPN11* | 1 | 2.00% (1/50) | 0 | 0 | 0 | 0 |
| *NRAS* | 1 | 2.00% (1/50) | 0 | 0 | 0 | 0 |
| *CTNNB1* | 3 | 6.00% (3/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *STK11* | 2 | 4.00% (2/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *AKT1* | 1 | 2.00% (1/50) | 1 | 3.70% (1/27) | 0 | 0 |
| *CDKN2A* | 3 | 6.00% (3/50) | 4 | 7.41% (2/27) | 3 | 5.26% (1/19) |
| *HRAS* | 21 | 42.00% (21/50) | 12 | 40.74% (11/27) | 8 | 42.11% (8/19) |
| *EGFR* | 2 | 4.00% (2/50) | 2 | 7.41% (2/27) | 1 | 5.26% (1/19) |
| *IDH1* | 2 | 4.00% (2/50) | 2 | 7.41% (2/27) | 1 | 5.26% (1/19) |
| *NOTCH1* | 2 | 4.00% (2/50) | 0 | 0 | 2 | 5.26% (1/19) |
| *VHL* | 0 | 0 | 1 | 3.70% (1/27) | 0 | 0 |
| *KIT* | 7 | 14.00% (7/50) | 2 | 7.41% (2/27) | 4 | 21.05% (4/19) |
| *MET* | 9 | 18.00% (9/50) | 5 | 18.52% (5/27) | 4 | 21.05% (4/19) |
| *MLH1* | 3 | 6.00% (3/50) | 2 | 7.41% (2/27) | 2 | 10.53% (2/19) |
| *MPL* | 0 | 0 | 0 | 0 | 1 | 5.26% (1/19) |

Total number of mutations: The overall number of mutations of any gene in various samples. Mutation frequency: The number of people with mutations in various samples/total number of people.

This study focused on analyzing the pathogenic mutation sites of various genes.

### Comparison between preoperative stool and tumor tissue results in CRC patients

As shown in Table 5, the *TP53*, *APC*, and *KRAS* genes had high incidences of pathogenic mutations in tumor tissues and preoperative stools among CRC patients. Despite high mutation frequency of *PIK3CA* in tumor tissues, its pathogenic mutation frequency in preoperative stools was only 3.70% (1/27). The possible causes of tumor heterogeneity or individual disparity could not be ruled out. Although other genes had pathogenic mutations, their mutation frequencies were rather low.

### Comparison between preoperative/postoperative stool results of CRC patients and control stool results

For CRC patients, the pathogenic mutation incidences of *APC*, *TP53*, and *KRAS* in the preoperative stools were 11.11% (3/27), 37.04% (10/27), and 25.93% (7/27), respectively. There were mutations of *PIK3CA*, *BRAF*, and *MPL* genes in the postoperative stools. Although they were pathogenic, only one

**Table 4 Fecal gene mutation results in the control groups**

| Group | Genes | Location | Amino acid mutation | Mutation frequency |
|---|---|---|---|---|
| Normal control | *PDGFRA* | Exon 19 | V824V | 2 |
| | *KIT* | Exon 10 | K546K | 1 |
| | *HRAS* | Exon 2 | H27H | 1 |
| Intestinal benign disease | *PDGFRA* | Exon 19 | V824V | 3 |
| | *HRAS* | Exon 2 | H27H | 1 |
| | *KIT* | Exon 10 | M541L | 1 |
| | *STK11* | Exon 8 | F354L | 1 |

**Table 5 Comparison of pathogenic mutation sites in preoperative stools *vs* tumor tissues**

| Gene | Positive rate of pathogenic gene mutation sites | |
|---|---|---|
| | Preoperative feces | Tumor tissues |
| *TP53* | 37.04% (10/27) | 46.00% (23/50) |
| *APC* | 11.11% (3/27) | 18.00% (9/50) |
| *KRAS* | 25.93% (7/27) | 54.00% (27/50) |
| *PIK3CA* | 3.70% (1/27) | 22.00% (11/50) |
| *FBXW7* | 3.70% (1/27) | 6.00% (3/50) |
| *GNAS* | 3.70% (1/27) | 4.00% (2/50) |
| *PTEN* | 3.70% (1/27) | 0 |
| *ABL1* | 3.70% (1/27) | 0 |
| *PDGFRA* | 3.70% (1/27) | 0 |
| *ATM* | 0 | 2.00% (1/50) |
| *SMAD4* | 0 | 2.00% (1/50) |
| *BRAF* | 0 | 2.00% (1/50) |
| *PTPN11* | 0 | 2.00% (1/50) |
| *NRAS* | 0 | 2.00% (1/50) |
| *CTNNB1* | 0 | 2.00% (1/50) |
| *VHL* | 3.70% (1/27) | 0 |

Positive rate of pathogenic gene mutation site: The number of people with mutations corresponding to the pathogenic mutation sites of any gene/total number of samples.

case was found for each gene. The possible cause of tumor heterogeneity could not be ruled out. No pathogenic gene mutation sites were detected in stool samples from the normal and benign control groups (Tables 6–8).

According to a combination of Tables 5–8, the *TP53*, *APC*, and *KRAS* genes all had high mutation frequencies in the preoperative stools and tumor tissues of CRC patients, while exhibiting no pathogenic mutations in the postoperative stools, or in the stool samples of the normal or benign control group. This indicates that tumor-specific DNA can be detected in the preoperative stools of CRC patients, and that the preoperative fecal expression of tumor-associated genes can reflect the gene mutations in tumor tissue to some extent. Compared to the postoperative stools and the stool samples of the two control groups, the pathogenic mutation frequencies of *TP53* and *KRAS* were significantly higher in the preoperative stools ($P < 0.05$), suggesting that fecal *TP53* and *KRAS* genes can be used for the screening, diagnosis, and prognostic prediction of CRC. Contrastively, the pathogenic mutation frequency of the *APC* gene in the preoperative stools differed insignificantly from that in the postoperative stool or the two control groups ($P > 0.05$), which was probably associated with the excessively small sample size.

**Table 6 Comparison of pathogenic mutation sites in preoperative stools *vs* normal control stools**

| Gene | Positive rate of pathogenic gene mutation sites | | $\chi^2$ | *P* value |
|---|---|---|---|---|
| | Preoperative feces | Normal control group | | |
| *TP53* | 37.04% (10/27) | 0 | 7.328 | 0.007 |
| *APC* | 11.11% (3/27) | 0 | 0.878 | 0.349 |
| *KRAS* | 25.93% (7/27) | 0 | 4.219 | 0.040 |
| *PIK3CA* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *FBXW7* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *GNAS* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PTEN* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *ABL1* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PDGFRA* | 3.70% (1/27) | 0 | Fisher exact test | 1 |

Positive rate of pathogenic gene mutation site: The number of people with mutations corresponding to the pathogenic mutation sites of any gene/total number of samples.

### *Mutation site analysis for TP53, KRAS, and APC genes*

As shown in Table 9, *TP53* mutations occurred in 31 of 50 tumor tissues, and the total mutation frequency was 40 times, of which pathogenic mutation sites accounted for 60.00% (24/40), with exon 5 R175H, exon 7 R248Q/W, and exon 8 R273H/C being the most common. The proportion of pathogenic mutation sites in 29 tumor tissues with *KRAS* mutations was 90.00% (27/30), most of which were located in exon 2, with G12D/V/S being predominant, followed by G13D/C. Additionally, there was 1 case of exon 3 Q61H mutation. As for the *APC* gene, all its pathogenic mutation sites were located in exon 17.

The 12 of 27 *TP53* mutations in preoperative stools were pathogenic mutations, which were mostly exon 7 R248Q and exon 8 R273H/C. The pathogenic mutation sites of the *KRAS* gene were primarily exon 2 G12D/V. As for the *APC* gene, all its pathogenic mutation sites were located in exon 17 (Table 10).

As are clear from Tables 9 and 10, the aforementioned pathogenic mutation sites occurred in both preoperative stools and tumor tissues, suggesting the possible correlation between preoperative stool and tumor tissue in CRC patients.

### *Combined TP53–KRAS detection*

Among the 27 preoperative stools of CRC patients, 16 had pathogenic mutations of the *TP53* gene, with a true positive rate of 59.26%, and 10 had pathogenic mutations of the *KRAS* gene, with a true positive rate of 37.04%. Pathogenic mutations of *TP53* or *KRAS* occurred in 18 preoperative stools, showing a true positive rate of 66.67% (18/27). According to Table 11, the sensitivity and negative predictive value of the combined TP53–KRAS detection were 66.67% and 68.97%, respectively, which were higher than that those of *TP53* or *KRAS* mutation detection alone. This suggests that the combined testing of *TP53* and *KRAS* genes can improve the detection rate.

### *Distribution of mutation sites of "unknown clinical significance"*

Mutation sites of "unknown clinical significance" occurred for such genes as TP53, *PTEN*, *KRAS*, *BRAF*, and *AKT1* in the tumor tissues of CRC patients. The five mutation sites of "unknown clinical significance" for *TP53* were respectively exon 5 A159V/V172G/G154D/P153S and exon 7 S241T. In the case of *PTEN*, 9 of 11 mutations were of "unknown clinical significance", all of which were exon 6 V133I. For the *KRAS* gene, the mutation sites of "unknown clinical significance" accounted for 10.00% (3/30), all of which were exon 4 A146T. In preoperative stools, the mutation sites of "unknown clinical significance" were scattered in *TP53*, *KRAS*, *AKT1*, and *STK11*. In the case of *TP53*, such mutation sites were exon 5 V172G and exon 7 I251T/S241T, which were identical to the mutation locations in tumor tissues. For the *KRAS* gene, the mutation site of "unknown clinical significance" was exon 4 A146T. As for *AKT1* and *STK11*, such mutation sites were located separately in exon 3 E17K and exon 4 E199K.

### *Distribution of "undetected" mutation sites*

As shown in Table 12, genes like *TP53*, *APC*, *EGFR*, *FBXW7*, and *NOTCH1* in tumor tissues all had "undetected" mutation sites, among which exon 4 A84G of *TP53* occurred more frequently (4/40). For the *APC* gene, "undetected" mutation sites were all found in exon 17. The pathogenic mutation sites of *APC* in Tables 9 and 10 were also located in exon 17, suggesting that the "undetected" mutation sites of

**Table 7 Comparison of pathogenic mutation sites in preoperative stools *vs* benign control group**

| Gene | Positive rate of pathogenic gene mutation sites | | $\chi^2$ | *P* value |
|---|---|---|---|---|
| | Preoperative feces | Benign control group | | |
| *TP53* | 37.04% (10/27) | 0 | 7.328 | 0.007 |
| *APC* | 11.11% (3/27) | 0 | 0.878 | 0.349 |
| *KRAS* | 25.93% (7/27) | 0 | 4.219 | 0.040 |
| *PIK3CA* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *FBXW7* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *GNAS* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PTEN* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *ABL1* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PDGFRA* | 3.70% (1/27) | 0 | Fisher exact test | 1 |

Positive rate of pathogenic gene mutation site: The number of people with mutations corresponding to the pathogenic mutation sites of any gene/total number of samples.

**Table 8 Comparison of pathogenic mutation sites in preoperative stools *vs* postoperative stools**

| Gene | Positive rate of pathogenic gene mutation sites | | $\chi^2$ | *P* value |
|---|---|---|---|---|
| | Preoperative feces | Postoperative stools | | |
| *TP53* | 37.04% (10/27) | 0 | 6.947 | 0.008 |
| *APC* | 11.11% (3/27) | 0 | 0.804 | 0.370 |
| *KRAS* | 25.93% (7/27) | 0 | 3.974 | 0.046 |
| *PIK3CA* | 3.70% (1/27) | 5.26% (1/19) | Fisher exact test | 1 |
| *FBXW7* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *GNAS* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PTEN* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *ABL1* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *PDGFRA* | 3.70% (1/27) | 0 | Fisher exact test | 1 |
| *BRAF* | 0 | 5.26% (1/19) | Fisher exact test | 1 |
| *MPL* | 0 | 5.26% (1/19) | Fisher exact test | 1 |

Positive rate of pathogenic gene mutation site: The number of people with mutations corresponding to the pathogenic mutation sites of any gene/total number of sample.

*APC* in Table 12 may be unreported new sites. They are probably associated with the carcinogenesis and progression of CRC, which require further exploration. Table 13 shows that 6 of 27 *TP53* mutation sites in the preoperative stools were "undetected" sites. They were distributed in exon 4, exon 5, exon 7, and exon 8, among which there were three exon 7 N247D (mutation start and stop positions were both 7577542). Additionally, genes like *CDKN2A*, *HRAS*, *EGFR*, *IDH1*, and *PDGFRA* also had "undetected" mutations sites.

## DISCUSSION

Studies have shown that the genetic factors coupled with prolonged unfavorable external factors induce the disturbance of homeostasis, which leads to a series of changes including angiogenesis, cell hyperproliferation, apoptosis evasion, and enhanced invasion capacity, ultimately resulting in the occurrence of malignancies. It is generally believed that CRC often develops slowly from adenomas, during which multiple steps are involved, including the activation of proto-oncogenes and the

**Table 9 Analysis of pathogenic mutation sites in tumor tissues**

| Gene | Location | Amino acid mutation | Mutation frequency | N | M |
|---|---|---|---|---|---|
| *TP53* | Exon 4 | R110L | 1 | | |
| | Exon 5 | R175H | 4 | | |
| | | Y163C | 1 | | |
| | | C176Y | 1 | | |
| | Exon 6 | R196[1] | 2 | | |
| | | Y220C | 2 | | |
| | Exon 7 | R248Q/W | 3 | | |
| | | G245S/C | 2 | | |
| | Exon 8 | R273H/C | 4 | | |
| | | R282W | 2 | | |
| | | R306[1] | 1 | | |
| | Exon 10 | R342[1] | 1 | 24/40 | 23/31 |
| *KRAS* | Exon 2 | G12D/V/S | 22 | | |
| | | G13D/C | 4 | | |
| | Exon 3 | Q61H | 1 | 27/30 | 27/29 |
| *APC* | Exon 17 | Q886[1] | 1 | | |
| | | S1483fs | 1 | | |
| | | R876[1] | 2 | | |
| | | R1450[1] | 2 | | |
| | | E1306[1] | 1 | | |
| | | Q1294[1] | 1 | | |
| | | G1312[1] | 1 | 9/15 | 9/12 |

[1]Indicates stop codon.

N: Pathogenic sites/total mutation sites; M: Number of pathogenic cases/total number of mutation cases.

inactivation of tumor suppressor genes. The mutation statuses of genes like *TP53*, *APC*, and *KRAS* have been reported to be closely associated with the carcinogenesis and progression of CRC[24,25]. With the introduction of the concept of "precision medicine", molecular targeted therapy has become a hot research topic in recent years. This NGS-based study investigated the value of fecal genetic testing in the CRC diagnosis.

As a tumor suppressor gene, *TP53* regulates downstream genes when cells are under stress, which induces apoptosis and cell cycle arrest *via* a series of processes, thereby preventing the growth of tumor cells[26,27]. Its mutation is a key step in facilitating the adenoma–adenocarcinoma transition. In malignancies, the p53 protein encoded by *TP53* is mutant. The mutant p53 protein can lose its tumor suppressor effect and acquire new oncogenic functions to promote the invasion and metastasis of tumor cells[28]. In this study, *TP53* had 60.00% (24/40) and 59.26% (16/27) of mutations in the tumor tissues and preoperative stools of CRC patients, respectively, among which pathogenic mutation sites accounted for 60.00% (24/40) and 40.74% (11/27) of total mutation sites. Such pathogenic mutation sites as exon 5 C176Y, exon 6 R196*, exon 7 R248Q, exon 7 G245C/S, and exon 8 R273H/C existed in both types of samples, showing consistency. Such mutation hotspots as *R273H*, *R248Q*, and *R282W* have been reported as typical "hotspots" in the DNA binding domain. Over 95% of missense *TP53* mutations occur in the DNA binding domain, which can affect the ability of proteins to bind to their target DNA sequences[29-31]. Lo *et al*[32] screened a patient with CRC lymphatic metastasis for tumor infiltrating lymphocytes. Since some tumor patients had TP53 R175H mutations during genetic testing, and HLA-A *0201 could limit the recognition of mutant TP53 R175H, they identified the T cell receptors capable of recognizing the TP53 R175H mutation hotspots, thereby finding patients with different tumor types who shared the same mutation hotspots. In their opinion, the mutant *TP53* could serve as an ideal common antigen target, and these specific hotspot mutations had potential as valuable therapeutic targets for a variety of tumor cells. A study by Olszewski *et al*[33] suggested that changes in the

**Table 10 Analysis of pathogenic mutation sites in preoperative feces**

| Gene | Location | Amino acid mutation | Mutation frequency | N | M |
|---|---|---|---|---|---|
| *TP53* | Exon 5 | C176Y | 1 | | |
| | | F134V | 1 | | |
| | Exon 6 | R196[1] | 1 | | |
| | Exon 7 | N235D | 1 | | |
| | | R248Q | 3 | | |
| | | G245C/S | 2 | | |
| | Exon 8 | R273H/C | 3 | 12/27 | 10/16 |
| *KRAS* | Exon 2 | G12D/V | 5 | | |
| | | G13D | 1 | | |
| | Exon 3 | Q61H | 1 | | |
| | | A59T | 1 | 8/11 | 7/10 |
| *APC* | Exon 17 | R1450[1] | 1 | | |
| | | Q886[1] | 1 | | |
| | | Q1294[1] | 1 | 3/3 | 3/3 |

[1]Indicates stop codon.
N: Pathogenic sites/total mutation sites; M: Number of pathogenic cases/total number of mutation cases.

**Table 11 Colorectal cancer diagnosis results by *TP53* and *KRAS* mutations in preoperative stools, *n* (%)**

| Gene | TP | FP | FN | TN | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| *TP53* | 16 | 0 | 11 | 20 | 59.26 | 100.00 | 100.00 | 64.52 |
| *KRAS* | 10 | 0 | 17 | 20 | 37.04 | 100.00 | 100.00 | 54.05 |
| *TP53* or *KRAS* | 18 | 0 | 9 | 20 | 66.67 | 100.00 | 100.00 | 68.97 |

TP: True positive; FP: False positive; FN: False negative; TN: True negative; PPV: Positive predictive value; NPV: Negative predictive value.

oncogenic activity of TP53 led to acquisition of new functions for the mutated tumor suppressor p53 protein, and that the overexpression of such mutation hotspots as codons 175, 248, or 273 might be associated with the chemotherapeutic resistance or invasive changes. Their analysis of *TP53* mutation database in human somatic cells revealed that patients carrying TP53 R248Q allele had earlier tumor onsets, but lower frequencies of metastasis.

The 21 kDa protein encoded by *KRAS*, a member of the ras proto-oncogene family, can participate in the G protein signaling pathway and regulate the proliferation and differentiation of cells. *KRAS* mutations can continuously activate the RAS-RAF-MAPK pathway, causing uncontrolled cellular proliferation and differentiation, thereby inducing CRC[14]. Research has demonstrated that the patients' resistance to EGFR-targeted drugs was closely associated with the activation of the *KRAS* gene[34]. In the present study, the incidences of *KRAS* mutations in tumor tissue and preoperative stools of CRC patients were 58.00% (29/50) and 37.04% (10/27), respectively. Among them, pathogenic mutation sites existed in 27 tissues and 7 preoperative stools, with the most common ones being G12D/V and G13D located in exon 2. Armengol *et al*[35] explored the gene mutations in stool samples of 52 CRC patients. They found 12 cases of *KRAS* gene mutations, of which 58.3% (7/12) were located in codons 12 and 13 of exon 2. Knight argued that when there was a KRAS G12D carcinogenic mutation in the body, the formation or growth of intestinal adenomas was not inhibited by rapamycin[36]. Compared to KRAS G12D, patients with KRAS A146T mutation could have stronger resistance to EGFR tolerance, and better survival rates[37]. Mulla *et al*[38] analyzed the RAS mutations in 51 CRC patients, finding that 43% of them had RAS mutations, 91% of which were *KRAS* mutations. The majority of *KRAS* mutations were located in G12D, followed by G13D. They also found that the incidence of *KRAS* mutations was slightly higher among individuals under 50 years of age than that of the elderly (> 50 years). Ottaiano *et al*[39] reported that mutant *KRAS* was a negative influencing factor of prognosis in CRC patients. Among these patients, those with G12C/S mutation showed the shortest survival and the worst prognosis.

**Table 12 "Undetected" gene mutation sites in tumor tissues**

| Gene | Location | Amino acid mutation | Mutation start position | Mutation end position | Number of cases | Undetected sites/total mutation sites |
|---|---|---|---|---|---|---|
| *TP53* | Exon 4 | A84G | 7579436 | 7579436 | 4 | |
| | Exon 5 | P152A | 7578476 | 7578476 | 1 | |
| | Exon 8 | L289P | 7577072 | 7577072 | 1 | 6/40 |
| *APC* | Exon 17 | S1346[1] | 112175328 | 112175328 | 1 | |
| | | K1573fs | 112175953 | 112175954 | 1 | |
| | | E1327fs | 112175213 | 112175217 | 1 | 3/15 |
| *FBXW7* | Exon 7 | R278[1] | 153258983 | 153258983 | 1 | |
| | Exon 12 | R266C | 153247289 | 153247289 | 1 | 2/5 |
| *NOTCH1* | Exon 26 | R1599P | 139399350 | 139399350 | 2 | 2/2 |
| *EGFR* | Exon 20 | I821T | 55249164 | 55249164 | 1 | 1/2 |

[1]Indicates stop codon.
fs: Frameshift mutation.

**Table 13 "Undetected" gene mutation sites in preoperative feces**

| Gene | Location | Amino acid mutation | Mutation start position | Mutation end position | Number of cases | Undetected sites/total mutation sites |
|---|---|---|---|---|---|---|
| *TP53* | Exon 4 | A84G | 7579436 | 7579436 | 1 | |
| | Exon 5 | S166P | 7578434 | 7578434 | 1 | |
| | Exon 7 | N247D | 7577542 | 7577542 | 3 | |
| | Exon 8 | L289P | 7577072 | 7577072 | 1 | 6/27 |
| *CDKN2A* | Exon 2 | V51A | 21971206 | 21971206 | 2 | |
| | | L63P | 21971170 | 21971170 | 1 | 3/4 |
| *HRAS* | Exon 2 | G12R | 534289 | 534289 | 1 | 1/10 |
| *EGFR* | Exon 20 | I821T | 55249164 | 55249164 | 1 | 1/2 |
| *IDH1* | Exon 4 | R119Q | 209113151 | 209113151 | 1 | 1/2 |
| *PDGFRA* | Exon 13 | G594fs | 55141059 | 55141059 | 1 | 1/8 |

fs: Frameshift mutation.

Varshavi *et al*[40] investigated how *KRAS* mutations in different codons affected the metabolic pathways. According to their findings, metabolic differences existed between various *KRAS* mutation sites, which might be one of the reasons for the varying efficacies of anti-cancer therapies across patients. Hence, more effective, individualized molecular targeted drugs can be searched based on such metabolic differences.

Mutations of *APC* were linked to familial adenomatous polyposis, and its inactivation and the activation of Wnt signaling pathway also played pivotal roles in the occurrence of CRC, which could affect the adhesion between tumor cells to accelerate the cellular migration[41,42]. It has been reported that in sporadic CRC, most of the *APC* mutations occurred around the mutation cluster region, especially between codons 1286 and 1513[43]. In this study, the incidences of *APC* mutations in tumor tissues and preoperative stools were 24.00% (12/50) and 11.11% (3/27), respectively. As shown in Tables 5 and 6, the pathogenic mutation frequency of the *APC* gene in preoperative stools differed insignificantly from that in control stools ($P$ = 0.370), which thus cannot serve as a specific gene for CRC screening and diagnosis. This may be attributed to the small sample size of this study, which remains further exploration. We found that all the pathogenic mutation sites of *APC* were located in exon 17, most of which used the mutations of certain amino acids as stop codons, thus allowing premature termination of mRNA translation (Tables 8 and 9). Luo's team performed NGS-based gene sequencing

on the blood and tissue samples of 22 CRC patients and 21 patients with intestinal polyps, in order to understand gene mutations. Through screening, they found that the *APC* mutations were most common in both types of samples, which were mostly pathogenic mutation sites. These pathogenic mutation sites of the *APC* gene accounted for 77.3% (17/22) of the total pathogenic mutation sites in all samples[44].

In addition to the pathogenic mutation sites of various samples, this study also found multiple mutation sites of "unknown clinical significance", which were scattered in such genes as *TP53*, *PTEN*, *KRAS*, *BRAF*, *AKT1*, and *PIK3CA*. There were also some gene mutation sites that had not been found in the Clinvar database. *TP53* exon4 A84G and *EGFR* exon20 I821T were present in both the preoperative stools and tumor tissues of CRC patients. The mutation start and stop positions were both 7579436 for the former, while 55249164 for the latter.

## CONCLUSION

Conclusively, NGS-based fecal DNA detection can be used as a complementary technique for the CRC diagnosis. Fecal *TP53* and *KRAS* can serve as a specific gene combination for the screening, diagnosis, prognostic prediction, and recurrence monitoring of CRC. Moreover, the combined TP53–KRAS testing can improve the CRC detection rate, which provides a molecular reference for developing personalized treatment programs. The mutation sites of "unknown clinical significance" and the "undetected" mutation sites may be unreported new mutation sites associated with CRC. Whether these genes are linked to the CRC diagnosis and prognosis remains to be further explored and researched.

## ARTICLE HIGHLIGHTS

### *Research background*

Colorectal cancer (CRC) is currently a health problem of global concern. In recent years, the incidence of CRC presents a trend of gradual increase. Most patients have unobvious early symptoms, and they are commonly in mid and advanced stages when the symptoms become evident, with rather high mortalities. Featuring high throughput, fastness, and rich information, next generation sequencing (NGS) can greatly shorten the detection time, which is a research hotspot at home and abroad at present.

### *Research motivation*

As we all know, histopathological examination is the gold standard of diagnosis, but its invasiveness limits its development. Therefore, it is imperative to explore the screening, diagnosis, and prognosis of CRC by strong specificity, high sensitivity, and non-invasive methods.

### *Research objectives*

In this study, NGS technology was used to conduct genetic testing on stool samples of CRC patients, and the results were compared with the corresponding tumor tissue genetic testing results. The aim was to find genes or gene combinations with high specificity and sensitivity in the stool and establish a technical platform for CRC screening and diagnosis and curative effect monitoring through fecal DNA detection, providing a strong basis and support for personalized diagnosis and treatment of CRC.

### *Research methods*

NGS was used to sequence the DNA in stools of patients with CRC, which were then compared with the genetic testing results of the stool samples of normal control and benign intestinal disease groups, as well as the tumor tissues of CRC patients. Specific genes or gene combinations in fecal DNA suitable for diagnosis and prognostic prediction of CRC were screened, and their significance in diagnosing CRC and predicting patients' prognosis was comprehensively evaluated.

### *Research results*

High mutation frequencies of *TP53*, *APC,* and *KRAS* were detected in the stools and tumor tissues of CRC patients prior to surgery. Contrastively, no pathogenic mutations of the above three genes were noted in the postoperative stools, or two control groups. This indicates that the tumor-specific DNA was detectable in the preoperative stools of CRC patients. Compared to the postoperative stools and the stools in the two control groups, the pathogenic mutation frequencies of *TP53* and *KRAS* were significantly higher for the preoperative stools ($P < 0.05$), suggesting that fecal *TP53* and *KRAS* genes can be used for CRC screening, diagnosis, and prognostic prediction. No significant difference in the pathogenic mutation frequency of the *APC* gene was found from the postoperative stools or the two control groups ($P > 0.05$), so further analysis with larger sample size is required. In 27 preoperative stools of CRC patients, the sensitivity and negative predictive value of *TP53*- *KRAS* gene combination detection were higher than those of *TP53* mutation or *KRAS* mutation alone, suggesting that *TP53*-*KRAS*

gene combination detection can improve the detection rate of CRC. The "undetected" mutation sites found in preoperative stools and tumor tissues may be new mutation types in the occurrence and development of CRC, which need to be further studied. In addition, some mutations of "unknown clinical significance" were found, and their clinical value is worth further study.

### Research conclusions

NGS-based fecal genetic testing can be used as a complementary technique for the CRC diagnosis. Fecal *TP53* and *KRAS* can be used as specific genes for the screening, diagnosis, prognostic prediction, and recurrence monitoring of CRC. Moreover, the combined testing of *TP53* and *KRAS* genes can improve the CRC detection rate.

### Research perspectives

Fecal genetic detection is a new method for CRC diagnosis, which has the advantages of non-invasiveness, convenient sampling, and dynamic monitoring. Although the sensitivity of fecal genetic test in CRC screening is low, it is certain that it has great potential and broad prospects in the diagnosis and prognosis assessment of CRC. In addition, the "undetected" mutation sites in preoperative stools of CRC patients and the "unknown clinical significance" mutation sites are related to the occurrence and development of CRC, which requires further research and exploration.

## FOOTNOTES

**Author contributions:** He SY performed most of the experiments, analyzed some data, and drafted the manuscript; Li YC and Wang Y provided specimens for the study; Peng HL and Zhou CL gave constructive guidance on the study; Zhang CM contributed to the statistics; Chen SL and Yin JF performed some of the experiments; Lin M designed and supervised the study and edited the manuscript; all authors approved the final version of the article.

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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 **Siegel R**, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 104-117 [PMID: 24639052 DOI: 10.3322/caac.21220]
3 **Huang GW**. Clinical Value of Combined Examination of Fecal Occult Blood and Tumor Markers in the Diagnosis of Colorectal Cancer. [cited 10 January 2022]. Available from: http://en.cnki.com.cn/Article_en/CJFDTotal-HZZZ201907062.htm
4 **Chen WQ**, Li XQ, Zhang XM, Wu JN, Zhao XH, Gu HG, Ren J, Wang DQ. Expression and clinical significance of C-

terminal tensin-like protein in colorectal cancer. *Zhonghua Xiaohua Waike Zazhi* 2016; **15**: 809-814 [DOI: 10.21767/2172-0479.100116]
5 **Croke L**. Colorectal Cancer Screening: ACS Updates Guideline for Adults with Average Risk. *Am Fam Physician* 2019; **99**: 129-130 [PMID: 30633474]
6 **van Lanschot MC**, Carvalho B, Coupé VM, van Engeland M, Dekker E, Meijer GA. Molecular stool testing as an alternative for surveillance colonoscopy: a cross-sectional cohort study. *BMC Cancer* 2017; **17**: 116 [PMID: 28173852 DOI: 10.1186/s12885-017-3078-y]
7 **Sun Y**, Yang B, Lin M, Yu H, Chen H, Zhang Z. Identification of serum miR-30a-5p as a diagnostic and prognostic biomarker in colorectal cancer. *Cancer Biomark* 2019; **24**: 299-305 [PMID: 30829615 DOI: 10.3233/CBM-182129]
8 **Loktionov A**. Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins? *World J Gastrointest Oncol* 2020; **12**: 124-148 [PMID: 32104546 DOI: 10.4251/wjgo.v12.i2.124]
9 **Al Buthi SA**, Bin Gheshayan SF, Al Samaani I, Al Ahmadi B, Al Selaim NA. A Mixed Neuroendocrine/Non-Neuroendocrine Neoplasm Arising in the Background of Ulcerative Colitis: A Case Report and Review of the Literature. *Am J Case Rep* 2022; **23**: e934396 [PMID: 35236818 DOI: 10.12659/AJCR.934396]
10 **Yan F**, Tu Z, Duan L, Wang D, Lin F. MicroRNA-383 suppresses cell proliferation and invasion in colorectal cancer by directly targeting paired box 6. *Mol Med Rep* 2018; **17**: 6893-6901 [PMID: 29512711 DOI: 10.3892/mmr.2018.8682]
11 **Yu CT**, Chen T, Lu S, Hu W, Zhang Q, Tan J, Sun D, Li L, Sun X, Xu C, Lai Y, Fan M, Shen Z, Shen W, Cheng H. Identification of Significant Modules and Targets of Xian-Lian-Jie-Du Decoction Based on the Analysis of Transcriptomics, Proteomics and Single-Cell Transcriptomics in Colorectal Tumor. *J Inflamm Res* 2022; **15**: 1483-1499 [PMID: 35256851 DOI: 10.2147/JIR.S344861]
12 **Wang B**, Wu S, Huang F, Shen M, Jiang H, Yu Y, Yu Q, Yang Y, Zhao Y, Zhou Y, Pan B, Liu T, Guo W. Analytical and clinical validation of a novel amplicon-based NGS assay for the evaluation of circulating tumor DNA in metastatic colorectal cancer patients. *Clin Chem Lab Med* 2019; **57**: 1501-1510 [PMID: 31339850 DOI: 10.1515/cclm-2019-0142]
13 **Ma CX**, Guan X, Wang S, Liu Z, Jiang Z, Wang XS. [Application and prospect of fecal DNA test in colorectal cancer screening]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2019; **22**: 491-494 [PMID: 31104434 DOI: 10.3760/cma.j.issn.1671-0274.2019.05.018]
14 **Mahasneh A**, Al-Shaheri F, Jamal E. Molecular biomarkers for an early diagnosis, effective treatment and prognosis of colorectal cancer: Current updates. *Exp Mol Pathol* 2017; **102**: 475-483 [PMID: 28506769 DOI: 10.1016/j.yexmp.2017.05.005]
15 **Carethers JM**. Fecal DNA Testing for Colorectal Cancer Screening. *Annu Rev Med* 2020; **71**: 59-69 [PMID: 31451044 DOI: 10.1146/annurev-med-103018-123125]
16 **Anderson BW**, Ahlquist DA. Molecular Detection of Gastrointestinal Neoplasia: Innovations in Early Detection and Screening. *Gastroenterol Clin North Am* 2016; **45**: 529-542 [PMID: 27546847 DOI: 10.1016/j.gtc.2016.04.009]
17 **Wakai T**, Prasoon P, Hirose Y, Shimada Y, Ichikawa H, Nagahashi M. Next-generation sequencing-based clinical sequencing: toward precision medicine in solid tumors. *Int J Clin Oncol* 2019; **24**: 115-122 [PMID: 30515675 DOI: 10.1007/s10147-018-1375-3]
18 **Del Vecchio F**, Mastroiaco V, Di Marco A, Compagnoni C, Capece D, Zazzeroni F, Capalbo C, Alesse E, Tessitore A. Next-generation sequencing: recent applications to the analysis of colorectal cancer. *J Transl Med* 2017; **15**: 246 [PMID: 29221448 DOI: 10.1186/s12967-017-1353-y]
19 **Hinrichs JW**, van Blokland WT, Moons MJ, Radersma RD, Radersma-van Loon JH, de Voijs CM, Rappel SB, Koudijs MJ, Besselink NJ, Willems SM, de Weger RA. Comparison of next-generation sequencing and mutation-specific platforms in clinical practice. *Am J Clin Pathol* 2015; **143**: 573-578 [PMID: 25780010 DOI: 10.1309/AJCP40XETVYAMJPY]
20 **Nagahashi M**, Shimada Y, Ichikawa H, Kameyama H, Takabe K, Okuda S, Wakai T. Next generation sequencing-based gene panel tests for the management of solid tumors. *Cancer Sci* 2019; **110**: 6-15 [PMID: 30338623 DOI: 10.1111/cas.13837]
21 **Xuan J**, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: promises and challenges. *Cancer Lett* 2013; **340**: 284-295 [PMID: 23174106 DOI: 10.1016/j.canlet.2012.11.025]
22 **Kraus C**, Rau TT, Lux P, Erlenbach-Wünsch K, Löhr S, Krumbiegel M, Thiel CT, Stöhr R, Agaimy A, Croner RS, Stürzl M, Hohenberger W, Hartmann A, Reis A. Comprehensive screening for mutations associated with colorectal cancer in unselected cases reveals penetrant and nonpenetrant mutations. *Int J Cancer* 2015; **136**: E559-E568 [PMID: 25142776 DOI: 10.1002/ijc.29149]
23 **Li W**, Qiu T, Guo L, Ying J, Zhou A. NGS-based oncogenic mutations analysis in advanced colorectal cancer patients improves targeted therapy prediction. *Pathol Res Pract* 2019; **215**: 483-489 [PMID: 30611619 DOI: 10.1016/j.prp.2018.12.037]
24 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-i]
25 **Kronborg O**, Fenger C, Olsen J, Jørgensen OD, Søndergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996; **348**: 1467-1471 [PMID: 8942774 DOI: 10.1016/S0140-6736(96)03430-7]
26 **Soussi T**, Wiman KG. Shaping genetic alterations in human cancer: the p53 mutation paradigm. *Cancer Cell* 2007; **12**: 303-312 [PMID: 17936556 DOI: 10.1016/j.ccr.2007.10.001]
27 **Li XL**, Zhou J, Chen ZR, Chng WJ. P53 mutations in colorectal cancer - molecular pathogenesis and pharmacological reactivation. *World J Gastroenterol* 2015; **21**: 84-93 [PMID: 25574081 DOI: 10.3748/wjg.v21.i1.84]
28 **Tong X**, Xu D, Mishra RK, Jones RD, Sun L, Schiltz GE, Liao J, Yang GY. Identification of a druggable protein-protein interaction site between mutant p53 and its stabilizing chaperone DNAJA1. *J Biol Chem* 2021; **296**: 100098 [PMID: 33208462 DOI: 10.1074/jbc.RA120.014749]
29 **Shah HD**, Saranath D, Murthy V. A molecular dynamics and docking study to screen anti-cancer compounds targeting mutated p53. *J Biomol Struct Dyn* 2022; **40**: 2407-2416 [PMID: 33111621 DOI: 10.1080/07391102.2020.1839559]
30 **Navalkar A**, Ghosh S, Pandey S, Paul A, Datta D, Maji SK. Prion-like p53 Amyloids in Cancer. *Biochemistry* 2020; **59**: 146-155 [PMID: 31603660 DOI: 10.1021/acs.biochem.9b00796]

31 **Cho Y**, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* 1994; **265**: 346-355 [PMID: 8023157 DOI: 10.1126/science.8023157]

32 **Lo W**, Parkhurst M, Robbins PF, Tran E, Lu YC, Jia L, Gartner JJ, Pasetto A, Deniger D, Malekzadeh P, Shelton TE, Prickett T, Ray S, Kivitz S, Paria BC, Kriley I, Schrump DS, Rosenberg SA. Immunologic Recognition of a Shared p53 Mutated Neoantigen in a Patient with Metastatic Colorectal Cancer. *Cancer Immunol Res* 2019; **7**: 534-543 [PMID: 30709841 DOI: 10.1158/2326-6066.CIR-18-0686]

33 **Olszewski MB**, Pruszko M, Snaar-Jagalska E, Zylicz A, Zylicz M. Diverse and cancer typespecific roles of the p53 R248Q gainoffunction mutation in cancer migration and invasiveness. *Int J Oncol* 2019; **54**: 1168-1182 [PMID: 30968154 DOI: 10.3892/ijo.2019.4723]

34 **Yun J**, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K, Roper J, Chio II, Giannopoulou EG, Rago C, Muley A, Asara JM, Paik J, Elemento O, Chen Z, Pappin DJ, Dow LE, Papadopoulos N, Gross SS, Cantley LC. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* 2015; **350**: 1391-1396 [PMID: 26541605 DOI: 10.1126/science.aaa5004]

35 **Armengol G**, Sarhadi VK, Ghanbari R, Doghaei-Moghaddam M, Ansari R, Sotoudeh M, Puolakkainen P, Kokkola A, Malekzadeh R, Knuutila S. Driver Gene Mutations in Stools of Colorectal Carcinoma Patients Detected by Targeted Next-Generation Sequencing. *J Mol Diagn* 2016; **18**: 471-479 [PMID: 27155048 DOI: 10.1016/j.jmoldx.2016.01.008]

36 **Knight JRP**, Alexandrou C, Skalka GL, Vlahov N, Pennel K, Officer L, Teodosio A, Kanellos G, Gay DM, May-Wilson S, Smith EM, Najumudeen AK, Gilroy K, Ridgway RA, Flanagan DJ, Smith RCL, McDonald L, MacKay C, Cheasty A, McArthur K, Stanway E, Leach JD, Jackstadt R, Waldron JA, Campbell AD, Vlachogiannis G, Valeri N, Haigis KM, Sonenberg N, Proud CG, Jones NP, Swarbrick ME, McKinnon HJ, Faller WJ, Le Quesne J, Edwards J, Willis AE, Bushell M, Sansom OJ. MNK Inhibition Sensitizes *KRAS*-Mutant Colorectal Cancer to mTORC1 Inhibition by Reducing eIF4E Phosphorylation and c-MYC Expression. *Cancer Discov* 2021; **11**: 1228-1247 [PMID: 33328217 DOI: 10.1158/2159-8290.CD-20-0652]

37 **Poulin EJ**, Bera AK, Lu J, Lin YJ, Strasser SD, Paulo JA, Huang TQ, Morales C, Yan W, Cook J, Nowak JA, Brubaker DK, Joughin BA, Johnson CW, DeStefanis RA, Ghazi PC, Gondi S, Wales TE, Iacob RE, Bogdanova L, Gierut JJ, Li Y, Engen JR, Perez-Mancera PA, Braun BS, Gygi SP, Lauffenburger DA, Westover KD, Haigis KM. Tissue-Specific Oncogenic Activity of $KRAS^{A146T}$. *Cancer Discov* 2019; **9**: 738-755 [PMID: 30952657 DOI: 10.1158/2159-8290.CD-18-1220]

38 **Mulla N**, Alshareef A, Syed AR, Al-Jahel M. Clinico-Pathological Study of K-ras Mutations in Colorectal Tumors: A Single-Center Retrospective Study of 51 Patients in Madinah, Saudi Arabia. *Cureus* 2020; **12**: e9978 [PMID: 32983680 DOI: 10.7759/cureus.9978]

39 **Ottaiano A**, Normanno N, Facchini S, Cassata A, Nappi A, Romano C, Silvestro L, De Stefano A, Rachiglio AM, Roma C, Maiello MR, Scala S, Delrio P, Tatangelo F, Di Mauro A, Botti G, Avallone A, Nasti G. Study of Ras Mutations' Prognostic Value in Metastatic Colorectal Cancer: STORIA Analysis. *Cancers (Basel)* 2020; **12** [PMID: 32708575 DOI: 10.3390/cancers12071919]

40 **Varshavi D**, Varshavi D, McCarthy N, Veselkov K, Keun HC, Everett JR. Metabolic characterization of colorectal cancer cells harbouring different KRAS mutations in codon 12, 13, 61 and 146 using human SW48 isogenic cell lines. *Metabolomics* 2020; **16**: 51 [PMID: 32300895 DOI: 10.1007/s11306-020-01674-2]

41 **Phull MS**, Jadav SS, Gundla R, Mainkar PS. A perspective on medicinal chemistry approaches towards adenomatous polyposis coli and Wnt signal based colorectal cancer inhibitors. *Eur J Med Chem* 2021; **212**: 113149 [PMID: 33445154 DOI: 10.1016/j.ejmech.2020.113149]

42 **Caspi M**, Wittenstein A, Kazelnik M, Shor-Nareznoy Y, Rosin-Arbesfeld R. Therapeutic targeting of the oncogenic Wnt signaling pathway for treating colorectal cancer and other colonic disorders. *Adv Drug Deliv Rev* 2021; **169**: 118-136 [PMID: 33346022 DOI: 10.1016/j.addr.2020.12.010]

43 **Aitchison A**, Hakkaart C, Day RC, Morrin HR, Frizelle FA, Keenan JI. *APC* Mutations Are Not Confined to Hotspot Regions in Early-Onset Colorectal Cancer. *Cancers (Basel)* 2020; **12** [PMID: 33352971 DOI: 10.3390/cancers12123829]

44 **Luo J**. Mutation analysis of sporadic colorectal cancer and polypby next-generation of sequencing. M.M. Thesis, Fujian Medical University. 2015. [cited 10 January 2022]. Available from: https://kns.cnki.net/kns8/defaultresult/index

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ORIGINAL ARTICLE

Basic Study

# Mechanism and therapeutic strategy of hepatic *TM6SF2*-deficient non-alcoholic fatty liver diseases *via in vivo* and *in vitro* experiments

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## Abstract

### *BACKGROUND*

The lack of effective pharmacotherapies for nonalcoholic fatty liver disease (NAFLD) is mainly attributed to insufficient research on its pathogenesis. The

pathogenesis of TM6SF2-efficient NAFLD remains unclear, resulting in a lack of therapeutic strategies for TM6SF2-deficient patients.

***AIM***

To investigate the role of TM6SF2 in fatty acid metabolism in the context of fatty liver and propose possible therapeutic strategies for NAFLD caused by TM6SF2 deficiency.

***METHODS***

Liver samples collected from both NAFLD mouse models and human participants (80 cases) were used to evaluate the expression of TM6SF2 by using western blotting, immunohistochemistry, and quantitative polymerase chain reaction. RNA-seq data retrieved from the Gene Expression Omnibus database were used to confirm the over-expression of TM6SF2. Knockdown and overexpression of TM6SF2 were performed to clarify the mechanistic basis of hepatic lipid accumulation in NAFLD. MK-4074 administration was used as a therapeutic intervention to evaluate its effect on NAFLD caused by TM6SF2 deficiency.

***RESULTS***

Hepatic TM6SF2 levels were elevated in patients with NAFLD and NAFLD mouse models. TM6SF2 overexpression can reduce hepatic lipid accumulation, suggesting a protective role for TM6SF2 in a high-fat diet (HFD). Downregulation of TM6SF2, simulating the TM6SF2 E167K mutation condition, increases intracellular lipid deposition due to dysregulated fatty acid metabolism and is characterized by enhanced fatty acid uptake and synthesis, accompanied by impaired fatty acid oxidation. Owing to the potential effect of TM6SF2 deficiency on lipid metabolism, the application of an acetyl-CoA carboxylase inhibitor (MK-4074) could reverse the NAFLD phenotypes caused by TM6SF2 deficiency.

***CONCLUSION***

TM6SF2 plays a protective role in the HFD condition; its deficiency enhanced hepatic lipid accumulation through dysregulated fatty acid metabolism, and MK-4074 treatment could alleviate the NAFLD phenotypes caused by TM6SF2 deficiency.

**Key Words:** TM6SF2; Nonalcoholic fatty liver disease; Fatty acid metabolism; Treatment; MK-4074

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**Core Tip:** In this study, we observed TM6SF2 overexpression in nonalcoholic fatty liver disease (NAFLD) cases. TM6SF2 overexpression can reduce hepatic lipid accumulation, suggesting a protective role for TM6SF2 in a high-fat diet. Meanwhile, there is an imbalance in the processes of uptake, synthesis, and intracellular expense of fatty acids in TM6SF2-deficient mouse models. Therefore, we propose possible therapeutic strategies for NAFLD caused by TM6SF2 deficiency.

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## INTRODUCTION

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rapidly increasing and is now more than one-quarter of the global population[1]. Approximately 6%–30% of patients with NAFLD evolve from simple steatosis (SS) to steatohepatitis, characterized by excessive hepatic lipid accumulation, inflammation, and hepatocyte damage[2,3]. Long-term steatohepatitis can cause liver fibrosis, and its adverse outcome may include liver dysfunction and hepatocellular carcinoma[4,5]. The prognosis of NAFLD and its related complications varies among individuals, given that genetic variants affect the occurrence and progression of NAFLD[6]. The development of genome-wide association studies and high-throughput technologies has allowed for in-depth analysis of genetic risk factors for NAFLD[7,8]. Identification of genes with high-risk single nucleotide polymorphisms (SNPs) has allowed the screening of individuals with a genetic predisposition to NAFLD[9], and several studies have reported some potential SNP loci (*e.g.*, PNPLA3 rs738409, MBOAT7 rs641738, and PPARGC1A rs2290602)

associated with the susceptibility and progression of fatty liver[10-12]. To further elucidate the mechanistic basis of the link between potential SNPs and susceptibility to NAFLD, the functions of many candidate genes have been thoroughly investigated, including *TM6SF2*.

TM6SF2 is a multi-transmembrane protein expressed predominantly in the intestine and liver, implying a metabolism-related function[13]. Our previous study and others have demonstrated that the rs58542926 locus of TM6SF2 confers susceptibility to NAFLD[14-16]. The nonsynonymous variant of TM6SF2, rs58542926 (E167K), leads to protein misfolding, acceleration of protein degradation, and, therefore, a reduction in TM6SF2 protein levels and gene function[15]. Recently, several studies have focused on the influence of TM6SF2 deficiency or its E167K mutant on cholesterol metabolism and its association with reduced very low-density lipoproteins (VLDL) content that causes a decrease in hepatic triglyceride (TG) output[17-19]. Considering that the liver is the central hub for lipid metabolism, there is a balance among the processes of uptake, synthesis, and intracellular expense of fatty acids under normal circumstances. Hepatic lipid accumulation may occur when this balance is disrupted[20]. However, the regulatory role of TM6SF2 in fatty acid metabolism in the context of NAFLD remains largely unknown. Therefore, our study aimed to elucidate the influence of TM6SF2 on fatty acid metabolism in experimental NAFLD models.

The present study revealed that hepatic TM6SF2 expression was elevated in patients with NAFLD and mouse models. Our results suggest that TM6SF2 elevation is a compensatory response to NAFLD. Physiologically, the reactive overexpression of TM6SF2 can protect the liver and reduce hepatic lipid accumulation under the condition of a high-fat diet (HFD). Loss of TM6SF2 exacerbates hepatic lipid accumulation. Further investigation revealed that the enhanced processes of uptake and synthesis of fatty acids and impaired oxidation processes were observed in hepatic TM6SF2 deficiency, suggesting that TM6SF2 deficiency caused a pathogenic link between the metabolic dysfunction of fatty acids and hepatic steatosis in NAFLD. The elucidation of metabolic alterations suggested the way of therapeutic intervention, and our results showed that liver-specific acetyl-CoA carboxylase (ACC) inhibitor (MK-4074) would block the enhanced synthesis of fatty acid, improve fatty acid β-oxidation, and then reverse the NAFLD phenotypes caused by TM6SF2 deficiency. Collectively, this study suggested a protective role of TM6SF2 in HFD conditions, revealed a pivotal role of TM6SF2 in fatty acid metabolism, and suggested a therapeutic intervention for TM6SF2-deficient exacerbated NAFLD.

## MATERIALS AND METHODS

### Cell culture

Knockdown of the *TM6SF2* gene was mediated by the lentiviral vectors pLenti6.3-MCS-TM6SF2-EGFP (sh-TM6SF2) or pLenti6.3-MCS-EGFP (sh-Ctrl). Target sequence for sh-TM6SF2: TGACCTGGCCCT-TGTCATATA. After two or three generations of antibiotic screening, the expression of TM6SF2 was evaluated by immunoblotting. All cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Cat. No. C125C5, NCM Biotech, Suzhou, China) at 37 ℃ and 5% $CO_2$. The cells were cultured under 1% $O_2$ and 5% $CO_2$ for 24 h to simulate hypoxic conditions. For starvation, cells were cultured in DMEM containing 0.1% FBS for 24 h.

### Western blot

Cells in a 6-cm dish at 80% confluence were lysed with 250 μL of RIPA lysis buffer and centrifuged at 13000 rpm. The quantification of the supernatant collected was assessed using the BCA method (Beyotime, Jiangsu, China). Proteins were transferred to a 0.45 μm PVDF membrane according to the standard immunoblotting protocol after electrophoresis. The membrane was blocked in 5% fat-free milk and then maintained in primary antibodies for 12 h at 4 ℃. After washing, the membrane was incubated with HRP-labeled goat anti-rabbit (mouse) antibody at 25 ℃ for 60 min and visualized using an ECL reagent (Millipore, MA, United States). Supplementary Table 1 lists the antibodies used in this study.

### Clinical specimens

Liver specimens were collected from the Shanghai General Hospital. Parts of the specimens were snap frozen in liquid nitrogen and then transferred to a -80℃ refrigerator to detect mRNA levels, and some were stored in formaldehyde solution for later pathological examination. When ≥ 5% of hepatocytes had steatosis, NAFLD diagnosis was confirmed. The diagnosis of the tissue sections was independently reviewed by two pathologists. This study was approved by the Ethics Committee of the Shanghai General Hospital and was performed strictly in accordance with the Declaration of Helsinki. All patients or their family members were fully informed of the study and signed written consent forms.

### Immunohistochemistry

Paraffin-embedded liver tissues were deparaffinized using standard protocols and rehydrated. After antigen retrieval, the tissue was blocked and incubated with an anti-TM6SF2 (1:200) antibody overnight at 4 ℃. Then the slides were washed and incubated with biotin-labeled goat anti-mouse IgG (H + L) at

37 ℃ for 15 min and developed with a DAB work solution. Images were obtained using a microscope (Leica, Germany).

### Real-time polymerase chain reaction assay

A total of 50 mg of liver samples from mice or humans was lysed with RNAiso Plus reagent (Takara Biotechnology, Otsu, Japan), and total RNA was extracted according to the standard protocol. Reverse transcription of 500ng of RNA was performed using the RNA polymerase chain reaction (PCR) Kit (Takara Biotechnology), and the resulting cDNA was used as the PCR template. Quantification of target gene expression was performed using a LightCycler® 96 (Roche, Switzerland) and a Hieff® quantitative PCR SYBR Green Master Mix (No Rox) kit (Yeasen Biotechnology Co., Ltd, China). The mRNA level of *β-actin* was used as the endogenous control. We chose the TM6SF2 level of one patient as the reference, and the TM6SF2 levels of all other patients were presented several times of this reference. The primers used are listed in Supplementary Table 1.

### Experimental animals

The AAV system (type 8) expressing TM6SF2 short hairpin RNA (shRNA) (AAV-shTm6sf2) or overexpressing human TM6SF2 (AAV8-TM6SF2) was used to regulate the levels of TM6SF2 in C57BL/6 mice. The corresponding controls were AAV-shNC and AAV8-vector, respectively. All mice were fed a normal chow diet (NCD) for three days, and then 100 μL of AAV8 virus ($2 \times 10^{11}$) was injected into the tail vein. Transfection efficiency was determined by immunoblotting. Male mice weighing 19–22 g (aged 4–6 wk) were housed in a 23 ± 2°C environment with a 12 h light/dark cycle. All mice were allowed water ad libitum and were continuously fed an HFD or NCD for 16 wk. For MK-4074 treatment, the mice were primarily fed an 8-wk HFD to induce NAFLD phenotypes and then administered MK4074 (10 mg/kg/day) or placebo (normal saline) for an additional eight weeks on the same diets.

### Evaluation of serum parameters and hepatic lipid content

The concentrations of cytokines, metabolites, and hepatic enzymes in serum were determined using commercial kits [ab208348 for tumor necrosis factor (TNF), ab197742 for interleukin (IL)-1β, ab222503 for IL-6, JLC049 for CCL2, JLC5800 for CXCL10, ab180875 for acetoacetate, ab180876 for β-hydroxybutyrate (β-OH), C010-2-1 for aspartate transaminase (AST), and C009-2-1 for alanine aminotransferase (ALT)] according to the manufacturer's instructions. The hepatic lipid content and malonyl-CoA levels were also measured using commercial kits [A110-1-1 for TG, A111-1-1 for total cholesterol (TC), A042-2-1 for non-esterified fatty acids (NEFA), and JL47416 for malonyl-CoA], and normalized by the total protein. The details of all the commercial kits used are listed in Supplementary Table 1.

### Microarrays

After palmitic acid (PA, 150 μmol/L) stimulation for 24 h, total RNA was extracted from both TM6SF2-knockdown L02 cells and the corresponding controls using RNAiso Plus (Takara Biotechnology). Microarray gene expression analysis was performed by Illumina sequencing. We analyzed differentially expressed genes (DEGs) between sh-Ctrl and sh-TM6SF2 cells by using the "edgeR" package based on the R platform[21]. Fold change > 1.2 and adjusted *P*-value < 0.05 were set as the screening cutoffs for the upregulated DEGs. These DEGs were further analyzed *via* DAVID 6.8 (https://david.ncifcrf.gov/) for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The "ggplot2" package in R is used for result visualization.

### Preparation of fatty acid solution

A 5 mmol/L stock solution of PA (Sigma-Aldrich) was obtained by dissolving PA in 3% of bovine serum albumin (BSA, Sigma-Aldrich) medium by continuous stirring for approximately 4 h in a 60 °C water bath. The stock solution was then diluted in DMEM to achieve the designated concentration, and 3% of BSA was used as a control. The concentrations of free fatty acids range from 0.1 to 0.7 mmol/L[22-24].

### Glucose and insulin tolerance test

Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed as previously described [25]. After the above steps were completed, the glucose concentrations of the blood collected from the tail vein at the indicated points were analyzed immediately using a glucose meter (Yuwell, China).

### Nile red staining

After sh-ctrl and sh-TM6SF2 L02 cells were constructed, they were incubated in DMEM containing 150 μmol/L of PA for 24 h to generate a steatosis cell model. Cells were then fixed with 4% paraformaldehyde and incubated with 0.1 μg/mL Nile Red for 15 min. After washing with PBS, the cells were incubated with DAPI (Solarbio) for 5 min. Images were taken with a Leica microscope (Leica, Germany) and quantified using the ImageJ software.

### Magnetic resonance imaging

Magnetic resonance imaging (MRI) imaging was performed using a 7.0 T Bruker BioSpec MRI scanner (Bruker, United States). Before imaging, the mice were fasted overnight to avoid disturbance of stomach contents during liver imaging. The mice were first anesthetized with oxygen containing 2%–3% isoflurane and then placed in a prone position with their heads facing inward. Throughout the imaging process, the mice were continuously provided with oxygen mixed with 1%-3% isoflurane through the nosecone to maintain the respiratory rate at 50-70 breaths/minute. All mice underwent abdominal MRI to determine the maximum cross-sectional area of the liver. The region of interest was analyzed using ImageJ software, and the size of the cross-sectional area was represented by the number of pixels.

### Histological analysis

Liver sections were embedded in paraffin and stained with hematoxylin and eosin (H&E), and frozen liver sections were stained with Oil Red O (O8010-5g; Solarbio) for lipid visualization. Images were obtained using a light microscope (Leica). Oil Red O- stained sections were quantified using ImageJ software.

### FAO measurement

A total of 6000 cells were seeded in an XF96 cell culture microplate, and each group was assayed in five or six replicate wells. After cell adherence (about 8 h), the growth medium was changed to a substrate-limited growth medium (0.5 mmol/L of glucose, 1 mmol/L of GlutaMAX, 0.5 mmol/L of XF L-carnitine, 1% FBS, pH 7.4) for overnight incubation. Before the Seahorse Bioscience XF96 instrument (Agilent, United States) was started, the medium was changed to a PA measurement medium. Different inhibitors (oligomycin 2 μmol/L, FCCP 2 μmol/L, and R/A 1.2 μmol/L) were added to each chamber when the instrument began to measure the oxygen change rate. The difference in each group's oxygen consumption rate (OCR) revealed the level of fatty acid oxidation (FAO) based on palmitate substrates. The basal respiration, maximal respiration, spare respiratory capacity, and ATP production were calculated.

### Cell viability assay

Cell viability was assessed using the CCK-8 assay (NCM Biotech, China). Sh-ctrl and sh-TM6SF2 cells were seeded in a 96-well plate at a density of 5000 cells *per* well, and a cell viability assay was performed after PA or fatty acid-free BSA treatment for 24 h. Absorbance (450 nm) was measured using a microplate reader (BioTek, United States). Each sample was assayed in five replicate wells, and the experiment was independently performed three times.

### Quantitation of intracellular TG levels in cell lines

After PA or BSA treatment for 24 h, cells were lysed on ice with RIPA buffer (Beyotime, Beijing, China) for 20 min and centrifuged at 13000 rpm for 20 min at 4 °C. Then the supernatant was transferred to a new tube. TG levels were evaluated using a TG detection kit (Nanjing Jiancheng, Jiangsu, China) and normalized to the total protein level. The experiment was independently performed three times.

### Bioinformatic analyses

Expression profiles of TM6SF2 in NAFLD cases were retrieved from the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) database. The accession numbers are GSE130970, GSE48452, GSE83452, and GSE89632. The details of the cohorts are presented in Supplementary Table 2.

### Statistical analysis

All data are presented as the mean ± SD. Student's t-test was used to evaluate the differences between the two groups. Differences between more than two groups were compared using one-way analysis of variance followed by Tukey's multiple comparisons test. *In vitro* studies, all experiments were independently performed three times in vitro. The SPSS software (version 25.0) was used for all statistical analyses. $P < 0.05$ was considered statistically significant and denoted as $^{a}P < 0.05$, $^{b}P < 0.01$, and $^{c}P < 0.001$; NS: Not significant.

## RESULTS

### Hepatic TM6SF2 expression is upregulated in patients with NAFLD and HFD-induced mice models

The mRNA levels of TM6SF2 were examined in 80 liver samples of patients with NAFLD and healthy subjects ($n = 40$), which showed that the transcriptional levels of TM6SF2 were both elevated in patients with SS or nonalcoholic steatohepatitis (NASH) (Figure 1A, SS: $n = 20$; NASH: $n = 20$). Additionally, we retrospectively analyzed another four transcriptomic datasets retrieved from the GEO database (GSE13970, GSE48452, GSE83452, and GSE89632, Figure 1B), which contained transcriptional profiles of both healthy participants and patients with NAFLD. Patients with SS or NASH displayed higher hepatic

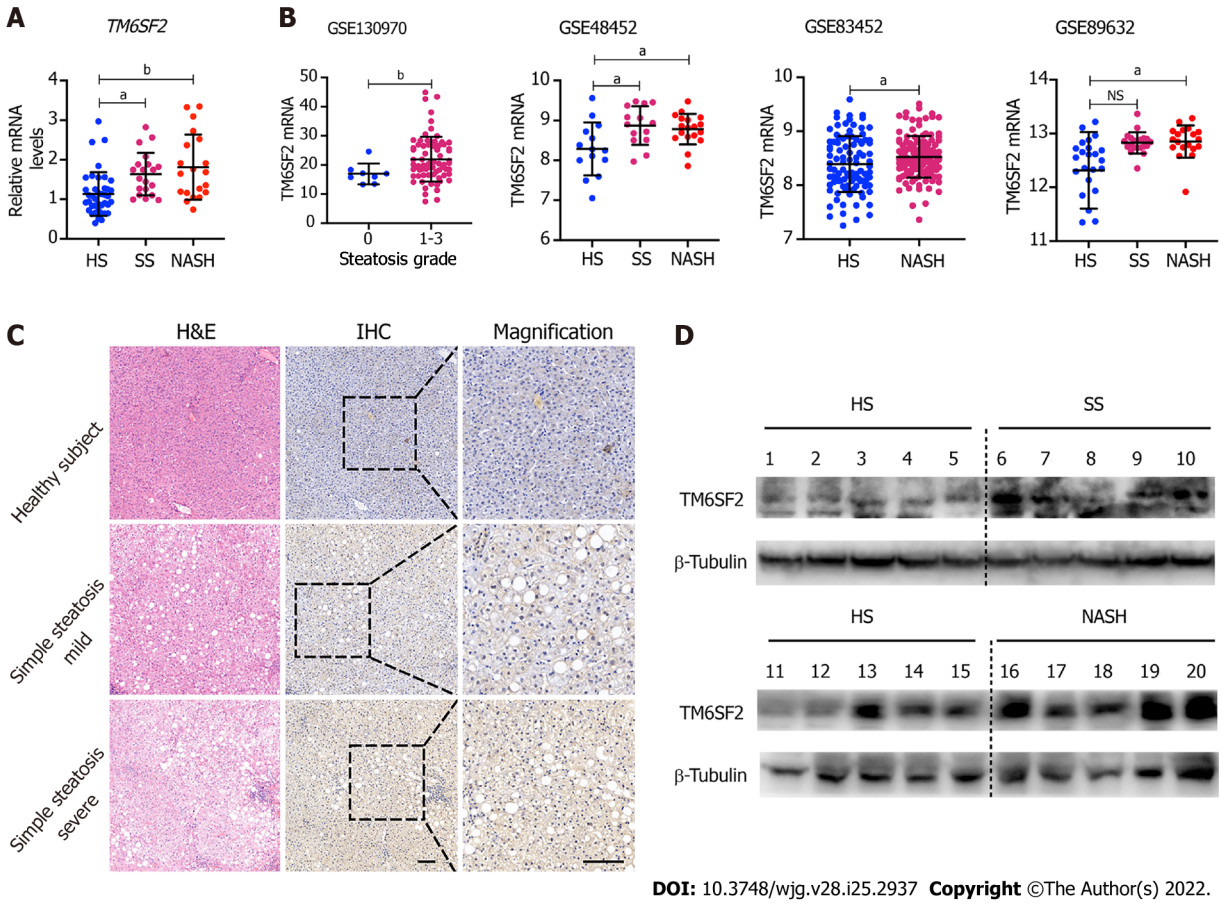


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**Figure 1 Hepatic TM6SF2 expression is upregulated in nonalcoholic fatty liver disease patients.** A: Hepatic mRNA levels of TM6SF2 in liver specimens, including healthy subjects (HS, *n* = 40) and patients with simple steatosis (SS, *n* = 20) or nonalcoholic steatohepatitis (NASH, *n* = 20); B: Hepatic mRNA levels of TM6SF2 in individuals with or without nonalcoholic fatty liver disease (NAFLD) from four expression microarray series retracted from Gene Expression Omnibus database; C: Immunohistochemical staining with TM6SF2 was performed on individuals with or without NAFLD. Representative images of normal liver tissues *vs* tissues of SS were shown. Scale bars: 50 μm; D: Immunoblot analysis of TM6SF2 expression in liver specimens. HS (*n* = 10), SS (*n* = 5) and NASH (*n* = 5). $^{a}P < 0.05$; $^{b}P < 0.01$, NS: Not significant; HS: Healthy subjects; SS: Simple steatosis; NASH: Nonalcoholic steatohepatitis; IHC: Immunohistochemistry; H&E: Hematoxylin and eosin.

TM6SF2 mRNA levels than healthy participants. Immunohistochemistry staining indicated that TM6SF2 tended to upregulate TM6SF2 expression from normal liver to severe SS (Figure 1C). We also noted upregulation of TM6SF2 protein in the liver samples of patients with SS or NASH (Figure 1D). To further confirm TM6SF2 overexpression under overnutrition, we evaluated the changes in TM6SF2 expression in and *in vitro* NAFLD cell model and stimulated two cell lines, L02 and HepG2, with PA, as it was reported that this *in vitro* model mimics steatosis *in vivo*[22-24]. The results showed that PA stimulation increased the protein levels of TM6SF2 in a time- and dose-dependent manner (Supplementary Figure 1A). Further, whether in protein or mRNA levels, the augmented TM6SF2 expression was also demonstrated in HFD-fed mice for 12 wk (Supplementary Figure 1B). These results revealed that hepatic TM6SF2 levels were upregulated in patients with NAFLD and HFD-induced mouse models.

### *TM6SF2 overexpression mitigates hepatic lipid accumulation in HFD-induced mice models*

As we had found that the levels of TM6SF2 were increased in participants with NAFLD and HFD-induced models, we wanted to determine the physiological significance of TM6SF2 elevation when NAFLD phenotypes occur. To this end, we used the AAV8 vector to deliver human TM6SF2 (AAV8-TM6SF2) in the livers of mice (AAV8-vector was used as a control) and fed them HFD for 16 wk (Figure 2A and B). The results showed that TM6SF2 overexpression in the liver significantly reduced the liver/body weight ratio, and its impact on body weight was limited (Figure 2C and D). Hepatic TM6SF2 overexpression in mice resulted in lower levels of hepatic lipid content as well as lower serum ALT and AST levels (Figure 2E and F). TM6SF2-overexpression groups relative to controls displayed equal body size (Figure 2G) but demonstrated a minor fatty liver and a smaller cross-sectional area of the fatty liver (Figure 2H). Hepatic lipid accumulation was also ameliorated in AAV8-TM6SF2 mice, as indicated by the pathological examination (Figure 2I). We also observed that TM6SF2 knocked-down cells reconstituted with wild-type TM6SF2 exhibited decreased intracellular lipid accumulation and

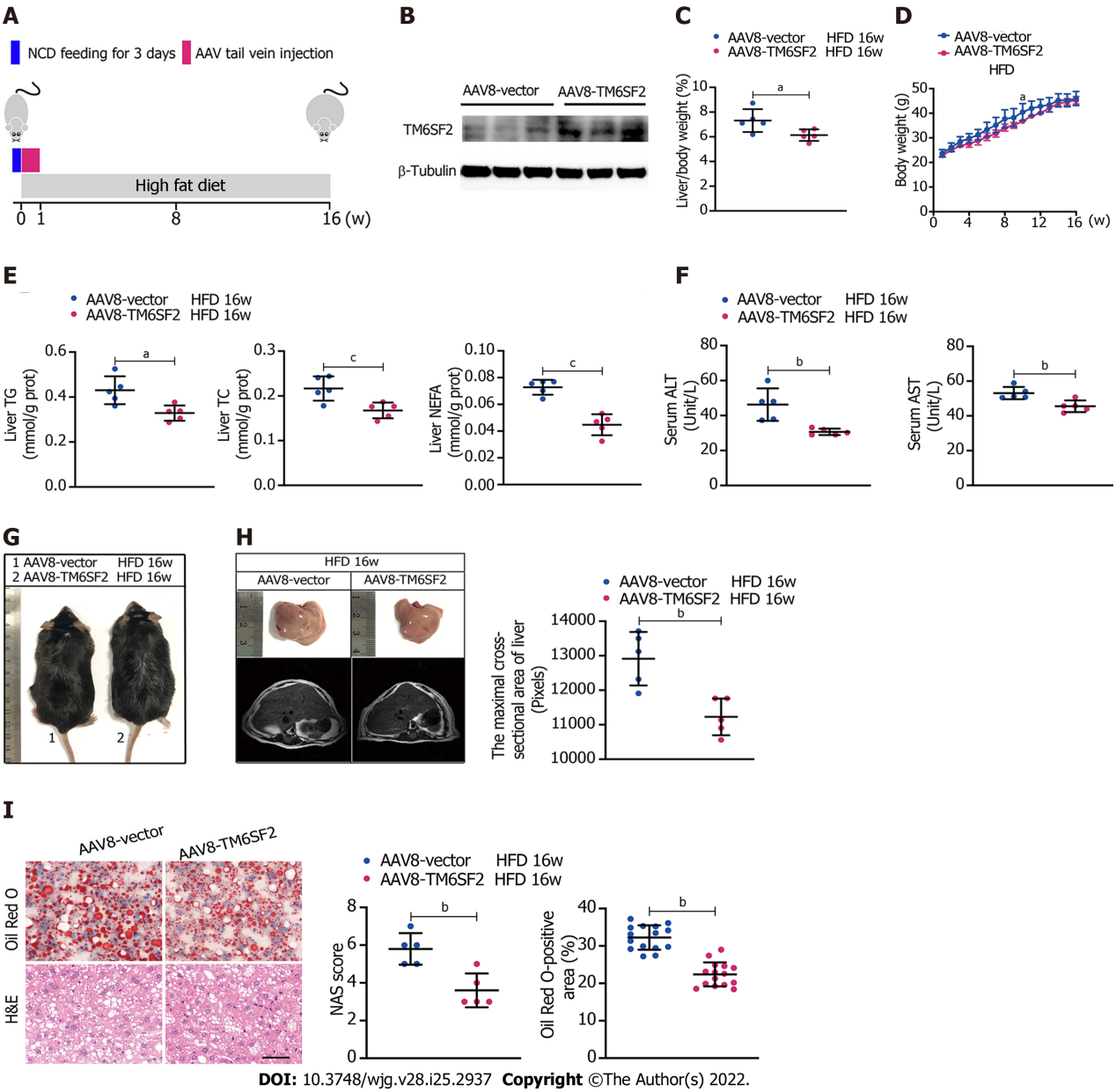


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**Figure 2 Overexpression of TM6SF2 improves hepatic lipid accumulation in high-fat diet-induced mice models.** A: Schematic representation of animal experiments. Mice were injected with AAV8-vector or AAV8-TM6SF2 virus *via* the tail vein and fed a high-fat diet for 16 wk ($n$ = 5 mice *per* group); B: The efficiency of TM6SF2 overexpression in liver were shown; C-F: The liver/body weight ratio (C), the body weight (D), hepatic lipid content (E), and serum levels of alanine aminotransferase and aspartate transaminase (F) were shown; G: Representative images of gross morphology; H: Left, representative photos of fatty livers (top) and livers subjected to magnetic resonance imaging scanning (bottom) were shown. Right, the maximal cross-sectional area of livers is quantified by the number of pixels; I: Left, representative images of hematoxylin and eosin-staining (left) and Oil Red O-staining (right) of liver sections. Right, the nonalcoholic fatty liver disease activity score (left) and quantification of lipid (right, 8 fields of each mouse were examined) were performed. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. H&E: Hematoxylin and eosin; NCD: Normal chow diet; HFD: High-fat diet; NAS: Nonalcoholic fatty liver disease activity score.

improved PA-induced cell death (Supplementary Figure 2). This evidence suggests that the elevation of TM6SF2 is a potential reaction to antagonize NAFLD phenotypes and serves a protective role during NAFLD.

### *Knockdown of TM6SF2 promotes hepatic lipid accumulation and inflammation in the HFD-induced mice model*

Pathologically, there exists a genetic E167K mutation in the TM6SF2 protein in humans, and those with the TM6SF2 E167K mutation are associated with an increased risk of NAFLD[26]. Ehrhardt *et al*[27] reported that the E167K mutation causes downregulation of TM6SF2 at the protein level. To simulate the E167K condition and explore the influence of TM6SF2 on lipid accumulation, we generated TM6SF2-knockdown cells in both the L02 and HepG2 cell lines (Figure 3A), which were incubated with PA (150 μmol/L) or BSA (fatty acid free) for 24 h. We found that TM6SF2-knockdown cells demonstrated higher levels of intracellular TG (Figure 3B) and more severe lipid accumulation (Figure 3C). Lipid overload

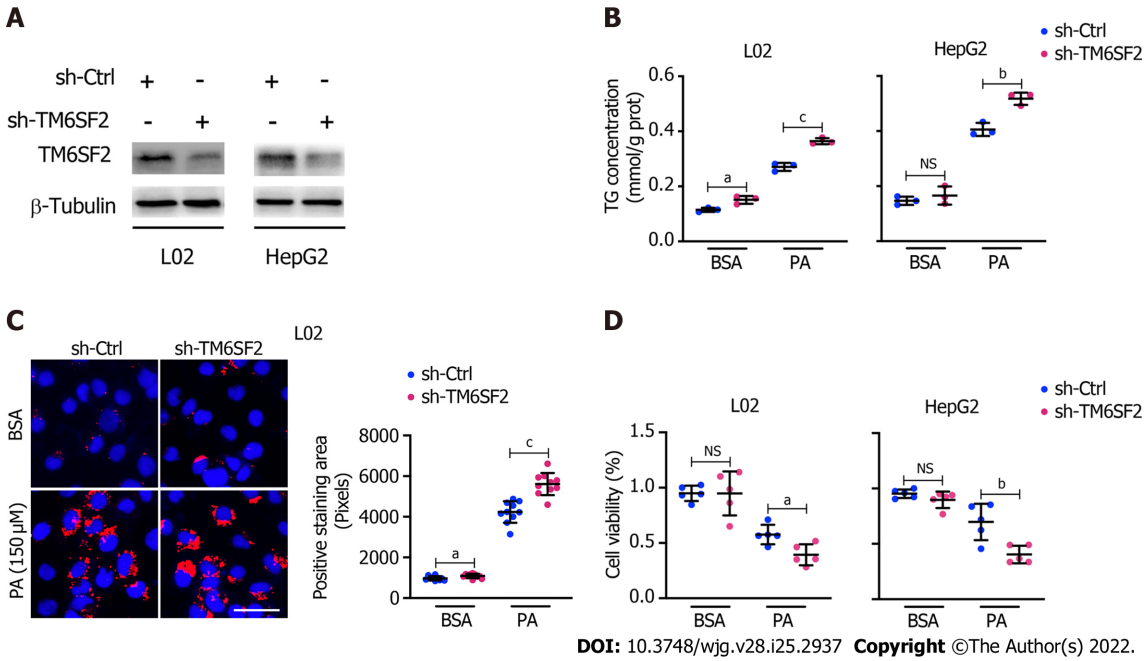


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**Figure 3 Knockdown of TM6SF2 promotes intracellular lipid accumulation and lipid-overload induced cell death.** A: The efficiency of TM6SF2 knockdown in two cell lines; B: Intracellular triglyceride levels ($n$ = 3) in TM6SF2-knockdown cells were examined after palmitic acid (PA) (150 μmol/L) or bovine serum albumin (BSA) (fatty acid free) treatment for 24 h; C: Nile red staining (left, nuclei labeled with DAPI, blue) and quantification of lipid accumulation (right) of sh-Ctrl or sh-TM6SF2 L02 cells after PA or BSA treatment. Scale bars: 50 μm; D: TM6SF2-knockdown cells were incubated with PA (150 μmol/L) or BSA for 24 h. The cellular viability was examined ($n$ = 5). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. NS: Not significant; BSA: Bovine serum albumin; PA: Palmitic acid; TG: Triglyceride; μM: μmol/L.

induces endoplasmic reticulum stress (ERS) and causes cell death[28,29]. The cell viability assay results proved that TM6SF2 knockdown markedly exacerbated PA-induced cell death (Figure 3D), which reflected the increase in lipid content in the TM6SF2-knockdown group. To determine whether TM6SF2 deficiency exacerbated hepatic steatosis *in vivo*, we generated hepatic TM6SF2-knockdown (hereafter referred to as AAV-shTm6sf2, AAV-shNC was used as a control) mice by injecting AAV vectors carrying TM6SF2-targeting shRNA, and mice were fed an HFD or NCD for 16 wk (Figure 4A and B). Compared to the control mice, the HFD-fed AAV-shTm6sf2 mice displayed severe hepatocellular ballooning degeneration and lipid accumulation, as shown by H&E and Oil Red O staining (Figure 4C); these mice had higher levels of hepatic TG, TC, and NEFA (Figure 4D). Similarly, AAV-shTm6sf2 mice had a higher liver/body weight ratio (Figure 4E) but exhibited a slightly higher body weight (Figure 4F) only at the initiation of feeding. In addition, hepatic TM6SF2-knockdown mice relative to controls displayed a modest increase in size (Figure 4G) but a significantly enlarged liver and a larger maximal cross-sectional area of the liver (Figure 4H). However, these parameters did not differ between the two groups when placed in a NCD environment (Figures 4D–F).

It is noteworthy that hepatic steatosis is often accompanied by inflammation, and the boundary between the two phases (SS and NASH) of NAFLD is blurred[30]. The hepatic TM6SF2-knockdown group displayed higher serum levels of inflammatory cytokines, such as TNF, IL-6, IL-1β, CCL2, and CXCL10 than control mice after 16 wk of HFD feeding (Supplementary Figure 3A). Consistently, hepatic expression of inflammation-related genes was also augmented in the AAV-shTm6sf2 group (Supplementary Figure 3B). Meanwhile, the AAV-shTm6sf2 group showed higher serum levels of ALT and AST (Supplementary Figure 3C) and more infiltrated neutrophils and macrophages than the AAV-shNC group (Supplementary Figure 3D). These results show that TM6SF2 knockdown promotes hepatic lipid accumulation and inflammation in an HFD-induced mouse model.

### *Hepatic knockdown of TM6SF2 enhances de novo lipogenesis*

Triplicate samples of PA-treated TM6SF2-knockdown L02 cells and their controls were used for transcriptome analysis to explore the changes in biological processes in TM6SF2 knockdown and identify upregulated DEGs. KEGG pathway enrichment analysis was applied to explore the function of these DEGs, which highlighted that fatty acid metabolism was the key process that changed in TM6SF2-knockdown cells (Figure 5A), and genes associated with fatty acid synthesis were found to be upregulated (Figure 5B). To confirm this, *in vitro* NAFLD models were used to verify our findings. PA is a potent agonist of fatty acid synthesis[31]. PA administration activated fatty acid synthesis *in vitro*, as indicated by increased protein levels of p-ACC, FASN, SCD-1, and sterol regulatory element-binding protein 1c (SREBP-1c) (Figure 5C). When comparing the levels of these proteins between TM6SF2-

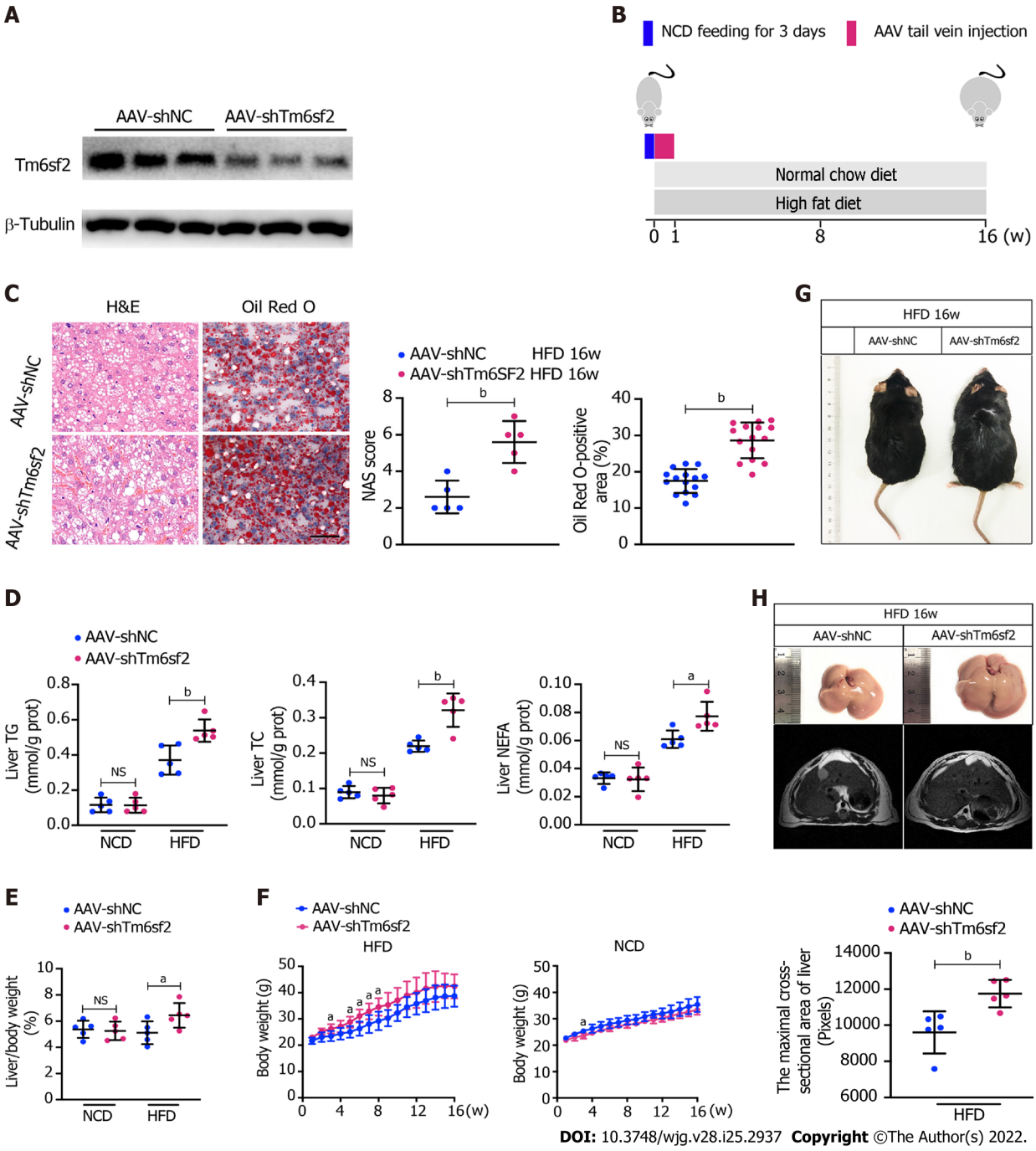


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**Figure 4 TM6SF2 deficiency exacerbates hepatic lipid accumulation and inflammation.** A: Mice were injected with AAV-shNC and AAV-shTm6sf2 viruses *via* the tail vein and fed a high-fat diet or normal chow diet for 16 wk ($n$ = 5 mice *per* group). The efficiency of TM6SF2 knockdown in liver were shown; B: Schematic representation of animal experiments; C: Left, representative images of hematoxylin and eosin-staining (left) and Oil Red O-staining (right) of liver sections. Right, the Nonalcoholic fatty liver disease activity score (left) and quantification of lipid (right, 8 fields of each mouse were examined) were performed; D-F: Hepatic lipid contents (triglyceride, total cholesterol and non-esterified fatty acids) (D), liver/body weight ratio (E) and body weight (F) were measured; G: Representative images of mice morphology; H: Top, representative photos of fatty livers (top) and livers subjected to magnetic resonance imaging scanning (bottom) of mice were shown. Bottom, the maximal cross-sectional area of livers is quantified by the number of pixels. $^{a}P < 0.05$; $^{b}P < 0.01$. NS: Not significant; H&E: Hematoxylin and eosin; NCD: Normal chow diet; HFD: High-fat diet; TG: Triglyceride; TC: Total cholesterol; NEFA: Non-esterified fatty acids.

knockdown cells and the corresponding controls, the results showed that these proteins were upregulated in TM6SF2-knockdown cells, suggesting that PA-induced fatty acid synthesis was enhanced by the knockdown of TM6SF2 (Figure 5D). *De novo* lipogenesis is mainly mediated by SREBP-1c on the transcriptional regulation of ACC and fatty acid synthase. The mature form of SREBP-1c translocates to the nucleus and activates its target genes, such as *Acaca* and *Fasn*[32]. This translocation-to-nucleus phenomenon was also enhanced in the TM6SF2-knockdown cells (Figure 5E). In addition, TM6SF2 knockdown increased hepatic malonyl-CoA, a metabolite of ACC[33], in HFD-fed AAV-shTm6sf2 mice (Figure 5F), which indicated that ACC activity was enhanced due to TM6SF2 deficiency. It is noteworthy that the transcriptional activity of hepatic SREBP-1c is also influenced by circulating

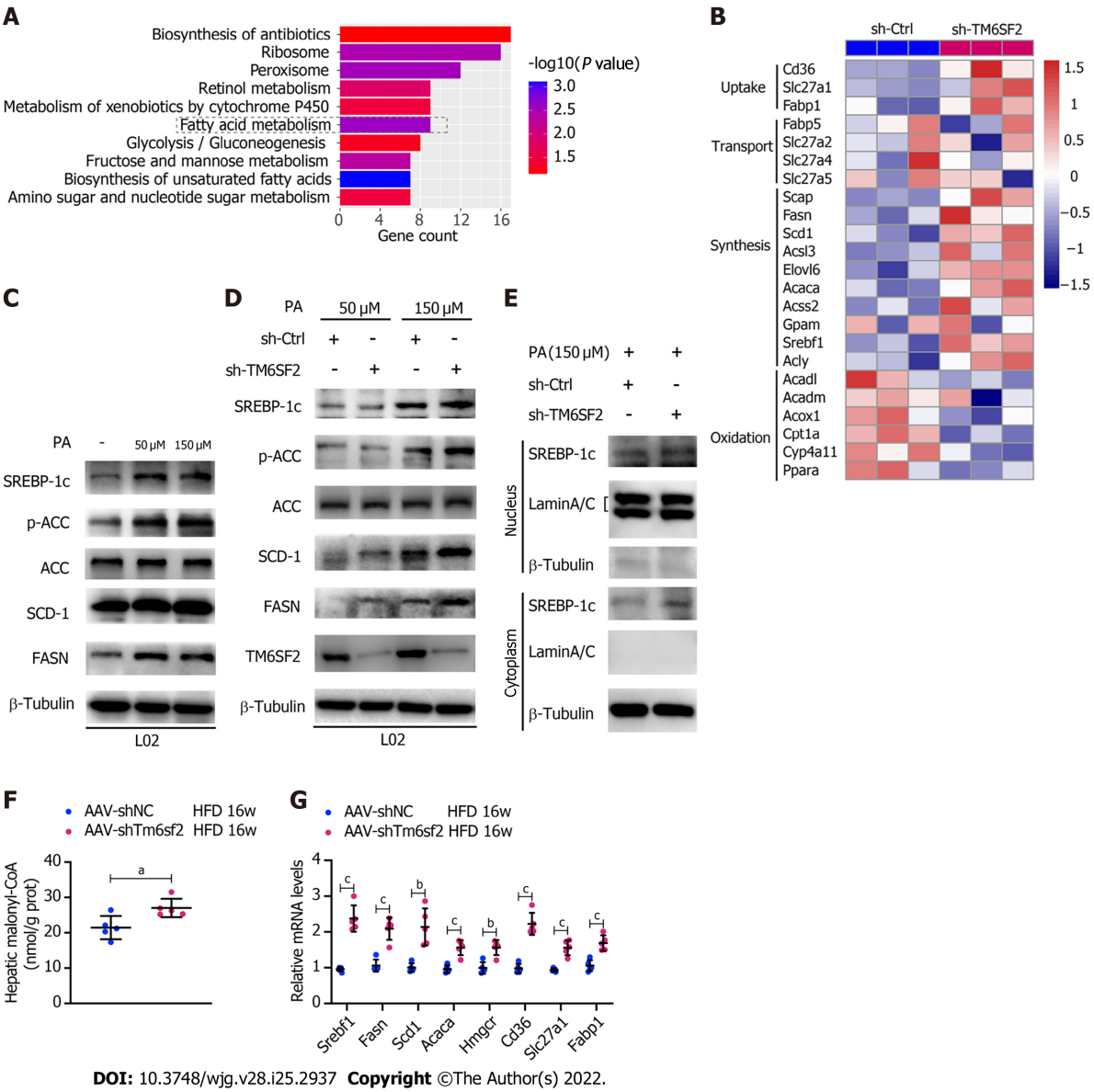


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**Figure 5 The activity of fatty acid synthesis was enhanced in TM6SF2 knockdown group.** A: Pathway enrichment of the up-regulated differentially expressed genes by Kyoto Encyclopedia of Genes and Genomes analysis; B: Microarray heatmap of genes involved in fatty acid metabolism in TM6SF2-knockdown cells; C: The SREBP-1c target protein levels in L02 and HepG2 cells after palmitic acid (PA) treatment; D: The SREBP-1c target protein levels in TM6SF2-knockdown cells after PA treatment; E: Immunoblot analysis of SREBP-1c in cytoplasmic and nuclear extracts; F: Hepatic malonyl-CoA contents of mice in two groups ($n$ = 5 mice *per* group); G: The mRNA levels of genes involved in fatty acid synthesis (*Srebf1*, *Fasn*, *Scd1*, *Acaca* and *Hmgcr*) and uptake (*Cd36*, *Slc27a1* and *Fabp1*). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. HFD: High-fat diet; PA: Palmitic acid; ACC: Acetyl-CoA carboxylase; μM: μmol/L.

insulin status. We evaluated the mice's blood glucose and insulin levels and found no differences between the two groups with or without HFD feeding (Supplementary Figure 4A). In addition, no obvious differences were noted in the GTT and ITT (Supplementary Figure 4B). These results suggest that the enhanced steatosis in hepatic TM6SF2-knockdown mice is unlikely to be induced by altering insulin sensitivity. Moreover, we also noted that TM6SF2 knockdown did not affect the fatty acid transport process but caused an increase in the mRNA levels of molecules involved in fatty acid uptake (*e.g.*, CD36 and FABP1) (Figure 5B). This increase was augmented when the TM6SF2-knockdown cells were under starvation or hypoxic conditions (Supplementary Figure 4C), as hypoxia and starvation have been reported to enhance lipid uptake[34-36]. In parallel, the levels of fatty acid synthesis- and uptake-related genes were elevated in the livers of AAV-shTm6sf2 mice compared to control mice (Figure 5G). This evidence suggests that the unfavorable fatty liver in hepatic TM6SF2-knowndown mice may be associated with enhanced *de novo* lipogenesis and lipid uptake.

### *TM6SF2 deficiency could lead to impaired FAO*

It was previously reported that NAFLD caused by TM6SF2 deficiency was partially due to impaired VLDL secretion, which is detrimental to the liver, as VLDL secretion is considered a significant pathway of lipid export in the liver[18]. FAO, another method of energy expenditure[37], has rarely been

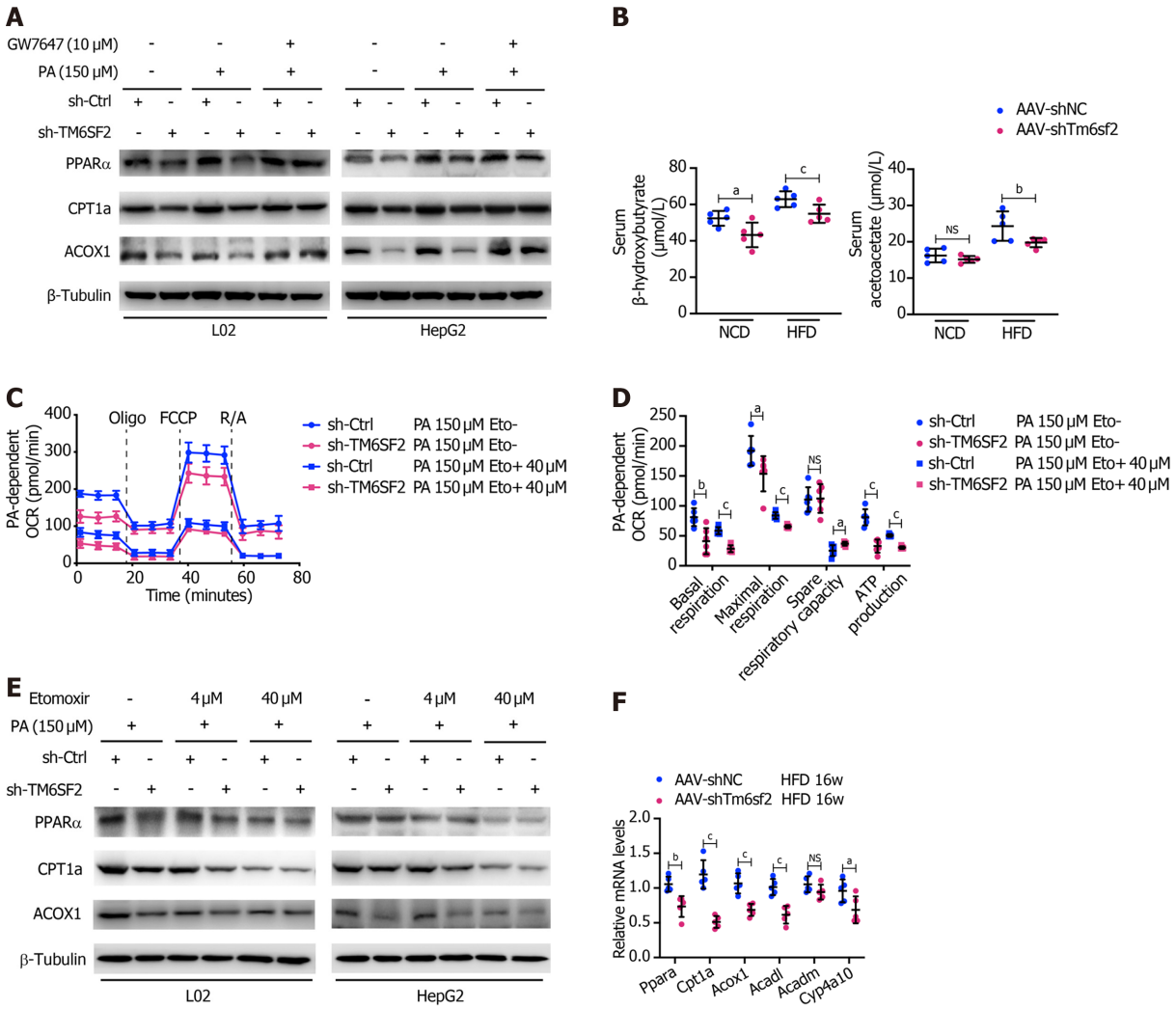


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**Figure 6 TM6SF2 deficiency caused the downregulation of fatty acid oxidation in liver.** A: Protein levels of PPARα, CPT1A and ACOX1 was analyzed in TM6SF2-knockdown cells after palmitic acid (PA) stimulation and their response to GW7647 (10 μmol/L) treatment; B: Serum levels of β-hydroxybutyrate and acetoacetate in normal chow diet- or high-fat diet-fed AAV-shNC or AAV-shTm6sf2 mice ($n$ = 5 mice *per* group); C and D: PA-dependent oxygen consumption rate in sh-Ctrl and sh-TM6SF2 L02 cells with or without etomoxir (ETO, 40 μmol/L) administration (C, $n$ = 6). The basal respiration, maximal respiration, spare respiratory capacity, and ATP production were calculated (D); E: Protein levels of PPARα, CPT1A and ACOX1 were determined in TM6SF2-knockdown cells with or without ETO treatment under PA (150 μmol/L) stimulation; F: The hepatic mRNA levels of fatty acid oxidation-related genes in the indicated mice. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. PA: Palmitic acid; NS: Not significant; NCD: Normal chow diet; HFD: High-fat diet; OCR: Oxygen consumption rate; μM: μmol/L.

reported in the absence of the TM6SF2 protein. Therefore, we evaluated and compared the expression of the key molecules involved in FAO. The results showed that TM6SF2-knockdown cells exhibited decreased levels of FAO-related proteins[38], including PPARα, CPT1A, and ACOX1, with or without PA stimulation (Figure 6A), which is consistent with the results of transcriptomic data (Figure 5B). Furthermore, PPARα signaling has been reported to function as a key factor in regulating FAO[39], and we found that the downregulated levels of FAO-related proteins in TM6SF2-knockdown cells could be reversed by PPARα activation through PPARα agonist (GW7647) stimulation (Figure 6A). We next tested hepatic FAO *in vivo* by evaluating the levels of serum ketone bodies, β-OH, and acetoacetate in both AAV-shTm6sf2 mice and control groups after a 16 h fast. The results showed that the levels of ketone bodies were significantly lower in the TM6SF2-knockdown group (Figure 6B), suggesting that TM6SF2 deficiency reduces FAO. In addition, compared to the control group, TM6SF2-knockdown L02 cells had a lower OCR with PA as the only substrate (Figures 6C and D). Meanwhile, the CPT1A inhibitor etomoxir inhibited the FAO process and diminished the difference in both groups, as well as the FAO-related proteins (Figure 6C-E). AAV-shTm6sf2 mice also showed a lower expression of FAO-related genes in the liver (Figure 6F). Together, these data indicate that TM6SF2 deficiency impairs cellular FAO.

### *MK4074 is effective in treating NAFLD exacerbated by TM6SF2 deficiency*

These results showed that TM6SF2 deficiency could lead to abnormal fatty acid metabolism within cells,

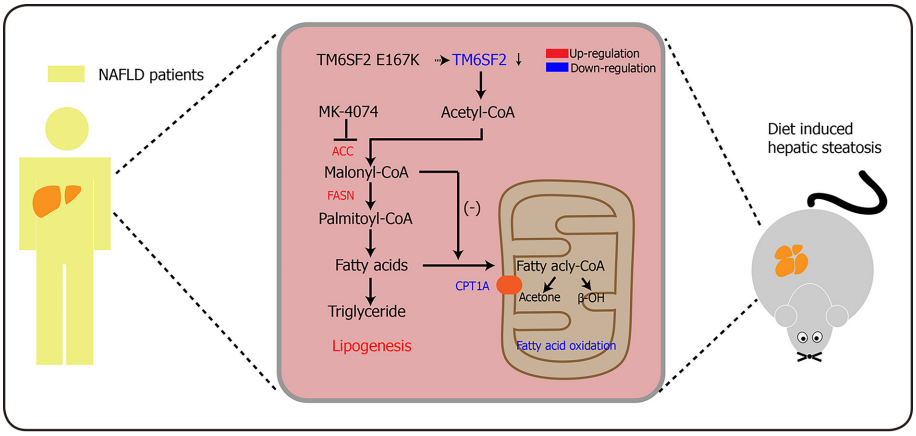


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**Figure 7 Schematic diagram of MK-4074 blocking acetyl-CoA carboxylase pathway and alleviating nonalcoholic fatty liver disease in TM6SF2-deficient mice.** ACC: Acetyl-CoA carboxylase; NAFLD: Nonalcoholic fatty liver disease.

characterized by enhanced lipogenesis and uptake, accompanied by impaired fatty acid β-oxidation (Figure 7). It is worth noting that the suppressed β-oxidation process may be associated with the increased malonyl-CoA content due to the enhanced activity of ACC in this situation. Malonyl-CoA is a natural CPT1A inhibitor, which causes a decrease in β-oxidation in mitochondria[40]. Therefore, inhibiting the activity of ACC may be a promising method to alleviate fatty liver disease in mice with hepatic TM6SF2 deficiency. To test this idea and rectify this metabolic abnormality, we thought of a way of inhibiting ACC, this rate-limiting enzyme, by using a previously reported ACC inhibitor, MK-4074, which could simultaneously limit the *de novo* lipid synthesis pathway and increase the level of intracellular lipid oxidation, to explore whether the drug has therapeutic effect on TM6SF2-deficient cases. To this end, both AAV-shTm6sf2 and control mice were first subjected to 8-wk HFD feeding to induce NAFLD phenotypes, and then two groups of mice received MK-4074 (MK-4074, 10 mg/kg/day) or placebo (normal saline) treatment and were fed with HFD for an additional eight weeks (Figure 8A). The results showed that MK-4074 treatment could diminish the increase in the levels of malonyl-CoA (Figure 8B), as well as hepatic TG, TC, and NEFA caused by TM6SF2 deficiency (Figure 8C), suggesting the success of blocking the SPREBP-1c/ACC pathway. Compared to placebo treatment, the HFD-fed AAV sh-Tm6sf2- mice under MK-4074 treatment exhibited mild hepatic ballooning degeneration and steatosis (Figure 8D). At the same time, MK-4074 treatment improved the liver/body weight ratio (Figure 8E), although the body weight improvement was rather limited (Figure 8F). In addition, the beneficial effect is also manifested *in vitro* experiments that MK-4074 could reduce lipid accumulation (Figure 9A and B) and improve PA-induced cell death in TM6SF2 knocked-down groups (Figure 9C). Meanwhile, MK-4074 inhibited malonyl-CoA production (Figure 8B) and improved the decrease in FAO caused by TM6SF2 deficiency, as evidenced by the increased FAO-related protein and ketone body levels in the TM6SF2-knockdown group (Figure 9D and E), which renders MK-4074 the prime option for treating this condition.

## DISCUSSION

The present study revealed that hepatic TM6SF2 expression was upregulated in both NAFLD mouse models and patients. TM6SF2 overexpression alleviated NAFLD phenotypes in HFD-induced models, suggesting a protective role for reactive TM6SF2 elevation under NAFLD conditions. Simulating the situation in patients with the E167K mutation, TM6SF2 knockdown exacerbated hepatic steatosis and inflammation. We noted a dysregulated fatty acid metabolism process under TM6SF2 deficiency, characterized by enhanced fatty acid uptake and fatty acid synthesis, accompanied by an impaired FAO process and insufficient ketone body production. Given the potential impact of TM6SF2 on fatty acid metabolism in NAFLD, exploring the effects of targeted NAFLD therapeutics on fatty liver is of the utmost importance, inspiring us to consider the impact of genetic variation on fatty liver. We used a previously reported liver-specific ACC inhibitor, MK-4074, which could reduce hepatic lipogenesis while increasing FAO, to explore the therapeutic effect on fatty liver caused by TM6SF2 deficiency (Figure 7). Our results show that MK-4074 has an encouraging effect on treating TM6SF2 knockdown-induced NAFLD, as indicated by the reduced hepatic lipid content, lower liver/body ratio, and alleviated PA-induced cell death suggesting the effectiveness of MK-4074 in treating this condition.

Previous studies have considered TM6SF2 as a pivotal molecule in hepatic lipid output in the form of VLDL-TG, and a majority of studies have focused on the association between the export of accumulated

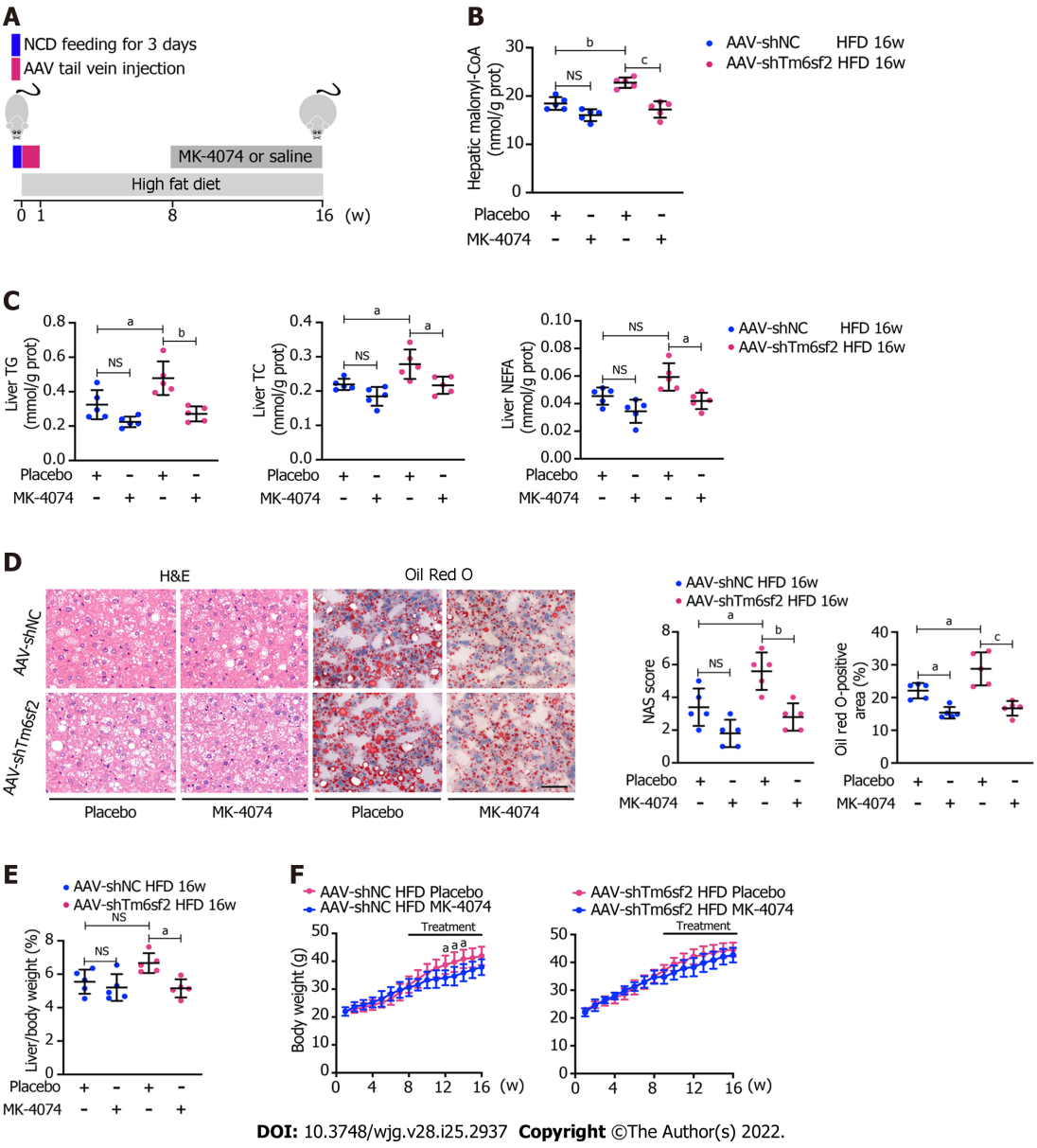


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**Figure 8 Therapeutic potential of MK-4074 on nonalcoholic fatty liver disease caused by TM6SF2 deficiency.** A: Schematic representation of animal experiments; B-D: Two groups (AAV-shNC and AAV-shTm6sf2) of mice were fed a high-fat diet (HFD) for 8 wk to induce nonalcoholic fatty liver disease phenotypes and then each subgroup was dosed with MK-4074 (10 mg/kg/day) or placebo orally for additional 8 wk. The hepatic malonyl-CoA levels (B) and lipid content (C) as well as results of hematoxylin and eosin-staining and Oil Red O-staining of liver sections (D) were shown; E and F: The liver/body weight ratio (E) and the body weight (F) of HFD-fed AAV-shNC and AAV-shTm6sf2 mice with or without MK-4074 treatment ($n$ = 5 mice *per* group) were shown. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. NS: Not significant; H&E: Hematoxylin and eosin; NCD: Normal chow diet; HFD: High-fat diet; TG: Triglyceride; TC: Total cholesterol; NEFA: Non-esterified fatty acids.

lipids and the TM6SF2 E167K mutant[15,18,41]. The TM6SF2 E167K mutant may result in a misfolded protein easily degraded within the cells, resulting in decreased TM6SF2 levels[42]. The liver is a pivotal hub for lipid metabolism, and its dysregulation could be a significant cause of NAFLD[43]. Therefore, we investigated the cause of TM6SF2 deficiency-induced NAFLD from another perspective, dysregulated fatty acid metabolism. Our results revealed that TM6SF2 knockdown significantly increased the expression of SREBP-1c and its target genes both *in vivo* and *in vitro*, suggesting abnormally enhanced *de novo* lipogenesis (DNL). Meanwhile, the results showed that the levels of OCR were decreased in the TM6SF2-knockdown groups, indicating that the FAO process was impaired under these circumstances. We further tested the transcriptional levels of FAO-related genes in hepatic TM6SF2-knockdown mice fed an HFD and showed lower levels of genes involving FAO than the control mice on the same diet for 16 wk. Previous studies showed that elevated levels of malonyl-CoA would suppress FAO by inhibiting CPT1A activity[44]. In our study, the malonyl-CoA content was higher in AAV-shTm6sf2 mice than in the control mice, which implies that the decreased FAO process is related to the enhanced DNL due to TM6SF2 knockdown.

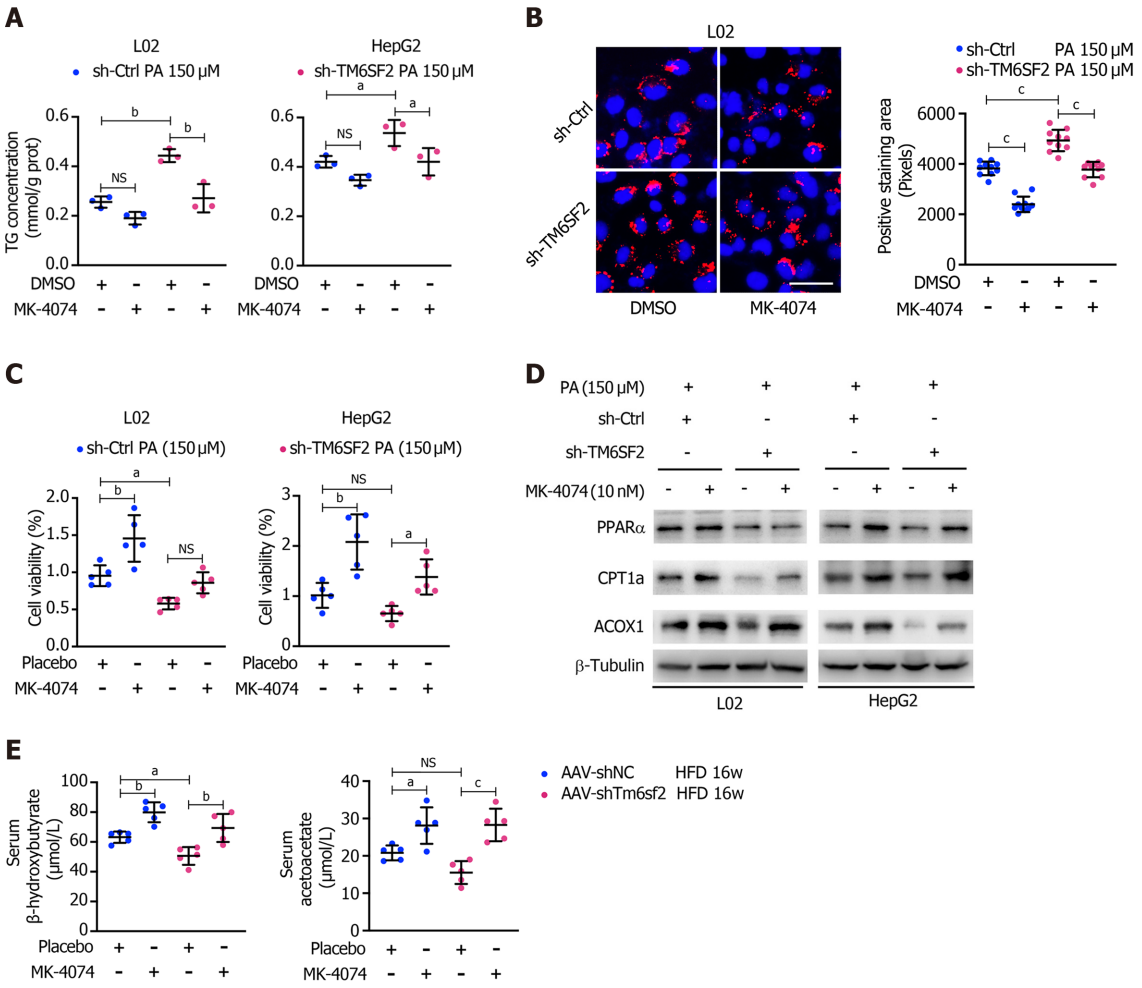


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**Figure 9 MK-4074 can improve intracellular lipid accumulation, lipid overload-induced cell death, and fatty acids β-oxidation.** A: Intracellular triglyceride levels ($n$ = 3) in TM6SF2-knockdown cells with palmitic acid (PA) (150 μmol/L) stimulation were examined after MK-4074 (10 nM) treatment for 24 h; B: Representative nile red staining images (left) and lipid droplet quantification (right) of sh-Ctrl or sh-TM6SF2 L02 cells after PA stimulation in response to MK-4074 (10 nM) treatment (10 fields of each sample were examined). Scale bars: 50 μm; C: The cellular viability was examined in TM6SF2-knockdown cells with or without MK-4074 treatment in response to PA-induced cell death ($n$ = 5); D: The sh-Ctrl and sh-TM6SF2 cells were cultured with PA (150 μmol/L) for 24 h. Cells were then treated with MK-4074 (10 nM) for 12 h. The expression of fatty acid oxidation-related proteins was determined; E: Serum levels of β-hydroxybutyrate and acetoacetate in the indicated mice with or without MK-4074 treatment. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. NS: Not significant; HFD: High-fat diet; TG: Triglyceride; PA: Palmitic acid; μM: μmol/L.

Currently, genetic technologies have already allowed for the identification of a subgroup of people with an increased risk of NAFLD or NAFLD-related complications associated with specific pathophysiological characteristics[45]. Unfortunately, there is no precise treatment for metabolic diseases with specific congenital genetic variations in NAFLD. However, much effort has been made to elucidate the mechanism of NAFLD caused by genetic mutations and implement precision medicine. For example, human genetics of NAFLD offers the opportunity to apply targeted therapy with the PNPLA3 I148M variant. This locus is the strongest genetic factor that increases susceptibility to NAFLD[10]. Recently, researchers used mice with the PNPLA3 variant knock-in and tried to improve NAFLD phenotypes through antisense oligonucleotides to downregulate the PNPLA3 mutant protein[46]. Although the mechanisms are not fully understood, accumulating evidence reveals that PNPLA3 I148M impairs lipid droplet remodeling in hepatocytes due to the loss of lipase activity[47]. Inhibition of PNPLA3 148M expression reduced liver fat content in these engineered mice and, specifically, had a beneficial effect in mitigating inflammation and fibrosis. If these applications are translated into humans, after safety and reliability examinations, downregulation of PNPLA3 148M may be the first case of human genetics-based targeted therapy. The importance of this method is to treat not only the phenotype of the disease but also the cause.

As described in the PNPLA3 mutant protein case, this evidence proved the possibility of targeted therapy to eliminate the cause of fatty liver in patients carrying a specific risk allele. However, there have been no reports of attempts to treat fatty liver caused by TM6SF2 deficiency or its E167K mutant. Based on our data, mice with TM6SF2 deficiency show enhanced DNL. Among the TM6SF2/SREBP-1c/ACC axis, ACC acts as the rate-limiting enzyme for converting acetyl-CoA to malonyl-CoA[48]. The

product, malonyl-CoA, was further catalyzed by FASN to form palmitate. TGs are one of the many lipid species that are integrated with DNL-derived palmitate. ACC inhibitors have the potential to mitigate hepatic steatosis in NAFLD, given that ACC is essential and pivotal in hepatic fatty acid metabolism. Independent studies have revealed that blocking DNL by ACC inhibition has a beneficial effect in reducing liver fat, steatosis, and inflammation in NAFLD models[44,49]. Among ACC inhibitors, MK-4074 has recently been tested for NAFLD treatment, and its effects have been encouraging. MK-4074 treatment decreased DNL and enhanced FAO in participants under overnutrition. Based on its function, we hypothesized that it could be used to treat fatty liver caused by the reduction of TM6SF2, and *in vivo* and *in vitro* experiments confirmed this hypothesis. ACC inhibitors have recently been shown to effectively mitigate liver fibrosis in NASH patients[49,50]. The accumulating evidence has shown that TM6SF2 E167K is also associated with NASH and fibrosis[18,51]. Whether ACC inhibitors can be applied to mitigate liver fibrosis in NASH models with TM6SF2 deficiency or the E167K mutant is unknown. This indicates the direction of our future work.

## CONCLUSION

This study showed that dysregulated fatty acid metabolism occurs in the context of TM6SF2 deficiency under overnutrition. Therapeutics aimed at abnormal fatty acid metabolism may be a promising strategy for improving the hepatic lipid profile of patients with the TM6SF2 E167K variant in the clinical setting. Furthermore, our study suggests that MK-4074 could be a potential drug to lower hepatic lipid content in a TM6SF2-knockdown NAFLD mouse model. Further investigations are required to test whether this approach would lower hepatic lipid levels in NAFLD patients with the E167K mutation.

## ARTICLE HIGHLIGHTS

### *Research background*

Previously, we found that the TM6SF2 E167K mutation was associated with susceptibility to nonalcoholic fatty liver disease (NAFLD) in a Chinese cohort, but the underlying mechanism is poorly understood.

### *Research motivation*

Because existing evidence has shown that *TM6SF2* gene mutation would reduce its expression at the protein level, we used NAFLD mouse models to explore the causes of NAFLD caused by TM6SF2 deficiency in the context of high-fat feeding and test possible treatment strategies. This would benefit our understanding of the mechanisms underlying NAFLD in humans.

### *Research objectives*

Mechanism and therapeutic strategy of hepatic TM6SF2-deficient NAFLD *via in vivo* and *in vitro* experiments.

### *Research methods*

This study mainly involved the knockdown and overexpression of the hepatic *TM6SF2* gene in NAFLD mice and cell models to explore its effects on pathological changes in the liver. In addition, RNA-seq, oxygen consumption rate technology, western blotting, and pathological examination were performed to investigate the underlying mechanisms of NALFD caused by TM6SF2 deficiency.

### *Research results*

Hepatic TM6SF2 expression was upregulated in patients with NAFLD and high-fat diet (HFD)-fed mice. Overexpression of TM6SF2 mitigated hepatic lipid accumulation in HFD-fed mouse models. Knockdown of TM6SF2 promoted hepatic lipid accumulation and inflammation. TM6SF2 deficiency promotes hepatic lipid accumulation through the dysregulation of fatty acid metabolism. MK-4074 administration may serve as a potential drug to improve NAFLD caused by TM6SF2 deficiency.

### *Research conclusions*

In this study, we found that the reactive overexpression of TM6SF2 under HFD conditions could alleviate hepatic lipid accumulation, the loss of which accelerated the NALFD phenotypes under HFD feeding. We also found that dysregulated fatty acid metabolism occurs in the context of TM6SF2 deficiency under overnutrition conditions. Therapeutics aimed at abnormal fatty acid metabolism may be a promising strategy for improving the hepatic lipid profile of patients with the TM6SF2 E167K variant in the clinical setting. Our study suggests that MK-4074 could be a potential drug for lowering hepatic lipid content in a TM6SF2-knockdown NAFLD mouse model.

### *Research perspectives*

TM6SF2 plays a protective role in the HFD condition; its deficiency enhanced hepatic lipid accumulation through dysregulated fatty acid metabolism, and MK-4074 treatment could alleviate the NAFLD phenotypes caused by TM6SF2 deficiency. The next step was to investigate whether MK-4074 has a therapeutic effect in patients with NAFLD harboring the TM6SF2 E167K mutation.

## FOOTNOTES

**Author contributions:** Li ZY and Wu G contributed equally to the work, Li ZY and Wu G performed the *in vitro* and *in vivo* experiments; Qiu C, Zhou ZJ, Wang YP, Song GH and Xiao C performed the analysis of GEO data; Deng GL and Wang RT provided the technical supports; Zhang X interpreted the data; Li ZY wrote the manuscript; Wang XL and Yang YL contributed equally, Wang XL and Yang YL conceived and supervised the study; all authors critically reviewed and edited the manuscript.

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## REFERENCES

1 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]
2 **Marengo A**, Jouness RI, Bugianesi E. Progression and Natural History of Nonalcoholic Fatty Liver Disease in Adults. *Clin Liver Dis* 2016; **20**: 313-324 [PMID: 27063271 DOI: 10.1016/j.cld.2015.10.010]
3 **Enomoto H**, Bando Y, Nakamura H, Nishiguchi S, Koga M. Liver fibrosis markers of nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; **21**: 7427-7435 [PMID: 26139988 DOI: 10.3748/wjg.v21.i24.7427]
4 **Younossi ZM**. Long-Term Outcomes of Nonalcoholic Fatty Liver Disease: From Nonalcoholic Steatohepatitis to Nonalcoholic Steatofibrosis. *Clin Gastroenterol Hepatol* 2017; **15**: 1144-1147 [PMID: 28549675 DOI: 10.1016/j.cgh.2017.05.029]
5 **Wong VW**, Chitturi S, Wong GL, Yu J, Chan HL, Farrell GC. Pathogenesis and novel treatment options for non-alcoholic steatohepatitis. *Lancet Gastroenterol Hepatol* 2016; **1**: 56-67 [PMID: 28404113 DOI: 10.1016/S2468-1253(16)30011-5]

6 **Carlsson B**, Lindén D, Brolén G, Liljeblad M, Bjursell M, Romeo S, Loomba R. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2020; **51**: 1305-1320 [PMID: 32383295 DOI: 10.1111/apt.15738]

7 **Kahali B**, Halligan B, Speliotes EK. Insights from Genome-Wide Association Analyses of Nonalcoholic Fatty Liver Disease. *Semin Liver Dis* 2015; **35**: 375-391 [PMID: 26676813 DOI: 10.1055/s-0035-1567870]

8 **Namjou B**, Lingren T, Huang Y, Parameswaran S, Cobb BL, Stanaway IB, Connolly JJ, Mentch FD, Benoit B, Niu X, Wei WQ, Carroll RJ, Pacheco JA, Harley ITW, Divanovic S, Carrell DS, Larson EB, Carey DJ, Verma S, Ritchie MD, Gharavi AG, Murphy S, Williams MS, Crosslin DR, Jarvik GP, Kullo IJ, Hakonarson H, Li R; eMERGE Network, Xanthakos SA, Harley JB. GWAS and enrichment analyses of non-alcoholic fatty liver disease identify new trait-associated genes and pathways across eMERGE Network. *BMC Med* 2019; **17**: 135 [PMID: 31311600 DOI: 10.1186/s12916-019-1364-z]

9 **Li YY**. Genetic and epigenetic variants influencing the development of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 6546-6551 [PMID: 23236228 DOI: 10.3748/wjg.v18.i45.6546]

10 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

11 **Thangapandi VR**, Knittelfelder O, Brosch M, Patsenker E, Vvedenskaya O, Buch S, Hinz S, Hendricks A, Nati M, Herrmann A, Rekhade DR, Berg T, Matz-Soja M, Huse K, Klipp E, Pauling JK, Wodke JA, Miranda Ackerman J, Bonin MV, Aigner E, Datz C, von Schönfels W, Nehring S, Zeissig S, Röcken C, Dahl A, Chavakis T, Stickel F, Shevchenko A, Schafmayer C, Hampe J, Subramanian P. Loss of hepatic Mboat7 leads to liver fibrosis. *Gut* 2021; **70**: 940-950 [PMID: 32591434 DOI: 10.1136/gutjnl-2020-320853]

12 **Yoneda M**, Hotta K, Nozaki Y, Endo H, Uchiyama T, Mawatari H, Iida H, Kato S, Hosono K, Fujita K, Yoneda K, Takahashi H, Kirikoshi H, Kobayashi N, Inamori M, Abe Y, Kubota K, Saito S, Maeyama S, Wada K, Nakajima A. Association between PPARGC1A polymorphisms and the occurrence of nonalcoholic fatty liver disease (NAFLD). *BMC Gastroenterol* 2008; **8**: 27 [PMID: 18588668 DOI: 10.1186/1471-230X-8-27]

13 **Mahdessian H**, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda A, Hamsten A, Eriksson P, van't Hooft F. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci U S A* 2014; **111**: 8913-8918 [PMID: 24927523 DOI: 10.1073/pnas.1323785111]

14 **Wang X**, Liu Z, Peng Z, Liu W. The TM6SF2 rs58542926 T allele is significantly associated with non-alcoholic fatty liver disease in Chinese. *J Hepatol* 2015; **62**: 1438-1439 [PMID: 25687425 DOI: 10.1016/j.jhep.2015.01.040]

15 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]

16 **Roh YS**, Loomba R, Seki E. The TM6SF2 variants, novel genetic predictors for nonalcoholic steatohepatitis. *Gastroenterology* 2015; **148**: 252-254 [PMID: 25451657 DOI: 10.1053/j.gastro.2014.11.014]

17 **Fan Y**, Lu H, Guo Y, Zhu T, Garcia-Barrio MT, Jiang Z, Willer CJ, Zhang J, Chen YE. Hepatic Transmembrane 6 Superfamily Member 2 Regulates Cholesterol Metabolism in Mice. *Gastroenterology* 2016; **150**: 1208-1218 [PMID: 26774178 DOI: 10.1053/j.gastro.2016.01.005]

18 **Dongiovanni P**, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, Motta BM, Kaminska D, Rametta R, Grimaudo S, Pelusi S, Montalcini T, Alisi A, Maggioni M, Kärjä V, Borén J, Käkelä P, Di Marco V, Xing C, Nobili V, Dallapiccola B, Craxi A, Pihlajamäki J, Fargion S, Sjöström L, Carlsson LM, Romeo S, Valenti L. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015; **61**: 506-514 [PMID: 25251399 DOI: 10.1002/hep.27490]

19 **Li BT**, Sun M, Li YF, Wang JQ, Zhou ZM, Song BL, Luo J. Disruption of the ERLIN-TM6SF2-APOB complex destabilizes APOB and contributes to non-alcoholic fatty liver disease. *PLoS Genet* 2020; **16**: e1008955 [PMID: 32776921 DOI: 10.1371/journal.pgen.1008955]

20 **Hodson L**, Gunn PJ. The regulation of hepatic fatty acid synthesis and partitioning: the effect of nutritional state. *Nat Rev Endocrinol* 2019; **15**: 689-700 [PMID: 31554932 DOI: 10.1038/s41574-019-0256-9]

21 **Robinson MD**, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010; **26**: 139-140 [PMID: 19910308 DOI: 10.1093/bioinformatics/btp616]

22 **Gómez-Lechón MJ**, Donato MT, Martínez-Romero A, Jiménez N, Castell JV, O'Connor JE. A human hepatocellular in vitro model to investigate steatosis. *Chem Biol Interact* 2007; **165**: 106-116 [PMID: 17188672 DOI: 10.1016/j.cbi.2006.11.004]

23 **Ricchi M**, Odoardi MR, Carulli L, Anzivino C, Ballestri S, Pinetti A, Fantoni LI, Marra F, Bertolotti M, Banni S, Lonardo A, Carulli N, Loria P. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. *J Gastroenterol Hepatol* 2009; **24**: 830-840 [PMID: 19207680 DOI: 10.1111/j.1440-1746.2008.05733.x]

24 **Alsabeeh N**, Chausse B, Kakimoto PA, Kowaltowski AJ, Shirihai O. Cell culture models of fatty acid overload: Problems and solutions. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018; **1863**: 143-151 [PMID: 29155055 DOI: 10.1016/j.bbalip.2017.11.006]

25 **Aji G**, Huang Y, Ng ML, Wang W, Lan T, Li M, Li Y, Chen Q, Li R, Yan S, Tran C, Burchfield JG, Couttas TA, Chen J, Chung LH, Liu D, Wadham C, Hogg PJ, Gao X, Vadas MA, Gamble JR, Don AS, Xia P, Qi Y. Regulation of hepatic insulin signaling and glucose homeostasis by sphingosine kinase 2. *Proc Natl Acad Sci U S A* 2020; **117**: 24434-24442 [PMID: 32917816 DOI: 10.1073/pnas.2007856117]

26 **Xu M**, Li Y, Zhang S, Wang X, Shen J. Interaction of TM6SF2 E167K and PNPLA3 I148M variants in NAFLD in northeast China. *Ann Hepatol* 2019; **18**: 456-460 [PMID: 31054977 DOI: 10.1016/j.aohep.2018.10.005]

27 **Ehrhardt N**, Doche ME, Chen S, Mao HZ, Walsh MT, Bedoya C, Guindi M, Xiong W, Ignatius Irudayam J, Iqbal J, Fuchs S, French SW, Mahmood Hussain M, Arditi M, Arumugaswami V, Péterfy M. Hepatic Tm6sf2 overexpression affects cellular ApoB-trafficking, plasma lipid levels, hepatic steatosis and atherosclerosis. *Hum Mol Genet* 2017; **26**: 2719-2731 [PMID: 28449094 DOI: 10.1093/hmg/ddx159]

28 **Zou Y**, Qi Z. Understanding the Role of Exercise in Nonalcoholic Fatty Liver Disease: ERS-Linked Molecular Pathways.

*Mediators Inflamm* 2020; **2020**: 6412916 [PMID: 32774148 DOI: 10.1155/2020/6412916]
29 **Yang WS**, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. *Trends Cell Biol* 2016; **26**: 165-176 [PMID: 26653790 DOI: 10.1016/j.tcb.2015.10.014]
30 **Anstee QM**, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 411-428 [PMID: 31028350 DOI: 10.1038/s41575-019-0145-7]
31 **Chen X**, Li L, Liu X, Luo R, Liao G, Liu J, Cheng J, Lu Y, Chen Y. Oleic acid protects saturated fatty acid mediated lipotoxicity in hepatocytes and rat of non-alcoholic steatohepatitis. *Life Sci* 2018; **203**: 291-304 [PMID: 29709653 DOI: 10.1016/j.lfs.2018.04.022]
32 **Li Y**, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 2011; **13**: 376-388 [PMID: 21459323 DOI: 10.1016/j.cmet.2011.03.009]
33 **Fadó R**, Rodríguez-Rodríguez R, Casals N. The return of malonyl-CoA to the brain: Cognition and other stories. *Prog Lipid Res* 2021; **81**: 101071 [PMID: 33186641 DOI: 10.1016/j.plipres.2020.101071]
34 **Tian Y**, Yang B, Qiu W, Hao Y, Zhang Z, Li N, Cheng S, Lin Z, Rui YC, Cheung OKW, Yang W, Wu WKK, Cheung YS, Lai PBS, Luo J, Sung JJY, Chen R, Wang HY, Cheng ASL, Yang P. ER-residential Nogo-B accelerates NAFLD-associated HCC mediated by metabolic reprogramming of oxLDL lipophagy. *Nat Commun* 2019; **10**: 3391 [PMID: 31358770 DOI: 10.1038/s41467-019-11274-x]
35 **Tomašić N**, Kotarsky H, de Oliveira Figueiredo R, Hansson E, Mörgelin M, Tomašić I, Kallijärvi J, Elmér E, Jauhiainen M, Eklund EA, Fellman V. Fasting reveals largely intact systemic lipid mobilization mechanisms in respiratory chain complex III deficient mice. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165573 [PMID: 31672551 DOI: 10.1016/j.bbadis.2019.165573]
36 **Chen X**, Gao Y, Wu G, Gu J, Cai Y, Xu J, Cheng H. Molecular cloning, tissue expression, and transcriptional regulation of fabp1 and fabp2 in javelin goby (Synechogobius hasta) in response to starvation stress. *Comp Biochem Physiol B Biochem Mol Biol* 2020; **250**: 110484 [PMID: 32745520 DOI: 10.1016/j.cbpb.2020.110484]
37 **Wang J**, Zhang Y, Zhuo Q, Tseng Y, Wang J, Ma Y, Zhang J, Liu J. TET1 promotes fatty acid oxidation and inhibits NAFLD progression by hydroxymethylation of PPARα promoter. *Nutr Metab (Lond)* 2020; **17**: 46 [PMID: 32577122 DOI: 10.1186/s12986-020-00466-8]
38 **Xu J**, Xiao G, Trujillo C, Chang V, Blanco L, Joseph SB, Bassilian S, Saad MF, Tontonoz P, Lee WN, Kurland IJ. Peroxisome proliferator-activated receptor alpha (PPARalpha) influences substrate utilization for hepatic glucose production. *J Biol Chem* 2002; **277**: 50237-50244 [PMID: 12176975 DOI: 10.1074/jbc.M201208200]
39 **Wang Y**, Nakajima T, Gonzalez FJ, Tanaka N. PPARs as Metabolic Regulators in the Liver: Lessons from Liver-Specific PPAR-Null Mice. *Int J Mol Sci* 2020; **21** [PMID: 32192216 DOI: 10.3390/ijms21062061]
40 **Foster DW**. Malonyl-CoA: the regulator of fatty acid synthesis and oxidation. *J Clin Invest* 2012; **122**: 1958-1959 [PMID: 22833869 DOI: 10.1172/jci63967]
41 **Borén J**, Adiels M, Björnson E, Matikainen N, Söderlund S, Rämö J, Ståhlman M, Ripatti P, Ripatti S, Palotie A, Mancina RM, Hakkarainen A, Romeo S, Packard CJ, Taskinen MR. Effects of TM6SF2 E167K on hepatic lipid and very low-density lipoprotein metabolism in humans. *JCI Insight* 2020; **5** [PMID: 33170809 DOI: 10.1172/jci.insight.144079]
42 **Prill S**, Caddeo A, Baselli G, Jamialahmadi O, Dongiovanni P, Rametta R, Kanebratt KP, Pujia A, Pingitore P, Mancina RM, Lindén D, Whatling C, Janefeldt A, Kozyra M, Ingelman-Sundberg M, Valenti L, Andersson TB, Romeo S. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. *Sci Rep* 2019; **9**: 11585 [PMID: 31406127 DOI: 10.1038/s41598-019-47737-w]
43 **Alves-Bezerra M**, Cohen DE. Triglyceride Metabolism in the Liver. *Compr Physiol* 2017; **8**: 1-8 [PMID: 29357123 DOI: 10.1002/cphy.c170012]
44 **Wu X**, Huang T. Recent development in acetyl-CoA carboxylase inhibitors and their potential as novel drugs. *Future Med Chem* 2020; **12**: 533-561 [PMID: 32048880 DOI: 10.4155/fmc-2019-0312]
45 **Trépo E**, Valenti L. Update on NAFLD genetics: From new variants to the clinic. *J Hepatol* 2020; **72**: 1196-1209 [PMID: 32145256 DOI: 10.1016/j.jhep.2020.02.020]
46 **Lindén D**, Ahnmark A, Pingitore P, Ciociola E, Ahlstedt I, Andréasson AC, Sasidharan K, Madeyski-Bengtson K, Zurek M, Mancina RM, Lindblom A, Bjursell M, Böttcher G, Ståhlman M, Bohlooly-Y M, Haynes WG, Carlsson B, Graham M, Lee R, Murray S, Valenti L, Bhanot S, Åkerblad P, Romeo S. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. *Mol Metab* 2019; **22**: 49-61 [PMID: 30772256 DOI: 10.1016/j.molmet.2019.01.013]
47 **Wang Y**, Kory N, BasuRay S, Cohen JC, Hobbs HH. PNPLA3, CGI-58, and Inhibition of Hepatic Triglyceride Hydrolysis in Mice. *Hepatology* 2019; **69**: 2427-2441 [PMID: 30802989 DOI: 10.1002/hep.30583]
48 **Brownsey RW**, Zhande R, Boone AN. Isoforms of acetyl-CoA carboxylase: structures, regulatory properties and metabolic functions. *Biochem Soc Trans* 1997; **25**: 1232-1238 [PMID: 9449982 DOI: 10.1042/bst0251232]
49 **Gao YS**, Qian MY, Wei QQ, Duan XB, Wang SL, Hu HY, Liu J, Pan CY, Zhang SQ, Qi LW, Zhou JP, Zhang HB, Wang LR. WZ66, a novel acetyl-CoA carboxylase inhibitor, alleviates nonalcoholic steatohepatitis (NASH) in mice. *Acta Pharmacol Sin* 2020; **41**: 336-347 [PMID: 31645659 DOI: 10.1038/s41401-019-0310-0]
50 **Bates J**, Vijayakumar A, Ghoshal S, Marchand B, Yi S, Kornyeyev D, Zagorska A, Hollenback D, Walker K, Liu K, Pendem S, Newstrom D, Brockett R, Mikaelian I, Kusam S, Ramirez R, Lopez D, Li L, Fuchs BC, Breckenridge DG. Acetyl-CoA carboxylase inhibition disrupts metabolic reprogramming during hepatic stellate cell activation. *J Hepatol* 2020; **73**: 896-905 [PMID: 32376414 DOI: 10.1016/j.jhep.2020.04.037]
51 **Liu Z**, Que S, Zhou L, Zheng S, Romeo S, Mardinoglu A, Valenti L. The effect of the TM6SF2 E167K variant on liver steatosis and fibrosis in patients with chronic hepatitis C: a meta-analysis. *Sci Rep* 2017; **7**: 9273 [PMID: 28839198 DOI: 10.1038/s41598-017-09548-9]

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ORIGINAL ARTICLE

Basic Study

# Upregulated adenosine 2A receptor accelerates post-infectious irritable bowel syndrome by promoting CD4+ T cells' T helper 17 polarization

Li-Wei Dong, Zhi-Chao Ma, Jiao Fu, Bai-Li Huang, Fu-Jin Liu, Deming Sun, Cheng Lan

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## Abstract

*BACKGROUND*

Post-infectious irritable bowel syndrome (PI-IBS) is generally regarded as a functional disease. Several recent studies have reported the involvement of low-grade inflammation and immunological dysfunction in PI-IBS. T helper 17 (Th17) polarization occurs in IBS. Adenosine and its receptors participate in intestinal inflammation and immune regulation.

*AIM*

To investigate the role of Th17 polarization of CD4+ T cells regulated by adenosine 2A receptor (A2AR) in PI-IBS.

*METHODS*

A PI-IBS model was established by infecting mice with *Trichinella spiralis*. The intestinal A2AR and CD4+ T lymphocytes were detected by immunohistochemistry, and the inflammatory cytokines were detected by enzyme-linked immunoassay. CD4+ T lymphocytes present in the animal's spleen were separated and cultured with or without A2AR agonist and antagonist. Western blotting and real-time quantitative polymerase chain reaction were performed to determine the effect of A2AR on the cells and intestinal tissue. Cytokine production was determined. The protein and mRNA levels of A2AR associated signaling pathway molecules were also evaluated. Furthermore, A2AR agonist and antagonist were injected into the mouse model and the clinical features were observed.

***RESULTS***

The PI-IBS mouse model showed increased expression of ATP and A2AR ($P < 0.05$), and inhibition of A2AR improved the clinical features in PI-IBS, including the abdominal withdrawal reflex and colon transportation test ($P < 0.05$). The number of intestinal CD4+ T cells and interleukin-17 (IL-17) protein levels increased during PI-IBS, which was reversed by administration of the A2AR antagonist ($P < 0.05$). CD4+ T cells expressed A2AR and produced IL-17 *in vitro*, which was regulated by the A2AR agonist and antagonist. The A2AR antagonist increased the production of IL-17 by CD4+ T cells *via* the Janus kinase-signal transducer and activator of transcription-receptor-related orphan receptor γ signaling pathway.

***CONCLUSION***

The results of the present study suggested that the upregulation of A2AR increases PI-IBS by promoting the Th17 polarization of CD4+ T cells.

**Key Words:** Adenosine 2A receptor; CD4+ T cells; T helper 17 polarization; Post-infectious irritable bowel syndrome; Regulation

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**Core Tip:** T helper 17 (Th17) polarization occurs during the development of irritable bowel syndrome (IBS). Adenosine and its receptors participate in the development of intestinal inflammation and immune regulation. Here, we found that the intestinal levels of ATP, adenosine 2A receptor (A2AR), and interleukin-17 (IL-17) and the number of CD4+ T cells increased in post-infectious IBS (PI-IBS) mice. In addition, A2AR promoted CD4+ T cells' IL-17 production. Upregulated A2AR accelerated PI-IBS by promoting CD4+ T cells' Th17 polarization, which was reversed by the A2AR antagonist. Thus, our results prove that the regulation of CD4+ T cells' Th17 polarization by A2AR plays a pathogenetic role in the development of PI-IBS.

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## INTRODUCTION

The predominant clinical features of irritable bowel syndrome (IBS) include abdominal pain, abnormal bowel habit, and stool changes. IBS occurs predominantly in developed countries. The incidence rate is about 10%-60%. During the past three decades, the incidence of IBS has rapidly increased in underdeveloped countries[1]. IBS can occur after an acute gastrointestinal infection, which is defined as post-infectious IBS (PI-IBS)[2]. The patients suffer from refractory discomfort, live with problems, and have to undergo high medical costs for their treatment. The laboratory reports of these patients do not show morphological findings, which leads to a shortage of time in targeting treatment[3]. Low-grade inflammation and immune activation in the colonic mucosa are observed during IBS[4]. PI-IBS might be alleviated due to regulation of the inflammatory response[5]. Upregulated ephrin type-A receptor 2 and activated nuclear factor kappa-light-chain-enhancer of activated B cells signaling pathway can alleviate PI-IBS[6]. Moreover, non-coding RNAs such as microRNA can modulate inflammation and immune response by targeting peroxiredoxin-1 in PI-IBS[7].

Recently, interleukin-17 (IL-17) raised concerns in PI-IBS. For instance, rifaximin protects mice against PI-IBS by suppressing the production of IL-17[8]. Our previous studies reported that heat shock protein 70 alleviated PI-IBS by upregulating T helper 17 (Th17) response in γδ T cells[9,10].

Adenosine originates from ATP in the local tissue during inflammation and regulates tissue blood flow[11]. As an endogenous molecule, adenosine exhibits anti-inflammatory and immunosuppressive effects by binding to the adenosine receptors (ARs), which are located on some immune and tissue cells and function as a G-protein coupled receptor[12-14]. ARs consist of the following four subtypes: A1R, adenosine A2a receptor, adenosine A2b receptor, and A3R, which are expressed at various locations in the intestine[15]. Different receptors induce different pathophysiological roles. A2BR inhibits the release of inflammatory cytokines, tumor necrosis factor-α, IL-6, and IL-12[16-18]. We previously reported that ARs contribute to the modulation of immune response by γ δ T cells[19,20]. In the intestine, adenosine

and its receptors regulate the enteric nervous system and visceral hypersensitivity. Endogenous adenosine affects the secretion of hydrocarbon by duodenum, whereas extracellular ATP induces the apoptosis of intestinal epithelial cells[21,22]. Adenosine inhibits inflammatory damage in the intestine, protects the intestine against ischemia, and reduces lesions in chronic septic mice by A1AR, A2AR, and A2BR[23,24]. A3R regulates distal colonic neuro-muscle function in rats with colitis[25]. Adenosine receptors also contribute to the inhibition of visceral pain by electroacupuncture in IBS[26]. The role of ARs in intestinal functions shows that they can be a potential target for intestinal inflammatory diseases. However, ARs are not widely studied in IBS, especially in PI-IBS. A2AR is reported to protect the enteric Glia barrier function against intestinal injury induced by hypoxia[27].

The relationship between A2AR, CD4+ T cells, and IL-17 is unknown. Whether A2AR is expressed in CD4+ T cells is not known. Whether A2AR affects IL-17 production directly or through other indirect pathways is unknown. Furthermore, the relationship between A2AR, IL-17, and CD4+ T cells in PI-IBS is unknown. Therefore, we investigated the role of Th17 polarization of CD4+ T cells regulated by A2AR in PI-IBS.

## MATERIALS AND METHODS

### Animal grouping

57BL/6 mice (female, mean age: 56 d, average weight: 15 g) were provided by the Hainan Provincial Experimental Animal Center and fed in pathogen-free, room temperature conditions under a normal 12-h light/dark diurnal rhythm. The animals were provided with a standard diet and water *ad libitum*. Four groups of mice were formed: Control, PI-IBS, PI-IBS+Sch58261, and PI-IBS+CGS21680 ($n$ = 24 in each group). Twelve mice were processed for the measurement of A2AR, cytokines, and signaling molecules protein and mRNA levels. Six mice were sacrificed in order to isolate, purify, and culture their CD4+ T cells. Visceral hypersensitivity and intestinal motility were evaluated in the remaining 6 animals.

### PI-IBS modeling

Infection with *Trichinella spiralis* (Lanzhou Veterinary Research Institute, Lanzhou, China) was performed to establish the PI-IBS model[28]. After 60 d of infection, the parasite larvae were digested from Sprague–Dawley rats with 1.5% gastric pepsin (Invitrogen, Carlsbad, CA, United States) and suspended in 0.2 mL of saline. The mice were fed the larvae (300 larvae/mouse).

### Histopathological examination

On the 56$^{th}$ day after infection, the animals' ileum was fixed in 10% formalin and embedded in paraffin wax. The tissues were mounted on slides after creating 5-μm-thick sections, followed by dewaxing and dehydration. The slides were stained with hematoxylin-eosin and used for further evaluation of the inflammatory score following previous scoring standards[29].

### Abdominal withdrawal reflex

Visceral hypersensitivity was evaluated with the abdominal withdrawal reflex (AWR)[30]. A gasbag was inserted into the anesthetized animal's anus, which was distended in volume from 0.25 mL to 0.65 mL for 15 min, thrice, followed by a recording of the animal's behavior. The AWR score was found to be in accordance with the standard from 0 to 4 points, as described previously[30]. The AWR scoring standard: when stimulated, the animals are in stable mood, 0 point; if the animals are in unstable mood, twisting their heads once in a while, 1 point; slightly contracting their abdominal and back muscles, 2 points; intensively contracting their abdominal muscles and uplifting the abdomen from the ground, 3 points; intensively contracting abdominal muscles, bowing the abdomen and uplifting the abdomen and perineum, 4 points.

### Colon transportation test

Intestinal motility was evaluated with the colon transportation test (CTT); 0.4 mL of activated carbon was placed in the animal's stomach, and the animal's initial time to black stool elimination was recorded. After 8 h, the Bristol stool scoring system was used to assess the total stool's characteristics [31]. Normal shaped stool, 1 point; soft or deformed stool, 2 points; water-like stool, 3 points.

### Cellular immunohistochemistry

Cellular immunohistochemistry was performed as reported elsewhere[32]. The suspended cells were fixed in 4% paraformaldehyde and uniformly placed onto slides. The sections were then incubated with primary antibody rabbit anti-mouse A2AR monoclonal antibody and a secondary antibody goat anti-rabbit HRP antibody (ab60032 and ab6721; Abcam, Cambridge, United Kingdom). DAB reagent sets (Beijing Sequoia Jinqiao, Beijing, China) were used to determine the reactivity. The quantitative expression of A2AR in the cellular membrane was calculated using a semi-quantitative integral method.

The staining intensity was scored on a scale of 0-3. The percentage area of positively stained cells was classified as 0-4 corresponding to the values of < 5% to > 75%. The evaluation with a score of ≥ 6 was expressed as a positive expression. The stain was analyzed by Nikon DR-Si2 cell count software and digital image analysis; two senior pathologists verified the results.

### Western blotting

Western blotting (Wuhan Boster Corporation, Wuhan, China) was performed to evaluate the protein level. RIPA (R0278; Sigma-Aldrich, Saint Louis, MO, United States) buffer was used to extract the protein from the ground ileum tissue samples. The Bradford assay was performed to determine the protein concentration. After separation on SDS-PAGE gel electrophoresis, 40 μg of the sample was transferred onto a polyvinylidene fluoride membrane. The following transmembrane conditions were set during the assay: (1) β-actin and A2AR: running at 200 mA for 90 min; (2) Janus kinase (JAK) and p-JAK: running at 200 mA for 120 min, followed by running at 300 mA for 30 min; (3) Signal transducer and activator of transcription (STAT3) and p-STAT3: running at 200 mA for 20 min, followed by running at 300 mA for 15 min; and (4) Receptor-related orphan receptor-γt (RORγt): running at 200 mA for 120 min.

The dilution rate of the primary antibodies rabbit anti-mouse multiple-clone antibodies is shown in Table 1.

Blotted in TBST for 60 min, the membrane was incubated with rabbit anti-mouse protein antibodies (ab2787; Abcam, United States) as the primary antibodies and with goat anti-rabbit antibodies as the secondary antibodies (ab8226; Abcam) at 4 °C for 12 h. ECL chemiluminescent assay was performed to autograph the membrane. The detected gray-scale ratio of protein/β-actin represented the relative protein level.

### Real-time quantitative PCR

Total RNA in the ileum tissues was extracted with the Trizol reagent set (Invitrogen). The primers were designed by Invitrogen (Table 2). β-actin served as an internal control.

RT-PCR was performed under the following protocol conditions: The samples were run by a pre-denaturation program (5 min at 94 °C), followed by a denaturation program (1 min at 94 °C). The amplification and qualification program was repeated for 30 cycles (50 s at 57 °C, 20 s at 60 °C), followed by an extension program (7 min at 72 °C).

The relative expression was expressed as the ratio of the target gene to the control.

### Determination of ATP

The ileum tissue sample was treated ultrasonically and centrifuged at 4 °C for 15 min, followed by the addition of double-distilled water (1:9). The 10% homogenate was heated in a boiling water bath for 10 min and then centrifuged at 3000 rpm for 10 min. The supernatant was collected for enumeration.

The concentration of ATP was measured by a colorimetric method. Briefly, the OD of the tube was measured at the wavelength of 636 nm. The ATP concentration in the sample μmol/gprot) = (measured OD - Control OD)/(Standard OD - OD) × standard concentration (1000 μmol/mL)/sample concentration (gprot/L).

### Isolation and purification of CD4+ T cells

CD4+ T cells were isolated from the animals' spleen[33]. Briefly, the animals were sacrificed and their spleens were collected and washed with PBS. The red blood cells were rinsed with red blood cell lysis buffer. The lymphocytes liquid overflowing from the cleaned spleen was repeatedly washed with PBS, centrifuged at 1960 rpm, and incubated with CD4 (FITC) 0.25 μg/test; the CD4+ T cells were selected by fluorescence-activated cell sorting (FACS).

### Evaluation of CD4+ T cells' function

The effect of A2AR on CD4 T cell function, including proliferation, apoptosis, ATP production, and Th17 polarization response was evaluated by the CCK8 assay, Annexin V staining assay, and ELISA. Furthermore, the intra-cellular signaling pathway was investigated by Western blotting and RT-PCR.

### Proliferation

The CCK8 assay was performed to determine proliferation of the isolated CD4+ T cells. The cell density was set to $5 \times 10^5$/mL. The cells were added to a 96 well culture plate at the concentration of 100 μL ($5 \times 10^4$ cells), followed by treatment with 100 nmol/L of CGS-21680 and 1 μmol/L Sch58261, cultured for 0.5 h at 37°C under 5% $CO_2$ atmosphere. Then, 20 μL/well of CCK8 was added into the system and continuously cultured for 2 h at 37°C under 5% $CO_2$ atmosphere. The $OD_{450}$ of the cultured cells was measured with an enzyme-labeled instrument.

### Apoptosis

The rate of apoptosis of CD4+ T cells was measured by Annexin V staining assay. Briefly, the collected CD4+ T cells were incubated in a 6-well plate ($5 \times 10^5$ cells/well), to which 100 nmol/L of CGS-21680

**Table 1 The dilution rate of primary antibodies**

| Primary antibodies | Dilution rate |
|---|---|
| β-actin | 1:1000 |
| A2AR | 1:500 |
| RORγt | 1:2000 |
| STAT3 | 1:1000 |
| p-STAT3 | 1:2000 |
| JAK2 | 1:1000 |
| p-JAK2 | 1:1000 |

**Table 2 Primers used in the present study**

| Name | Primer | Sequence | Size |
|---|---|---|---|
| *mus β-actin* | Forward | 5′-CACGATGGAGGGGCCGGACTCATC-3′ | 240 bp |
| | Reverse | 5′-TAAAGACCTCTATGCCAACACAGT-3′ | |
| *musRORγt* | Forward | 5′-CGCACCAACCTCTTTTCA-3′ | 274 bp |
| | Reverse | 5′-GCTCCACACCACCGTATTT-3′ | |
| *mus JAK* | Forward | 5′-AGTGGCGGCATGATTTTGTT-3′ | 181 bp |
| | Reverse | 5′-GCTCGAACGCACTTTGGTAA-3′ | |
| *mus STAT3* | Forward | 5′-GTAGAGCCATACACCAAGCAGCAG-3′ | 123 bp |
| | Reverse | 5′-AATGTCGGGGTAGAGGTAGACAAGT-3′ | |
| *mus A2AR* | Forward | 5′-GCCTCTTCTTCGCCTGCTTTGTCC-3′ | 140 bp |
| | Reverse | 5′-GCCCTTCGCCCTCATACCCGTCAC-3′ | |

and 1 μmol/L of Sch58261 were added and cultured at 37°C under 5% $CO_2$ for 0.5 h. Then, 5 μL of Annexin V-APC and 5 μL of 7-AAD staining liquid were added to the wells. FACS was performed to detect the rate of apoptosis.

### *Th17 polarization*

ELISA was performed to determine the concentration of cytokines in the supernatant of CD4+ T cells. A high-sensitivity (sensitivity range: 0.25-16 pg/mL) ELISA kit (R&D Systems, Minneapolis, MI, United States) was used to analyze the IL-17 and IFNγ protein concentrations according to the manufacturer's protocol.

### *Signaling pathway*

The protein and mRNA of the signaling-associated molecules were determined by Western blotting and RT-qPCR, as described previously.

### *Statistical analysis*

The Kolmogorov–Smirnov test was performed using SPSS 22.0 software to indicate normal distribution of the data. Data were expressed as the mean ±standard error of the mean. The differences between the two groups were analyzed by the Student's *t*-test. Values in the same row with different superscripts indicate significance ($P < 0.05$), otherwise no significance ($P > 0.05$).

### *Ethical considerations*

The experimental protocol was approved by the Animal Care and Use Committee of Hainan Province and conducted in accordance with the Chinese guidelines for animal welfare.

**Table 3 The effect of adenosine 2A receptor on the abdominal withdrawal reflex score in the post-infectious irritable bowel syndrome mouse**

| Distending air volume (mL) | AWR | | | |
|---|---|---|---|---|
| | 0.25 | 0.35 | 0.5 | 0.65 |
| Control ($n$ = 6) | 0.00 ± 0.00 | 1.67 ± 0.53 | 2.56 ± 0.62 | 3.67 ± 0.57 |
| PI-IBS ($n$ = 6) | 0.00 ± 0.00 | 2.11 ± 0.49[a] | 3.22 ± 0.58 | 3.89 ± 0.46 |
| IBS + Sch58261 ($n$ = 6) | 0.00 ± 0.00 | 1.89 ± 0.40[c] | 2.89 ± 0.45 | 3.67 ± 0.52 |
| IBS + CGS21680 ($n$ = 6) | 0.00 ± 0.00 | 2.00 ± 0.41 | 3.11 ± 0.54 | 3.78 ± 0.57 |

[a]$P$ < 0.05 *vs* control group.
[c]$P$ < 0.05 *vs* post-infectious irritable bowel syndrome group. AWR: Abdominal withdrawal reflex; PI-IBS: Post-infectious irritable bowel syndrome.

**Table 4 The effect of adenosine 2A receptor on intestinal mobility in the post-infectious irritable bowel syndrome mouse**

| Group | Time to first black stool (min) | Bristol stool grade |
|---|---|---|
| Control ($n$ = 6) | 105.39 ± 15.16 | 1.01 ± 0.12 |
| PI-IBS ($n$ = 6) | 69.45 ± 8.72[a] | 2.54 ± 0.48[a] |
| IBS + Sch58261 ($n$ = 6) | 94.70 ± 14.56[c] | 1.29 ± 0.35[c] |
| IBS + CGS21680 ($n$ = 6) | 78.91 ± 9.74 | 3.11 ± 0.54 |

[a]$P$ < 0.05 *vs* control group.
[c]$P$ < 0.05 *vs* post-infectious irritable bowel syndrome group. PI-IBS: Post-infectious irritable bowel syndrome.

## RESULTS

### *PI-IBS mouse model and the effect of A2AR on the clinical features of PI-IBS*

On the 56th day of infection with *T. spiralis*, the experimental mice showed no significant colonic inflammation (Figure 1), accompanied by a higher AWR score, longer CTT, and higher Bristol stool grade (Tables 3 and 4), suggesting that the PI-IBS mouse model was successfully established. Injection of the A2AR agonist CGS21680 significantly increased the clinical features and slightly accelerated intestinal inflammation. On the other hand, the A2AR antagonist Sch58261 markedly improved the model animal's clinical manifestations. These results confirm the important role of A2AR in the development of PI-IBS. However, the underlying mechanism behind this role warrants further investigation.

### *Changes in intestinal ATP and A2AR in the PI-IBS mouse*

When compared with the control group, intestinal ATP significantly increased in the PI-IBS mouse ($P$ < 0.05; Figure 2) along with an increase in A2AR protein expression ($P$ < 0.05; Figure 3A and B). The A2AR antagonist Sch58261 significantly decreased intestinal ATP and A2AR protein levels, while the A2AR antagonist CGS21680 significantly increased these protein levels ($P$ < 0.05; Figure 2, Figure 3A and B). These results suggest that adenosine and A2AR are involved in the development of PI-IBS.

### *CD4+ T cells' isolation and A2AR expression*

CD4+ T cells were isolated and purified from PI-IBS mice spleens by FACS (Figure 3A) for further functional evaluation *in vitro*. Cellular immunohistochemistry results revealed that the CD4+ T cells from PI-IBS mice obviously increased their A2AR protein and mRNA expression, which could have been regulated by the A2AR antagonist and agonist, respectively ($P$ < 0.05; Figure 3C-E). These results suggest that CD4+ T cells could express A2AR, and their expression was regulated by A2AR.

CD4+ T cells from PI-IBS mice showed obvious proliferation, decreased apoptosis, and increased ATP and IL-17 production, which could have been regulated by the A2AR antagonist and agonist, respectively ($P$ < 0.05; Figure 4A-D). These results suggest that CD4+ T cell function, especially Th17 polarization was regulated by A2AR.

### *CD4+ T cells signaling pathway*

The JAK/p-JAK protein and mRNA expression in CD4+ T cells from PI-IBS mice were detected by Western blotting and RT-PCR. The A2AR antagonist Sch58261 decreased JAK/p-JAK protein and mRNA expression, while the A2AR agonist CGS21680 increased JAK/p-JAK protein and mRNA

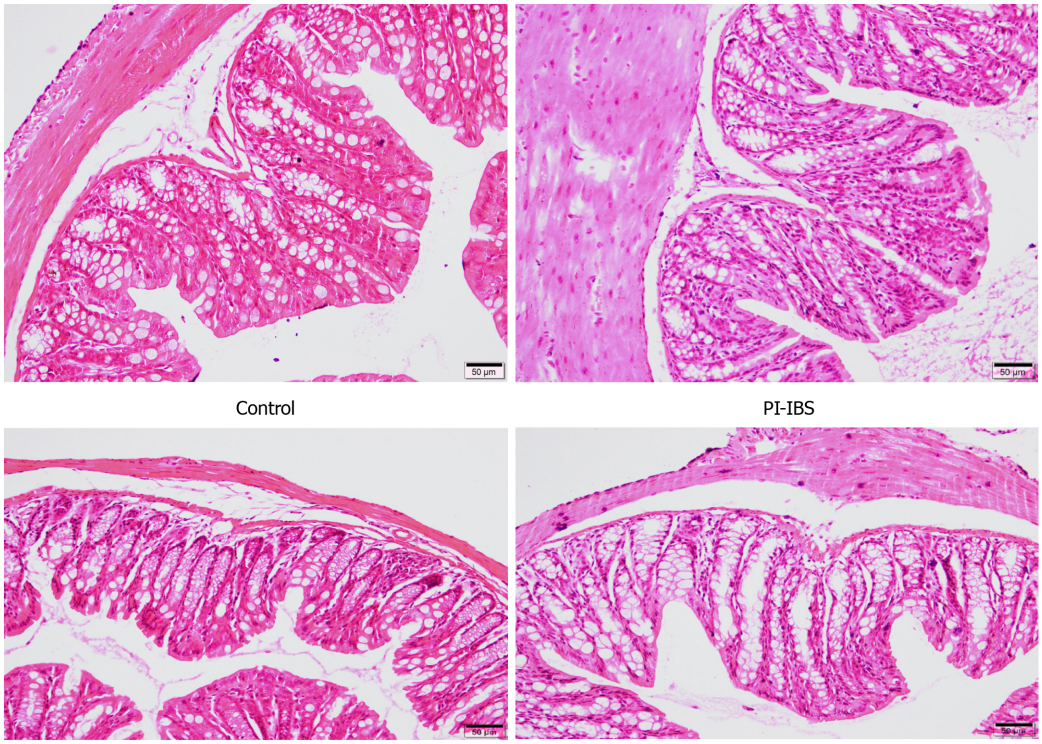


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**Figure 1 The effect of adenosine 2A receptor on the histopathological changes in the post-infectious irritable bowel syndrome mouse.** On the 56th day of infection with *T. spiralis*, colon tissues from the infected mice were collected, fixed, sectioned, stained with hematoxylin-eosin, and evaluated by light microscopy. Compared with the control mouse, the post-infectious irritable bowel syndrome (PI-IBS) mouse model showed no significant inflammatory changes in colon tissues. CGS21680, an agonist of adenosine 2A receptor, induced subtle inflammatory changes in the colon tissues of the PI-IBS model mouse, such as slight infiltration of inflammatory cells. IBS: Irritable bowel syndrome.

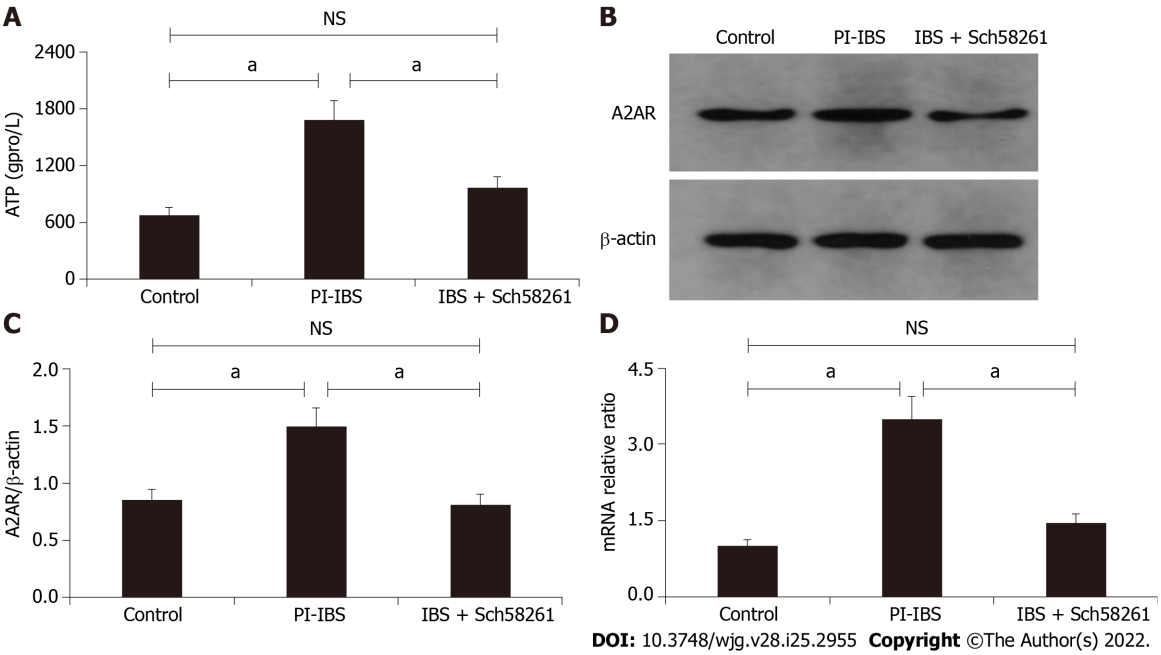


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**Figure 2 The expression of intestinal ATP and adenosine 2A receptor in the post-infectious irritable bowel syndrome mouse.** A: The expression level of ATP in different groups; B and C: Western blot analysis showed the expression level of adenosine 2A receptor (A2AR) in the three different groups; D: Relative intestinal A2AR mRNA level in the three groups. $^{a}P < 0.05$. A2AR: Adenosine 2A receptor; PI-IBS: Post-infectious irritable bowel syndrome; NS: Not significant.

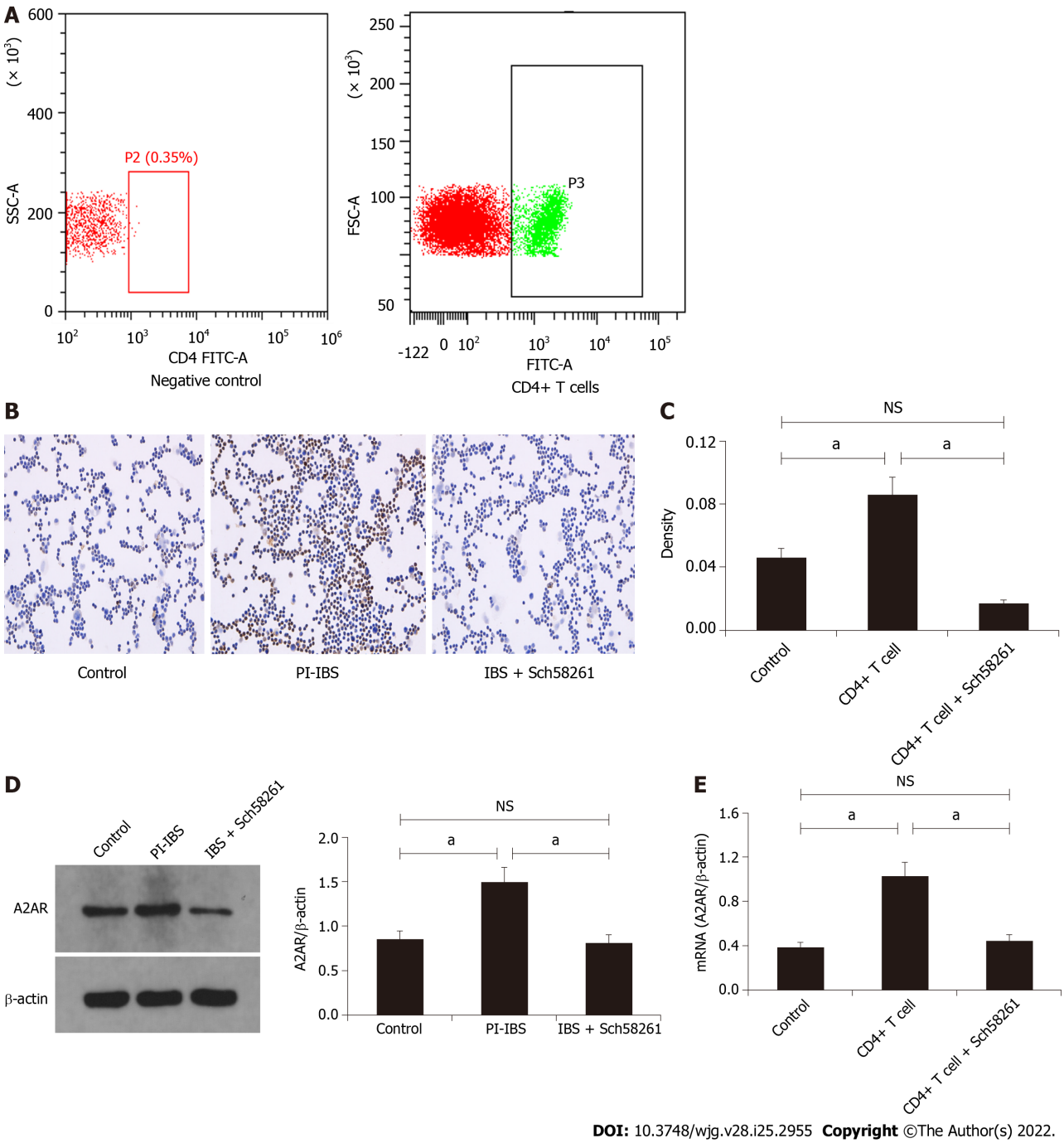


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**Figure 3 Adenosine 2A receptor expression level in CD4+ T cells isolated and purified from the post-infectious irritable bowel syndrome mouse.** A: Fluorescence-activated cell sorting results showed that CD4+ T cells (P3) were successfully isolated and purified; B-C: Immunohistochemistry staining of adenosine 2A receptor (A2AR) in CD4+ T cells; D: Western blot assay evaluated A2AR expression levels in CD4+ T cells isolated from different groups; E: Relative A2AR mRNA levels in the three groups. $^{a}P < 0.05$. A2AR: Adenosine 2A receptor; PI-IBS: Post-infectious irritable bowel syndrome; NS: Not significant.

expression. These results suggest that, during PI-IBS, the JAK pathway participates in CD4+ T cell A2AR-associated biological behavior (Figure 5A and B).

The STAT3/P-STAT3 protein and mRNA expression in CD4+ T cells from PI-IBS mice were detected by Western blotting and RT-PCR. The A2AR antagonist Sch58261 decreased STAT3/P-STAT3 protein and mRNA expression, while the A2AR agonist CGS21680 increased STAT3/P-STAT3 protein and mRNA expression. These results suggest that, during PI-IBS, the STAT3 pathway participated in CD4+ T-cell A2AR-associated biological behavior (Figure 5A and B).

The RORγt protein and mRNA expression in CD4+ T cells from PI-IBS mice were detected by Western blotting and RT-PCR. The A2AR antagonist Sch58261 decreased RORγt protein and mRNA expression, while the A2AR agonist CGS21680 increased RORγt protein and mRNA expression. These results suggest that, during PI-IBS, the RORγt pathway participated in CD4+ T cell A2AR-associated biological behavior (Figure 5A and B).

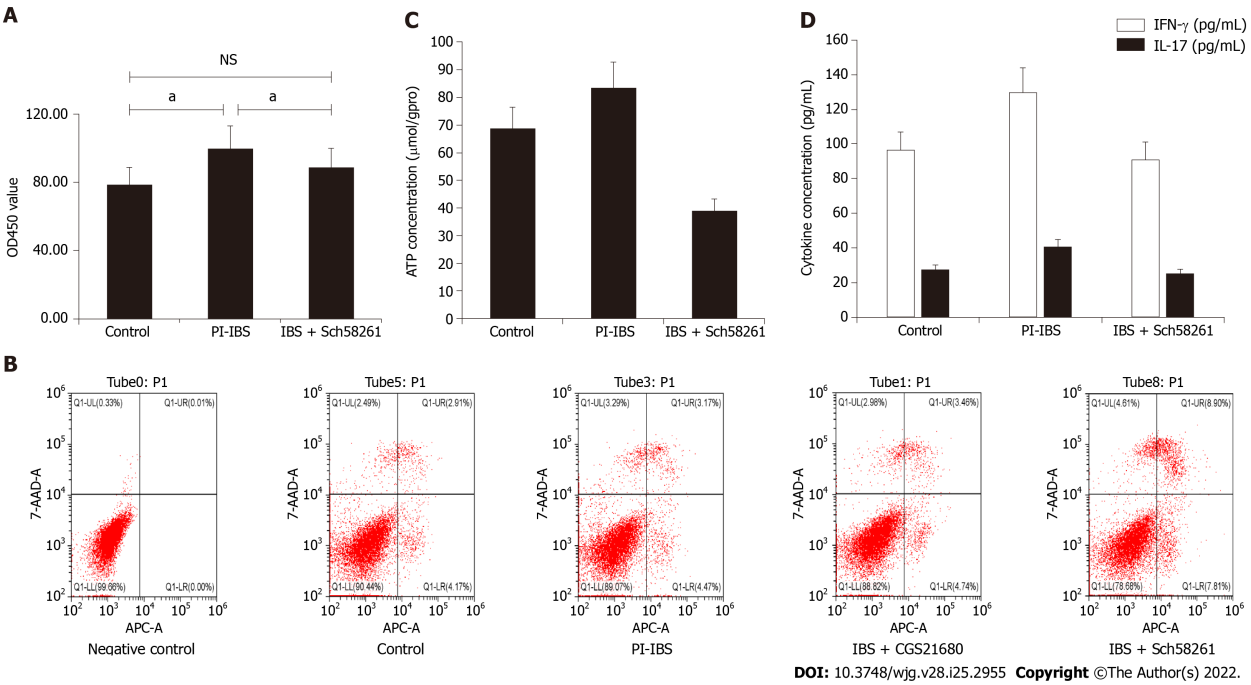


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**Figure 4 Functional evaluation of CD4+ T cells *in vitro*.** A: CCK-8 assay indicated that CD4+ T cells from post-infectious irritable bowel syndrome mice showed increased proliferation ability; B: Apoptosis analysis of CD4+ T cells between the three groups; C: ATP expression in CD4+ T cells between the three groups; D: Expression level of IFN-γ and IL-17 in the three groups. [a]$P < 0.05$. A2AR: Adenosine 2A receptor; PI-IBS: Post-infectious irritable bowel syndrome; NS: Not significant.

## DISCUSSION

Lasting low level inflammation occurs in the intestine in IBS and especially in PI-IBS due to complex immune abnormalities. Therefore, patients show continuous clinical symptoms and not distinct biochemical and pathological changes. However, the precise mechanism underlying immune regulation in PI-IBS is unclear. Therefore, the relationship between ARs and IL-17 response in the inflammation and immune response in PI-IBS should be investigated.

Our results showed that increasing intestinal ATP level in PI-IBS was accompanied by increasing intestinal A2AR level, which suggested that ATP and A2AR could participate in the pathogenesis of PI-IBS. The quantitative changes that lead to the development of PI-IBS, resulted from PI-IBS, or increased PI-IBS is not known. The animal's intestinal tissue remained unchanged following injection of the A2AR antagonist, but the clinical symptoms improved. This result was contradictory to the previous hypotheses which state that adenosine and its receptors play a protective role in inflammation. During the inflammatory process, adenosine and its receptors could trigger another unknown pathway to maintain the balance of inflammation and the immune response, otherwise, a prolonged presence of increased concentrations of adenosine can be harmful and can induce an immunosuppressed environment that is ideal for the onset and development of neoplasia[34]. Our study reports the complex condition in PI-IBS.

We elucidated the mechanism underlying the role of A2AR in PI-IBS. CD4+ T cells act as an important regulator in inflammation and immune response. Th17 polarization is one of the most important pathways of the immune event associated with CD4+ T cells. We first found that the number of CD4+ T cells distinctly increased, accompanied by an increase in the expression of A2AR on these cells. We then found that the agonist of A2AR increased the number of CD4+ T cells accompanied by an increase in the expression of A2AR on these cells. The A2AR agonist increased the proliferation of CD4+ T cells and inhibited their apoptosis. On the contrary, the A2AR antagonist decreased the number of CD4+ T cells and inhibited their function. These results suggested that CD4+ T cells participate in A2AR-induced intestinal inflammation.

Th17 polarization was associated with A2AR. IL-17 plays a dual role in inflammation and the immune response. IL-17 can improve inflammation in some conditions. For instance, HSP70 induces γ δ T cells to produce IL-17 that protects mice against PI-IBS[10]. IL-17 protects mice against colitis induced by adherent-invasive *Escherichia coli*[35]. In other conditions, IL-17 can increase inflammation. For instance, Th17 cells and IL-17 promotes skin and lung inflammation and fibrosis in the bleomycin-induced murine model of systemic sclerosis[36].

Unfortunately, the studies involving animal models have not determined the efficacy of anti-IL-17 antibodies in improving intestinal inflammation[37]. Our results showed that the increase in A2AR expression and chronic inflammation is associated with the Th17 polarization of CD4+ T cells, which

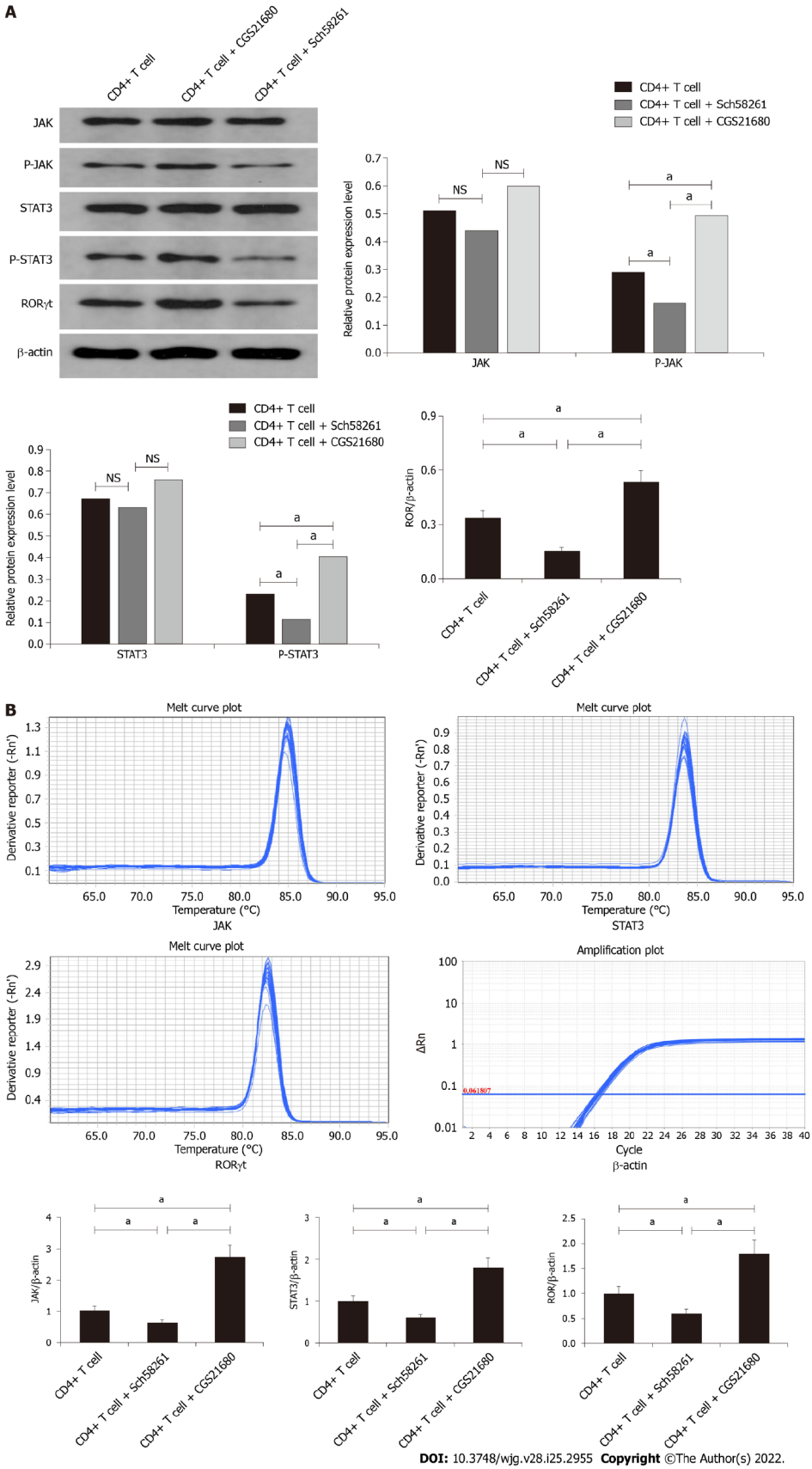


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**Figure 5 Signaling pathway analysis by Western blot assay.** A: Western blot results indicate that the expression level of p-JAK, p-STAT3, and receptor-related orphan receptor (ROR) were significantly increased in the CD4+ T cell + CGS21680 group and decreased in CD4+ T cell + Sch58261 group; B: The mRNA expression level of p-JAK, p-STAT3, and ROR between the different groups. [a]*P* < 0.05. A2AR: Adenosine 2A receptor; PI-IBS: Post-infectious irritable bowel syndrome; NS: Not significant.

suggested that the A2AR-CD4+ T cells-IL-17 polarization pathway is present in PI-IBS.

We investigated the intracellular signaling pathway of the Th17 polarization of CD4+ T cells induced by A2AR. We found that JAK and STAT3 protein and their phosphorylated products were significantly increased in CD4+ T cells from the PI-IBS mouse model. The A2AR antagonist reduced the protein and mRNA level of JAK and STAT3, which was increased by the A2AR agonist. Our results suggested that the JAK-STAT3 pathway is an important intracellular signaling pathway in the A2AR-induced Th17 polarization of CD4+ T cells in PI-IBS.

The present study has some limitations. The type of visceral hypersensitivity in the PI-IBS mouse model was not investigated. The water content in the Bristol stool grade was not analyzed. The direct isolation of CD4+ T cells from the intestine is difficult. Therefore, we used CD4+ T cells isolated from the spleen, which might have resulted in some variation compared with CD4+ T cells from the intestine.

## CONCLUSION

In the present study, we established a mouse model of PI-IBS and the mice were administered an A2AR agonist and antagonist. Interestingly, we found that A2AR increased in PI-IBS accompanied by CD4+ T cell activation, proliferation and production of IL-17. A2AR antagonist improved the clinical symptoms and the cellular and molecular events during PI-IBS. This study showed that the upregulation of A2AR increases PI-IBS by promoting the Th17 polarization of CD4+ T cells.

## ARTICLE HIGHLIGHTS

### *Research background*

It has been proved that low-grade inflammation and immunological dysfunction are involved in post-infectious irritable bowel syndrome (PI-IBS). T helper 17 (Th17) polarization occurs in IBS. Adenosine and its receptors participate in intestinal inflammation and immune regulation.

### *Research motivation*

To elucidate the pathogenesis of PI-IBS and identify a potential target for the treatment of this disease.

### *Research objectives*

This study aims to explore the role of adenosine 2A receptor (A2AR) in PI-IBS and its underlying mechanism, especially the relationship between A2AR and Th17 response.

### *Research methods*

A PI-IBS model was established by infecting mice with *Trichinella spiralis*. The expression and function of A2AR and CD4+ T lymphocytes were examined. Furthermore, the effect of A2AR on CD4+ T lymphocyte Th17 polarization was observed and the clinical features of PI-IBS were evaluated.

### *Research results*

The main results can be summarized as follow: (1) expression of ATP and A2AR and inhibition of A2AR improved the clinical features in PI-IBS; (2) CD4+ T cells expressed A2AR and produced IL-17 *in vitro*, which was regulated by the A2AR agonist and antagonist; and (3) The A2AR antagonist increased the production of IL-17 by CD4+ T cells *via* the Janus kinase-signal transducer and activator of transcription-receptor-related orphan receptor γ signaling pathway.

### *Research conclusions*

The upregulation of A2AR increases PI-IBS by promoting the Th17 polarization of CD4+ T cells.

### *Research perspectives*

The present study could provide a new pathway to elucidate the pathogenesis of PI-IBS and identify a novel therapy target for this disease.

## FOOTNOTES

**Author contributions:** Dong LW and Lan C designed the study and interpreted the findings; Dong LW and Ma ZC performed the experiments, analyzed the data, and drafted the manuscript; Fu J, Huang BL and Liu FJ helped collect and analyze the data; Sun D and Lan C critically revised the manuscript for important intellectual content and reviewed the article; all authors have read and approved the final manuscript.

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## REFERENCES

1 **Mayer EA**, Labus JS, Tillisch K, Cole SW, Baldi P. Towards a systems view of IBS. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 592-605 [PMID: 26303675 DOI: 10.1038/nrgastro.2015.121]
2 **Chey WD**, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *JAMA* 2015; **313**: 949-958 [PMID: 25734736 DOI: 10.1001/jama.2015.0954]
3 **Hellström PM**. Pathophysiology of the irritable bowel syndrome - Reflections of today. *Best Pract Res Clin Gastroenterol* 2019; **40-41**: 101620 [PMID: 31594651 DOI: 10.1016/j.bpg.2019.05.007]
4 **Barbara G**, Grover M, Bercik P, Corsetti M, Ghoshal UC, Ohman L, Rajilić-Stojanović M. Rome Foundation Working Team Report on Post-Infection Irritable Bowel Syndrome. *Gastroenterology* 2019; **156**: 46-58.e7 [PMID: 30009817 DOI: 10.1053/j.gastro.2018.07.011]
5 **Balemans D**, Mondelaers SU, Cibert-Goton V, Stakenborg N, Aguilera-Lizarraga J, Dooley J, Liston A, Bulmer DC, Vanden Berghe P, Boeckxstaens GE, Wouters MM. Evidence for long-term sensitization of the bowel in patients with post-infectious-IBS. *Sci Rep* 2017; **7**: 13606 [PMID: 29051514 DOI: 10.1038/s41598-017-12618-7]
6 **Zeng L**, Li K, Wei H, Hu J, Jiao L, Yu S, Xiong Y. A Novel EphA2 Inhibitor Exerts Beneficial Effects in PI-IBS *in Vivo* and *in Vitro* Models *via* Nrf2 and NF-κB Signaling Pathways. *Front Pharmacol* 2018; **9**: 272 [PMID: 29662452 DOI: 10.3389/fphar.2018.00272]
7 **Zhang Y**, Wu X, Wu J, Li S, Han S, Lin Z, Ding S, Jia X, Gong W. Decreased expression of microRNA-510 in intestinal tissue contributes to post-infectious irritable bowel syndrome *via* targeting PRDX1. *Am J Transl Res* 2019; **11**: 7385-7397 [PMID: 31934286]
8 **Xu D**, Gao J, Gillilland M 3rd, Wu X, Song I, Kao JY, Owyang C. Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and visceral hyperalgesia in rats. *Gastroenterology* 2014; **146**: 484-96.e4 [PMID: 24161699 DOI: 10.1053/j.gastro.2013.10.026]
9 **Yang B**, Zhou X, Lan C. Changes of cytokine levels in a mouse model of post-infectious irritable bowel syndrome. *BMC Gastroenterol* 2015; **15**: 43 [PMID: 25886744 DOI: 10.1186/s12876-015-0272-8]
10 **He Z**, Sun X, Ma Z, Fu J, Huang B, Liu F, Chen Y, Deng T, Han X, Sun D, Lan C. Heat shock protein 70 protects mouse against post-infection irritable bowel syndrome *via* up-regulating intestinal γδ T cell's Th17 response. *Cell Biosci* 2018; **8**: 38 [PMID: 29928498 DOI: 10.1186/s13578-018-0237-z]
11 **Aslam M**, Gündüz D, Troidl C, Heger J, Hamm CW, Schulz R. Purinergic Regulation of Endothelial Barrier Function. *Int J Mol Sci* 2021; **22** [PMID: 33530557 DOI: 10.3390/ijms22031207]
12 **Effendi WI**, Nagano T, Kobayashi K, Nishimura Y. Focusing on Adenosine Receptors as a Potential Targeted Therapy in Human Diseases. *Cells* 2020; **9** [PMID: 32213945 DOI: 10.3390/cells9030785]
13 **Burnstock G**. Purinergic Signalling: Therapeutic Developments. *Front Pharmacol* 2017; **8**: 661 [PMID: 28993732 DOI: 10.3389/fphar.2017.00661]
14 **Colgan SP**, Fennimore B, Ehrentraut SF. Adenosine and gastrointestinal inflammation. *J Mol Med (Berl)* 2013; **91**: 157-164 [PMID: 23296303 DOI: 10.1007/s00109-012-0990-0]
15 **Fredholm BB**, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001; **53**: 527-552 [PMID: 11734617]
16 **Kolachala VL**, Bajaj R, Chalasani M, Sitaraman SV. Purinergic receptors in gastrointestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G401-G410 [PMID: 18063703 DOI: 10.1152/ajpgi.00454.2007]

17 **Antonioli L**, Colucci R, Pellegrini C, Giustarini G, Tuccori M, Blandizzi C, Fornai M. The role of purinergic pathways in the pathophysiology of gut diseases: pharmacological modulation and potential therapeutic applications. *Pharmacol Ther* 2013; **139**: 157-188 [PMID: 23588157 DOI: 10.1016/j.pharmthera.2013.04.002]

18 **Belikoff BG**, Hatfield S, Georgiev P, Ohta A, Lukashev D, Buras JA, Remick DG, Sitkovsky M. A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. *J Immunol* 2011; **186**: 2444-2453 [PMID: 21242513 DOI: 10.4049/jimmunol.1001567]

19 **Liang D**, Zuo A, Shao H, Chen M, Kaplan HJ, Sun D. A2B adenosine receptor activation switches differentiation of bone marrow cells to a CD11c(+)Gr-1(+) dendritic cell subset that promotes the Th17 response. *Immun Inflamm Dis* 2015; **3**: 360-373 [PMID: 26734458 DOI: 10.1002/iid3.74]

20 **Chen M**, Liang D, Zuo A, Shao H, Kaplan HJ, Sun D. An A2B Adenosine Receptor Agonist Promotes Th17 Autoimmune Responses in Experimental Autoimmune Uveitis (EAU) *via* Dendritic Cell Activation. *PLoS One* 2015; **10**: e0132348 [PMID: 26147733 DOI: 10.1371/journal.pone.0132348]

21 **Vuerich M**, Mukherjee S, Robson SC, Longhi MS. Control of Gut Inflammation by Modulation of Purinergic Signaling. *Front Immunol* 2020; **11**: 1882 [PMID: 33072065 DOI: 10.3389/fimmu.2020.01882]

22 **Longhi MS**, Moss A, Jiang ZG, Robson SC. Purinergic signaling during intestinal inflammation. *J Mol Med (Berl)* 2017; **95**: 915-925 [PMID: 28547076 DOI: 10.1007/s00109-017-1545-1]

23 **Sitaraman SV**, Merlin D, Wang L, Wong M, Gewirtz AT, Si-Tahar M, Madara JL. Neutrophil-epithelial crosstalk at the intestinal lumenal surface mediated by reciprocal secretion of adenosine and IL-6. *J Clin Invest* 2001; **107**: 861-869 [PMID: 11285305 DOI: 10.1172/JCI11783]

24 **Belikoff B**, Hatfield S, Sitkovsky M, Remick DG. Adenosine negative feedback on A2A adenosine receptors mediates hyporesponsiveness in chronically septic mice. *Shock* 2011; **35**: 382-387 [PMID: 21192284 DOI: 10.1097/SHK.0b013e3182085f12]

25 **Guzman J**, Yu JG, Suntres Z, Bozarov A, Cooke H, Javed N, Auer H, Palatini J, Hassanain HH, Cardounel AJ, Javed A, Grants I, Wunderlich JE, Christofi FL. ADOA3R as a therapeutic target in experimental colitis: proof by validated high-density oligonucleotide microarray analysis. *Inflamm Bowel Dis* 2006; **12**: 766-789 [PMID: 16917233 DOI: 10.1097/00054725-200608000-00014]

26 **Hou T**, Xiang H, Yu L, Su W, Shu Y, Li H, Zhu H, Lin L, Hu X, Liang S, Zhang H, Li M. Electroacupuncture inhibits visceral pain *via* adenosine receptors in mice with inflammatory bowel disease. *Purinergic Signal* 2019; **15**: 193-204 [PMID: 31187350 DOI: 10.1007/s11302-019-09655-4]

27 **Sun L**, Li X, Guan H, Chen S, Fan X, Zhou C, Yang H, Xiao W. A Novel Role of $A_{2A}R$ in the Maintenance of Intestinal Barrier Function of Enteric Glia from Hypoxia-Induced Injury by Combining with mGluR5. *Front Pharmacol* 2021; **12**: 633403 [PMID: 34093180 DOI: 10.3389/fphar.2021.633403]

28 **Zhou X**, Dong L, Yang B, He Z, Chen Y, Deng T, Huang B, Lan C. Preinduction of heat shock protein 70 protects mice against post-infection irritable bowel syndrome *via* NF-κB and NOS/NO signaling pathways. *Amino Acids* 2015; **47**: 2635-2645 [PMID: 26215736 DOI: 10.1007/s00726-015-2056-4]

29 **Suzuki R**, Kohno H, Sugie S, Tanaka T. Dose-dependent promoting effect of dextran sodium sulfate on mouse colon carcinogenesis initiated with azoxymethane. *Histol Histopathol* 2005; **20**: 483-492 [PMID: 15736053 DOI: 10.14670/HH-20.483]

30 **Lan C**, Sun XN, Zhou XC, Yang B, Huang BL, Deng TZ, He ZT, Han XY. Preinduced intestinal HSP70 improves visceral hypersensitivity and abnormal intestinal motility in PI-IBS mouse model. *Asian Pac J Trop Med* 2016; **9**: 302-305 [PMID: 26972407 DOI: 10.1016/j.apjtm.2016.01.022]

31 **Vork L**, Wilms E, Penders J, Jonkers DMAE. Stool Consistency: Looking Beyond the Bristol Stool Form Scale. *J Neurogastroenterol Motil* 2019; **25**: 625 [PMID: 31587553 DOI: 10.5056/jnm19086]

32 **Wu Z**, Yang L, Shi L, Song H, Shi P, Yang T, Fan R, Jiang T, Song J. Prognostic Impact of Adenosine Receptor 2 (A2aR) and Programmed Cell Death Ligand 1 (PD-L1) Expression in Colorectal Cancer. *Biomed Res Int* 2019; **2019**: 8014627 [PMID: 31275983 DOI: 10.1155/2019/8014627]

33 **Sauer KA**, Scholtes P, Karwot R, Finotto S. Isolation of CD4+ T cells from murine lungs: a method to analyze ongoing immune responses in the lung. *Nat Protoc* 2006; **1**: 2870-2875 [PMID: 17406546 DOI: 10.1038/nprot.2006.435]

34 **D'Antongiovanni V**, Fornai M, Pellegrini C, Benvenuti L, Blandizzi C, Antonioli L. The Adenosine System at the Crossroads of Intestinal Inflammation and Neoplasia. *Int J Mol Sci* 2020; **21** [PMID: 32708507 DOI: 10.3390/ijms21145089]

35 **Zhang HJ**, Xu B, Wang H, Wang GD, Jiang MZ, Lei C, Ding ML, Yu PF, Nie YZ, Wu KC, Sha SM, Li MB. IL-17 is a protection effector against the adherent-invasive Escherichia coli in murine colitis. *Mol Immunol* 2018; **93**: 166-172 [PMID: 29195141 DOI: 10.1016/j.molimm.2017.11.020]

36 **Lei L**, Zhao C, Qin F, He ZY, Wang X, Zhong XN. Th17 cells and IL-17 promote the skin and lung inflammation and fibrosis process in a bleomycin-induced murine model of systemic sclerosis. *Clin Exp Rheumatol* 2016; **34** Suppl 100: 14-22 [PMID: 26750756]

37 **Fauny M**, Moulin D, D'Amico F, Netter P, Petitpain N, Arnone D, Jouzeau JY, Loeuille D, Peyrin-Biroulet L. Paradoxical gastrointestinal effects of interleukin-17 blockers. *Ann Rheum Dis* 2020; **79**: 1132-1138 [PMID: 32719044 DOI: 10.1136/annrheumdis-2020-217927]

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ORIGINAL ARTICLE

Retrospective Study

# Four-year experience with more than 1000 cases of total laparoscopic liver resection in a single center

Xiang Lan, Hai-Li Zhang, Hua Zhang, Yu-Fu Peng, Fei Liu, Bo Li, Yong-Gang Wei

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## Abstract

***BACKGROUND***

Laparoscopic liver resection (LLR) has become a safe surgical procedure that needs additional summarization.

***AIM***

To review 4 years of total LLR surgeries, exceeding 1000 cases, which were performed at a single center.

***METHODS***

Patients who underwent LLR at West China Hospital of Sichuan University between January 2015 and December 2018 were identified. Surgical details, including the interventional year, category of liver disease, and malignant liver tumors prognosis, were evaluated. The learning curve for LLR was evaluated using the cumulative sum method. The Kaplan-Meier method was used to perform survival analysis.

***RESULTS***

Ultimately, 1098 patients were identified. Hepatocellular carcinoma (HCC) was the most common disease that led to the need for LLR at the center ($n$ = 462, 42.08%). The average operation time was 216.94 ± 98.51 min. The conversion rate was 1.82% (20/1098). The complication rate was 9.20% (from grade II to V). The 1-year and 3-year overall survival rates of HCC patients were 89.7% and 81.9%, respectively. The learning curve was grouped into two phases for local resection (cases 1-106 and 107-373), three phases for anatomical segmentectomy (cases 1-44, 45-74 and 75-120), and three phases for hemihepatectomy (cases 1-17, 18-48 and

49-88).

*CONCLUSION*
LLR may be considered a first-line surgical intervention for liver resection that can be performed safely for a variety of primary, secondary, and recurrent liver tumors and for benign diseases once technical competence is proficiently attained.

**Key Words:** Laparoscopic liver resection; Single-center experience; Learning curve; Liver

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**Core Tip:** West China Hospital of Sichuan University is the biggest and most advanced one in the western region of China. About 1500-2500 cases of liver resection have been performed in our center every year. Since the first laparoscopic liver resection (LLR) was performed in 2015, we have accumulated more than 1000 cases rapidly, including hemi-hepatectomy, mesohepatectomy, anatomical segmentectomy from segment I to VIII and even the first case of laparoscopic donor hepatectomy in Mainland China. In this study, we want to share our experiences and introduce technical innovation of LLR.

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## INTRODUCTION

Laparoscopic liver resection (LLR) has become a safe approach for liver resection, following advances in surgical techniques, anesthesiology, and perioperative care. Since its first use was reported in 1996 by Azagra *et al*[1], LLR has evolved to become a primary choice of intervention for many patients with liver tumors[2-6]. Experience with laparoscopic procedures has allowed for the development of complex liver resection techniques using laparoscopy, including mesohepatectomy, caudate lobectomy, and even combined resection of several hepatic segments[7-9].

Total LLR has been performed since 2015 at the West China Hospital of Sichuan University. Although total LLR has been performed for a shorter period of time at the West China Hospital of Sichuan University compared to other centers, a large number of clinical patients in the western region of China have been treated with LLR at the West China Hospital of Sichuan University, conveying a rich experience in performing open liver resection to surgeons at the center. Over the past 4 years, LLR, including hemihepatectomy, mesohepatectomy, anatomical segmentectomy from segment I to VIII, and even the first case of laparoscopic donor hepatectomy in mainland China, has been performed successfully at the center[10]. The present study reviews the single center's 4-year experience with LLR in more than 1000 patients and discusses its safety and feasibility, the learning curve for performing laparoscopic surgery, LLR technical innovations, and patient outcomes.

## MATERIALS AND METHODS

### *Patient characteristics*

Patients who underwent LLR between January 2015 and December 2018 at the West China Hospital of Sichuan University were studied retrospectively. Inclusion criteria were as follows: patients who had undergone LLR due to liver disease; patients with hemangioma who met the following criteria: (1) Symptomatic hemangioma; (2) Increasing tumor size; (3) Heavy psychological burden and anxiety symptoms that affect daily life and require surgical treatment; (4) Tumor located in special segments ( *e.g.*, the caudate lobe or the porta hepatis) that are difficult to treat as tumor size increases; and (5) Unclear diagnosis in which the possibility of a malignant tumor cannot be completely ruled out; and patients with hepatocellular carcinoma (HCC) who met the following criteria: Barcelona Clinic Liver Cancer 0-B stage HCC[11]; A3 stage with normal liver function after conservative treatment; and A4 or B stage with a tumor located in the same hemiliver. Exclusion criteria were as follows: patients who only received laparoscopic surgery without hepatectomy.

Patient data (age, sex, liver function before and after the operation, complications, hospital stay after the operation, operation time, hemorrhage, ascites, and perioperative mortality) were collected. All

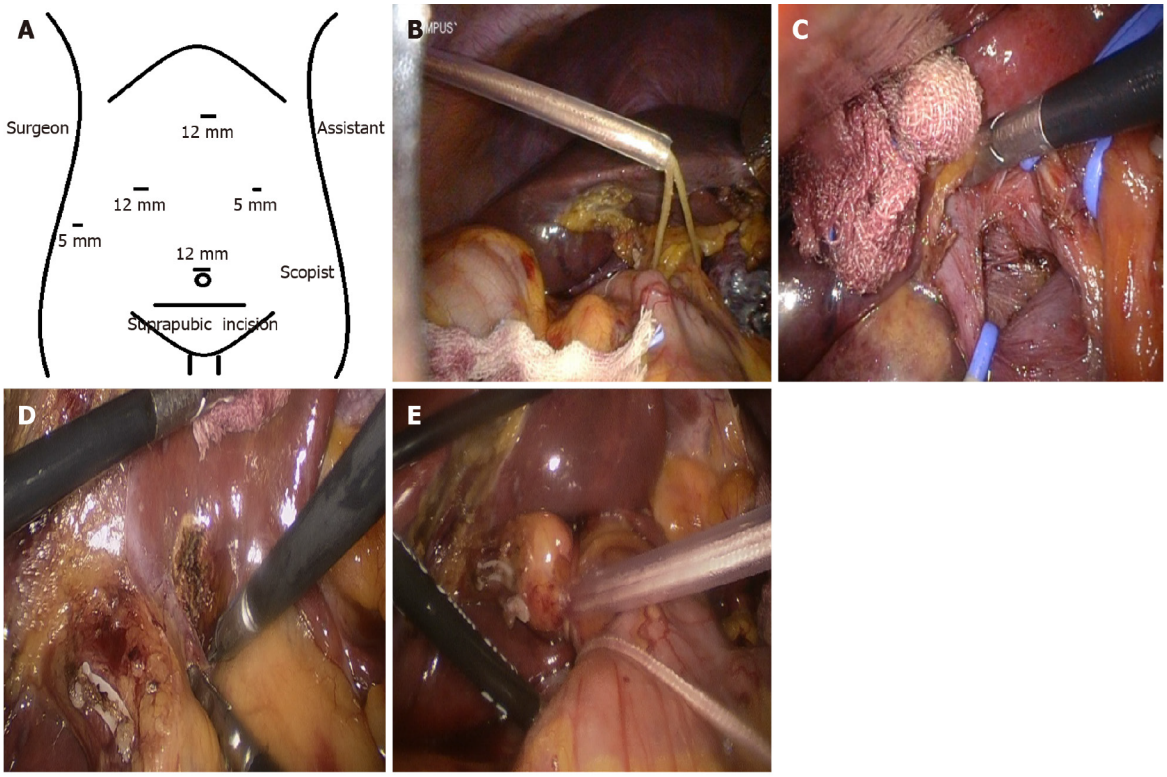


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**Figure 1 Hepatic inflow occlusion methods.** A: Trocar position; B: Extracorporeal intermittent pringle maneuver; C: Anatomical continuous hemihepatic vascular inflow occlusion (CHVIO; intro-Glissonian methods); D and E: Extra-Glissonian CHVIO.

patients provided written informed consent for surgery, and this study was approved by the Ethical Review Board of West China Hospital of Sichuan University.

### *Surgical technique*

Surgical procedures were described in our previous study[12]. A five-trocar approach was used at the tumor location (Figure 1A). Two hepatic inflow occlusion methods were adopted at the center[12]. Intermittent pringle (IP): During the operation, hepatic inflow was blocked for 15 min and released for 5 min (Figure 1B) and continuous hemihepatic vascular inflow occlusion (CHVIO) (Figure 1C-E).

### *Histopathology*

**Liver capsule invasion and tumor location:** Liver capsule invasion was determined by the histopathological report. In this study, "central tumor" was defined as a tumor within the main hepatic body, located in segments I/IV/V/VIII; otherwise, the tumor was considered a "marginal tumor". If the tumor spanned across regions I/IV/V/VIII and another region, it was considered a "central tumor".

**Diagnosis of cirrhosis:** Liver cirrhosis was diagnosed by histological examination of hepatic tissues by two experienced pathologists. The histopathology diagnostic standard used to diagnose chronic hepatitis was previously described by Desmet *et al*[13]. G0-4/S1-3 was not considered liver cirrhosis, whereas G0-4/S4 was diagnosed as liver cirrhosis.

**HCC differentiation:** HCC differentiation was classified according to the World Health Organization Classification of Tumors of the Digestive System[14], with grade 1 being well differentiated, grade 2 moderately differentiated, and grade 3 poorly differentiated. At the center, pathological reports sometimes describe grade 1-2 or grade 2-3 HCC in a single patient. Patients with grades 1, 2 and 1-2 were allocated to the "well-to-moderately differentiated" group, and patients with grades 2-3 and 3 were allocated to the "poorly differentiated" group in this study.

**Classification of complications:** The Clavien–Dindo classification of surgical complications was adopted[15]. Grade I was regarded as the absence of complications, while Grades II-V were regarded as the presence of complications.

### *The learning curve*

The learning curve of LLR was evaluated using the cumulative sum (CUSUM) method. The CUSUM procedure is a well-established method that detects data changes and monitors surgical performance. In

**Table 1 The demographic data of all 1098 patients**

| Demographic data | Frequencies | Range/percentage |
|---|---|---|
| Sex | | |
| Male | 655 | 59.65% |
| Female | 443 | 40.35% |
| Age (yr) | 50.28 ± 13.01 | 15-86 |
| Disease | | |
| HCC | 462 | 42.08% |
| ICC | 58 | 5.28% |
| HCC and ICC mixed carcinoma | 5 | 0.46% |
| Metastasis | 92 | 8.38% |
| Hepatolithiasis | 59 | 5.37% |
| Hemangioma | 213 | 19.40% |
| FNH and hyperplastic disease | 88 | 8.01% |
| Parasitic disease | 30 | 2.73% |
| Living donors | 15 | 1.37% |
| Liver abscess | 11 | 1.00% |
| Caroli disease | 5 | 0.46% |
| Hepatic cyst | 6 | 0.55% |
| Trauma | 2 | 0.18% |
| Other benign liver occupancy | 52 | 4.73% |

ICC: Intrahepatic cholangiocarcinoma; FNH: Focal nodular hyperplasia; HCC: Hepatocellular carcinoma.

this study, the operation time of each case was ordered chronologically (date of surgery). The formulation of the CUSUM was defined as: Where was an individual operation time, and was the mean of the overall operation time[16]. The learning curve is presented as a broken line graph according to the above formulation.

### Follow-up

Patients were followed up 1, 3, 6, 12, 24 and 36 mo postoperatively and assessed by computerized tomography or magnetic resonance imaging, liver function testing, and serum tumor marker measurement. All patients were followed until confirmation of death. If a patient lost contact during follow-up, the survival time was divided into the former interval of follow-up and was included in the censored data.

### Statistical analysis

Measurement data between 2 comparative intervals were evaluated by independent-samples *t* test. Pearson's chi-square test and Fisher's exact test (when expected cell frequencies were less than 5) were used to determine significant differences in categorical parameters. The Kaplan-Meier method was used to perform survival analysis. Multiple regression analysis was used to assess risk factors determining the patient's disease prognosis. A $P$ value < 0.05 was considered to represent a statistically significant difference.

## RESULTS

### Demographic data

A total of 1148 consecutive patients who received laparoscopic surgery (except laparoscopic cholecystectomy) between January 2015 and December 2018 were identified. Fifty patients did not meet the inclusion criteria. The remaining 1098 patients underwent LLR, and their demographic data were collected (Table 1).

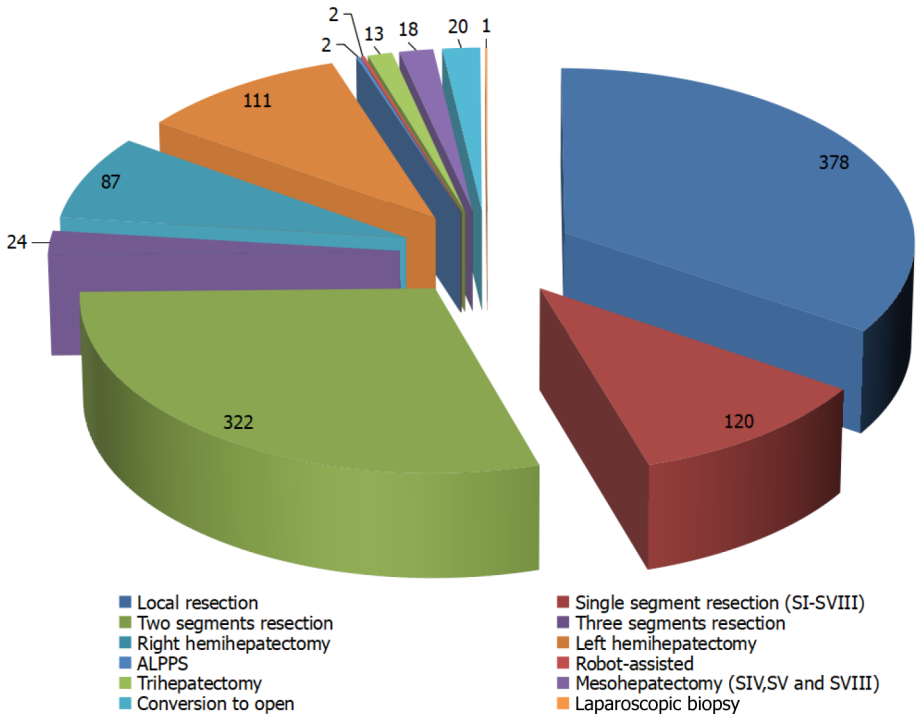


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**Figure 2 The details of different types of liver resection at the center.** ALPPS: Associating liver partition and portal vein ligation for staged hepatectomy.

The average operation time was 216.95 ± 98.51 min (range, 31-650 min). The longest operation time was observed in LLR in which resection of a tumor in segments I, IV, V and VIII was combined with resection in the caudate lobe invading the middle hepatic vein[9]. The average blood loss was 242.51 ± 143.23 mL (range, 5-4000 mL). The greatest blood loss, 4000 mL was observed in a patient who had liver paragonimiasis and that underwent conversion to open left hemihepatectomy with a splenectomy. A total of 22 patients (2.00%) underwent blood transfusion. The rate of conversion to open surgery was 1.82% (20/1098). With regard to methods of hepatic inflow occlusion, the IP maneuver was employed in 77.14% of patients (847/1098), and CHVIO was used in 21.58% of patients (237/1098). Local resection, 2 segmental resections and anatomical hepatectomy of a single segment represented the top 3 categories of LLR at the center (Figure 2).

Compared with preoperative data, alanine transaminase and aspartate aminotransferase levels peaked at postoperative day (POD) 1 and then gradually decreased ($P < 0.01$, Figure 3A and B). However, the average total bilirubin level tended to increase postoperatively and peaked at POD 5 ($P < 0.01$, Figure 3C). Albumin (ALB) levels and drainage decreased quickly following operation, and ALB levels were maintained at a lower level for a longer period of time ($P < 0.01$, Figure 3D and E).

### *The progression of surgical outcomes each year*

Since the first LLR case in 2015, surgical procedures at the center have been continuously improved according to accumulated experiences. For example, the method of hepatic inflow occlusion was changed from CHVIO to IP, as the latter resulted in less blood loss and a clearer view of the operation site[12].

To further examine technique progression, surgical outcomes, including intraoperative data, mortality on POD 90, and surgical complications, were analyzed by year (Table 2). The inpatient time, postoperative inpatient time, operative time, postoperative liver function, mortality, complications, and drainage significantly decreased from year to year. Although some data (hospitalization expenses and blood loss) did not change significantly, the data trended toward decreasing over time. These results imply that surgeons at the center conquered the LLR learning curve, resulting in the LLR technique advancements.

### *The learning curves for different types of LLR*

Considering that the surgical difficulty of different types of LLR is distinct, 3 representative LLR techniques were selected for investigation of their learning curves: local resection, anatomical resection, and right hemihepatectomy.

For local resection, there was one peak point observed in the 106$^{th}$ case. Therefore, 2 phases were initially differentiated on the graph: Phase I, cases 1-106, and phase II, cases 107-373 (Figure 4A). For anatomical resection, 2 peak points were observed at the 44$^{th}$ and 74$^{th}$ cases. Therefore, 3 phases were

**Table 2 Main operation-related data for different years**

| Index/year | 2015 (*n* = 88) | 2016 (*n* = 279) | 2017 (*n* = 351) | 2018 (*n* = 380) | *F*/$x^2$ | *P* value |
|---|---|---|---|---|---|---|
| Age (yr) | 51.97 ± 11.35 | 49.34 ± 12.60 | 49.88 ± 13.05 | 50.95 ± 13.58 | 1.415 | - |
| Sex (M/F) | 55/34 | 159/120 | 203/148 | 239/141 | 3.134 | - |
| LLR types | | | | | | |
| Local resection | 2.27% (2/88) | 35.13% (98/279) | 39.60% (139/351) | 36.58% (139/380) | 45.306 | < 0.01[b] |
| Anatomical resection | 23.86% (21/88) | 8.24% (23/279) | 11.40% (40/351) | 9.47% (36/380) | 18.096 | < 0.01[b] |
| Right hemi-hepatectomy | 14.77% (13/88) | 9.32% (26/279) | 7.41% (26/351) | 5.79% (22/380) | 8.903 | < 0.05[a] |
| Pre-operative ALT (U/L) | 36.82 ± 28.17 | 36.67 ± 20.05 | 35.71 ± 25.37 | 43.58 ± 33.39 | 1.667 | - |
| Pre-operative TBL (umol/L) | 14.76 ± 5.81 | 13.55 ± 5.83 | 13.51 ± 6.21 | 14.63 ± 7.78 | 2.519 | - |
| Inpatient time (d) | 12.48 ± 6.23 | 11.77 ± 6.67 | 9.53± 5.04 | 8.03 ± 3.20 | 37.583 | < 0.01[b] |
| Post operative inpatient time (d) | 7.98 ± 5.00 | 6.87 ± 4.82 | 5.39 ± 3.70 | 4.70 ± 2.22 | 29.781 | < 0.01[b] |
| Hospitalization expense (RMB, Yuan) | 48708.55 ± 20939.76 | 47280.53 ± 17249.26 | 46468.97 ± 35172.25 | 43016.31 ± 14509.30 | 2.448 | - |
| Operation time (min) | 257.98 ± 114.25 | 236.85 ± 100.40 | 203.81 ± 85.83 | 205.61 ± 99.54 | 12.596 | < 0.01[b] |
| Blood loss (mL) | 275.65 ± 39.19 | 245.53 ± 19.10 | 232.54 ± 12.34 | 241.84 ± 20.32 | 0.413 | - |
| Post operative ALT (U/L) | 355.19 ± 44.44 | 288.22 ± 19.17 | 237.16 ± 10.66 | 251.93 ± 16.03 | 5.262 | < 0.01[b] |
| Post operative TBL (umol/L) | 36.07 ± 3.49 | 27.86 ± 1.01 | 28.31 ± 0.92 | 24.61 ± 1.02 | 7.443 | < 0.01[b] |
| Mortality of 90 d | 2.27% (2/88) | 0.36% (1/279) | 0 (0/351) | 0 (0/380) | 14.989 | < 0.01[b] |
| Complications | 13.64% (12/88) | 13.98% (39/279) | 7.98% (28/351) | 5.79% (22/380) | 15.621 | < 0.01[b] |
| Drainage (mL) | 133.12 ± 20.44 | 111.88 ± 7.62 | 103.10 ± 6.63 | 82.44 ± 8.27 | 3.433 | < 0.05[a] |
| Conversion rate | 0 (0/88) | 2.15% (6/279) | 2.56% (9/351) | 1.32% (5/380) | 3.427 | - |

[a]$P < 0.05$.
[b]$P < 0.01$.
ALT: Alanine transaminase; LLR: Laparoscopic liver resection; TBL: Total bilirubin.

initially differentiated on the graph: Phase I, cases 1-44; phase II, cases 45-74; and phase III, cases 75-120 (Figure 4B). For right hemihepatectomy, there were 2 peak points observed at the 17$^{th}$ and 48$^{th}$ cases. Therefore, 3 phases were initially differentiated on the graph: Phase I, cases 1-17; phase II, cases 18-48; and phase III, cases 49-88 (Figure 4C).

These results indicate that the learning processes of anatomical resection and right hemihepatectomy were more complicated than the learning process of local resection and involved an initial period, increased competence in LLR, and then mastery and the challenging period.

### *HCC patient characteristics and disease prognosis*

Among the 1098 patients enrolled in the study, 462 patients suffered from HCC, and 5 patients suffered from mixed carcinoma (Table 1). The details of all 467 patients with HCC-related disease are shown in Table 3.

Follow-up data were obtained for 438 patients, and disease-free survival (DFS) and overall survival (OS) were assessed. The 3-year DFS and OS rates were 69.4% and 81.9%, respectively. The 1-year DFS and OS rates were 76.3% and 89.7%, respectively (Figure 5).

## DISCUSSION

The present study shows that LLR is a safe and efficient treatment for a variety of primary, secondary, and recurrent liver tumors and for benign diseases. LLR has several advantages, including minimal damage to the abdominal wall, faster postoperative recovery, and fewer patient complaints[17,18]. Currently, LLR is widely performed in many liver surgery centers. The College of Medicine, Zhejiang University, was one of the first medical centers to perform LLR in China. They reported a 14-year, single-center experience with 365 cases and claimed that LLR has several advantages: lower economic burden, time saving, less blood loss, minimal harm, and improved safety[19]. Researchers also found that surgeons who use LLR must have extensive experience in performing open hepatectomy and that

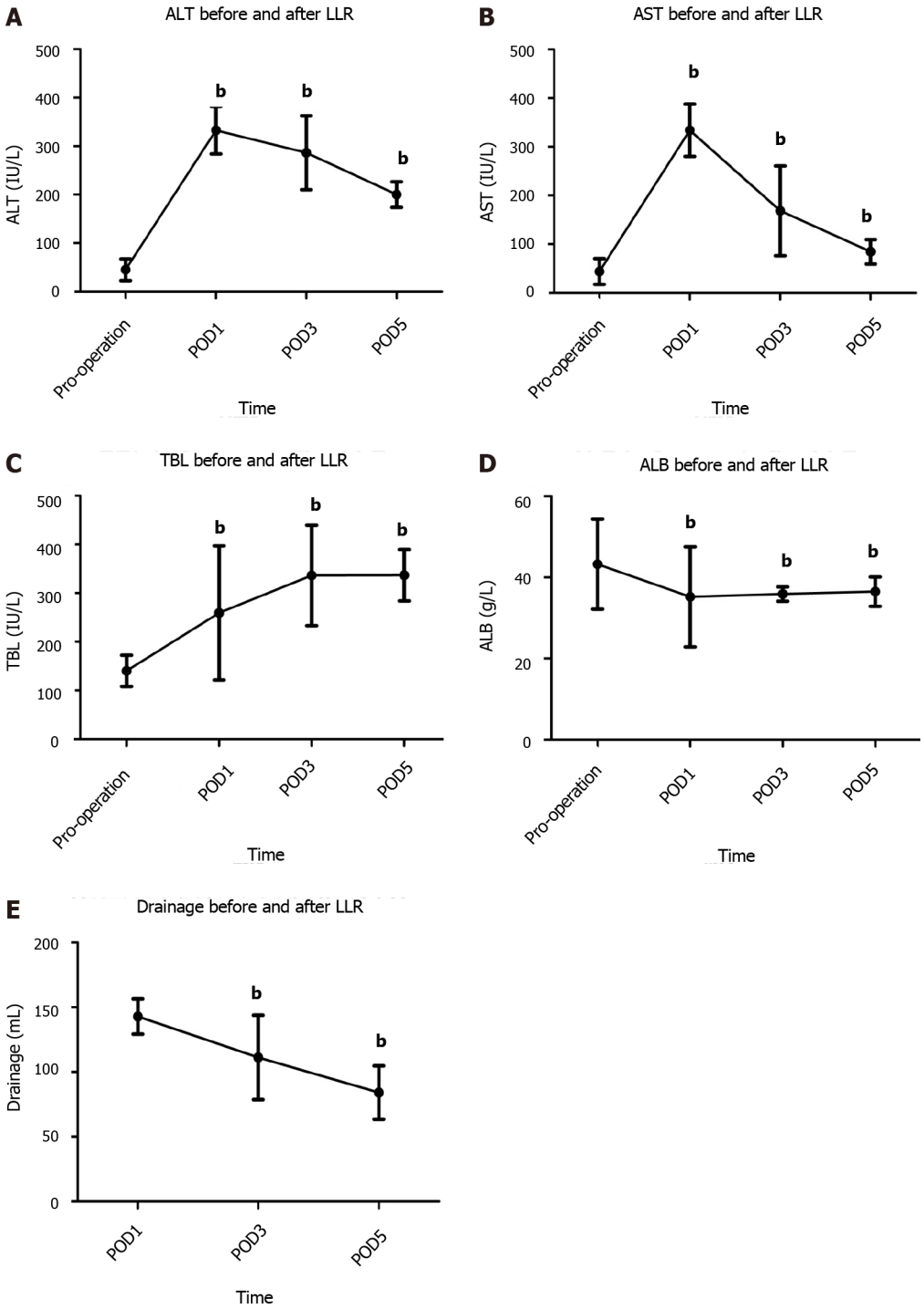


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**Figure 3 Liver function and drainage trends in the perioperative period ($^{b}P < 0.01$).** A: Alanine transaminase before and after laparoscopic liver resection (LLR); B: Aspartate aminotransferase before and after LLR; C: Total bilirubin before and after LLR; D: Albumin before and after LLR; E: Drainage before and after LLR. ALT: Alanine transaminase; AST: Aspartate aminotransferase; LLR: Laparoscopic liver resection; ALB: Albumin; TBL: Total bilirubin; POD: Postoperative day.

these surgeons will experience a learning curve when performing LLR. The need for a stepwise progression through the learning curve to minimize morbidity and mortality has been highlighted by many centers[20-22]. Furthermore, using laparoscopic surgery for major liver resections and liver resections for lesions adjacent to major vessels has been confirmed to be both feasible and safe[23-25]. Previous researchers have claimed that the use of LLR for major liver resection has similar morbidity and mortality rates as open surgery. Moreover, tumor recurrence rates following LLR for lesions adjacent to major vessels was not increased[24]. Recent studies support the consensus that LLR is feasible, safe, and minimally invasive. Once surgeons progress through the learning curve, this procedure can offer benefits to patients. Halls *et al*[26] and Ban *et al*[27] established a difficulty score

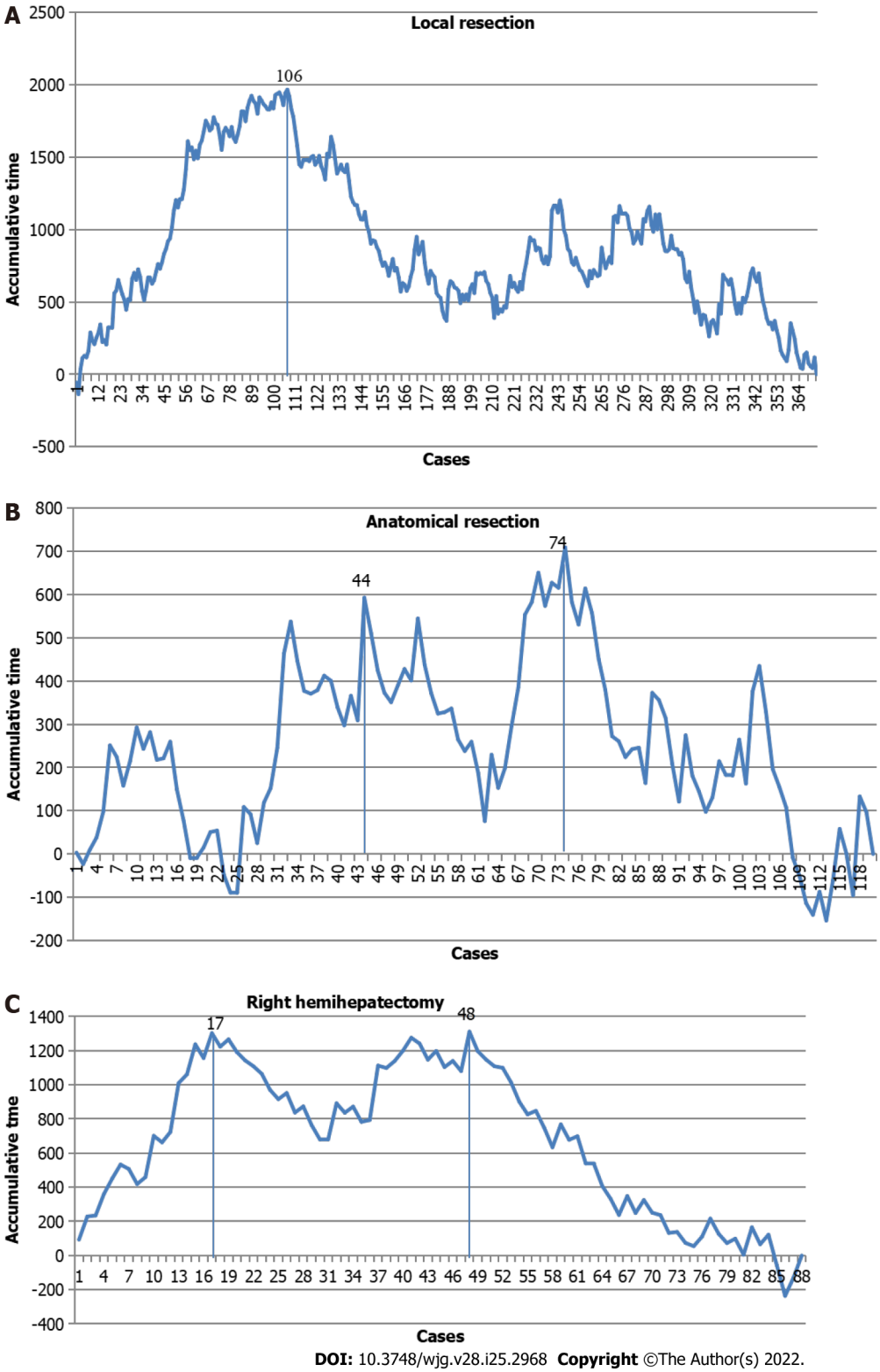


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**Figure 4 The learning curves for different types of laparoscopic liver resection.** A: The learning curve for local resection; B: The learning curve for anatomical resection; C: The learning curve for right hemihepatectomy.

model to predict intraoperative complications during LLR. Patient characteristics such as neoadjuvant chemotherapy, lesion type and size, classification of resection, and previous open liver resection were associated with a higher risk of surgery-related complications after LLR when compared to surgery-related complications after open liver resection. These findings may provide insight for surgeons when making treatment decisions to obtain better patient outcomes following LLR.

The large Chinese population, especially in Sichuan Province, allowed the center in the present study to accumulate over 1000 LLR cases in a 4-year period. These cases involved all types of LLR, including laparoscopic living donor resection. As reported, the increase in the volume of LLRs performed in 2009-

**Table 3 Characteristics of patients with hepatocellular carcinoma and mixed carcinoma**

| | Frequencies | Range/percentage |
|---|---|---|
| Incision margin (cm) | 1.18 ± 1.0 | 0-20 |
| Liver cirrhosis | | |
| Cirrhosis | 201 | 43.04% |
| Non | 266 | 56.96% |
| Tumor location | | |
| Central | 229 | 49.03% |
| Marginal | 238 | 50.97% |
| BCLC stage | | |
| 0 | 81 | 17.34% |
| A1 | 245 | 52.46% |
| A2 | 40 | 8.57% |
| A3 | 22 | 4.71% |
| A4 | 14 | 3.00% |
| B | 48 | 10.27% |
| C | 17 | 3.64% |
| D | 0 | 0 |
| TNM stage | | |
| Ia | 302 | 64.66% |
| Ib | 99 | 21.20% |
| IIa | 43 | 9.21% |
| IIb | 15 | 3.21% |
| IIIa | 7 | 1.50% |
| IIIb | 1 | 0.21% |
| IV | 0 | 0 |
| Liver capsule invasion | | |
| Positive | 156 | 33.40% |
| Differentiation | 311 | 66.60% |
| Well-moderate | 275 | 58.87% |
| Poor | 192 | 41.13% |

BCLC: Barcelona Clinic Liver Cancer; TNM: Tumor-node-metastasis.

2012 *vs* 2000-2008 may be partially attributed to the Louisville 2009 Consensus[28]. Therefore, approximately 22 out of the total of 160 cases were necessary to overcome the learning curve[22,29,30]. A decrease in blood loss during LLR was observed after a minimum of performing 50 cases[29]; and at least 25 cases were needed to master laparoscopic living donor resection[31]. At the center, 106 cases were needed to optimize local resection *via* LLR. However, for complex LLR, 2 peak points were observed in the learning curve. Repetitive training (an initial period, increased competence in LLR, and then mastery and the challenging period) was necessary for surgeons to master hemihepatectomy and anatomical resection *via* LLR. Furthermore, an increase in the conversion rate in 2016/2017 was noted. This time period corresponded to the second and third years of performing LLR at the center when surgeons were overcoming the second peak of the LLR learning curve and at which time there was a huge increase in the number of LLR cases requiring more complex surgeries. After the LLR technology was mastered, the conversion rate at the center decreased significantly in 2018.

Some studies have claimed that continuous hemi-hepatic vascular inflow occlusion is associated with similar outcomes as IP[32,33]. However, at the center in the present study, the IP method was adopted instead of hemihepatic vascular inflow occlusion once the LLR learning curve was overcome, as the IP

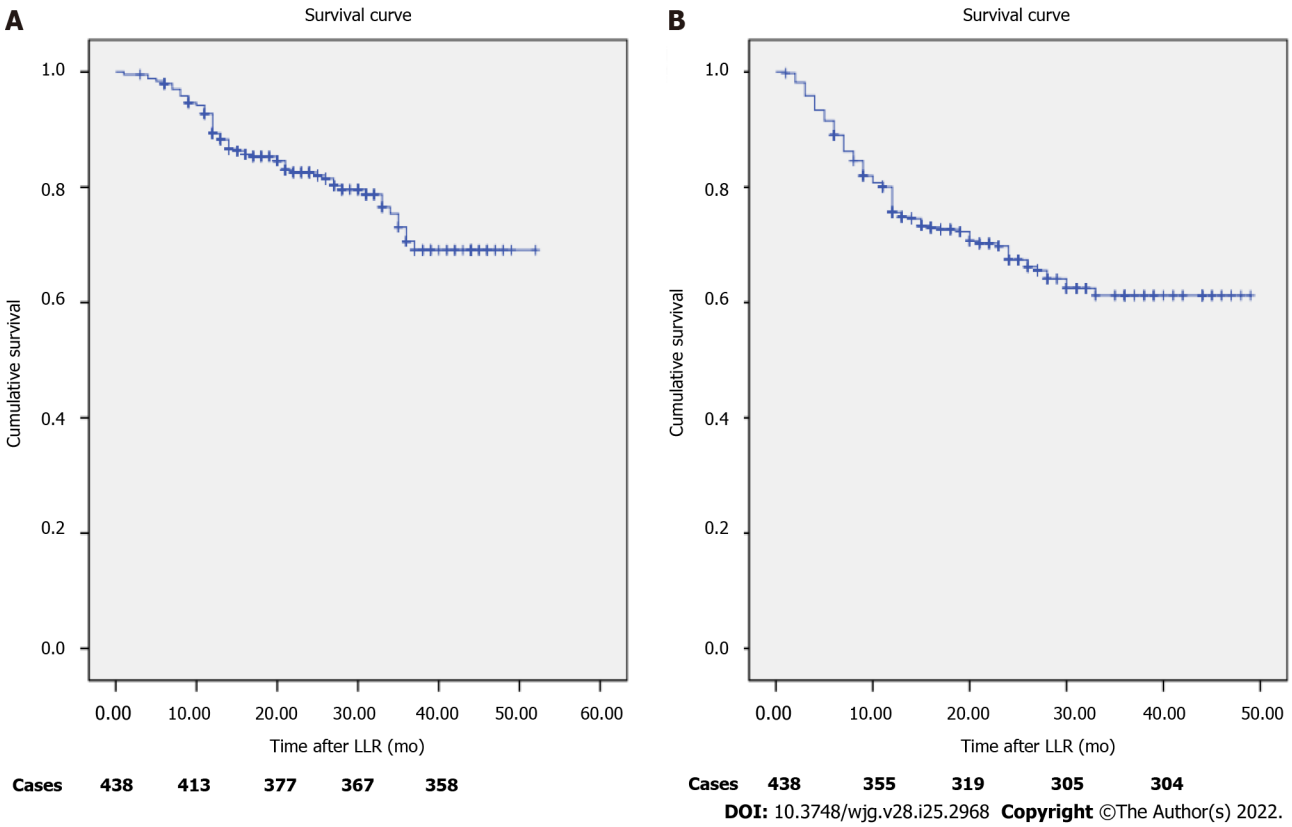


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**Figure 5 Survival rates of patients with hepatocellular carcinoma.** A: Overall survival rates; B: Disease-free survival rates. LLR: Laparoscopic liver resection.

method resulted in less blood loss than the hemihepatic vascular inflow occlusion method[12]. Additionally, the intrahepatic Glissonian approach was adopted to replace the extra-Glissonian approach.

The morbidity and mortality rates at the center are similar to those in other centers. One study reviewed 2804 patients who received LLR and found a cumulative mortality rate of 0.3% and a morbidity rate of 10.5%. Liver-specific complications included bile leaks (1.5%), transient liver ascites (1%), and abscesses (2%)[34]. At the center in the present study, in addition to observing these common complications, 4 patients who underwent right anterior lobectomy suffered from right posterior branch injury. One of these 4 patients suffered from liver failure and ultimately died. Due to this complication the LLR technique was improved so that a longer right anterior pedicle is now exposed prior to transection. Similarly, for patients who undergo right hemihepatectomy, the right anterior and posterior pedicle must be transected to prevent left pedicle injury. For patients who undergo left hemihepatectomy, the presence of ischemia in the right lobe following ligation of the left Glissonian sheath must be determined. The cause of death in the other 2 patients was refractory hypernatremia and ascites with liver failure. The most common postoperative complications at the center were pneumonia, hypohepatia, ascites, and bile leakage. Postoperative bleeding requiring a second operation was rare in these cases. Once the LLR learning curve was conquered and the LLR technique advanced and was optimized, postoperative complications significantly decreased from year to year.

## CONCLUSION

In conclusion, LLR may be performed safely for a variety of primary, secondary and recurrent liver tumors and for benign diseases. Once the learning curve is overcome, LLR can offer short-term benefits for patients.

## ARTICLE HIGHLIGHTS

### *Research background*

Laparoscopic liver resection (LLR) has become a safe approach but still need to be further summarized.

### *Research motivation*
The present study reviews the 4-year experience of total LLR in a single center, which exceeded 1000 cases.

### *Research objectives*
Summarize the past, in order to obtain better progress in this technology in the future.

### *Research methods*
Patients who underwent LLR at West China Hospital of Sichuan University between January 2015 and December 2018 were identified. Surgical details in different years, categories of liver disease and prognosis of malignant liver tumors were evaluated. The learning curve for LLR was evaluated using the cumulative sum method. The Kaplan-Meier method was used to perform survival analysis.

### *Research results*
Ultimately, 1098 patients were identified. Hepatocellular carcinoma (HCC) was the most common disease that led to the need for LLR in our center ($n$ = 462, 42.08%). The average operation time was 216.94 min ± 98.51 min. The conversion rate was 1.82% (20/1098). The complication rate was 9.20% (from grade II to V). The 1-year and 3-year overall survival rates of HCC patients were 89.7% and 81.9%, respectively. The learning curve was grouped into two phases for local resection (cases 1-106 and 107-373), three phases for anatomical segmentectomy (cases 1-44, 45-74 and 75-120) and three phases for hemi-hepatectomy (cases 1-17, 18-48 and 49-88).

### *Research conclusions*
LLR may be considered a first-line surgical intervention for liver resection that can be performed safely for a variety of primary, secondary, and recurrent liver tumors and for benign diseases once technical competence is proficiently attained.

### *Research perspectives*
It is a very promising surgical procedure that can give patients a faster recovery time.

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## FOOTNOTES

**Author contributions:** Lan X and Zhang HL contributed equally to this study and participated in the research design and preparation of the paper; Liu F, Zhang H and Zhang HL performed the statistical analysis; Li B revised this article and Wei YG performed the operation; all the authors contributed to this study.

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**Informed consent statement:** Written informed consent for surgery was obtained from both patient and her family in this study.

**Conflict-of-interest statement:** All the authors have no conflicts of interest or financial ties to disclose.

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## REFERENCES

1 **Azagra JS**, Goergen M, Gilbart E, Jacobs D. Laparoscopic anatomical (hepatic) left lateral segmentectomy-technical aspects. *Surg Endosc* 1996; **10**: 758-761 [PMID: 8662435 DOI: 10.1007/BF00193052]
2 **Feldbrügge L**, Wabitsch S, Benzing C, Krenzien F, Kästner A, Haber PK, Atanasov G, Andreou A, Öllinger R, Pratschke J, Schmelzle M. Safety and feasibility of laparoscopic liver resection in patients with a history of abdominal surgeries. *HPB (Oxford)* 2020; **22**: 1191-1196 [PMID: 31831317 DOI: 10.1016/j.hpb.2019.11.006]
3 **Schmelzle M**, Krenzien F, Schöning W, Pratschke J. Laparoscopic liver resection: indications, limitations, and economic aspects. *Langenbecks Arch Surg* 2020; **405**: 725-735 [PMID: 32607841 DOI: 10.1007/s00423-020-01918-8]
4 **Kaibori M**, Ichihara N, Miyata H, Kakeji Y, Nanashima A, Kitagawa Y, Yamaue H, Yamamoto M, Endo I. Surgical outcomes of laparoscopic versus open repeat liver resection for liver cancers: A report from a nationwide surgical database in Japan. *J Hepatobiliary Pancreat Sci* 2022 [PMID: 35445565 DOI: 10.1002/jhbp.1156]
5 **Cai LX**, Tong YF, Yu H, Liang X, Liang YL, Cai XJ. Is Laparoscopic Hepatectomy a Safe, Feasible Procedure in Patients with a Previous Upper Abdominal Surgery? *Chin Med J (Engl)* 2016; **129**: 399-404 [PMID: 26879012 DOI: 10.4103/0366-6999.176068]
6 **Monden K**, Sadamori H, Hioki M, Ohno S, Takakura N. Short-term outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma in older patients: a propensity score matching analysis. *BMC Surg* 2022; **22**: 63 [PMID: 35197022 DOI: 10.1186/s12893-022-01518-x]
7 **Coelho FF**, Kruger JA, Fonseca GM, Araújo RL, Jeismann VB, Perini MV, Lupinacci RM, Cecconello I, Herman P. Laparoscopic liver resection: Experience based guidelines. *World J Gastrointest Surg* 2016; **8**: 5-26 [PMID: 26843910 DOI: 10.4240/wjgs.v8.i1.5]
8 **Ho KM**, Han HS, Yoon YS, Cho JY, Choi YR, Jang JS, Kwon SU, Kim S, Choi JK. Laparoscopic Total Caudate Lobectomy for Hepatocellular Carcinoma. *J Laparoendosc Adv Surg Tech A* 2017; **27**: 1074-1078 [PMID: 27855267 DOI: 10.1089/lap.2016.0459]
9 **Li H**, Wei Y, Li B, Peng B. Laparoscopic hepatectomy for segments I, IV, V and VIII. *Surg Endosc* 2017; **31**: 3028-3029 [PMID: 27822585 DOI: 10.1007/s00464-016-5319-6]
10 **Li H**, Wei Y, Li B, Peng B. The First Case of Total Laparoscopic Living Donor Right Hemihepatectomy in Mainland China and Literature Review. *Surg Laparosc Endosc Percutan Tech* 2016; **26**: 172-175 [PMID: 26974531 DOI: 10.1097/SLE.0000000000000256]
11 **Marrero JA**, Kudo M, Bronowicki JP. The challenge of prognosis and staging for hepatocellular carcinoma. *Oncologist* 2010; **15** Suppl 4: 23-33 [PMID: 21115578 DOI: 10.1634/theoncologist.2010-S4-23]
12 **Lan X**, Li H, Liu F, Li B, Wei Y, Zhang H, Xu H. Does liver cirrhosis have an impact on the results of different hepatic inflow occlusion methods in laparoscopic liver resection? *HPB (Oxford)* 2019; **21**: 531-538 [PMID: 30342833 DOI: 10.1016/j.hpb.2018.09.009]
13 **Desmet VJ**, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513-1520 [PMID: 8188183]
14 **Li ZS**, Li Q. [The latest 2010 WHO classification of tumors of digestive system]. *Zhonghua Bing Li Xue Za Zhi* 2011; **40**: 351-354 [PMID: 21756837]
15 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
16 **Steiner SH**, Cook RJ, Farewell VT, Treasure T. Monitoring surgical performance using risk-adjusted cumulative sum charts. *Biostatistics* 2000; **1**: 441-452 [PMID: 12933566 DOI: 10.1093/biostatistics/1.4.441]
17 **Chen J**, Li H, Liu F, Li B, Wei Y. Surgical outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma for various resection extent. *Medicine (Baltimore)* 2017; **96**: e6460 [PMID: 28328863 DOI: 10.1097/MD.0000000000006460]
18 **Morise Z**, Ciria R, Cherqui D, Chen KH, Belli G, Wakabayashi G. Can we expand the indications for laparoscopic liver resection? *J Hepatobiliary Pancreat Sci* 2015; **22**: 342-352 [PMID: 25663288 DOI: 10.1002/jhbp.215]
19 **Cai X**, Li Z, Zhang Y, Yu H, Liang X, Jin R, Luo F. Laparoscopic liver resection and the learning curve: a 14-year, single-center experience. *Surg Endosc* 2014; **28**: 1334-1341 [PMID: 24399518 DOI: 10.1007/s00464-013-3333-5]
20 **Ibuki S**, Hibi T, Tanabe M, Geller DA, Cherqui D, Wakabayashi G; INSTALL-2 Collaborative Study Group. Short-term Outcomes of "Difficult" Laparoscopic Liver Resection at Specialized Centers: Report From INSTALL (International Survey on Technical Aspects of Laparoscopic Liver Resection)-2 on 4478 Patients. *Ann Surg* 2022; **275**: 940-946 [PMID: 32889884 DOI: 10.1097/SLA.0000000000004434]
21 **Sultana A**, Nightingale P, Marudanayagam R, Sutcliffe RP. Evaluating the learning curve for laparoscopic liver resection: a comparative study between standard and learning curve CUSUM. *HPB (Oxford)* 2019; **21**: 1505-1512 [PMID: 30992198 DOI: 10.1016/j.hpb.2019.03.362]
22 **Shanti H**, Raman R, Chakravartty S, Belgaumkar AP, Patel AG. Effect of the learning curve on survival after laparoscopic liver resection for colorectal metastases. *BJS Open* 2022; **6** [PMID: 35380619 DOI: 10.1093/bjsopen/zrac020]

23 **Wang ZY**, Chen QL, Sun LL, He SP, Luo XF, Huang LS, Huang JH, Xiong CM, Zhong C. Laparoscopic versus open major liver resection for hepatocellular carcinoma: systematic review and meta-analysis of comparative cohort studies. *BMC Cancer* 2019; **19**: 1047 [PMID: 31694596 DOI: 10.1186/s12885-019-6240-x]

24 **Abu Hilal M**, van der Poel MJ, Samim M, Besselink MG, Flowers D, Stedman B, Pearce NW. Laparoscopic liver resection for lesions adjacent to major vasculature: feasibility, safety and oncological efficiency. *J Gastrointest Surg* 2015; **19**: 692-698 [PMID: 25564324 DOI: 10.1007/s11605-014-2739-2]

25 **Morise Z**, Kawabe N, Tomishige H, Nagata H, Kawase J, Arakawa S, Isetani M. How Far Can We Go with Laparoscopic Liver Resection for Hepatocellular Carcinoma? *Biomed Res Int* 2015; **2015**: 960752 [PMID: 26448949 DOI: 10.1155/2015/960752]

26 **Halls MC**, Berardi G, Cipriani F, Barkhatov L, Lainas P, Harris S, D'Hondt M, Rotellar F, Dagher I, Aldrighetti L, Troisi RI, Edwin B, Abu Hilal M. Development and validation of a difficulty score to predict intraoperative complications during laparoscopic liver resection. *Br J Surg* 2018; **105**: 1182-1191 [PMID: 29737513 DOI: 10.1002/bjs.10821]

27 **Ban D**, Tanabe M, Ito H, Otsuka Y, Nitta H, Abe Y, Hasegawa Y, Katagiri T, Takagi C, Itano O, Kaneko H, Wakabayashi G. A novel difficulty scoring system for laparoscopic liver resection. *J Hepatobiliary Pancreat Sci* 2014; **21**: 745-753 [PMID: 25242563 DOI: 10.1002/jhbp.166]

28 **Chen TH**, Yang HR, Jeng LB, Hsu SC, Hsu CH, Yeh CC, Yang MD, Chen WT. Laparoscopic Liver Resection: Experience of 436 Cases in One Center. *J Gastrointest Surg* 2019; **23**: 1949-1956 [PMID: 30421118 DOI: 10.1007/s11605-018-4023-3]

29 **Tomassini F**, Scuderi V, Colman R, Vivarelli M, Montalti R, Troisi RI. The single surgeon learning curve of laparoscopic liver resection: A continuous evolving process through stepwise difficulties. *Medicine (Baltimore)* 2016; **95**: e5138 [PMID: 27787369 DOI: 10.1097/MD.0000000000005138]

30 **Efanov M**, Alikhanov R, Tsvirkun V, Kazakov I, Melekhina O, Kim P, Vankovich A, Grendal K, Berelavichus S, Khatkov I. Comparative analysis of learning curve in complex robot-assisted and laparoscopic liver resection. *HPB (Oxford)* 2017; **19**: 818-824 [PMID: 28599892 DOI: 10.1016/j.hpb.2017.05.003]

31 **Broering DC**, Berardi G, El Sheikh Y, Spagnoli A, Troisi RI. Learning Curve Under Proctorship of Pure Laparoscopic Living Donor Left Lateral Sectionectomy for Pediatric Transplantation. *Ann Surg* 2020; **271**: 542-548 [PMID: 29995683 DOI: 10.1097/SLA.0000000000002948]

32 **Ni JS**, Lau WY, Yang Y, Pan ZY, Wang ZG, Liu H, Wu MC, Zhou WP. A prospective randomized controlled trial to compare pringle manoeuvre with hemi-hepatic vascular inflow occlusion in liver resection for hepatocellular carcinoma with cirrhosis. *J Gastrointest Surg* 2013; **17**: 1414-1421 [PMID: 23715650 DOI: 10.1007/s11605-013-2236-z]

33 **Fu SY**, Lau WY, Li GG, Tang QH, Li AJ, Pan ZY, Huang G, Yin L, Wu MC, Lai EC, Zhou WP. A prospective randomized controlled trial to compare Pringle maneuver, hemihepatic vascular inflow occlusion, and main portal vein inflow occlusion in partial hepatectomy. *Am J Surg* 2011; **201**: 62-69 [PMID: 20409520 DOI: 10.1016/j.amjsurg.2009.09.029]

34 **Nguyen KT**, Gamblin TC, Geller DA. World review of laparoscopic liver resection-2,804 patients. *Ann Surg* 2009; **250**: 831-841 [PMID: 19801936 DOI: 10.1097/SLA.0b013e3181b0c4df]

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SCIENTOMETRICS

# Mapping the global research landscape on nutrition and the gut microbiota: Visualization and bibliometric analysis

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## Abstract

### *BACKGROUND*

Nutrition is a significant modifiable element that influences the composition of the gastrointestinal microbiota, implying the possibility of therapeutic diet

methods that manipulate the composition and diversity of the microbial.

***AIM***
To overview research papers on nutrition and gut microbiota and determines the hotspots in this field at the global level.

***METHODS***
Scopus and Reference Citation Analysis were used to construct a bibliometric technique. It was decided to create bibliometric indicators and mapping as in most previous studies. 2012 through 2021 served as the study's timeframe.

***RESULTS***
A total of 5378 documents from the Scopus database were selected for analysis. Of all retrieved studies, 78.52% were research papers ($n$ = 4223), followed by reviews ($n$ = 820; 15.25%). China ranked first with a total number of articles of 1634 (30.38%), followed by the United States in second place with a total number of articles of 1307 (24.3%). In the last decade, emerging hotspots for gut microbiota and nutrition research included "gut microbiota metabolism and interaction with dietary components", "connection between the gut microbiota and weight gain", and "the influence of high-fat diet and gut microbiota on metabolic disorders".

***CONCLUSION***
This is the first thorough bibliometric analysis of nutrition and gut microbiota publications conducted on a global level. Investigation of the association between nutrition/diet and the gut microbiota is still in its infancy and will be expanded in the future. However, according to recent trends, the “effect of gut microbiota and high-fat diet on metabolic disorders" will be an increasing concern in the future.

**Key Words:** Gut microbiota; Microbiome; Nutrition; Diet; Bibliometric; Scopus; VOSviewer

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**Core Tip:** The number of publications on the gut microbiota has progressively expanded as the potential clinical implications of the gut microbiota have become better known, and this has become an emerging research subject. Therefore, this comprehensive bibliometric study intends to provide an overview of the current state of worldwide nutrition and gut microbiota research and to stay ahead of emerging trends and important turns in the field's evolution. Investigation on the association between nutrition/diet and the gut microbiota is still in its infancy and will be expanded in the future. However, according to recent trends, the “effect of gut microbiota and high-fat diet on metabolic disorders" will be a bigger worry in the future.

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## INTRODUCTION

The microbiome is a term that refers to the collective genomes of microorganisms living in a certain habitat, and the gut microbiota is a complex collection of microorganisms that includes archaea, bacteria, fungus, viruses, and protozoa, among others[1]. These communities interact with each other and their host, with the majority of them being bacteria that reside mainly in the colon of the gastrointestinal tract and interact with them. The human genome has thousands of genes; however, the microbiome contains millions of genes that produce a large number of metabolites that alter host activities, resulting in a substantial impact on the host at the physiological level[2]. Different levels of evidence show that functional or compositional changes in the microbiota present in the human gut contribute to human disease and health, including metabolic, immunological and neuro-behavioral traits that support the role of the microbiota in human health[3,4].

The composition of the gut microbiota varies greatly between individuals. Several studies have found that dietary variations between people are one of the external variables that influence and define the gut microbiota structure[5-8]. Studies in recent years have shown that diet or food as the main source of

energy for gut microbes can greatly affect the diversity and components of the gut microbiota, which affects host health[9-12]. In addition to diet, the gut microbiota is affected by different external variables, such as lifestyle and medication (*e.g.*, antibiotics), which mainly affect the microbiota. There are also intrinsic factors that can affect the gut microbiota, such as host genetics, immune and metabolic regulations[13]. Dietary modifications can account for more than 50% of the changes in the gut microbiota changes; however, host genes account for only 10%[14].

Research showed that disturbances in the interaction between nutrients in food and the microbiome affect its structure, which has been shown to be linked to the development of a variety of diseases[15, 16]. Long-term or, to a lesser extent, short-term dietary intake modifications could determine each human's stable microbiota profile. For example, regardless of the stage of life, the intake of protein, fat, fermentable or non-fermentable carbs, probiotic, and polyphenol effectively modulates the host's gut microbiota, metabolic, and immunological parameters. Compared to high animal protein consumption and low carbohydrate intake, high consumption increases the risk of various health conditions, such as diabetes, cancer, cardiovascular disease, and irritable bowel syndrome[7,10]. Another evidence on the diversity of bacterial content in the gastrointestinal tract of humans revealed that lower bacterial diversity has been found in individuals with various diseases such as arthritis, inflammatory bowel disease, type 1 and type 2 diabetes, eczema, and obesity than in healthy individuals who support the role of the microbiota in human health and diseases[17-21]. Accordingly, diversity appears as a positive indicator of a healthy gastrointestinal tract[22,23].

The results have been applied to numerous domains connected to the gut microbiota over the last years[24-28]. However, there are no bibliometric analyses of nutrition/diet and gut microbiota publications. As a result, the objective of the present study was to carefully review publications related to nutrition and gut microbiota to assess the current state and hotspots in this field. Therefore, this comprehensive bibliometric study intends to provide an overview of the current state of worldwide nutrition and gut microbiota research and to stay ahead of emerging trends and important turns in the field's evolution.

## MATERIALS AND METHODS

### Data sources

We performed this bibliometric analysis using the Scopus bibliographic database, which Elsevier B.V. owns. In bibliometric research, only one database is used because bibliometric indicators and literature mapping are difficult to execute on documents acquired from several databases. Furthermore, the grey literature cannot be included in the data retrieved[29,30]. The current analysis was conducted using the SciVerse Scopus database for numerous reasons. First, Scopus has a much larger number and diversity of indexed publications compared to other databases like PubMed or Web of Science. Scopus has nearly the same number of journals indexed as PubMed and Web of Science combined[31]. Second, because all articles listed in PubMed are simultaneously indexed in Scopus, PubMed is completely comprehensive in Scopus. As a result, Scopus is considered to be comprehensive, as it includes papers from both PubMed and Web of Science[29,30]. Third, Scopus publishes publications in a wide range of disciplines, including science, technology, medicine, social science, and the arts and humanities. Fourth, Scopus enables academics to create sophisticated and extensive search queries by combining various Boolean operators. Finally, Scopus enables the researcher to export and examine the data that have been retrieved. This comprises mapping and statistical analysis. Due to the rapid update of the database, the literature retrieval was carried out on a single day (January 7, 2022). Therefore, it was decided that the publication period for this study would be between 2012 and 2021.

### Goal definition

The primary goal of this study is to assess the current state and hotspots in the field of nutrition and gut microbiota by examining the scientific production data set related to nutrition and gut microbiota with the goal of characterisation and understanding it in terms of scientometric indices. Based on bibliometric and/or scientometric indicators, the mapping and analysis of scientific knowledge allow the identification of the dynamics and/or expansion of a specific field of study and the support of strategic decisions related to various research institutions[32].

### Search strategy

We used the key terms "microbiome or microbiota" and "nutrition or diet" and its synonyms because we are interested in nutrition and gut microbiota *per se* as a novel concept in scientific research rather than related subjects. The current study focuses on research papers containing "nutrt* or nutrient* or diet* or eat* or feeding" and "microbiome or microbiota or *flora" to recognize items based on their search in the fields topic and the time was ten years between 2012 and 2021. The asterisk (*) was used in search query to retrieve documents with keywords such as diet or dietary.

### Validation of the search strategy

The search query was repeatedly fine-tuned in the current study until the top 100 cited publications in the retrieved literature were free of false-positive results. Furthermore, the search query was checked for missing data (false-negative) using a previously published method that depends on the association between the retrieved research output and the actual research output in this field[29,30]. Instead of a title/abstract search, keywords were used in the title search. As a result, the title search will produce the fewest false positive documents, making it a reliable method[29,33-35]. However, a title/abstract search will show many false positives in which the main focus is not on nutrition and the microbiota *per se*.

### Bibliometric analysis

The Scopus advanced search tool was used to implement the method. Using the "analyze" function, the retrieved documents were examined. The abstained findings were saved as.csv files in Microsoft Excel. Annual growth, document types, journal names with impact factor (IF), funding agencies, country names, institution names, and document citations were all included in the exported findings. This data was displayed as a linear graph of the annual growth of publication and tables for the core of (top 10 active) journals, funding agencies, countries, institutions, and cited documents in the results section.

The *Impact Index Per Article* for the top 10 high-cited papers collected from Reference Citation Analysis (RCA) is presented. RCA is an open multidisciplinary citation analysis database owned by Baishideng Publishing Group Inc. (Pleasanton, CA 94566, United States)[36].

### Visualized analysis

Data on terms in titles and abstracts and cross-country collaboration were visualized using the free Web tool VOSviewer 1.6.17 to determine a worldwide scientific cooperation network across countries/regions and examine hotspots and research trends. Nodes in VOSviewer maps are colored and sized differently. The size is related to the number of times it occurs. The node's color shows its relationship to other nodes with similar colors[37].

## RESULTS

### Number of publications and evolution over time

A total of 5378 documents from the Scopus database were selected for analysis. The documents retrieved were of 11 types, mainly research articles ($n$ = 4223; 78.52%), followed by reviews ($n$ = 820; 15.25%). Our findings revealed that the scientific advancement of articles on nutrition and gut microbiota has grown in the past ten years. For example, we found 1295 documents in 2021, compared to only 120 documents in 2012 (Figure 1). Of the 5378 documents retrieved, 89 were clinical trials. Fifteen publications on nutrition and gut microbiota were case reports or case series.

### Contributions of countries to global publications

Table 1 Lists the contributions of the top 10 countries in publications related to nutrition and gut microbiota from 2012 to 2021. Topping the list was China, with 1634 items (30.38% of the total), followed by the United States, with 1307 items (second place) (24.3%). Italy ($n$ = 346, 6.43%) and Spain ($n$ = 313, 5.82%) ranked third and fourth positions, respectively. An examination of countries with more than 20 publications on nutrition and gut microbiota was conducted. Using data from 44 eligible nations, five VOSviewer clusters were used to examine their centrality and connections to other countries (Figure 2). The United States and China had the highest number of publications in the clusters (green cluster). There is a direct correlation between the number and width of the lines on this map with the degree of interaction and strength between countries.

### Analysis of affiliations

As shown in Table 2, the top ten affiliations with the highest number of publications relevant to nutritional science and the gut microbiota were identified. The country with the highest number of affiliations was China. The *Chinese Academy of Sciences* had the highest number of publications ($n$ = 138; 2.57%), followed by the *China Agricultural University* ($n$ = 111; 2.06%), the *Chinese Academy of Agricultural Sciences* ($n$ = 95; 1.77%), and the *University College Cork* ($n$ = 81; 1.51%).

### Analysis of funding agencies

Of the documents retrieved 3271 (60.82%) were publications that were part of funded projects. Again, *Chinese funding agencies* were the most active in this field, the *National Natural Science Foundation of China* ($n$ = 711; 13.22%) being the most active, followed by the *National Institutes of Health* ($n$ = 327; 6.08%), and the *National Institute of Diabetes and Digestive and Kidney Diseases* ($n$ = 190; 3.53%) (Table 3).

**Table 1 List of the top ten countries publishing research on nutrition and the gut microbiota from 2012 to 2021**

| Ranking | Country | Number of documents | % |
|---|---|---|---|
| 1st | China | 1634 | 30.38 |
| 2nd | United States | 1307 | 24.30 |
| 3rd | Italy | 346 | 6.43 |
| 4th | Spain | 313 | 5.82 |
| 5th | United Kingdom | 259 | 4.82 |
| 6th | Canada | 246 | 4.57 |
| 7th | Germany | 240 | 4.46 |
| 8th | Australia | 227 | 4.22 |
| 9th | France | 216 | 4.02 |
| 10th | South Korea | 204 | 3.79 |

**Table 2 The top 10 productive affiliations publishing research on nutrition and the gut microbiota from 2012 to 2021**

| Ranking | Institute | Country | *n* | % |
|---|---|---|---|---|
| 1st | *Chinese Academy of Sciences* | China | 138 | 2.57 |
| 2nd | *China Agricultural University* | China | 111 | 2.06 |
| 3rd | *Chinese Academy of Agricultural Sciences* | China | 95 | 1.77 |
| 4th | *University College Cork* | Ireland | 81 | 1.51 |
| 5th | *APC Microbiome Ireland* | Ireland | 80 | 1.49 |
| 6th | *Nanjing Agricultural University* | China | 74 | 1.38 |
| 7th | *Københavns Universitet* | Denmark | 73 | 1.36 |
| 8th | *Wageningen University & Research* | Netherlands | 62 | 1.15 |
| 9th | *Consejo Superior de Investigaciones Cientificas* | Spain | 55 | 1.02 |
| 10th | *Dankook University* | South Korea | 54 | 1.00 |

**Table 3 The top ten funding agencies having the most publications on nutrition and the gut microbiota from 2012 to 2021**

| Ranking | Funding agencies | Country | No. of publication | % |
|---|---|---|---|---|
| 1st | *National Natural Science Foundation of China* | China | 711 | 13.22 |
| 2nd | *National Institutes of Health* | United States | 327 | 6.08 |
| 3rd | *National Institute of Diabetes and Digestive and Kidney Diseases* | United States | 190 | 3.53 |
| 4th | *National Key Research and Development Program of China* | China | 184 | 3.42 |
| 5th | *European Commission* | European Union | 102 | 1.90 |
| 6th | *Ministry of Science and Technology of the People's Republic of China* | China | 86 | 1.60 |
| 6th | *National Institute of General Medical Sciences* | China | 86 | 1.60 |
| 8th | *European Regional Development Fund* | European Union | 80 | 1.49 |
| 9th | *National Institute of Allergy and Infectious Diseases* | United States | 75 | 1.39 |
| 10th | *Horizon 2020 Framework Programme* | European Union | 74 | 1.38 |

### *Journal analysis*

As presented in Table 4, *Nutrients* (*n* = 271 publications, IF: 5.717) published the most papers on nutrition and the gut microbiota, followed by *Frontiers in Microbiology* (*n* = 167 publications, IF: 5.64), and *Scientific Reports* (*n* = 136 publications, IF: 4.379). The top ten academic journals published approx-

**Table 4 The top 10 most active journals publishing research on nutrition and the gut microbiota from 2012 to 2021**

| Ranking | Journal | *n* | % | IF[1] |
|---|---|---|---|---|
| 1st | *Nutrients* | 271 | 5.04 | 5.717 |
| 2nd | *Frontiers in Microbiology* | 167 | 3.11 | 5.64 |
| 3rd | *Scientific Reports* | 136 | 2.53 | 4.379 |
| 4th | *Plos One* | 124 | 2.31 | 3.24 |
| 5th | *Food and Function* | 116 | 2.16 | 5.396 |
| 6th | *Journal of Functional Foods* | 87 | 1.62 | 4.451 |
| 7th | *Molecular Nutrition and Food Research* | 82 | 1.52 | 5.914 |
| 8th | *Poultry Science* | 78 | 1.45 | 3.352 |
| 9th | *Animals* | 70 | 1.30 | 2.752 |
| 10th | *Journal of Agricultural and Food Chemistry* | 68 | 1.26 | 5.279 |

[1]Impact factor (IF) was extracted from Journal Citation Reports (Source Clarivate, 2021).

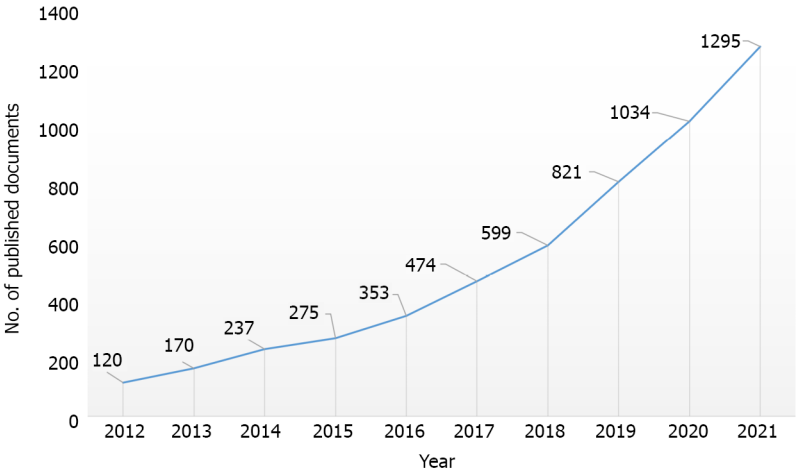


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**Figure 1 Growth trends of publications on the nutrition and the gut microbiota from 2012 to 2021.**

imately 22.3% of the publications.

### *Analysis of research hotspots*

To detect trends and topics in most articles on nutrition and gut microbiota, we used VOSviewer software to look at the distribution of cooccurrence terms in the titles and abstracts of 5378 papers. By mapping terms in titles and abstracts with a minimum frequency of 100 occurrences, three major clusters emerged, highlighting three remarkable research themes. For example, in Figure 3, of the 85327 terms, 293 terms appeared at least 100 times, distributed in three clusters. Cluster 1, indicated by red frames, includes those terms commonly seen in publications related to interaction of the gut microbiota with dietary components; Cluster 2, shown by green frames, contains terms commonly seen in publications related tothe correlation between weight gain and the gut microbiota; and Cluster 3, marked by green frames, includes terms commonly observed in studies related to the effect of the gut microbiota and a high-fat diet and on metabolic syndromes. Figure 4 illustrates an overlay visualization in which the VOSviewer was used to color the terms based on their publication year. The blue and green colors indicate that the terminology appeared first, while the yellow colors indicate that they appeared later. Most studies before 2019 focused on the "connection between the gut microbiota and weight gain" and the "interaction of the gut microbiota with dietary components". However, according to recent trends, the "effect of high-fat diet and the gut microbiota on metabolic syndromes" will be a bigger concern in the future.

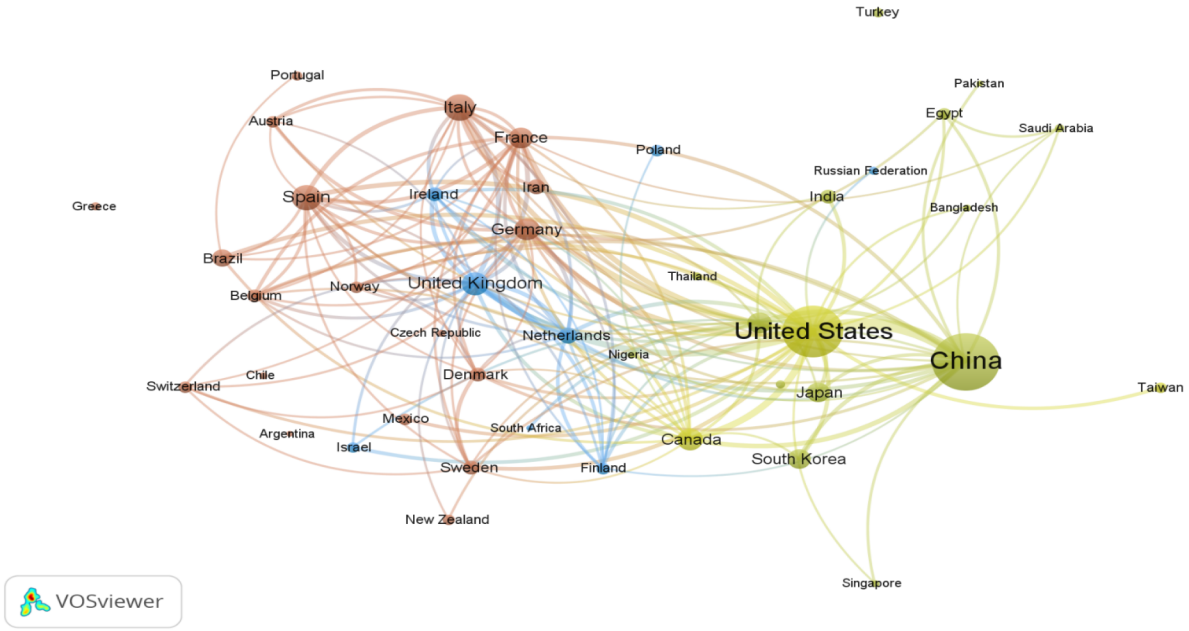


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**Figure 2 A network visualization map depicting international collaboration in nutrition and gut microbiota research during 2012-2021.** This visualized map of collaborations was created after each country had at least 20 publications. Of 154 countries active in this field, 44 countries meet this threshold. The node's size indicates the number of publications for that country.

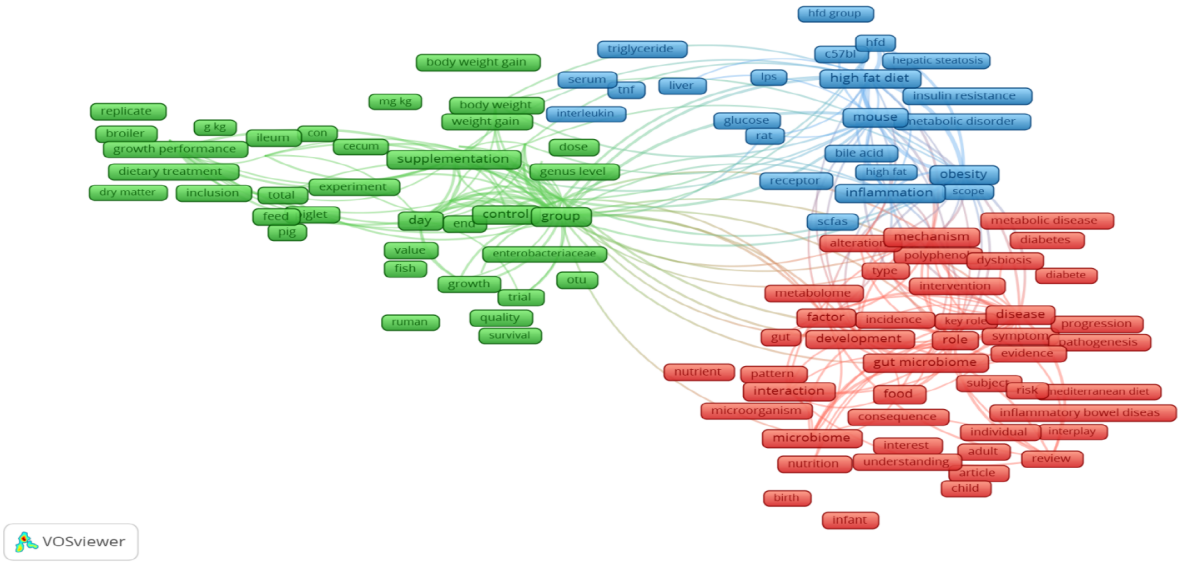


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**Figure 3 Network visualization map of terms in the title/abstract fields of publications relevant to nutrition and gut microbiota research from 2012 to 2021.** When the minimum-term occurrences were put 100 times, this graphical map of terms was created. Of the 85327 terms in this field, 293 achieve this threshold, which were grouped into three clusters and colored differently. The size of the frame shows the number of publications that contain that term.

### *Analysis of citations*

According to citation analysis, the retrieved articles garnered 146101 citations, averaging 27.17 *per* document, and an h-index of 154. Citations ranged from 0 to 4588. Two thousand four hundred forty-two items were indexed with ten or more citations, while 852 were indexed without citations. Table 5 lists the top ten articles with the highest number of articles on research related to nutrition and gut microbiota from 2012 to 2021. The 10 highest citations ranged from 4588 to 796. The impact index *per* article of the 10 most cited articles ranged from 96.3 to 506.2 (Table 5).

**Table 5 The top 10 high cited papers on research related to nutrition and the gut microbiota from 2012 to 2021**

| Ranking | Ref. | Year | Source title | IF[1] | Impact index *per* article[2] | Cited by |
|---|---|---|---|---|---|---|
| 1st | David *et al*[56] | 2014 | *Nature* | 49.962 | 506.2 | 4588 |
| 2nd | Koeth *et al*[57] | 2013 | *Nature Medicine* | 53.44 | 242.8 | 2294 |
| 3rd | Den Besten *et al*[58] | 2013 | *Journal of Lipid Research* | 5.922 | 202.2 | 1921 |
| 4th | Claesson *et al*[59] | 2012 | *Nature* | 49.962 | 168.0 | 1793 |
| 5th | Trompette *et al*[60] | 2014 | *Nature Medicine* | 53.44 | 165.6 | 1368 |
| 6th | Flint *et al*[61] | 2012 | *Nature Reviews Gastroenterology and Hepatology* | 46.802 | 96.3 | 1011 |
| 7th | Desai *et al*[62] | 2016 | *Cell* | 41.582 | 182.0 | 948 |
| 8th | Sonnenburg and Bäckhed[63] | 2016 | *Nature* | 49.962 | 150.3 | 914 |
| 9th | Chassaing *et al*[64] | 2015 | *Nature* | 49.962 | 122.1 | 889 |
| 10th | Dao *et al*[65] | 2016 | *Gut* | 23.059 | 111.3 | 796 |
| 10th | Singh *et al*[10] | 2017 | *Journal of Translational Medicine* | 5.531 | 148.0 | 796 |

[1]Impact factor (IF) was extracted from Journal Citation Reports (Source Clarivate, 2021).
[2]The impact index *per* article is presented based on Reference Citation Analysis [Source: Baishideng Publishing Group Inc. (Pleasanton, CA 94566, United States)].

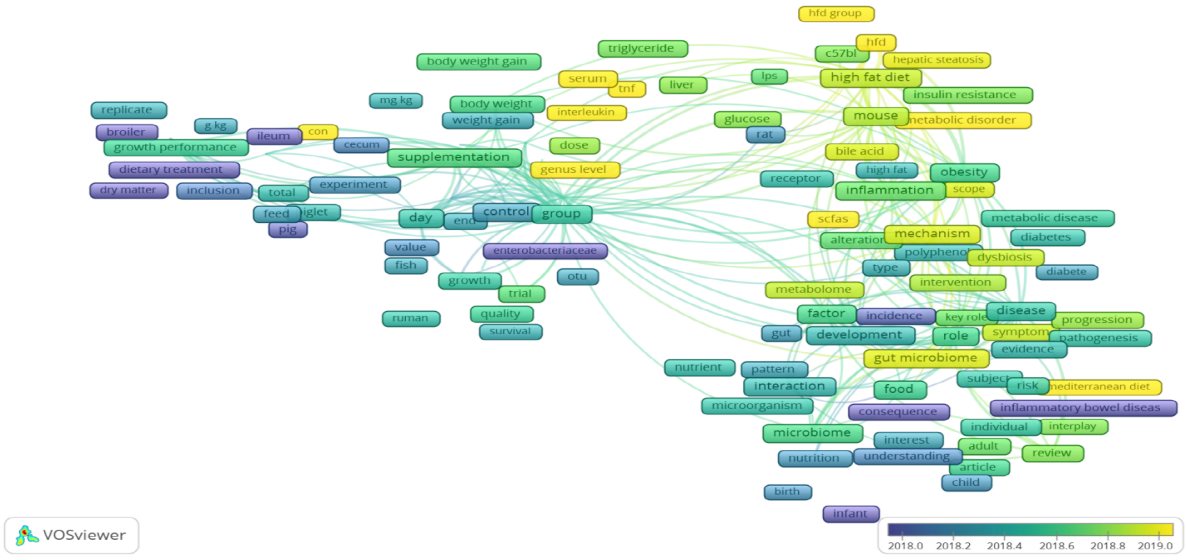


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**Figure 4 Network visualization map of terms in the title/abstract and their frequency of appearance of keywords is distributed according to the mean frequency of appearance.** Blue-colored and green-colored terms first showed, and then yellow terms appeared later.

## DISCUSSION

This study has developed research techniques to thoroughly analyze the current status and direction of development of studies related to nutrition and the gut microbiota from 2012 to 2021 using a bibliometric and visualization analysis. The number of publications on the gut microbiota has progressively expanded as the potential clinical implications of the gut microbiota have become better known, and this has become an emerging research subject. Our review of research on global nutrition and gut microbiota found that relevant publications have expanded in the last ten years. A possible explanation for this finding might be that our understanding of the role of the gut microbiota has advanced significantly due to the advancement of scientific tools.

The examination of collaboration networks can provide rich data for evaluating research collaborations and characterizing essential collaborators. China and the United States had a clear advantage in this field, possibly due to their superior economics and investment in the scientific field; for example, in 2013, a notable research project from the United States was launched on the intestinal microbiota-brain

axis[38], which resulted in a positive impact on the quality and productivity. In addition, Chinese scientists have made significant advances in the field of gut microbiota[39]. Fecal microbiota transplantation (FMT) has attracted remarkable interest in the treatment of extraintestinal health problems. China registered 84 (24%) of the 367 clinical investigations employing FMT[40]. This is a significant contribution by Chinese researchers to this field and the clinical transformation of studies on the gut microbiota. In addition to FMT research, Chinese researchers are also interested in the development and clinical application of probiotics[41]. Furthermore, one possible reason for the increase in Chinese publication numbers, the *National Natural Science Foundation of China* launched a fund for the role and mechanism of the 'Gut-Liver Axis' in gastrointestinal diseases (H03)[42]. This research should focus on the gut microbiota and metabolites, nutrition, neurotransmitters, gastrointestinal hormones, and bile acid, particularly their interactions with the gastrointestinal tract or liver-localized immune cells/factors to mediate a variety of functional roles. Expand on the "gut-liver axis" and its role in the mechanisms of gastrointestinal disease mechanisms to develop novel diagnostic and therapeutic strategies[42].

Furthermore, the increase in publications on the microbiota and nutrition can be attributed to the fact that numerous hot topics were published during this period of time, exposing new theories and establishing new research fields such as the "interaction of the gut microbiota with dietary components", "connection between the gut microbiota and weight gain", and "the impact of a high-fat diet and the gut microbiota on metabolic syndrome". The effect of diet on the gut microbiota and, consequently, on human health suggests new therapeutic and diagnostic concepts and guides to develop probiotic and prebiotic strategies and functional nutrition[43-47].

The 'effect of a high-fat diet and the gut microbiota on metabolic syndromes' as a theme was among the main hot topics in the current study. Most publications on nutrition and gut microbiota before 2019 used words such as "connection between the gut microbiota and weight gain" and "interaction of the gut microbiota with dietary components". This was in the early stages of this research area. The "effect of a high-fat diet and gut microbiota on metabolic syndrome" as a theme was one of the main hot topics in this work and was considered the current trend after 2019. As a result, the evidence that emerges proposes that the gut microbiota may play a role in maintaining body weight and energy balance. It is assumed to be fueled, at least in part, by changes in gut microbiota composition caused by high-fat diets (HFDs); HFDs have been correlated with changes in gut microbial profile as well as decreased diversity [48,49]. Furthermore, metabolic syndrome and its associated disorders have been linked to increasing evidence suggesting that the gut microbiota in the digestive tract plays a significant role. Numerous studies in animals and humans have shown that high-fat and high-sugar (fructose) diets are responsible for the three hallmarks of metabolic syndrome: Obesity, dyslipidemia, and insulin resistance by altering the composition and diversity of the gut microbiota[50-53]. Furthermore, there is evidence that HFD is associated with cardiovascular disease[54] because the intestinal microbiota converts dietary choline to trimethylamine, which is absorbed in the gut and converted to trimethylamine N-oxygen. This metabolite causes atherosclerosis in the liver[55].

### *Strengths and limitations*

This is the first thorough bibliometric analysis of nutrition and gut microbiota research conducted on a global level. Research on the relationship between nutrition/diet and gut microbiota is still in its infancy and will expand in the future. The aforementioned growing hotspots serve as a foundation and guide for the development of new projects by scientific researchers, physicians, and medical educators.

Our study has several limitations. First, although Scopus is indeed the largest database available, the search for studies was carried out only from the Scopus database, which could have limited the generalizability of the findings due to the loss of certain documents published in unindexed journals. Second, the accuracy and comprehensiveness of the keywords have a significant impact on the reliability of the data retrieved in our study. As a result, some important and influential articles may be omitted from a representative list due to the use of other terms in their titles. Third, the current study only looked for terms such as "nutrition", "gut microbiota" and other related terms in the title search only. Papers with these terms in the abstract or text may have been missed in this study. Furthermore, while most publications were published and indexed in 2021, the amount of scientific research productivity this year could be higher because new journal issues are still being published and indexed.

## CONCLUSION

To our knowledge, this is the first bibliometric study of trends in publications on microbiota and nutrition. We are entering an era in which we can more easily alter our health through diet and track results using our microbiota or their metabolites. Over the last decade, there has been an increase in the number of publications on the microbiota and nutrition. The top three contributors to microbiota and nutrition research were China, the United States, and Italy. China is the leading country in this area of study, achieving some significant research findings and playing a key role in the progress of the field. "Connection between the gut microbiota and weight gain', 'interaction with dietary components" and

"the effect of a high-fat diet and the gut microbiota on metabolic syndromes' were hotspots for gut microbiota and nutrition research in the last 10 years. As a result, our timely examination and analysis of hotspots and research trends may aid in the growth of this discipline. Furthermore, these findings offer a fresh perspective on the study of the microbiota and nutrition/diet, which could help with future etiological research, diagnosis, and treatment of metabolic syndromes.

## ARTICLE HIGHLIGHTS

### *Research background*

Dietary composition is crucial in regulating the gut microbiota and, as a result, in the prevention, management, and treatment of diseases including diabetes and cancer.

### *Research motivation*

According to a growing body of evidence, the gut microbiota appears to considerably impact human health. Intestinal homeostasis, physiology, gut microbiome, immune system, and host metabolic pathways are all affected by changes in the gut microbiota. However, there are no bibliometric analyses of nutrition/diet and gut microbiota publications.

### *Research objectives*

The current study gives an overview of research publications on nutrition and gut microbiota and identifies global hotspots on this topic.

### *Research methods*

A comprehensive, validated search query was used to find relevant publications on nutrition and gut microbiota. SciVerse Scopus was the database used.

### *Research results*

This is the first bibliometric analysis of trends in microbiota and nutrition publications. The number of publications on microbiota and nutrition has increased over the last decade. China, the United States, and Italy were the top three contributors to microbiota and nutrition research.

### *Research conclusions*

In conclusion with our timely examination and analysis of hotspots and research trends, we believe that this discipline will grow. Furthermore, these findings shed new light on the study of the microbiota and nutrition/diet, which could aid in future etiological research, diagnosis, and treatment of metabolic syndromes.

### *Research perspectives*

This comprehensive bibliometric study aims to provide an overview of the current state of global nutrition and gut microbiota research and stay ahead of emerging trends and critical turns in the field's evolution.

## FOOTNOTES

**Author contributions:** Zyoud SH designed the study, collected the data, analyzed the data, made major contributions to the manuscript's existing literature search and interpretation, and drafted the manuscript; Shakhshir M, Abushanab AS, Al-Jabi SW, Jairoun AA, Shahwan M, Koni A and Abu Taha A were involved in the interpretation of the data, and made revisions to the initial draft; all authors provided a critical review and approved the final manuscript before submission.

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## REFERENCES

1 **Berg G**, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, Kazou M, Kinkel L, Lange L, Lima N, Loy A, Macklin JA, Maguin E, Mauchline T, McClure R, Mitter B, Ryan M, Sarand I, Smidt H, Schelkle B, Roume H, Kiran GS, Selvin J, Souza RSC, van Overbeek L, Singh BK, Wagner M, Walsh A, Sessitsch A, Schloter M. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 2020; **8**: 103 [PMID: 32605663 DOI: 10.1186/s40168-020-00875-0]

2 **Rath CM**, Dorrestein PC. The bacterial chemical repertoire mediates metabolic exchange within gut microbiomes. *Curr Opin Microbiol* 2012; **15**: 147-154 [PMID: 22209085 DOI: 10.1016/j.mib.2011.12.009]

3 **Jackson MA**, Verdi S, Maxan ME, Shin CM, Zierer J, Bowyer RCE, Martin T, Williams FMK, Menni C, Bell JT, Spector TD, Steves CJ. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nat Commun* 2018; **9**: 2655 [PMID: 29985401 DOI: 10.1038/s41467-018-05184-7]

4 **Valdes AM**, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ* 2018; **361**: k2179 [PMID: 29899036 DOI: 10.1136/bmj.k2179]

5 **Zhang C**, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, Zhao G, Chen Y, Zhao L. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J* 2010; **4**: 232-241 [PMID: 19865183 DOI: 10.1038/ismej.2009.112]

6 **Kolodziejczyk AA**, Zheng D, Elinav E. Diet-microbiota interactions and personalized nutrition. *Nat Rev Microbiol* 2019; **17**: 742-753 [PMID: 31541197 DOI: 10.1038/s41579-019-0256-8]

7 **Mills S**, Stanton C, Lane JA, Smith GJ, Ross RP. Precision Nutrition and the Microbiome, Part I: Current State of the Science. *Nutrients* 2019; **11** [PMID: 31022973 DOI: 10.3390/nu11040923]

8 **Donovan SM**. Introduction to the special focus issue on the impact of diet on gut microbiota composition and function and future opportunities for nutritional modulation of the gut microbiome to improve human health. *Gut Microbes* 2017; **8**: 75-81 [PMID: 28282253 DOI: 10.1080/19490976.2017.1299309]

9 **McKenney PT**, Pamer EG. From Hype to Hope: The Gut Microbiota in Enteric Infectious Disease. *Cell* 2015; **163**: 1326-1332 [PMID: 26638069 DOI: 10.1016/j.cell.2015.11.032]

10 **Singh RK**, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017; **15**: 73 [PMID: 28388917 DOI: 10.1186/s12967-017-1175-y]

11 **Conlon MA**, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014; **7**: 17-44 [PMID: 25545101 DOI: 10.3390/nu7010017]

12 **Li YD**, Liu BN, Zhao SH, Zhou YL, Bai L, Liu EQ. Changes in gut microbiota composition and diversity associated with post-cholecystectomy diarrhea. *World J Gastroenterol* 2021; **27**: 391-403 [PMID: 33584071 DOI: 10.3748/wjg.v27.i5.391]

13 **Rothschild D**, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018; **555**: 210-215 [PMID: 29489753 DOI: 10.1038/nature25973]

14 **Clark A**, Mach N. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *J Int Soc Sports Nutr* 2016; **13**: 43 [PMID: 27924137 DOI: 10.1186/s12970-016-0155-6]

15 **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]

16 **Garn H**, Neves JF, Blumberg RS, Renz H. Effect of barrier microbes on organ-based inflammation. *J Allergy Clin Immunol* 2013; **131**: 1465-1478 [PMID: 23726530 DOI: 10.1016/j.jaci.2013.04.031]

17 **Vijay A**, Valdes AM. Role of the gut microbiome in chronic diseases: a narrative review. *Eur J Clin Nutr* 2022; **76**: 489-501 [PMID: 34584224 DOI: 10.1038/s41430-021-00991-6]

18 **Zheng L**, Wen XL. Gut microbiota and inflammatory bowel disease: The current status and perspectives. *World J Clin Cases* 2021; **9**: 321-333 [PMID: 33521100 DOI: 10.12998/wjcc.v9.i2.321]

19 **Xia F**, Wen LP, Ge BC, Li YX, Li FP, Zhou BJ. Gut microbiota as a target for prevention and treatment of type 2 diabetes: Mechanisms and dietary natural products. *World J Diabetes* 2021; **12**: 1146-1163 [PMID: 34512884 DOI: 10.4239/wjd.v12.i8.1146]

20 **Li WZ**, Stirling K, Yang JJ, Zhang L. Gut microbiota and diabetes: From correlation to causality and mechanism. *World J Diabetes* 2020; **11**: 293-308 [PMID: 32843932 DOI: 10.4239/wjd.v11.i7.293]

21 **LaCourse KD**, Johnston CD, Bullman S. The relationship between gastrointestinal cancers and the microbiota. *Lancet Gastroenterol Hepatol* 2021; **6**: 498-509 [PMID: 33743198 DOI: 10.1016/S2468-1253(20)30362-9]

22 **Dogra SK**, Doré J, Damak S. Gut Microbiota Resilience: Definition, Link to Health and Strategies for Intervention. *Front Microbiol* 2020; **11**: 572921 [PMID: 33042082 DOI: 10.3389/fmicb.2020.572921]

23 **Voigt RM**, Bishehsari F. The intestinal microbiota: determinants of resiliency? *Lancet Healthy Longev* 2021; **2**: e2-e3 [DOI: 10.1016/s2666-7568(20)30048-9]

24 **Yuan X**, Chang C, Chen X, Li K. Emerging trends and focus of human gastrointestinal microbiome research from 2010-2021: a visualized study. *J Transl Med* 2021; **19**: 327 [PMID: 34332587 DOI: 10.1186/s12967-021-03009-8]

25 **Zhu X**, Hu J, Deng S, Tan Y, Qiu C, Zhang M, Ni X, Lu H, Wang Z, Li L, Luo Y, Huang S, Xiao T, Liu S, Li X, Shang D, Wen Y. Comprehensive Bibliometric Analysis of the Kynurenine Pathway in Mood Disorders: Focus on Gut Microbiota Research. *Front Pharmacol* 2021; **12**: 687757 [PMID: 34239441 DOI: 10.3389/fphar.2021.687757]

26 **Xavier-Santos D**, Padilha M, Fabiano GA, Vinderola G, Gomes Cruz A, Sivieri K, Costa Antunes AE. Evidences and perspectives of the use of probiotics, prebiotics, synbiotics, and postbiotics as adjuvants for prevention and treatment of COVID-19: A bibliometric analysis and systematic review. *Trends Food Sci Technol* 2022; **120**: 174-192 [PMID: 35002079 DOI: 10.1016/j.tifs.2021.12.033]

27 **Yue YY**, Fan XY, Zhang Q, Lu YP, Wu S, Wang S, Yu M, Cui CW, Sun ZR. Bibliometric analysis of subject trends and knowledge structures of gut microbiota. *World J Clin Cases* 2020; **8**: 2817-2832 [PMID: 32742991 DOI: 10.12998/wjcc.v8.i13.2817]

28 **Zyoud SH**, Smale S, Waring WS, Sweileh W, Al-Jabi SW. Global research trends in the microbiome related to irritable bowel syndrome: A bibliometric and visualized study. *World J Gastroenterol* 2021; **27**: 1341-1353 [PMID: 33833487 DOI: 10.3748/wjg.v27.i13.1341]

29 **Sweileh WM**. Bibliometric analysis of peer-reviewed literature on antimicrobial stewardship from 1990 to 2019. *Global Health* 2021; **17**: 1 [PMID: 33397377 DOI: 10.1186/s12992-020-00651-7]

30 **Sweileh WM**. Bibliometric analysis of global scientific literature on vaccine hesitancy in peer-reviewed journals (1990-2019). *BMC Public Health* 2020; **20**: 1252 [PMID: 32807154 DOI: 10.1186/s12889-020-09368-z]

31 **Falagas ME**, Pitsouni EI, Malietzis GA, Pappas G. Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. *FASEB J* 2008; **22**: 338-342 [PMID: 17884971 DOI: 10.1096/fj.07-9492LSF]

32 **Santos BS**, Silva I, Lima L, Endo PT, Alves G, Ribeiro-Dantas MDC. Discovering temporal scientometric knowledge in COVID-19 scholarly production. *Scientometrics* 2022; **127**: 1609-1642 [PMID: 35068619 DOI: 10.1007/s11192-021-04260-y]

33 **Sweileh WM**. Global research activity on antimicrobial resistance in food-producing animals. *Arch Public Health* 2021; **79**: 49 [PMID: 33849636 DOI: 10.1186/s13690-021-00572-w]

34 **Sweileh WM**. Global research publications on systemic use of off-label and unlicensed drugs: A bibliometric analysis (1990-2020). *Int J Risk Saf Med* 2022; **33**: 77-89 [PMID: 34275912 DOI: 10.3233/JRS-210012]

35 **Sweileh WM**. Global Research Activity on Elder Abuse: A Bibliometric Analysis (1950-2017). *J Immigr Minor Health* 2021; **23**: 79-87 [PMID: 32488667 DOI: 10.1007/s10903-020-01034-1]

36 **Baishideng Publishing Group Inc**. Reference Citation Analysis. 2022. [cited 29 April 2022]. Available from: https://www.referencecitationanalysis.com/

37 **van Eck NJ**, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 2010; **84**: 523-538 [PMID: 20585380 DOI: 10.1007/s11192-009-0146-3]

38 **Schmidt C**. Mental health: thinking from the gut. *Nature* 2015; **518**: S12-S15 [PMID: 25715275 DOI: 10.1038/518S13a]

39 **Wang H**, Yang X. China's contribution to gut microbiota research. *Arch Community Med Public Health* 2020; **6**: 049-052

40 **Olesen SW**, Panchal P, Chen J, Budree S, Osman M. Global disparities in faecal microbiota transplantation research. *Lancet Gastroenterol Hepatol* 2020; **5**: 241 [PMID: 32061326 DOI: 10.1016/S2468-1253(19)30452-2]

41 **Zhang B**, Wang Y, Tan Z, Li Z, Jiao Z, Huang Q. Screening of Probiotic Activities of Lactobacilli Strains Isolated from Traditional Tibetan Qula, A Raw Yak Milk Cheese. *Asian-Australas J Anim Sci* 2016; **29**: 1490-1499 [PMID: 26954218 DOI: 10.5713/ajas.15.0849]

42 **National Natural Science Foundation of China**. National Natural Science Fund Guide to Programs 2020. [cited 15 January 2022]. Available from: https://www.nsfc.gov.cn/english/site_1/pdf/NationalNaturalScienceFundGuidetoPrograms2020.pdf

43 **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]

44 **Kim KA**, Gu W, Lee IA, Joh EH, Kim DH. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice *via* the TLR4 signaling pathway. *PLoS One* 2012; **7**: e47713 [PMID: 23091640 DOI: 10.1371/journal.pone.0047713]

45 **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]

46 **Rinninella E**, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. What is the Healthy Gut Microbiota Composition? *Microorganisms* 2019; **7** [PMID: 30634578 DOI: 10.3390/microorganisms7010014]

47 **Wu WC**, Inui A, Chen CY. Weight loss induced by whole grain-rich diet is through a gut microbiota-independent mechanism. *World J Diabetes* 2020; **11**: 26-32 [PMID: 32064033 DOI: 10.4239/wjd.v11.i2.26]

48 **Murphy EA**, Velazquez KT, Herbert KM. Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care* 2015; **18**: 515-520 [PMID: 26154278 DOI: 10.1097/MCO.0000000000000209]

49 **He C**, Cheng D, Peng C, Li Y, Zhu Y, Lu N. High-Fat Diet Induces Dysbiosis of Gastric Microbiota Prior to Gut Microbiota in Association With Metabolic Disorders in Mice. *Front Microbiol* 2018; **9**: 639 [PMID: 29686654 DOI: 10.3389/fmicb.2018.00639]

50 **O'Donovan AN**, Herisson FM, Fouhy F, Ryan PM, Whelan D, Johnson CN, Cluzel G, Ross RP, Stanton C, Caplice NM. Gut microbiome of a porcine model of metabolic syndrome and HF-pEF. *Am J Physiol Heart Circ Physiol* 2020; **318**: H590-H603 [PMID: 32031871 DOI: 10.1152/ajpheart.00512.2019]

51 **Velasquez MT**. Altered Gut Microbiota: A Link Between Diet and the Metabolic Syndrome. *Metab Syndr Relat Disord* 2018; **16**: 321-328 [PMID: 29957105 DOI: 10.1089/met.2017.0163]

52 **Guevara-Cruz M**, Flores-López AG, Aguilar-López M, Sánchez-Tapia M, Medina-Vera I, Díaz D, Tovar AR, Torres N. Improvement of Lipoprotein Profile and Metabolic Endotoxemia by a Lifestyle Intervention That Modifies the Gut

Microbiota in Subjects With Metabolic Syndrome. *J Am Heart Assoc* 2019; **8**: e012401 [PMID: 31451009 DOI: 10.1161/JAHA.119.012401]

53 **Cândido FG**, Valente FX, Grześkowiak ŁM, Moreira APB, Rocha DMUP, Alfenas RCG. Impact of dietary fat on gut microbiota and low-grade systemic inflammation: mechanisms and clinical implications on obesity. *Int J Food Sci Nutr* 2018; **69**: 125-143 [PMID: 28675945 DOI: 10.1080/09637486.2017.1343286]

54 **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]

55 **Yoo W**, Zieba JK, Foegeding NJ, Torres TP, Shelton CD, Shealy NG, Byndloss AJ, Cevallos SA, Gertz E, Tiffany CR, Thomas JD, Litvak Y, Nguyen H, Olsan EE, Bennett BJ, Rathmell JC, Major AS, Bäumler AJ, Byndloss MX. High-fat diet-induced colonocyte dysfunction escalates microbiota-derived trimethylamine *N*-oxide. *Science* 2021; **373**: 813-818 [PMID: 34385401 DOI: 10.1126/science.aba3683]

56 **David LA**, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559-563 [PMID: 24336217 DOI: 10.1038/nature12820]

57 **Koeth RA**, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013; **19**: 576-585 [PMID: 23563705 DOI: 10.1038/nm.3145]

58 **den Besten G**, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**: 2325-2340 [PMID: 23821742 DOI: 10.1194/jlr.R036012]

59 **Claesson MJ**, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488**: 178-184 [PMID: 22797518 DOI: 10.1038/nature11319]

60 **Trompette A**, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, Marsland BJ. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014; **20**: 159-166 [PMID: 24390308 DOI: 10.1038/nm.3444]

61 **Flint HJ**, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 577-589 [PMID: 22945443 DOI: 10.1038/nrgastro.2012.156]

62 **Desai MS**, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Núñez G, Martens EC. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* 2016; **167**: 1339-1353.e21 [PMID: 27863247 DOI: 10.1016/j.cell.2016.10.043]

63 **Sonnenburg JL**, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature* 2016; **535**: 56-64 [PMID: 27383980 DOI: 10.1038/nature18846]

64 **Chassaing B**, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, Gewirtz AT. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 2015; **519**: 92-96 [PMID: 25731162 DOI: 10.1038/nature14232]

65 **Dao MC**, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyles L; MICRO-Obes Consortium, Dumas ME, Rizkalla SW, Doré J, Cani PD, Clément K. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 2016; **65**: 426-436 [PMID: 26100928 DOI: 10.1136/gutjnl-2014-308778]

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*CASE REPORT*

# Early gastric cancer presenting as a typical submucosal tumor cured by endoscopic submucosal dissection: A case report

Joon Hyun Cho, Si Hyung Lee

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**Peer-review model:** Single blind

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## Abstract

*BACKGROUND*

Submucosal tumor (SMT)-like gastric cancer is rare, and almost all cases undergo curative surgical treatment because the submucosal layer is usually deeply invaded by tumor cells or because histopathologic types of SMT-like gastric cancer are undifferentiated or poorly differentiated. No report has been issued on an SMT-like gastric cancer cured by endoscopic resection alone or on changes in the endoscopic features of this type of tumor over several years.

*CASE SUMMARY*

We describe an exceptional case of a 53-year-old male with a 1.5 cm-sized SMT-like lesion covered by normal-appearing mucosa discovered by esophagogastroduodenoscopy (EGD) at the gastric antrum. Endoscopic ultrasound (EUS) visualized a homogeneous, well-circumscribed hypoechogenic lesion arising from the second sonographic layer with associated subtle obliteration of the third sonographic layer. Initial endoscopic biopsy was negative for neoplasm. The patient refused to undergo an invasive procedure and was subsequently lost to follow-up. Three years after initial detection, EGD revealed the lesion had become markedly erythematous, and at 4 years after initial EGD it had increased in size to 1.8 cm and developed a central ulcer and a heterogeneous EUS echo. Finally, endoscopic submucosal dissection (ESD) was performed, and histopathologic examination revealed a moderately differentiated adenocarcinoma had minutely invaded the submucosal layer (invasion depth 169 μm) but without lymphovascular invasion and with negative resection margins. Fortunately, no additional surgical treatment was required. He has been followed for 4 years after ESD without any evidence of local or distant recurrence.

*CONCLUSION*

This report describes an extremely rare case of early gastric cancer presenting as SMT that was cured by ESD after a treatment delay of 4 years and the endoscopic changes that occurred during this period. The report highlights the importance of

considering the possibility of gastric cancer when SMT is encountered in clinical practice.

**Key Words:** Subepithelial lesion; Submucosal tumor; Early gastric cancer; Adenocarcinoma; Endoscopic mucosal dissection; Case report

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**Core Tip:** We experienced an exceptionally rare case of early gastric cancer presenting as submucosal tumor (SMT) that was successfully treated by endoscopic submucosal dissection (ESD) alone, although the procedure was performed four years after first detection due to patient refusal and follow-up loss. The present case cautions that SMT-like gastric cancer should be included in the differential diagnosis when a hypoechogenic mass is detected in the 2nd or 3rd layer by endoscopic ultrasound, regardless of size and the absence of findings suggesting malignancy. When diagnosis is uncertain, invasive techniques such as diagnostic endoscopic mucosal resection or ESD, which can potentially be used for therapeutic purposes, should be considered and close follow-up is recommended.

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## INTRODUCTION

Gastric carcinoma is an epithelial tumor exposed to the mucosal surface that occurs in lamina propria with variable gross findings. However, cases of gastric carcinoma with features of submucosal tumor (SMT) are rarely encountered in routine clinical settings and reportedly account for only 0.2% to 0.62% of all resected gastric cancers[1]. The majority of patients with gastric adenocarcinoma resembling SMT are not indicated for endoscopic resection or offered non-curative resection after endoscopic resection. Submucosal layer is deeply invaded by tumor cells, even in cases of early gastric cancer (EGC)[2], and histopathologically, most adenocarcinomas presenting as SMTs are undifferentiated or poorly differentiated[3,4], and as a result, almost all undergo curative surgical treatment. Accordingly, no case has been reported on SMT-like gastric cancer cured by endoscopic resection alone. In addition, no report has been issued on changes in the endoscopic features of this type of tumor over several years. Here, we report an exceptionally rare case of EGC presenting as SMT that was cured by endoscopic submucosal dissection (ESD) and describe changes in the endoscopic features of this tumor over a period of 4 years.

## CASE PRESENTATION

### *Chief complaints*

A 53-year-old male Korean patient was referred to our institution for further evaluation and treatment of a gastric SMT discovered by esophagogastroduodenoscopy (EGD) during a routine medical check-up.

### *History of present illness*

The patient had no abdominal pain or related discomfort.

### *History of past illness*

He had a history of abdominal surgery due to duodenal ulcer perforation 30 years previously.

### *Personal and family history*

The patient had diabetes that was being treated with oral hypoglycemic agents. He was a smoker (30 pack-years) and social-alcohol drinker and had no significant family history.

### *Physical examination*

Physical examination was unremarkable, and his abdomen was soft, nontender, and nondistended with no palpable mass.

### *Laboratory examinations*

Laboratory tests, which included tests for common serum tumor markers such as CEA and CA 19-9, revealed no abnormalities.

### *Imaging examinations*

At initial EGD, an SMT-like elevated lesion of diameter 15-mm was observed at the great curvature side of the proximal part of the gastric antrum (Figure 1A). The lesion was covered with normal-appearing mucosa without any erosion, ulcer, or mucosal erythema. Mild-atrophic gastritis, confined to antrum, was observed in background mucosa. Endoscopic ultrasound (EUS; GF-UM2000, Olympus, Tokyo, Japan) demonstrated a 15 mm × 7 mm homogeneous, well-circumscribed hypoechogenic lesion arising from the second sonographic layer with associated subtle obliteration of the third sonographic layer (Figure 2A). The EUS appearance of the lesion was suggestive of a gastric neuroendocrine tumor (NET) or ectopic pancreas. Initial biopsy specimens were negative for neoplasm. Computed tomography (CT) of the abdomen showed a 15 mm protruding intraluminal mass at the gastric antrum and no evidence of lymph node enlargement or distant metastasis (Figure 3). After this initial work-up, endoscopic resection of the lesion was planned for definitive histopathologic examination and treatment. However, the patient declined any invasive procedure and was subsequently lost to follow-up.

### *Further diagnostic work-up*

About 3 years after initial EGD, he underwent a national health screening examination at our hospital, and the previously noted gastric SMT was detected again by EGD. The lesion showed no change in size as compared with its size 3 years previously, but marked erythema of overlying mucosa was observed (Figure 1B). Endoscopic biopsy was performed, but specimens were negative for neoplasm. During consultation regarding his health examination results, he was recommended for further evaluation and treatment at the gastroenterology department but again refused and was lost to follow-up.

About a year later, he revisited our hospital with mild indigestion. Follow-up EGD then revealed the SMT-like lesion had enlarged (to a greatest diameter of approximately 18 mm) and that a 5 mm central ulcer had developed on the top of the lesion (Figure 1C). EUS also demonstrated an 18 mm × 9 mm homogeneous, well-circumscribed hypoechogenic mass arising from the second sonographic layer with associated subtle obliteration of the third sonographic layer (Figure 2B). Echogenicity at this time was slightly more heterogeneous than that observed by initial EUS. CT of the abdomen showed no evidence of lymph node enlargement or distant metastasis, and endoscopic biopsy specimens taken from the lesion revealed tubular adenoma with high-grade dysplasia.

## FINAL DIAGNOSIS

The patient was then admitted for further endoscopic treatment. A serum anti-*Helicobacter pylori* (*H. pylori*) IgG assay was negative on the day of endoscopic examination. ESD of the lesion was performed, and histopathologic examination of the resected specimen revealed moderately differentiated adenocarcinoma that invaded the submucosal layer (depth of invasion 169 μm) with no lymphovascular invasion (Figure 4).

## TREATMENT

Furthermore, resection margins were all negative for cancer. Also, rapid urease test and Giemsa staining were negative for *H. pylori* infection.

## OUTCOME AND FOLLOW-UP

His post-ESD course was uneventful, and he has since been followed up at our outpatient department for 4 years without any evidence of local or distant recurrence. The latest follow-up EGD performed (approximately 3.5 years after ESD) showed only post-ESD scarring (Figure 1D).

## DISCUSSION

This case report is meaningful for the following reasons. First, to the best of our knowledge, this is the first case report of SMT-like gastric cancer cured by ESD alone, and this result was obtained even though ESD was performed four years after initial detection. Second, this case report describes the natural course of SMT-like gastric cancer and the endoscopic changes that occurred after a treatment

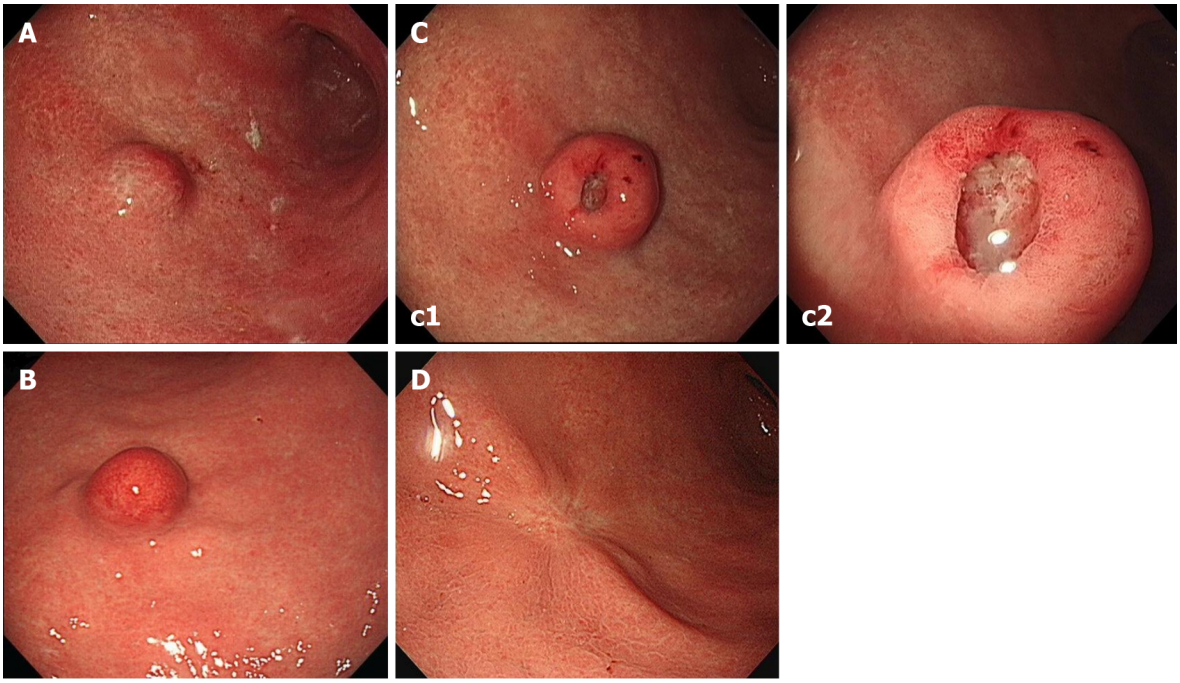


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**Figure 1 Endoscopy images.** A: Initial endoscopic image showing a 15 mm-sized submucosal tumor-like elevated lesion with normally appearing mucosa at the great curvature side of the proximal part of gastric antrum; B: Endoscopic image obtained 3 years later showing the tumor had not changed in size but that marked erythema had developed on overlying mucosa; C: Endoscopic image (c2 shows a higher magnification image of the mass) obtained 4 years after initial examination [immediately before endoscopic submucosal dissection (ESD)] showing the tumor had increased in size to 18 mm and developed a 5-mm-sized central ulcer and overlying reddish mucosa of fine granularity; D: Endoscopic image obtained approximately 2.5 years after ESD showing post-ESD scarring.

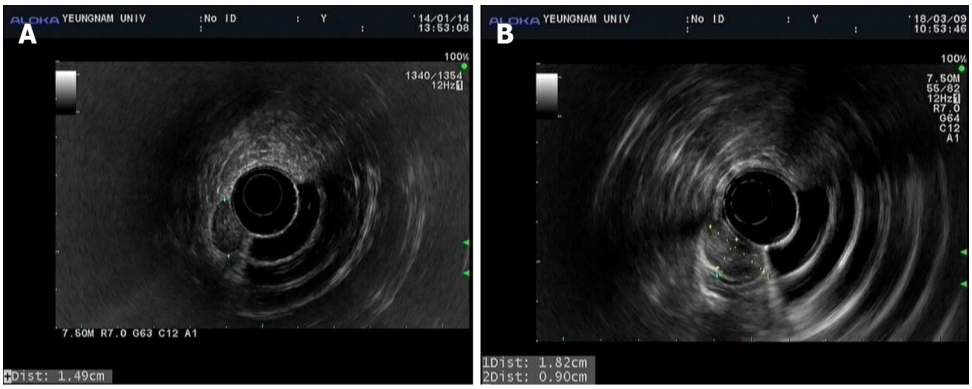


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**Figure 2 Endoscopic ultrasound images.** A: Initial endoscopic ultrasound (EUS) image showing a well-circumscribed homogeneous and hypoechogenic mass measuring about 15 mm × 7 mm originating from the second sonographic layer with associated subtle obliteration of the third sonographic layer; B: EUS image obtained 4 years after the initial examination showing a well-circumscribed, hypoechogenic mass of greater size (18 mm × 9 mm) with slightly more heterogeneous echogenicity, and that the mass originated from the second sonographic layer with associated subtle obliteration of the third sonographic layer.

delay of 4 years. Furthermore, unlike previous reports, mucosa overlying the SMT appeared completely normal when the tumor was first detected.

Gastric cancer usually derives from the lamina propria layer and has macroscopic appearances ranging from well-defined protuberant to diffuse infiltrating. Approximately 95% of gastric cancers are adenocarcinomas[5] but only rarely appear in the form of SMT. According to the literature[2], fewer than 0.5% of gastric cancer cases present as SMT. However, when an SMT-like lesion is encountered in clinical practice, the possibility of gastric cancer should be carefully considered and the lesion differentiated from other submucosal lesions, such as gastric NET[6], leiomyoma, lymphoma, gastrointestinal stromal tumor (GIST), lipoma, ectopic pancreas, and other unusual manifestations, such as metastatic carcinoma[7] and gastric glomus tumor[8], which require completely different treatment strategies.

Pathologic diagnoses of reported cases of gastric cancer presenting as SMT include gastric adenocarcinoma[9], gastric mucinous adenocarcinoma[10,11], and gastric lymphoepithelioma-like carcinoma

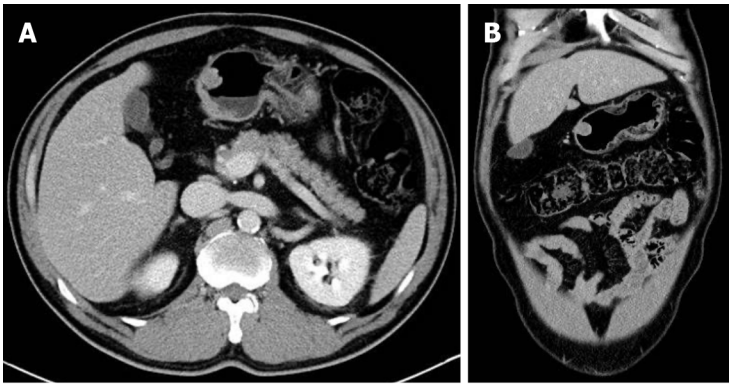


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**Figure 3 Abdominal computed tomography images.** Initial axial (A) and coronal (B) computed tomography images showing a 15 mm protruding intraluminal mass at gastric antrum.

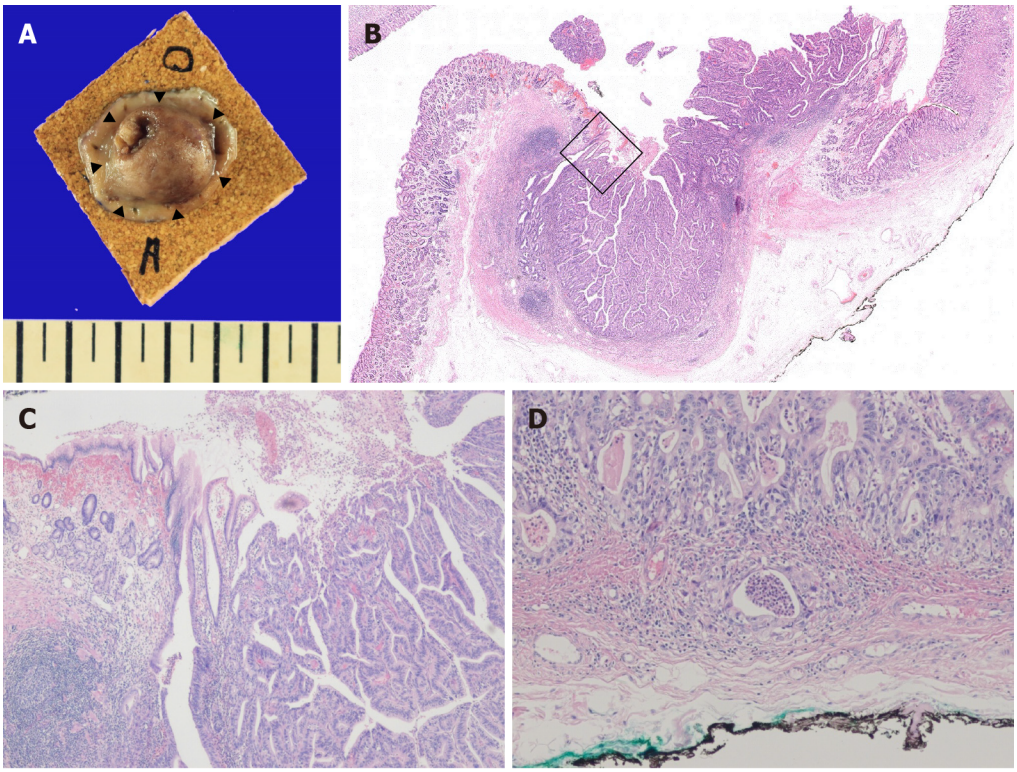


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**Figure 4 Pathology of the endoscopically resected lesion.** A: Gross appearance of the resected lesion, which had negative lateral margins. The black triangles indicate the border of the cancerous region; B: Hematoxylin & eosin stained section showing gastric wall mucosal layer elevation by the adenocarcinoma, central erosion, and exposed tumor cells (original magnification × 10); C: Microscopic finding of the boxed area in Figure B showing the periphery of the tumor mass, which demonstrated moderately differentiated adenocarcinoma (original magnification × 40); D: Microscopic finding of the vertical resection margin showing penetration of the muscularis mucosa and minute invasion of submucosa (depth of invasion 169 μm) by tumor cells (original magnification × 100).

[12]. The most common histopathologic type is undifferentiated to poorly differentiated adenocarcinoma, which constitutes 68.8% to 71.4% of these cancers[3,4]. The mechanism of SMT-like gastric cancer development is obscure, but several pathologic mechanisms have been suggested. These mechanisms include the biologic tendencies of poorly differentiated adenocarcinomas to exhibit invasion of a deeper layer at an early stage, marked lymphocyte infiltration around tumors[1], the production of large amounts of mucin by mucinous adenocarcinoma[10,11], substantial amounts of surrounding fibrosis due to repetitive inflammation, abundant edematous fibrosis[13], and adenocarcinomas arising from a submucosal heterotopic gastric gland[9,14], which have been recognized as aberrant lamina propria components associated with repeated erosion and regeneration. These factors may facilitate a predominance of submucosal growth and penetration of muscularis mucosa during the early stage of carcinogenesis and contribute to a macroscopic appearance indistinguishable from SMT.

However, the tumor in our case had a moderately differentiated histology, which was not in line with any of these mechanisms.

Although the diagnosis of SMT-like gastric cancer is usually difficult due to a deep tumor location and non-specific and overlapping imaging features, some EGD characteristics of SMT-like gastric cancer have been reported. In particular, SMT with central ulceration or depression has been described as common for SMT-like gastric cancer[10,14-16]. Erythematous surface change is another reported characteristic[14,15]. Fujiyoshi *et al*[16] concluded that a small SMT (3-5 cm) with a central ulcer or irregular erythematous change should raise suspicion of malignancy. However, since our case originally appeared as SMT with completely normal overlying mucosa, we suspected SMT originating from the second or third layer rather than cancer. However, EGD at 3 and 4 years after initial detection of the SMT-like lesion, showed erythematous change and central ulceration. Interestingly, definite changes were observed in the endoscopic features of the tumor 4 years after initial detection, but it was not possible to determine when these morphologic changes occurred precisely due to follow-up loss.

Histological diagnosis of gastric SMT by endoscopic biopsy is often difficult and detailed imaging usually fails to provide sufficient evidence to differentiate benign and malignant tumors. Furthermore, endoscopic biopsy specimens, even those taken from a central ulcer, may be unhelpful[17], and when a tumor is completely covered with normal mucosa, it is extremely difficult to obtain an adequate sample of the underlying lesion. In the present case, negative results for neoplasm were reported for biopsies performed during EGD on overlying normal mucosa at initial presentation and on overlying erythematous mucosa 3 years later.

EUS is useful for evaluating gastric SMT. Findings that suggest malignant SMT include[18] a size > 3 cm or > 5 cm, rapid growth, heterogeneous echogenicity, and irregular margins, whereas GIST or leiomyoma may present as a homogeneous, well-demarcated, submucosal mass with smooth margins. However, these imaging characteristics are non-specific, and EUS images alone are insufficient for accurate diagnosis. More invasive techniques such as EUS-guided fine-needle aspiration or biopsy and endoscopic mucosal resection may aid differential diagnoses[18]. In our patient, initial EUS depicted a 15 mm-sized well-circumscribed homogeneous mass, which did not suggest malignant SMT. However, EUS performed 4 years later showed the mass had increased in size to 18 mm and had slightly more heterogeneous echogenicity.

Gastric cancers resembling SMT are characterized by a predominance of submucosal or sometimes deeper invasion into the gastric wall[2], which suggests they are likely to be more advanced and pose a greater risk of metastasis than ordinary gastric cancers of similar size[13]. Furthermore, most adenocarcinomas presenting as SMTs are of the undifferentiated or poorly differentiated histopathologic types[3,4], and thus, in almost all case reports, SMT-like gastric cancer, even when small, has been treated in the same way as advanced gastric cancer. Although ESD can potentially be used for therapeutic purposes, the pathologic results of most specimens resected by ESD indicate non-curative resection, and thus, additional gastrectomy with lymphadenectomy is required[19]. To date, no case report of cure by endoscopic resection has been published in the English literature. However, in the present case, ESD was performed to provide a definitive histopathologic diagnosis and a treatment strategy, and additional surgery was not needed, based on a final pathologic report that the tumor was not poorly differentiated, did not invade deep submucosa, and had negative lateral and deep margins.

## CONCLUSION

The present case emphasizes that although SMT-like gastric cancer is rare, it should be included in the differential diagnosis when a hypoechogenic mass is visualized in the 2nd or 3rd layer by EUS, regardless of lesion size and the absence of findings suggesting malignancy. In addition, if diagnosis is uncertain, the use of techniques more aggressive than EUS alone, such as diagnostic endoscopic mucosal resection or ESD, which can potentially be used for therapeutic purposes, should be considered and close follow-up is recommended.

## FOOTNOTES

**Author contributions:** Cho JH and Lee SH were responsible for the design of this report, the acquisition of clinical data, and writing and revision of the manuscript.

**Informed consent statement:** Informed written consent was obtained from the patient regarding the publication of this report and accompanying images.

**Conflict-of-interest statement:** The authors have no conflict of interest to declare.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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## REFERENCES

1 **Umehara Y**, Kimura T, Okubo T, Sano Y, Nakai K, Oi S, Higashi Y, Funai K. Gastric carcinoma resembling submucosal tumor. *Gastric Cancer* 1999; **2**: 191-193 [PMID: 11957095 DOI: 10.1007/s101200050045]
2 **Ishiguro S**, Tsukamoto Y, Kasugai T, Yasuhara Y, Tsuji N, Fujii Y, Nishizawa Y, Yamato N. Characteristic feature of the gastric carcinoma with submucosal tumor like appearance. *Stomach Intest* 2003; **38**: 1519-1526
3 **Takemoto N**, Baba T. Kaku Y, Takekoshi T, Maruyama M, Takahashi T, Kato H, Yanagisawa A. Radiologic diagnosis of gastric cancer morphologically mimicking submucosal tumor. *Stomach Intest* 1995; **30**: 759-768 [DOI: 10.11641/pdensks.53.0_156]
4 **Mitsunaga A**, Futami S, Murata Y, Suzuki A. Uchida KJ, Nemoto Y, Ikeda I, Nakamura S, Murata Y, Suzuki H. Endoscopic diagnosis of the gastric cancer showing the features of a submucosal tumor. *tomach Intest* 1995; **30**: 769-776 [DOI: 10.1055/s-2007-1001465]
5 **Nagini S**. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. *World J Gastrointest Oncol* 2012; **4**: 156-169 [PMID: 22844547 DOI: 10.4251/wjgo.v4.i7.156]
6 **Gheorghe AV**, Rimbas M, Ginghina O, Spanu A, Voiosu TA. An atypical type I gastric neuroendocrine tumor. *Rom J Intern Med* 2017; **55**: 253-256 [PMID: 28710883 DOI: 10.1515/rjim-2017-0029]
7 **Inagaki C**, Suzuki T, Kitagawa Y, Hara T, Yamaguchi T. A case report of prostate cancer metastasis to the stomach resembling undifferentiated-type early gastric cancer. *BMC Gastroenterol* 2017; **17**: 93 [PMID: 28784100 DOI: 10.1186/s12876-017-0655-0]
8 **Handa Y**, Kano M, Kaneko M, Hirabayashi N. Gastric Glomus Tumor: A Rare Cause of Upper Gastrointestinal Bleeding. *Case Rep Surg* 2015; **2015**: 193684 [PMID: 26697255 DOI: 10.1155/2015/193684]
9 **Manabe S**, Mukaisho KI, Yasuoka T, Usui F, Matsuyama T, Hirata I, Boku Y, Takahashi S. Gastric adenocarcinoma of fundic gland type spreading to heterotopic gastric glands. *World J Gastroenterol* 2017; **23**: 7047-7053 [PMID: 29097877 DOI: 10.3748/wjg.v23.i38.7047]
10 **Yu BC**, Lee WK. Two cases of mucinous adenocarcinoma of the stomach mistaken as submucosal tumor. *J Korean Surg Soc* 2013; **84**: 118-122 [PMID: 23396274 DOI: 10.4174/jkss.2013.84.2.118]
11 **Yoo CH**, Park SJ, Park MI, Moon W, Kim HH, Lee JS, Song JY, Jang HK. Submucosal tumor-like early-stage mucinous gastric carcinoma: a case study. *Korean J Gastroenterol* 2013; **62**: 122-125 [PMID: 23981947 DOI: 10.4166/kjg.2013.62.2.122]
12 **Takahashi T**, Otani Y, Yoshida M, Furukawa T, Kameyama K, Akiba Y, Saikawa Y, Kubota T, Kumai K, Kuramochi S, Mukai M, Ishii H, Kitajima M. Gastric cancer mimicking a submucosal tumor diagnosed by laparoscopic excision biopsy. *J Laparoendosc Adv Surg Tech A* 2005; **15**: 51-56 [PMID: 15772477 DOI: 10.1089/Lap.2005.15.51]
13 **Ohara N**, Tominaga O, Uchiyama M, Nakano H. A case of advanced gastric cancer resembling submucosal tumor of the stomach. *Jpn J Clin Oncol* 1997; **27**: 423-426 [PMID: 9438007 DOI: 10.1093/jjco/27.6.423]
14 **Hashimoto R**, Hamamoto H, Omori Y, Tanuma T. Early gastric cancer on submucosal heterotopic gastric glands. *Gastrointest Endosc* 2017; **85**: 851-852 [PMID: 27179691 DOI: 10.1016/j.gie.2016.05.002]
15 **Teraishi F**, Uno F, Kagawa S, Fujiwara T, Gouchi A, Tanaka N. Advanced gastric adenocarcinoma mimicking a submucosal tumor. *Endoscopy* 2007; **39** Suppl 1: E191-E192 [PMID: 17614044 DOI: 10.1055/s-2007-966403]
16 **Fujiyoshi A**, Kawamura M, Ishitsuka S. Gastric adenocarcinoma mimicking a submucosal tumor: case report. *Gastrointest Endosc* 2003; **58**: 633-635 [PMID: 14560759]
17 **Tio TL**, Tytgat GN, den Hartog Jager FC. Endoscopic ultrasonography for the evaluation of smooth muscle tumors in the upper gastrointestinal tract: an experience with 42 cases. *Gastrointest Endosc* 1990; **36**: 342-350 [PMID: 2210274 DOI: 10.1016/s0016-5107(90)71061-9]
18 **Papanikolaou IS**, Triantafyllou K, Kourikou A, Rösch T. Endoscopic ultrasonography for gastric submucosal lesions. *World J Gastrointest Endosc* 2011; **3**: 86-94 [PMID: 21772939 DOI: 10.4253/wjge.v3.i5.86]
19 **Kim HI**, Shim KN, Yoon SY, Song EM, Cho WY, Kim SE, Jung HK, Jung SA. A case of early gastric adenocarcinoma resembling subepithelial tumor. *Korean J Helicobacter Up Gastrointest Res* 2013; **13**: 60-63 [DOI: 10.7704/kjhugr.2013.13.1.60]

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LETTER TO THE EDITOR

# Acupuncture and moxibustion for treatment of Crohn's disease: A brief review

Jing Xie, Yan Huang, Huan-Gan Wu, Jing Li

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**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

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Grade C (Good): C
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## Abstract

Crohn's disease (CD) is a kind of intestinal inflammatory disease that can affect any part of the gastrointestinal tract and the incidence rate of CD is gradually increasing worldwide. Acupuncture and moxibustion have unique curative effects on gastrointestinal diseases and can be new options for the treatment of CD.

**Key Words:** Crohn's disease; Acupuncture; Moxibustion; Treatment; Review

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**Core Tip:** Crohn's disease (CD) can affect any part of the gastrointestinal tract, especially the terminal ileum and the ascending colon, and is collectively known as inflammatory bowel disease with ulcerative colitis. The incidence rate of CD is gradually increasing worldwide. This letter mainly introduces that acupuncture has a unique curative effect on CD and can provide a new research direction for the treatment of CD.

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## TO THE EDITOR

Crohn's disease (CD) can affect any part of the gastrointestinal tract, especially the terminal ileum and the ascending colon, and is collectively known as inflammatory bowel disease (IBD) with ulcerative colitis. The main symptoms include abdominal pain, diarrhea, fistula, intestinal obstruction, fever, emaciation, and nutritional disorders. In the past three decades, the incidence rate of CD has gradually increased worldwide at an annual growth rate between 4%-15%, which warrants our attention[1]. Currently, biological therapy is the preferred treatment for CD, and the commonly used biological agents such as infliximab and adalimumab have brought significant benefits to the patients. With multiple biological agents at their disposal, clinicians can, according to different symptoms, choose drugs with different anti-inflammatory mechanisms. However, despite the undisputed efficacy of these agents, a significant proportion of patients fail to receive effective treatments. For example, many patients do not respond to induction therapy or lose the response after initial improvements. As traditional Chinese medicine therapies, acupuncture and moxibustion have a long history in treating gastrointestinal diseases and significant advantages against symptoms such as abdominal pain, diarrhea, fatigue, and anorexia. Therefore, we summarize articles about clinical acupuncture and moxibustion treatment for CD to explore their unique advantages.

Using keywords such as acupuncture, CD, and clinical trials, we searched PubMed for clinical trials of acupuncture and moxibustion in the treatment of CD up to February 28, 2020, and found four articles that met the criteria. The four articles that we found and retrieved showed that acupuncture and moxibustion had curative effects on CD. Joos *et al*'s randomized controlled trial confirmed that after 4 wk of treatment, the CD activity index (CDAI) of the acupuncture group showed a significantly larger ( $P = 0.003$) decrease (from 250 ± 51 to 163 ± 56) than that of the groups without acupuncture (from 220 ± 42 to 181 ± 46)[2]. Bao *et al*'s 12-wk trial with the treatment group undergoing herb-partitioned moxibustion combined with acupuncture (HMA) and the control group receiving wheat bran partitioned moxibustion combined with superficial acupuncture showed a significantly higher ($P = 0.000$) total treatment efficacy of the treatment group at 83.72% compared to 40.48% of the control group and a significantly lower ($P = 0.000$) CDAI in the treatment group than that of the control group[3]. Both clinical trials demonstrated that acupuncture combined with moxibustion significantly reduced the CDAI in the patients and significantly improved their quality of life. Shang *et al*[4] conducted a clinical trial with one group receiving HMA and the other group treated with mesalazine (MESA). After 12 wk of treatment, the expression of *ZO-1* mRNA in the HMA group was significantly improved compared with the MESA group (2378.17 ± 308.77 *vs* 2200.56 ± 281.88; $P = 0.023$), confirming that HMA can repair the intestinal epithelial barrier by up-regulating the expression of tight junction protein and its mRNA, thus reducing the intestinal inflammatory response in CD patients. Horta *et al*[5] investigated whether electroacupuncture (EAc) therapy could relieve fatigue in patients with IBD by allocating 52 patients into the EAc, sham EAc (ShEAc), and waitlist (WL) groups. Evaluation with the IBD-validated Functional Assessment of Chronic Illness Therapy-Fatigue Scale showed reduced fatigue scores in patients of the EAc and ShEAc groups after 8 wk of treatment and significantly better curative effects in the two groups than that in the WL group.

According to these four clinical trials, acupuncture treatment tends to attenuate symptoms such as abdominal pain, diarrhea, and fatigue in patients with CD. Therefore, acupuncture and moxibustion therapy may potentially improve the physical and mental health of CD patients. Unfortunately, clinical trials of acupuncture for CD with relatively long-term observation and a large sample are still lacking. Except those conducted by Chinese researchers, there are few studies on treating CD with acupuncture, and more evidence is required to support the application of acupuncture and moxibustion therapies in treating CD and verify their potential benefits.

## FOOTNOTES

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## REFERENCES

1 **Adamina M**, Bonovas S, Raine T, Spinelli A, Warusavitarne J, Armuzzi A, Bachmann O, Bager P, Biancone L, Bokemeyer B, Bossuyt P, Burisch J, Collins P, Doherty G, El-Hussuna A, Ellul P, Fiorino G, Frei-Lanter C, Furfaro F, Gingert C, Gionchetti P, Gisbert JP, Gomollon F, González Lorenzo M, Gordon H, Hlavaty T, Juillerat P, Katsanos K, Kopylov U, Krustins E, Kucharzik T, Lytras T, Maaser C, Magro F, Marshall JK, Myrelid P, Pellino G, Rosa I, Sabino J, Savarino E, Stassen L, Torres J, Uzzan M, Vavricka S, Verstockt B, Zmora O. ECCO Guidelines on Therapeutics in Crohn's Disease: Surgical Treatment. *J Crohns Colitis* 2020; **14**: 155-168 [PMID: 31742338 DOI: 10.1093/ecco-jcc/jjz187]

2 **Joos S**, Brinkhaus B, Maluche C, Maupai N, Kohnen R, Kraehmer N, Hahn EG, Schuppan D. Acupuncture and moxibustion in the treatment of active Crohn's disease: a randomized controlled study. *Digestion* 2004; **69**: 131-139 [PMID: 15114043 DOI: 10.1159/000078151]

3 **Bao CH**, Zhao JM, Liu HR, Lu Y, Zhu YF, Shi Y, Weng ZJ, Feng H, Guan X, Li J, Chen WF, Wu LY, Jin XM, Dou CZ, Wu HG. Randomized controlled trial: moxibustion and acupuncture for the treatment of Crohn's disease. *World J Gastroenterol* 2014; **20**: 11000-11011 [PMID: 25152604 DOI: 10.3748/wjg.v20.i31.11000]

4 **Shang HX**, Wang AQ, Bao CH, Wu HG, Chen WF, Wu LY, Ji R, Zhao JM, Shi Y. Moxibustion combined with acupuncture increases tight junction protein expression in Crohn's disease patients. *World J Gastroenterol* 2015; **21**: 4986-4996 [PMID: 25945013 DOI: 10.3748/wjg.v21.i16.4986]

5 **Horta D**, Lira A, Sanchez-Lloansi M, Villoria A, Teggiachi M, García-Rojo D, García-Molina S, Figuerola A, Esteve M, Calvet X. A Prospective Pilot Randomized Study: Electroacupuncture vs. Sham Procedure for the Treatment of Fatigue in Patients With Quiescent Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2020; **26**: 484-492 [PMID: 31091322 DOI: 10.1093/ibd/izz091]

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CORRECTION

# Correction to "Aberrant methylation of secreted protein acidic and rich in cysteine gene and its significance in gastric cancer"

Shuai Shao, Nuo-Ming Zhou, Dong-Qiu Dai

**Specialty type:** Oncology

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

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## Abstract

We have replaced the misapplied images and the revised Figure 6C is provided.

**Key Words:** Correction

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**Core Tip:** This is a correction to "Aberrant methylation of secreted protein acidic and rich in cysteine gene and its significance in gastric cancer".

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## CORRECTION

Correction to: Shao S, Zhou NM, Dai DQ. Aberrant methylation of secreted protein acidic and rich in cysteine gene and its significance in gastric cancer. *World J Gastroenterol* 2019; 25: 6713-6727 PMID: 31857774 DOI: 10.3748/wjg.v25.i46.6713.

We are deeply regretful that we have misapplied one inappropriate image in this article. We have replaced the misapplied image and the corrected pictures are shown in Figure 1. This correction will have no influence on the interpretation of the entire results and conclusion in this study. We apologize for any inconvenience this may

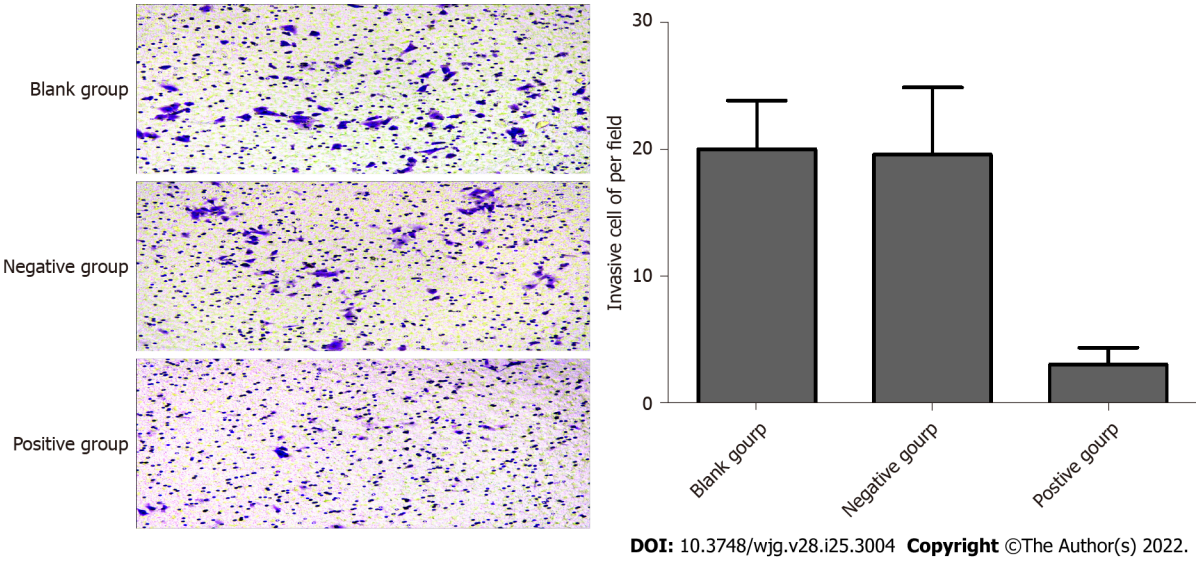


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**Figure 1 Modification of the Figure 6C in original manuscript.**

cause.

We have provided the correction document, and we would like to express our sincere apologies to the editors and readers for our mistakes. We are looking forward to hearing from you at your earliest convenience.

## FOOTNOTES

**Author contributions:** Shao S, Zhou NM and Dai DQ contributed to this paper.

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## REFERENCES

1 **Shao S**, Zhou NM, Dai DQ. Aberrant methylation of secreted protein acidic and rich in cysteine gene and its significance in gastric cancer. *World J Gastroenterol* 2019; **25**: 6713-6727 [PMID: 31857774 DOI: 10.3748/wjg.v25.i46.6713]

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CORRECTION

# Correction to "Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study"

Xia Liu, Yi-Wen Cheng, Li Shao, Shu-Hong Sun, Jian Wu, Qing-Hai Song, Hong-Sheng Zou, Zong-Xin Ling

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## Abstract

Liu X, Cheng YW, Shao L, Sun SH, Wu J, Song QH, Zou HS, Ling ZX. Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study. *World J Gastroenterol* 2021; 27(19): 2394-2414 [PMID: 34040330 DOI: 10.3748/wjg.v27.i19.2394]. In this article, the affiliation of the first author was wrong. Xia Liu, the first author, belonged to Department of Intensive Care Unit, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310003, China.

**Key Words:** Correction

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**Core Tip:** This manuscript is an author's affiliation correction for Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study. *World J Gastroenterol* 2021; 27: 2394-2414.

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## CORRECTION

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In this article[1], the affiliation of the first author was wrong. Xia Liu, the first author, belonged to Department of Intensive Care Unit, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310003, China.

## FOOTNOTES

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## REFERENCES

1 **Liu X**, Cheng YW, Shao L, Sun SH, Wu J, Song QH, Zou HS, Ling ZX. Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study. *World J Gastroenterol* 2021; **27**: 2394-2414 [PMID: 34040330 DOI: 10.3748/wjg.v27.i19.2394]

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