ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2022 March 15; 14(3): 547-747

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

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World Journal of Gastrointestinal Oncology

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NAME OF JOURNAL

World Journal of Gastrointestinal Oncology

ISSN

ISSN 1948-5204 (online)

LAUNCH DATE

February 15, 2009

FREQUENCY

Monthly

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Monjur Ahmed, Florin Burada

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PUBLICATION DATE

March 15, 2022

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https://www.wjgnet.com/bpg/gerinfo/242

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World Journal of Gastrointestinal Oncology

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World J Gastrointest Oncol 2022 March 15; 14(3): 547-567

DOI: 10.4251/wjgo.v14.i3.547

ISSN 1948-5204 (online)

REVIEW

Inflammatory bowel disease-related colorectal cancer: Past, present and future perspectives

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Sano W, Japan

Received: March 15, 2021
Peer-review started: March 15, 2021
First decision: April 6, 2021
Revised: April 21, 2021
Accepted: February 25, 2022
Article in press: February 25, 2022
Published online: March 15, 2022

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Abstract

Inflammatory bowel disease-related colorectal cancer (IBD-CRC) is one of the most serious complications of IBD contributing to significant mortality in this cohort of patients. IBD is often associated with diet and lifestyle-related gut microbial dysbiosis, the interaction of genetic and environmental factors, leading to chronic gut inflammation. According to the "common ground hypothesis", microbial dysbiosis and intestinal barrier impairment are at the core of the chronic inflammatory process associated with IBD-CRC. Among the many underlying factors known to increase the risk of IBD-CRC, perhaps the most important factor is chronic persistent inflammation. The persistent inflammation in the colon results in increased proliferation of cells necessary for repair but this also increases the risk of dysplastic changes due to chromosomal and microsatellite instability. Multiple pathways have been identified, regulated by many positive and negative factors involved in the development of cancer, which in this case follows the 'inflammation-dysplasia-carcinoma' sequence. Strategies to lower this risk are extremely important to reduce morbidity and mortality due to IBD-CRC, among which colonoscopic surveillance is the most widely accepted and implemented modality, forming part of many national and international guidelines. However, the effectiveness of surveillance in IBD has been a topic of

much debate in recent years for multiple reasons — cost-benefit to health systems, resource requirements, and also because of studies showing conflicting long-term data. Our review provides a comprehensive overview of past, present, and future perspectives of IBD-CRC. We explore and analyse evidence from studies over decades and current best practices followed globally. In the future directions section, we cover emerging novel endoscopic techniques and artificial intelligence that could play an important role in managing the risk of IBD-CRC.

Key Words: Inflammatory bowel disease; Colorectal cancer; Colitis-associated cancer; Surveillance in inflammatory bowel disease; Dye-spray colonoscopy; Adenomas; Dysplasia; Colectomy

Core Tip: The focus of the review is on the evolution of inflammatory bowel disease-related colorectal cancer (IBD-CRC) based on literature over the past decades to the present day. We provide a comprehensive overview of risk factors associated with IBD-CRC, molecular pathways identified and current strategies used to reduce incidence globally. We also touch upon the history of surveillance practice, its effectiveness, and the latest guidance on IBD surveillance by international societies. In a section on future directions, we discuss introduction of novel endoscopic technologies, artificial intelligence, and potential use of microbiota modulation, all of which could help reduce the risk of IBD-CRC.

Citation: Majumder S, Shivaji UN, Kasturi R, Sigamani A, Ghosh S, Iacucci M. Inflammatory bowel disease-related colorectal cancer: Past, present and future perspectives. *World J Gastrointest Oncol* 2022; 14(3): 547-567
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/547.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.547

INTRODUCTION

Chronic inflammation is known to be a major risk factor in the pathogenesis of cancer. Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal (GI) tract and IBD-related colorectal cancer (IBD-CRC) is one of its major and serious complications. Although only 1%-2% of IBD patients develop IBD-CRC, it contributes to about 15% of IBD-related mortality[1]. As per current long-term epidemiological data, the risk of CRC in IBD patients is high, particularly in patients with extensive ulcerative colitis (UC). Eaden *et al*[2] in their meta-analysis of 116 studies found that the incidence of IBD-CRC was 2%, 8% and 18% at 10, 20, and 30 years after the onset of UC, respectively. The role of Crohn's disease (CD) in the development of CRC is debatable and considered modest in comparison to UC. Canavan *et al*[3] in their meta-analysis estimated cumulative risk of 2.9% at 10 years, 5.6% at 20 years, and 8.3% at 30 years in patients with CD. However, factors like patient selection, sample size, duration of follow-up, completeness of case recruitment, and geographical differences may have influenced these estimates.

The severity of inflammation is a significant risk factor that increases the risk of IBD-CRC. Inflammation-related oxidative stress leading to genomic instability is considered the main trigger for the development of CRC. Studies on colonic tissue in IBD-CRC at the cellular and molecular level have found that the sequence of development of carcinogenesis is different from that observed in sporadic cancer in the non-inflamed colon. IBD-associated carcinogenesis follows an 'inflammation-dysplasia-carcinoma' sequence instead of the 'adenoma-carcinoma' sequence seen in sporadic CRC[4].

In this review article, we present a comprehensive overview of the literature on the epidemiology of IBD-CRC over decades, risk factors and pathogenesis including molecular pathways implicated. In addition, we present preventive strategies, current evidence for surveillance, the evolution of surveillance techniques with time, chemoprevention and explored which endoscopic technologies are likely to become standard for surveillance in the future. We have also touched upon the emergence of using diet and faecal microbiota modulation as a potential strategy in the future.

Trends in the incidence of IBD related CRC over decades

Many recent population-based studies and meta-analyses have shown that the risk of IBD-CRC is lower than what has been previously reported, most of which were from studies done in tertiary referral centres. One probable reason for this difference could be that recent studies are more focused on selecting the right study population (included more severe cases), sample size and are more thorough with follow-up; completeness of study recruitment and geographical differences were perhaps taken into consideration while analysing their findings. The details of the study results that reported the

incidence of IBD-CRC over the last four decades are summarized in Table 1.

RISK FACTORS OF IBD RELATED CRC

The risk factors of IBD-CRC can be broadly classified as factors that are genetic or familial and factors related to diet and lifestyle. These are illustrated in Figure 1A.

Age and disease duration

The association between disease duration in IBD and probability of CRC is controversial. Studies published over the decades report different conclusions. Several studies have found that the incidence of IBD-CRC is higher among patients who develop IBD at a young age making duration of disease an important risk factor[2,5,6]. Another surveillance study published in 2015 by investigators at St Mark's Hospital, London followed up 1375 UC patients for 15234 patient-years (median, 11 years per patient) and IBD-CRC was detected in 72 patients [incidence rate (IR), 4.7 per 1000 patient-years]. Although the IR of early IBD-CRC was noted to have increased by 2.5-fold in the current decade compared with the past decade (P = 0.045) it is reassuring that the 10-year survival rate was high (79.6%)[7]. A number of studies have concluded that in Crohn's colitis risk of CRC is similar to UC if the extension and duration of the disease are comparable[8,9].

Geographic variation risk

In a meta-analysis, Zhou *et al*[10] found that Oceania has a higher incidence than other continents. In Asia, it was found that the risk of CRC among UC patients increased after 10-20 years of disease duration, whereas in Europe, the risk of CRC in UC showed no statistical difference in disease duration for 1-9 years, 10-20 years, 21-30 years, or more than 30 years. In North America, the risk of CRC among UC patients increased significantly after more than 30 years of disease detection.

Gender

Gender is reported to be an important risk factor for IBD-CRC. In a large population-based cohort (n = 7607) of individuals diagnosed with IBD from 1954 to 1989, the risk of CRC was found to be 60% higher in males aged < 45 years at diagnosis, with a relative risk (RR) of 1.6 [95% confidence interval (CI): 1.2-2.2] compared to females[11]. Similar findings were noted in a meta-analysis conducted by Jess *et al*[12] where men had a greater risk with a standardized IR (SIR) of 2.6 (95%CI: 2.2-3.0) compared to women (SIR of 1.9; 95%CI: 1.5-2.3).

Extensive UC

In a study published in 1994, Gillen *et al*[13] reported a 19-fold increase in the risk of CRC in extensive UC compared to the general population (matched for age, sex, and disease duration). Similar findings were reported by Zhou *et al*[10] in a large meta-analysis that included 58 studies and 267566 UC patients; they found that disease extent-specific risk estimates for CRC in UC were reported in 21 of the 58 studies and that extensive UC and left-sided UC had a higher risk of CRC (SIR: 1.42, 95%CI: 0.83-2.42; SIR: 0.56, 95%CI: 0.38-0.83 respectively) compared to proctitis (SIR: 0.18, 95%CI: 0.01-0.03).

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease and a significant proportion of patients with PSC also develop IBD[14], often characterized by pancolitis, rectal sparing, backwash ileitis, and importantly, a threefold increased risk of colorectal dysplasia[15,16]. PSC with CD phenotype has been observed to be less severe than PSC with underlying UC[15-17].

A multicentric retrospective cohort study involving 277 PSC-IBD patients found that the IR of CRC since PSC diagnosis at 3.3 cases per 1000 patient-years (95%CI: 1.9-5.6), with an IR of 61 PSC cases per 100000 IBD patient-years. Of these, 69.7% were male, 67.5% had UC, and the mean age at PSC diagnosis was 40 ± 16 years. PSC-IBD patients with symptoms of PSC at diagnosis were noted to have an increased risk of CRC[16].

Gut microbial dysbiosis

Recent evidence suggests that intestinal microbiota; particularly the bacterial component plays a fundamental role in the health and progression of diseases such as IBD and CRC. The factors that are known to influence the gut microbiome are illustrated in Figure 1B.

The development of IBD is often associated with altered microbial communities (dysbiosis) in the gut, interaction of genetic and environmental factors leading to chronic inflammation in the intestine. According to the "common ground hypothesis", microbial dysbiosis and a leaky gut (due to intestinal barrier impairment)[18-20] are at the core of the chronic inflammatory process associated with IBD-CRC [21]. Several studies involving patient and gnotobiotic mouse models[22,23] have substantiated this hypothesis[24].

Table 1 The difference in the incidence of inflammatory bowel disease related colorectal cancer, past and present

Epidemiology		CRC in ulcerative colitis	CRC in indeterminate colitis	CRC in Crohn's disease
Annual incidence	Past	Stewénius *et al*[111], 1995, 1.4/1000 PYD; Eaden *et al*[2], 2001, 2/1000 PYD-after 10 yr of initial onset; 7/1000 PY (patients with extensive colitis 90%) 30 yr of initial onset; Castaño-Milla *et al*[112], 2012, 1.01/1000 PYD - after 10 yr of initial onset; 3.75/1000 PYD - after 20 yr of initial onset; 5.85/1000 PYD - after 30 yr of initial onset	Stewénius *et al*[111], 1995, 2.4/1000 PYD	Olén *et al*[113], 2020, a Scandinavian population-based cohort study 0.31 per 1000 PY(1968); Laukoetter *et al*[114], 2011, 0.5/1000 PYD
	Present	Fumery *et al*[115], 2017, the annual incidence of CRC was 0.8% (95%CI: 0.4-1.3)		Olén *et al*[113], 2020, a Scandinavian population-based cohort study 0.47 per 1000 person-years (2017)
Risk	Past	Eaden *et al*[2], 2001, 0.3% after 30 yr of initial onset		Canavan *et al*[3], 2006, 2.9% after 10 yr of initial onset; 5.6% after 20 yr; 8.3% after 30 yr. Friedman *et al*[116], 2008, 7% by 10th surveillance (patients with extensive colitis 90%). Basseri *et al*[117], 2012, 5.6% by 10th surveillance (patients with extensive colitis 55%)
	Present	Fumery *et al*[115], 2017, the risk of CRC was higher when LGD was diagnosed by an expert gastrointestinal pathologist (1.5%) than by community pathologists (0.2%). Factors significantly associated with dysplasia progression were concomitant: PSC (OR, 3.4; 95% CI: 1.5-7.8); Invisible dysplasia (*vs* visible dysplasia; OR, 1.9; 95% CI: 1.0-3.4), distal location (*vs* proximal location; OR, 2.0; 95% CI: 1.1-3.7); Multifocal dysplasia (*vs* unifocal dysplasia; OR, 3.5; 95% CI: 1.5-8.5)	Keller *et al*[118], 2019, IBD-CRC is responsible for approximately 2% of the annual mortality from CRC overall, but 10%-15% of the annual deaths in IBD patients	Olén *et al*[113], a Scandinavian population-based cohort study. Patients with Crohn's disease who were diagnosed with CRC were at increased risk of CRC mortality compared with reference individuals also diagnosed with CRC [HR 1.42 (1.16-1.75) when adjusted for tumour stage]

PYD: Patient year days; LGD: Low-grade dysplasia; CRC: Colorectal cancer; IBD-CRC: Inflammatory bowel disease-related colorectal cancer; PSC: Primary sclerosing cholangitis; HR: Hazard ratio; OR: Odds ratio; CI: Confidence interval.

Studies have shown high densities of mucosa-associated bacteria[21,25], with the ability to produce a greater mass of biofilm and extracellular matrix were present in IBD patients[26]. These mucosae associated highly virulent bacteria are suspected to play a pivotal role in gut inflammation and tumorigenesis[21]. Some of the common gut commensals like *Helicobacter pylori*[27], *Fusobacterium nucleatum*[28], *Bacteriodes fragilis*[29], and *Campylobacter* species[30] have been implicated in gastric tumorigenesis and CRC[23]. In a study by Gevers *et al*[31] on new-onset treatment-naïve pediatric patients with CD and UC, an abundance of *Enterobacteriaceae*, *Bacteroides/Prevotella*, *Veillonellaceae*, and *Fusobacteriaceae* were seen in ileal and colonic biopsies. Although the role of microbial and host factors in disease pathogenesis has not been established in chronic gut inflammation and IBD-CRC, it can be hypothesized that the combined effect of host barrier defects and bacterial invasiveness may evoke a massive amount of immune hyperactivation in the gut mucosa. This is likely to ultimately lead to a vicious cycle of chronic inflammation driven by the malignant transformation of the gut epithelium[21].

PATHOGENESIS OF CRC IN IBD

Molecular pathways and mechanisms

The molecular pathogenesis of IBD-CRC is very different from sporadic CRC[32]. With the advent of molecular technology in recent years, the pathophysiology of the development of IBD-CRC has been extensively studied, and has led to better understanding of molecular mechanisms and identification of new biomarkers[32-34].

Numerous positive and negative regulators in the development of IBD-CRC have been identified which are illustrated in Figure 2. The process of development of IBD-CRC is triggered probably due to chromosomal and microsatellite instability through well-defined pathways (*Wnt* pathway, CIMP pathway), causing mucosal dysplasia[32]. The involvement of these pathways suggests that persistent inflammation plays a prominent role in carcinogenesis. The changes occurring in the micro-environment due to chronic inflammation are thought to be responsible for the increased risk. The chronic proliferation necessary to repair epithelial layer damage (caused by constant inflammation) enhances the risk of dysplasia[35]. Although multiple cytokines and pathways have been identified in the pathogenesis of IBD-CRC[32-34], it continues to be a topic of ongoing research. Further research will not only enhance our understanding but also help identify non-invasive biomarkers and targets of therapy. A summary of currently known molecular mechanisms is summarized in Table 2.

Table 2 Cytokines implicated in tumorigenesis in the colon

Cytokines	The mechanism	Potential target of therapy?	Ref.
TNF-α	Triggers systemic inflammation and is one of the cytokines that make up the acute phase reaction in IBD and other chronic inflammatory diseases TNF-α regulates the induction MACC1 *via* the NF-κB subunit p65 and the transcription factor *c-Jun* in CRC cells	Yes: Anti TNF used to control inflammation in IBD; hence may reduce incidence of CRC but this is debatable	Pache *et al*[119], Kobelt *et al*[120]
IL-6 family	In the chronic phase of inflammation, IL-6 is able to activate almost all the cells of the body: trans-signalling-Increased formations of IL-6-sIL-6R complexes interact with gp130 on the membrane of CD4+T-cells and leads to an increased expression and nuclear translocation of STAT3, which causes the induction of anti-apoptotic genes, *e.g.*, *Bcl-xl*. This leads to resistance of lamina propria T-cells to apoptosis. T-cell expansion contributes to chronic intestinal inflammation	No: Anti IL-6 antibodies not successfully used in IBD. Unlikely to be useful in reducing risk of IBD-CRC	Atreya and Neurath[121], Allocca *et al*[122], Coskun *et al*[123], Danese *et al*[124]
IL-11	IL-11 belongs to the IL-6 family of cytokines. IL-11 has pro-tumorigenic activities such as proliferation, self-renewal, invasion and angiogenesis	No: No evidence to suggest it could be used as therapeutic agent. Could be useful as a diagnostic and prognostic biomarker	Murakami *et al*[125], Johnstone *et al*[126], Ren *et al*[127], Unver and McAllister[128], Pastor *et al*[129], Putoczki *et al*[130]
IL-17	IL-7 is a cytokine that helps the long-term survival of Th17 cells and innate lymphoid cells that express the transcription factor RORγt. It is suspected to be important for maintaining populations of T cells that induce and induce mucosal inflammation in IBD. IL-7 also maintains NKT cells that produce IL-17, using the PI3K/AKT/*mTOR* pathway	No: Anti-IL-17 medications are associated with IBD exacerbation	Hohenberger *et al*[131], Moschen *et al*[132]
IL-21	IL21 plays a dual role: IL-21 deficiency as a novel cause of early-onset IBD in human subjects accompanied by defects in B-cell development. Reduced numbers of circulating CD19 (+) B cells, including IgM (+) naive and class-switched IgG memory B cells, with a concomitant increase in transitional B-cell numbers. IL-21 Overproduction: IL-21 plays an important role in sustaining tissue-damaging immune responses	Yes: Could be used as a potential new therapeutic target in CD but unclear if it will influence IBD-CRC	Di Fusco *et al*[133], Salzer *et al*[134]
IL-23	IL-23R signalling affects disease susceptibility increased production of IL-23 by macrophages, dendritic cells or granulocytes has been observed in various mouse models of colitis, colitis-associated cancer and IBD patients	Yes: Currently in clinical trials for CD but too early to comment on effect on IBD-CRC	Moschen *et al*[132], Neurath[135]

NKT: Natural killer cells; MACC1: MET transcriptional regulator; UC: Ulcerative colitis; IBD-CRC: Inflammatory bowel disease-related colorectal cancer; AKT/PKB: Protein kinase B; IL: Interleukins; TNF-α: Tumor necrosis factor-α; RORγ: DNA-binding transcription factor; *mTOR*: Mechanistic target of rapamycin; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: Phosphoinositide 3-kinases; gp130: Glycoprotein 130; *Bcl-xl*: B-cell lymphoma-extra large; *c-Jun*: c-Jun proto-oncogene; p65: Nuclear factor NF-kappa-B p65 subunit.

TP53 and KRAS mutations

Earlier studies have found *TP53* and *KRAS* mutations in IBD-CRC and sporadic CRC. However, the molecular pathway towards the progression of carcinogenesis is different[32,36,37]. Recent studies have shown that *TP53* mutations were detected among 70% of sporadic colorectal carcinomas[38] and that both loss and gain of function of *TP53* might promote malignancy at the late phase of carcinogenesis [39].

It has been reported that the adenomatous polyposis coli (*APC*) and *KRAS* mutations were significantly less common in IBD-CRCs than in sporadic CRCs (15% *vs* 53%, $P < 0.001$ and 20% *vs* 38%, $P = 0.02$, respectively)[38].

STAT3 and IL-6/p-STAT3 pathway

The signal transducer and activator of transcription 3 (STAT3) pathway has been identified as an important one in the development of both sporadic CRC as well as IBD-CRC (Figure 3A). The exact mechanism is still not very well understood but it is reported to be due to signalling protein dysregulation and constitutive activation of STAT3[33,40]. Corvinus *et al*[41] showed in their murine model that constitutive STAT3 activation was persistent and important in CRC cells, possibly triggered by IL-6. Further, studies by other investigators have reported subsequently that STAT3 activation is associated with invasion, survival, and growth of CRC cells in mice *in vivo*[40,42]. Lin *et al*[40] have also demonstrated using both *in vitro* and *in vivo* models that blockade of IL-6/p-STAT3 (phosphorylated-STAT3) using an inhibitor suppressed tumour cell growth in colon cancer cells.

In human colonic tissue, patients with active colitis had significantly more IL-6 and p-STAT3-positive epithelial cells than both inactive UC and controls; in addition, they found that the proportion of suppressor of cytokine signalling 3 (SOCS3)-positive cells was lower in patients with dysplastic lesions and CRC. A study by Gui *et al*[43] that compared the expression of IL-6, STAT3, and SOCS3 in adenomas from IBD and non-IBD patients found significantly lower IL6, lower IL6R, higher STAT3, and lower SOCS3 expression in IBD associated dysplastic lesions.

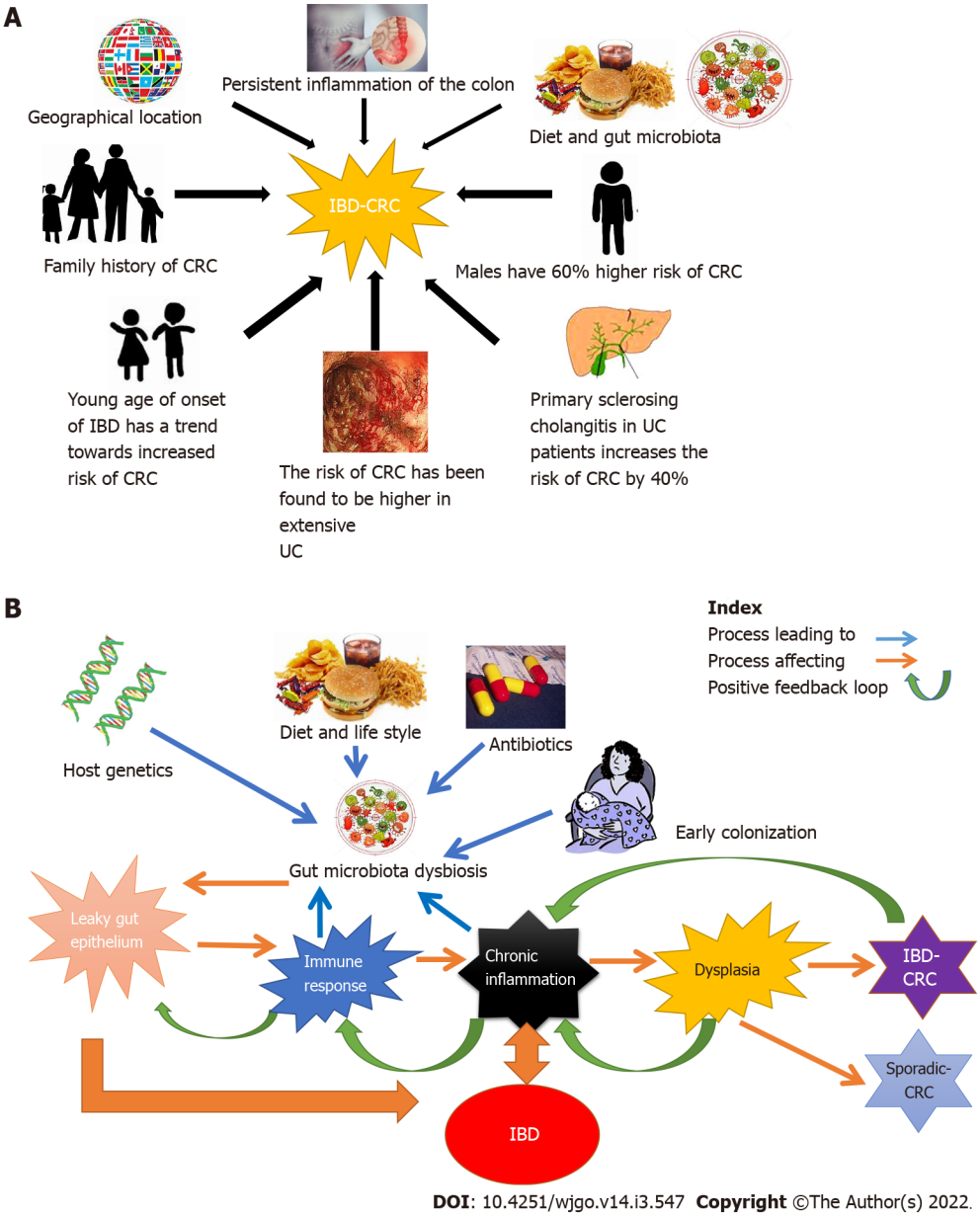
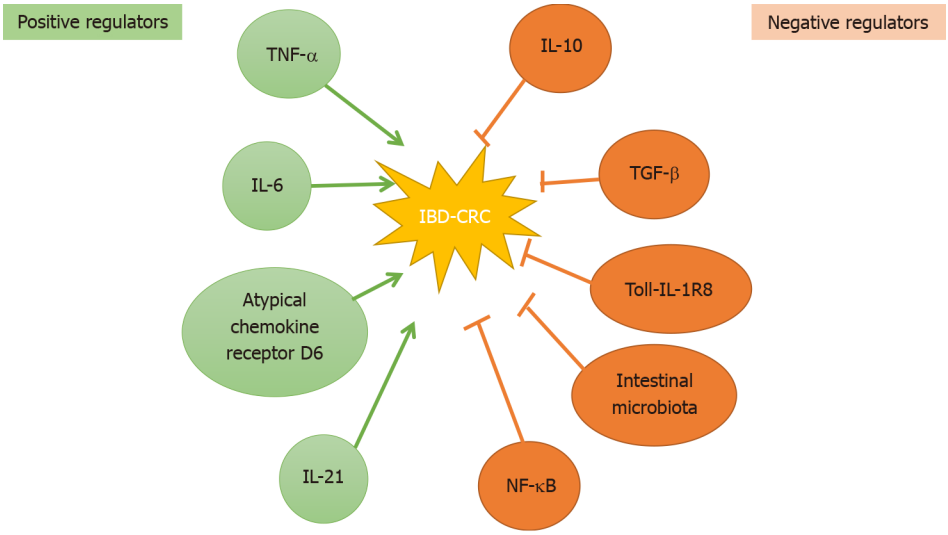


Figure 1 Risk factors leading to the development of inflammatory bowel disease-related colorectal cancer and the role of gut microbiota in inflammatory bowel disease-related colorectal cancer. A: Risk factors leading to the development of inflammatory bowel disease-related colorectal cancer (IBD-CRC). Risk factors are classified as familial and genetic. The factors are depicted in clockwise order: Genetic factors include a person's genetic makeup, family history of IBD and rarely monogenic causes of IBD. Younger age at diagnosis, male gender and durations of the disease has been identified as strong risk factors for IBD-CRC in longitudinal studies. The geographical location of the person, their diet, lifestyle, underlying diseases like extensive or left-sided ulcerative colitis, Primary sclerosing cholangitis, and other conditions causing persistent colon inflammation, are also known to increase the risk of development of IBD-CRC; B: Role of gut microbiota in IBD-CRC. Multiple factors such as diet, antibiotic use, and mode of birth and host genetic makeup influence/modulate the gut microbiota. This can lead to microbial dysbiosis mediated intestinal tissue damage causing intestinal barrier leak and mobilization of gut microbiota into host mucosa. The gut microbiota mediated break in the mucosal barrier, in turn, triggers an aggravated immune response leading to chronic inflammation. IBD, driven by an aberrant autoimmune response also leads to inflammation of the gut. This chronic inflammatory state leads to tissue damage causing dysplasia that can progress to cancer over time. CRC: Colorectal cancer; IBD: Inflammatory bowel disease; UC: Ulcerative colitis.

Overall, dysregulation of this signalling pathway plays an important role in triggering neoplasia. STAT3 pathway driven by IL-6 continues to be a topic of ongoing research and likely to be an attractive target with therapeutic potential.

The Wnt pathway

The canonical *Wnt*-pathway (β-catenin mediated *Wnt*-signalling) regulates the proliferation and differentiation of colonic stem cells in the normal colon[44,45]. However, the loss of *APC* gene results in the shift of β-catenin from membrane to the nucleus. This causes increased transcription of cyclin D1 and *c-myc* genes, leading to carcinogenesis (Figure 3B)[32,46]. Claessen *et al*[32] in their study reported that the *Wnt* pathway was activated in the early phase of colitis-associated CRC, and in about 50% of IBD-



DOI: 10.4251/wjgo.v14.i3.547

Figure 2 Molecular regulators of development of inflammatory bowel disease-related colorectal cancer. Over the years numerous biological molecules and pathways have been identified that positively or negatively regulate the development of inflammatory bowel disease-related colorectal cancer. Microbial dysbiosis in conjunction with cytokines [tumor necrosis factor-α, interleukin (IL)-6 and IL-21] and chemokines (atypical chemokine receptor D6) drive intestinal immune response, in turn leading to chronic inflammation, tissue injury, dysplasia and cancer. The negative regulators including cytokines IL-10 and transforming growth factor-β, nuclear factor-kappa beta, Toll-like receptors, along with healthy gut microbiota prevent gut inflammation-mediated tissue injury and promote healing of damaged tissue. NF-κβ: Nuclear factor-kappa beta; TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor β; IL: Interleukin; CRC: Colorectal cancer; IBD: Inflammatory bowel disease.

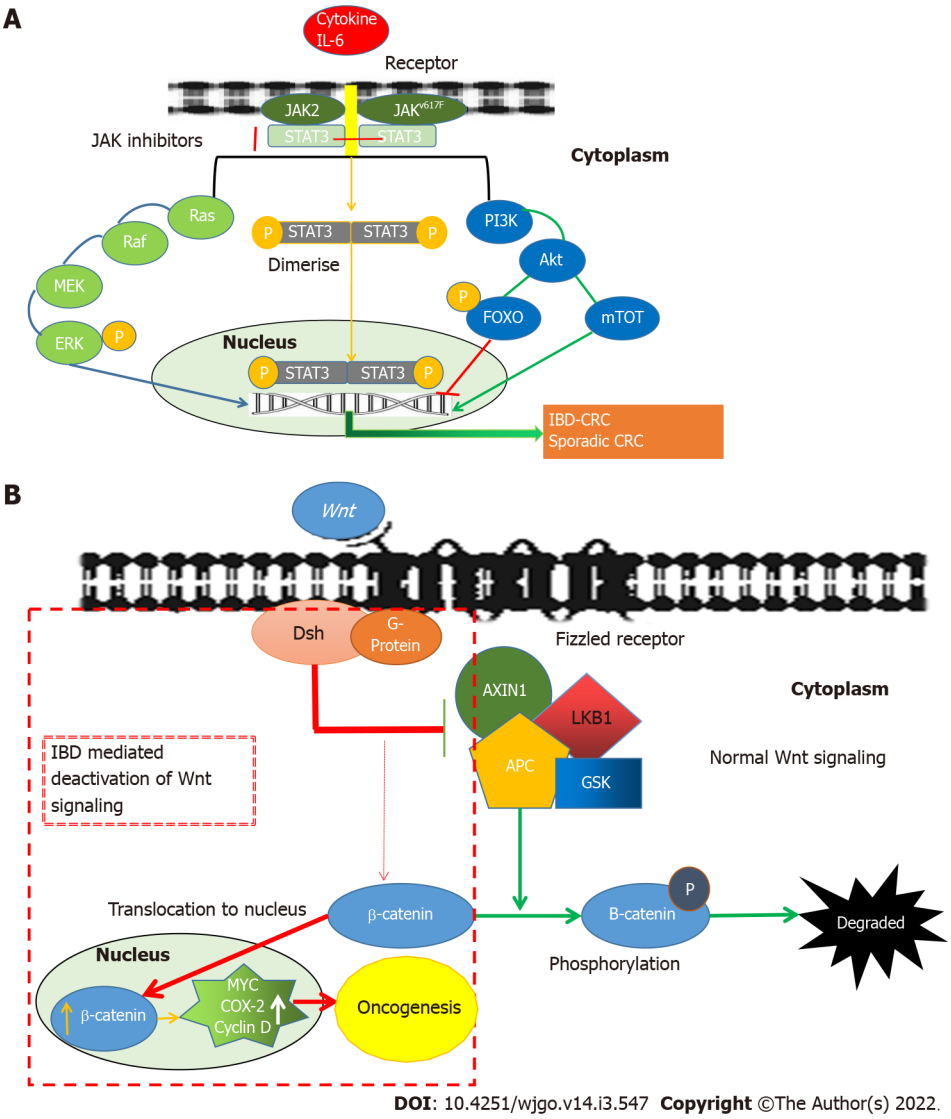
associated neoplasia cases. Another significant finding was that the pathway was also activated in the surrounding regions of dysplasia associated with IBD, a phenomenon termed as "field-cancerization" [32]. They suggest that estimation of β-catenin can be used as a biomarker for colonic field cancerization, facilitating early detection of neoplasia during colonic surveillance[32]. It has been shown in other studies that β-catenin could potentially be used as a marker of survival[47,48] and prognosis[47].

Dysplasia

CRC results from a series of genetic mutations that alter the normal growth pattern of cells, as a consequence of which, affected cells acquire a growth advantage over other cells. This aberration leads to morphological changes termed dysplasia. It was postulated that colorectal dysplasia could represent a premalignant lesion in IBD as early as 1949 by Warren and Sommers[49] and some years later in 1967 important observations that dysplasia originated from nonpolypoid mucosa were also reported by another group.

Historically, an elevated lesion containing dysplasia was referred to as a dysplasia-associated lesion or mass (DALM)[50]. The diagnosis of DALM became complicated over time because of the inconsistent criteria used in describing IBD-related dysplasia. The term DALM was also was being inaccurately always linked to colectomy[50]. However, with the advent of fibre optic endoscopic visualization techniques and improvement in localized surgical resection procedures the definition, classification, and management of dysplasia became more systematic[50-52]. The SCENIC guidelines in 2015 made important recommendations to standardize how lesions are described during surveillance. It was recommended that the term DALM be abandoned[53]. The term dysplasia redefined as an abnormal growth of cells, tissues, or organs leading to the development of abnormal histological or anatomical structures has now replaced the previously used term DALM[52] dysplasia is categorized as low-grade dysplasia (LGD) and high-grade dysplasia (HGD) based on the degree of histological abnormalities.

The identification of dysplastic changes is important as this is an important stage in the development of cancer and considered a strong predictor of CRC in IBD. Chronic intestinal inflammation is the primary risk factor that leads to LGD, which can then progress to HGD and eventually CRC[54] (Figure 4). This sequence of events is thought to be accelerated in IBD-CRC compared to sporadic CRC [54]. In a study de Jong *et al*[55] investigated the long-term risk of HGD and CRC following the development of LGD using a nationwide database identifying a large IBD patient cohort. The risk factors for advanced neoplasia progression were found to be age > 55 years at the time of LGD detection, male gender and follow-up at a tertiary IBD referral centre. The study also found that the incidence rate of progression to advanced neoplasia was 22% after 15 years of detection of IBD. Dysplasia in colonic strictures and epithelial dysplasia are both well-documented risk factors and considered to be precursors to the development of IBD-CRC[55,56]. In a case-control study among 53568 IBD patients undergoing colonoscopy, Sonnenberg and Genta[56] found that the prevalence of dysplasia was 3.22% and 2.08% in UC and CD respectively [odds ratio (OR) = 0.75, 95%CI: 0.65-0.86],



DOI: 10.4251/wjgo.v14.i3.547

Figure 3 The inflammatory pathways leading to the development of IBD-related colorectal cancer. A: The role of the JAK/STAT3 pathway in the development of IBD-related colorectal cancer. Various *in vivo* and *in vitro* models have shown that the JAK/STAT3 pathway plays a vital role in oncogenesis. The signal transduction of IL-6 involves the activation of JAK, activating transcription factors of the signal transducers and activators of STAT3. This is followed by its phosphorylation, dimerization and nuclear translocation of STAT3, initiating transcription of STAT3 target genes (including cyclin D1, *Bcl-xL*, *c-myc*, *Mcl1*, surviving and VEGF) leading to carcinogenesis. PI3K mediated activation of Forkhead box O3 (*FOXO*) leads to inhibition of gene transcription, whereas PI3K mediated activation of *mTOT* leads to oncogene transcription-mediated development of oncogenesis; B: The canonical *Wnt*-pathway in the development of IBD-related colorectal cancer. The canonical *Wnt*-pathway (*β-catenin* mediated *Wnt*-signaling) regulates proliferation and differentiation of the colonic stem cell in the normal colon. However, the loss of the adenomatous polyposis coli (*APC*) gene results in the shift of β-catenin from the membrane to the nucleus leading to increased transcription of cyclin D1 and *c-myc* genes thereby triggering carcinogenesis. IBD: Inflammatory bowel disease; CRC: Colorectal cancer; JAK: Juan kinase; P: Phosphorylation; STAT3: Signal transducer and activator of transcription proteins 3; PI3K: Phosphoinositide-3-kinases; Akt: RAC-alpha serine/threonine-protein kinase; FOXO: Forkhead box; *mTOT*: Mechanistic target of rapamycin; Ras: Small GTPase; Raf: Rapidly accelerated fibrosarcoma; MEK: Mitogen-activated extracellular signal-regulated kinase; ERK: Extracellular-signal-regulated kinase; Wnt: Wingless and int-1; Dsh: Dishevelled; AXIN: Axin-related protein 1; LKB1: Liver kinase B1; APC: Anaphase-promoting complex; GSK: Glycogensynthase kinase; MYC: *C-myc*; COX-2: Cyclooxygenase-2.

with a small increase in the prevalence of dysplasia within a stricture. The prevalence of cancer was higher in IBD patients with stricture compared to that without-0.78% and 0.11%, respectively (OR = 6.87, 95%CI: 3.30-12.89). A thirty-six-year analysis of a colonoscopic surveillance program found that in patients with UC who had undergone a colectomy due to HGD, 46% had a cancerous growth in the colon[57], thereby suggesting that the presence of HGD confers a high risk of synchronous cancer in the colon. Overall, dysplasia is a well-established histological stage in the development of IBD-CRC, and its detection during colonoscopy should prompt appropriate management to prevent progression to CRC. Surveillance programs are intended for the early detection of dysplastic lesions and strictures. In high-risk patients, surveillance helps in tracking disease progression in IBD patients. The intervals at which surveillance should take place vary from region to region, and based on guidelines by national societies. The British Society of Gastroenterology (BSG) recommends an intensified surveillance endoscopy program or a colectomy after the first 5 years of detection of LGD[58]. Other recommendations include

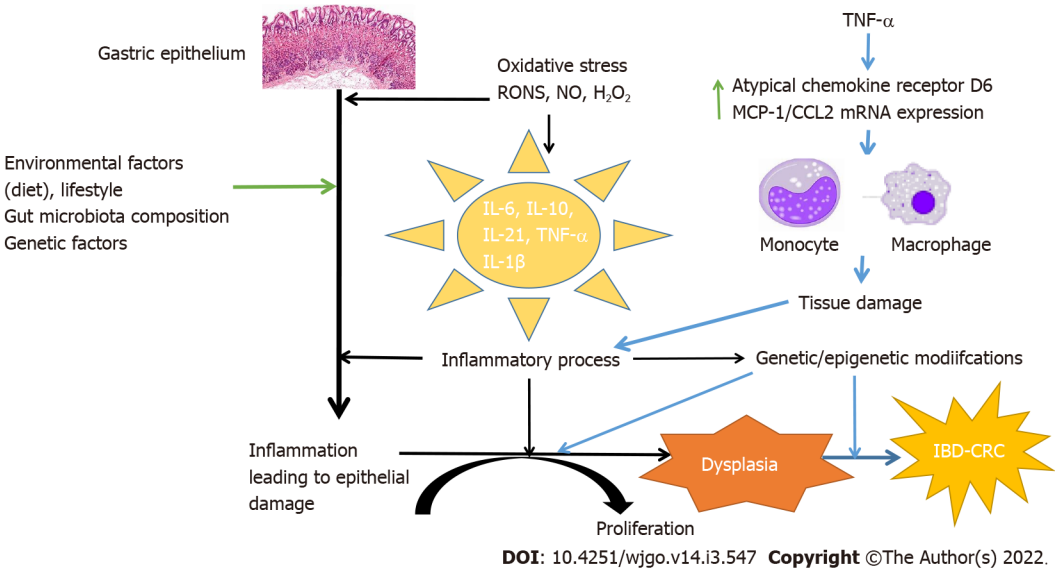


Figure 4 Pathophysiology of inflammatory bowel disease-related colorectal cancer. The pathophysiology of inflammatory bowel disease-related colorectal cancer (IBD-CRC) is different from sporadic IBD. IBD-CRC follows an "inflammation-dysplasia-carcinoma" sequence instead of the "adenoma-carcinoma" sequence as is seen in sporadic CRC. The pathophysiology associated with inflammation is at the heart of IBD-CRC. Various factors including genetic, familial along with numerous positive and negative molecular regulators and pathways have been identified which influence the development and maintenance of an inflammatory state. Inflammation leads to aberrant immune response leading to a chronic inflammatory state and gut tissue damage. Tissue damage and inflammation lead to dysplasia mediated carcinogenesis. CRC: Colorectal cancer; IBD: Inflammatory bowel disease; TNF-α: Tumor necrosis factor-α.

re-evaluation by a second pathologist if LGD is detected and further assessment by an expert endoscopist. The details of surveillance techniques are discussed in detail in the next section.

STRATEGIES USED TO REDUCE THE INCIDENCE OF CRC IN IBD

Surveillance colonoscopy

Surveillance in IBD — the evolution of guidance and practice over time: Surveillance in IBD could be described as the process of careful examination of the colon to detect early mucosal changes that may herald possible neoplasia. The mucosal changes/lesions (dysplasia of varying degrees) or adenomas provide an opportunity for early diagnosis and management of these lesions. There have been multiple studies in the past which have supported the use of surveillance as a tool to reduce cancer incidence in IBD. With the wider adoption of surveillance programmes over many years, long-term data have been in favour of regular surveillance of at-risk patients[7,59]. Over the last 2 decades, the practice of surveillance in IBD has largely been in line with guidance, which was mainly based on their large meta-analysis on the risk of IBD related CRC in 2001[2]. This landmark study, in particular, helped strengthen guidelines for regular surveillance. The summaries of recommendations are: Screening colonoscopy after 8-10 years that will also clarify disease extent for all patients; Regular surveillance to begin after 8-10 years for pancolitis and after 15-20 years for the left-sided disease; Reduced screening interval with increasing disease duration (due to increased risk in pancolitis); In the second decade of disease a colonoscopy to be conducted every three years, every two years for the third decade, and yearly by the fourth decade of disease; Two to four random biopsy specimens every 10 cm should be taken from the entire colon with additional samples of suspicious areas; Patients with PSC (including those with an orthotopic liver transplant) represent a subgroup at higher risk of cancer and they should have an annual colonoscopy.

These recommendations have been adopted by both the BSG and European Crohn's and Colitis Organisation (ECCO), with some minor differences and recent updates[60,61]. The core recommendations for surveillance remained stagnant for about twenty years. Recent advances in endoscopic technology and the use of new methods have meant that surveillance practices have started to change but can vary depending on the centre, availability of equipment, and expertise. The introduction of new technology has been matched by sound recommendations by the SCENIC guidelines and availability of newer endoscopic classification systems to help clinicians describe IBD-related dysplastic lesions whilst using these techniques. *e.g.*, the Frankfurt Advanced Chromoendoscopic IBD LEsions (FACILE) classification that has been developed, validated, and shown to be reproducible[62].

Although the SCENIC guidelines do not recommend routine use of Narrow Band Imaging (NBI) for surveillance, recent studies have shown that this could be a reliable modality. A large multicentre study by Watanabe *et al*[63] randomised 263 surveillance patients to either chromoendoscopy or surveillance

using NBI. The results showed no significant difference in lesion detection rates (10.7% *vs* 11.9%) and the duration of procedure was shorter with NBI (by 4 min; $P < 0.001$)[63]. Further, a study by Bisschops *et al*[64] found NBI to be significantly better than high definition chromoendoscopy images to differentiate neoplastic from non-neoplastic lesions among experts. The results of these studies indicate that the NBI may have a potential role in surveillance in the future and is likely to find a place in updated guidelines.

How effective is surveillance?

The effectiveness of surveillance in IBD has been a topic of much debate over years for multiple reasons — cost-benefit to health systems, resource requirements, and also because studies show many conflicting data.

A Cochrane review by Collins *et al*[65] from 2006 looked into the effectiveness of surveillance in reducing the death rate from CRC in IBD. This study included a combination of prospective and retrospective studies that looked at the impact of surveillance on IBD-CRC. They reported on direct and indirect evidence to answer the question of the effectiveness of surveillance. The details of the studies included are given in Table 3. In summary, one study showed a dose-response to survival wherein a higher number of surveillance procedures were protective and increased survival, one showed that surveillance picked up CRC at an earlier stage and 5-year survival was better in the surveillance group compared to the non-surveillance group and another showed improved survival in the surveillance group compared to non-surveillance, but no improvement in mortality due to CRC. Some other studies have tried to estimate the economic benefits of surveillance. However, these models were calculated for sporadic cancers, and conclusions extended to IBD-CRC. It was shown that screening programs for normal individuals in the community have financial gains and therefore an argument has been made in favour of surveillance of high-risk patients with IBD. A more recent systematic review and meta-analysis by Bye *et al*[66] included observational studies of patients that included patients undergoing surveillance. Their pooled analysis showed a reduction in IBD-CRC in patients undergoing surveillance by 42% and IBD-CRC-related death by 64%, compared to those who did not undergo surveillance[66]. Current literature appears to favour surveillance and therefore it is part of standard service provision in many endoscopy centres.

Impact of using different biopsy techniques and endoscopic modalities

Random biopsies during surveillance colonoscopy had been standard practice, which was a labour-intensive process not only for the endoscopist but also the pathologist. Studies that looked at accuracy of targeted biopsies changed the landscape of surveillance making it more efficient without compromising on the accuracy of detecting neoplasia.

Targeted biopsies and white light endoscopy

In a key prospective exploratory trial, Watanabe *et al*[67] randomised chronic UC patients undergoing surveillance to either have targeted biopsies (from lesions detected) or step-wise multiple biopsies (random biopsies every 10 cm). The patients underwent high-definition white-light endoscopy (HD-WLE) in most cases. The investigators found that the detection of neoplasia was significantly higher in the target biopsy group compared to random biopsies (6.9% *vs* 0.5%), with a lower mean number of biopsies in the targeted group (34.8 *vs* 3.1; $P < 0.001$) and shorter examination time, concluding that targeted biopsies were as effective as random biopsies and more cost-effective[67]. This finding has been suggested in other studies, thereby indicating random biopsies could still be useful in select high risk patients, in line with the 2019 European Society of Gastrointestinal Endoscopy (ESGE) recommendations [68].

Dye-chromoendoscopy

Dye-chromoendoscopy (DCE) is currently the standard of care for surveillance colonoscopy in IBD as it has been reported to aid the detection of subtle mucosal lesions. A prospective randomised trial that compared DCE using methylene blue with conventional endoscopy reported more accurate findings with better ability to differentiate between neoplastic and non-neoplastic lesions in patients with long-standing UC. Another prospective study by Marion *et al*[69] in 2008 compared the same techniques with randomised and targeted biopsies in a cohort of 102 patients with IBD. DCE detected significantly higher number of dysplastic lesions compared to random biopsies[69]. A large systematic review and network meta-analysis found DCE to have a significantly higher diagnostic yield for neoplastic lesions compared to WLE[70]. This technique is therefore recommended for surveillance endoscopy by the ESGE.

Virtual chromoendoscopy

Virtual electronic chromoendoscopy (VCE) or dyeless virtual chromoendoscopy uses image enhanced technology (I scan) that has been introduced in recent years but already increasingly adopted by expert endoscopists for surveillance colonoscopy. A retrospective study by Gasia *et al*[71] compared various technologies namely standard WLE, high definition WLE, DCE, VCE, and also strategies of targeted

Table 3 Summary of studies over decades reporting on surveillance in inflammatory bowel disease

Ref.	Number of patients and cohort	Results	Conclusions benefit-yes/no
Rosenstock *et al*[136], 1985, Retrospective Review	248 chronic UC patients	In this cohort of patients: Overall incidence of HGD was 6%; HGD or carcinoma found in 24 procedures in 16 patients, mean disease duration of 16 yr, 15 patients had HGD; DALM most consistent indicator of carcinoma. > 95% of cancers 6 recognized at colonoscopy	The presence/absence of dysplasia a reliable histological marker that correlates with the presence/absence of cancer in UC. DALM with HGD had the strongest indication for surgery. Benefit- yes
Lashner *et al*[137], 1990, Prospective surveillance programme	99 patients with pancolitis	In this cohort of patients: Both groups comparable in terms of age at onset, disease duration and gender; Total 8 fewer deaths in the surveillance group ($P < 0.05$); Colectomy was less common and was performed 4 yr later in the surveillance group ($P < 0.05$)	Screening in UC associated with improved survival and delayed colectomy. Findings did not show improvement in cancer-related survival. Benefit-equivocal
Löfberg *et al*[138], 1990, 15-yr Prospective surveillance programme	72 UC, 12 patients developed definite dysplasia	In this cohort of patients: LGD detected in 7 patients; HGD in 4 and 1 Dukes' Stage-A cancer at operation; The cumulative risk of developing at least LGD was 14% after 25 yr of disease; Abnormal, aneuploid DNA content detected in biopsies of 12/59 patients (20.3%) this correlated significantly with LGD and HGD	Long-term use of surveillance in UC is reliable in detecting dysplasia and identify patients for prophylactic surgery. Benefit-yes; Earlier detection of neoplasia
Nugent *et al*[139], 1991, 13-yr Prospective surveillance programme	213 UC patients	In this cohort of patients: A total of 15 patients underwent colectomy; A total of 7 patients had unsuspected carcinoma at various stages; Dysplasia detected among 11 patients; No difference in the prevalence of dysplasia between left-sided v/s extensive disease; No carcinoma detected among 175 patients without dysplasia on initial biopsies	Surveillance programme effective aid in reducing the risk of carcinoma in UC. Short term risk of CRC low if biopsy negative. Colectomy deferred in this group. Benefit-yes
Lynch *et al*[140], 1993, Prospective surveillance(between 1978 and 1990)	160 UC patients	In this cohort of patients: A total of 739 colonoscopies carried out (4.6 colonoscopies/per patient); A 709 patient-years follow-up was carried out; In 1 patient Dukes's A cancer was detected; IBD-CRC caused the death of 1 patient; Overall, 9 IBD-CRC cases were diagnosed during the study period but only 1 case was detected by way of the surveillance programme	Results of this large study with long follow-up cast doubts on the effect-iveness of the surveillance programmes in detecting CRC in patients with UC. Benefit-no
Jonsson *et al*[141], 1994, Prospective, longitudinal study between 1977 and 1991	131 patients with UC	In this cohort of patients: A total of 632 colonoscopies performed, dysplasia was diagnosed in 24 (4 HGD), other than those with cancer; CRC diagnosed in 4 patients, of whom 2 included in the programme with a diagnosis of cancer; CRC and dysplasia are seen mainly in the left colon and in pancolitis patients	The surveillance programme was resource consuming and the cost-benefit must be questioned. Benefit-no. No cost-benefit as per authors
Karlén *et al*[142], 1998, Prospective case-control study	4664 patients with UC, 142 patients with definite UC	In this cohort of patients: In 2 out of 40 patients with UC and 18/102 controls had at least one-surveillance colonoscopy (RR 0.29, 95% CI: 0.06-1.31); Out of 12 controls, only one patient with UC had two or more surveillance colonoscopies (RR 0.22, 95%CI: 0.03-1.74), indicating a protective dose-response relation	Surveillance may be associated with decreased risk of death from CRC in patients with long-standing UC. Benefit-yes. May improve survival
Friedman *et al*[143], 2001, Prospective Longitudinal study	259 patients with chronic Crohn's colitis	In this cohort of patients: A total of 663 examinations were performed on 259 patients; The median interval between examinations was 24 mo; More frequent examinations were carried out(1-6 mo) in patients with dysplasia; Dysplasia or cancer was detected in 16% (10 indefinite, 23 LGD, 4 HGD and 5 cancers); Definite dysplasia or cancer was associated with age > 45 yr and had increased symptoms	Colonoscopic surveillance should be strongly considered in chronic extensive Crohn's colitis. Benefit-yes. May improve survival
Biasco *et al*[144], 2002, Prospective Longitudinal study (20 yr duration)	65 patients with UC > 7 yr	In this cohort of patients: A total of 23 (35.3%) patients had surgery; A total of 29 (44.66%) patients discontinued the programme; Only 11 (16.9%) patients have remained in the programme	Results cast some doubts on the significance of such a programme and on its long-term feasibility. Benefit-no. Long-term feasibility doubtful
Hata *et al*[145] 2003, Retrospective January 1979 and December 2001	217 UC patients	In this cohort of patients: A total of 15 patients were detected to have definite dysplasia; Among 5/15 proved to have invasive cancer in resected specimens; cumulative risk for development of definite dysplasia at 10, 20 and 30 yr was 3.1%, 10.0%, and 15.6% respectively; A cumulative risk for the development of invasive cancer at 10, 20, and 30 yr was 0.5%, 4.1%, and 6.1%, respectively	The surveillance programme is useful for detecting IBD-CRC and survival may be improved by surveillance colonoscopy. Benefit-yes. May improve survival

UC: Ulcerative colitis; IBD-CRC: Inflammatory bowel disease related colorectal cancer; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; DALM: Dysplasia-associated lesion/mass; RR: Relative risk; CI: Confidence interval.

biopsies *vs* random. They found targeted biopsies to be better at neoplasia detection across all technologies except standard WLE. In a prospective randomised trial by the same investigating group, Iacucci *et al*[72] randomised patients with long-standing colitis into three arms: WLE, DCE, and VCE. In this non-inferiority study, VCE was found to be non-inferior to DCE in the detection of all neoplastic lesions. ESGE now strongly recommends the use of VCE or dye-spray with targeted biopsies for surveillance of

colon with quiescent disease[68].

Chemoprevention of CRC

Chemoprevention in cancer is a term used for the use of pharmacological agents to reduce or delay the risk of carcinogenesis or progression of the disease[73,74]. Although there have been multiple drugs investigated for their potential, mesalazine currently has the largest evidence base to support its use for chemoprevention in CRC[74-76].

Mesalazine: Mesalazine or 5-aminosalicylic acid (5-ASA), a structural analogue of aspirin, has been used for many decades as first-line therapy for mild-to-moderate UC in oral and topical forms. In addition to its anti-inflammatory properties, it has received much attention for its chemopreventive effects. The drug appears to exert its effects through multiple mechanisms. A systematic review that looked into molecular mechanisms of chemoprevention of CRC was published in 2009. Lyakhovich and Gasche[74] in this study summarised that 5-ASA inhibits cyclooxygenase-2 (COX-2)/prostaglandin E2 synthesis, decreases the transcriptional activity of NF-κB by modulating RelA/p65 phosphorylation, and interferes with the Wnt pathway through protein phosphatase 2A. Multiple other systematic reviews have reported on the chemoprotective effects of 5-ASA. Velayos *et al*[77] included nine studies with 1932 UC patients in their systematic review and meta-analysis and reported a protective effect of 5-ASA in IBD-CRC and CRC/dysplasia. A large meta-analysis by Qiu *et al*[76] comprising of 26 studies with > 15000 patients (UC + CD) reported a chemopreventive effect on CRC but not dysplasia. A dose of > 1.2 g/d was effective to reduce the risk. Another meta-analysis reported that 5-ASA was protective against CRC and dysplasia with a strong protective effect noted in UC but a non-significant effect in CD [78].

With many reporting on the mechanisms of 5-ASA in reducing the risk of CRC, it is plausible that it has a chemopreventive effect in IBD and can be used in this cohort of patients.

Thiopurines: Thiopurines have been used for many decades in the management of IBD. There have been no randomised studies to investigate the efficacy of thiopurine therapy and current evidence is from cohort, case-control or population-based studies, with conflicting reports. A systematic review by Jess *et al*[79] in 2014 reported no protective effect of thiopurine therapy on CRC in IBD patients. The studies included carried heterogeneity and included clinic-based cohort and case-control studies, but no population-based studies. The lack of protective effect may be explained due to the inclusion of studies with patients at a severe spectrum of disease[79].

Another systematic review and meta-analysis by Lu *et al*[80] reported in 2018 on 24 observational studies involving 76999 participants to evaluate the risks of developing CRC in IBD patients on thiopurines. The authors found an overall protective effect of thiopurine use on CRC in patients with IBD (OR = 0.63, 95%CI: 0.46-0.86) in a pooled estimate and the effect was significant in UC patients (OR = 0.67, 95%CI: 0.45-0.98), but not in CD patients (OR = 1.06, 95%CI: 0.54-2.09). Interestingly, the authors also reported that the protective effect was limited to clinic-based and case-control studies but no population-based studies.

Aspirin/non-steroidal anti-inflammatory drugs: Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) have been studied for their chemo-preventive properties in the context of sporadic CRC. The elevated levels of COX-2 expression found in most CRC meant that NSAIDs and selective COX-2 inhibitors (COXIBs)[81] carry the potential for use in chemoprevention. A large, randomised study reported a reduction in metastatic disease in CRC with aspirin use[82]. Although the mechanisms make these medications attractive options, there have been no prospective studies done to study their efficacy in the context of IBD-CRC. A systematic review and meta-analysis by Burr *et al*[83] reported on the effect of aspirin and NSAIDs on IBD-CRC. They found only 9 retrospective studies in IBD which included the use of either or both these drugs with CRC as one of the outcomes. The authors concluded that the studies presented several limitations including selection bias as well as confounding. Also, the number of patients included was a small and overall large variation in studies that led to no strong conclusions[83].

At present, the use of aspirin or NSAIDs as chemopreventive agents is not part of any guidelines. This is unlikely to change as large prospective studies that study IBD-CRC are unlikely to be carried out.

Folic acid: IBD leads to impaired folate absorption. Folate is involved in DNA methylation and may produce epigenetic changes that affect the gut microbial and host immune interactions[84]. Folic acid has been investigated in the past as a chemopreventive agent. The effect of folate supplementation on dysplasia and cancer in IBD was first reported by Lashner *et al*[85] in a case-control study. In this study, all patients with pancolitis of > 7-year duration (except those with known HGD and invasive cancer) in the surveillance program exposed to folate supplements were compared to the control group (patients in the surveillance program, no dysplasia and not exposed to folate). Although folate supplementation was associated with a 62% lower incidence of dysplasia or cancer, the duration, and dose of folate intake were unclear, and results did not reach statistical significance probably because it was underpowered. Another retrospective study by the same group reported that the relative risk of neoplasia was lower (0.54) with folate supplementation (after at least 6-mo of exposure). The authors concluded that daily

folate supplementation may protect against the development of neoplasia in UC, although the results did not reach statistical significance.

The effects of folate supplementation were best summarised by a systematic review and meta-analysis by Burr *et al*[86] in which they included ten studies with low to moderate heterogeneity and a total of 4517 patients. The authors concluded that the results showed a pooled hazard ratio of 0.58 (95%CI: 0.37-0.80) suggesting an overall protective effect for folate supplementation on the development of IBD-CRC[86].

While there is weak evidence from retrospective studies in favour of folate supplementation, in the absence of prospective randomised data to support this, it is unlikely that folic acid will be used routinely for chemoprevention. At present, it is not part of guidelines by most national and international societies despite it being a cheap, safe, and well-tolerated supplement.

Surgery

Surgery in the form of colectomy remains an important and effective strategy in preventing IBD-CRC, particularly in patients who have HGD or 'indefinite' dysplasia or invisible dysplasia detected on biopsies. Among visible lesions seen during endoscopy, polypoid lesions and some non-polypoid lesions with LGD in selective cases can generally be managed with endoscopic resection if full resection can be achieved, and further surveillance may be a reasonable option as per current guidelines[61].

However, non-polypoid lesions that cannot be managed endoscopically or the presence of invisible dysplasia regardless of degree are considered high-risk for progression to cancer and therefore recommended to undergo surgery[61].

The presence of visible dysplasia perhaps is relatively straightforward with the grade of dysplasia determining the intensity of future surveillance or need for surgery, but invisible dysplasia poses a challenge. Although the proportion of invisible dysplastic lesions is low due to the use of advanced endoscopic techniques[87], the detection of such lesions can present a dilemma in management, particularly because patients do not readily accept colectomy despite physician recommendations[88].

The risk of cancer with visible LGD has been known to be low with a larger body of evidence. In a retrospective study by Ten Hove *et al*[89], the incidence rate of advanced cancer was low at 1.34 per 100 years in patients with LGD after a follow-up of nearly 5 years, with no significant difference between chromoendoscopy and WLE and a systematic review by Kabir *et al*[90] reported a pooled estimated rate of cancer in visible LGD at 2.7%. However, there is very little data available on invisible dysplasia. In their systematic review, Kabir *et al*[90] reported that pooled estimates of cancer due to invisible HGD and invisible LGD were 11.4% and 2.4% respectively, based on two cohort studies and one case series. With such a high risk of progression to cancer, surgery should be considered as a serious and realistic option in reducing the risk of IBD-CRC.

Diet therapy and gut microbiota modulation

Our better understanding of the human gut microbiome has opened up a new possibility of treatment for IBD and IBD-CRC[91]. Recent molecular level research on the gut microbiota using whole-genome sequencing technology has proved that some factors can alter the microbiome and the pathogenesis of IBD[92].

It has been hypothesized that diet plays a key role in the modulation of the gut microbiota composition. Gut microbiota in turn plays a major role in maintaining gut homeostasis and is associated with the modulation of host inflammatory and immune responses[93]. Studies have shown that nutritional components (added sugars, trans-fats, omega-6 fatty acids, red processed meat *etc*.) contribute to a chronic inflammatory condition by regulating various immune and inflammatory pathways[94,95]. Diet has been identified as one of the vital factors associated with CRC etiology[94,96].

Dietary therapy is also considered to be helpful, especially in children with CD who receive exclusive enteral nutrition[95,97]. Therefore, microbiome-modulating interventions like the application of probiotics[98,99], prebiotics[97,100,101], antibiotics, faecal microbiota transplantation (FMT)[102,103], and gene manipulation is being widely explored as new treatment options for a large number of chronic inflammatory diseases including UC, CD, and CRC. Genetic studies involving IBD patients reported 163 IBD susceptibility gene loci. These loci were found to be involved in regulating the host and gut microbes' interactions[104,105]. Mechanistically, it is plausible that by correcting the gut microbiota composition, the innate immune system can be modulated, leading to lesser inflammatory damage to the gut epithelium. This could enhance gut barrier function, prevent pathogen colonization and exert selective cytotoxicity against tumour cells[92]. These actions could break the vicious cycle of inflammation-mediated dysplasia.

Future directions

Advanced endoscopic technologies: There have been several recent advances made with novel endoscopic technologies such as endocytoscopy, confocal laser endomicroscopy (CLE), both of which allow examination of the bowel mucosa with histology-like images at 500-fold to 1000-fold magnification, allowing *in vivo* evaluation in real-time.

Endocytoscopy has been reported to be effective in recognising low-grade adenoma in the colon [106]. Its utility in IBD surveillance has not been evaluated thoroughly yet and is a subject of research. There is evidence that CLE is a useful tool in assessing dysplasia, with a stronger evidence base in the evaluation of Barrett's oesophagus. It has been studied in the context of IBD and shown to increase the rate of detection of neoplastic lesions. In a consensus-based report on the applications of CLE, although there was wide agreement that CLE can detect dysplasia effectively in IBD[107], its adoption is limited by cost and lack of expertise. This is likely to change in the future as endoscopists become more familiar with the technology and wider use may drive down costs.

Full-spectrum endoscopy (FUSE) is an emerging technique that employs two lateral additional cameras to a standard colonoscope, allowing operators to view behind folds and blind spots. Leong and Koo[1] investigated its ability to detect dysplastic lesions in a robust study design involving patients undergoing surveillance. They prospectively randomised 52 patients to either standard colonoscopy or FUSE and then crossed over to the other group for a repeat procedure. FUSE missed significantly fewer dysplastic lesions compared to standard (25% *vs* 71.4%) with a slightly longer withdrawal time. Kudo *et al*[108] reported similar findings in their tandem colonoscopy trial. The advantages of this technique are apparent but are currently not part of guidelines and recommendations by relevant societies. Further research and familiarity with the technique are likely to encourage more clinicians to use this for surveillance.

Artificial intelligence: The next generation of advancement comes in the form of using artificial intelligence (AI) in endoscopy. AI is currently being used widely in innumerable areas and its applications are seemingly unlimited. AI in IBD has been evaluated by Stidham *et al*[109] where they found that performance of deep learning models was similar to experienced human reviewers when grading endoscopic severity in UC. AI built into endoscopic systems to aid detection of dysplastic lesions is currently a subject of research globally, with few early reports available in literature[110].

Microbiota modulation: The discovery of microbiota-regulated mucosal and systemic immune response pathways have opened up avenues to explore the impact of this response on the development of cancer immunotherapies. However, it should also be considered that an individual's commensal gut microbiota keeps evolving and changing throughout the lifetime based on various environmental factors[23]. This phenomenon plays a pivotal role in phenotypic variation in disease development, progression, and therapeutic success among individuals. Therefore, it will not be wrong to hypothesize that future gut microbiota modulating therapies need to be personalized according to an individual's microbiota.

CONCLUSION

IBD-related CRC is a serious complication that deserves attention. The evolution of strategies in reducing this risk over decades is interesting. Although surveillance is now the cornerstone of early detection of neoplasia, the key to reducing this risk is keeping patients in remission. It is encouraging that there are some signals of lowered risk of IBD-CRC recently but with increasing disease burden, we have to remain vigilant. Further research into exploring pathways involved in CRC will provide a better understanding and potential new targets to exploit, be it for new or repurposed drugs. The expansion in the use of advanced endoscopic techniques is likely to improve neoplasia detection and help patients. AI carries the potential to bring about a paradigm shift in endoscopy and surveillance but needs rigorous evaluation before it is deployed for routine clinical use. Lastly, modulation of microbiota may well be something to watch out for in the future as a reliable intervention in this cohort.

FOOTNOTES

Author contributions: Majumder S and Shivaji UN contributed literature search, data collection, data analysis, writing and editing manuscript, revision and final approval; Kasturi R, Sigamani A, Ghosh S and Iacucci M contributed writing and editing manuscript, revision and final approval.

Conflict-of-interest statement: The authors have none to declare.

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S-Editor: Gao CC
L-Editor: A
P-Editor: Gao CC

REFERENCES

1 **Leong RW**, Koo JH. Colorectal cancer in inflammatory bowel disease. *J Gastroenterol Hepatol* 2009; **24**: 503-505 [PMID: 19368629 DOI: 10.1111/j.1440-1746.2009.05790.x]
2 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535 [PMID: 11247898 DOI: 10.1136/gut.48.4.526]
3 **Canavan C**, Abrams KR, Mayberry J. Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. *Aliment Pharmacol Ther* 2006; **23**: 1097-1104 [PMID: 16611269 DOI: 10.1111/j.1365-2036.2006.02854.x]
4 **Itzkowitz SH**, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G7-17 [PMID: 15194558 DOI: 10.1152/ajpgi.00079.2004]
5 **Kim ER**, Chang DK. Colorectal cancer in inflammatory bowel disease: the risk, pathogenesis, prevention and diagnosis. *World J Gastroenterol* 2014; **20**: 9872-9881 [PMID: 25110418 DOI: 10.3748/wjg.v20.i29.9872]
6 **Beaugerie L**, Itzkowitz SH. Cancers Complicating Inflammatory Bowel Disease. *N Engl J Med* 2015; **373**: 195 [PMID: 26154801 DOI: 10.1056/NEJMc1505689]
7 **Choi CH**, Rutter MD, Askari A, Lee GH, Warusavitarne J, Moorghen M, Thomas-Gibson S, Saunders BP, Graham TA, Hart AL. Forty-Year Analysis of Colonoscopic Surveillance Program for Neoplasia in Ulcerative Colitis: An Updated Overview. *Am J Gastroenterol* 2015; **110**: 1022-1034 [PMID: 25823771 DOI: 10.1038/ajg.2015.65]
8 **Averboukh F**, Ziv Y, Kariv Y, Zmora O, Dotan I, Klausner JM, Rabau M, Tulchinsky H. Colorectal carcinoma in inflammatory bowel disease: a comparison between Crohn's and ulcerative colitis. *Colorectal Dis* 2011; **13**: 1230-1235 [PMID: 21689324 DOI: 10.1111/j.1463-1318.2011.02639.x]
9 **Kiran RP**, Khoury W, Church JM, Lavery IC, Fazio VW, Remzi FH. Colorectal cancer complicating inflammatory bowel disease: similarities and differences between Crohn's and ulcerative colitis based on three decades of experience. *Ann Surg* 2010; **252**: 330-335 [PMID: 20622662 DOI: 10.1097/SLA.0b013e3181e61e69]
10 **Zhou Q**, Shen ZF, Wu BS, Xu CB, He ZQ, Chen T, Shang HT, Xie CF, Huang SY, Chen YG, Chen HB, Han ST. Risk of Colorectal Cancer in Ulcerative Colitis Patients: A Systematic Review and Meta-Analysis. *Gastroenterol Res Pract* 2019; **2019**: 5363261 [PMID: 31781191 DOI: 10.1155/2019/5363261]
11 **Söderlund S**, Granath F, Broström O, Karlén P, Löfberg R, Ekbom A, Askling J. Inflammatory bowel disease confers a lower risk of colorectal cancer to females than to males. *Gastroenterology* 2010; **138**: 1697-1703 [PMID: 20167217 DOI: 10.1053/j.gastro.2010.02.007]
12 **Jess T**, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol* 2012; **10**: 639-645 [PMID: 22289873 DOI: 10.1016/j.cgh.2012.01.010]
13 **Gillen CD**, Walmsley RS, Prior P, Andrews HA, Allan RN. Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. *Gut* 1994; **35**: 1590-1592 [PMID: 7828978 DOI: 10.1136/gut.35.11.1590]
14 **Stiehl A**, Benz C, Sauer P. Primary sclerosing cholangitis. *Can J Gastroenterol* 2000; **14**: 311-315 [PMID: 10799084 DOI: 10.1155/2000/983681]
15 **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]
16 **Guerra I**, Bujanda L, Castro J, Merino O, Tosca J, Camps B, Gutiérrez A, Gordillo Ábalos J, de Castro L, Iborra M, Carbajo AY, Taxonera C, Rodríguez-Lago I, Mesonero F, de Francisco R, Gómez-Gómez GJ, Chaparro M, Tardillo CA, Rivero M, Algaba A, Martín Arranz E, Cañete F, Vicente R, Sicilia B, Antolín B, Prieto V, Márquez L, Benítez JM, Camo P, Piqueras M, Gargallo CJ, Hinojosa E, Huguet JM, Pérez Calle JL, Van Domselaar M, Rodriguez C, Calvet X, Muñoz-Villafranca C, García-Sepulcre MF, Munoz-Garrido P, Fernández-Clotet A, Gómez Irwin L, Hernández S, Guardiola J, Sempere L, González Muñoza C, Hernández V, Beltrán B, Barrio J, Alba C, Moraleja I, López-Sanromán A, Riestra S, Martínez Montiel P, Garre A, Arranz L, García MJ, Martín Arranz MD, Corsino P, Arias L, Fernández-Salazar L, Fernández-Pordomingo A, Andreu M, Iglesias E, Ber Y, Mena R, Arroyo Villarino MT, Mora M, Ruiz L, López-Serrano P, Blazquez I, Villoria A, Fernández M, Bermejo F, Banales JM, Domènech E, Gisbert JP; Spanish GETECCU group (ENEIDA Project). Clinical Characteristics, Associated Malignancies and Management of Primary Sclerosing Cholangitis in Inflammatory Bowel Disease Patients: A Multicentre Retrospective Cohort Study. *J Crohns Colitis* 2019; **13**: 1492-1500 [PMID: 31063540 DOI: 10.1093/ecco-jcc/jjz094]
17 **Mertz A**, Nguyen NA, Katsanos KH, Kwok RM. Primary sclerosing cholangitis and inflammatory bowel disease comorbidity: an update of the evidence. *Ann Gastroenterol* 2019; **32**: 124-133 [PMID: 30837784 DOI: 10.20524/aog.2019.0344]
18 **Lynch SV**, Pedersen O. The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* 2016; **375**: 2369-2379 [PMID: 27974040 DOI: 10.1056/NEJMra1600266]

19 **Ni J**, Shen TD, Chen EZ, Bittinger K, Bailey A, Roggiani M, Sirota-Madi A, Friedman ES, Chau L, Lin A, Nissim I, Scott J, Lauder A, Hoffmann C, Rivas G, Albenberg L, Baldassano RN, Braun J, Xavier RJ, Clish CB, Yudkoff M, Li H, Goulian M, Bushman FD, Lewis JD, Wu GD. A role for bacterial urease in gut dysbiosis and Crohn's disease. *Sci Transl Med* 2017; **9** [PMID: 29141885 DOI: 10.1126/scitranslmed.aah6888]

20 **Balzan S**, de Almeida Quadros C, de Cleva R, Zilberstein B, Cecconello I. Bacterial translocation: overview of mechanisms and clinical impact. *J Gastroenterol Hepatol* 2007; **22**: 464-471 [PMID: 17376034 DOI: 10.1111/j.1440-1746.2007.04933.x]

21 **Yu LC**. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. *J Biomed Sci* 2018; **25**: 79 [PMID: 30413188 DOI: 10.1186/s12929-018-0483-8]

22 **Rogala AR**, Oka A, Sartor RB. Strategies to Dissect Host-Microbial Immune Interactions That Determine Mucosal Homeostasis vs. Intestinal Inflammation in Gnotobiotic Mice. *Front Immunol* 2020; **11**: 214 [PMID: 32133003 DOI: 10.3389/fimmu.2020.00214]

23 **Matson V**, Chervin CS, Gajewski TF. Cancer and the Microbiome-Influence of the Commensal Microbiota on Cancer, Immune Responses, and Immunotherapy. *Gastroenterology* 2021; **160**: 600-613 [PMID: 33253684 DOI: 10.1053/j.gastro.2020.11.041]

24 **Kåhrström CT**. Host response: Phagocytosis runs like clockwork. *Nat Rev Microbiol* 2012; **10**: 162 [PMID: 22330881 DOI: 10.1038/nrmicro2751]

25 **Swidsinski A**, Göktas O, Bessler C, Loening-Baucke V, Hale LP, Andree H, Weizenegger M, Hölzl M, Scherer H, Lochs H. Spatial organisation of microbiota in quiescent adenoiditis and tonsillitis. *J Clin Pathol* 2007; **60**: 253-260 [PMID: 16698947 DOI: 10.1136/jcp.2006.037309]

26 **Motta JP**, Flannigan KL, Agbor TA, Beatty JK, Blackler RW, Workentine ML, Da Silva GJ, Wang R, Buret AG, Wallace JL. Hydrogen sulfide protects from colitis and restores intestinal microbiota biofilm and mucus production. *Inflamm Bowel Dis* 2015; **21**: 1006-1017 [PMID: 25738373 DOI: 10.1097/MIB.0000000000000345]

27 **Díaz P**, Valenzuela Valderrama M, Bravo J, Quest AFG. *Helicobacter pylori* and Gastric Cancer: Adaptive Cellular Mechanisms Involved in Disease Progression. *Front Microbiol* 2018; **9**: 5 [PMID: 29403459 DOI: 10.3389/fmicb.2018.00005]

28 **Flanagan L**, Schmid J, Ebert M, Soucek P, Kunicka T, Liska V, Bruha J, Neary P, Dezeeuw N, Tommasino M, Jenab M, Prehn JH, Hughes DJ. Fusobacterium nucleatum associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 1381-1390 [PMID: 24599709 DOI: 10.1007/s10096-014-2081-3]

29 **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]

30 **Wu N**, Yang X, Zhang R, Li J, Xiao X, Hu Y, Chen Y, Yang F, Lu N, Wang Z, Luan C, Liu Y, Wang B, Xiang C, Wang Y, Zhao F, Gao GF, Wang S, Li L, Zhang H, Zhu B. Dysbiosis signature of fecal microbiota in colorectal cancer patients. *Microb Ecol* 2013; **66**: 462-470 [PMID: 23733170 DOI: 10.1007/s00248-013-0245-9]

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

32 **Claessen MM**, Schipper ME, Oldenburg B, Siersema PD, Offerhaus GJ, Vleggaar FP. WNT-pathway activation in IBD-associated colorectal carcinogenesis: potential biomarkers for colonic surveillance. *Cell Oncol* 2010; **32**: 303-310 [PMID: 20208143 DOI: 10.3233/CLO-2009-0503]

33 **Ma XT**, Wang S, Ye YJ, Du RY, Cui ZR, Somsouk M. Constitutive activation of Stat3 signaling pathway in human colorectal carcinoma. *World J Gastroenterol* 2004; **10**: 1569-1573 [PMID: 15162527 DOI: 10.3748/wjg.v10.i11.1569]

34 **Lin L**, Hron JD, Peng SL. Regulation of NF-kappaB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. *Immunity* 2004; **21**: 203-213 [PMID: 15308101 DOI: 10.1016/j.immuni.2004.06.016]

35 **Romano M**, DE Francesco F, Zarantonello L, Ruffolo C, Ferraro GA, Zanus G, Giordano A, Bassi N, Cillo U. From Inflammation to Cancer in Inflammatory Bowel Disease: Molecular Perspectives. *Anticancer Res* 2016; **36**: 1447-1460 [PMID: 27069120]

36 **Laurent C**, Svrcek M, Flejou JF, Chenard MP, Duclos B, Freund JN, Reimund JM. Immunohistochemical expression of CDX2, β-catenin, and TP53 in inflammatory bowel disease-associated colorectal cancer. *Inflamm Bowel Dis* 2011; **17**: 232-240 [PMID: 20815042 DOI: 10.1002/ibd.21451]

37 **Kanaan Z**, Rai SN, Eichenberger MR, Barnes C, Dworkin AM, Weller C, Cohen E, Roberts H, Keskey B, Petras RE, Crawford NP, Galandiuk S. Differential microRNA expression tracks neoplastic progression in inflammatory bowel disease-associated colorectal cancer. *Hum Mutat* 2012; **33**: 551-560 [PMID: 22241525 DOI: 10.1002/humu.22021]

38 **Alpert L**, Yassan L, Poon R, Kadri S, Niu N, Patil SA, Mujacic I, Montes D, Galbo F, Wurst MN, Zhen CJ, Cohen RD, Rubin DT, Pekow JR, Weber CR, Xiao SY, Hart J, Segal J, Setia N. Targeted mutational analysis of inflammatory bowel disease-associated colorectal cancers. *Hum Pathol* 2019; **89**: 44-50 [PMID: 31054900 DOI: 10.1016/j.humpath.2019.04.013]

39 **Watanabe S**, Tsuchiya K, Nishimura R, Shirasaki T, Katsukura N, Hibiya S, Okamoto R, Nakamura T, Watanabe M. *TP53* Mutation by CRISPR System Enhances the Malignant Potential of Colon Cancer. *Mol Cancer Res* 2019; **17**: 1459-1467 [PMID: 30988165 DOI: 10.1158/1541-7786.MCR-18-1195]

40 **Lin L**, Liu A, Peng Z, Lin HJ, Li PK, Li C, Lin J. STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. *Cancer Res* 2011; **71**: 7226-7237 [PMID: 21900397 DOI: 10.1158/0008-5472.CAN-10-4660]

41 **Corvinus FM**, Orth C, Moriggl R, Tsareva SA, Wagner S, Pfitzner EB, Baus D, Kaufmann R, Huber LA, Zatloukal K, Beug H, Ohlschläger P, Schütz A, Halbhuber KJ, Friedrich K. Persistent STAT3 activation in colon cancer is associated with enhanced cell proliferation and tumor growth. *Neoplasia* 2005; **7**: 545-555 [PMID: 16036105 DOI:

10.1593/neo.04571]
42 **Lin Q**, Lai R, Chirieac LR, Li C, Thomazy VA, Grammatikakis I, Rassidakis GZ, Zhang W, Fujio Y, Kunisada K, Hamilton SR, Amin HM. Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. *Am J Pathol* 2005; **167**: 969-980 [PMID: 16192633 DOI: 10.1016/s0002-9440(10)61187-x]
43 **Gui X**, Iacucci M, Ghosh S. Dysregulation of IL6/IL6R-STAT3-SOCS3 signaling pathway in IBD-associated colorectal dysplastic lesions as compared to sporadic colorectal adenomas in non-IBD patients. *Pathol Res Pract* 2020; **216**: 153211 [PMID: 32979687 DOI: 10.1016/j.prp.2020.153211]
44 **de Lau W**, Barker N, Clevers H. WNT signaling in the normal intestine and colorectal cancer. *Front Biosci* 2007; **12**: 471-491 [PMID: 17127311 DOI: 10.2741/2076]
45 **Pinto D**, Clevers H. Wnt control of stem cells and differentiation in the intestinal epithelium. *Exp Cell Res* 2005; **306**: 357-363 [PMID: 15925592 DOI: 10.1016/j.yexcr.2005.02.022]
46 **Aust DE**, Terdiman JP, Willenbucher RF, Chang CG, Molinaro-Clark A, Baretton GB, Loehrs U, Waldman FM. The APC/beta-catenin pathway in ulcerative colitis-related colorectal carcinomas: a mutational analysis. *Cancer* 2002; **94**: 1421-1427 [PMID: 11920497 DOI: 10.1002/cncr.10334]
47 **Nazemalhosseini Mojarad E**, Kashfi SM, Mirtalebi H, Almasi S, Chaleshi V, Kishani Farahani R, Tarban P, Molaei M, Zali MR, J K Kuppen P. Prognostic Significance of Nuclear β-Catenin Expression in Patients with Colorectal Cancer from Iran. *Iran Red Crescent Med J* 2015; **17**: e22324 [PMID: 26421170 DOI: 10.5812/ircmj.22324v2]
48 **Mårtensson A**, Oberg A, Jung A, Cederquist K, Stenling R, Palmqvist R. Beta-catenin expression in relation to genetic instability and prognosis in colorectal cancer. *Oncol Rep* 2007; **17**: 447-452 [PMID: 17203186]
49 **Warren S**, Sommers SC. Pathogenesis of ulcerative colitis. *Am J Pathol* 1949; **25**: 657-679 [PMID: 18152861]
50 **Chiu K**, Riddell RH, Schaeffer DF. DALM, rest in peace: a pathologist's perspective on dysplasia in inflammatory bowel disease in the post-DALM era. *Mod Pathol* 2018; **31**: 1180-1190 [PMID: 29789648 DOI: 10.1038/s41379-018-0068-9]
51 **Mark-Christensen A**, Laurberg S, Haboubi N. Dysplasia in Inflammatory Bowel Disease: Historical Review, Critical Histopathological Analysis, and Clinical Implications. *Inflamm Bowel Dis* 2018; **24**: 1895-1903 [PMID: 29668897 DOI: 10.1093/ibd/izy075]
52 **Iacucci M**, Uraoka T, Fort Gasia M, Yahagi N. Novel diagnostic and therapeutic techniques for surveillance of dysplasia in patients with inflammatory bowel disease. *Can J Gastroenterol Hepatol* 2014; **28**: 361-370 [PMID: 25157526 DOI: 10.1155/2014/825947]
53 **Laine L**, Kaltenbach T, Barkun A, McQuaid KR, Subramanian V, Soetikno R; SCENIC Guideline Development Panel. SCENIC international consensus statement on surveillance and management of dysplasia in inflammatory bowel disease. *Gastroenterology* 2015; **148**: 639-651.e28 [PMID: 25702852 DOI: 10.1053/j.gastro.2015.01.031]
54 **Wu XR**, Zheng XB, Huang Y, Cao Q, Zhang HJ, Miao YL, Zou KF, Chen M, Zhang FM, Mei Q, Gonzalo D, Allende D, Hu PJ, Shen B, Liu XL, Lan P. Risk factors for colorectal neoplasia in patients with underlying inflammatory bowel disease: a multicenter study. *Gastroenterol Rep (Oxf)* 2019; **7**: 67-73 [PMID: 30792868 DOI: 10.1093/gastro/goy039]
55 **de Jong ME**, Kanne H, Nissen LHC, Drenth JPH, Derikx LAAP, Hoentjen F. Increased risk of high-grade dysplasia and colorectal cancer in inflammatory bowel disease patients with recurrent low-grade dysplasia. *Gastrointest Endosc* 2020; **91**: 1334-1342.e1 [PMID: 31923409 DOI: 10.1016/j.gie.2019.12.041]
56 **Sonnenberg A**, Genta RM. Epithelial Dysplasia and Cancer in IBD Strictures. *J Crohns Colitis* 2015; **9**: 769-775 [PMID: 26079724 DOI: 10.1093/ecco-jcc/jjv108]
57 **Rutter MD**, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC, Forbes A. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. *Gastroenterology* 2006; **130**: 1030-1038 [PMID: 16618396 DOI: 10.1053/j.gastro.2005.12.035]
58 **Cairns SR**, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, Eaden JA, Rutter MD, Atkin WP, Saunders BP, Lucassen A, Jenkins P, Fairclough PD, Woodhouse CR; British Society of Gastroenterology; Association of Coloproctology for Great Britain and Ireland. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut* 2010; **59**: 666-689 [PMID: 20427401 DOI: 10.1136/gut.2009.179804]
59 **Lutgens MW**, Oldenburg B, Siersema PD, van Bodegraven AA, Dijkstra G, Hommes DW, de Jong DJ, Stokkers PC, van der Woude CJ, Vleggaar FP. Colonoscopic surveillance improves survival after colorectal cancer diagnosis in inflammatory bowel disease. *Br J Cancer* 2009; **101**: 1671-1675 [PMID: 19826420 DOI: 10.1038/sj.bjc.6605359]
60 **Lamb CA**, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**: s1-s106 [PMID: 31562236 DOI: 10.1136/gutjnl-2019-318484]
61 **Magro F**, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, Burisch J, Gecse KB, Hart AL, Hindryckx P, Langner C, Limdi JK, Pellino G, Zagórowicz E, Raine T, Harbord M, Rieder F; European Crohn's and Colitis Organisation [ECCO]. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J Crohns Colitis* 2017; **11**: 649-670 [PMID: 28158501 DOI: 10.1093/ecco-jcc/jjx008]
62 **Iacucci M**, McQuaid K, Gui XS, Iwao Y, Lethebe BC, Lowerison M, Matsumoto T, Shivaji UN, Smith SCL, Subramanian V, Uraoka T, Sanduleanu S, Ghosh S, Kiesslich R. A multimodal (FACILE) classification for optical diagnosis of inflammatory bowel disease associated neoplasia. *Endoscopy* 2019; **51**: 133-141 [PMID: 30541154 DOI: 10.1055/a-0757-7759]
63 **Watanabe K**, Nishishita M, Shimamoto F, Fukuchi T, Esaki M, Okamoto Y, Maehata Y, Oka S, Nishiyama S, Fujii S, Hirai F, Matsui T, Kakimoto K, Okada T, Inoue T, Hida N, Goto H, Nozaki R, Sakurai T, Kashida H, Takeuchi K, Ohmiya N, Saruta M, Saito S, Saito Y, Tanaka S, Fujiwara Y, Arakawa T, Suzuki Y, Ajioka Y, Tajiri H. 722 Comparison Between Newly-Developed Narrow Band Imaging and Panchromoendoscopy for Surveillance Colonoscopy in Patients With Longstanding Ulcerative Colitis: A Prospective Multicenter Randomized Controlled Trial, Navigator Study.

Gastrointestinal Endoscopy 2016; **83**: AB172 [DOI: 10.1016/j.gie.2016.03.147]

64 **Bisschops R**, Bessissow T, Dekker E, East JE, Para-Blanco A, Ragunath K, Bhandari P, Rutter M, Schoon E, Wilson A, John JM, Van Steen K, Baert F, Ferrante M. Pit pattern analysis with high-definition chromoendoscopy and narrow-band imaging for optical diagnosis of dysplasia in patients with ulcerative colitis. *Gastrointest Endosc* 2017; **86**: 1100-1106.e1 [PMID: 28986266 DOI: 10.1016/j.gie.2017.09.024]

65 **Collins PD**, Mpofu C, Watson AJ, Rhodes JM. Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. *Cochrane Database Syst Rev* 2006; CD000279 [PMID: 16625534 DOI: 10.1002/14651858.CD000279.pub3]

66 **Bye WA**, Ma C, Nguyen TM, Parker CE, Jairath V, East JE. Strategies for Detecting Colorectal Cancer in Patients with Inflammatory Bowel Disease: A Cochrane Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2018; **113**: 1801-1809 [PMID: 30353058 DOI: 10.1038/s41395-018-0354-7]

67 **Watanabe T**, Ajioka Y, Mitsuyama K, Watanabe K, Hanai H, Nakase H, Kunisaki R, Matsuda K, Iwakiri R, Hida N, Tanaka S, Takeuchi Y, Ohtsuka K, Murakami K, Kobayashi K, Iwao Y, Nagahori M, Iizuka B, Hata K, Igarashi M, Hirata I, Kudo SE, Matsumoto T, Ueno F, Watanabe G, Ikegami M, Ito Y, Oba K, Inoue E, Tomotsugu N, Takebayashi T, Sugihara K, Suzuki Y, Watanabe M, Hibi T. Comparison of Targeted *vs* Random Biopsies for Surveillance of Ulcerative Colitis-Associated Colorectal Cancer. *Gastroenterology* 2016; **151**: 1122-1130 [PMID: 27523980 DOI: 10.1053/j.gastro.2016.08.002]

68 **Bisschops R**, East JE, Hassan C, Hazewinkel Y, Kamiński MF, Neumann H, Pellisé M, Antonelli G, Bustamante Balen M, Coron E, Cortas G, Iacucci M, Yuichi M, Longcroft-Wheaton G, Mouzyka S, Pilonis N, Puig I, van Hooft JE, Dekker E. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - Update 2019. *Endoscopy* 2019; **51**: 1155-1179 [PMID: 31711241 DOI: 10.1055/a-1031-7657]

69 **Marion JF**, Waye JD, Present DH, Israel Y, Bodian C, Harpaz N, Chapman M, Itzkowitz S, Steinlauf AF, Abreu MT, Ullman TA, Aisenberg J, Mayer L; Chromoendoscopy Study Group at Mount Sinai School of Medicine. Chromoendoscopy-targeted biopsies are superior to standard colonoscopic surveillance for detecting dysplasia in inflammatory bowel disease patients: a prospective endoscopic trial. *Am J Gastroenterol* 2008; **103**: 2342-2349 [PMID: 18844620 DOI: 10.1111/j.1572-0241.2008.01934.x]

70 **Imperatore N**, Castiglione F, Testa A, De Palma GD, Caporaso N, Cassese G, Rispo A. Augmented Endoscopy for Surveillance of Colonic Inflammatory Bowel Disease: Systematic Review With Network Meta-analysis. *J Crohns Colitis* 2019; **13**: 714-724 [PMID: 30597029 DOI: 10.1093/ecco-jcc/jjy218]

71 **Gasia MF**, Ghosh S, Panaccione R, Ferraz JG, Kaplan GG, Leung Y, Novak KL, Seow CH, Iacucci M. Targeted Biopsies Identify Larger Proportions of Patients With Colonic Neoplasia Undergoing High-Definition Colonoscopy, Dye Chromoendoscopy, or Electronic Virtual Chromoendoscopy. *Clin Gastroenterol Hepatol* 2016; **14**: 704-12.e4 [PMID: 26804384 DOI: 10.1016/j.cgh.2015.12.047]

72 **Iacucci M**, Kaplan GG, Panaccione R, Akinola O, Lethebe BC, Lowerison M, Leung Y, Novak KL, Seow CH, Urbanski S, Minoo P, Gui X, Ghosh S. A Randomized Trial Comparing High Definition Colonoscopy Alone With High Definition Dye Spraying and Electronic Virtual Chromoendoscopy for Detection of Colonic Neoplastic Lesions During IBD Surveillance Colonoscopy. *Am J Gastroenterol* 2018; **113**: 225-234 [PMID: 29134964 DOI: 10.1038/ajg.2017.417]

73 **Lippman SM**, Benner SE, Hong WK. Cancer chemoprevention. *J Clin Oncol* 1994; **12**: 851-873 [PMID: 8151328 DOI: 10.1200/jco.1994.12.4.851]

74 **Lyakhovich A**, Gasche C. Systematic review: molecular chemoprevention of colorectal malignancy by mesalazine. *Aliment Pharmacol Ther* 2010; **31**: 202-209 [PMID: 19891667 DOI: 10.1111/j.1365-2036.2009.04195.x]

75 **Andrews JM**, Travis SP, Gibson PR, Gasche C. Systematic review: does concurrent therapy with 5-ASA and immunomodulators in inflammatory bowel disease improve outcomes? *Aliment Pharmacol Ther* 2009; **29**: 459-469 [PMID: 19077129 DOI: 10.1111/j.1365-2036.2008.03915.x]

76 **Qiu X**, Ma J, Wang K, Zhang H. Chemopreventive effects of 5-aminosalicylic acid on inflammatory bowel disease-associated colorectal cancer and dysplasia: a systematic review with meta-analysis. *Oncotarget* 2017; **8**: 1031-1045 [PMID: 27906680 DOI: 10.18632/oncotarget.13715]

77 **Velayos FS**, Terdiman JP, Walsh JM. Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: a systematic review and metaanalysis of observational studies. *Am J Gastroenterol* 2005; **100**: 1345-1353 [PMID: 15929768 DOI: 10.1111/j.1572-0241.2005.41442.x]

78 **Bonovas S**, Fiorino G, Lytras T, Nikolopoulos G, Peyrin-Biroulet L, Danese S. Systematic review with meta-analysis: use of 5-aminosalicylates and risk of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2017; **45**: 1179-1192 [PMID: 28261835 DOI: 10.1111/apt.14023]

79 **Jess T**, Lopez A, Andersson M, Beaugerie L, Peyrin-Biroulet L. Thiopurines and risk of colorectal neoplasia in patients with inflammatory bowel disease: a meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 1793-1800.e1 [PMID: 24907505 DOI: 10.1016/j.cgh.2014.05.019]

80 **Lu MJ**, Qiu XY, Mao XQ, Li XT, Zhang HJ. Systematic review with meta-analysis: thiopurines decrease the risk of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2018; **47**: 318-331 [PMID: 29205426 DOI: 10.1111/apt.14436]

81 **Wang D**, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 2010; **29**: 781-788 [PMID: 19946329 DOI: 10.1038/onc.2009.421]

82 **Rothwell PM**, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012; **379**: 1591-1601 [PMID: 22440947 DOI: 10.1016/S0140-6736(12)60209-8]

83 **Burr NE**, Hull MA, Subramanian V. Does aspirin or non-aspirin non-steroidal anti-inflammatory drug use prevent colorectal cancer in inflammatory bowel disease? *World J Gastroenterol* 2016; **22**: 3679-3686 [PMID: 27053860 DOI: 10.3748/wjg.v22.i13.3679]

84 **Leddin D**, Tamim H, Levy AR. Is folate involved in the pathogenesis of inflammatory bowel disease? *Med Hypotheses*

2013; **81**: 940-941 [PMID: 24045091 DOI: 10.1016/j.mehy.2013.08.025]

85 **Lashner BA**, Heidenreich PA, Su GL, Kane SV, Hanauer SB. Effect of folate supplementation on the incidence of dysplasia and cancer in chronic ulcerative colitis. A case-control study. *Gastroenterology* 1989; **97**: 255-259 [PMID: 2568304 DOI: 10.1016/0016-5085(89)90058-9]

86 **Burr NE**, Hull MA, Subramanian V. Folic Acid Supplementation May Reduce Colorectal Cancer Risk in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol* 2017; **51**: 247-253 [PMID: 26905603 DOI: 10.1097/MCG.0000000000000498]

87 **Rutter MD**. Importance of nonpolypoid (flat and depressed) colorectal neoplasms in screening for CRC in patients with IBD. *Gastrointest Endosc Clin N Am* 2014; **24**: 327-335 [PMID: 24975524 DOI: 10.1016/j.giec.2014.03.002]

88 **Siegel CA**, Schwartz LM, Woloshin S, Cole EB, Rubin DT, Vay T, Baars J, Sands BE. When should ulcerative colitis patients undergo colectomy for dysplasia? *Inflamm Bowel Dis* 2010; **16**: 1658-1662 [PMID: 20186940 DOI: 10.1002/ibd.21233]

89 **Ten Hove JR**, Mooiweer E, van der Meulen de Jong AE, Dekker E, Ponsioen CY, Siersema PD, Oldenburg B. Clinical implications of low grade dysplasia found during inflammatory bowel disease surveillance: a retrospective study comparing chromoendoscopy and white-light endoscopy. *Endoscopy* 2017; **49**: 161-168 [PMID: 27951611 DOI: 10.1055/s-0042-119394]

90 **Kabir M**, Fofaria R, Arebi N, Bassett P, Tozer PJ, Hart AL, Thomas-Gibson S, Humphries A, Suzuki N, Saunders B, Warusavitarne J, Faiz O, Wilson A. Systematic review with meta-analysis: IBD-associated colonic dysplasia prognosis in the videoendoscopic era (1990 to present). *Aliment Pharmacol Ther* 2020; **52**: 5-19 [PMID: 32432797 DOI: 10.1111/apt.15778]

91 **Fong W**, Li Q, Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 2020; **39**: 4925-4943 [PMID: 32514151 DOI: 10.1038/s41388-020-1341-1]

92 **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]

93 **Shaoul R**, Day AS. Nutritional regulators of intestinal inflammation. *Curr Opin Gastroenterol* 2019; **35**: 486-490 [PMID: 31464809 DOI: 10.1097/MOG.0000000000000585]

94 **López-Alarcón M**, Perichart-Perera O, Flores-Huerta S, Inda-Icaza P, Rodríguez-Cruz M, Armenta-Álvarez A, Bram-Falcón MT, Mayorga-Ochoa M. Excessive refined carbohydrates and scarce micronutrients intakes increase inflammatory mediators and insulin resistance in prepubertal and pubertal obese children independently of obesity. *Mediators Inflamm* 2014; **2014**: 849031 [PMID: 25477716 DOI: 10.1155/2014/849031]

95 **De Almeida CV**, de Camargo MR, Russo E, Amedei A. Role of diet and gut microbiota on colorectal cancer immunomodulation. *World J Gastroenterol* 2019; **25**: 151-162 [PMID: 30670906 DOI: 10.3748/wjg.v25.i2.151]

96 **Demeyer D**, Honikel K, De Smet S. The World Cancer Research Fund report 2007: A challenge for the meat processing industry. *Meat Sci* 2008; **80**: 953-959 [PMID: 22063824 DOI: 10.1016/j.meatsci.2008.06.003]

97 **Limketkai BN**, Wolf A, Parian AM. Nutritional Interventions in the Patient with Inflammatory Bowel Disease. *Gastroenterol Clin North Am* 2018; **47**: 155-177 [PMID: 29413010 DOI: 10.1016/j.gtc.2017.09.007]

98 **Batista D**, Raffals L. Role of intestinal bacteria in the pathogenesis of pouchitis. *Inflamm Bowel Dis* 2014; **20**: 1481-1486 [PMID: 25046009 DOI: 10.1097/MIB.0000000000000055]

99 **Konstantinov SR**, Kuipers EJ, Peppelenbosch MP. Functional genomic analyses of the gut microbiota for CRC screening. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 741-745 [PMID: 24042452 DOI: 10.1038/nrgastro.2013.178]

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

101 **Fukuda S**, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 2011; **469**: 543-547 [PMID: 21270894 DOI: 10.1038/nature09646]

102 **Costello SP**, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, Katsikeros R, Makanyanga J, Campaniello MA, Mavrangelos C, Rosewarne CP, Bickley C, Peters C, Schoeman MN, Conlon MA, Roberts-Thomson IC, Andrews JM. Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis: A Randomized Clinical Trial. *JAMA* 2019; **321**: 156-164 [PMID: 30644982 DOI: 10.1001/jama.2018.20046]

103 **Paramsothy S**, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, Leong RWL, Connor S, Ng W, Paramsothy R, Xuan W, Lin E, Mitchell HM, Borody TJ. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 2017; **389**: 1218-1228 [PMID: 28214091 DOI: 10.1016/S0140-6736(17)30182-4]

104 **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]

105 **Liu JZ**, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, Ripke S, Lee JC, Jostins L, Shah T, Abedian S, Cheon JH, Cho J, Dayani NE, Franke L, Fuyuno Y, Hart A, Juyal RC, Juyal G, Kim WH, Morris AP, Poustchi H, Newman WG, Midha V, Orchard TR, Vahedi H, Sood A, Sung JY, Malekzadeh R, Westra HJ, Yamazaki K, Yang SK; International Multiple Sclerosis Genetics Consortium; International IBD Genetics Consortium, Barrett JC, Alizadeh BZ, Parkes M, Bk T, Daly MJ, Kubo M, Anderson CA, Weersma RK. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015; **47**: 979-986 [PMID: 26192919 DOI: 10.1038/ng.3359]

106 **Kudo T**, Suzuki K, Mori Y, Misawa M, Ichimasa K, Takeda K, Nakamura H, Maeda Y, Ogawa Y, Hayashi T, Wakamura K, Ishida F, Inoue H, Kudo SE. Endocytoscopy for the differential diagnosis of colorectal low-grade adenoma: a novel possibility for the "resect and discard" strategy. *Gastrointest Endosc* 2020; **91**: 676-683 [PMID: 31785276 DOI: 10.1016/j.gie.2019.11.029]

107 **Wang KK**, Carr-Locke DL, Singh SK, Neumann H, Bertani H, Galmiche JP, Arsenescu RI, Caillol F, Chang KJ, Chaussade S, Coron E, Costamagna G, Dlugosz A, Ian Gan S, Giovannini M, Gress FG, Haluszka O, Ho KY, Kahaleh M, Konda VJ, Prat F, Shah RJ, Sharma P, Slivka A, Wolfsen HC, Zfass A. Use of probe-based confocal laser endomicroscopy (pCLE) in gastrointestinal applications. A consensus report based on clinical evidence. *United European Gastroenterol J* 2015; **3**: 230-254 [PMID: 26137298 DOI: 10.1177/2050640614566066]

108 **Kudo T**, Saito Y, Ikematsu H, Hotta K, Takeuchi Y, Shimatani M, Kawakami K, Tamai N, Mori Y, Maeda Y, Yamada M, Sakamoto T, Matsuda T, Imai K, Ito S, Hamada K, Fukata N, Inoue T, Tajiri H, Yoshimura K, Ishikawa H, Kudo SE. New-generation full-spectrum endoscopy *vs* standard forward-viewing colonoscopy: a multicenter, randomized, tandem colonoscopy trial (J-FUSE Study). *Gastrointest Endosc* 2018; **88**: 854-864 [PMID: 29908178 DOI: 10.1016/j.gie.2018.06.011]

109 **Stidham RW**, Liu W, Bishu S, Rice MD, Higgins PDR, Zhu J, Nallamothu BK, Waljee AK. Performance of a Deep Learning Model *vs* Human Reviewers in Grading Endoscopic Disease Severity of Patients With Ulcerative Colitis. *JAMA Netw Open* 2019; **2**: e193963 [PMID: 31099869 DOI: 10.1001/jamanetworkopen.2019.3963]

110 **Maeda Y**, Kudo SE, Ogata N, Misawa M, Mori Y, Mori K, Ohtsuka K. Can artificial intelligence help to detect dysplasia in patients with ulcerative colitis? *Endoscopy* 2021; **53**: E273-E274 [PMID: 33003217 DOI: 10.1055/a-1261-2944]

111 **Stewénius J**, Adnerhill I, Anderson H, Ekelund GR, Florén CH, Fork FT, Janzon L, Lindström C, Ogren M. Incidence of colorectal cancer and all cause mortality in non-selected patients with ulcerative colitis and indeterminate colitis in Malmö, Sweden. *Int J Colorectal Dis* 1995; **10**: 117-122 [PMID: 7636371 DOI: 10.1007/bf00341210]

112 **Castaño-Milla C**, Chaparro M, Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther* 2014; **39**: 645-659 [PMID: 24612141 DOI: 10.1111/apt.12651]

113 **Olén O**, Erichsen R, Sachs MC, Pedersen L, Halfvarson J, Askling J, Ekbom A, Sørensen HT, Ludvigsson JF. Colorectal cancer in Crohn's disease: a Scandinavian population-based cohort study. *Lancet Gastroenterol Hepatol* 2020; **5**: 475-484 [PMID: 32066530 DOI: 10.1016/S2468-1253(20)30005-4]

114 Laukoetter MG, Mennigen R, Hannig CM, Osada N, Rijcken E, Vowinkel T, Krieglstein CF, Senninger N, Anthoni C, Bruewer M. Intestinal cancer risk in Crohn's disease: a meta-analysis. *J Gastrointest Surg* 2011; **15**: 576-583 [PMID: 21152994 DOI: 10.1007/s11605-010-1402-9]

115 **Fumery M**, Dulai PS, Gupta S, Prokop LJ, Ramamoorthy S, Sandborn WJ, Singh S. Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients With Ulcerative Colitis With Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 665-674.e5 [PMID: 27916678 DOI: 10.1016/j.cgh.2016.11.025]

116 **Friedman S**, Rubin PH, Bodian C, Harpaz N, Present DH. Screening and surveillance colonoscopy in chronic Crohn's colitis: results of a surveillance program spanning 25 years. *Clin Gastroenterol Hepatol* 2008; **6**: 993-8; quiz 953 [PMID: 18585966 DOI: 10.1016/j.cgh.2008.03.019]

117 **Basseri RJ**, Basseri B, Vassilaki ME, Melmed GY, Ippoliti A, Vasiliauskas EA, Fleshner PR, Lechago J, Hu B, Berel D, Targan SR, Papadakis KA. Colorectal cancer screening and surveillance in Crohn's colitis. *J Crohns Colitis* 2012; **6**: 824-829 [PMID: 22398087 DOI: 10.1016/j.crohns.2012.01.005]

118 **Keller DS**, Windsor A, Cohen R, Chand M. Colorectal cancer in inflammatory bowel disease: review of the evidence. *Tech Coloproctol* 2019; **23**: 3-13 [PMID: 30701345 DOI: 10.1007/s10151-019-1926-2]

119 **Pache I**, Rogler G, Felley C. TNF-alpha blockers in inflammatory bowel diseases: practical consensus recommendations and a user's guide. *Swiss Med Wkly* 2009; **139**: 278-287 [PMID: 19452290]

120 **Kobelt D**, Dahlmann M, Dumbani M, Güllü N, Kortüm B, Vílchez MEA, Stein U, Walther W. Small Ones to Fight a Big Problem-Intervention of Cancer Metastasis by Small Molecules. *Cancers (Basel)* 2020; **12** [PMID: 32503267 DOI: 10.3390/cancers12061454]

121 **Atreya R**, Neurath MF. Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. *Clin Rev Allergy Immunol* 2005; **28**: 187-196 [PMID: 16129903 DOI: 10.1385/criai:28:3:187]

122 **Allocca M**, Jovani M, Fiorino G, Schreiber S, Danese S. Anti-IL-6 treatment for inflammatory bowel diseases: next cytokine, next target. *Curr Drug Targets* 2013; **14**: 1508-1521 [PMID: 24102406 DOI: 10.2174/13894501113146660224]

123 **Coskun M**, Vermeire S, Nielsen OH. Novel Targeted Therapies for Inflammatory Bowel Disease. *Trends Pharmacol Sci* 2017; **38**: 127-142 [PMID: 27916280 DOI: 10.1016/j.tips.2016.10.014]

124 **Danese S**, Vermeire S, Hellstern P, Panaccione R, Rogler G, Fraser G, Kohn A, Desreumaux P, Leong RW, Comer GM, Cataldi F, Banerjee A, Maguire MK, Li C, Rath N, Beebe J, Schreiber S. Randomised trial and open-label extension study of an anti-interleukin-6 antibody in Crohn's disease (ANDANTE I and II). *Gut* 2019; **68**: 40-48 [PMID: 29247068 DOI: 10.1136/gutjnl-2017-314562]

125 **Murakami M**, Kamimura D, Hirano T. Pleiotropy and Specificity: Insights from the Interleukin 6 Family of Cytokines. *Immunity* 2019; **50**: 812-831 [PMID: 30995501 DOI: 10.1016/j.immuni.2019.03.027]

126 **Johnstone CN**, Chand A, Putoczki TL, Ernst M. Emerging roles for IL-11 signaling in cancer development and progression: Focus on breast cancer. *Cytokine Growth Factor Rev* 2015; **26**: 489-498 [PMID: 26209885 DOI: 10.1016/j.cytogfr.2015.07.015]

127 **Ren C**, Chen Y, Han C, Fu D, Chen H. Plasma interleukin-11 (IL-11) levels have diagnostic and prognostic roles in patients with pancreatic cancer. *Tumour Biol* 2014; **35**: 11467-11472 [PMID: 25123265 DOI: 10.1007/s13277-014-2459-y]

128 **Unver N**, McAllister F. IL-6 family cytokines: Key inflammatory mediators as biomarkers and potential therapeutic targets. *Cytokine Growth Factor Rev* 2018; **41**: 10-17 [PMID: 29699936 DOI: 10.1016/j.cytogfr.2018.04.004]

129 **Pastor MD**, Nogal A, Molina-Pinelo S, Quintanal-Villalonga Á, Meléndez R, Ferrer I, Romero-Romero B, De Miguel MJ, López-Campos JL, Corral J, García-Carboner R, Carnero A, Paz-Ares L. IL-11 and CCL-1: Novel Protein Diagnostic Biomarkers of Lung Adenocarcinoma in Bronchoalveolar Lavage Fluid (BALF). *J Thorac Oncol* 2016; **11**: 2183-2192 [PMID: 27524264 DOI: 10.1016/j.jtho.2016.07.026]

130 **Putoczki TL**, Thiem S, Loving A, Busuttil RA, Wilson NJ, Ziegler PK, Nguyen PM, Preaudet A, Farid R, Edwards KM, Boglev Y, Luwor RB, Jarnicki A, Horst D, Boussioutas A, Heath JK, Sieber OM, Pleines I, Kile BT, Nash A, Greten FR, McKenzie BS, Ernst M. Interleukin-11 is the dominant IL-6 family cytokine during gastrointestinal tumorigenesis and can

be targeted therapeutically. *Cancer Cell* 2013; **24**: 257-271 [PMID: 23948300 DOI: 10.1016/j.ccr.2013.06.017]

131 **Hohenberger M**, Cardwell LA, Oussedik E, Feldman SR. Interleukin-17 inhibition: role in psoriasis and inflammatory bowel disease. *J Dermatolog Treat* 2018; **29**: 13-18 [PMID: 28521565 DOI: 10.1080/09546634.2017.1329511]

132 **Moschen AR**, Tilg H, Raine T. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 185-196 [PMID: 30478416 DOI: 10.1038/s41575-018-0084-8]

133 **Di Fusco D**, Izzo R, Figliuzzi MM, Pallone F, Monteleone G. IL-21 as a therapeutic target in inflammatory disorders. *Expert Opin Ther Targets* 2014; **18**: 1329-1338 [PMID: 25162763 DOI: 10.1517/14728222.2014.945426]

134 **Salzer E**, Kansu A, Sic H, Májek P, Ikincioğullari A, Dogu FE, Prengemann NK, Santos-Valente E, Pickl WF, Bilic I, Ban SA, Kuloğlu Z, Demir AM, Ensari A, Colinge J, Rizzi M, Eibel H, Boztug K. Early-onset inflammatory bowel disease and common variable immunodeficiency-like disease caused by IL-21 deficiency. *J Allergy Clin Immunol* 2014; **133**: 1651-9.e12 [PMID: 24746753 DOI: 10.1016/j.jaci.2014.02.034]

135 **Neurath MF**. IL-23 in inflammatory bowel diseases and colon cancer. *Cytokine Growth Factor Rev* 2019; **45**: 1-8 [PMID: 30563755 DOI: 10.1016/j.cytogfr.2018.12.002]

136 **Rosenstock E**, Farmer RG, Petras R, Sivak MV Jr, Rankin GB, Sullivan BH. Surveillance for colonic carcinoma in ulcerative colitis. *Gastroenterology* 1985; **89**: 1342-1346 [PMID: 4054527 DOI: 10.1016/0016-5085(85)90653-5]

137 **Lashner BA**, Kane SV, Hanauer SB. Colon cancer surveillance in chronic ulcerative colitis: historical cohort study. *Am J Gastroenterol* 1990; **85**: 1083-1087 [PMID: 2389720]

138 **Löfberg R**, Broström O, Karlén P, Tribukait B, Ost A. Colonoscopic surveillance in long-standing total ulcerative colitis--a 15-year follow-up study. *Gastroenterology* 1990; **99**: 1021-1031 [PMID: 2394325 DOI: 10.1016/0016-5085(90)90622-8]

139 **Nugent FW**, Haggitt RC, Gilpin PA. Cancer surveillance in ulcerative colitis. *Gastroenterology* 1991; **100**: 1241-1248 [PMID: 2013371]

140 **Lynch DA**, Lobo AJ, Sobala GM, Dixon MF, Axon AT. Failure of colonoscopic surveillance in ulcerative colitis. *Gut* 1993; **34**: 1075-1080 [PMID: 8174957 DOI: 10.1136/gut.34.8.1075]

141 **Jonsson B**, Ahsgren L, Andersson LO, Stenling R, Rutegård J. Colorectal cancer surveillance in patients with ulcerative colitis. *Br J Surg* 1994; **81**: 689-691 [PMID: 8044548 DOI: 10.1002/bjs.1800810520]

142 **Karlén P**, Kornfeld D, Broström O, Löfberg R, Persson PG, Ekbom A. Is colonoscopic surveillance reducing colorectal cancer mortality in ulcerative colitis? *Gut* 1998; **42**: 711-714 [PMID: 9659169 DOI: 10.1136/gut.42.5.711]

143 **Friedman S**, Rubin PH, Bodian C, Goldstein E, Harpaz N, Present DH. Screening and surveillance colonoscopy in chronic Crohn's colitis. *Gastroenterology* 2001; **120**: 820-826 [PMID: 11231935 DOI: 10.1053/gast.2001.22449]

144 **Biasco G**, Rossini FP, Hakim R, Brandi G, Di Battista M, Di Febo G, Calabrese C, Santucci R, Miglioli M. Cancer surveillance in ulcerative colitis: critical analysis of long-term prospective programme. *Dig Liver Dis* 2002; **34**: 339-342 [PMID: 12118951 DOI: 10.1016/s1590-8658(02)80127-x]

145 **Hata K**, Watanabe T, Kazama S, Suzuki K, Shinozaki M, Yokoyama T, Matsuda K, Muto T, Nagawa H. Earlier surveillance colonoscopy programme improves survival in patients with ulcerative colitis associated colorectal cancer: results of a 23-year surveillance programme in the Japanese population. *Br J Cancer* 2003; **89**: 1232-1236 [PMID: 14520452 DOI: 10.1038/sj.bjc.6601247]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 568-586

DOI: 10.4251/wjgo.v14.i3.568

ISSN 1948-5204 (online)

REVIEW

Barrett's esophagus: Review of natural history and comparative efficacy of endoscopic and surgical therapies

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Xu H, Yalçınkaya İ

Received: May 22, 2021
Peer-review started: May 22, 2021
First decision: October 3, 2021
Revised: November 12, 2021
Accepted: February 15, 2022
Article in press: February 15, 2022
Published online: March 15, 2022

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Abstract

Barrett's esophagus (BE) is the precursor to esophageal adenocarcinoma (EAC). Progression to cancer typically occurs in a stepwise fashion through worsening dysplasia and ultimately, invasive neoplasia. Established EAC with deep involvement of the esophageal wall and/or metastatic disease is invariably associated with poor long-term survival rates. This guides the rationale of surveillance of Barrett's in an attempt to treat lesions at an earlier, and potentially curative stage. The last two decades have seen a paradigm shift in management of Barrett's with rapid expansion in the role of endoscopic eradication therapy (EET) for management of dysplastic and early neoplastic BE, and there have been substantial changes to international consensus guidelines for management of early BE based on evolving evidence. This review aims to assist the physician in the therapeutic decision-making process with patients by comprehensive review and summary of literature surrounding natural history of Barrett's by histological stage, and the effectiveness of interventions in attenuating the risk posed by its natural history. Key findings were as follows. Non-dysplastic Barrett's is associated with extremely low risk of progression, and interventions cannot be justified. The annual risk of cancer progression in low grade dysplasia is between 1%-3%; EET can be offered though evidence for its benefit remains confined to highly select settings. High-grade dysplasia progresses to cancer in 5%-10% *per* year; EET is similarly effective to and less morbid than surgery and should be routinely performed for this indication. Risk of nodal metastases in intramucosal cancer is 2%-4%, which is comparable to operative mortality rate, so EET is usually preferred. Submucosal cancer is associated with nodal metastases in 14%-41% hence surgery remains standard of care, except for select situations.

Key Words: Barrett's esophagus; Endoscopic eradication therapy; Dysplasia; Adenocarcinoma; Natural history; Radiofrequency ablation

Core Tip: Barrett's esophagus (BE) is an important premalignant condition. The last two decades have seen treatment paradigms increasingly shift towards endoscopic eradication therapy for dysplastic and early neoplastic cases, where it appears safe and effective. We herein provide a comprehensive review of the literature relating to Barrett's natural history and comparative efficacy of surveillance, endoscopic and surgical therapies for BE by histological stage.

Citation: Choi KKH, Sanagapalli S. Barrett's esophagus: Review of natural history and comparative efficacy of endoscopic and surgical therapies. *World J Gastrointest Oncol* 2022; 14(3): 568-586
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/568.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.568

INTRODUCTION

Barrett's esophagus (BE) is an acquired condition characterised by metaplastic change of esophageal mucosal cells in response to chronic gastro-esophageal reflux. While the very definition of BE is variable (and controversial), it is most commonly diagnosed in the presence of salmon-colored mucosa extending at least 1 cm proximal to the gastroesophageal junction, where there is histopathological confirmation of replacement of normal squamous epithelium by metaplastic intestinal-type columnar epithelium. Its clinical importance primarily relates to its established status as a precursor lesion to esophageal adenocarcinoma (EAC)[1]. Worldwide, esophageal cancer ranks seventh in incidence and sixth in overall mortality[2], and is subdivided into squamous cell carcinoma and adenocarcinoma. The incidence of EAC is rising in both Western and Eastern parts of the world[3,4], with EAC now becoming the dominant type of esophageal cancer in high-income, Western countries[3-7]. EAC is associated with high morbidity and mortality, and is commonly diagnosed late with metastatic disease[5,8].

On the other hand, it is clear that in the majority of cases, EAC arises within a segment of pre-existing BE[9]. BE is thought to progress to EAC in a stepwise fashion *via* the development of dysplasia and finally, neoplasia. Hence it is logical that surveillance of patients with established BE may prevent the poor outcomes associated with EAC by the detection of treatable premalignant or earlier stage localized malignant lesions, and this seems to have been borne out in some data[10]. Recent data from the United States show metastatic EAC at diagnosis has a 5-year survival rate of 4.3%, whereas local disease has a 40.3% 5-year survival[11]. This forms the rationale for Barrett's surveillance programs that are recommended by international societies[12-14]. Concomitant with increased surveillance of BE, recent decades have also seen significant advances in therapeutic options for premalignant Barrett's, with endoscopic therapies now having entered widespread use for premalignant BE and for some cases of early EAC. This has led to significant changes in international consensus recommendations for management of BE, though these are not always entirely in agreement with each other. Controversy in management of BE persists, primarily arising from persistent uncertainties regarding natural history and identification of dysplasia.

The purpose of this review therefore is to assist the treating physician in efficient decision making in patients with BE or early EAC by reviewing the current literature regarding natural history of BE, and comparing this to our current understanding of the risks and expected efficacy of current management options including surveillance, endoscopic therapy and surgery.

LITERATURE

A comprehensive Medline search was performed using the following keywords and phrases: "Barrett's esophagus, non-dysplastic Barrett's esophagus/oesophagus, low grade dysplasia, high grade dysplasia, surveillance, esophageal cancer, Barrett's endoscopic therapy, endoscopic eradication therapy, radiofrequency ablation, endoscopic resection, esophagectomy, lymph node metastasis, adenocarcinoma, intramucosal adenocarcinoma, T1a esophageal/oesophageal adenocarcinoma, submucosa adenocarcinoma T1b esophageal/oesophageal adenocarcinoma, meta-analysis, systematic review". There was a focus on original and high-quality research. In addition, we manually reviewed reference lists of all citing references to ensure no relevant articles were excluded.

STAGES OF BARRETT'S ESOPHAGUS AND NEOPLASIA

Since EAC is thought to arise in a stepwise histopathological progression from BE (Figure 1)[15], the optimal management strategy is primarily dependent on the degree of dysplastic and neoplastic stage. There is considerable variability in nomenclature, but for the purposes of this review the following classification will be used[16].

Non-invasive neoplasia

Non-dysplastic BE: Intestinal metaplasia without histological features of dysplasia.

BE with low grade dysplasia (LGD): Intestinal metaplasia with histological features of low-grade dysplasia or intra-epithelial neoplasia.

BE with high grade dysplasia (HGD): Intestinal metaplasia with histological features of high-grade dysplasia or intra-epithelial neoplasia. HGD is synonymous with carcinoma in situ, Tis[17], non-invasive carcinoma, suspicion of invasive carcinoma, or defined by malignant cells confined by the basement membrane.

Invasive neoplasia

Intramucosal EAC: Invasion of neoplastic cells beyond the basement membrane into the mucosa but not into the submucosa (T1a[17]).

Submucosal EAC: Invasion of neoplastic cells beyond the basement membrane into the submucosa (T1b[17]).

T2 EAC: Invasion beyond submucosa but confined to the muscularis propia[17].

NATURAL HISTORY OF BARRETT'S ESOPHAGUS AND EARLY STAGE ESOPHAGEAL ADENOCARCINOMA

Consideration of natural history is essential when evaluating the utility of interventions for any condition. In BE, the most important and clinically relevant endpoint is development of adenocarcinoma, and will be the focus of review for natural history studies of premalignant BE. Where early stage adenocarcinoma has already developed within BE, the endpoint of interest is nodal metastases, since this is the major factor determining appropriateness of endoscopic or surgical therapy.

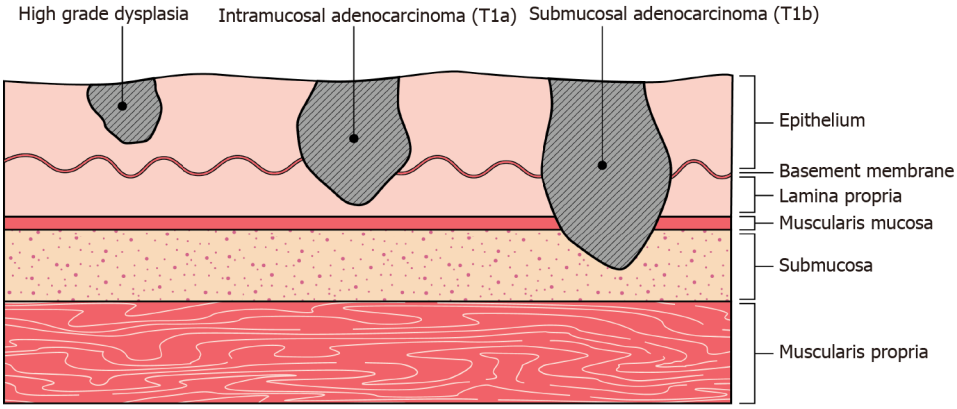
Risk of adenocarcinoma

Non-dysplastic Barrett's: There is a large body of data examining the risk of cancer progression for non-dysplastic BE. Several meta analyses incorporating multiple retrospective case series have reported annual progression rates of non-dysplastic BE to cancer of between 0.33%–0.70%[18-41]. Within this range, Shaheen *et al*[18] showed an inverse relationship between study size and cancer risk whereby small studies tended report higher progression rates[18]. Meta-analyses reporting higher progression rates also tended to incorporate a significant minority of LGD cases that were not separated in analyses [20,21,23] (Supplementary Table 1).

Population-based studies have reported rates of progression at the lower end of the abovementioned range. De Jonge *et al*[29] showed from a registry in the Netherlands including more than 38000 subjects an annual progression rates of 0.39% after careful exclusion of prevalent HGD and EAC cases[29] and even lower rates have been found in other national databases[30,31]. The only prospective natural history study in patients with non-dysplastic BE followed 150 subjects over 5.5 years that led to 3 cases of EAC (annual progression rate 0.36%)[25]. Taken in sum, the annual risk of cancer in those with non-dysplastic BE is felt to be below 0.5%.

Barrett's with low grade dysplasia: There is marked heterogeneity in the reported rate of progression of LGD-BE to EAC. This is now thought to primarily relate to the significant variability in the classification of LGD by pathologists. Traditionally, the risk of progression of LGD was deemed low. Several large, multicenter series suggested that the annualized risk of progression to EAC was less than 1%[25-27,34,42]. However, due to concerns including non-centralized histopathology reading, marked interobserver variability in dysplasia diagnosis, short follow up duration and significant rates of regression of dysplasia in follow up, the possibility of overstaging of dysplasia in these studies was raised. Several population-based studies based on national registry data from the United States and Europe also reported similar rates of between 0.24% and 0.92%[29,31,33,43]. Such data is subject to the same limitations as cohort studies as they include patients from smaller centers where overdiagnosis of LGD is even more likely to occur.

Several studies have attempted to address the issue of overstaging of dysplasia and suggested that the true rate of progression to EAC may be higher. Curvers *et al*[24] had pathology specimens from 147 subjects with LGD re-examined by an expert panel who downstaged the diagnosis in 85%. Of the minority who were confirmed to have LGD by the expert panel, the annualized risk of progression to



DOI: 10.4251/wjgo.v14.i3.568

Figure 1 Progression from high grade dysplasia to intramucosal adenocarcinoma to submucosal adenocarcinoma in each respective layer.

EAC was 3.3%. This was significantly higher than in those who were downstaged to non-dysplastic BE where progression rate to EAC or HGD was only 0.49%, thus providing a convincing argument that inconsistency in pathological diagnosis was the major factor in variability in reported progression rates. Duits *et al*[37,44] similarly demonstrated that the majority of cases of LGD-BE diagnosed in community centers are downstaged by a centralized expert panel; but those who are confirmed dysplastic have a higher rate of progression than previously thought[37,44]. The control arm of the SURF trial, examining outcomes in LGD-BE, also found that when LGD was confirmed by an expert panel of experienced pathologists, the rate of progression to EAC was 2.9% *per* annum[45]. In contrast, a recent well-designed RCT with expert GI pathologists and central pathology review, showed that even after downstaging 26% of patients initially thought to have LGD, progression of 'true' LGD to EAC in those under surveillance was a low 2.4% at three years[46]. The authors identified nearly 1/3 of their initial diagnosis of LGD spontaneously regressed raising the issue of potential consensual misclassification of the diagnosis of LGD. Even in the presence of agreement between multiple expert pathologists k-values may still be suboptimal[37,47,48], thus not completely eliminating the issue of overdiagnosis in LGD.

Therefore, the risk of progression of LGD-BE depends upon the rigor by which it is diagnosed. There can be significant variability in diagnosis depending on local expertise and experience. It is clear that diagnosis in community centers can be unreliable, and when the diagnosis of LGD is made, biopsies should be repeated and examined by at least two expert gastrointestinal pathologists. If a conclusive diagnosis of LGD remains, then the annualized risk of progression to cancer may approximate 1%–3% (Supplementary Table 2).

Barrett's with high grade dysplasia: There is a paucity of high-quality literature describing true progression rates of HGD-BE to EAC. Only three small single center observational studies exist reporting annual incidence rates of cancer between 5% and 8.7%[49-51]. Two well-conducted meta-analyses primarily comprising the abovementioned studies reported identical weighted risks of cancer of 6.6% *per* annum. A single randomized controlled trial included in the meta-analyses followed 70 subjects with HGD-BE over the course of 3.3 years. 19 of these patients developed EAC giving an annual progression rate of 8.14%[52].

While not as significant of an issue as for LGD, pathological overstaging of dysplasia may also be a problem in HGD-BE[53]. Two additional studies suggested that when HGD-BE was characterized following consensus amongst more than one pathologist, the annual risk of progression was higher and between 19%-31.25%[32,42]. Regardless, the annual risk of cancer in HGD-BE is certainly high and is at least in the range of 5%-10% (Supplementary Table 3).

Rate of lymph node metastasis

Conceptually, lymph node metastasis is the major factor that precludes the curative potential of endoscopic therapy for early adenocarcinoma in BE. Lymph node metastasis is also an important outcome as it leads to higher mortality[54-59], tumor recurrence[57,60,61] and is an indication for systemic therapy[62,63]. Most data that assesses risk of lymph node metastases comes from retrospective studies with histopathological lymph node dissection samples from esophagectomy specimens, therefore is significantly limited by selection bias.

Barrett's with high grade dysplasia: HGD, that is, neoplasia confined to the mucosa that does not extend through the basement membrane confirmed by expert gastrointestinal pathologists, has a negligible lymph node metastasis rate[54,60,64-67]. This was confirmed by a systematic review in 2012 which compiled 524 subjects with HGD-BE showing a lymph node metastasis rate of 0%[68].

Intramucosal adenocarcinoma: There is a wide range in the reported rate of lymph node metastasis in intramucosal cancer, ranging between 0% and 9.5%[55-61,64,67-78]. However most of these studies arise from retrospective surgical series suffering from small sample sizes and selection bias[55-61,64,67,69-78]. Larger population database studies tend to suggest much lower rates of lymph node metastasis. A recent retrospective cohort study comprising 782 patients and using the National Cancer Database capturing 70% of all cancers in the United States, showed a relatively low lymph node metastasis rate of 3.6% for intramucosal adenocarcinoma[54]. The reliability of this study stems from including patients with clear staging and adequate lymph node sampling[54]. Another large United States database identified 3595 individuals with intramucosal adenocarcinoma who had undetected lymph node metastatic rate of 8.7%[74], though 16% of the cohort had squamous cell carcinoma which may tend to metastasize to lymph nodes earlier[79]. Further, a systematic review including 1350 patients with intramucosal adenocarcinoma identified 26 individuals with metastasis to surrounding lymph nodes. After prevalence rates were weighted for study sample size, a lymph node metastasis rate of 1.93% was reported.

It appears intramucosal adenocarcinoma has an approximate lymph node metastasis risk between 2%–4% (Table 1). Those with high risk features (invasion into the muscularis mucosae, poor differentiation, and lymphovascular invasion) may have greater risk of metastasis[61].

Submucosal adenocarcinoma: An even wider discrepancy exists in lymph node metastasis for submucosal adenocarcinoma ranging from 14% to 41% (Table 2)[55-61,64,67,69-78]. Such variation is explained by a number of factors. The number of lymph nodes resected during esophagectomy vary widely, and are often not reported; those with greater numbers of nodes excised tend to show higher metastasis rates[73].

Further, other factors may significantly impact rates of lymph node metastases in submucosal disease. Lymphovascular invasion, poor differentiation and size (2 cm) are prognostic factors known to increase the risk of lymph node metastasis[54,55,57,58,60,64,65,67,69,70,72,73,80]**.** A study by Sepesi *et al*[72] contained a cohort of submucosal adenocarcinoma patients with almost a third exhibiting poor differentiation and found a lymph node metastasis rate of 31%[72]. In contrast, a large retrospective study containing 14000 subjects identified a lymph node metastasis rate of 8.6% when tumors were smaller than 2 cm and well to moderately differentiated[64]. Even lower rates of 1.9% have been reported where invasion depth into the submucosa was shallow and no other poor prognostic features were present [81]. Several other studies identify depth of submucosal invasion as another independent risk factor for nodal metastasis in submucosal disease, but this is not a universal finding[56-58].

MANAGEMENT STRATEGIES

Surveillance

Surveillance of BE is recommended by all international societies for all patients who have a history of non-dysplastic BE, and is one of the strategies available for LGD-BE[12-14]. Surveillance involves dye-based[82] or virtual chromoendoscopy[83] in combination with white light endoscopy[13] using a systematic 4-quadrant biopsy protocol (Seattle protocol)[84]. The surveillance interval is determined by a risk appraisal based on the prior endoscopic and histological findings.

Barrett's endoscopic eradication therapy

Barrett's endoscopic eradication therapy (EET) has become an established therapeutic modality for dysplastic and early neoplastic BE. EET is an umbrella term given to a multimodal therapeutic strategy whereby nodular components of the BE segment are endoscopically resected, with subsequent treatment of residual flat components of the segment with ablative therapies.

Resection: Resection is the first component to successful EET. It relies on a careful high-quality endoscopic examination with white light as well as an enhanced imaging modality (dye-based or virtual) for detection of nodular or irregular lesions. Resection is vital from a therapeutic standpoint but also assists in staging by providing depth of tumor invasion that cannot be ascertained from mucosal biopsies alone[85]. The most widely used resection technique in BE is endoscopic mucosal resection (EMR). EMR can be performed using the cap and snare technique or by multi-band mucosectomy[86,87].

Endoscopic submucosal dissection (ESD) is an advanced resection technique that has theoretical advantages of allowing en bloc resection and thorough assessment of lateral and deep margins of the specimen. However, ESD is technically challenging, time consuming, has a steep learning curve, and is not as widely available. Further, it has not been clearly shown to be superior to EMR for neoplasia remission, recurrence or need for surgery in BE[88]. At present, it is usually reserved for large lesions with endoscopic evidence of submucosal invasion[89].

Table 1 Efficacy of surgery for Barrett's esophagus with intramucosal adenocarcinoma

Ref.	Type	*n*[1]	LNMrate	5-yr DFS or DSS	5-yr OS
Rice *et al*[97]	Retrospective	53	2%	-	77%
Liu *et al*[61]	Retrospective	53	-	100%	91%
Prasad *et al*[75]	Retrospective	46	8.6%	97%	95%
Pennathur *et al*[59]	Retrospective	29	7%	82%	73%
Wang *et al*[109]	Retrospective	60; T1a 32%; HGD 68%	-	-	88%
Sepesi *et al*[72]	Retrospective	25	0%	-	85%
Zehetner *et al*[96]	Retrospective	48	-	88%	94% (3 yr)
Hölscher *et al*[56]	Retrospective	70; SCC 29%	0%	-	87%
Leers *et al*[55]	Retrospective	75	1.3%	98%	82%
Pech *et al*[95]	Retrospective	38	-	100% (3.7 yr)	93%
Ngamruengphong *et al*[120]	Retrospective	671	-	-	76%
Lorenz *et al*[57]	Retrospective	42	8.7%	93.4%	91%
Newton *et al*[54]	Retrospective	303	3.6%	-	80%
Marino *et al*[121]	Retrospective	1317	-	-	79%
Semenkovich *et al*[74]	Retrospective	428; SCC 16%	8.7%	-	80%

[1]Pure T1a cohort unless otherwise stated.
DFS: Disease free survival; DSS: Disease specific survival; HGD: High grade dysplasia; LNM: Lymph node metastasis; OS: overall survival; SCC: Squamous cell carcinoma.

Table 2 Efficacy of surgery for Barrett's esophagus with submucosal adenocarcinoma

Ref.	Type	*n*	LNM rate	5-yr DFS	5-yr OS
Rice *et al*[97]	Retrospective	31	5%	-	60%
Liu *et al*[61]	Retrospective	37	-	60%	58%
Pennathur *et al*[59]	Retrospective	71	27%	62%	60%
Sepesi *et al*[72]	Retrospective	29	31%	-	60%
Hölscher *et al*[56]	Retrospective	101; SCC 35%	34%	-	66%
Leers *et al*[55]	Retrospective	51	22%	79%DSS	71%
Ngamruengphong *et al*[120]	Retrospective	523	-	-	64%
Lorenz *et al*[57]	Retrospective	168	20.6%	85%	74%
Schölvinck *et al*[78]	Retrospective	26	17% (n = 69 including EET group)	-	Median survival: 51 mo
Schwameis *et al*[76]	Retrospective	32	22%	-	84%
Newton *et al*[54]	Retrospective (NCDB)	512	23.4%	-	64.4%
Semenkovich *et al*[74]	Retrospective (NCDB)	1146; SCC 16%	14%	-	60%
Otaki *et al*[77]	Retrospective	68	14.7%	92%	89%

DSS: Disease specific survival; EET: Endoscopic eradication therapy; NCDB: National cancer database; DFS: Disease free survival; OS: Overall survival; SCC: Squamous cell carcinoma.

Ablative therapy: Ablation always follows resection other than in the scenario where all visible intestinal metaplasia has been endoscopically resected. It is typically applied to LGD-BE or flat HGD-BE. There are numerous modalities of ablative therapy, however the technique with the best efficacy, ease of use and favorable safety profile is radiofrequency ablation (RFA)[90]. RFA is applied using a

catheter with distal balloon or other attachment bringing electrodes in contact with the esophageal mucosa[42].

A recent meta-analysis assessing adverse events of EET with most included studies using a combination of RFA and EMR showed an overall adverse event rate of 8.8%. The most noteworthy is stricture formation, which represented 5.6% of all patients, although strictures can almost always be treated safely with endoscopic dilatation with durable response[42,45,85,91,92]. Other serious adverse events included bleeding in 1% and 0.6% rate of perforation. Post-procedural chest pain in the absence of other serious complication occurs in 1.5%–5.4%[42,45,46]. No deaths attributable to endoscopic therapy were recorded[93].

Surgery

En-bloc esophagectomy and lymphadenectomy of the mediastinal and abdominal nodes *via* an abdominal or right transthoracic approach is the standard surgical approach to adenocarcinoma arising within a Barrett's segment[57,73]. For tumors in the distal two thirds of the esophagus, esophagectomy is typically performed with the Ivor-Lewis technique, *via* laparotomy and right thoracotomy. Tumors located in the upper third of the esophagus are typically managed *via* the McKeown technique[58].

Esophagectomy has traditionally been considered a relatively high-risk surgery with significant morbidity and mortality rates. Adenocarcinoma specific 90-d mortality has been reported in up to 8.7% [94]. However, early stage carcinoma limited up to submucosa tends to be associated with much more favorable operative risks. When esophagectomy is performed for such early disease, operative mortality ranges between 0% and 5% (Supplementary Table 4)[54,57,59,75-78,95-97]. Serious adverse events, however, remain relatively common and include anastomotic leaks and tracheal injury[98]. Even when the immediate postoperative course is benign, foregut function is permanently altered, and there can be long-term (and in some cases, permanent) impairment of quality of life due to dysphagia, vomiting, reflux symptoms, abdominal pain, and dumping syndrome[99].

THERAPEUTIC EFFICACY

Non-dysplastic Barrett's

Endoscopic eradication therapy: Surveillance with repeat endoscopy every 3–5 years is recommended for non-dysplastic BE[12-14], however there is little data examining ablative therapy. Wani *et al*[22] suggested in a meta analyses that ablative therapies reduced the annual incidence of EAC from 0.60% to 0.16%[22], though the included studies were of varying quality. A single prospective multi-center trial including 50 patients reported complete eradication of intestinal metaplasia rate of 92% at 5 years of follow up. Of the 8% who recurred, all were retreated and eradication of intestinal metaplasia re-achieved. There was no progression to EAC for the duration of the study with no recorded mortalities, serious adverse effects or strictures[90].

Due to the low progression rates of non-dysplastic BE to cancer it is unlikely that any study will ever demonstrate a benefit of ablative therapy in preventing progression to cancer, let alone a mortality benefit. Due to the very low risk profile of non-dysplastic BE, EET is not indicated given that it is not entirely devoid of risk.

Barrett's with low grade dysplasia

Endoscopic eradication therapy: The management of LGD-BE is the most controversial aspect of the management of BE. Retrospective data suggested that EET was highly effective in eradicating intestinal metaplasia in LGD-BE[92]. In terms of the efficacy of RFA for preventing progression of LGD-BE to cancer, a systematic review and meta-analysis including 19 studies and a total of 2700 patients found that compared to surveillance, RFA was associated with relative risk of disease progression to HGD or EAC of 0.14[100]. Three randomized controlled trials examining this question have been published to date. The SURF trial compared RFA against surveillance in patients with LGD-BE without visible lesions. Progression to EAC was reduced by 7.4% (1.5% in RFA arm *vs* 8.8% in control arm) over a median 3 year follow up period[45]. Long term, no further EAC occurred in the ablation arm compared with 10.3% rate of cancer observed in the control arm over 73 mo. On intention to treat analysis, the number needed to treat was 11.4 to prevent cancer. Notably, all 23 progressors to HGD or EAC subsequently achieved complete eradication of cancer and dysplasia by the end of the extended study [101]. Subsequently the AIM DYSPLASIA study showed 5% of LGD-BE patients receiving RFA progressed to HGD compared with 14% in the sham arm over the 12-mo study period. No cancers developed in either arm[42]. The study was extended for 3 more years and only 1 subject from the sham arm developed intramucosal adenocarcinoma, which was cured with EMR[91]. However, these studies are limited by roughly half of subjects not reaching their third year of follow up. A recent multi-center RCT by Barret *et al*[46] retained a near entirety of their cohort of 82 patients for up to 3 years and did not show statistical significance in neoplastic progression rates (12.5% RFA *vs* 26.2% surveillance, $P = 0.15$). The most notable finding, and likely explanation for the negative result, was that RFA was much less

effective with significantly lower rates of eradication of dysplasia and intestinal metaplasia (55% and 35% respectively) compared to the earlier studies (Table 3). The lower efficacy of RFA in this study may be attributed to several factors, most importantly a less aggressive protocol (maximum number of ablation sessions was capped at 4). There was a suggestion of a learning curve and operator effect, with significant difference in success rates between low and high-volume centers[46]. Further, this seemed to be a less 'aggressive' cohort of LGD-BE with much lower rate of neoplastic progression, and higher rate of spontaneous remission of LGD-BE, compared to the former studies. There is no data on the surgical efficacy of LGD-BE.

With conflicting findings from high-quality randomized controlled trials, the decision to offer EET for LGD-BE remains nuanced and several factors need to be considered in the decision-making process. Firstly, RFA only provides a benefit when LGD cases are carefully confirmed by expert pathologists to avoid overdiagnosis and identifying a highly select LGD-BE cohort with rates of progression comparable to that typically associated with HGD-BE[42,45]. This is not representative of most patients diagnosed with LGD-BE. Second, a commitment to an aggressive RFA protocol with potential for several sessions (often 4 or more) needs to be made in order for RFA to be successful in reducing risk of cancer progression. Third, it appears that RFA is more likely to be successful in high-volume centers. Fourth, one must bear in mind that when under surveillance by experts, cancers that evolve from LGD-BE tend to be early and appear to be amenable to curable therapy. Therefore, based on available data, one could argue that the long-term outcome for those under surveillance is no worse, even if HGD or cancer developed.

Barrett's with high grade dysplasia

Endoscopic eradication therapy: Studies of varying quality demonstrate that RFA reduces annual progression of HGD to EAC to a range between 0.6%–2.4%[22,42,91,102-105] compared to no treatment, which has an estimated rate of 5%–10% as described above. To date, there is one RCT that has randomized RFA of HGD against a control arm. RFA reduced progression to cancer from 19% to 2.4%, and the number needed to treat was six[42]. This trial was extended by 3 more years with cross-over from the sham arm to RFA, leading durable remission and an annual progression rate to EAC of 0.60% [91]. Only one other prospective study recruited 75 consecutive subjects with HGD-BE, finding that all patients who achieved complete eradication of BE with EET had no progression to EAC over a follow up period of 31 mo[85].

Once the threshold of HGD has been reached, subjects are also at risk of developing other areas of HGD within their Barrett's mucosa[50]. Further, those who achieve complete endoscopic eradication of Barrett's mucosa are far less likely to progress to cancer compared to those where this is only partially achieved[85,103-105]. Thus, a logical step is to eradicate all surrounding Barrett's tissue once a diagnosis of HGD has been made. In patients with HGD, EET is effective in eradicating all dysplasia in 79%–97%, and intestinal metaplasia in 51.2%–94%[85,92,96,102-105] (Table 4). Low eradication rates are explained by non-standardized and incomplete RFA treatment sessions[102] and inclusion of treatment-experienced subjects representing resistant disease due to fibrosis[103]. Furthermore, experienced centers and contemporary data report higher rates of complete eradication of dysplasia and intestinal metaplasia above 90%[92]. Haidry *et al*[104] compared early and late cohorts, finding rise in eradication of intestinal metaplasia from 57% to 83%[104].

Therefore, EET is effective in reducing annual cancer progression risk by 5-fold, to approximately 2% by eradicating areas of HGD as well as surrounding Barrett's mucosa. The risk of cancer appears to be reliably attenuated when all residual Barrett's mucosa is completely treated. Overall 5-year survival rate appears to be very high at 90%, even in those who do not achieve complete eradication of Barrett's mucosa[85].

Surgery: We can presume individuals have complete eradication of dysplasia and intestinal metaplasia on the day of esophagectomy. Nevertheless, there is paucity of high quality evidence of overall survival and recurrence following esophagectomy for HGD-BE, as most studies are retrospective with small numbers. Five retrospective studies that had referred patients for surgery after biopsy or esophagectomy confirmation of HGD-BE showed promising 5-year survival rates ranging from 83%-97%[97,106-109] with disease free survival at 5 years surpassing 94% in 2 of these studies[106,107]. Another study by Edwards *et al*[110] reported an 82% survival rate after a median of 2.7 years in a small cohort of eleven[110]. These disease free survival rates are not markedly different to those following EET for HGD. However, rates in surgical series incorporate a mixed population, with up to 40% of HGD-BE subjects referred for esophagectomy having evidence of infiltration past the basement membrane corresponding to intramucosal adenocarcinoma[97,106,108,110], reflecting a period where endoscopic assessment was not as accurate as the modern era with subtle lesions likely missed.

Therefore, esophagectomy for HGD shows 5-year overall survival rates above 83% and there is some data to suggest disease-free survival at 5 years exceeds 94%.

Intramucosal esophageal adenocarcinoma (T1a)

Endoscopic eradication therapy: Successful endoscopic eradication rates of intramucosal adenocarcinoma is reported to occur in between 82%–100%[75,92,95,96,104,105,111-119]. Pech's large cohort

Table 3 Efficacy of endoscopic eradication therapy for Barrett's esophagus with low-grade dysplasia

Ref.	Type	*n*	CE-IM	CE-D	NNT to prevent disease progression	Annual disease progression, treatment *vs* placebo (*P* value)
Wani *et al*[22]	Meta-analysis	1512	-	-	65.5 (EAC)	0.16% *vs* 1.7% (*P* = 0.99) (EAC)
Shaheen *et al*[42]	RCT	64	81%	90.5%	11.3 (HGD)	5% *vs* 14% (HGD) (*P* = 0.33)
Shaheen *et al*[91]	Retrospective	52	98%	98%	NA	NA
Bulsiewicz *et al*[92]	Retrospective	41	93%	100%	NA	NA
Phoa *et al*[45]	RCT	136	88.2%	92.6%	13.6 (EAC)	1.5% *vs* 8.8% at 3 yr (EAC) (*P* = 0.03)
Qumseya *et al*[100]	Meta-analysis	2746	-	-	16 (EAC)	NA
Pouw *et al*[101]	Retrospective	83	90%	90%	11.4 (EAC)	NA
Barret *et al*[46]	RCT	82	37.5%	52.5%	-	5% *vs* 2.4% at 3 yr: (EAC) (*P* = 0.52)

n: Patient number; CE-D/IM: Complete eradication of dysplasia/intestinal metaplasia; EAC: Esophageal adenocarcinoma; HGD: High grade dysplasia; NNT: Number needed to treat; NA: Not application.

Table 4 Efficacy of endoscopic eradication therapy for Barrett's esophagus with high-grade dysplasia

Ref.	Type[1]	*n*	CE-IM	CE-D	NNT to prevent disease progression	Annual disease progression, treatment *vs* placebo (*P* value)
Overholt *et al*[52]	RCT (*PDT*)	208	52%	77% (including HGD)	22	3.6% *vs* 8.14% (*P* = 0.006)
Ganz *et al*[102]	Retrospective	92	54%	80%	NA	1.4%
Wani *et al*[22]	Meta-analysis	236	-	-	20.4	1.7% *vs* 6.6% (*P* = 0.02)
Shaheen *et al*[42]	RCT	63	73.8%	81%	6	2.4% *vs* 19% (*P* = 0.04)
Shaheen *et al*[91]	Retrospective	54	89%	93%	NA	0.6%
Moss *et al*[85]	Prospective (*SRER*)	35	94%	94%	NA	Nil
Zehetner *et al*[96]	Retrospective	22	89%	89.5%	NA	Nil
Okoro *et al*[103]	Retrospective	35	51.2%	79%	NA	2.3% (2 yr)
Bulsiewicz *et al*[92]	Retrospective	118	90%	97%	NA	NA
Haidry *et al*[104]	Retrospective	122	85%	92%	NA	2.5% (3 yr)
Li *et al*[105]	Retrospective	832	83.4%	92.1%	NA	3% (2.8 yr)

[1]Studies used endoscopic mucosal resection and radiofrequency ablation unless otherwise stated.
CE-D/IM: Complete eradication of dysplasia/intestinal metaplasia; EAC: Esophageal adenocarcinoma; NNT: Number needed to treat; PDT: Photodynamic therapy; SRER: Stepwise radical endoscopic resection; NA: Not application.

involved 1000 prospective patients over a 15-year period with successful endoscopic resection of cancer and HGD in 96.3%. These patients were closely followed up giving rise to a long term remission rate of 93.8% at 5 years[117]. Another prospective study by Phoa *et al*[119] followed 132 subjects with a significant proportion having intramucosal adenocarcinoma. 92% achieved cure of cancer and dysplasia with a quarter of patients reaching 3 year follow up having durable response rate of 95%[119]. A number of other prospective trials have shown successful endoscopic eradication rates of intramucosal adenocarcinoma exceeding 97%[111-115].

Although initial remission rates are promising, long term outcomes may be more relevant. Three prospective studies exceeding 100 subjects show durability rates of 93.8%–100% over a follow up period ranging between 3–5 years[95,111,117]. Remaining data showing endoscopic eradication rates are displayed in Table 5. Despite these limitations it is clear that residual or recurrent EAC is easily managed by further EET[75,95,96,111,112,115,117,119]. Pech *et al*[117] showed retreatment with EET was successful in 115 out of 140 subjects[117]. Further, esophagectomy also appears to remain a valid

Table 5 Efficacy of endoscopic eradication therapy for Barrett's esophagus with intramucosal adenocarcinoma

Ref.	Type[1]	*n*[2]	Eradication of T1a	5-yr OS
Ell *et al*[111]	Prospective	100	99%	98%
Pech *et al*[112]	Prospective (*EMR +/- PDT*)	349; HGD 17.5%	97.4% (including HGD)	NA
Pouw *et al*[113]	Prospective (*RFA +/- EMR*)	44; HGD up to 27%	100%	NA
Prasad *et al*[75]	Retrospective (*PDT*)	132	94%	83%
Pouw *et al*[114]	Prospective (*EMR + RFA*)	24; HGD 25%; T1b 8%	100%	NA
Pech *et al*[95]	Retrospective (*EMR +/- APC*)	79	98.7%	96%
Van Vilsteren *et al*[115]	RCT	47; HGD up to 40%	97.9%	NA
Zehetner *et al*[96]	Retrospective	18	82% (14/17); 3/17 subsequently successfully treated under surveillance	NA
Bulsiewicz *et al*[92]	Retrospective	29	93%	NA
Ngamruengphong *et al*[120]	Retrospective	229; HGD 24%	-	60%
Saligram *et al*[116]	Retrospective	54	96%	89% (over 2 yr)
Pech *et al*[117]	Prospective	1000	96.3% (including HGD)	91.5%
Haidry *et al*[104]	Retrospective	63	97.5% (combined with HGD cohort)	NA
Agoston *et al*[118]	Retrospective	79	86%	NA
Li *et al*[105]	Retrospective	162	97.5%	NA
Phoa *et al*[119]	Prospective	132; ND/LGD 8.4%; HGD 30%; T1b 1.7%	92%	NA
Marino *et al*[121]	Retrospective	856	-	71.8%
Semenkovich *et al*[74]	Retrospective	1123	-	70%

[1]Studies use endoscopic mucosal resection/radiofrequency ablation unless otherwise stated.
[2]Pure T1a cohort unless otherwise stated.
n: Patient number; APC: Argon plasma coagulation; OS: Overall survival; PDT: Photodynamic therapy; NA: Not application.

treatment option for treatment failures with minimal risk of lymph node metastasis[75,115-117,119].

Reported survival rates of subjects with intramucosal adenocarcinoma who have undergone EET are between 60%–100%[74,75,95,111,120,121]. Lower survival rates are felt to be secondary to selection bias in these observational studies whereby those with frailty, age and comorbidities are more likely to receive less invasive EET than surgery[74,120]. Further, deaths are predominantly due to causes unrelated to EAC[75,95,112,115-117], for example, Pech *et al*[117] reported only 2 in 1000 subjects with tumor-associated deaths[117].

Intramucosal adenocarcinoma can be successfully treated with EET in greater than 90% of cases with durable remission in the vast majority. 5-year overall survival is an estimated 80%, with deaths predominantly attributable to other causes.

Surgery: There are several large surgical series reporting overall survival rates whose findings are severely limited by lack of data on follow up protocols, imaging modalities for surveillance and comorbidities[54,74]. However, at least eight good quality retrospective studies with follow up of 4 years or more reported estimated 5-year overall survival between 73%-93% (Table 1)[55-57,59,72,75,95,97]. The largest of these retrospective studies contained 75 subjects with intramucosal adenocarcinoma from a single center with detailed follow up protocol over a median duration of 50 mo. The 5-year overall survival rate was 92% with 5 year disease specific survival an estimated 98%[55].

Surgery provides definitive therapy of cancer as well as Barrett's mucosa leading to high 5-year overall survival rates of approximately 80%. Most deaths are attributable to non-EAC related causes and correlate to even greater rates of disease-free survival approaching 100%.

Submucosal esophageal adenocarcinoma

Endoscopic eradication therapy: There are no prospective or randomized controlled studies that assess the survival benefit of endoscopic therapy for submucosal adenocarcinoma. Endoscopic eradication of submucosally invasive adenocarcinoma is reportedly achieved in 63%-100% following EET[77,81,122] (Table 6). Manner *et al*[81] retrospectively studied efficacy of EET in 61 subjects with low risk submucosal disease, defined as macroscopically polypoid or flat, minor invasion depth into the submucosa, good to moderate differentiation and with no lymphovascular invasion. Cancer eradication was achieved in 87% and durable response was sustained in 83.6% over a mean reaching 4 years. 5-year overall survival was 84%. Only 1 patient required esophagectomy for lymph node metastasis found during surveillance after complete endoscopic remission was achieved[81]. However, this study and others did not uniformly apply ablative therapy following endoscopic resection[77,81,122], thus possibly underreporting true eradication rates. When disease recurrence occurs after initial EET, successful retreatment appears to be achievable with minimal risk of lymph node metastasis[81,122].

Of 5-year overall survival rates of submucosal adenocarcinoma undergoing EET range from 50%–87% [74,77,81]. Low survival rates were associated with several factors including high risk histological features and extensive comorbidities, with EET often performed in patients deemed unfit for surgery with the majority of subsequent deaths attributable to other causes[77,78].

Complete eradication of cancer may be achievable in up to 87% in low risk submucosal adenocarcinoma. Reported overall survival is very low, though this primarily relates to the frail and comorbid demographic that typically is selected for EET. There remains a role for endoscopic therapy with curative intent in low-risk submucosal disease. Especially in those with comorbidities, EET is a reasonable option in the setting of low-risk histological features.

Surgery: There are numerous retrospective studies of varying size and quality that report overall survival and recurrence rates of submucosal EAC. 5-year overall survival rates for submucosal adenocarcinoma range between 58%–89%[55-57,59,61,72,76,77,97]. Four studies report 5-year disease free survival rates between 60%–92%[57,59,61,77], with contemporary series typically reporting higher overall and cancer-specific survival rates[56,59,61,72,97] . Disease-free survival is typically significantly higher than overall survival given the high rates of non-cancer related deaths in this cohort[61,72,78]. Esophagectomy appears effective in treating submucosal tumors regardless of the presence of high risk features. Otaki *et al*[77] showed a 5-year overall survival rate of 89% despite the majority of patients having at least 1 high risk feature[77].

Surgery appears to be a very effective and curative option in submucosal EAC. Survival rates may reach up to 80% in appropriate surgical candidates, with a significant portion of deaths being unrelated to EAC.

CONCLUSION

Non-dysplastic Barrett's

We recommend surveillance endoscopy for patients with non-dysplastic BE. EET is not justified in non-dysplastic BE due to the extremely low rates of cancer progression (Table 7).

Barrett's with low grade dysplasia

We recommend that LGD be always confirmed by expert gastrointestinal pathologists. If confirmed, such patients should all enter a close surveillance program at a high-volume specialized Barrett's center. EET can be offered, as long as the following caveats are understood: (1) Only a small minority will progress; (2) Benefit of RFA seems confined to aggressive RFA protocols performed in expert centers; (3) It appears that in patients under surveillance by expert hands any progression to HGD or cancer can be detected early and completely treated without any adverse consequences; and (4) Adverse events occur following RFA in an estimated 10%, however rarely severe.

Barrett's with high grade dysplasia

After the confirmation of HGD-BE by expert gastrointestinal pathologists, we recommend referral to an expert Barrett's center with repeat endoscopy within 4 wk of diagnosis. We recommend all visible lesions be treated with EMR initially which provides additional staging information, followed by sequential RFA until eradication of all visible intestinal metaplasia is achieved. HGD-BE without visible lesions should commence treatment with RFA. The risk of lymph node metastasis is negligible in HGD-BE[68], and surgery should not be offered.

Intramucosal adenocarcinoma of the esophagus

We recommend EET for management of intramucosal adenocarcinoma over surgery. While the literature suggests that cancer-free survival may be modestly higher for surgery, EET is far less morbid, recurrences following EET can usually be managed endoscopically, and for persistent failures salvage

Table 6 Efficacy of endoscopic eradication therapy for Barrett's esophagus with submucosal adenocarcinoma

Ref.	Type	*n*	Eradication of cancer	Survival
Manner *et al*[81]	Retrospective	61	87% (including HGD)	5-yr OS 84%
Ngamruengphong *et al*[120]	Retrospective	39	-	5-yr OS 66%
Schölvinck *et al*[78]	Retrospective	43	-	Median survival: 46 mo
Künzli *et al*[122]	Retrospective (RFA or APC)	35	100%	-
Semenkovich *et al*[74]	Retrospective	588	-	5-yr OS 50%
Otaki *et al*[77]	Retrospective (RFA/APC/Cryo)	73	63% (including HGD)	5-yr OS 59%

APC: Argon plasma coagulation; Cryo: Cryotherapy; HGD: High grade dysplasia; OS: Overall survival; RFA: Radiofrequency ablation.

Table 7 Recommendations for non-invasive Barrett's esophagus

Stage	Annualized risk of cancer	Recommended management	Risks of intervention	Post-intervention cancer risk
NDBE	0.5%	Surveillance	Negligible	NA
LGD[1]	1%–3%	Surveillance or EET	Stricture 6%; Chest pain 5%; Bleeding 1%; Perforation 1%	1% *per* year
HGD	5%–10%	EET	Stricture 6%; Chest pain 5%; Bleeding 1%; Perforation 1%	2% *per* year

[1]Low grade dysplasia diagnosed by an expert gastrointestinal pathologist.
HGD: High grade dysplasia; EET: Endoscopic eradication therapy; LGD: Low grade dysplasia; NDBE: Non-dysplastic Barrett's esophagus; NA: Not application.

Table 8 Recommendations for invasive adenocarcinoma arising from Barrett's esophagus

Invasive Barrett's esophagus by stage	Risk of nodal metastases	Recommended management	Risks of intervention	5-yr disease free survival	5-yr overall survival
Intramucosal adenocarcinoma	2%–4%	EET	Stricture 6%; Chest pain 5%; Bleeding 1%; Perforation 1%	NA	Estimated 80%
Submucosal adenocarcinoma	14%–41%	Surgery	Mortality 3%; Adverse events up to 62%; Long-term symptoms due to altered upper gut function	Estimated 70%	Estimated 75%

EET: Endoscopic eradication therapy; NA: Not application.

esophagectomy is not precluded. Where high-risk histological features are present, surgery may be a greater consideration (Table 8).

Submucosal adenocarcinoma of the esophagus

We recommend surgery as standard therapy for submucosal adenocarcinoma due to high risk of lymph node metastasis. The role of EET is confined to comorbid or elderly patients at high surgical risk, especially where there are low risk histological features.

FOOTNOTES

Author contributions: Choi KKH collected and analyzed the data and wrote the manuscript; Sanagapalli S conceived the review and critically revised the manuscript.

Conflict-of-interest statement: There were no conflicts-of-interest in the writing of the article.

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S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **Cameron AJ**, Souto EO, Smyrk TC. Small adenocarcinomas of the esophagogastric junction: association with intestinal metaplasia and dysplasia. *Am J Gastroenterol* 2002; **97**: 1375-1380 [PMID: 12094853 DOI: 10.1111/j.1572-0241.2002.05669.x]
2 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
3 **Arnold M**, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. *Gut* 2015; **64**: 381-387 [PMID: 25320104 DOI: 10.1136/gutjnl-2014-308124]
4 **Edgren G**, Adami HO, Weiderpass E, Nyrén O. A global assessment of the oesophageal adenocarcinoma epidemic. *Gut* 2013; **62**: 1406-1414 [PMID: 22917659 DOI: 10.1136/gutjnl-2012-302412]
5 **Hur C**, Miller M, Kong CY, Dowling EC, Nattinger KJ, Dunn M, Feuer EJ. Trends in esophageal adenocarcinoma incidence and mortality. *Cancer* 2013; **119**: 1149-1158 [PMID: 23303625 DOI: 10.1002/cncr.27834]
6 **Rubenstein JH**, Shaheen NJ. Epidemiology, Diagnosis, and Management of Esophageal Adenocarcinoma. *Gastroenterology* 2015; **149**: 302-17.e1 [PMID: 25957861 DOI: 10.1053/j.gastro.2015.04.053]
7 **Rustgi AK**, El-Serag HB. Esophageal carcinoma. *N Engl J Med* 2014; **371**: 2499-2509 [PMID: 25539106 DOI: 10.1056/NEJMra1314530]
8 **Hagen JA**, DeMeester SR, Peters JH, Chandrasoma P, DeMeester TR. Curative resection for esophageal adenocarcinoma: analysis of 100 en bloc esophagectomies. *Ann Surg* 2001; **234**: 520-30; discussion 530 [PMID: 11573045 DOI: 10.1097/00000658-200110000-00011]
9 **Sawas T**, Killcoyne S, Iyer PG, Wang KK, Smyrk TC, Kisiel JB, Qin Y, Ahlquist DA, Rustgi AK, Costa RJ, Gerstung M, Fitzgerald RC, Katzka DA; OCCAMS Consortium. Identification of Prognostic Phenotypes of Esophageal Adenocarcinoma in 2 Independent Cohorts. *Gastroenterology* 2018; **155**: 1720-1728.e4 [PMID: 30165050 DOI: 10.1053/j.gastro.2018.08.036]
10 **El-Serag HB**, Naik AD, Duan Z, Shakhatreh M, Helm A, Pathak A, Hinojosa-Lindsey M, Hou J, Nguyen T, Chen J, Kramer JR. Surveillance endoscopy is associated with improved outcomes of oesophageal adenocarcinoma detected in patients with Barrett's oesophagus. *Gut* 2016; **65**: 1252-1260 [PMID: 26311716 DOI: 10.1136/gutjnl-2014-308865]
11 **NIH**. Cancer Stats Facts: Esophageal Cancer. [cited 1 May 2021]. Available from: https://seer.cancer.gov/statfacts/html/esoph.html
12 **Shaheen NJ**, Falk GW, Iyer PG, Gerson LB; American College of Gastroenterology. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. *Am J Gastroenterol* 2016; **111**: 30-50; quiz 51 [PMID: 26526079 DOI: 10.1038/ajg.2015.322]
13 **ASGE Standards of Practice Committee**, Qumseya B, Sultan S, Bain P, Jamil L, Jacobson B, Anandasabapathy S, Agrawal D, Buxbaum JL, Fishman DS, Gurudu SR, Jue TL, Kripalani S, Lee JK, Khashab MA, Naveed M, Thosani NC, Yang J, DeWitt J, Wani S; ASGE Standards of Practice Committee Chair. ASGE guideline on screening and surveillance of Barrett's esophagus. *Gastrointest Endosc* 2019; **90**: 335-359.e2 [PMID: 31439127 DOI: 10.1016/j.gie.2019.05.012]
14 **Weusten B**, Bisschops R, Coron E, Dinis-Ribeiro M, Dumonceau JM, Esteban JM, Hassan C, Pech O, Repici A, Bergman J, di Pietro M. Endoscopic management of Barrett's esophagus: European Society of Gastrointestinal Endoscopy (ESGE) Position Statement. *Endoscopy* 2017; **49**: 191-198 [PMID: 28122386 DOI: 10.1055/s-0042-122140]
15 **Hameeteman W**, Tytgat GN, Houthoff HJ, van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. *Gastroenterology* 1989; **96**: 1249-1256 [PMID: 2703113 DOI: 10.1016/s0016-5085(89)80011-3]
16 **Schlemper RJ**, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Fléjou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255 [PMID: 10896917 DOI: 10.1136/gut.47.2.251]
17 **Rice TW**, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. *Ann Cardiothorac Surg* 2017; **6**: 119-130 [PMID: 28447000 DOI: 10.21037/acs.2017.03.14]
18 **Shaheen NJ**, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology* 2000; **119**: 333-338 [PMID: 10930368 DOI: 10.1053/gast.2000.9302]
19 **Chang EY**, Morris CD, Seltman AK, O'Rourke RW, Chan BK, Hunter JG, Jobe BA. The effect of antireflux surgery on esophageal carcinogenesis in patients with barrett esophagus: a systematic review. *Ann Surg* 2007; **246**: 11-21 [PMID:

17592284 DOI: 10.1097/01.sla.0000261459.10565.e9]

20 **Thomas T**, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett's oesophagus. *Aliment Pharmacol Ther* 2007; **26**: 1465-1477 [PMID: 17900269 DOI: 10.1111/j.1365-2036.2007.03528.x]

21 **Yousef F**, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008; **168**: 237-249 [PMID: 18550563 DOI: 10.1093/aje/kwn121]

22 **Wani S**, Puli SR, Shaheen NJ, Westhoff B, Slehria S, Bansal A, Rastogi A, Sayana H, Sharma P. Esophageal adenocarcinoma in Barrett's esophagus after endoscopic ablative therapy: a meta-analysis and systematic review. *Am J Gastroenterol* 2009; **104**: 502-513 [PMID: 19174812 DOI: 10.1038/ajg.2008.31]

23 **Sikkema M**, de Jonge PJF, Steyerberg EW, Kuipers EJ. Risk of Esophageal Adenocarcinoma and Mortality in Patients With Barrett's Esophagus: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2010; **8**: 235-244 [PMID: 19850156 DOI: 10.1016/j.cgh.2009.10.010]

24 **Curvers WL**, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, Bohmer C, Mallant-Hent RC, van Oijen A, Naber AH, Scholten P, Busch OR, Blaauwgeers HG, Meijer GA, Bergman JJ. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *Am J Gastroenterol* 2010; **105**: 1523-1530 [PMID: 20461069 DOI: 10.1038/ajg.2010.171]

25 **Conio M**, Blanchi S, Lapertosa G, Ferraris R, Sablich R, Marchi S, D'Onofrio V, Lacchin T, Iaquinto G, Missale G, Ravelli P, Cestari R, Benedetti G, Macrì G, Fiocca R, Munizzi F, Filiberti R. Long-term endoscopic surveillance of patients with Barrett's esophagus. Incidence of dysplasia and adenocarcinoma: a prospective study. *Am J Gastroenterol* 2003; **98**: 1931-1939 [PMID: 14499768 DOI: 10.1111/j.1572-0241.2003.07666.x]

26 **Dulai GS**, Shekelle PG, Jensen DM, Spiegel BM, Chen J, Oh D, Kahn KL. Dysplasia and risk of further neoplastic progression in a regional Veterans Administration Barrett's cohort. *Am J Gastroenterol* 2005; **100**: 775-783 [PMID: 15784018 DOI: 10.1111/j.1572-0241.2005.41300.x]

27 **Sharma P**, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2006; **4**: 566-572 [PMID: 16630761 DOI: 10.1016/j.cgh.2006.03.001]

28 **Lim CH**, Treanor D, Dixon MF, Axon AT. Low-grade dysplasia in Barrett's esophagus has a high risk of progression. *Endoscopy* 2007; **39**: 581-587 [PMID: 17611911 DOI: 10.1055/s-2007-966592]

29 **de Jonge PJ**, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut* 2010; **59**: 1030-1036 [PMID: 20639249 DOI: 10.1136/gut.2009.176701]

30 **Bhat S**, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, Murray LJ. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011; **103**: 1049-1057 [PMID: 21680910 DOI: 10.1093/jnci/djr203]

31 **Hvid-Jensen F**, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011; **365**: 1375-1383 [PMID: 21995385 DOI: 10.1056/NEJMoa1103042]

32 **Montgomery E**, Goldblum JR, Greenson JK, Haber MM, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Washington K, Zahurak ML, Hart J. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: a follow-up study based on 138 cases from a diagnostic variability study. *Hum Pathol* 2001; **32**: 379-388 [PMID: 11331954 DOI: 10.1053/hupa.2001.23511]

33 **Schouten LJ**, Steevens J, Huysentruyt CJ, Coffeng CE, Keulemans YC, van Leeuwen FE, Driessen AL, van den Brandt PA. Total cancer incidence and overall mortality are not increased among patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2011; **9**: 754-761 [PMID: 21570484 DOI: 10.1016/j.cgh.2011.04.008]

34 **Wani S**, Falk G, Hall M, Gaddam S, Wang A, Gupta N, Singh M, Singh V, Chuang KY, Boolchand V, Gavini H, Kuczynski J, Sud P, Reddymasu S, Bansal A, Rastogi A, Mathur SC, Young P, Cash B, Lieberman DA, Sampliner RE, Sharma P. Patients with nondysplastic Barrett's esophagus have low risks for developing dysplasia or esophageal adenocarcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 220-7; quiz e26 [PMID: 21115133 DOI: 10.1016/j.cgh.2010.11.008]

35 **Desai TK**, Krishnan K, Samala N, Singh J, Cluley J, Perla S, Howden CW. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012; **61**: 970-976 [PMID: 21997553 DOI: 10.1136/gutjnl-2011-300730]

36 **Gaddam S**, Singh M, Balasubramanian G, Thota P, Gupta N, Wani S, Higbee AD, Mathur SC, Horwhat JD, Rastogi A, Young PE, Cash BD, Bansal A, Vargo JJ, Falk GW, Lieberman DA, Sampliner RE, Sharma P. Persistence of nondysplastic Barrett's esophagus identifies patients at lower risk for esophageal adenocarcinoma: results from a large multicenter cohort. *Gastroenterology* 2013; **145**: 548-53.e1 [PMID: 23714382 DOI: 10.1053/j.gastro.2013.05.040]

37 **Duits LC**, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, Offerhaus GJ, Visser M, Meijer SL, Krishnadath KK, Tijssen JG, Mallant-Hent RC, Bergman JJ. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut* 2015; **64**: 700-706 [PMID: 25034523 DOI: 10.1136/gutjnl-2014-307278]

38 **Pereira AD**, Chaves P. Low risk of adenocarcinoma and high-grade dysplasia in patients with non-dysplastic Barrett's esophagus: Results from a cohort from a country with low esophageal adenocarcinoma incidence. *United European Gastroenterol J* 2016; **4**: 343-352 [PMID: 27403300 DOI: 10.1177/2050640615612409]

39 **Visrodia K**, Iyer PG, Schleck CD, Zinsmeister AR, Katzka DA. Yield of Repeat Endoscopy in Barrett's Esophagus with No Dysplasia and Low-Grade Dysplasia: A Population-Based Study. *Dig Dis Sci* 2016; **61**: 158-167 [PMID: 25956705 DOI: 10.1007/s10620-015-3697-6]

40 **Krishnamoorthi R**, Lewis JT, Krishna M, Crews NJ, Johnson ML, Dierkhising RA, Ginos BF, Wang KK, Wolfsen HC, Fleischer DE, Ramirez FC, Buttar NS, Katzka DA, Iyer PG. Predictors of Progression in Barrett's Esophagus with Low-Grade Dysplasia: Results from a Multicenter Prospective BE Registry. *Am J Gastroenterol* 2017; **112**: 867-873 [PMID: 28374813 DOI: 10.1038/ajg.2017.84]

41 **Peters Y**, Honing J, Kievit W, Kestens C, Pestman W, Nagtegaal ID, van der Post RS, Siersema PD. Incidence of Progression of Persistent Nondysplastic Barrett's Esophagus to Malignancy. *Clin Gastroenterol Hepatol* 2019; **17**: 869-877.e5 [PMID: 30213587 DOI: 10.1016/j.cgh.2018.08.033]

42 **Shaheen NJ**, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, Galanko JA, Bronner MP, Goldblum JR, Bennett AE, Jobe BA, Eisen GM, Fennerty MB, Hunter JG, Fleischer DE, Sharma VK, Hawes RH, Hoffman BJ, Rothstein RI, Gordon SR, Mashimo H, Chang KJ, Muthusamy VR, Edmundowicz SA, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Falk GW, Kimmey MB, Madanick RD, Chak A, Lightdale CJ. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009; **360**: 2277-2288 [PMID: 19474425 DOI: 10.1056/NEJMoa0808145]

43 **Jung KW**, Talley NJ, Romero Y, Katzka DA, Schleck CD, Zinsmeister AR, Dunagan KT, Lutzke LS, Wu TT, Wang KK, Frederickson M, Geno DM, Locke GR, Prasad GA. Epidemiology and natural history of intestinal metaplasia of the gastroesophageal junction and Barrett's esophagus: a population-based study. *Am J Gastroenterol* 2011; **106**: 1447-55; quiz 1456 [PMID: 21483461 DOI: 10.1038/ajg.2011.130]

44 **Duits LC**, van der Wel MJ, Cotton CC, Phoa KN, Ten Kate FJW, Seldenrijk CA, Offerhaus GJA, Visser M, Meijer SL, Mallant-Hent RC, Krishnadath KK, Pouw RE, Tijssen JGP, Shaheen NJ, Bergman JJGHM. Patients With Barrett's Esophagus and Confirmed Persistent Low-Grade Dysplasia Are at Increased Risk for Progression to Neoplasia. *Gastroenterology* 2017; **152**: 993-1001.e1 [PMID: 28012849 DOI: 10.1053/j.gastro.2016.12.008]

45 **Phoa KN**, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, Fullarton G, Di Pietro M, Ravi N, Visser M, Offerhaus GJ, Seldenrijk CA, Meijer SL, ten Kate FJ, Tijssen JG, Bergman JJ. Radiofrequency ablation *vs* endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. *JAMA* 2014; **311**: 1209-1217 [PMID: 24668102 DOI: 10.1001/jama.2014.2511]

46 **Barret M**, Pioche M, Terris B, Ponchon T, Cholet F, Zerbib F, Chabrun E, Le Rhun M, Coron E, Giovannini M, Caillol F, Laugier R, Jacques J, Legros R, Boustiere C, Rahmi G, Metivier-Cesbron E, Vanbiervliet G, Bauret P, Escourrou J, Branche J, Jilet L, Abdoul H, Kaddour N, Leblanc S, Bensoussan M, Prat F, Chaussade S. Endoscopic radiofrequency ablation or surveillance in patients with Barrett's oesophagus with confirmed low-grade dysplasia: a multicentre randomised trial. *Gut* 2021; **70**: 1014-1022 [PMID: 33685969 DOI: 10.1136/gutjnl-2020-322082]

47 **Wani S**, Falk GW, Post J, Yerian L, Hall M, Wang A, Gupta N, Gaddam S, Singh M, Singh V, Chuang KY, Boolchand V, Gavini H, Kuczynski J, Sud P, Bansal A, Rastogi A, Mathur SC, Young P, Cash B, Goldblum J, Lieberman DA, Sampliner RE, Sharma P. Risk factors for progression of low-grade dysplasia in patients with Barrett's esophagus. *Gastroenterology* 2011; **141**: 1179-1186, 1186.e1 [PMID: 21723218 DOI: 10.1053/j.gastro.2011.06.055]

48 **Kerkhof M**, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruïne A, Driessen A, ten Kate FJ, Kusters JG, Kuipers EJ, Siersema PD; CYBAR study group. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. *Histopathology* 2007; **50**: 920-927 [PMID: 17543082 DOI: 10.1111/j.1365-2559.2007.02706.x]

49 **Schnell TG**, Sontag SJ, Chejfec G, Aranha G, Metz A, O'Connell S, Seidel UJ, Sonnenberg A. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 2001; **120**: 1607-1619 [PMID: 11375943 DOI: 10.1053/gast.2001.25065]

50 **Weston AP**, Sharma P, Topalovski M, Richards R, Cherian R, Dixon A. Long-term follow-up of Barrett's high-grade dysplasia. *Am J Gastroenterol* 2000; **95**: 1888-1893 [PMID: 10950031 DOI: 10.1111/j.1572-0241.2000.02234.x]

51 **Reid BJ**, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000; **95**: 1669-1676 [PMID: 10925966 DOI: 10.1111/j.1572-0241.2000.02196.x]

52 **Overholt BF**, Lightdale CJ, Wang KK, Canto MI, Burdick S, Haggitt RC, Bronner MP, Taylor SL, Grace MG, Depot M; International Photodynamic Group for High-Grade Dysplasia in Barrett's Esophagus. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. *Gastrointest Endosc* 2005; **62**: 488-498 [PMID: 16185958 DOI: 10.1016/j.gie.2005.06.047]

53 **Sangle NA**, Taylor SL, Emond MJ, Depot M, Overholt BF, Bronner MP. Overdiagnosis of high-grade dysplasia in Barrett's esophagus: a multicenter, international study. *Mod Pathol* 2015; **28**: 758-765 [PMID: 25676554 DOI: 10.1038/modpathol.2015.2]

54 **Newton AD**, Predina JD, Xia L, Roses RE, Karakousis GC, Dempsey DT, Williams NN, Kucharczuk JC, Singhal S. Surgical Management of Early-Stage Esophageal Adenocarcinoma Based on Lymph Node Metastasis Risk. *Ann Surg Oncol* 2018; **25**: 318-325 [PMID: 29147928 DOI: 10.1245/s10434-017-6238-z]

55 **Leers JM**, DeMeester SR, Oezcelik A, Klipfel N, Ayazi S, Abate E, Zehetner J, Lipham JC, Chan L, Hagen JA, DeMeester TR. The prevalence of lymph node metastases in patients with T1 esophageal adenocarcinoma a retrospective review of esophagectomy specimens. *Ann Surg* 2011; **253**: 271-278 [PMID: 21119508 DOI: 10.1097/SLA.0b013e3181fbad42]

56 **Hölscher AH**, Bollschweiler E, Schröder W, Metzger R, Gutschow C, Drebber U. Prognostic impact of upper, middle, and lower third mucosal or submucosal infiltration in early esophageal cancer. *Ann Surg* 2011; **254**: 802-7; discussion 807 [PMID: 22042472 DOI: 10.1097/SLA.0b013e3182369128]

57 **Lorenz D**, Origer J, Pauthner M, Graupe F, Fisseler-Eckhoff A, Stolte M, Pech O, Ell C. Prognostic risk factors of early esophageal adenocarcinomas. *Ann Surg* 2014; **259**: 469-476 [PMID: 24096754 DOI: 10.1097/SLA.0000000000000217]

58 **Ancona E**, Rampado S, Cassaro M, Battaglia G, Ruol A, Castoro C, Portale G, Cavallin F, Rugge M. Prediction of lymph node status in superficial esophageal carcinoma. *Ann Surg Oncol* 2008; **15**: 3278-3288 [PMID: 18726651 DOI: 10.1245/s10434-008-0065-1]

59 **Pennathur A**, Farkas A, Krasinskas AM, Ferson PF, Gooding WE, Gibson MK, Schuchert MJ, Landreneau RJ, Luketich JD. Esophagectomy for T1 esophageal cancer: outcomes in 100 patients and implications for endoscopic therapy. *Ann Thorac Surg* 2009; **87**: 1048-1054; discussion 1054 [PMID: 19324126 DOI: 10.1016/j.athoracsur.2008.12.060]

60 **Westerterp M**, Koppert LB, Buskens CJ, Tilanus HW, ten Kate FJ, Bergman JJ, Siersema PD, van Dekken H, van Lanschot JJ. Outcome of surgical treatment for early adenocarcinoma of the esophagus or gastro-esophageal junction. *Virchows Arch* 2005; **446**: 497-504 [PMID: 15838647 DOI: 10.1007/s00428-005-1243-1]

61 **Liu L**, Hofstetter WL, Rashid A, Swisher SG, Correa AM, Ajani JA, Hamilton SR, Wu TT. Significance of the depth of tumor invasion and lymph node metastasis in superficially invasive (T1) esophageal adenocarcinoma. *Am J Surg Pathol* 2005; **29**: 1079-1085 [PMID: 16006804]

62 **van Hagen P**, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hospers GA, Bonenkamp JJ, Cuesta MA, Blaisse RJ, Busch OR, ten Kate FJ, Creemers GJ, Punt CJ, Plukker JT, Verheul HM, Spillenaar Bilgen EJ, van Dekken H, van der Sangen MJ, Rozema T, Biermann K, Beukema JC, Piet AH, van Rij CM, Reinders JG, Tilanus HW, van der Gaast A; CROSS Group. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012; **366**: 2074-2084 [PMID: 22646630 DOI: 10.1056/NEJMoa1112088]

63 **Ajani JA**, D'Amico TA, Bentrem DJ, Chao J, Corvera C, Das P, Denlinger CS, Enzinger PC, Fanta P, Farjah F, Gerdes H, Gibson M, Glasgow RE, Hayman JA, Hochwald S, Hofstetter WL, Ilson DH, Jaroszewski D, Johung KL, Keswani RN, Kleinberg LR, Leong S, Ly QP, Matkowskyj KA, McNamara M, Mulcahy MF, Paluri RK, Park H, Perry KA, Pimiento J, Poultsides GA, Roses R, Strong VE, Wiesner G, Willett CG, Wright CD, McMillian NR, Pluchino LA. Esophageal and Esophagogastric Junction Cancers, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2019; **17**: 855-883 [PMID: 31319389 DOI: 10.6004/jnccn.2019.0033]

64 **Gamboa AM**, Kim S, Force SD, Staley CA, Woods KE, Kooby DA, Maithel SK, Luke JA, Shaffer KM, Dacha S, Saba NF, Keilin SA, Cai Q, El-Rayes BF, Chen Z, Willingham FF. Treatment allocation in patients with early-stage esophageal adenocarcinoma: Prevalence and predictors of lymph node involvement. *Cancer* 2016; **122**: 2150-2157 [PMID: 27142247 DOI: 10.1002/cncr.30040]

65 **Ishihara R**, Oyama T, Abe S, Takahashi H, Ono H, Fujisaki J, Kaise M, Goda K, Kawada K, Koike T, Takeuchi M, Matsuda R, Hirasawa D, Yamada M, Kodaira J, Tanaka M, Omae M, Matsui A, Kanesaka T, Takahashi A, Hirooka S, Saito M, Tsuji Y, Maeda Y, Yamashita H, Oda I, Tomita Y, Matsunaga T, Terai S, Ozawa S, Kawano T, Seto Y. Risk of metastasis in adenocarcinoma of the esophagus: a multicenter retrospective study in a Japanese population. *J Gastroenterol* 2017; **52**: 800-808 [PMID: 27757547 DOI: 10.1007/s00535-016-1275-0]

66 **Williams VA**, Watson TJ, Herbella FA, Gellersen O, Raymond D, Jones C, Peters JH. Esophagectomy for high grade dysplasia is safe, curative, and results in good alimentary outcome. *J Gastrointest Surg* 2007; **11**: 1589-1597 [PMID: 17909921 DOI: 10.1007/s11605-007-0330-9]

67 **Buskens CJ**, Westerterp M, Lagarde SM, Bergman JJ, ten Kate FJ, van Lanschot JJ. Prediction of appropriateness of local endoscopic treatment for high-grade dysplasia and early adenocarcinoma by EUS and histopathologic features. *Gastrointest Endosc* 2004; **60**: 703-710 [PMID: 15557945 DOI: 10.1016/s0016-5107(04)02017-6]

68 **Dunbar KB**, Spechler SJ. The risk of lymph-node metastases in patients with high-grade dysplasia or intramucosal carcinoma in Barrett's esophagus: a systematic review. *Am J Gastroenterol* 2012; **107**: 850-62; quiz 863 [PMID: 22488081 DOI: 10.1038/ajg.2012.78]

69 **Davison JM**, Landau MS, Luketich JD, McGrath KM, Foxwell TJ, Landsittel DP, Gibson MK, Nason KS. A Model Based on Pathologic Features of Superficial Esophageal Adenocarcinoma Complements Clinical Node Staging in Determining Risk of Metastasis to Lymph Nodes. *Clin Gastroenterol Hepatol* 2016; **14**: 369-377.e3 [PMID: 26515637 DOI: 10.1016/j.cgh.2015.10.020]

70 **Lee L**, Ronellenfitsch U, Hofstetter WL, Darling G, Gaiser T, Lippert C, Gilbert S, Seely AJ, Mulder DS, Ferri LE. Predicting lymph node metastases in early esophageal adenocarcinoma using a simple scoring system. *J Am Coll Surg* 2013; **217**: 191-199 [PMID: 23659947 DOI: 10.1016/j.jamcollsurg.2013.03.015]

71 **Gockel I**, Domeyer M, Sgourakis GG, Schimanski CC, Moehler M, Kirkpatrick CJ, Lang H, Junginger T, Hansen T. Prediction model of lymph node metastasis in superficial esophageal adenocarcinoma and squamous cell cancer including D2-40 immunostaining. *J Surg Oncol* 2009; **100**: 191-198 [PMID: 19548259 DOI: 10.1002/jso.21336]

72 **Sepesi B**, Watson TJ, Zhou D, Polomsky M, Litle VR, Jones CE, Raymond DP, Hu R, Qiu X, Peters JH. Are endoscopic therapies appropriate for superficial submucosal esophageal adenocarcinoma? *J Am Coll Surg* 2010; **210**: 418-427 [PMID: 20347733 DOI: 10.1016/j.jamcollsurg.2010.01.003]

73 **Bollschweiler E**, Baldus SE, Schröder W, Prenzel K, Gutschow C, Schneider PM, Hölscher AH. High rate of lymph-node metastasis in submucosal esophageal squamous-cell carcinomas and adenocarcinomas. *Endoscopy* 2006; **38**: 149-156 [PMID: 16479422 DOI: 10.1055/s-2006-924993]

74 **Semenkovich TR**, Hudson JL, Subramanian M, Mullady DK, Meyers BF, Puri V, Kozower BD. Trends in Treatment of T1N0 Esophageal Cancer. *Ann Surg* 2019; **270**: 434-443 [PMID: 31274653 DOI: 10.1097/SLA.0000000000003466]

75 **Prasad GA**, Wu TT, Wigle DA, Buttar NS, Wongkeesong LM, Dunagan KT, Lutzke LS, Borkenhagen LS, Wang KK. Endoscopic and surgical treatment of mucosal (T1a) esophageal adenocarcinoma in Barrett's esophagus. *Gastroenterology* 2009; **137**: 815-823 [PMID: 19524578 DOI: 10.1053/j.gastro.2009.05.059]

76 **Schwameis K**, Green KM, Worrell SG, Samaan J, Cooper S, Tatishchev S, Oh DS, Hagen JA, DeMeester SR. Outcome with Primary En-bloc Esophagectomy for Submucosal Esophageal Adenocarcinoma. *Ann Surg Oncol* 2017; **24**: 3921-3925 [PMID: 28975518 DOI: 10.1245/s10434-017-6091-0]

77 **Otaki F**, Ma GK, Krigel A, Dierkhising RA, Lewis JT, Blevins CH, Gopalakrishnan NP, Ravindran A, Johnson ML, Leggett CL, Wigle D, Wang KK, Falk GW, Abrams JA, Nakagawa H, Rustgi AK, Wang TC, Lightdale CJ, Ginsberg GG, Iyer PG. Outcomes of patients with submucosal (T1b) esophageal adenocarcinoma: a multicenter cohort study. *Gastrointest Endosc* 2020; **92**: 31-39.e1 [PMID: 31953189 DOI: 10.1016/j.gie.2020.01.013]

78 **Schölvinck D**, Künzli H, Meijer S, Seldenrijk K, van Berge Henegouwen M, Bergman J, Weusten B. Management of patients with T1b esophageal adenocarcinoma: a retrospective cohort study on patient management and risk of metastatic disease. *Surg Endosc* 2016; **30**: 4102-4113 [PMID: 27357927 DOI: 10.1007/s00464-016-5071-y]

79 **Gockel I**, Sgourakis G, Lyros O, Polotzek U, Schimanski CC, Lang H, Hoppo T, Jobe BA. Risk of lymph node metastasis in submucosal esophageal cancer: a review of surgically resected patients. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 371-384 [PMID: 21651355 DOI: 10.1586/egh.11.33]

80 **Shimada H**, Nabeya Y, Matsubara H, Okazumi S, Shiratori T, Shimizu T, Aoki T, Shuto K, Akutsu Y, Ochiai T. Prediction of lymph node status in patients with superficial esophageal carcinoma: analysis of 160 surgically resected

cancers. *Am J Surg* 2006; **191**: 250-254 [PMID: 16442955 DOI: 10.1016/j.amjsurg.2005.07.035]

81 **Manner H**, Pech O, Heldmann Y, May A, Pohl J, Behrens A, Gossner L, Stolte M, Vieth M, Ell C. Efficacy, safety, and long-term results of endoscopic treatment for early stage adenocarcinoma of the esophagus with low-risk sm1 invasion. *Clin Gastroenterol Hepatol* 2013; **11**: 630-5; quiz e45 [PMID: 23357492 DOI: 10.1016/j.cgh.2012.12.040]

82 **Sharma P**, Savides TJ, Canto MI, Corley DA, Falk GW, Goldblum JR, Wang KK, Wallace MB, Wolfsen HC; ASGE Technology and Standards of Practice Committee. The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on imaging in Barrett's Esophagus. *Gastrointest Endosc* 2012; **76**: 252-254 [PMID: 22817781 DOI: 10.1016/j.gie.2012.05.007]

83 **Sharma P**, Bergman JJ, Goda K, Kato M, Messmann H, Alsop BR, Gupta N, Vennalaganti P, Hall M, Konda V, Koons A, Penner O, Goldblum JR, Waxman I. Development and Validation of a Classification System to Identify High-Grade Dysplasia and Esophageal Adenocarcinoma in Barrett's Esophagus Using Narrow-Band Imaging. *Gastroenterology* 2016; **150**: 591-598 [PMID: 26627609 DOI: 10.1053/j.gastro.2015.11.037]

84 **Levine DS**, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. *Gastroenterology* 1993; **105**: 40-50 [PMID: 8514061 DOI: 10.1016/0016-5085(93)90008-z]

85 **Moss A**, Bourke MJ, Hourigan LF, Gupta S, Williams SJ, Tran K, Swan MP, Hopper AD, Kwan V, Bailey AA. Endoscopic resection for Barrett's high-grade dysplasia and early esophageal adenocarcinoma: an essential staging procedure with long-term therapeutic benefit. *Am J Gastroenterol* 2010; **105**: 1276-1283 [PMID: 20179694 DOI: 10.1038/ajg.2010.1]

86 **Pouw RE**, van Vilsteren FG, Peters FP, Alvarez Herrero L, Ten Kate FJ, Visser M, Schenk BE, Schoon EJ, Peters FT, Houben M, Bisschops R, Weusten BL, Bergman JJ. Randomized trial on endoscopic resection-cap *vs* multiband mucosectomy for piecemeal endoscopic resection of early Barrett's neoplasia. *Gastrointest Endosc* 2011; **74**: 35-43 [PMID: 21704807 DOI: 10.1016/j.gie.2011.03.1243]

87 **May A**, Gossner L, Behrens A, Kohnen R, Vieth M, Stolte M, Ell C. A prospective randomized trial of two different endoscopic resection techniques for early stage cancer of the esophagus. *Gastrointest Endosc* 2003; **58**: 167-175 [PMID: 12872081 DOI: 10.1067/mge.2003.339]

88 **Terheggen G**, Horn EM, Vieth M, Gabbert H, Enderle M, Neugebauer A, Schumacher B, Neuhaus H. A randomised trial of endoscopic submucosal dissection *vs* endoscopic mucosal resection for early Barrett's neoplasia. *Gut* 2017; **66**: 783-793 [PMID: 26801885 DOI: 10.1136/gutjnl-2015-310126]

89 **Sun F**, Yuan P, Chen T, Hu J. Efficacy and complication of endoscopic submucosal dissection for superficial esophageal carcinoma: a systematic review and meta-analysis. *J Cardiothorac Surg* 2014; **9**: 78 [PMID: 24885614 DOI: 10.1186/1749-8090-9-78]

90 **Fleischer DE**, Overholt BF, Sharma VK, Reymunde A, Kimmey MB, Chuttani R, Chang KJ, Muthasamy R, Lightdale CJ, Santiago N, Pleskow DK, Dean PJ, Wang KK. Endoscopic radiofrequency ablation for Barrett's esophagus: 5-year outcomes from a prospective multicenter trial. *Endoscopy* 2010; **42**: 781-789 [PMID: 20857372 DOI: 10.1055/s-0030-1255779]

91 **Shaheen NJ**, Overholt BF, Sampliner RE, Wolfsen HC, Wang KK, Fleischer DE, Sharma VK, Eisen GM, Fennerty MB, Hunter JG, Bronner MP, Goldblum JR, Bennett AE, Mashimo H, Rothstein RI, Gordon SR, Edmundowicz SA, Madanick RD, Peery AF, Muthusamy VR, Chang KJ, Kimmey MB, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Dumot JA, Falk GW, Galanko JA, Jobe BA, Hawes RH, Hoffman BJ, Sharma P, Chak A, Lightdale CJ. Durability of radiofrequency ablation in Barrett's esophagus with dysplasia. *Gastroenterology* 2011; **141**: 460-468 [PMID: 21679712 DOI: 10.1053/j.gastro.2011.04.061]

92 **Bulsiewicz WJ**, Kim HP, Dellon ES, Cotton CC, Pasricha S, Madanick RD, Spacek MB, Bream SE, Chen X, Orlando RC, Shaheen NJ. Safety and efficacy of endoscopic mucosal therapy with radiofrequency ablation for patients with neoplastic Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013; **11**: 636-642 [PMID: 23103824 DOI: 10.1016/j.cgh.2012.10.028]

93 **Qumseya BJ**, Wani S, Desai M, Qumseya A, Bain P, Sharma P, Wolfsen H. Adverse Events After Radiofrequency Ablation in Patients With Barrett's Esophagus: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2016; **14**: 1086-1095.e6 [PMID: 27068041 DOI: 10.1016/j.cgh.2016.04.001]

94 **Bollschweiler E**, Schröder W, Hölscher AH, Siewert JR. Preoperative risk analysis in patients with adenocarcinoma or squamous cell carcinoma of the oesophagus. *Br J Surg* 2000; **87**: 1106-1110 [PMID: 10931059 DOI: 10.1046/j.1365-2168.2000.01474.x]

95 **Pech O**, Bollschweiler E, Manner H, Leers J, Ell C, Hölscher AH. Comparison between endoscopic and surgical resection of mucosal esophageal adenocarcinoma in Barrett's esophagus at two high-volume centers. *Ann Surg* 2011; **254**: 67-72 [PMID: 21532466 DOI: 10.1097/SLA.0b013e31821d4bf6]

96 **Zehetner J**, DeMeester SR, Hagen JA, Ayazi S, Augustin F, Lipham JC, DeMeester TR. Endoscopic resection and ablation *vs* esophagectomy for high-grade dysplasia and intramucosal adenocarcinoma. *J Thorac Cardiovasc Surg* 2011; **141**: 39-47 [PMID: 21055772 DOI: 10.1016/j.jtcvs.2010.08.058]

97 **Rice TW**, Blackstone EH, Goldblum JR, DeCamp MM, Murthy SC, Falk GW, Ormsby AH, Rybicki LA, Richter JE, Adelstein DJ. Superficial adenocarcinoma of the esophagus. *J Thorac Cardiovasc Surg* 2001; **122**: 1077-1090 [DOI: 10.1067/mtc.2001.113749]

98 **Gurusamy KS**, Pallari E, Midya S, Mughal M. Laparoscopic *vs* open transhiatal oesophagectomy for oesophageal cancer. *Cochrane Database Syst Rev* 2016; **3**: CD011390 [PMID: 27030301 DOI: 10.1002/14651858.CD011390.pub2]

99 **Blazeby JM**, Farndon JR, Donovan J, Alderson D. A prospective longitudinal study examining the quality of life of patients with esophageal carcinoma. *Cancer* 2000; **88**: 1781-1787 [PMID: 10760752]

100 **Qumseya BJ**, Wani S, Gendy S, Harnke B, Bergman JJ, Wolfsen H. Disease Progression in Barrett's Low-Grade Dysplasia With Radiofrequency Ablation Compared With Surveillance: Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2017; **112**: 849-865 [PMID: 28374819 DOI: 10.1038/ajg.2017.70]

101 **Pouw RE**, Klaver E, Phoa KN, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Pech O, Manner H, Ragunath K,

Fernández-Sordo JO, Fullarton G, Di Pietro M, Januszewicz W, O'Toole D, Bergman JJ. Radiofrequency ablation for low-grade dysplasia in Barrett's esophagus: long-term outcome of a randomized trial. *Gastrointest Endosc* 2020; **92**: 569-574 [PMID: 32217112 DOI: 10.1016/j.gie.2020.03.3756]

102 **Ganz RA**, Overholt BF, Sharma VK, Fleischer DE, Shaheen NJ, Lightdale CJ, Freeman SR, Pruitt RE, Urayama SM, Gress F, Pavey DA, Branch MS, Savides TJ, Chang KJ, Muthusamy VR, Bohorfoush AG, Pace SC, DeMeester SR, Eysselein VE, Panjehpour M, Triadafilopoulos G; U. S. Multicenter Registry. Circumferential ablation of Barrett's esophagus that contains high-grade dysplasia: a U.S. Multicenter Registry. *Gastrointest Endosc* 2008; **68**: 35-40 [PMID: 18355819 DOI: 10.1016/j.gie.2007.12.015]

103 **Okoro NI**, Tomizawa Y, Dunagan KT, Lutzke LS, Wang KK, Prasad GA. Safety of prior endoscopic mucosal resection in patients receiving radiofrequency ablation of Barrett's esophagus. *Clin Gastroenterol Hepatol* 2012; **10**: 150-154 [PMID: 22056303 DOI: 10.1016/j.cgh.2011.10.030]

104 **Haidry RJ**, Butt MA, Dunn JM, Gupta A, Lipman G, Smart HL, Bhandari P, Smith L, Willert R, Fullarton G, Di Pietro M, Gordon C, Penman I, Barr H, Patel P, Kapoor N, Hoare J, Narayanasamy R, Ang Y, Veitch A, Ragunath K, Novelli M, Lovat LB; UK RFA Registry. Improvement over time in outcomes for patients undergoing endoscopic therapy for Barrett's oesophagus-related neoplasia: 6-year experience from the first 500 patients treated in the UK patient registry. *Gut* 2015; **64**: 1192-1199 [PMID: 25539672 DOI: 10.1136/gutjnl-2014-308501]

105 **Li N**, Pasricha S, Bulsiewicz WJ, Pruitt RE, Komanduri S, Wolfsen HC, Chmielewski GW, Corbett FS, Chang KJ, Shaheen NJ. Effects of preceding endoscopic mucosal resection on the efficacy and safety of radiofrequency ablation for treatment of Barrett's esophagus: results from the United States Radiofrequency Ablation Registry. *Dis Esophagus* 2016; **29**: 537-543 [PMID: 26121935 DOI: 10.1111/dote.12386]

106 **Reed MF**, Tolis G Jr, Edil BH, Allan JS, Donahue DM, Gaissert HA, Moncure AC, Wain JC, Wright CD, Mathisen DJ. Surgical treatment of esophageal high-grade dysplasia. *Ann Thorac Surg* 2005; **79**: 1110-5; discussion 1110 [PMID: 15797034 DOI: 10.1016/j.athoracsur.2004.09.006]

107 **Prasad GA**, Wang KK, Buttar NS, Wongkeesong LM, Krishnadath KK, Nichols FC 3rd, Lutzke LS, Borkenhagen LS. Long-term survival following endoscopic and surgical treatment of high-grade dysplasia in Barrett's esophagus. *Gastroenterology* 2007; **132**: 1226-1233 [PMID: 17408660 DOI: 10.1053/j.gastro.2007.02.017]

108 **Heitmiller RF**, Redmond M, Hamilton SR. Barrett's esophagus with high-grade dysplasia. An indication for prophylactic esophagectomy. *Ann Surg* 1996; **224**: 66-71 [PMID: 8678620 DOI: 10.1097/00000658-199607000-00010]

109 **Wang VS**, Hornick JL, Sepulveda JA, Mauer R, Poneros JM. Low prevalence of submucosal invasive carcinoma at esophagectomy for high-grade dysplasia or intramucosal adenocarcinoma in Barrett's esophagus: a 20-year experience. *Gastrointest Endosc* 2009; **69**: 777-783 [PMID: 19136106 DOI: 10.1016/j.gie.2008.05.013]

110 **Edwards MJ**, Gable DR, Lentsch AB, Richardson JD. The rationale for esophagectomy as the optimal therapy for Barrett's esophagus with high-grade dysplasia. *Ann Surg* 1996; **223**: 585-9; discussion 589 [PMID: 8651749 DOI: 10.1097/00000658-199605000-00014]

111 **Ell C**, May A, Pech O, Gossner L, Guenter E, Behrens A, Nachbar L, Huijsmans J, Vieth M, Stolte M. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). *Gastrointest Endosc* 2007; **65**: 3-10 [PMID: 17185072 DOI: 10.1016/j.gie.2006.04.033]

112 **Pech O**, Behrens A, May A, Nachbar L, Gossner L, Rabenstein T, Manner H, Guenter E, Huijsmans J, Vieth M, Stolte M, Ell C. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. *Gut* 2008; **57**: 1200-1206 [PMID: 18460553 DOI: 10.1136/gut.2007.142539]

113 **Pouw RE**, Gondrie JJ, Sondermeijer CM, ten Kate FJ, van Gulik TM, Krishnadath KK, Fockens P, Weusten BL, Bergman JJ. Eradication of Barrett esophagus with early neoplasia by radiofrequency ablation, with or without endoscopic resection. *J Gastrointest Surg* 2008; **12**: 1627-36; discussion 1636 [PMID: 18704598 DOI: 10.1007/s11605-008-0629-1]

114 **Pouw RE**, Wirths K, Eisendrath P, Sondermeijer CM, Ten Kate FJ, Fockens P, Devière J, Neuhaus H, Bergman JJ. Efficacy of radiofrequency ablation combined with endoscopic resection for barrett's esophagus with early neoplasia. *Clin Gastroenterol Hepatol* 2010; **8**: 23-29 [PMID: 19602454 DOI: 10.1016/j.cgh.2009.07.003]

115 **van Vilsteren FG**, Pouw RE, Seewald S, Alvarez Herrero L, Sondermeijer CM, Visser M, Ten Kate FJ, Yu Kim Teng KC, Soehendra N, Rösch T, Weusten BL, Bergman JJ. Stepwise radical endoscopic resection *vs* radiofrequency ablation for Barrett's oesophagus with high-grade dysplasia or early cancer: a multicentre randomised trial. *Gut* 2011; **60**: 765-773 [PMID: 21209124 DOI: 10.1136/gut.2010.229310]

116 **Saligram S**, Chennat J, Hu H, Davison JM, Fasanella KE, McGrath K. Endotherapy for superficial adenocarcinoma of the esophagus: an American experience. *Gastrointest Endosc* 2013; **77**: 872-876 [PMID: 23472998 DOI: 10.1016/j.gie.2013.01.014]

117 **Pech O**, May A, Manner H, Behrens A, Pohl J, Weferling M, Hartmann U, Manner N, Huijsmans J, Gossner L, Rabenstein T, Vieth M, Stolte M, Ell C. Long-term efficacy and safety of endoscopic resection for patients with mucosal adenocarcinoma of the esophagus. *Gastroenterology* 2014; **146**: 652-660.e1 [PMID: 24269290 DOI: 10.1053/j.gastro.2013.11.006]

118 **Agoston AT**, Strauss AC, Dulai PS, Hagen CE, Muzikansky A, Fudman DI, Abrams JA, Forcione DG, Jajoo K, Saltzman JR, Odze RD, Lauwers GY, Gordon SR, Lightdale CJ, Rothstein RI, Srivastava A. Predictors Of Treatment Failure After Radiofrequency Ablation For Intramucosal Adenocarcinoma in Barrett Esophagus: A Multi-institutional Retrospective Cohort Study. *Am J Surg Pathol* 2016; **40**: 554-562 [PMID: 26645729 DOI: 10.1097/PAS.0000000000000566]

119 **Phoa KN**, Pouw RE, Bisschops R, Pech O, Ragunath K, Weusten BL, Schumacher B, Rembacken B, Meining A, Messmann H, Schoon EJ, Gossner L, Mannath J, Seldenrijk CA, Visser M, Lerut T, Seewald S, ten Kate FJ, Ell C, Neuhaus H, Bergman JJ. Multimodality endoscopic eradication for neoplastic Barrett oesophagus: results of an European multicentre study (EURO-II). *Gut* 2016; **65**: 555-562 [PMID: 25731874 DOI: 10.1136/gutjnl-2015-309298]

120 **Ngamruengphong S**, Wolfsen HC, Wallace MB. Survival of patients with superficial esophageal adenocarcinoma after endoscopic treatment *vs* surgery. *Clin Gastroenterol Hepatol* 2013; **11**: 1424-1429.e2; quiz e81 [PMID: 23735443 DOI: 10.1016/j.cgh.2013.05.025]

121 **Marino KA**, Sullivan JL, Weksler B. Esophagectomy *vs* endoscopic resection for patients with early-stage esophageal adenocarcinoma: A National Cancer Database propensity-matched study. *J Thorac Cardiovasc Surg* 2018; **155**: 2211-2218.e1 [PMID: 29455958 DOI: 10.1016/j.jtcvs.2017.11.111]

122 **Künzli HT**, Belghazi K, Pouw RE, Meijer SL, Seldenrijk CA, Weusten B, Bergman J. Endoscopic management and follow-up of patients with a submucosal esophageal adenocarcinoma. *United European Gastroenterol J* 2018; **6**: 669-677 [PMID: 30083328 DOI: 10.1177/2050640617753808]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 587-606

DOI: 10.4251/wjgo.v14.i3.587

ISSN 1948-5204 (online)

REVIEW

Gut and liver involvement in pediatric hematolymphoid malignancies

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Abdelbasset WK, Saudi Arabia; Delgado-Gallegos JL, Mexico; Saito R, Japan

Received: September 3, 2021
Peer-review started: September 3, 2021
First decision: October 3, 2021
Revised: October 22, 2021
Accepted: February 27, 2022
Article in press: February 27, 2022
Published online: March 15, 2022

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Abstract

Hematolymphoid malignancies are common neoplasms in childhood. The involvement of the gastrointestinal (GI) tract, liver, biliary system, pancreas, and peritoneum are closely interlinked and commonly encountered. In leukemias, lymphomas, and Langerhans cell histiocytosis (LCH), the manifestations result from infiltration, compression, overwhelmed immune system, and chemotherapy-induced drug toxicities. In acute leukemias, major manifestations are infiltrative hepatitis, drug induced gastritis, neutropenic typhlitis and chemotherapy related pancreatitis. Chronic leukemias are rare. Additional presentation in lymphomas is cholestasis due to infiltration or biliary obstruction by lymph nodal masses. Presence of ascites needs a thorough workup for the underlying pathophysiology that may modify the therapy and affect the outcome. Uncommon hemato-lymphoid malignancies are primary hepatic, hepatosplenic, and GI lymphomas which have strict definitions. In advanced diseases with extensive spread, it may be impossible to distinguish these diseases from the primary site of origin. LCH produces biliary strictures that mimic as sclerosing cholangitis. Liver infiltration is associated with poor liver recovery even after chemotherapy. The heterogeneity of gut and liver manifestations in hematolymphoid malignancies has a clinical impact on their management. Though chemotherapy is the mainstay of therapy in all hematolymphoid malignancies, debulking surgery and radiotherapy have an adjuvant role in specific clinical scenarios. Rare situations presenting as liver failure or end-stage liver disease require liver transplantation. At their initial presentation to a primary care physician, given the ambiguity in clinical manifestations and the prognostic difference with time-bound management, it is vital to recognize them early for optimal outcomes. Pooled data from robust registries across the world is required for better understanding of these complications.

Key Words: Leukemia; Lymphoma; Langerhans cell histiocytosis; Gastrointestinal; Hepatobiliary

Core Tip: Pediatric hematolymphoid malignancies commonly are leukemias, lymphomas, and Langerhans cell histiocytosis. Their gut and liver involvement are seldom discussed due to a lack of literature in children. Manifestations result from infiltration, compression, overwhelmed immune system, and chemotherapy-induced drug toxicities. In this review, we will discuss the diverse abdominal manifestations and challenges from a pediatric gastroenterologist's perspective.

Citation: Devarapalli UV, Sarma MS, Mathiyazhagan G. Gut and liver involvement in pediatric hematolymphoid malignancies. *World J Gastrointest Oncol* 2022; 14(3): 587-606
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/587.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.587

INTRODUCTION

Hematolymphoid malignancies in children are characterized by uncontrolled clonal proliferation of hematopoietic progenitor cells with resultant infiltration of bone marrow and involvement of lymphoreticular system including lymph nodes, Waldeyer's ring, thymus, liver, spleen and gut. Their diverse complications are seen mainly in the abdomen which is often challenging for a pediatric gastroenterologist (Table 1). The liver is a part of the reticuloendothelial system and is hence invariably involved. Hematologic malignancies disseminate to infiltrate vital organs such as the gastrointestinal (GI) tract and pancreas. Nodal compressions and drug toxicities can often involve the pancreatobiliary system. Rarely the peritoneum is also infiltrated and complicates the clinical scenario. As the systemic complaints overwhelm the clinical picture, the abdominal manifestations are undermined and underreported leading to a lack of robust data in children. This review collates the majority of the available literature and sheds light on their diverse abdominal manifestations which range from asymptomatic involvement to life-threatening conditions. This review is limited to the discussion of the important hematolymphoid malignancies such as leukemias, lymphomas, and Langerhans cell histiocytosis (LCH). Discussion on detailed chemotherapeutic management and various opportunistic infectious complications of hematolymphoid malignancies affecting the abdomen are beyond the scope of this manuscript.

Rationale of the review

The gut and liver involvement in hematolymphoid malignancies frequently manifests with features that overlap with chronic infections and inflammatory conditions. The review may help readers in tackling the common clinical dilemmas and give a clear outline of various abdominal complications encountered in hematolymphoid malignancies. This may in turn improvise interdisciplinary referrals between a hematologist and pediatric gastroenterologist during the management for better outcomes.

LEUKEMIAS IN CHILDREN

The acute leukemias of childhood collectively represent about 32% of malignancies in children younger than 15 years of age[1]. Of all the leukemias in childhood, 97% are due to acute leukemias constituting lymphoid (76%), myeloid (20%), or undifferentiated lineage (1%) variants. Chronic myeloid leukemia (CML) and juvenile myelomonocytic leukemia (JMML) constitute the remaining 3%[2]. Infiltration of lymphoreticular organs, mainly the spleen, liver, and lymph nodes is a characteristic feature of acute leukemias[3].

Acute lymphoid leukemia

Liver involvement: The most common hepatic involvement at initial presentation is asymptomatic hepatomegaly, reported in up to 68% of acute lymphoid leukemia (ALL) pediatric patients. At the initial diagnosis, 34%-61% of children have enlargement of the liver or spleen (palpable organomegaly approximately > 4 cm below the costal margin)[1]. Abnormal liver biochemistry is seen in two settings of ALL: At initial diagnosis and/or during the treatment course. It is speculated that in ALL, there is a direct portal and sinusoidal infiltration by leukemic cells. The rise in liver transaminases is a consequence of hepatocellular necrosis due to leukemic infiltrates[4]. In a study by Segal *et al*[4], elevated liver transaminases were overall found in 34% of patients at presentation. Hepatosplenomegaly on physical examination was noted in 34% with hepatitis *vs* 56% of patients without hepatitis (P = 0.014). Additionally, 3.4% had conjugated hyperbilirubinemia along with abnormal liver enzymes. There were

Table 1 Overview of gut and liver manifestations of hematolymphoid malignancies in children

	Overview of gut and liver manifestations
Infiltration	Intussusception; Adynamic ileus; Mucosal ulceration; Hemorrhage; Perforation; Protein-losing enteropathy; Jaundice (biliary wall infiltration); Hepatosplenomegaly; Acute liver failure; Vanishing bile duct syndrome; Portal hypertension; Ascites (peritoneal seeding, peritoneal lymphomatosis); Splenic infarction, rupture
Immunodeficiency	Necrotizing enterocolitis (typhlitis); Appendicitis; Wound infections; Perirectal abscess; Sepsis; Opportunistic infections; Esophageal and hepatic candidiasis; Herpes infections; Cytomegalovirus infections; Pseudomembranous colitis; Protozoal infections; Invasive fungemia; Hepatitis B and C reactivation
Drug toxicity	Mucositis; Gastritis and gastroparesis; Ileus; Pseudoobstruction; Bowel necrosis; Pancreatitis; Hepatotoxicity
Compression	Gastric outlet obstruction; Biliary obstruction; Secondary Budd-Chiari syndrome; Chylous Ascites
Miscellaneous:	Gastrointestinal haemorrhage (thrombocytopenia, coagulopathy, secondary hemophagocytic lymphohistiocytosis)

no significant differences in the overall outcome among those who had normal *vs* elevated transaminases. Hence it was inferred that hepatitis at the time of diagnosis of ALL does not significantly affect the outcome or the induction chemotherapy success. Liver biopsy is not routinely advocated in ALL patients presenting with hepatitis. Due to concomitant bone marrow depression, these children carry a higher risk for bleeding complications as compared to patients having non-malignant liver disease[5]. The usual resolution of hepatitis with treatment suggests that liver biopsies should be reserved in patients with abnormal liver biochemistry who are either refractory to induction chemotherapy or have persistent hepatitis despite normal viral studies and abdominal imaging. The indications and timing of the liver biopsy should be individualized on a case-to-case basis and performed only if necessary. Liver failure as the presentation has been rarely reported in ALL[5]. This entity is commonly associated with T-cell ALL and has an overall poor prognosis despite the initiation of chemotherapy or liver transplantation. Anecdotal reports suggest short-term success after liver transplantation[6]. Rarely ischemia may be seen in hypovolemic conditions secondary to sepsis or volume loss. Occult chronic hepatitis B or C may reactivate during chemotherapy. Acute and chronic infection of hepatitis B or C may occur due to unscreened blood product transfusion. In countries with a high seroprevalence of cyto-megalo-virus (CMV), hepatic reactivations are often noted during the induction or maintenance phase of chemotherapy with steroids as they impair cellular immunity against CMV. Other systemic viral, bacterial, fungal, or parasitic infections may ensue due to an overall immunocompromised state.

Implications of chemotherapy on the liver: It is observed that the use of steroids during pre-phase induction chemotherapy normalizes the abnormal liver biochemistry in ALL patients presenting with hepatitis without features of fulminant liver failure. This allows full induction chemotherapy to be delivered under the routine protocol. In those with significant liver dysfunction, current ALL induction protocols suggest a dose reduction in chemotherapy agents or withholding a dose[4]. On chemotherapy, any rise in liver enzymes merits a thorough workup. They usually reflect liver injury secondary to chemotherapeutic agents such as methotrexate, L-asparaginase, 6-mercaptopurine (6-MP), daunorubicin, and vincristine. Persistent elevation of liver enzymes particularly occurring after 6-MP exposure necessitates evaluation for thiopurine methyltransferase activity. Those with abnormally low levels should not be given 6-MP and require either dose modification or replacement with other chemotherapeutic agents[7]. High dose methotrexate (> 500 mg/m^2), anthracyclines and L-asparaginase can cause liver cell failure in a predisposed patient with underlying liver disease. Genetic polymorphisms of various enzymes are involved in the drug metabolism during the therapy of ALL[8-10].

GI involvement: Pathogenesis of GI involvement is multifactorial. Various hypotheses include leukemic infiltration of gut mucosa, mucosal injury by chemotherapeutic agents (*e.g.*, methotrexate), gut neuropathy (*e.g.*, vincristine), and cancer cachexia. In the latter, there is a reduced anti-oxidant pool to support epithelial regeneration leading to the disrupted mucosal surface, intestinal edema and engorged vessels. These pathophysiologic alterations along with neutropenia and immune dysregulation make the gut more vulnerable to bacterial intramural invasion[11,12]. In a study of 273 children with ALL, GI symptoms at initial presentation were abdominal pain (19.5%), abdominal distension (18.5%), vomiting (14.9%) and bleeding (7.9%)[13]. In a retrospective analysis of 129 children with ALL who underwent upper GI endoscopy before chemotherapy, overall 82% had features suggestive of GI inflammation. Lesions in the esophagus, stomach, and duodenal bulb were 8.5%, 78%, 39.5% respectively. Concomitant *Helicobacter pylori* infection was also found to be an uncommon cause of gastritis in leukemic children as compared to adults[14]. Leukemic infiltrates in the GI tract initially remain clinically silent. Necrotizing enteropathy or typhlitis may occur in terminal stages[2]. Typhlitis is more common with acute myeloid leukemia (AML) than ALL (cumulative risk of 28.5% *vs* 7.4% respectively)[12]. Typhlitis is diagnosed by the presence of a clinical triad of abdominal pain, fever, and neutropenia or imaging signs (thickened bowel wall)[12]. Gram-negative rods, gram-positive cocci, enterococci, fungi, and viruses have been implicated as causes[11]. Fungal infections can play an

important role in necrotizing enteropathy. A systematic review of published case studies found significantly lower mortality rate in patients receiving antifungal agents for the treatment of necrotizing enteropathy[15]. In such situations, it may not be often possible to distinguish from other differential diagnoses such as pseudomembranous colitis, appendicitis, or ischemic colitis. Frank or localized intestinal perforation can culminate rapidly. Abdominal plain X-rays may show a dilated atonic cecum and ascending colon filled with liquid or gas, signs of intramural gas, and small bowel dilatation. However, X-rays have limited value due to their poor sensitivity and specificity[16]. On contrast-enhanced computed tomography (CT), bowel wall thickening is significantly more prominent in *Clostridiodes difficile* (*C. difficile*) colitis (mean wall thickness, 12 mm; range, 8-20 mm) than in neutropenic enterocolitis (mean wall thickness, 7 mm; range, 4-15 mm; $P < 0.01$)[17]. Inflammatory mass, pericolonic inflammation, and pneumatosis intestinalis may be rarely seen[11]. A barium enema is contraindicated due to its potential in causing colonic perforation and septicemia. Colonoscopy is best avoided unless biopsies are required to differentiate the condition from *C. difficile* colitis[18]. Medical therapy is the mainstay of management. Though most children with typhlitis respond to broad-spectrum antibiotics along with the anaerobic cover, granulocyte colony-stimulating factor support, intensive fluid replacement, and bowel rest, there is still a risk of mortality in 20%[12]. Initial empiric coverage for antifungal agents is not routinely recommended but they can be considered if the initial therapy does not show optimal response in 72 h[11]. Indications for surgery include bowel perforation, uncontrolled massive GI bleeding, abscess or appendicitis which occurs in 0.5%-1.5% of patients[11,19].

Implications of the chemotherapy on the GI tract: On a background of an already inflamed gastric mucosa, steroids and anthracyclines can predispose to further gastritis. In addition, vincristine used during the induction phase can lead to neuropathy of the GI tract causing gastroparesis, paralytic ileus and colonic atony. Changes in the gut microbial flora, small intestinal bacterial overgrowth and post-chemotherapy pro-inflammatory state cumulatively predispose the GI tract to be the source of febrile neutropenia in children with leukemia[20].

Pancreatic involvement: Pancreatitis has been rarely reported due to a progressive infiltrative disease or hypercalcemia[21]. The natural history of acute pancreatitis in hematolymphoid malignancies is modified due to the immunocompromised state when compared to normal individuals. The systemic inflammatory response syndrome may often be masked and the compensated anti-inflammatory response may occur earlier in the course. Abdominal complications include abdominal compartment syndrome, hemorrhage, infected necrosis, walled-off collections, and bowel obstruction. Endoscopic and radiological interventions in the induction phase may be precluded by thrombocytopenia due to disease or septicemia related. In a recent study, most children with treatment-related pancreatitis had genetic polymorphisms in 4-aminobutyrate aminotransferase (ABAT), asparagine synthetase, and cystic fibrosis transmembrane conductance regulator (CFTR) genes. Notably, these children harboured many more CFTR variants (71.4%) when compared to controls (39.1%). Identifying correlative variants in ethnically vulnerable populations may improve screening to identify which subgroup of patients with ALL are at the greatest risk for pancreatitis[22].

Implications of chemotherapy on the pancreas: Acute pancreatitis is most commonly described with the use of L-asparaginase in the induction period[13,14]. L-asparaginase-associated pancreatitis had an earlier incidence ranging from 0.7% to 24% and mortality rates of 2%-5%[23]. Incidence of acute pancreatitis is between 7%-18% in ALL. Due to the high recurrence rates of acute pancreatitis after rechallenge, it is one of the most common causes of truncation of asparaginase therapy during chemotherapy[23]. Studies assessing asparaginase-associated pancreatitis in children with ALL retrospectively reported incidences between 6.7% to 18%. Severity can range from mild to severe pancreatitis, which could be influenced by their immune suppression, frequent microbial translocation from the gut, coagulation disturbances, hyperlipidaemia associated with asparaginase-containing combination chemotherapy and the presence of leukemic infiltrations in the pancreas altering micro-architecture with most cases improving by withdrawal of the drug and conservative management[24]. Chronic complications rarely occur in the form of chronic pancreatitis or diabetes mellitus[23]. Acute pancreatitis has been reported after treatment with all asparaginase formulations[23]. A retrospective analysis of 403 children with ALL who developed acute pancreatitis after Peg-asparaginase administration revealed that patients with higher median age (10-18 years) have 2.4 times increased risk of pancreatitis than the younger ones. There was a non-statistically significant trend towards inferior 5-year event-free survival. Also 29% of patients with a known history of acute pancreatitis subsequently relapsed compared to only 14% with no prior history of acute pancreatitis[25]. ATF5 362TT and CT genotypes were associated with decreased risk of developing acute pancreatitis and have better disease outcomes demonstrating a low risk for events and superior survival[26]. Pancreatitis was more common in asparaginase-containing blocks *vs* non-asparaginase containing blocks (83% *vs* 17%; $P < 0.0001$). The median interval between receiving Peg-asparaginase dose and developing acute pancreatitis was 10 d. In a recent systematic review, older age, asparaginase formulation, higher ALL risk stratification, and higher asparaginase dosing appear to play a limited role in the development of acute pancreatitis. The Ponte di Legno Toxicity Working Group reviewed a large number of trials to investigate the risk of complications and

risk of re-exposing patients with acute pancreatitis[27]. Complications noted in the 465 patients with acute pancreatitis included mechanical ventilation (8%), pseudocysts (26%), acute insulin need (21%), and death (2%). Older age was associated with more complications (10.5 years *vs* 6.1 years without complications; $P < 0.0001$). One year after diagnosis of acute pancreatitis, 11% of patients continued to need insulin, had recurrent abdominal pain, or both. Ninety-six patients were re-exposed to asparaginase, including 59 after severe acute pancreatitis. Forty-four (46%) patients developed a second episode, 22 (52%) were severe, suggesting a high risk of recurrence[27]. Presently most oncologists agree that after recovery from a documented episode of pancreatitis, re-challenge with L-asparaginase should be an absolute contraindication. Re-challenge in mild pancreatitis is fraught with a high risk of recurrence, sometimes more severe than the first episode. Hypertriglyceridemia from L-asparaginase can also predispose to acute pancreatitis. Hypertriglyceridemia can also result in gall stones causing cholangitis and biliary pancreatitis[25].

AML

In AML, the extra-medullary manifestations are seen in 20%-25% of children. These include chloromas (tumor nodules), skin infiltration, cerebrospinal disease, gingival infiltration, hepatosplenomegaly, or testicular involvement. In particular, AML-M4 and AML-M5 present similar to ALL with lymphadenopathy and hepatosplenomegaly. Morphology and immunohistochemistry can distinguish the diagnosis[28].

Liver involvement: Though liver involvement as hepatomegaly is lesser than ALL clinically, postmortem studies have demonstrated up to 75% involvement of the liver[29]. Acute hepatitis may present as conjugated jaundice due to granulocytic sarcoma impeding bile flow or due to myeloid cell infiltration[30]. Acute liver failure at presentation is rarely reported with pediatric AML posing significant challenges to chemotherapy administration and invariably had poor outcomes in the cases described in the literature[31]. Coagulopathy and bleeding manifestations without other features of liver failure are the presenting features of the AML-M3 variant. This condition requires prompt institution of all-trans-retinoic acid (ATRA) along with cryoprecipitate transfusions to surpass the consumptive coagulopathy.

Involvement of other abdominal organs: Acute abdominal pain in AML is a significant problem. Spontaneous atraumatic rupture of the spleen presents as acute abdominal pain often mimicking a surgical abdomen or gut ischemia. Kehr's sign (acute pain in left shoulder tip) and hemoperitoneum are the hallmarks of this condition. It is more commonly associated with AML than ALL with worse outcomes. Its frequency is approximately 0.18%[32]. Possible mechanisms are rapid splenic enlargement outgrowing the vascular supply, leukemic infiltration of the splenic capsule, splenic infarction, and leukemia-associated thrombocytopenia-coagulopathy. Due to improvement in imaging techniques, the frequency of detection is 9-fold higher (0.55%) presently than in the earlier era (0.06%). There is also an increased incidental detection of "preclinical" splenic rupture. In this subset, 30% do not have palpable spleens[32]. Adolescent age group, acute promyelocytic leukemia variant, high leukocyte count at presentation, fungal infection, thrombocytopenia, and coagulopathy may predispose to pathologic splenic rupture[32]. The overall incidence of typhlitis in acute leukemias in children is 4%-5% with a higher cumulative risk in AML than ALL[12].

Implications of chemotherapy in AML: Chemotherapeutic agents used for AML include cytarabine, mitoxantrone, and daunorubicin, they can cause liver injury manifesting as hepatitis, cholestasis, and/or biliary stricture[33]. Furthermore, tretinoin and arsenic trioxide used in AML-M3 can cause hepatic impairment requiring dose modifications. Pancreatitis is seen with cytarabine therapy in 5% of AML[34].

Infantile leukemias

Infantile leukemias constitute a distinct subset of hematological malignancies characterized by aggressive presentation with high leukocyte counts, infiltration of extramedullary organs, and central nervous system involvement. Contrary to the epidemiology in older children where ALL predominates AML, in infants, the incidence is equal[35]. They are characterized by mixed-lineage leukemia gene rearrangements and frequently present with massive hepatosplenomegaly. They can present with cholestasis, elevated transaminases, or if untreated can lead to acute liver failure. Myeloid sarcoma may involve the liver particularly with chromosomal translocations involving t(1:22). Bone marrow examination may be unyielding due to marrow fibrosis, hence biopsy of the liver may clinch the diagnosis[36]. Outcomes are universally poor. Transient abnormal myelopoiesis (TAM) affects 10%-15% of neonates with down syndrome (trisomy 21), with mutations in the GATA-1 gene. Immature megakaryoblasts are seen in the liver, bone marrow, and peripheral blood. The clinical presentation can be highly variable ranging from incidentally detected in an otherwise well infant to a disseminated leukemic infiltration (10%-20 % of neonates) presenting with hepatomegaly (40%), splenomegaly (30%), jaundice (70%), hepatitis (25%) and coagulation disturbances (10%-25%)[37]. In TAM, liver failure can occur due to idiopathic progressive fibrosis, leukemic infiltration or iron deposition. Observation is

recommended for asymptomatic cases. Chemotherapy is indicated in those with a total blast count higher than 100000/μL, organomegaly causing respiratory compromise, significant anemia resulting in cardiac failure, hydrops fetalis, life-threatening hepatic dysfunction, hyperbilirubinemia, ascites, hepatitis, and disseminated intravascular coagulation[28]. The disorder usually spontaneously regresses within 3 mo. However, in 20%-30% of TAM patients, acute megakaryoblastic leukemia subsequently develops in 1-3 years[37].

Chronic leukemias

CML and JMML are chronic leukemias commonly described in children. Hepatomegaly is seen in 85% of pediatric CML and JMML. Liver dysfunction is uncommon. They have a more aggressive course in children than adults with higher leucocyte counts, larger spleen size, and an increased frequency of blast crisis[38]. Resolution of spleen size is an important follow-up criterion in CML. JMML has a relatively younger age at onset than CML with lymphadenopathy, hepatosplenomegaly, thrombocytopenia, increased fetal hemoglobin, and a lesser overall survival than CML[28].

Clinical impact of leukemias for the gastroenterologist

Acute leukemias that present with febrile hepatitis are often initially mistaken for infectious and immunological causes. Most patients have elaborate workup and multiple failed antimicrobial therapy before the diagnosis is ascertained. The diagnosis is often made on peripheral smear by a seasoned hematopathologist when atypical cells are identified. Drug-induced hepatotoxicity is a serious concern as many drugs are precluded and the outcome of the disease is modified. GI symptoms require prolonged proton pump inhibitor therapy till the end of the induction phase. An acute abdomen may need to be evaluated for typhlitis, pancreatitis, or spontaneous splenic rupture. Typhlitis in leukemias often leads to a complicated course requiring gut rest, prolonged antibiotic therapy. Since appendicitis is a close differential diagnosis, there is a considerable dilemma for the surgeon whether to perform a laparotomy in a state of neutropenia[39]. Pancreatitis during chemotherapy may have an underlying genetic predisposition and may need exploration before induction. Bloody diarrhea is ominous for a colonic involvement such as a superinfection with *C. difficile* in a neutropenia state. These complications need to be timely managed to avoid prolonged chemotherapy interruptions which may otherwise impact relapse rates. CML presenting with massive splenomegaly is often worked up for other differential diagnoses such as tropical splenomegaly syndrome, kala-azar, and extrahepatic portal hypertension in developing countries.

LYMPHOMAS IN CHILDREN

Combined, Hodgkin's disease (HD) and non-Hodgkin lymphomas (NHL) are the third most common malignancies in children and adolescents, with HD being the most common cancer in children between the ages of 15-18 years[40]. It is important to determine whether extranodal involvement represents a primary manifestation or is a part of a disseminated disease, which may have a poorer prognosis[41]. Extranodal disease is present in 12% of HD. Extranodal involvement is more common in older (10-17 years: 14%) than younger children (0-9 year: 4%)[42].

Hepatobiliary involvement

Hepatobiliary involvement in HD: Liver involvement is less frequent in HD than in NHL. Five percent of patients with HD have liver involvement at the time of diagnosis[43]. Usually, hepatomegaly is present when the liver is involved. However, liver size can rarely be normal despite infiltration. Smaller lesions are more common than large masses[41]. HD of the liver is almost invariably associated with disease of the spleen. The more extensive the splenic disease, the greater the likelihood of hepatic involvement[43]. In HD, lymphomatous cells may infiltrate the liver in up to 15% of patients with hepatomegaly and 45% in the later stages of the disease. Jaundice as a presenting symptom in HD is seen in 3%-13% of patients[44]. On many occasions, the diagnosis of HD can be misled by the presence of granulomas in liver biopsy specimens. A false impression of tuberculosis and unwarranted empirical antitubercular therapy is commonly encountered where there is a high community prevalence. Excision biopsy of palpable lymph nodes and imaging-guided biopsy of representative areas distinguishes the condition[45]. Acute liver failure can rarely occur in the setting of hepatic infiltration. One of the mechanisms by which malignant infiltration may cause liver failure is ischemia secondary to the compression of the hepatic sinusoids by the infiltrating cells[46]. Cholestasis can occur as a result of direct infiltration, extrahepatic biliary obstruction, hemolysis, viral hepatitis, or drug hepatotoxicity[44, 47]. In HD, cholestasis in zone 3 has also been described due to vanishing bile duct syndrome where there is an irreversible destruction of the small intrahepatic bile ducts and significant liver damage[47]. The mechanism by which this syndrome occurs is poorly understood but may be a paraneoplastic effect, a defect in liver microsomal function, or a toxic effect of cytokines released from lymphoma cells[48]. Other causes of this vanishing bile duct syndrome should be considered in the differential diagnosis before attributing it to HD[44]. Even with adequate treatment of lymphoma, most of these patients die

of progressive liver dysfunction and failure. Their course becomes further tenacious due to the preclusion of potentially hepatotoxic agents[48].

Hepatobiliary involvement in NHL: The four common childhood NHL include Burkitt lymphoma, lymphoblastic lymphoma, diffuse large B-cell lymphoma (DLBCL), and anaplastic large cell lymphoma. Lymphomatous infiltration and extrahepatic obstruction occur more commonly in NHL than in HD; 16% to 43% of patients with NHL have liver involvement. Mild to moderate increases in alkaline phosphatase level and hepatomegaly commonly occur in NHL even without lymphomatous hepatic involvement[47]. As described earlier with HD, acute liver failure can also occur in NHL. The mechanism by which this occurs is likely similar to that in HD, with sudden ischemia related to massive infiltration of the sinusoids or replacement of liver parenchyma by malignant cells[46]. This condition should be suspected when a patient presents with new-onset hepatomegaly and lactic acidosis. Prompt evaluation including liver biopsy should ensue[47]. Although the prognosis is poor, there have been reports of successful treatment with immediate initiation of chemotherapy in this subset of patients. Cholestasis may be additionally be caused by compression of enlarged periportal and peribiliary lymph nodes. Ghosh *et al*[49] described a cohort of nine children with NHL who presented with jaundice as the primary presentation which constituted 11.2% of all NHL. Total bilirubin and liver enzymes ranged from 2.9-19.6 mg/dL and 55-654 U/L respectively. All had raised alkaline phosphatase ranging from 957-3786 U/L. Seven patients had biliary obstruction with periampullary, periportal, gastroduodenal, or subhepatic masses on imaging. Two patients had liver parenchymal infiltration without biliary obstruction. Histology of these patients with biliary obstruction was anaplastic large cell, high-grade B-cell, and Burkitt lymphoma. Biliary drainage was performed in one patient. Seven patients had amelioration of jaundice with chemotherapy alone in 10-46 d [49]. Published case reports and small series have used surgery, biliary drainage, steroids, and cytotoxic agents in various combinations and sequences. Waiting for serum bilirubin to normalize after biliary drainage or treating only with steroids may compromise the outcome of patients. Also, chemotherapy after biliary drainage has been associated with complications such as biliary leak and peritonitis[50].

Radiological patterns of hepatobiliary involvement: In NHL, discrete lesions can be noted on CT scans. Liver biopsy is the most accurate method for confirmation of liver involvement[51]. Diffusely increased or focal uptake, with or without focal or disseminated nodules supports liver involvement (Figure 1). Discrete nodular lesions are seen in only 10% of cases. HD manifests more often as miliary lesions (< 1 cm in diameter) than as masses. The diffuse or infiltrative form of the disease results in patchy, irregular infiltrates originating primarily in the portal areas[41]. In current practice, fluorodeoxyglucose positron emission tomography with computerized tomography (FDG-PET-CT) is the reference standard for both staging and follow-up of HD and NHL. If an FDG-PET-CT is performed, a bone marrow examination can be avoided for HL. Bone marrow examination may be only needed for suspected DLBCL where there is a discordance between suggestive histology but negative PET[52].

Implications of chemotherapy: Empiric dose reductions in chemotherapy are usually recommended in jaundiced patients. Guidelines recommend a 50% dose reduction of etoposide in the presence of serum bilirubin 1.5-3 mg/dL and omitting the drug if serum bilirubin is greater than 3 mg/dL. For etoposide, pharmacokinetics in the presence of hepatic dysfunction has been determined in very few studies. In the presence of elevated serum bilirubin, there is no robust data to guide the dose modification of ifosfamide and cytarabine[53]. Neurotoxicity of high-dose cytarabine may worsen with elevated serum bilirubin but there are no guidelines to suggest a modification. Methotrexate is metabolized by the liver and excreted through the kidney. In those with preexisting hepatic dysfunction, methotrexate has the potential to cause further hepatic insult and hence is mostly withheld or administered in reduced doses [53]. However, in the pediatric NHL series, some authors have used doses higher than that recommended without reporting any organ dysfunction or mortality due to drug toxicity[49]. Ballonoff *et al* [54] reviewed 37 adults with NHL and found an association between an improvement in cholestasis and complete response to chemotherapy or radiation therapy (or both).

GI and pancreatic involvement

Pancreatic HD is extremely rare and, in almost all cases, secondary to contiguous lymph node disease. Since the pancreas has no definable capsule, it may be difficult to distinguish an adjacent lymph node disease from intrinsic pancreatic infiltration[41]. The prevalence of pancreatic involvement in pediatric Burkitt lymphoma is between 4% to 10%[55]. CT shows focal pancreatic enlargement with patchy areas of non-enhancement. Marked dilatation of the biliary system can occur when a mass infiltrates the pancreatic head[56]. HD involving the GI tract is rare as compared to NHL. Extra-nodal involvement (except in the spleen, Waldeyer's ring, and thymus) indicates stage IV HD[41].

Ascites in abdominal lymphoma

Ascites is an uncommon manifestation in lymphoma. Secondary Budd-Chiari syndrome can result from compression of the hepatic veins and/or the inferior vena cava by enlarged lymph nodes. Chylous ascites (defined as ascitic fluid triglyceride more than 200 mg/dL) may rarely occur due to malignant

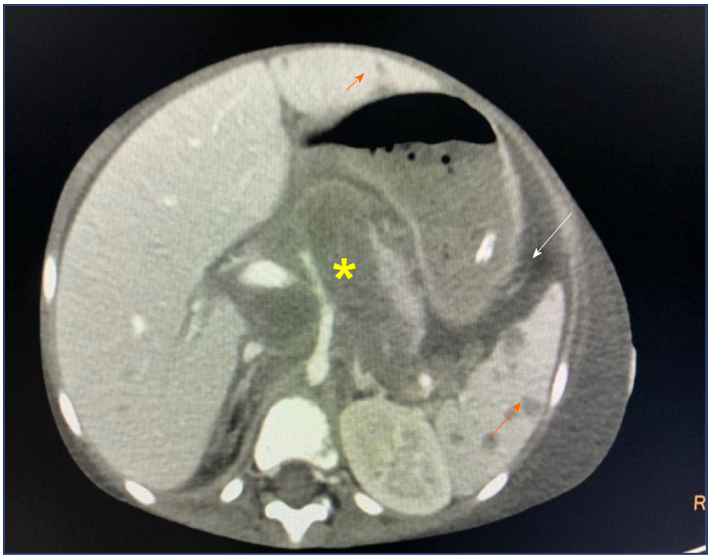

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Figure 1 Axial post contrast computed tomography image showing retroperitoneal lymphadenopathy with encasement of celiac artery and portal vein (yellow asterisk). There are multiple hypoenhancing lesions in liver, spleen (orange arrow) and presence of chylous ascites (white arrow).

lymph nodes obstructing the lymph flow from the gut to the cisterna chyli, resulting in leakage from the dilated subserosal lymphatics into the peritoneal cavity[57,58]. The presence of chylous ascites in lymphoma portends a poor prognosis[59]. Peritoneal lymphomatosis (PL) is a rare tumor originating in the peritoneum and quickly engulfs the gut, closely mimicking an acute abdomen. Most cases of primary PL have been reported in adults; however, the youngest case was seen in a 4-year-old[60,61]. The differential diagnoses of peritoneal thickening include tuberculosis, pseudomyxoma peritonei, lymphomatosis, mesenteric sarcoma, and desmoid tumors. Ascites occurs in 25% of patients with Burkitt lymphoma[62]. The diagnosis can often be made by paracentesis. Ascitic fluid is characteristically white (mimicking chylous ascites) with elevated lactate dehydrogenase, protein and atypical cells. Sometimes atypical cells may be missed because exfoliated mesothelial cells may predominate the cytological picture[63]. Sonography in PL shows thickened lamellar omentum, hypoechoic thickened mesentery that encases vessels, and non-septate, echogenic ascites[64]. CT abdomen shows omental caking, mesenteric soft-tissue nodularity along the vessels, lymphadenopathy, hepatosplenomegaly, hypoattenuating lesions in solid organs, and thickened bowel wall[60]. The most common histology encountered in peritoneal involvement in adults is DLBCL and in children is Burkitt lymphoma[63]. The treatment of PL is similar to Burkitt lymphoma. Although Burkitt lymphoma is chemosensitive, a large tumor burden in PL can lead to tumor lysis syndrome and rapid death[61].

Clinical impact of lymphomas for the gastroenterologist

Lymphomas presenting as cholestasis need a liver biopsy for diagnosis. In a setting of thrombocytopenia, a plugged percutaneous transhepatic or transjugular liver biopsy may be required. The presence of a granuloma is misleading for other differential diagnoses. In developing countries, many months of exposure to antitubercular therapy (for assumed disseminated tuberculosis) is common before a diagnosis of abdominal lymphoma is made. Vanishing bile duct syndrome has a poor prognosis. Often major lymph nodes are found around the periportal or peripancreatic areas in cholestasis. CT-guided biopsies risk chances of gut perforation of the overlapping small bowel. In such situations, tissue sampling can be challenging. Peribiliary and upper abdominal retroperitoneal lymph nodes are best accessed by pediatric endosonography in a specialized center. Linear endosonography is difficult to perform in a very young child as appropriately sized scopes are unavailable. Hence laparoscopy or laparotomy-based sampling is the final choice especially if the above techniques fail or lymph nodes are deep mesenteric or perivascular. Therapeutic endoscopic cholangiopancreatography and stent placement for biliary drainage are challenging in children. Ascites in lymphoma requires careful evaluation for an underlying source. PL has a universally poor prognosis.

PRIMARY LYMPHOMAS IN CHILDREN

Primary lymphomas in children can involve the GI tract [primary GI lymphoma (PGIL)], spleen (primary splenic lymphoma), liver [primary hepatic lymphoma (PHL)], and combination of spleen and liver [hepatosplenic T-cell lymphoma (HSTCL)]. The diagnosis of primary lymphomas is considered if

the bulk of the tumor is restricted to the organ of its origin after thorough staging and in the absence of distant lymphadenopathy or blood involvement (peripheral blood smear or bone marrow).

PHL

Criteria for diagnosis of PHL include symptoms caused mainly by liver involvement (palpable clinically at presentation or detected during staging radiologic studies) at presentation, absence of distant lymphadenopathy, and absence of leukemic blood involvement in the peripheral blood smear. PHL usually occurs in the 5th-6th decade and has been reported rarely in children[65,66]. The most common presentation is abdominal pain due to hepatomegaly. B symptoms of fever and weight loss occur in one-third of patients. Citak *et al*[67] in a review of literature of 10 children with PHL reported male preponderance. The presenting features were enlarging hepatomegaly, nonspecific symptoms of anorexia, fatigue and abdominal pain. Serum alkaline phosphatase and bilirubin levels are increased in 70% of cases. The most common type of primary hepatic NHL is DLBCL, comprising 80%-90% of the cases (Figure 2). This disease may present with nodules in the liver or diffuse portal infiltration and sinusoidal spread[68].

Most cases of PHL present with solitary or multiple mass lesions in the liver on imaging, diffuse involvement can also occur but is less common[65]. A solitary lesion is the most frequent finding which is encountered in 50% to 60% of cases. Estimating the prognosis of PHL is difficult because the condition is rare. Nodular, as opposed to diffusely infiltrative disease, may have a more favorable outcome with chemotherapy, with 3-year survival rates of 57% and 18%, respectively. PHL has also been described more often in those with immunodeficiency states, systemic lupus erythematosus, chronic hepatitis B, and chronic hepatitis C in adults[66]. Differential diagnoses of nodular PHL would be hepatocellular carcinoma, hepatoblastoma, liver embryonal sarcoma, metastatic neuroblastoma, liver rhabdomyosarcoma and hepatic Ewing's sarcoma[67]. There is a shift in paradigm from primary surgery to primary chemotherapy avoiding extensive hepatic lobectomy[65]. Early and aggressive anthracycline-based combination chemotherapy may result in prolonged remissions in PHL patients[69]. In situations where lymphoma presents as acute liver failure, it is often difficult to distinguish from PHL. Liver transplantation and subsequent chemotherapy are viable options in such scenarios[70].

HSTCL

HSTCL is a rare, aggressive lymphoma that infiltrates the hepatic sinusoids. In HSTCL, there is a diffuse hepatic sinusoidal and splenic sinus infiltration with clonal populations of gamma-delta T cell receptor expressing cells. Cytogenetic analysis commonly reveals an isochromosome 7q and trisomy 8[71]. Male patients younger than 35 years with inflammatory bowel disease and at least a 2-year history of exposure to combined thiopurine and biologic therapy may be at increased risk for developing HSTCL. Diak *et al*[72] reported 9 cases of HSTCL in the age group of 12-22 years receiving biological therapy and concomitant immunosuppression with thiopurines with or without steroids. During immunosuppression, thiopurines induce apoptosis, and this feature allows escape from tumor surveillance possibly leading to the development of malignancy. This situation, together with the effect of biological therapy on T cells (complement-mediated lysis and apoptosis), may partially explain as to why patients treated with these agents are at risk of HSTCL. Patients typically have hepatosplenomegaly, abnormal liver function tests, fever, weight loss, night sweats, pancytopenia, and peripheral lymphocytosis[72]. Bone marrow is involved in virtually all patients at the time of diagnosis. If HSTCL is suspected, a bone marrow biopsy (including immunophenotyping) should be performed to confirm the diagnosis[71]. Lymphadenopathy is usually absent. Histology can mimic autoimmune hepatitis and may often lead to misdiagnosis. With the increasing number of pediatric inflammatory bowel disease cases and the poor outcome of HSTCL, practice guidelines suggest thiopurines should be withdrawn from combination therapy after 6 mo in ulcerative colitis and 6-12 mo in Crohn's disease. This is preferably performed after checking adequate trough levels of anti-tumor necrosis factor agent and treatment target has been achieved[73,74].

PGIL

PGIL represents less than 5% of all pediatric neoplasms and is the most common bowel malignancy in childhood[75] (Table 2). Among its types, NHL is the most common malignancy of the GI tract in children. PGIL is less frequent than secondary GI involvement of nodal lymphoma. They are important since their evaluation, diagnosis, management, and prognosis are distinct from that of lymphoma at other sites and other cancers of the GI tract[75]. Criteria for PGIL suggested by Dawson is the presence of tumor bulk in the GI tract with minimal locoregional abdominal lymphadenopathy and characteristic absence of peripheral, mediastinal lymphadenopathy, hepatosplenomegaly, and normal blood cell counts[76]. The peak age for NHL of GI tract in children is 5-15 years. Surveillance, Epidemiology and End Results (SEER) registry from United States (1973-2006) showed the frequency of PGIL < 10 years and > 10 years as 44% and 56% respectively with higher male predilection (3:1)[75]. Small followed by large bowel are the most common sites in children, unlike adult patients where the stomach (50%-60%) is the most common site[75] (Figure 3A).The terminal ileum is the most commonly reported location in children, due to the high concentration of lymph tissue in that region of the bowel[76]. The most

Table 2 Outcome of gastrointestinal lymphomas in children from world global registries

	SEER registry, United States (1973-2006)[75]	West midlands, United Kingdom (1957-2000)[82]	Egypt cancer registry; (1997-2003)[77]
n	265	44	43
Incidence	0.199/100000	0.9 million/yr	-
Age (yr)	< 10: 44%; > 10: 56%	3-14	0.4-17
Male: female	3:1	5.7:1	2.3:1
Distant involvement	7.5%	-	-
Surgery	83.4%	96%	91%
Radiation	12.5%	71%	-

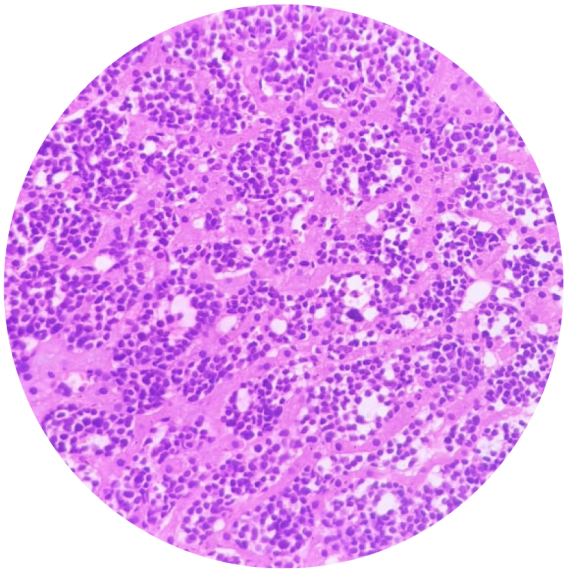

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Figure 2 Liver biopsy tissue showing normal hepatocytes with the sinusoids infiltrated by monomorphic round cell with darkly staining nuclei, small inconspicuous nucleoli and a narrow rim of cytoplasm. The infiltrating cells were positive for CD20, nuclear positivity for terminal deoxynucleotidyl transferase and high MIB1 index of 90%, suggestive of a B cell lymphoblastic lymphoma.

common presenting features are abdominal pain (81.4%) and abdominal lump (76.7%). Intestinal obstruction at presentation is seen in 11.6%[77]. Due to the absence of a desmoplastic response, the tumor grows along with walls. Bowel obstruction is initially uncommon until intussusceptions occur or tumor bulk becomes proliferative towards the lumen in the later part of the disease[78]. Of the PGIL, Burkitt lymphoma is the most frequent histological subtype in children[75] (Figure 3B). A typical morphological feature in Burkitt lymphoma is lymphoblastic cells having round nuclei with clumped chromatin and multiple, centrally located nucleoli giving the characteristic starry sky- appearance[79]. Sometimes it may not be possible to distinguish PGIL from secondary involvement in advanced cases when tumor bulk is high. Nearly half of children with GI NHL have tumor infiltrate confined to the GI tract with possible regional lymph node involvement. Imaging findings on CT include a diffuse or focal thickening of the stomach and/or bowel wall (Figures 4A, 4B and 4C). Aneurysmal dilatation of the bowel can be seen in nearly one-third of patients due to irregular growth in the muscularis propria and/or destruction of the autonomic nerve plexus[76]. Almost all the reported cases of appendicular lymphoma are due to NHL, seen as young as 3 years of age[80]. Pediatric NHL staging systems are St. Jude's and Revised International Pediatric NHL staging system[81]. The treatment approach in PGIL is debatable.

Proponents of surgery in the past argued that the disease is better debulked before chemotherapy to decrease tumor lysis syndrome, lessen the spread, and lessen the cumulative chemotherapy. Opponents who favored chemotherapy felt that upfront surgery had higher post-operative complications, effectively delayed starting of chemotherapy, and lead to poor long-term outcomes. Systematic review and meta-analysis compared surgery *vs* chemotherapy. It was seen that upfront surgery and

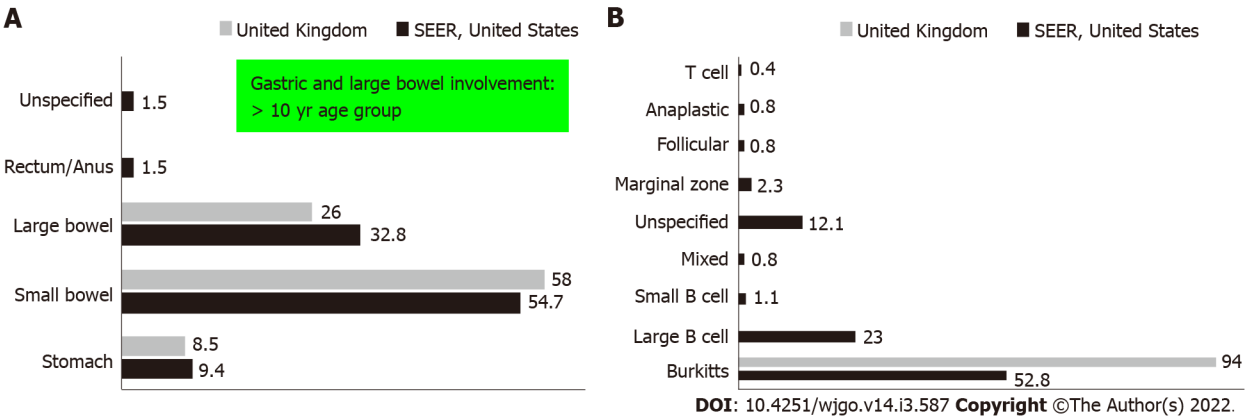

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Figure 3 Primary gastrointestinal lymphoma. A: Frequency of site involvement; B: Frequency of histological appearance. SEER: Surveillance, Epidemiology and End Results.

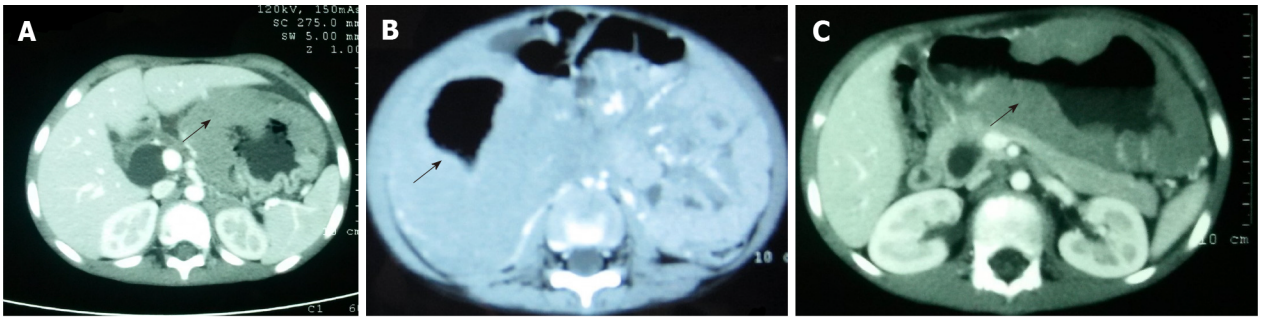

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Figure 4 Primary gastrointestinal lymphoma. Axial post contrast computed tomography abdomen with primarygastric lymphoma showing diffuse circumferential enhancing wall thickening (black arrow) and aneursymal dilatation of: A: Stomach; B: Ascending colon; C: Splenic flexure of colon.

chemotherapy in < 10 years of age showed near similar 5 and 10-year survival (83%-85%) in both groups. In those > 10 years of age, there was a significant statistical difference in the 5 and 10-year survival rates in the upfront surgery (79%) *vs* upfront chemotherapy (100%) groups[75]. Systematic reviews showed better 10-year disease-free survival and lesser recurrence in the medical group but higher mortality in the surgical group[75,77,82]. Chemotherapy alone seems to be the most effective treatment option in all stages of PGIL. Presently surgery is not indicated unless there are complications like perforation, hemorrhage, or obstruction which cannot be managed conservatively[83]. Further studies are needed to evaluate outcomes for patients with localized or distant disease, partial or complete resection, and the effect of adjuvant radiotherapy[75]. In terms of location, tumors located in the stomach, small bowel, colon, and rectum have 10-year survival as 64%, 86%, 83%, and 100%, respectively[75].

Immunoproliferative small intestinal disease

Immunoproliferative small intestinal disease (IPSID) is a unique lymphoproliferative disease that presents as malabsorption, anemia, pain abdomen, and protein-losing enteropathy. It is predominantly seen in adults but rarely also in adolescents. Blunting of villi and lymphoplasmacytic infiltrate with atypical lymphoid cells is the characteristic of small bowel histology. Diagnostic laparotomy for full-thickness small bowel biopsy and adjacent lymph nodes sampling is required with various systems of grading. Serum alpha heavy chain abnormal immunoglobulin A is pathognomonic by immunoelectrophoresis. Advanced IPSID presenting as abdominal mass is indistinguishable from lymph nodal lymphomas with secondary bowel involvement. Early disease is treated with antibiotics with a 33%-71% response[84]. Advanced IPSID needs chemotherapy and is associated with a poor prognosis[85]. IPSID is a close differential diagnosis for enteropathy-associated T-cell lymphoma which is seen in adults with underlying celiac disease.

Post-transplant lymphoproliferative disorder

Lymphoma in post-transplant recipients has been reported with the possible mechanism of continuous B-cell proliferation, which is normally inhibited by T-lymphocytes. Both solid organ and hematopoietic stem-cell transplant recipients are at risk for post-transplant lymphoproliferative disorder (PTLD), a

type of NHL driven mostly by Epstein-Barr virus infection. PTLD is associated highest with heart-lung, small bowel, and liver transplantations[86-88]. Liver and spleen involvement in PTLD is not unusual, occurring in 16% of patients over 20-years[85]. If present in the liver, PTLD can cause intrahepatic cholestasis or extrahepatic cholestasis from bulky lymphadenopathy around the porta hepatis.

Clinical impact of primary lymphomas for the gastroenterologist

Lymphomas originating from the GI or HB tract have a unique presentation. Due to their rapid infiltrative capacity, the disease is usually contained within their sites of origin before spillover into the blood or distant sites. Hence organomegaly and abdominal lump are the main presentations. PHL mimics storage disorders and congestive livers. PGIL mimics abdominal tuberculosis, intra-abdominal tumors, and large fungal masses such as basidiobolomycosis[89]. PGIL located in the upper GI tract or colon is amenable to endoscopic mucosal biopsies though often unyielding. Hence radiological guided needle biopsies are necessary to sample from deeper layers. A similar problem is encountered in IPSID. Diagnostic laparotomy is often required.

LCH

The hallmark of LCH is the accumulation of LC-like dendritic cells in one or more tissues or organs. The clinical manifestations of LCH are highly diverse and result both from direct (local effects of the growth and accumulation of pathologic LCs) and indirect effects (secondary changes on normal tissues, particularly cells of the immune system). The reported incidence of LCH ranges from 2.6 to 8.9 cases per million children younger than 15 years per year, with a median age at diagnosis of 3 years[90]. Pathologic LCs are clonal and nearly 60% of LCH samples carry the oncogenic BRAF V600E variant[90]. The characteristic histopathology required for a "presumptive diagnosis" usually shows a granulomatous-like lesion with immature dendritic-appearing cells that have characteristic bean-shaped, folded nuclei and pale cytoplasm. Often, multinucleated giant cells are present. A "definitive diagnosis" of LCH requires the immunohistochemical identification of the presence of Langerhans cell antigen expression of cell surface CD1a, CD207 (langerin), or by the presence of cells with Birbeck granules by electron microscopy. Early in the course of the disease, the lesions are usually proliferative and locally destructive. In later or healing stages, they can become more fibrotic[91]. The skeleton is the most commonly affected system, as bone lesions are present in approximately 80% of patients with LCH, and in half of them, lesions are single[90,92]. In infants with initially localized disease, progression to a multisystemic involvement (Figures 5A, 5B and 5C) has been observed in up to 40% of cases[90]. LCH can involve almost all organ systems and clinically risk organ involvement is defined as infiltration of the liver, spleen, bone marrow, and lung with the former three constituting high risk organs[93].

Hepatobiliary involvement

Liver is considered as a risk organ in staging of LCH with reported involvement ranging from 15%-60% and portends poor prognosis[94]. Liver involvement in LCH needs fulfillment of one or more of the following: Liver enlargement > 3 cm below the costal margin in the midclavicular line, liver dysfunction (*i.e.,*: Hypoproteinemia < 55 g/L, hypoalbuminemia < 25 g/L, hyperbilirubinemia > 1.5 mg/dL, edema or ascites, not as a result of other causes) or histopathological findings of active disease[90]. Yi *et al*[95] in a study of 31 children with LCH described hepatomegaly in 42%, jaundice in 16%, and splenomegaly in 19%. Hepatic involvement in LCH typically presents with hepatomegaly due to the direct infiltration by Langerhans cells. Hepatomegaly may also be due to Kupffer cell hypertrophy and hyperplasia secondary to a generalized immune reaction or by enlarged portal lymph nodes causing an obstruction. Presentation is typical with cholestasis and recurrent cholangitis. Biochemically there is a significantly elevated serum gamma-glutamyl transferase. Liver biopsy shows features of sclerosing cholangitis, periductal fibrosis, and bile duct proliferation. Liver biopsy is only recommended if there is clinically significant liver involvement and if the results of the same are likely to alter treatment in an otherwise diagnosed case of LCH (*i.e.,* to differentiate between active LCH and other causes of sclerosing cholangitis)[91]. The absence of Langerhans cells or CD1a positivity usually represents a burnt-out liver and has a poor response to chemotherapy. Since the disease process is usually found around the major bile ducts, a blind liver biopsy may miss the LCH infiltration[94]. Diagnostic imaging can visualize areas of LCH infiltration that may be missed on biopsy. Findings on liver imaging correspond to four progressive histological phases: Proliferative, granulomatous, xanthomatous, and the final fibrous phase. The proliferative and granulomatous phases show periportal histiocyte infiltration, inflammation, and edema which appear as periportal hypoechoic and relatively well-demarcated lesions on sonography. On CT, these lesions appear hypodense, and post-contrast enhancement is thought to reflect portal triaditis. Magnetic resonance imaging (MRI) shows hypointensities on T1-W images and hyperintensities on T2-W images. In the xanthomatous stage, periportal fatty lesions appear hyperechoic on sonography. They remain hypodense on CT images. On MRI, these lesions are hyperintense on T1 and hypointense on T2[96]. The fibrous stage is characterized by progression to periductal fibrosis and micronodular biliary cirrhosis which results from sclerosing cholangitis. In this

Table 3 Workup in Langerhans cell histiocytosis

Workup	
Mandatory investigations	Hemogram, complete liver function test (including coagulation), abdominal ultrasonography, chest Xray, skeletal survey (radiologic/nuclear), bone marrow examination
Optional	Complete body PET scan (at baseline and follow-up to monitor response and recurrence)
Special investigations	
Liver/biliary dysfunction	Liver biopsy, magnetic resonance cholangiography
Lung involvement	HRCT, pulmonary function test, bronchioalveolar lavage, lung biopsy (if necessary)
Craniofacial involvement, aural discharge, visual anomalies	MRI head, HRCT temporal bone
Diabetes inspidus	Urine specific gravity, water deprivation test, MRI head
Short stature, pubertal issues	Hormonal assessment, MRI head
Spinal involvement	MRI spine, spinal biopsy

PET: Positron emission tomography; HRCT: High-resolution computed tomography; MRI: Magnetic resonance imaging.

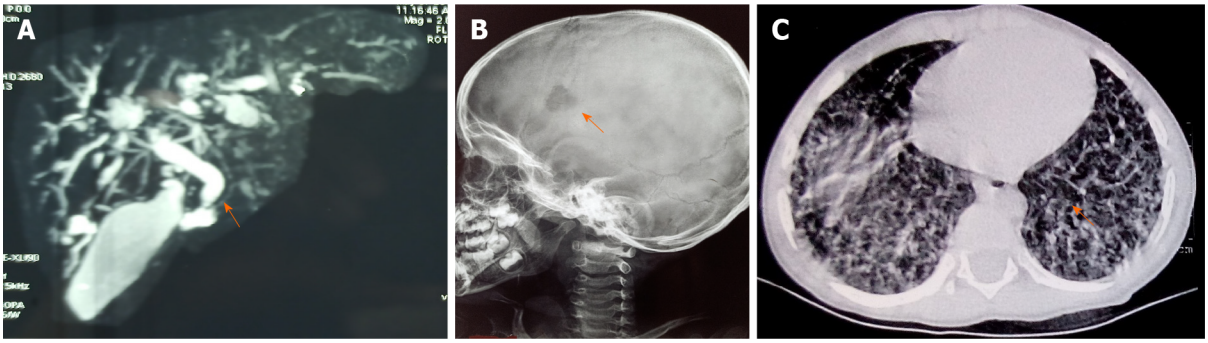

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Figure 5 In infants with initially localized disease, progression to a multisystemic involvement. A: Lower common bile duct stricture (orange arrow) with dilated duct, beading and dilatation of intrahepatic ducts on magnetic resonance cholangiography; B: Punched out lytic lesion in skull (orange arrow) on skeletal survey; C: Honeycombing cystic lesions in lung parenchyma on high-resolution computed tomography chest.

stage, sonography demonstrates well-demarcated periportal hypoechoic lesions with spotty calcification. Dilatation and beading of the biliary ducts, consistent with sclerosing cholangitis, can be seen with conventional cholangiography and MR cholangiopancreatography[96]. The hallmark of biliary involvement is an extrahepatic or intrahepatic sclerosing cholangitis that may occur in 10%-18% of patients with the multisystem form of LCH[97]. Sclerosing cholangitis may lead to secondary biliary cirrhosis, portal hypertension, and liver failure. Variceal bleeding is a frequent issue in portal hypertension requiring variceal banding or sclerotherapy. Braier *et al*[97] showed a 25% response to chemotherapy in LCH-sclerosing cholangitis. The rest either underwent liver transplantation or died. Sclerosing cholangitis is usually progressive and in these children, often the only successful treatment is liver transplantation[91,97]. Several poor prognostic factors are associated with survival like hepatic involvement, age < 1 year, and incomplete response to treatment. Three-year survival rates with and without liver involvement are 51.8% and 96.7% respectively[95]. The dominant extrahepatic biliary strictures persist despite chemotherapy and may need repeated endoscopic biliary dilatation. Therapeutic endoscopic retrograde cholangiopancreatography and biliary stent placement may be daunting in younger children where appropriate-sized endoscopes and accessories are not available. Repeated procedures in children expose them to greater radiation from fluoroscopy and adversely affect their long-term outcomes.

GI involvement

GI involvement is seen in less than 5% of cases commonly presenting as diarrhea, malabsorption, hematochezia, anemia and hypoproteinemia[98]. In a review of literature by Hait *et al*[99], of 22 children with LCH having GI involvement, 86% of patients presented before 1 year and 95% before 18 mo of age with 91% showing biopsy suggestive of LCH. Those with multiorgan involvement had higher mortality. Diarrhea, malabsorption, protein-losing enteropathy, and hematochezia are common manifestations of

Table 4 Common gastrointestinal manifestations with their clinical possibilities and recommendations for practice

Gut and liver manifestation	Possible reasons at diagnosis	Possible reasons during therapy	Recommendations
Jaundice	Tumor infiltration and necrosis of hepatocytes; Obstruction of biliary system by enlarged lymph nodes; Transfusion related viral hepatitis; Consider possibility of HLH as atypical presentation of hemato-lymphoid malignancies; Sclerosing cholangitis in a case of LCH	Chemotherapy induced liver injury (*e.g.*, 6-MP, Methotrexate); Reactivation of viral infections (*e.g.*, HBV, HCV, CMV, EBV)	Screen for HBV, HCV, HIV before starting chemotherapy; Safe transfusion practices; Exercise pharmacovigilance (chemotherapeutic drug dose modifications with underlying hepatic impairment, therapeutic drug monitoring-*e.g.*, Methotrexate); Screen for genetic polymorphisms (*e.g.*, TPMT, NUDT15 genotype for 6-MP)[104]; Abdominal imaging either CT angio or MRCP with MRI on clinical basis; Prioritize chemotherapy initiation for underlying malignancy over waiting for resolution of HLH with HLH treatment protocol [105]; Initiation of antivirals before chemotherapy for hepatitis B, hepatitis C infection as per standard guidelines [106,107]
Liver failure	Peculiar presentation with T-ALL, AML, TAM of newborn; Overwhelming sepsis at baseline due to poor immune reserve	Peculiar toxicity with L-asparaginase, high dose methotrexate and anthracyclines in predisposed individuals; Viral hepatitis especially hepatitis B reactivation	Early steroid initiation at presentation for preventing further liver cell necrosis in a case of ALL; Considering chemotherapy for TAM; FFP, cryoprecipitate product transfusions for hemostasis; Screen for hepatotrophic viral markers and appropriate antiviral therapy
Visceral perforation	Advanced stage lymphomas causing gut obstruction; Typhlitis due to severe neutropenia (*e.g.*, AML); Appendicitis as presentation (especially with ALL)	Post chemotherapy initiation with high grade lymphomas of stomach or intestine; *C. difficile* infection	Abdominal girth, bowel sound monitoring stringently in suspect cases; Abdominal imaging by CECT enterography with oral positive contrast; Stool examination in colitis for *C. difficile*; Anticipatory surgical consultation in advanced lymphomas
Bowel obstruction	High grade lymphomas causing intussusception; Extrinsic nodal compression of gut	Vinca alkaloid induced paralytic ileus during therapy; Septic ileus during periods of neutropenia	Abdominal imaging with CECT enterography; Adequate broad spectrum antibiotic cover; Surgical consultation for intussusception; Continuous gastric /bowel drainage above the level of obstruction
GI bleed	Mucosal bleed due to thrombocyt-openia at presentation; GI lymphoma[77]	Thrombocytopeniainduced mucosal bleeds; Drug induced coagulopathy (*e.g.*, peg-asparaginase); Typhlitis; *C. difficile* colitis	Conservative management with blood products; Laparotomy only in uncontrolled bleed for surgical resection; In suspect cases of *C.difficile* colitis, stool for toxin assay, GDH and consider colonoscopy
Pancreatitis	Rare as initial presentation	Drug induced (Asparaginase preparations, cytarabine)	Do not rechallenge with the same drug in case of AAP; Genetic testing could have a future role in predicting the risk of drug induced pancreatitis
Ascites	High grade lymphomas at presentation; Peritoneal lympho-matosis; Chylous ascites in prolonged untreated Hodgkins lymphomas; Reported cases of secondary BCS due to Burkitts lymphoma; Pancreatic ascites in severe pancreatitis	Drug induced liver failure (ex. Anthracyclines at toxic dose, L-asparaginase)	Ascitic fluid for flow cytometry and malignant cytology can provide rapid diagnosis; MCT supplementation for chylous ascites; Octreotide and TPN for refractory chylous ascites; Lymphangiography if refractory chylous ascites

GI: Gastrointestinal; 6MP: 6Mercaptopurine; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CMV: Cyto-megalo-virus; EBV: Epstein-Barr virus; HIV: Human immunodeficiency virus; LCH: Langerhans cell histiocytosis; T-ALL: T-cell acute lympoblastic leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; CT: Computed tomography; MRCP: Magnetic resonance cholangio-pancreatography; CECT: Contrast enhanced computerized tomography; FFP: Fresh frozen plasma; HLH: Hemophagocyticlymphohistiocytosis; TPMT: Thiopurinemethyltransferase; NUDT15: Nudix hydrolase-15; TAM: Transient abnormal myelopoiesis; GDH: Glutamate dehydrogenase; AAP: Asparaginase associated pancreatitis; MCT: Medium chain triglycerides; TPN: Total parenteral nutrition; BCS: Budd-Chiari syndrome; *C. difficile*: *Clostridiodes difficile*.

LCH involving the GI tract[91].

Clinical impact of LCH for the gastroenterologist

LCH is a complex disorder that is easier to diagnose clinically if there is a multisystemic presentation than isolated GI or HB involvement. Tissue diagnosis is definitive and a detailed workup for multisystemic involvement is important for management and prognosis (Table 3). The current standard of care for front-line therapy of patients with multifocal LCH or unifocal disease in CNS-risk sites is vinblastine/prednisone for 1 year, with the potential addition of 6-MP for high-risk LCH. Newer drugs such as vemurafenib are used to treat BRAF V600E mutation-positive, refractory, childhood LCH[100]. For those developing life-threatening diseases during treatment, alternative aggressive treatment should be considered, including hematopoietic stem cell transplantation. In the natural history, isolated HB disease is often treated as primary sclerosing cholangitis for many years till the disease evolves into a full-blown systemic problem or involves another site (bones, lungs, *etc.*). Hence guidelines for sclerosing

cholangitis in children require ruling out LCH effectively[101]. Recurrence rates in multisystem low-risk disease are lower in 12 mo than 6 mo of therapy. It is unclear whether further prolongation of therapy will ameliorate sclerosing cholangitis[91]. Since the majority of HB involvement is a reflection of a burnt-out disease, children on chemotherapy must be preemptively waitlisted for tentative liver transplantation. Dominant extrahepatic biliary strictures require biliary drainage for control of cholangitis and intractable pruritus that affect the quality of life.

NON-PHARMACOLOGICAL INTERVENTIONS IN PEDIATRIC HEMATOLYMPHOID MALIGNANCIES

The advances in supportive care have uplifted the survival curves dramatically in childhood hematolymphoid malignancies. Non-pharmacological management includes the appropriate use of interventional and non-interventional approaches tailored to the clinical scenario. The decision of proceeding with endoscopic or surgical intervention for an acutely ill child with cancer requires improved interdisciplinary communication and logistics in place. The various endoscopic interventions include biliary stenting by endoscopic retrograde cholangiopancreatography (ERCP) for cholangitis due to lymph nodal biliary obstruction and endoscopic balloon dilatation for dominant biliary strictures in sclerosing cholangitis[50]. Endoscopic percutaneous gastrostomy can be considered for optimizing nutrition in children with severe malnutrition, severe oral mucositis or anorexia. Similarly, antral stents can be deployed endoscopically for improving nutrition and palliation in malignant gastric outlet obstruction by lymphoma. Endoscopic closure with hemoclips (through the scope or over the scope) has been attempted in luminal perforations in GI lymphoma but this may be technically difficult and unrewarding. In ductal disruption/disconnected duct syndrome due to pancreatitis, pancreatic duct stenting can be attempted. Endoscopic or endoscopic ultrasound guided drainage for symptomatic pancreatic collections is performed in drug-induced severe pancreatitis. Radiological interventions include percutaneous transhepatic biliary drainage (for strictures proximal to liver hilum or after failed ERCP) and drainage of abdominal collections (after a sealed perforation or if the child is not a candidate for surgery). Pediatric hematolymphoid malignancies are both chemo and radiosensitive, hence the role of debulking surgery is limited to high-grade GI lymphomas presenting with emergencies such as bowel obstruction due to intussusception and extrinsic nodal compression[102]. Gut perforation, typhlitis and uncontrolled GI bleed may necessitate emergency laparotomy. In children with LCH, sclerosing cholangitis can progress to biliary cirrhosis necessitating salvage by a liver transplant. In liver failure, the use of liver assist devices like Prometheus, molecular adsorbent recirculation system can act as a bridge for liver transplantation.

Non-interventional approaches aim at optimizing nutrition, psychosocial support and judicious use of blood products. Nutritional compromise is multi-factorial and occurs mostly due to cancer-induced anorexia and emetogenic chemotherapeutic drugs. Hence ensuring nutritional rehabilitation through an energy-rich high protein diet and promoting a good psychosocial environment can lead to early recovery of these children[103]. In special circumstances like chylous ascites due to lymphatic infiltration by neoplastic cells, transient dietary modifications include the use of medium-chain triglycerides and restricting intake of long-chain triglycerides.

APPROACH TO COMMON GUT AND LIVER MANIFESTATIONS OF HEMATOLYMPHOID MALIGNANCIES IN CHILDREN

A summary of the clinical features and the recommendations are listed below (Table 4).

CONCLUSION

GI and hepatobiliary manifestations of hematolymphoid malignancies in children present with a multitude of symptoms ranging from subtle manifestations like asymptomatic organomegaly to moribund presentations like acute liver failure. The crux lies in establishing a diagnosis of malignancy while differentiating it from chronic infectious and inflammatory conditions. Use of invasive procedures such as guided biopsies and endoscopic interventions should be urged at the earliest window to avoid delay in therapy initiation in hematolymphoid malignancies with such atypical presentations. Pharmacovigilance should be practiced while using chemotherapeutic drugs to avoid hepatic and GI impairment. A prior thorough overview of specific manifestations and distinct drug toxicities by the physician would aid in optimal clinical management.

FOOTNOTES

Author contributions: Devarapalli UV, Sarma MS and Mathiyazhagan G contributed to the literature review; Devarapalli UV and Mathiyazhagan G contributed to the primary drafting; Sarma MS reviewed concept and design, intellectual inputs, and final drafting.

Conflict-of-interest statement: Authors declare no conflict of interest for this article.

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 **Gutierrez A**, Silverman LB. Acute Lymphoblastic leukemia. In: Orkin HS, Fisher DE, Ginsburg D, Look T, Lux SE, Nathan DG, editors. Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Philadelphia: Elsevier, 2015: 1527-1554
2 **Carroll WL**, BhatlaT. Acute Lymphoblastic leukemia. In: Lanzkowsky P, Lipton JM, Fish JD, editors. Manual of Pediatric Hematology and Oncology. London: Elsevier, 2016: 367-389
3 **Ebert EC**, Hagspiel KD. Gastrointestinal manifestations of leukemia. *J Gastroenterol Hepatol* 2012; **27**: 458-463 [PMID: 21913980 DOI: 10.1111/j.1440-1746.2011.06908.x]
4 **Segal I**, Rassekh SR, Bond MC, Senger C, Schreiber RA. Abnormal liver transaminases and conjugated hyperbilirubinemia at presentation of acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2010; **55**: 434-439 [PMID: 20658613 DOI: 10.1002/pbc.22549]
5 **Gu RL**, Xiang M, Suo J, Yuan J. Acute lymphoblastic leukemia in an adolescent presenting with acute hepatic failure: A case report. *Mol Clin Oncol* 2019; **11**: 135-138 [PMID: 31316772 DOI: 10.3892/mco.2019.1877]
6 **Reddi DM**, Barbas AS, Castleberry AW, Rege AS, Vikraman DS, Brennan TV, Ravindra KV, Collins BH, Sudan DL, Lagoo AS, Martin AE. Liver transplantation in an adolescent with acute liver failure from acute lymphoblastic leukemia. *Pediatr Transplant* 2014; **18**: E57-E63 [PMID: 24438382 DOI: 10.1111/petr.12221]
7 **Abaji R**, Krajinovic M. Thiopurine S-methyltransferase polymorphisms in acute lymphoblastic leukemia, inflammatory bowel disease and autoimmune disorders: influence on treatment response. *Pharmgenomics Pers Med* 2017; **10**: 143-156 [PMID: 28507448 DOI: 10.2147/PGPM.S108123]
8 **DeLeve LD**. Cancer chemotherapy. In: Kaplowitz N, DeLeve LD, editors. Drug-induced liver disease. Amsterdam: Elsevier, 2013: 541-568
9 **Hijiya N**, van der Sluis IM. Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. *Leuk Lymphoma* 2016; **57**: 748-757 [PMID: 26457414 DOI: 10.3109/10428194.2015.1101098]
10 **Schmiegelow K**. Advances in individual prediction of methotrexate toxicity: a review. *Br J Haematol* 2009; **146**: 489-503 [PMID: 19538530 DOI: 10.1111/j.1365-2141.2009.07765.x]
11 **Rodrigues FG**, Dasilva G, Wexner SD. Neutropenic enterocolitis. *World J Gastroenterol* 2017; **23**: 42-47 [PMID: 28104979 DOI: 10.3748/wjg.v23.i1.42]
12 **Altınel E**, Yarali N, Isık P, Bay A, Kara A, Tunc B. Typhlitis in acute childhood leukemia. *Med Princ Pract* 2012; **21**: 36-39 [PMID: 22024548 DOI: 10.1159/000331587]
13 **Robazzi TC**, Silva LR, Mendonça N, Barreto JH. Gastrointestinal manifestations as initial presentation of acute leukemias in children and adolescents. *Acta Gastroenterol Latinoam* 2008; **38**: 126-132 [PMID: 18697407]
14 **Guan X**, An X, Yu J, Xu Y. Incidence of upper digestive tract inflammation in children with acute lymphoblastic leukemia at diagnosis. *Int J Clin Exp Pathol* 2018; **11**: 3671-3677 [PMID: 31949748]
15 **Cardona Zorrilla AF**, Reveiz Herault L, Casasbuenas A, Aponte DM, Ramos PL. Systematic review of case reports concerning adults suffering from neutropenic enterocolitis. *Clin Transl Oncol* 2006; **8**: 31-38 [PMID: 16632437 DOI: 10.1007/s12094-006-0092-y]
16 **Tamburrini S**, Setola FR, Belfiore MP, Saturnino PP, Della Casa MG, Sarti G, Abete R, Marano I. Ultrasound diagnosis of typhlitis. *J Ultrasound* 2019; **22**: 103-106 [PMID: 30367357 DOI: 10.1007/s40477-018-0333-2]
17 **Kirkpatrick ID**, Greenberg HM. Gastrointestinal complications in the neutropenic patient: characterization and differentiation with abdominal CT. *Radiology* 2003; **226**: 668-674 [PMID: 12601214 DOI: 10.1148/radiol.2263011932]
18 **Mullassery D**, Bader A, Battersby AJ, Mohammad Z, Jones EL, Parmar C, Scott R, Pizer BL, Baillie CT. Diagnosis, incidence, and outcomes of suspected typhlitis in oncology patients--experience in a tertiary pediatric surgical center in the

United Kingdom. *J Pediatr Surg* 2009; **44**: 381-385 [PMID: 19231539 DOI: 10.1016/j.jpedsurg.2008.10.094]

19 **Ali A**, Alhindi S, Alalwan AA. Acute Appendicitis in a Child With Acute Leukemia and Chemotherapy-Induced Neutropenia: A Case Report and Literature Review. *Cureus* 2020; **12**: e8858 [PMID: 32617245 DOI: 10.7759/cureus.8858]

20 **Lakshmaiah KC**, Malabagi AS, Govindbabu, Shetty R, Sinha M, Jayashree RS. Febrile Neutropenia in Hematological Malignancies: Clinical and Microbiological Profile and Outcome in High Risk Patients. *J Lab Physicians* 2015; **7**: 116-120 [PMID: 26417163 DOI: 10.4103/0974-2727.163126]

21 **Wang Y**, Zhang X, Dong L, Tang K, Fang H, Tang Z, Zhang B. Acute lymphoblastic leukemia with pancreas involvement in an adult patient mimicking pancreatic tumor: A case report. *Medicine (Baltimore)* 2019; **98**: e15685 [PMID: 31169671 DOI: 10.1097/MD.0000000000015685]

22 **Grimes AC**, Chen Y, Bansal H, Aguilar C, Perez Prado L, Quezada G, Estrada J, Tomlinson GE. Genetic markers for treatment-related pancreatitis in a cohort of Hispanic children with acute lymphoblastic leukemia. *Support Care Cancer* 2021; **29**: 725-731 [PMID: 32447501 DOI: 10.1007/s00520-020-05530-w]

23 **Stefanović M**, Jazbec J, Lindgren F, Bulajić M, Löhr M. Acute pancreatitis as a complication of childhood cancer treatment. *Cancer Med* 2016; **5**: 827-836 [PMID: 26872431 DOI: 10.1002/cam4.649]

24 **Raja RA**, Schmiegelow K, Frandsen TL. Asparaginase-associated pancreatitis in children. *Br J Haematol* 2012; **159**: 18-27 [PMID: 22909259 DOI: 10.1111/bjh.12016]

25 **Bender C**, Maese L, Carter-Febres M, Verma A. Clinical Utility of Pegaspargase in Children, Adolescents and Young Adult Patients with Acute Lymphoblastic Leukemia: A Review. *Blood Lymphat Cancer* 2021; **11**: 25-40 [PMID: 33907490 DOI: 10.2147/BLCTT.S245210]

26 **Youssef YH**, Makkeyah SM, Soliman AF, Meky NH. Influence of genetic variants in asparaginase pathway on the susceptibility to asparaginase-related toxicity and patients' outcome in childhood acute lymphoblastic leukemia. *Cancer Chemother Pharmacol* 2021; **88**: 313-321 [PMID: 33959786 DOI: 10.1007/s00280-021-04290-6]

27 **Wolthers BO**, Frandsen TL, Baruchel A, Attarbaschi A, Barzilai S, Colombini A, Escherich G, Grell K, Inaba H, Kovacs G, Liang DC, Mateos M, Mondelaers V, Möricke A, Ociepa T, Samarasinghe S, Silverman LB, van der Sluis IM, Stanulla M, Vrooman LM, Yano M, Zapotocka E, Schmiegelow K; Ponte di Legno Toxicity Working Group. Asparaginase-associated pancreatitis in childhood acute lymphoblastic leukaemia: an observational Ponte di Legno Toxicity Working Group study. *Lancet Oncol* 2017; **18**: 1238-1248 [PMID: 28736188 DOI: 10.1016/S1470-2045(17)30424-2]

28 **Berman JN**, Look AT. Pediatric Myeloid Leukemia, Myelodysplasia, and Myeloproliferative Disease. In: Orkin HS, Fisher DE, Ginsburg D, Look T, Lux SE, Nathan DG, editors. Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Philadelphia: Elsevier, 2015: 1555-1613

29 **Van de Louw A**, Lewis AM, Yang Z. Autopsy findings in patients with acute myeloid leukemia and non-Hodgkin lymphoma in the modern era: a focus on lung pathology and acute respiratory failure. *Ann Hematol* 2019; **98**: 119-129 [PMID: 30218164 DOI: 10.1007/s00277-018-3494-3]

30 **Walsh LR**, Yuan C, Boothe JT, Conway HE, Mindiola-Romero AE, Barrett-Campbell OO, Yerrabothala S, Lansigan F. Acute myeloid leukemia with hepatic infiltration presenting as obstructive jaundice. *Leuk Res Rep* 2021; **15**: 100251 [PMID: 34141563 DOI: 10.1016/j.lrr.2021.100251]

31 **Nakano Y**, Yamasaki K, Otsuka Y, Ujiro A, Kawakita R, Tamagawa N, Okada K, Fujisaki H, Yorifuji T, Hara J. Acute Myeloid Leukemia With *RBM15-MKL1* Presenting as Severe Hepatic Failure. *Glob Pediatr Health* 2017; **4**: 2333794X16689011 [PMID: 28239626 DOI: 10.1177/2333794X16689011]

32 **Athale UH**, Kaste SC, Bodner SM, Ribeiro RC. Splenic rupture in children with hematologic malignancies. *Cancer* 2000; **88**: 480-490 [PMID: 10640983]

33 **Vincenzi B**, Armento G, Spalato Ceruso M, Catania G, Leakos M, Santini D, Minotti G, Tonini G. Drug-induced hepatotoxicity in cancer patients - implication for treatment. *Expert Opin Drug Saf* 2016; **15**: 1219-1238 [PMID: 27232067 DOI: 10.1080/14740338.2016.1194824]

34 **Trivedi CD**, Pitchumoni CS. Drug-induced pancreatitis: an update. *J Clin Gastroenterol* 2005; **39**: 709-716 [PMID: 16082282 DOI: 10.1097/01.mcg.0000173929.60115.b4]

35 **Kobos R**, Shukla N, Armstrong SA. Infant leukemias. In: Orkin HS, Fisher DE, Ginsburg D, Look T, Lux SE, Nathan DG, editors. Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Phiadelphia: Elsevier, 2015: 1614-1625

36 **Hang XF**, Xin HG, Wang L, Xu WS, Wang JX, Ni W, Cai X, Zhang RQ. Nonleukemic myeloid sarcoma of the liver: a case report and review of literature. *Hepatol Int* 2011; **5**: 747-750 [PMID: 21484146 DOI: 10.1007/s12072-010-9233-z]

37 **Bhatnagar N**, Nizery L, Tunstall O, Vyas P, Roberts I. Transient Abnormal Myelopoiesis and AML in Down Syndrome: an Update. *Curr Hematol Malig Rep* 2016; **11**: 333-341 [PMID: 27510823 DOI: 10.1007/s11899-016-0338-x]

38 **Hijiya N**, Millot F, Suttorp M. Chronic myeloid leukemia in children: clinical findings, management, and unanswered questions. *Pediatr Clin North Am* 2015; **62**: 107-119 [PMID: 25435115 DOI: 10.1016/j.pcl.2014.09.008]

39 **Many BT**, Lautz TB, Dobrozsi S, Wilkinson KH, Rossoff J, Le-Nguyen A, Dakhallah N, Piche N, Weinschenk W, Cooke-Barker J, Goodhue C, Zamora A, Kim ES, Talbot LJ, Quevedo OG, Murphy AJ, Commander SJ, Tracy ET, Short SS, Meyers RL, Rinehardt HN, Aldrink JH, Heaton TE, Ortiz MV, Baertschiger R, Wong KE, Lapidus-Krol E, Butter A, Davidson J, Stark R, Ramaraj A, Malek M, Mastropolo R, Morgan K, Murphy JT, Janek K, Le HD, Dasgupta R, Lal DR; PEDIATRIC SURGICAL ONCOLOGY RESEARCH COLLABORATIVE. Appendectomy Versus Observation for Appendicitis in Neutropenic Children With Cancer. *Pediatrics* 2021; **147** [PMID: 33504609 DOI: 10.1542/peds.2020-027797]

40 **Alexander S**, FerrandoAA. Pediatric Lymphoma. In: Orkin HS, Fisher DE,Ginsburg D, Look T, Lux SE, Nathan DG, editors.Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Philadelphia: Elsevier, 2015: 1626-1672

41 **Guermazi A**, Brice P, de Kerviler E E, Fermé C, Hennequin C, Meignin V, Frija J. Extranodal Hodgkin disease: spectrum of disease. *Radiographics* 2001; **21**: 161-179 [PMID: 11158651 DOI: 10.1148/radiographics.21.1.g01ja02161]

42 **Englund A**, Glimelius I, Rostgaard K, Smedby KE, Eloranta S, Molin D, Kuusk T, Brown PN, Kamper P, Hjalgrim H,

Ljungman G, Hjalgrim LL. Hodgkin lymphoma in children, adolescents and young adults - a comparative study of clinical presentation and treatment outcome. *Acta Oncol* 2018; **57**: 276-282 [PMID: 28760045 DOI: 10.1080/0284186X.2017.1355563]

43 **Hagleitner MM**, Metzger ML, Flerlage JE, Kelly KM, Voss SD, Kluge R, Kurch L, Cho S, Mauz-Koerholz C, Beishuizen A. Liver involvement in pediatric Hodgkin lymphoma: A systematic review by an international collaboration on Staging Evaluation and Response Criteria Harmonization (SEARCH) for Children, Adolescent, and Young Adult Hodgkin Lymphoma (CAYAHL). *Pediatr Blood Cancer* 2020; **67**: e28365 [PMID: 32491274 DOI: 10.1002/pbc.28365]

44 **Guliter S**, Erdem O, Isik M, Yamac K, Uluoglu O. Cholestatic liver disease with ductopenia (vanishing bile duct syndrome) in Hodgkin's disease: report of a case. *Tumori* 2004; **90**: 517-520 [PMID: 15656342]

45 **Shah KK**, Pritt BS, Alexander MP. Histopathologic review of granulomatous inflammation. *J Clin Tuberc Other Mycobact Dis* 2017; **7**: 1-12 [PMID: 31723695 DOI: 10.1016/j.jctube.2017.02.001]

46 **Rich NE**, Sanders C, Hughes RS, Fontana RJ, Stravitz RT, Fix O, Han SH, Naugler WE, Zaman A, Lee WM. Malignant infiltration of the liver presenting as acute liver failure. *Clin Gastroenterol Hepatol* 2015; **13**: 1025-1028 [PMID: 25277846 DOI: 10.1016/j.cgh.2014.09.040]

47 **Shimizu Y**. Liver in systemic disease. *World J Gastroenterol* 2008; **14**: 4111-4119 [PMID: 18636653 DOI: 10.3748/wjg.14.4111]

48 **Bakhit M**, McCarty TR, Park S, Njei B, Cho M, Karagozian R, Liapakis A. Vanishing bile duct syndrome in Hodgkin's lymphoma: A case report and literature review. *World J Gastroenterol* 2017; **23**: 366-372 [PMID: 28127210 DOI: 10.3748/wjg.v23.i2.366]

49 **Ghosh I**, Bakhshi S. Jaundice as a presenting manifestation of pediatric non-Hodgkin lymphoma: etiology, management, and outcome. *J Pediatr Hematol Oncol* 2010; **32**: e131-e135 [PMID: 20445407 DOI: 10.1097/MPH.0b013e3181d640c5]

50 **Odemiş B**, Parlak E, Başar O, Yüksel O, Sahin B. Biliary tract obstruction secondary to malignant lymphoma: experience at a referral center. *Dig Dis Sci* 2007; **52**: 2323-2332 [PMID: 17406815 DOI: 10.1007/s10620-007-9786-4]

51 **Huang MS**, Weinstein H. Non-HodgkinsLymphoma.In: Lanzkowsky P, Lipton JM, Fish JD, editors. Manual of Pediatric Hematology and Oncology. London: Elsevier, 2016: 442-452

52 **Cheson BD**, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, Lister TA; Alliance, Australasian Leukaemia and Lymphoma Group; Eastern Cooperative Oncology Group; European Mantle Cell Lymphoma Consortium; Italian Lymphoma Foundation; European Organisation for Research; Treatment of Cancer/Dutch Hemato-Oncology Group; Grupo Español de MédulaÓsea; German High-Grade Lymphoma Study Group; German Hodgkin's Study Group; Japanese Lymphoma Study Group; Lymphoma Study Association; NCIC Clinical Trials Group; Nordic Lymphoma Study Group; Southwest Oncology Group; United Kingdom National Cancer Research Institute. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol* 2014; **32**: 3059-3068 [PMID: 25113753 DOI: 10.1200/JCO.2013.54.8800]

53 **Superfin D**, Iannucci AA, Davies AM. Commentary: Oncologic drugs in patients with organ dysfunction: a summary. *Oncologist* 2007; **12**: 1070-1083 [PMID: 17914077 DOI: 10.1634/theoncologist.12-9-1070]

54 **Ballonoff A**, Kavanagh B, Nash R, Drabkin H, Trotter J, Costa L, Rabinovitch R. Hodgkin lymphoma-related vanishing bile duct syndrome and idiopathic cholestasis: statistical analysis of all published cases and literature review. *Acta Oncol* 2008; **47**: 962-970 [PMID: 17906981 DOI: 10.1080/02841860701644078]

55 **Aftandilian CC**, Friedmann AM. Burkitt lymphoma with pancreatic involvement. *J Pediatr Hematol Oncol* 2010; **32**: e338-e340 [PMID: 20930650 DOI: 10.1097/MPH.0b013e3181ed1178]

56 **Pietsch JB**, Shankar S, Ford C, Johnson JE. Obstructive jaundice secondary to lymphoma in childhood. *J Pediatr Surg* 2001; **36**: 1792-1795 [PMID: 11733908 DOI: 10.1053/jpsu.2001.28840]

57 **Almakdisi T**, Massoud S, Makdisi G. Lymphomas and chylous ascites: review of the literature. *Oncologist* 2005; **10**: 632-635 [PMID: 16177287 DOI: 10.1634/theoncologist.10-8-632]

58 **Bhardwaj R**, Vaziri H, Gautam A, Ballesteros E, Karimeddini D, Wu GY. Chylous Ascites: A Review of Pathogenesis, Diagnosis and Treatment. *J Clin Transl Hepatol* 2018; **6**: 105-113 [PMID: 29577037 DOI: 10.14218/JCTH.2017.00035]

59 **Steinemann DC**, Dindo D, Clavien PA, Nocito A. Atraumatic chylous ascites: systematic review on symptoms and causes. *J Am Coll Surg* 2011; **212**: 899-905.e1 [PMID: 21398159 DOI: 10.1016/j.jamcollsurg.2011.01.010]

60 **Curakova E**, Genadieva-Dimitrova M, Misevski J, Caloska-Ivanova V, Andreevski V, Todorovska B, Isahi U, Trajkovska M, Misevska P, Joksimovic N, Genadieva-Stavric S, Antovic S, Jankulovski N. NonHodgkin's Lymphoma with Peritoneal Localization. *Case Rep Gastrointest Med* 2014; **2014**: 723473 [PMID: 24711934 DOI: 10.1155/2014/723473]

61 **Ravindranath A**, Srivastava A, Seetharaman J, Pandey R, Sarma MS, Poddar U, Yachha SK. Peritoneal Lymphomatosis Masquerading as Pyoperitoneum in a Teenage Boy. *ACG Case Rep J* 2019; **6**: e00116 [PMID: 31616776 DOI: 10.14309/crj.0000000000000116]

62 **Biko DM**, Anupindi SA, Hernandez A, Kersun L, Bellah R. Childhood Burkitt lymphoma: abdominal and pelvic imaging findings. *AJR Am J Roentgenol* 2009; **192**: 1304-1315 [PMID: 19380555 DOI: 10.2214/AJR.08.1476]

63 **James KM**, Bogue CO, Murphy AJ, Navarro OM. Peritoneal Malignancy in Children: A Pictorial Review. *Can Assoc Radiol J* 2016; **67**: 402-408 [PMID: 27523447 DOI: 10.1016/j.carj.2016.03.003]

64 **Que Y**, Wang X. Sonography of peritoneal lymphomatosis: some new and different findings. *Ultrasound Q* 2015; **31**: 55-58 [PMID: 25706365 DOI: 10.1097/RUQ.0000000000000085]

65 **Ugurluer G**, Miller RC, Li Y, Thariat J, Ghadjar P, Schick U, Ozsahin M. Primary Hepatic Lymphoma: A Retrospective, Multicenter Rare Cancer Network Study. *Rare Tumors* 2016; **8**: 6502 [PMID: 27746888 DOI: 10.4081/rt.2016.6502]

66 **Delemos AS**, Friedman LS. Systemic causes of cholestasis. *Clin Liver Dis* 2013; **17**: 301-317 [PMID: 23540504 DOI: 10.1016/j.cld.2012.11.001]

67 **Citak EC**, Sari I, Demirci M, Karakus C, Sahin Y. Primary hepatic Burkitt lymphoma in a child and review of literature. *J Pediatr Hematol Oncol* 2011; **33**: e368-e371 [PMID: 22042287 DOI: 10.1097/MPH.0b013e31822ea131]

68 **Baumhoer D**, Tzankov A, Dirnhofer S, Tornillo L, Terracciano LM. Patterns of liver infiltration in lymphoproliferative disease. *Histopathology* 2008; **53**: 81-90 [PMID: 18540976 DOI: 10.1111/j.1365-2559.2008.03069.x]

69 **Noronha V**, Shafi NQ, Obando JA, Kummar S. Primary non-Hodgkin's lymphoma of the liver. *Crit Rev Oncol Hematol* 2005; **53**: 199-207 [PMID: 15718146 DOI: 10.1016/j.critrevonc.2004.10.010]

70 **Cameron AM**, Truty J, Truell J, Lassman C, Zimmerman MA, Kelly BS Jr, Farmer DG, Hiatt JR, Ghobrial R, Busuttil RW. Fulminant hepatic failure from primary hepatic lymphoma: successful treatment with orthotopic liver transplantation and chemotherapy. *Transplantation* 2005; **80**: 993-996 [PMID: 16249751 DOI: 10.1097/01.tp.0000173999.09381.95]

71 **Yabe M**, Miranda RN, Medeiros LJ. Hepatosplenic T-cell Lymphoma: a review of clinicopathologic features, pathogenesis, and prognostic factors. *Hum Pathol* 2018; **74**: 5-16 [PMID: 29337025 DOI: 10.1016/j.humpath.2018.01.005]

72 **Diak P**, Siegel J, La Grenade L, Choi L, Lemery S, McMahon A. Tumor necrosis factor alpha blockers and malignancy in children: forty-eight cases reported to the Food and Drug Administration. *Arthritis Rheum* 2010; **62**: 2517-2524 [PMID: 20506368 DOI: 10.1002/art.27511]

73 **van Rheenen PF**, Aloi M, Assa A, Bronsky J, Escher JC, Fagerberg UL, Gasparetto M, Gerasimidis K, Griffiths A, Henderson P, Koletzko S, Kolho KL, Levine A, van Limbergen J, Martin de Carpi FJ, Navas-López VM, Oliva S, de Ridder L, Russell RK, Shouval D, Spinelli A, Turner D, Wilson D, Wine E, Ruemmele FM. The Medical Management of Paediatric Crohn's Disease: an ECCO-ESPGHAN Guideline Update. *J Crohns Colitis* 2020 [PMID: 33026087 DOI: 10.1093/ecco-jcc/jjaa161]

74 **Turner D**, Ruemmele FM, Orlanski-Meyer E, Griffiths AM, de Carpi JM, Bronsky J, Veres G, Aloi M, Strisciuglio C, Braegger CP, Assa A, Romano C, Hussey S, Stanton M, Pakarinen M, de Ridder L, Katsanos K, Croft N, Navas-López V, Wilson DC, Lawrence S, Russell RK. Management of Paediatric Ulcerative Colitis, Part 1: Ambulatory Care-An Evidence-based Guideline From European Crohn's and Colitis Organization and European Society of Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2018; **67**: 257-291 [PMID: 30044357 DOI: 10.1097/MPG.0000000000002035]

75 **Kassira N**, Pedroso FE, Cheung MC, Koniaris LG, Sola JE. Primary gastrointestinal tract lymphoma in the pediatric patient: review of 265 patients from the SEER registry. *J Pediatr Surg* 2011; **46**: 1956-1964 [PMID: 22008334 DOI: 10.1016/j.jpedsurg.2011.06.006]

76 **Kumar P**, Singh A, Deshmukh A, Chandrashekhara SH. Imaging of Bowel Lymphoma: A Pictorial Review. *Dig Dis Sci* 2021 [PMID: 33877497 DOI: 10.1007/s10620-021-06979-3]

77 **Morsi A**, Abd El-Ghani Ael-G, El-Shafiey M, Fawzy M, Ismail H, Monir M. Clinico-pathological features and outcome of management of pediatric gastrointestinal lymphoma. *J Egypt Natl Canc Inst* 2005; **17**: 251-259 [PMID: 17102814]

78 **Qadri SK**, Shah A, Hamdani NH, Baba KM. Primary gastrointestinal lymphomas in children: An experience of 12 years from a tertiary care center of North India. *Indian J Cancer* 2016; **53**: 300-303 [PMID: 28071632 DOI: 10.4103/0019-509X.197718]

79 **Bellan C**, Stefano L, Giulia de F, Rogena EA, Lorenzo L. Burkitt lymphoma *vs* diffuse large B-cell lymphoma: a practical approach. *Hematol Oncol* 2009; **27**: 182-185 [PMID: 19670467 DOI: 10.1002/hon.914]

80 **Ghosal A**, Acharyya S. A rare presentation of Hodgkin's lymphoma in a very young child, with involvement of the appendix. *BMJ Case Rep* 2014; **2014** [PMID: 25015165 DOI: 10.1136/bcr-2014-204027]

81 **Rosolen A**, Perkins SL, Pinkerton CR, Guillerman RP, Sandlund JT, Patte C, Reiter A, Cairo MS. Revised International Pediatric Non-Hodgkin Lymphoma Staging System. *J Clin Oncol* 2015; **33**: 2112-2118 [PMID: 25940716 DOI: 10.1200/JCO.2014.59.7203]

82 **Hameed R**, Parkes S, Davies P, Morland BJ. Paediatric malignant tumours of the gastrointestinal tract in the West Midlands, UK, 1957-2000: a large population based survey. *Pediatr Blood Cancer* 2004; **43**: 257-260 [PMID: 15266410 DOI: 10.1002/pbc.20086]

83 **Erkurt MA**, Aydogdu I, Kuku I, Kaya E, Basaran Y. Clinicopathologic characteristics and therapeutic outcomes of primary gastrointestinal non-Hodgkin's lymphomas: 10 years of experience from a single center in eastern Anatolia. *Med Princ Pract* 2009; **18**: 399-406 [PMID: 19648764 DOI: 10.1159/000226295]

84 **Evangelista-Leite D**, Affonso Madaloso B, Shouta Yamashita B, Enrico Aloise F, Polito Verdasca L, Lopes de Mello M, Murata Hayashi R, Zimberg Chehter E. Treating chronic diarrhea: A systematic review on Immunoproliferative Small Intestinal Disease (IPSID). *PLoS One* 2021; **16**: e0253695 [PMID: 34270561 DOI: 10.1371/journal.pone.0253695]

85 **Geethakumari PR**, Rizvi SM. Gastrointestinal Lymphoma. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. Philadelphia: Elsevier, 2021: 442-457

86 **Offor UT**, Bacon CM, Roberts J, Powell J, Brodlie M, Wood K, Windebank KP, Flett J, Hewitt T, Rand V, Hasan A, Parry G, Gennery AR, Reinhardt Z, Bomken S. Transplantation for congenital heart disease is associated with an increased risk of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in children. *J Heart Lung Transplant* 2021; **40**: 24-32 [PMID: 33339556 DOI: 10.1016/j.healun.2020.10.006]

87 **Stanley K**, Friehling E, Ranganathan S, Mazariegos G, McAllister-Lucas LM, Sindhi R. Post-transplant lymphoproliferative disorder in pediatric intestinal transplant recipients: A literature review. *Pediatr Transplant* 2018; **22**: e13211 [PMID: 29745058 DOI: 10.1111/petr.13211]

88 **Liu Y**, Sun LY, Zhu ZJ, Wei L, Qu W, Wang L, Yuan LL, Zeng ZG. Post-transplant lymphoproliferative disorder after paediatric liver transplantation. *Int J Clin Pract* 2021; **75**: e13843 [PMID: 33222369 DOI: 10.1111/ijcp.13843]

89 **Ravindranath A**, Pai G, Sen Sarma M, Srivastava A, Lal R, Agrawal V, Marak R, Yachha SK, Poddar U, Prasad P. Gastrointestinal Basidiobolomycosis: A Mimic of Lymphoma. *J Pediatr* 2021; **228**: 306-307 [PMID: 32916142 DOI: 10.1016/j.jpeds.2020.09.009]

90 **Rodriguez-Galindo C**, Allen CE. Langerhans cell histiocytosis. *Blood* 2020; **135**: 1319-1331 [PMID: 32106306 DOI: 10.1182/blood.2019000934]

91 **Arceci RJ**. Histiocytosis syndromes. In: Lanzkowsky P, Lipton JM, Fish JD, editors. Manual of Pediatric Hematology and Oncology. London: Elsevier, 2016: 407-428

92 **Heisig A**, Sörensen J, Zimmermann SY, Schöning S, Schwabe D, Kvasnicka HM, Schwentner R, Hutter C, Lehrnbecher T. Vemurafenib in Langerhans cell histiocytosis: report of a pediatric patient and review of the literature. *Oncotarget* 2018; **9**: 22236-22240 [PMID: 29774135 DOI: 10.18632/oncotarget.25277]

93 **Allen CE**, Ladisch S, McClain KL. How I treat Langerhans cell histiocytosis. *Blood* 2015; **126**: 26-35 [PMID: 25827831 DOI: 10.1182/blood-2014-12-569301]

94 **Fu Z**, Li H, Arslan ME, Ells PF, Lee H. Hepatic Langerhans cell histiocytosis: A review. *World J Clin Oncol* 2021; **12**: 335-341 [PMID: 34131565 DOI: 10.5306/wjco.v12.i5.335]

95 **Yi X**, Han T, Zai H, Long X, Wang X, Li W. Liver involvement of Langerhans' cell histiocytosis in children. *Int J Clin Exp Med* 2015; **8**: 7098-7106 [PMID: 26221247]

96 **Faden I**, Avitzur Y, Abla O, Parra DA. Findings encountered in percutaneous cholangiography in a case of post-transplant recurrence of hepatic Langerhans cell histiocytosis with biliary involvement. *Pediatr Transplant* 2021; **25**: e13838 [PMID: 32985784 DOI: 10.1111/petr.13838]

97 **Braier J**, Ciocca M, Latella A, de Davila MG, Drajer M, Imventarza O. Cholestasis, sclerosing cholangitis, and liver transplantation in Langerhans cell Histiocytosis. *Med Pediatr Oncol* 2002; **38**: 178-182 [PMID: 11836717 DOI: 10.1002/mpo.1306]

98 **Jezierska M**, Stefanowicz J, Romanowicz G, Kosiak W, Lange M. Langerhans cell histiocytosis in children - a disease with many faces. Recent advances in pathogenesis, diagnostic examinations and treatment. *Postepy Dermatol Alergol* 2018; **35**: 6-17 [PMID: 29599667 DOI: 10.5114/pdia.2017.67095]

99 **Hait E**, Liang M, Degar B, Glickman J, Fox VL. Gastrointestinal tract involvement in Langerhans cell histiocytosis: case report and literature review. *Pediatrics* 2006; **118**: e1593-e1599 [PMID: 17030599 DOI: 10.1542/peds.2006-0708]

100 **Donadieu J**, Larabi IA, Tardieu M, Visser J, Hutter C, Sieni E, Kabbara N, Barkaoui M, Miron J, Chalard F, Milne P, Haroche J, Cohen F, Hélias-Rodzewicz Z, Simon N, Jehanne M, Kolenova A, Pagnier A, Aladjidi N, Schneider P, Plat G, Lutun A, Sonntagbauer A, Lehrnbecher T, Ferster A, Efremova V, Ahlmann M, Blanc L, Nicholson J, Lambilliote A, Boudiaf H, Lissat A, Svojgr K, Bernard F, Elitzur S, Golan M, Evseev D, Maschan M, Idbaih A, Slater O, Minkov M, Taly V, Collin M, Alvarez JC, Emile JF, Héritier S. Vemurafenib for Refractory Multisystem Langerhans Cell Histiocytosis in Children: An International Observational Study. *J Clin Oncol* 2019; **37**: 2857-2865 [PMID: 31513482 DOI: 10.1200/JCO.19.00456]

101 **Abdalian R**, Heathcote EJ. Sclerosing cholangitis: a focus on secondary causes. *Hepatology* 2006; **44**: 1063-1074 [PMID: 17058222 DOI: 10.1002/hep.21405]

102 **Mehendale S**, Kumar CP, Venkatasubramanian S, Prasanna T. Intussusception in Children Aged Less than Five years. *Indian J Pediatr* 2016; **83**: 1087-1092 [PMID: 27211600 DOI: 10.1007/s12098-016-2152-9]

103 **Rogers PC**. Importance of nutrition in pediatric oncology. *Indian J Cancer* 2015; **52**: 176-178 [PMID: 26853391 DOI: 10.4103/0019-509X.175830]

104 **Relling MV**, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM, Moyer AM, Evans WE, Klein TE, Antillon-Klussmann FG, Caudle KE, Kato M, Yeoh AEJ, Schmiegelow K, Yang JJ. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clin Pharmacol Ther* 2019; **105**: 1095-1105 [PMID: 30447069 DOI: 10.1002/cpt.1304]

105 **Lehmberg K**, Nichols KE, Henter JI, Girschikofsky M, Greenwood T, Jordan M, Kumar A, Minkov M, La Rosée P, Weitzman S; Study Group on Hemophagocytic Lymphohistiocytosis Subtypes of the Histiocyte Society. Consensus recommendations for the diagnosis and management of hemophagocytic lymphohistiocytosis associated with malignancies. *Haematologica* 2015; **100**: 997-1004 [PMID: 26314082 DOI: 10.3324/haematol.2015.123562]

106 **Indolfi G**, Abdel-Hady M, Bansal S, Debray D, Smets F, Czubkowski P, van der Woerd W, Samyn M, Jahnel J, Gupte G, Zellos A, Mozer-Glassberg Y, Verkade HJ, Sokal E, Fischler B. Management of Hepatitis B Virus Infection and Prevention of Hepatitis B Virus Reactivation in Children With Acquired Immunodeficiencies or Undergoing Immune Suppressive, Cytotoxic, or Biological Modifier Therapies. *J Pediatr Gastroenterol Nutr* 2020; **70**: 527-538 [PMID: 31977956 DOI: 10.1097/MPG.0000000000002628]

107 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [PMID: 31816111 DOI: 10.1002/hep.31060]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 607-627

DOI: 10.4251/wjgo.v14.i3.607

ISSN 1948-5204 (online)

REVIEW

Pathological, molecular, and clinical characteristics of cholangiocarcinoma: A comprehensive review

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gu J, Rompianesi G, Shi YJ

Received: September 25, 2021
Peer-review started: September 25, 2021
First decision: December 4, 2021
Revised: December 13, 2021
Accepted: February 23, 2022
Article in press: February 23, 2022
Published online: March 15, 2022

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Abstract

Cholangiocarcinomas are a heterogeneous group of highly aggressive cancers that may arise anywhere within the biliary tree. There is a wide geographical variation with regards to its incidence, and risk-factor associations which may include liver fluke infection, primary sclerosing cholangitis, and hepatolithiasis amongst others. These tumours are classified into intrahepatic, perihilar and distal based on their anatomical location. Morphologically, intrahepatic cholangiocarcinomas are further sub-classified into small and large duct variants. Perihilar and distal cholangiocarcinomas are usually mucin-producing tubular adenocarcinomas. Cholangiocarcinomas develop through a multistep carcinogenesis and are preceded by dysplastic and in situ lesions. While clinical characteristics and management of these tumours have been extensively elucidated in literature, their ultra-structure and tumour biology remain relatively unknown. This review focuses on the current knowledge of pathological characteristics, molecular alterations of cholangiocarcinoma, and its precursor lesions (including biliary intraepithelial neoplasia, intraductal papillary neoplasms of the bile duct, intraductal tubulopapillary neoplasms and mucinous cystic neoplasm).

Key Words: Cholangiocarcinoma; Classification; Pathology; Molecular features; Precursors lesions; Treatment

Core Tip: Cholangiocarcinoma is a heterogeneous and aggressive epithelial malignancy of the biliary system. The majority of cholangiocarcinomas are diagnosed at an advanced stage when choice of treatment is limited. Cholangiocarcinoma is classified into intrahepatic, perihilar, and distal bile duct cancer, according to the anatomical location. This review focuses on the current knowledge of histopathological features, molecular alterations and clinical characteristics of cholangiocarcinoma and its precursor lesions (including biliary intraepithelial neoplasia, intraductal papillary neoplasms of the bile duct, intraductal tubulopapillary neoplasms and hepatobiliary mucinous cystic neoplasm. Recently, actionable genetic alterations, mainly IDH1 mutations and FGFR2 fusions have been described, in cholangiocarcinoma.

Citation: Vij M, Puri Y, Rammohan A, G G, Rajalingam R, Kaliamoorthy I, Rela M. Pathological, molecular, and clinical characteristics of cholangiocarcinoma: A comprehensive review. *World J Gastrointest Oncol* 2022; 14(3): 607-627
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/607.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.607

INTRODUCTION

Cholangiocarcinoma, although rare, is the second most common primary hepatic cancer after hepatocellular carcinoma (HCC). It accounts for approximately 15% of cases and represents 3% of all gastrointestinal malignancies[1-3] These are a diverse group of highly fatal cancers that arise along the biliary tree[4]. Majority of cholangiocarcinoma are diagnosed in older individuals, with a peak incidence in the 7th decade. It afflicts both genders almost equally (slight male preponderance)[5]. Cholangiocarcinomas encompasse three distinct anatomical categories based on the site of biliary tract involvement, namely intrahepatic (IHCC), perihilar (PHCC) and distal cholangiocarcinoma (DCC). Each of these categories differ in their risk factors, clinical presentations, epidemiological features, morphologic and molecular characteristics[6,7]. Approximately 6%-10% are intrahepatic, 30% are distal and a majority (60%) are PHCC[8]. IHCC are tumours located proximal to the second-order bile ducts within the liver and thus arise from segmental or smaller intrahepatic biliary channels[9]. PHCC are localized to an area between the second-order bile ducts and the insertion of the cystic duct into the common bile duct[10]. DCC include tumours between the origin of the cystic duct and ampulla of Vater[11].

EPIDEMIOLOGY

Due to a wide geographical variation in the risk factors (both environmental and genetic), incidence and mortality rates of cholangiocarcinoma vary across regions[12]. The highest incidences of cholangiocarcinoma are reported in the northeast provinces of Thailand where the liver fluke *Opisthorchis viverrini* is endemic[13,14]. Age-standardized incidence rates in this region is an alarming 85/100000 population. Lowest incidences of this tumour are in Israel and Costa Rica (0.3/100000 population), while in the United States it is 1.6/100000 population[1,5]. Studies in the last few decades have reported increasing incidence of IHCC and a stable or decreasing incidence of PHCC and/or DCC in many European countries (Italy, Germany, England and Wales), United States, Australia and Japan[5,6,12]. The incidence of IHCC, PHCC and DCC has remained stable in France, and decreasing incidence of IHCC have been reported in Denmark[12].

RISK FACTORS

Most cases (70%) of cholangiocarcinoma are sporadic, occurring without any probable or known risk factors. Table 1 Lists all known risk factors for cholangiocarcinoma. Parasitic infections like *Opisthorchis viverrini* and *Clonorchis sinensis* (liver flukes) induce chronic bile duct inflammation, and periductal scarring which increase the risk of biliary tract malignancy[14]. In the Western world, primary sclerosing cholangitis (PSC) remains the most prevalent risk factor[4]. PSC induces chronic inflammation, biliary epithelial proliferation, and production of endogenous bile mutagens leading to biliary tumorigenesis[5]. Malignant transformation in epithelial lining of biliary cysts can occur as there is reflux of pancreatic enzymes, bile stasis and increased bile acid concentration[5]. Increased risk is also reported in Caroli disease and hepatolithiasis where there is bile stasis, chronic inflammation, bacterial infection, and recurrent cholangitis[12]. In cirrhotic patients, an increased risk of cholangiocarcinoma is observed due to the presence of amplified cell proliferation, release of inflammatory cytokines and

Table 1 Definite and probable risk factors for cholangiocarcinoma

Definite risk factors
Primary sclerosing cholangitis
Liver fluke infection (*Opisthorchis viverrine*, *Clonorchis sinensis*)
Hepatolithiasis
Biliary malformation (choledochal cysts, Caroli's disease, congenital hepatic fibrosis)
Thorotrast
Probable risk factors
Alcohol
Hepatitis B
Hepatitis C
Cirrhosis
Toxins (dioxin, polyvinyl chloride)
Biliary–enteric drainage procedures
Inflammatory bowel disease
Asbestos
Non-alcoholic fatty liver disease
Metabolic syndrome, type 2 diabetes, obesity
Smoking
Chronic pancreatitis

scarring[5]. Apart from the presence of cirrhosis, hepatitis B (HBV) and hepatitis C (HCV) viruses have a direct carcinogenic effect on hepatic progenitor cells resulting in an increased risk of cholangiocarcinoma in these patients. Obesity increases the risk of cancer by affecting the levels of leptin, adiponectin and proinflammatory cytokines[15]. Non-alcoholic fatty liver disease may promote cholangiocarcinoma development directly by induction of hepatic inflammation or, indirectly, by resulting in cirrhosis.

BILIARY TRACT ANATOMY

The biliary tract extends from the canals of Hering to the common bile duct and are broadly subdivided into extrahepatic and intrahepatic segments[16,17]. It is a complex structure showing wide variation in anatomy and histology[18]. The intrahepatic bile ducts (ducts proximal to the right and left duct confluence) are further subclassified into large and small intrahepatic bile ducts. Large intrahepatic bile ducts (> 300 μm in diameter) are also referred to as the 'perihilar' bile ducts and consist of the segment, right and left hepatic bile ducts. These ducts are lined by tall columnar epithelium with basally placed hyperchromatic-isomorphic nuclei, mucin filled cytoplasm and a fibro-collagenous duct wall[18]. The intrahepatic biliary tree begins at the level of canals of Hering which are lined partly by biliary epithelium and hepatocytes[19]. The canals of Hering continue into a channel, termed the ductule (< 20 μm in diameter). These are entirely lined by cholangiocytes and continue as the interlobular bile ducts (20-100 μm in diameter). The interlobular bile ducts are lined by cuboidal cells resting on a basement membrane. Multiple Interlobular bile ducts fuse to form septal (> 100 μm in diameter), area and segmental bile ducts. The septal ducts are lined by tall columnar cells with fibro-collagenous duct walls [18].

Embryologically, small intrahepatic bile ducts evolve from hepatoblasts through a process of ductal plate formation. During the first weeks of gestation, the ductal plate develops as a cylindrical, double-layered sleeve of cholangiocytes with a slit-like lumen surrounding a portal vein branch. Remodelling and partial involution of these cylindrical ductal plates give rise to bile ducts. Large ducts are formed from the caudal portion of the hepatic diverticulum[18]. The exact process of fusion of these ducts has not been entirely elucidated, but they appear in continuity throughout development. Peribiliary glands are physiologically distributed within the fibromuscular walls of extrahepatic bile ducts and large intrahepatic bile ducts[20].

GROWTH PATTERNS

Macroscopic pattern

Based on their macroscopic growth patterns, IHCC are classified into mass forming, periductal infiltrating, and intraductal growth types[21,22]. Mass forming lesions are the predominant type, accounting for 60-80% of IHCC[23-26]. These are firm, solid tumours with a white or greyish cut-surface, with well-defined borders within the hepatic parenchyma (Figure 1A). Intrahepatic metastases or coalescing lesions may be observed. Mucin may also be identified along the cut-surface. These tumours are thought to arise in small intrahepatic bile ducts and are commonly characterized by central necrosis or scaring. The periductal infiltrating type accounts for 15%-35% of IHCC and extends along the portal tracts presenting as bile duct strictures with luminal narrowing. The intraductal growth variant of IHCC is characterized by a papillary or polypoid lesion within a dilated bile duct and most often represent a malignant progression of intraductal papillary mucinous neoplasm (IPNB)[26]. They are the least common variant and account for 8-29% of cases. IHCC can have mixed growth patterns, for example, mass forming and periductal infiltrating[27]. Rarer undefined patterns have also been reported[22].

Macroscopically, PHCC and DCC have similar growth patterns; they present as flat or poorly defined nodular sclerosing tumours with thickening of the duct wall, often with diffuse infiltration into adjacent structures (approximately 80%) (Figure 1B) and, less frequently, as intraductal papillary tumours[26,28]. American Joint Committee on Cancer (AJCC)/ Union for International Cancer Control (UICC) and College of American Pathologists (CAP) recognize only the mass forming and periductal infiltrating types (or mixed types); they do not recognize intraductal or undefined growth patterns. This adds to the uncertainty in classifying these tumours[29].

Microscopic pattern

The histological classification of cholangiocarcinoma is highlighted in Table 2.

IHCC

Traditionally, IHCC are sub-classified into two broad sub-groups: well, moderately (Figure 1), poorly differentiated tubular/acinar adenocarcinoma or the uncommon morphological variants[21,30]. These tumours are lined by cuboidal to columnar epithelial cells, resembling biliary lining epithelial cells and demonstrate stromal desmoplasia and variable inflammation[26]. Tumour cells may show mucin production into the lumen of tubular structures on their apical aspect, and within the cell cytoplasm (Figure 1C). Poorly differentiated tumours demonstrate solid, cord-like, or cribriform growth patterns with variable cytological and nuclear pleomorphisms (Figure 1D). Variable necrosis has also been demonstrated. The cancer may show compression of hepatic parenchyma and no evidence of fibrous capsule. The neoplastic cells display invasion between hepatocytes and appear to infiltrate the sinusoids [31-33]. IHCC demonstrate a prominent desmoplastic microenvironment characterized by a dense collagenized stroma and abundance of cancer-associated fibroblasts and, to a lesser extent, tumour-associated histiocytes with a varying number of innate immune cells.

Large and small duct variants of IHCC

The large duct variant of IHCC (LD-IHCC) arises from intrahepatic bile ducts or its associated peribiliary glands. The neoplastic cells lining the malignant acini are cuboidal to tall columnar containing cytoplasmic mucin and usually form large acini within open luminal spaces associated with abundant desmoplastic stroma (Figure 1E)[34,35]. Sites of hepatic parenchymal infiltration show variable tumour histology, some resemble the small duct type or bile ductular type.

Small bile duct type of IHCC (SD-IHCC) arise from progenitor cells and mature hepatocytes and resemble -cholangiolar cells. These tumours show small monotonous or anastomosing glands which are lined with cuboidal cells. The cells have uniform nuclei with scant to moderate eosinophilic or amphophilic cytoplasm and no mucin production (Figure 2A, 2B). This classification into large and small duct types also has clinicopathologic, immunohistochemical, and molecular importance[22,26]. SD-IHCC are associated with chronic liver disease/cirrhosis (especially viral hepatitis), whereas LD-IHCC are linked to chronic biliary disease, precursor lesions, and hepatolithiasis. SD-IHCC nearly always has a mass forming macroscopic growth pattern and often with a central scar, whereas LD-IHCC have variable macroscopic growth patterns with mucin production (Figure 2C, 2D), poorer differentiation, perineural invasion, and lymph node metastases[26].

Ductal plate malformation type of IHCC

This subtype was first reported in 2012. The tumour was noted to mimic ductal plate malformations (DPM)[36]. DPM are developmental anomalies resulting from a lack of ductal plate remodelling during bile duct morphogenesis. Common examples of DPM include fibrocystic diseases such as Caroli's disease, congenital hepatic fibrosis, and von Meyenburg complex. Majority of patients with DPM type

Table 2 Histological classification of cholangiocarcinoma

Based on histological differentiation
Well (> 95% of tumour composed of glands)
Moderately (50%-95% of tumour composed of glands)
Poor (5%-49% of tumour composed of glands)
Undifferentiated type (< 5% of tumor composed of glands)
Based on glandular features
Conventional type (bile duct type)
Small bile duct type (intrahepatic)
Large bile duct type
Cholangiocellular (intrahepatic)
Uncommon variants
Ductal plate malformation type (intrahepatic)
Lymphoepithelioma type
Clear cell type
Squamous/adenosquamous type
Mucinous carcinoma
Sarcomatoid
Signet ring carcinoma
Neuroendocrine
HCC-like

HCC: Hepatocellular carcinoma.

of IHCC are males aged over 60 years at diagnosis. Sixty percent of cases are associated with chronic liver disease and the remaining show mild steatosis and/or portal inflammation. The tumours are usually solitary with whitish solid cut surface. Microscopically, the tumours are arranged in small nodules with desmoplastic stroma. The malignant acini show irregularly dilated lumen lined with a single layer of cuboidal or low columnar neoplastic cells with mild nuclear isomorphism and irregular protrusions and bulges (Figure 3A). The neoplastic cells are frequently positive for CK19, epithelial cell adhesion molecule (EpCAM), and epithelial membrane antigen (EMA). Neural cell adhesion molecule (NCAM) may also be variably expressed. These tumours show low proliferation with Ki67 index (< 10%) and p53 is scarcely expressed.

Cholangiocellular or bile ductular type of IHCC

This is a distinct biliary derived tumour and grossly, this subtype shows mass forming growth pattern [37]. Brightfield microscopy of these tumours show homogeneous morphology with well to moderately differentiated tumour cells forming small tubules, acini or cord-like structures with a slit-like lumen along with arborization resembling proliferating reactive bile ductules (Figure 3B)[38-39]. The neoplastic cells are small in size (small compared to conventional IHCC cells). The non-neoplastic hepatocytes are extensively replaced by tumour cells in the hepatic lobules. Marked collagenisation is also noted around the tumour cells. Immunopositivity for NCAM and EpCAM are observed in the tumour cells. DPM pattern has also been described in cholangiocellular IHCC[40].

Lymphoepithelioma-like cholangiocarcinoma

These are rare tumours resembling undifferentiated nasopharyngeal cancers. They have clusters of large cells with vesicular nuclei and prominent lymphoid cell inflammatory infiltrates. These cancers have been reported in various organs including salivary glands, stomach, lung, and the liver, where they present with hepatocellular or biliary features. The latter is labelled as lymphoepithelioma like cholangiocarcinoma (LLCC)[41,42]. These tumours are often associated with Epstein-Barr virus (EBV). 70% of the cases are EBV positive based on Epstein-Barr encoding region (EBER) in situ hybridization. This is contrary to what is observed in conventional biliary tract cancers, which are not associated with EBV infection[43].

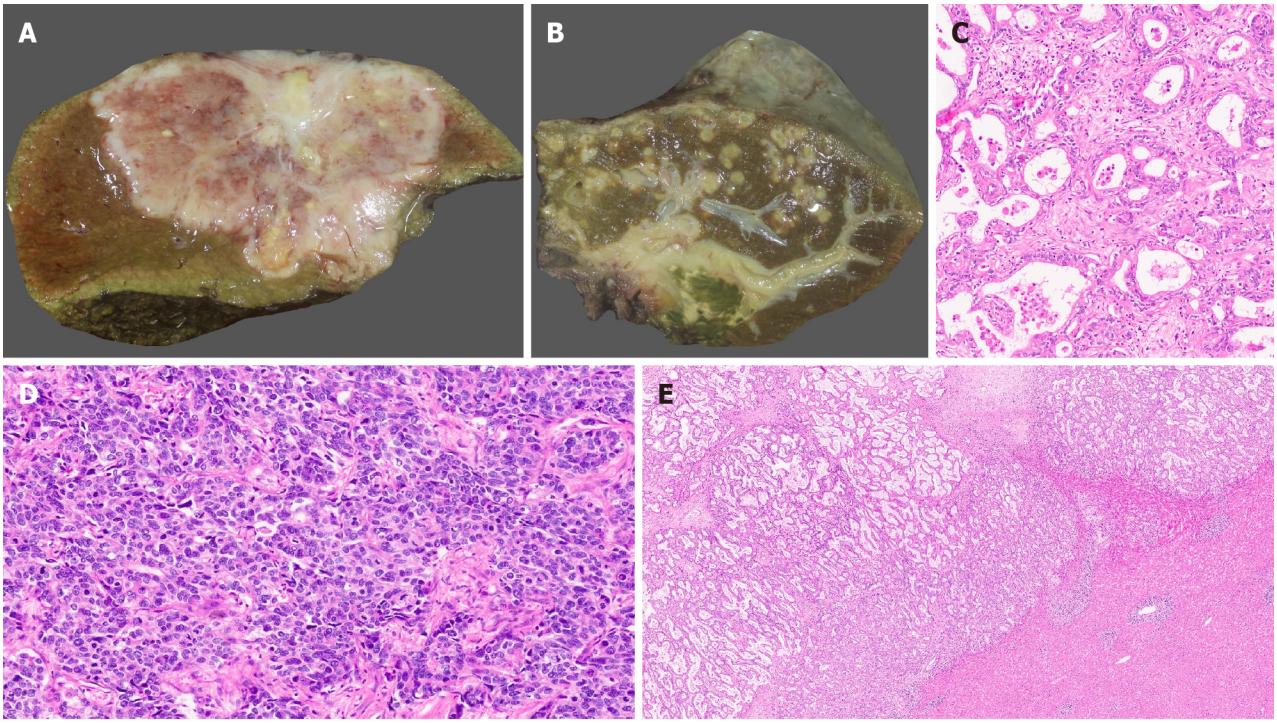

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Figure 1 Gross features and morphology of cholangiocarcinoma. A: Mass forming intrahepatic cholangiocarcinoma (IHCC); B: Extrahepatic cholangiocarcinoma with periductal infiltrating growth (arrow) and markedly greenish liver; C: Well differentiated cholangiocarcinoma [hematoxylin and eosin (H&E, × 25)]; D: Poorly differentiated cholangiocarcinoma (H&E, × 25); E: Large duct variant of IHCC (H&E, × 8).

Hsu *et al*[45] reported the first case of LLCC in 1996[44]. Compared to conventional cholangiocarcinoma, LLCC present at a younger age and have a female preponderance (female-to-male ratio > 3:1). Histologically, these tumours are composed of acini, clusters and cords of neoplastic cells associated with prominent lymphoplasmacytic infiltration (Figure 3C)[41]. The intimate relationship between the cancer cells and numerous lymphoid cells can make a pathologic diagnosis challenging. Pathologic tools which help confirm the diagnosis of LLCC in the midst of dense lymphoid tissue include a low threshold for cytokeratin immunohistochemistry and EBER in situ hybridization[45].

Clear cell cholangiocarcinoma

These are exceedingly rare liver tumours and are recognized as a special variant of IHCC[46,47]. Diagnostic difficulties may occur in differentiating this carcinoma from other types of clear cell cancers (clear cell HCCs and metastatic clear cell cancers from the kidney, ovary, thyroid, or gastrointestinal tract)[47]. Patients are usually in the 5th or 6th decade of life and there is no gender predilection. Predisposing factors for this tumour are as yet unknown, and there is no report on its relation with hepatitis. The prognosis is relatively better than conventional cholangiocarcinoma. The mechanism for the clear cell change has been speculated to involve glycogen, mucin, or lipid[48]. CD56 immunostaining is useful in diagnosis, as it is frequently expressed in clear cell cholangiocarcinoma, and scarcely in clear cell renal cell carcinoma or lung tumours.

Sarcomatoid cholangiocarcinoma

Intrahepatic sarcomatoid cholangiocarcinoma is an extremely rare tumour accounting for less than 1% of hepatobiliary system malignancies[49]**.** Light microscopy reveals a relatively well-delineated tumour characterized by spindle to epithelioid cancer cells having variable nuclear pleomorphism with hyperchromatic nuclei and inconspicuous-to-prominent nucleoli (Figure 3D). The tumour cells are interspersed with stromal tissue. Occasionally, cancer cells with mucin are observed. Inflammatory cell infiltration was present in the abundant stroma[49]**.** Pleomorphic giant cells have also been reported in the tumour[50]. The sarcomatoid subtype is an independent predictor of tumour recurrence, and has a poorer overall survival among IHCC sub-types[50].

Other rare subtypes

Other rare histological subtypes include squamous and adenosquamous carcinoma, mucinous carcinoma, signet ring cell carcinoma, undifferentiated, HCC-like, and mucoepidermoid carcinoma.

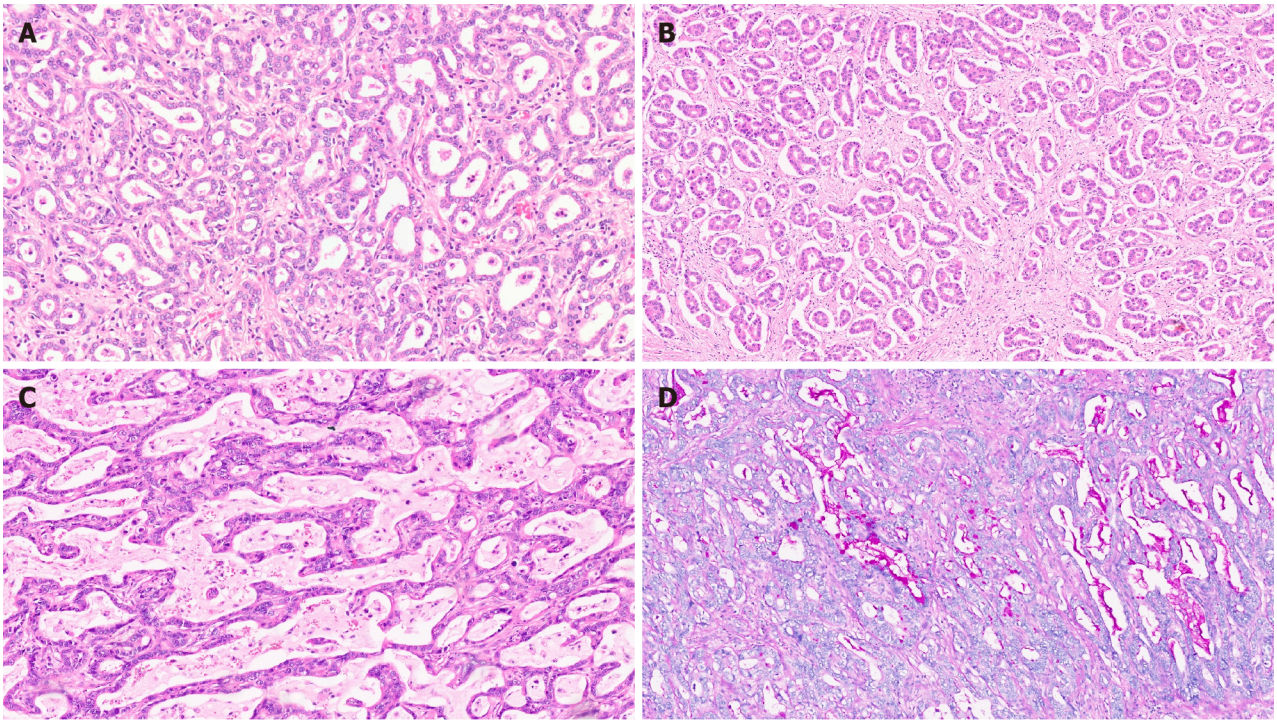

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Figure 2 Intrahepatic cholangiocarcinoma. A: Small duct variant of Intrahepatic cholangiocarcinoma (IHCC) (SD-IHCC) with closely packed small glands [hematoxylin and eosin (H&E, × 15)]; B: SD-IHCC with desmoplastic stroma (H&E, × 15); C: Large duct variant of IHCC (LD-IHCC) with mucin production (H&E, × 20); D: LD-IHCC with mucin [Periodic acid Schiff after diastase, × 15].

PHCC AND DCC

PHCC and DCC are histologically similar to LD-IHCC. The conventional types show well to moderately differentiated acinar structures (Figure 4A). Rarely, tumours with poor differentiation with cells arranged in solid sheets or cords may also be seen. Associated sclerotic desmoplastic stroma is usually identified. The nodular-sclerosing type demonstrates marked cancerous thickening of the affected bile ducts. Micropapillary or flat adenocarcinoma are observed on the luminal surface. Perineural invasion is common (Figure 4B), and lymphovascular invasion is variably observed. Mucin-producing epithelial cells lining the large bile duct and/or the hepatic progenitor cells are the purported cells of origin for DCC and PHCC[51]. Extrahepatic cholangiocarcinoma may also demonstrate intraductal growth with papillary, tubular or superficial spreading patterns. Adenosquamous carcinoma (Figure 4C) displaying mixed, isolated, or adjoining keratinizing squamous and tubular components have rarely been reported. Keratinizing squamous cell carcinoma, mucinous carcinoma, signet ring cell carcinoma, clear cell carcinoma, hepatoid and neuroendocrine tumours (Figure 4D) have also been reported.

IMMUNOHISTOCHEMICAL FEATURES

Cholangiocarcinomas (intrahepatic and extrahepatic) show immunopositivity for CK7, CK19, and EMA (Figure 5A, B and C)[29]. Positivity for Hepatocyte nuclear factor-1β (HNF-1β) (Figure 5D) and C-reactive protein have also been observed[52]. They are usually negative for CDX2 and SAT-B2, however some cases of IHCC may show focal mild positivity for CDX2 and SAT-B2. CK20 immunostain is typically negative or focally positive. These immunostains can help exclude a diagnosis of metastatic colorectal adenocarcinoma, which are typically strong positive for CK20, CDX2, and SAT-B2 but negative for CK7 and CK19. Differentiating cholangiocarcinomas from metastatic pancreatic ductal adenocarcinoma and upper gastrointestinal tract cancers using immunostains is difficult, as these tumours are also positive for CK7 and CK19. Several studies have shown that the LD-IHCC and SD-IHCC have distinct immunohistochemical characteristics[53-56]. LD-IHCC are positive for S100P and TFF1, whereas SD-IHCC tend to be positive for NCAM(CD56) and N-cadherin. NCAM and EMA are often negative or weakly positive for tumour cell cytoplasm in HCC-like IHCC, however strong expression for stem cell makers, including TROP2, EpCAM and Nestin have been reported[57] Ferná ndez Moro *et al*[58] proposed a comprehensive immunohistochemical panel including CK19, CK20, MUC2, MUC5AC, CA19–9, mCEA, CA125 and SMAD4 to aid in the differentiation of metastatic and pancreatobiliary adenocarcinomas.

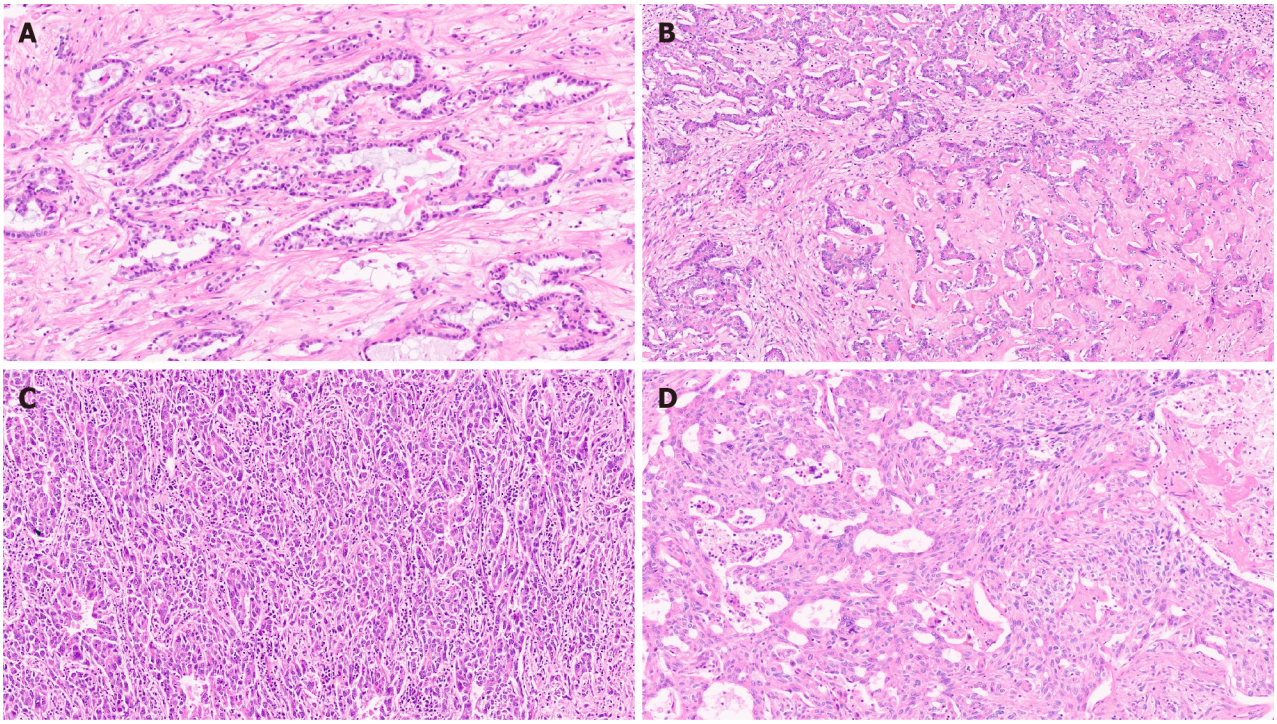

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Figure 3 Intrahepatic cholangiocarcinoma. A: Intrahepatic cholangiocarcinoma (IHCC) with ductal plate malformation phenotype [hematoxylin and eosin (H&E, × 20)]; B: Cholangiocellular or bile ductular type of IHCC (H&E, × 20); C: IHCC lymphoepithelioma subtype (H&E, × 20); D: IHCC with sarcomatoid areas (H&E, × 20).

PRECURSORS LESIONS OF CHOLANGIOCARCINOMA

Carcinogenesis of cholangiocarcinoma is a multistep process beginning with transformed biliary epithelial cells or from stem/progenitor cells. Table 3 describes precursor lesions of both intrahepatic and extrahepatic cholangiocarcinoma.

Biliary epithelial neoplasia

Biliary intraepithelial neoplasia (BilIN) represents the most frequent precursor lesion of invasive adenocarcinoma in the biliary tract[59]. BilIN are non-invasive microscopic flat, micropapillary (papillary projection with fibrovascular stalk) or pseudopapillary (papillary projection without fibrovascular stalk) lesions with dysplasia. BilIN do not produce clinical symptoms and are not detectable on imaging studies[60]. The 2019 WHO classification histologically stratifies BilIN into a two-tiered classification based on the tumour grade (high *vs* low) and intraepithelial extent of cellular and nuclear atypia (Figure 6A). This replaces an earlier classification which was three-tiered (BilIN-1, BilIN-2, and BilIN-3). BilIN-1 and BilIN-2 categories from the previous classification are now classified as low-grade and the former BilIN-3 is now classified as high-grade[61]. Furthermore, high grade BilIN is considered as carcinoma in situ.

The presence of BilIN have been associated with hepatolithiasis, PSC and choledochal cysts. It has also been observed in the mucosa adjacent to invasive adenocarcinoma. They have also been detected in cirrhotic livers from nonbiliary diseases (*e.g.*, alcoholic liver disease and hepatitis C)[61,62]. Multicentricity is common in BilIN. Macroscopically, BilIN may manifest as fine granularity, thickened velvety mucosa, or effacement of underlying tissue layers. However, it often appears grossly normal. Low grade BilIN are usually flat lesions with high N:C ratio, hyperchromatic stratified nuclei, and nucleoli. High grade lesions have papillary projections with loss of polarity, marked nuclear atypia and frequent mitosis[61]. BilIN may further be sub-classified as the classic type demonstrating columnar/cuboidal cells with eosinophilic cytoplasm and round nuclei, and the intestinal type characterized by columnar cells with elongated and hyperchromatic, pseudostratified nuclei along with occasional goblet-type cytoplasmic mucin resembling intestinal adenoma[60]. The classic type shows CK7 immunopositivity whereas intestinal type shows immunopositivity for any of the intestinal immunomarkers (CK20, CDX2, or MUC2)[63]. Distinguishing low-grade dysplasia from reactive atypia can occasionally be difficult. The presence of intraepithelial neutrophils which are observed in reactive changes help solve this conundrum. The term 'indefinite for dysplasia' has been proposed in cases where sufficient doubt precludes a definitive classification.

Table 3 Clinicopathologic, immunohistochemical, and molecular characteristics

	Large duct type	Small duct type
Location	Proximal to hepatic hilum	Peripheral
Risk factors	PSC, Liver fluke infection, Hepatolithiasis	Chronic liver disease, viral hepatitis
Gross features	Periductal infiltrating, Mixed pattern	Mass forming
Precursor lesion	BilIN, IPNB, ITPN	Unknown
Pathology	Large, widely spaced glands, Columnar with mucin production, desmoplastic stroma	Small tubules, fused or anastomosing glands, cuboidal to low columnar, central scarring, minimal to no mucin
Perinerual invasion	Common	Rare
Lymphovascular invasion/lymph node metastases	Common	Rare
Tumour border	Infiltrative	Expansile or pushing, rarelyinfiltrative
Immunohistochemical features	S100P and TFF1	CD56, N-cadherin, CRP
Molecular alterations	KRAS and GNAS mutationsCOX2 upregulations	IDH1/IDH2 and BRAF mutations, FGFR2 fusion

BilIN: Biliary intraepithelial neoplasia; CRP: C-reactive protein; IPNB: Intraductal papillary neoplasm of the bile duct; ITPN: Intraductal tubulopapillary neoplasms; PSC: Primary sclerosing cholangitis.

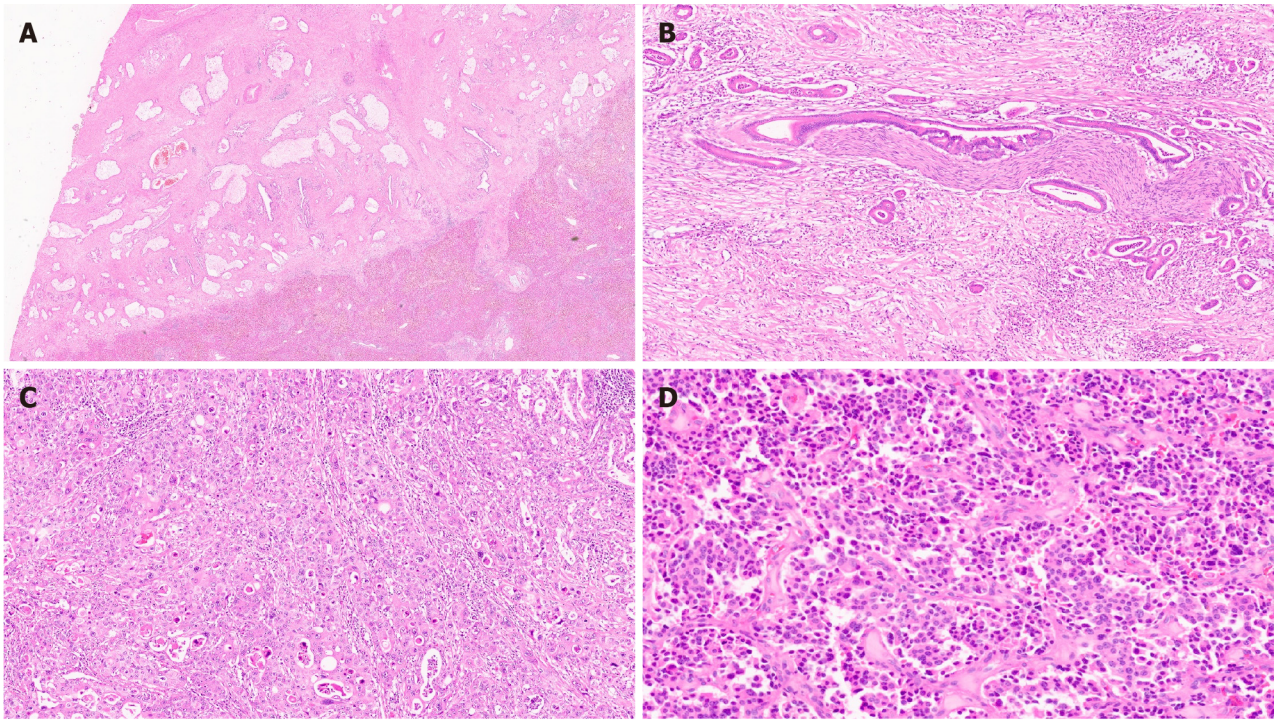

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Figure 4 Extrahepatic cholangiocarcinoma. A: Extrahepatic cholangiocarcinoma (EHCC) with large mucin producing malignant glands and abundant desmoplastic stroma [haematoxylin and eosin (H&E, × 8)]; B: EHCC with perineural invasion (H&E, × 20); C: EHCC adenosquamous subtype (H&E, × 15); D: Well differentiated neuroendocrine tumour of the bile duct (H&E, × 20).

Intraductal papillary neoplasms of the bile duct

Intraductal papillary neoplasia of the bile duct (IPNB) is a unique macroscopic premalignant neoplasm that may arise within intra- or extrahepatic bile ducts[64]. IPNB is typically diagnosed in middle-aged or elderly adults and has a slight male predominance[65]. IPNB is a rare disease entity with a prevalence of 4% to 15% among bile duct tumours, and higher incidence is noted in south-east Asian countries[65,66]. Risk factors include hepatolithiasis, liver fluke infections, PSC and congenital biliary tract disease. These tumours may be single or multiple and can present clinically as large duct obstruction with recurrent abdominal pain, cholangitis and cholestatic hepatic dysfunction[22]. Macroscopically, IPNBs present as visible polypoid, papillary, greyish white or brownish, soft tissue growths within a dilated bile duct

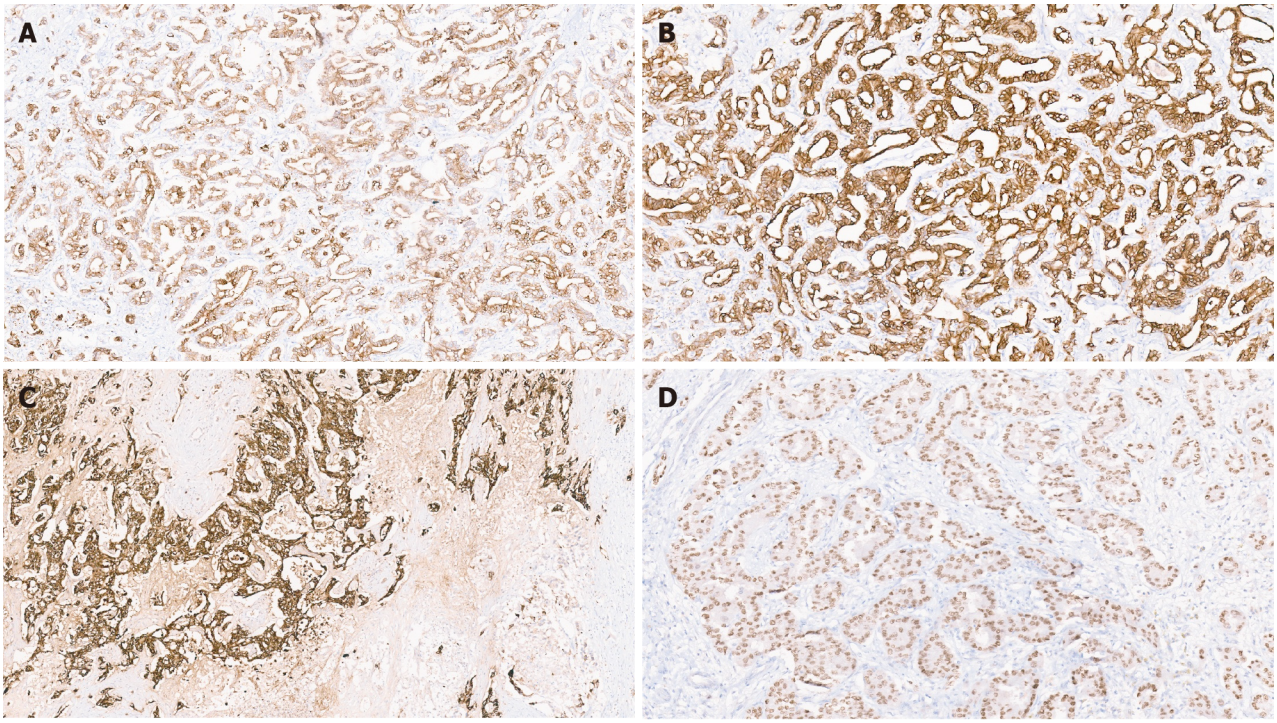

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Figure 5 Immunohistochemistry in cholangiocarcinoma. A: Positive CK7 immunostaining; B: Positive CK19 immunostaining; C: Positive epithelial membrane antigen immunostaining; D: Hepatocyte nuclear factor-1 β nuclear immunostaining in a small duct variant of intrahepatic cholangiocarcinoma.

lumen (Figure 6B). Some patients may present with mucus hypersecretion. Like intraductal papillary mucinous neoplasm (IPMN) of the pancreas, IPNB is histologically classified into four types based on their histological and immunohistochemical features: pancreaticobiliary (Figure 6C), intestinal, gastric and oncocytic types[67]. Pancreaticobiliary and intestinal subtypes are the most common types, although its frequency varies across geographical regions. Mixed subtypes are observed frequently in these neoplasms, and hence their classification is based on the most prevalent subtype. High-grade dysplasia is often extensive and invasive carcinomas are identified in approximately half the cases.

Carcinomas that arise from these lesions are usually the pancreatobiliary-type cholangiocarcinomas with tubular growth pattern, although other rare variants including neuroendocrine and mucinous tumours have been reported. Recently, a panel of Japanese and Korean biliary pathologists proposed a consensus classification for IPNB. These lesions are grouped into types 1 and 2, supplementing the traditional two-tiered grading system (low-grade and high-grade dysplasia[68]. Type 1 IPNB is characterized by regular structures, whereas type 2 show irregular structures. Foci of complicated lesions, such as cribriform or solid structures, are frequently observed in type 2. Pancreatobiliary type shows MUC1 immunostaining, while MUC2 is observed in the intestinal type[69]. MUC5AC is positive in all four types.

Intraductal tubulopapillary neoplasms of the bile duct

This is a recently identified distinct intraductal neoplasm with a predominantly tubular growth pattern. It occurs in the large intrahepatic and extrahepatic bile ducts and is often associated with invasive adenocarcinoma at the time of diagnosis (Figure 6D)[21,22]. ITPNs are rare premalignant lesions characterised by polypoid or solid tumours inside a dilated bile duct[70]. The mean age at presentation is 60 years, with no gender predilection. Purely intraductal tumours appear to have favourable outcomes, but metastases are known to occur in the presence of invasive carcinoma[21]. The tumour shows high cellularity with back-to-back tubular glands and solid sheets with minimal papillary architecture. The cells are cuboidal to columnar with mild to moderate cytological atypia[71]. Despite being associated with invasive carcinoma, overall, ITPNs have a better prognosis than IPNBs. This may be due to an earlier diagnosis resulting from a large in situ intraductal component, or could possibly be due to the inherent differences in the molecular background[72].

Hepatobiliary mucinous cystic neoplasm

Hepatobiliary mucinous cystic neoplasm (HMCN) are lesions characterized by neoplastic mucinous and/or nonmucinous biliary epithelium surrounded by ovarian-type mesenchymal stroma (Figure 7A)[73]. This is a rare tumour representing less than 5% of all cystic neoplasms of the liver and is diagnosed almost exclusively in women in their fourth or fifth decade of life[73,74]. HMCN display no

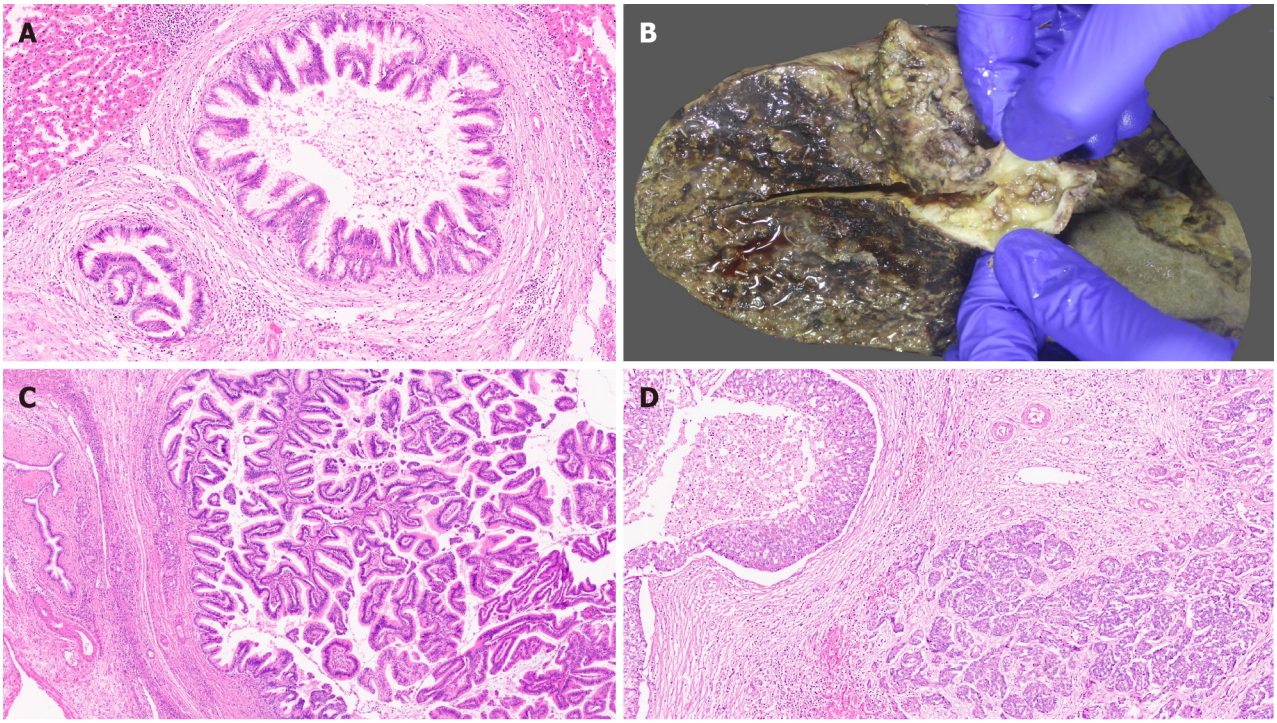

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Figure 6 Precursor lesions of cholangiocarcinoma. A: Biliary intraepithelial neoplasia with low grade dysplasia [haematoxylin and eosin (H&E, × 15)]; B: Intraductal papillary neoplasm of the bile duct (IPNB) (arrow); C: IPNB pancreaticobiliary subtype (H&E, × 10); D: Intraductal tubulopapillary neoplasms of the bile duct with invasive carcinoma (H&E, × 10).

communication with bile ducts and were previously included in the biliary cystadenoma/adenocarcinoma type[21]. Grossly, they are multilocular neoplasms ranging in size from 5 to 29 cm and show a cyst-in-cyst appearance on pre-operative imaging[75]. HMCNs present either as low- or intermediate-grade dysplasia or malignant features with high-grade dysplasia[75]. The benign and borderline categories are however, more common. Their ovarian-type stroma is positive for estrogen and progesterone receptor, inhibin-α and FOXL2[73]. Non-invasive HMCNs have an excellent prognosis, especially when resected completely[76].

Other precursors to lesions

Premalignant lesions of IHCC, particularly those of the mass forming type remain relatively undefined. Hepatic adenofibroma is a benign tumour similar to biliary micro hamartoma with abundant fibrotic stroma and glandular cystic dilatation. They have potential for malignant transformation[77]. Biliary hamartoma, also known as the von Meyenburg complex are histopathological lesions composed of irregular small bile ducts or dilated ductular structures, frequently containing bile, with a fibrous stroma (Figure 7B). The epithelial lining cells are flattened or cuboidal, monomorphic, and lack mitoses. Biliary hamartomas are typically found adjacent to a portal area and may be multiple. Biliary hamartomas are generally regarded as benign. Few reports of cholangiocarcinoma arising from biliary hamartomas raise the question of its potential role as being a precursor lesion[78,79]. Bile duct adenomas (BDA) and atypical epithelial lesions of small bile ducts have occasionally been reported as candidate preinvasive lesions of peripheral IHCC[26]. BDAs are usually solitary and subcapsular (nearly 90% of the cases), and over 90% are less than 1cm in size. They are composed of small, normal looking bile ducts (Figure 7C). In small biopsies differentiation between a well-differentiated IHCC and BDA may be difficult. Both p53 and p16 immunohistochemistry can be helpful to distinguish these two lesions. P53 shows a strong and diffuse expression in malignant lesions while p16 is constantly expressed in bile duct adenoma.

MOLECULAR PATHOMECHANISMS

The knowledge of molecular pathology of cholangiocarcinoma has markedly evolved over the past decade. With the advent of high-throughput gene sequencing technologies, multiple new genetic and epigenetic alterations in cholangiocarcinoma have been uncovered[80]. In-depth sequencing has also highlighted the molecular complexity and heterogeneity of these tumours. A better understanding of

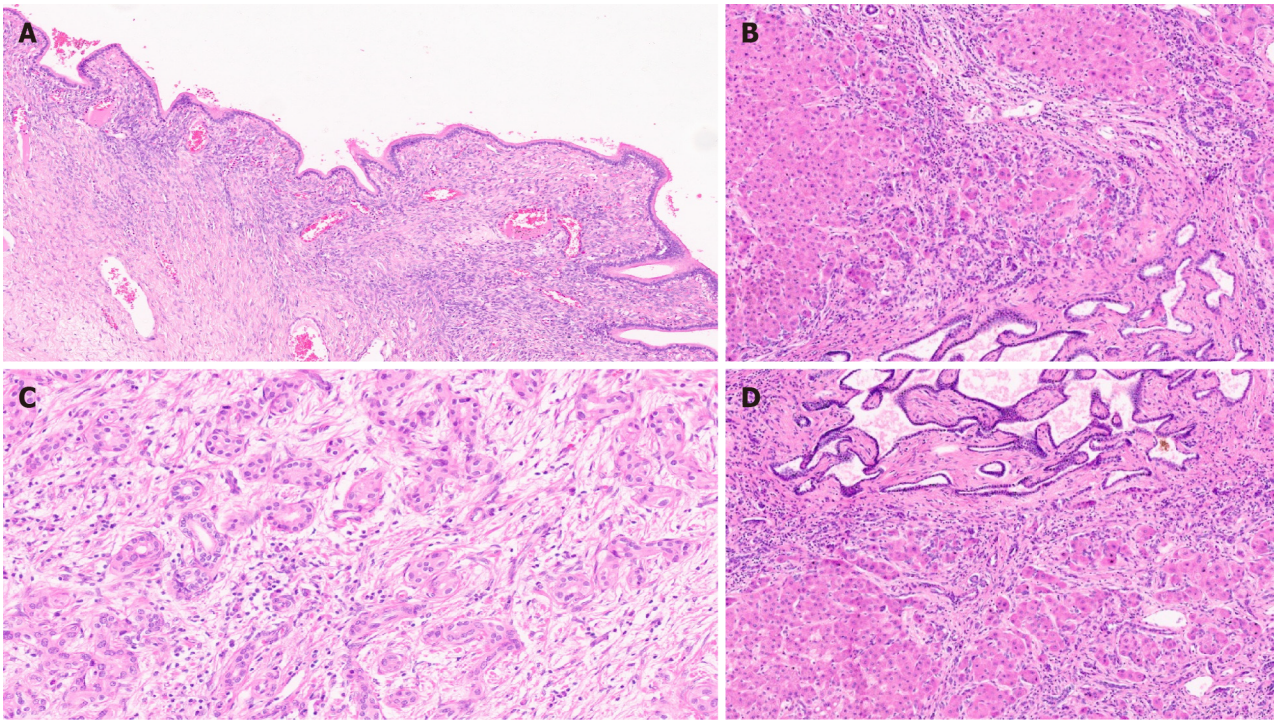

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Figure 7 Other precursor lesions. A: Hepatobiliary mucinous cystic neoplasm with mucinous lining epithelial and ovarian stroma [haematoxylin and eosin (H&E, × 10)]; B: Von Mayenberg complex (H&E, × 20); C: Bile duct adenoma (H&E, × 20).

the underlying pathomechanisms of cholangiocarcinogenesis will help to improve the description of the tumour and its subtypes. Moreover, it will also pave the way for personalized treatment for these rare primary liver cancers[17]. It is important for future studies to search for distinct subgroups within the subtypes on a morphomolecular basis.

Molecular characteristics of intrahepatic cholangiocarcinoma

Table 3 summarizes the clinicopathologic, immunohistochemical, and molecular characteristics of IHCC [53]. Mutation analysis of both LD-IHCC and SD-IHCC reveal KRAS as the most frequently mutated oncogene in LD-IHCC[54]. Large-duct type also show a high mutation frequency of tumour suppressor genes (*e.g.*, p53). SD-IHCC show higher frequency mutations of IDH1 and IDH2[81,82]. IDH1 and IDH2 are relevant in carcinogenesis due to their involvement in cell metabolism[83]. Nakamura described FGFR2 fusion genes in SD-IHCC[81]. With a prevalence of 14%–23% in IHCC, FGFR2 rearrangement is the most common type of FGFR aberration[84]. Lowery *et al*[85] performed targeted next-generation sequencing assay and reported alterations in ARID1A, BAP1, and TP53, along with IDH1, and FGFR2 gene fusions. They also reported a tendency toward mutual exclusivity between multiple genes including TP53:IDH1, IDH1:KRAS, TP53:BAP1, and IDH1:FGFR2. FGFR2 rearrangements seem to occur more frequently in younger patients and possibly confer a better prognosis[86]. Rarely NTRK fusions have also been reported[86].

Jang *et al*[87] investigated the molecular landscape of IHCC in both histologically unremarkable livers and in those with chronic liver disease (CLD). They employed a high throughput mass spectrometry-based platform and compared the mutation profiles of 43 IHCC with histologically unremarkable livers and 38 with CLD[87] The most commonly mutated gene was KRAS followed by MLH1, NRAS, GNAS and EGFR. The frequency of BRAF, APC, PIK3CA, CDKN2A, PTEN, and TP53 mutations was < 5%. Overall mutation rates of biliary cancer with CLD were lower than that of cancers in a histologically unremarkable liver. Sia *et al*[88] classified IHCC into two unique subclasses: inflammation and proliferation, each with distinct features, activated genes, and clinical outcomes. The inflammation class demonstrated activated inflammatory signalling pathways, with overexpression of cytokines, and STAT3 activation, while proliferation class was characterized by the activation of RAS, MAPK and MET oncogenic signalling pathways, mutations in KRAS and BRAF as well as expression of genes that were previously associated with worse outcome in patients with HCC. Kim *et al*[89] classified IHCC into two classes, those primarily driven by either somatic mutations (M class) or by DNA copy number alterations (C class). Compared to M class IHCC with a relative deficit of copy number alterations, C class IHCC harbour recurrent focal copy number alterations including deletions involving CDKN2A, ROBO1, ROBO2, RUNX3, and SMAD4.

DNA mismatch repair (MMR) deficiency leading to microsatellite instability (MSI) have been demonstrated as a distinct pathway for carcinogenesis[90]. MSI is clinically relevant, since these cancers are responsive to immune checkpoint inhibitor therapy[91]. Although MSI most commonly occurs in colorectal and endometrial cancers, a wide variety of other cancers, including biliary cancer exhibit MSI. Goeppert *et al*[91] analysed the mononucleotide MSI marker panel consisting of BAT25, BAT26, and CAT25 in 159 IHCC and detected high-level of MSI (MSI-H) in 2 cases. Patients affected by MSI-H cholangiocarcinoma were younger and showed atypical histomorphology along with a longer overall survival and high tumour stage. Correlation analysis of MSI status with tumour-infiltrating immune cells, MHC I, and PD-L1 expression in the same cholangiocarcinoma cohort showed increased numbers of CD8, FOXP3, CD20 positive cells and moderate or high MHC I expression levels in MSI-H IHCC[90]. Overall, the frequency of MSI-H based on various studies is 10%[90]. Very recently Zhou *et al*[92] evaluated the role of Brahma-related gene 1 (Brg1) in IHCC and demonstrated that a high Brg1 expression in hepatic progenitor cells (HPC) promoted HPC expansion, liver cirrhosis, and IHCC development in response to chronic biliary injury.

Intratumoural heterogeneity in intrahepatic cholangiocarcinoma

Dong *et al*[93] performed multiregional whole-exome sequencing to investigate intratumoural heterogeneity (ITH) and its impact on IHCC progression. They demonstrated many factors, such as parallel evolution and chromosome instability may participate and promote the branch diversity of IHCC. In primary and recurrent metastatic tumours, they found evidence of polyclonal metastatic seeding, indicating that symbiotic communities of multiple clones existed and were maintained during metastasis.

Molecular alterations of extrahepatic cholangiocarcinoma

These lesions show similar molecular profiles as LD-IHCC and have the presence of KRAS mutation. As demonstrated in a recent study, KRAS, TP53, ARID1A, and SMAD4 are the most prevalent mutations [94]. Mutations in IDH1/2 and BAP1 and FGFR2-fusions reported in SD-IHCC have not been identified in these tumours. Four distinct transcriptome-based molecular classes of EHCC were identified. Metabolic class showed a hepatocyte-like phenotype with activation of the transcription factor HNF4A and enrichment in gene signatures related to bile acid metabolism. The proliferation class was characterized by enrichment of MYC targets, ERBB2 mutations/ amplifications and activation of mTOR signalling and was more common in patients with DCC. The mesenchymal class was defined by signatures of epithelial mesenchymal transition, aberrant TGFβ signalling and poor overall survival and immune class showed lymphocyte infiltration of the tumour, overexpression of PD-1/PD-L1 and molecular features associated with a better response to immune checkpoint inhibitors. Kim *et al*[95] investigated and found MSI-H in 1 of 18 EHCC (6%). Overall, the reported frequency of MSI-H in carcinomas of the large bile ducts is estimated to be 5%[90].

Epigenetics of cholangiocarcinoma

Epigenetics are heritable elements that regulate gene expression without modifying the nucleotide sequence of the DNA. They play an important role in cholangiocarcinogenesis[96]. A multitude of alterations of key epigenetic players have been observed in cholangiocarcinoma: DNA methylation, histone modifications, chromatin remodelling and noncoding RNAs (ncRNAs). In tumours, the aberrant DNA methylation occur at the 50 methylcytosine (5-mc) in CpG rich area in the promotor sequence of tumour suppressor genes resulting in gene inactivation[80]. A study investigating 489 cases identified four clusters (cluster 1 to cluster 4) of cholangiocarcinoma based on their DNA methylation pattern with different clinical outcomes[97]. Cluster 1 and 4 were clearly distinguished by their highly distinctive patterns of genome-wide DNA hypermethylation, targeting either promoter CpG islands or promoter CpG shores. Further analysis demonstrated that Cluster 1 cholangiocarcinoma were fluke-positive with increased mutation rates (mutation signature 1 enrichment, and increased point-mutation subclonality), while Cluster 4 were fluke-negative and by comparison relatively clonal. Cluster 1 showed downregulation of the DNA demethylation enzyme *TET1* and upregulation of the histone methyltransferase *EZH2* . Hypermethylation of CpG sites was also observed in cluster 4 with enrichment of FGFR translocations and IDH1/2 and BAP1 mutations.

Several histone deacetylase (HDAC) enzymes are overexpressed in cholangiocarcinoma[98,99]. Overexpression of HDAC6 is associated with shortening and/or loss of ciliary appendages, an important feature of malignant transformation of cholangiocytes[100] HDAC1 was found to be overexpressed in IHCC cells *in vitro* as a result of elevated SPRR2A, a gene involved in maintenance of epithelial barriers and wound repair, resulting in deacetylation of p53[101]. Evidence also suggests that a variety of HDAC inhibitors, such as valproic acid (VPA) and vorinostat can *in vitro* and *in vivo* inhibit the growth of cholangiocarcinoma individually or in combination with chemotherapeutic agents[99]. ncRNAs are the newly defined players in cholangiocarcinogenesis, being able to act as tumour suppressor genes or oncogenes. Therefore, representing potentially valuable tools in diagnosis and targets for treatment[102].

Molecular alterations of precursor lesions

Genomic alterations accumulate in precursor lesions during the multistep biliary carcinogenesis. An increased expression of p21, p53, cyclin D1 along with a decreased expression of Dcp4 is observed in a histological progression of BilIN[103]. Expression of EZH2 shows a stepwise increase from low grade to high grade to invasive cancer[104]. Molecular alterations of KRAS have also been reported in BilIN [105]. Molecular analysis of IPNBs reveal KRAS mutations, over-expressions of TP53 and losses of p16 in low-grade dysplasia. A loss of SMAD4 is noted in late phases of tumour development[106]. Another study investigated the genetic landscape of biliary papillary neoplasms by whole exome sequencing. Mutations in either APC or CTNNB1 were detected in 4 of 7 cases. Somatic mutations were also identified in KRAS, BRAF, CDC27, KMT2C, KMT2D, and MSH3, MSH6, PMS1[107]. Genetic alterations reported in ITPN are CDKN2A/p16 and TP53[108]. Very recently, Gross *et al*[109] performed whole exome sequencing of ITPN and demonstrated a high genetic diversity with recurrent copy number variants (CNVs) (loss of chromosome 1p36 and others), and only a few recurrent somatic mutations in TG, SLIT2, FGFR2, and HMCN1. They also identified cell cycle, chromatin remodelling, and DNA damage/repair as key signalling pathways in these neoplasms. In HMCN, there is activation of hedgehog and wnt pathways; and downregulation of T-helper 1 and 2 pathways[73].

Role of molecular pathology in diagnosis and management

Molecular characterisation of cholangiocarcinoma is now being considered a way to differentiate benign and cancerous biliary strictures. It will potentially help clinicians decide optimal treatment plan. Recently, a study evaluated a 28-gene next-generation sequencing panel (genes that are commonly mutated, amplified, and/or deleted in malignant biliary neoplasms) named BiliSeq using endoscopic retrograde cholangiopancreatography-obtained biliary specimens from patients with bile duct strictures [110]. Combining BiliSeq with pathological evaluation of biliary tissue improved the detection of malignant biliary strictures and allowed for the identification of potentially targetable molecular alterations, thus guiding treatment decisions.

LIQUID BIOPSY IN CHOLANGIOCARCINOMA

The term liquid biopsies comprise a diverse group of methodologies centring around the detection and analysis of tumour cells or tumour cell products obtained from blood or other body fluids[111]. Different types of liquid biopsies include circulating tumour cells (CTCs), cell free nucleic acids (cfDNA, mRNA, non-coding RNA such as micro-RNA or long non-coding RNA), "tumour-educated platelets" (TEPs) and vesicles such as exosomes[112]. The clinical application of liquid biopsies includes early detection of cancer or tumour recurrence, individual risk-assessment and treatment monitoring. Few studies have evaluated role of liquid biopsies in cholangiocarcinoma. Yang *et al*[113] showed that CTCs were associated with more-aggressive tumour characteristics and were independently associated with a poorer survival in patients with cholangiocarcinoma. Wintachai *et al*[114] investigated the diagnostic and prognostic values of plasma cfDNA levels from 62 cholangiocarcinoma patients, 33 benign biliary disease patients and 30 normal controls. They demonstrated a superior diagnostic efficacy of cfDNA in detecting cholangiocarcinoma than CEA and CA19-9. Most commonly identified genetic alterations were in ARID1A (30%), PBRM1 (30%), mTOR (30%), and FGFR3 (30%). The current role of liquid biopsies in cholangiocarcinoma remain limited and further research is required to appreciate its full potential[111].

CLINICAL FEATURES AND MANAGEMENT

Clinically and management-wise cholangiocarcinomas can be classified according to anatomical location of lesion along the biliary tract. IHCC arise proximal to second order of biliary tree and hence are harder to diagnose before they become symptomatic. Symptoms usually arise from the size and pressure on vascular and biliary structures. Compression on the biliary system leads to jaundice. Occasionally, IHCC can co-exist with HCC in a cirrhotic liver and are usually incidentally diagnosed in liver transplant (LT) recipients. In a non-cirrhotic liver, standard of care remains anatomical liver resection with an aim to achieve microscopically negative (R0) resection margins. Radiologically, hypervascular IHCC associated with high microvascular density, arterial vessel density, and cholangiocellular or bile ductular subtype on pathology have a more favourable outcome. These tumours are less aggressive in nature than those with hypovascular features[57]. The roles of adjuvant and neoadjuvant chemo-radiotherapy have not been entirely defined. However, gemcitabine-based adjuvant chemotherapy has shown to improve the overall and disease-free survival. Nonetheless, overall survival remains dismal due to a delay in the diagnosis[115]. Early results from targeted therapies including inhibitors of IDH or fibroblast growth factor receptor (FGFR) in IHCC have been promising[116]. Emerging clinical data from immune checkpoint inhibitors therapy suggest modest efficacy in cholangiocarcinoma[116]. Role

of NTRK, BRAF and MEK inhibitors are also being investigated in cholangiocarcinoma[117].

Due to their anatomical location, PHCC and DCC usually present earlier than IHCC with symptoms of vascular or biliary compression. The most common presentation is in the form of obstructive jaundice. PHCC also known as Klatskin's tumour have been variously classified based on the anatomical location, extent of tumour involvement and resectability[118-121]. Bismuth's classification is based on the anatomical location of the tumour is the most commonly used classification[121]. Standard of care is again R0 resection followed by adjuvant therapy with Gemcitabine based on stage of the lesion [122]. Newer modalities in terms of focussed radiation therapy has shown some promise[123]. LT has been successfully performed in selected cases of unresectable cholangiocarcinoma. Several series have shown good 5-year overall survival in highly selected patients who have undergone neo-adjuvant therapy as a part of specially designed algorithms (*e.g.,* Mayo protocol)[124]. DCC behave akin to periampullary carcinoma and the treatment is mainly surgical in the form of a pancreaticoduodenectomy (Whipple's procedure)[125]. Surgical resection is the standard therapy for IPNBs confined to the liver. IPNB-associated invasive adenocarcinoma has demonstrated a better prognosis than conventional IHCC[126]. Complete surgical resection is also the treatment of choice for ITPNs.

Surgical resection and lymphadenectomy

Treatment of choice as mentioned above, is surgical resection for these tumours. Proximal and distal extent of the lesion, along with the degree of vascular involvement combined with the quality and volume of the liver are crucial factors in the surgical management algorithm in cholangiocarcinoma [127]. Arterial and portal vein local resections are indicated when R0 resection can be potentially achieved. Anatomical resection of the liver is sufficient in IHCC, however for PHCC & DCC an extensive locoregional lymphadenectomy is indicated. Lymphadenectomy for these tumours have shown to improve survival, and furthermore allow accurate staging, prognostication and institution of adjuvant therapy. Although data on the extent of lymphadenectomy is not sufficient and conclusive, the involvement of para-aortic lymph nodes is unequivocally a bad prognostic indicator and lymphadenectomy should not be extended to the same[128,129].

CONCLUSION

Cholangiocarcinomas are a heterogeneous group of cancers arising from the biliary tree demonstrating marked geographical variation due to regional differences in risk factors. Traditionally considered as a single disease, extensive genomic and epigenomic characterization in the last decade have uncovered various molecular alterations associated with specific subtypes of cholangiocarcinoma. Mutated genes may be specifically targeted for therapeutic intervention, a few of which include inhibitors of IDH and FGFR in intrahepatic cancers and trastuzumab in HER2-positive extrahepatic cancers. Despite recent advances in our understanding of biliary cancer, many important questions remain for the prevention and treatment of this lethal disease. Currently, there is no international consensus on the histological classification of cholangiocarcinoma, and there remains a need for standardization of nomenclature and diagnostic criteria of these tumours.

FOOTNOTES

Author contributions: Vij M conceptualized the review, performed literature search and drafting of the manuscript; Puri Y, Rammohan A, G G, Rajalingam R, Kaliamoorthy I and Rela M edited and revised the review; All authors read and approved the manuscript for publication.

Conflict-of-interest statement: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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S-Editor: Wang LL

L-Editor: Filipodia
P-Editor: Wang LL

REFERENCES

1 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]
2 **Rizvi S**, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol* 2018; **15**: 95-111 [PMID: 28994423 DOI: 10.1038/nrclinonc.2017.157]
3 **Sia D**, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. *Gastroenterology* 2017; **152**: 745-761 [PMID: 28043904 DOI: 10.1053/j.gastro.2016.11.048]
4 **Kam AE**, Masood A, Shroff RT. Current and emerging therapies for advanced biliary tract cancers. *Lancet Gastroenterol Hepatol* 2021; **6**: 956-969 [PMID: 34626563 DOI: 10.1016/S2468-1253(21)00171-0]
5 **Khan SA**, Tavolari S, Brandi G. Cholangiocarcinoma: Epidemiology and risk factors. *Liver Int* 2019; **39** Suppl 1: 19-31 [PMID: 30851228 DOI: 10.1111/Liv.14095]
6 **Marcano-Bonilla L**, Mohamed EA, Mounajjed T, Roberts LR. Biliary tract cancers: epidemiology, molecular pathogenesis and genetic risk associations. *Chin Clin Oncol* 2016; **5**: 61 [PMID: 27829275 DOI: 10.21037/cco.2016.10.09]
7 **Sempoux C**, Jibara G, Ward SC, Fan C, Qin L, Roayaie S, Fiel MI, Schwartz M, Thung SN. Intrahepatic cholangiocarcinoma: new insights in pathology. *Semin Liver Dis* 2011; **31**: 49-60 [PMID: 21344350 DOI: 10.1055/s-0031-1272839]
8 **Razumilava N**, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; **383**: 2168-2179 [PMID: 24581682 DOI: 10.1016/S0140-6736(13)61903-0]
9 **Hennedige TP**, Neo WT, Venkatesh SK. Imaging of malignancies of the biliary tract- an update. *Cancer Imaging* 2014; **14**: 14 [PMID: 25608662 DOI: 10.1186/1470-7330-14-14]
10 **Kendall T**, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B, Carpino G. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int* 2019; **39** Suppl 1: 7-18 [PMID: 30882996 DOI: 10.1111/Liv.14093]
11 **Oliveira IS**, Kilcoyne A, Everett JM, Mino-Kenudson M, Harisinghani MG, Ganesan K. Cholangiocarcinoma: classification, diagnosis, staging, imaging features, and management. *Abdom Radiol (NY)* 2017; **42**: 1637-1649 [PMID: 28271275 DOI: 10.1007/s00261-017-1094-7]
12 **Bergquist A**, von Seth E. Epidemiology of cholangiocarcinoma. *Best Pract Res Clin Gastroenterol* 2015; **29**: 221-232 [PMID: 25966423 DOI: 10.1016/j.bpg.2015.02.003]
13 **Sriamporn S**, Pisani P, Pipitgool V, Suwanrungruang K, Kamsa-ard S, Parkin DM. Prevalence of Opisthorchis viverrini infection and incidence of cholangiocarcinoma in Khon Kaen, Northeast Thailand. *Trop Med Int Health* 2004; **9**: 588-594 [PMID: 15117303 DOI: 10.1111/j.1365-3156.2004.01234.x]
14 **Sithithaworn P**, Yongvanit P, Duenngai K, Kiatsopit N, Pairojkul C. Roles of liver fluke infection as risk factor for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2014; **21**: 301-308 [PMID: 24408775 DOI: 10.1002/jhbp.62]
15 **Choi J**, Ghoz HM, Peeraphatdit T, Baichoo E, Addissie BD, Harmsen WS, Therneau TM, Olson JE, Chaiteerakij R, Roberts LR. Aspirin use and the risk of cholangiocarcinoma. *Hepatology* 2016; **64**: 785-796 [PMID: 26940227 DOI: 10.1002/hep.28529]
16 **Cardinale V**, Wang Y, Carpino G, Mendel G, Alpini G, Gaudio E, Reid LM, Alvaro D. The biliary tree--a reservoir of multipotent stem cells. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 231-240 [PMID: 22371217 DOI: 10.1038/nrgastro.2012.23]
17 **Roskams T**, Desmet V. Embryology of extra- and intrahepatic bile ducts, the ductal plate. *Anat Rec (Hoboken)* 2008; **291**: 628-635 [PMID: 18484608 DOI: 10.1002/ar.20710]
18 **Zen Y**, Hübscher SG, Nakanuma Y. Bile duct diseases. In: Burt AD, Ferrell LD, Hubscher SG, editors. MacSween's Pathology of the Liver. 7th edition. Philadelphia: Elsevier, 2018: 516-593
19 **Roskams TA**, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004; **39**: 1739-1745 [PMID: 15185318 DOI: 10.1002/hep.20130]
20 **Cardinale V**, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 2011; **54**: 2159-2172 [PMID: 21809358 DOI: 10.1002/hep.24590]
21 **Nakanuma Y**, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2010; **2**: 419-427 [PMID: 21191517 DOI: 10.4254/wjh.v2.i12.419]
22 **Lendvai G**, Szekerczés T, Illyés I, Dóra R, Kontsek E, Gógl A, Kiss A, Werling K, Kovalszky I, Schaff Z, Borka K. Cholangiocarcinoma: Classification, Histopathology and Molecular Carcinogenesis. *Pathol Oncol Res* 2020; **26**: 3-15 [PMID: 30448973 DOI: 10.1007/s12253-018-0491-8]
23 **Dodson RM**, Weiss MJ, Cosgrove D, Herman JM, Kamel I, Anders R, Geschwind JF, Pawlik TM. Intrahepatic

cholangiocarcinoma: management options and emerging therapies. *J Am Coll Surg* 2013; **217**: 736-750.e4 [PMID: 23890842 DOI: 10.1016/j.jamcollsurg.2013.05.021]

24 **Vyas M**, Deshpande V. How do I distinguish cholangiocarcinoma from metastatic carcinoma and why does it matter? Diagnostic Histopathology, 2021

25 **Nakanuma Y**, Klimstra DS, Komuta M, Zen Y. Intrahepatic Cholangiocarcinoma. In: WHO Classification of Tumours Editorial Board, Editor. Digestive system tumours. WHO classification of tumours series. 5th ed. Lyon: International Agency for Research on Cancer, 2019: 254-259

26 **Nakanuma Y**, Kakuda Y. Pathologic classification of cholangiocarcinoma: New concepts. *Best Pract Res Clin Gastroenterol* 2015; **29**: 277-293 [PMID: 25966428 DOI: 10.1016/j.bpg.2015.02.006]

27 **Yamasaki S**. Intrahepatic cholangiocarcinoma: macroscopic type and stage classification. *J Hepatobiliary Pancreat Surg* 2003; **10**: 288-291 [PMID: 14598147 DOI: 10.1007/s00534-002-0732-8]

28 **Nakanuma Y**, Sato Y, Ojima H, Kanai Y, Aishima S, Yamamoto M, Ariizumi S, Furukawa T, Hayashi H, Unno M, Ohta T; Hepatolithiasis Subdivision of Intractable Hepatobiliary Diseases Study Group of Japan (Chairman, Hirohito Tsubouchi). Clinicopathological characterization of so-called "cholangiocarcinoma with intraductal papillary growth" with respect to "intraductal papillary neoplasm of bile duct (IPNB)". *Int J Clin Exp Pathol* 2014; **7**: 3112-3122 [PMID: 25031730]

29 **Krasinskas AM**. Cholangiocarcinoma. *Surg Pathol Clin* 2018; **11**: 403-429 [PMID: 29751883 DOI: 10.1016/j.path.2018.02.005]

30 **Nakanuma Y**, Curado MP, Franceschi S, Gores G, Paradis V, Sripa B, et al Intrahepatic cholangiocarcinoma. In: Bosman FT, Carnoiro F, Hruba RH, Theise ND, editors. WHO classification of tumors of the digestive system. Lyon: IARC Press, 2010: 217-224

31 **Nakanuma Y**, Sasaki M, Ikeda H, Sato Y, Zen Y, Kosaka K, Harada K. Pathology of peripheral intrahepatic cholangiocarcinoma with reference to tumorigenesis. *Hepatol Res* 2008; **38**: 325-334 [PMID: 18093122 DOI: 10.1111/j.1872-034X.2007.00312.x]

32 **Kozaka K**, Sasaki M, Fujii T, Harada K, Zen Y, Sato Y, Sawada S, Minato H, Matsui O, Nakanuma Y. A subgroup of intrahepatic cholangiocarcinoma with an infiltrating replacement growth pattern and a resemblance to reactive proliferating bile ductules: 'bile ductular carcinoma'. *Histopathology* 2007; **51**: 390-400 [PMID: 17553067 DOI: 10.1111/j.1365-2559.2007.02735.x]

33 **Vijgen S**, Terris B, Rubbia-Brandt L. Pathology of intrahepatic cholangiocarcinoma. *Hepatobiliary Surg Nutr* 2017; **6**: 22-34 [PMID: 28261592 DOI: 10.21037/hbsn.2016.11.04]

34 **Sigel CS**, Drill E, Zhou Y, Basturk O, Askan G, Pak LM, Vakiani E, Wang T, Boerner T, Do RKG, Simpson AL, Jarnagin W, Klimstra DS. Intrahepatic Cholangiocarcinomas Have Histologically and Immunophenotypically Distinct Small and Large Duct Patterns. *Am J Surg Pathol* 2018; **42**: 1334-1345 [PMID: 30001234 DOI: 10.1097/PAS.0000000000001118]

35 **Akita M**, Fujikura K, Ajiki T, Fukumoto T, Otani K, Azuma T, Itoh T, Ku Y, Zen Y. Dichotomy in intrahepatic cholangiocarcinomas based on histologic similarities to hilar cholangiocarcinomas. *Mod Pathol* 2017; **30**: 986-997 [PMID: 28338651 DOI: 10.1038/modpathol.2017.22]

36 **Nakanuma Y**, Sato Y, Ikeda H, Harada K, Kobayashi M, Sano K, Uehara T, Yamamoto M, Ariizumi S, Park YN, Choi JH, Yu E. Intrahepatic cholangiocarcinoma with predominant "ductal plate malformation" pattern: a new subtype. *Am J Surg Pathol* 2012; **36**: 1629-1635 [PMID: 23073321 DOI: 10.1097/PAS.0b013e31826e0249]

37 **Moeini A**, Sia D, Zhang Z, Camprecios G, Stueck A, Dong H, Montal R, Torrens L, Martinez-Quetglas I, Fiel MI, Hao K, Villanueva A, Thung SN, Schwartz ME, Llovet JM. Mixed hepatocellular cholangiocarcinoma tumors: Cholangiolocellular carcinoma is a distinct molecular entity. *J Hepatol* 2017; **66**: 952-961 [PMID: 28126467 DOI: 10.1016/j.jhep.2017.01.010]

38 **Vogel A**, Saborowski A. Cholangiocellular Carcinoma. *Digestion* 2017; **95**: 181-185 [PMID: 28288474 DOI: 10.1159/000454763]

39 **Komuta M**, Govaere O, Vandecaveye V, Akiba J, Van Steenbergen W, Verslype C, Laleman W, Pirenne J, Aerts R, Yano H, Nevens F, Topal B, Roskams T. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology* 2012; **55**: 1876-1888 [PMID: 22271564 DOI: 10.1002/hep.25595]

40 **Sasaki M**, Sato Y, Nakanuma Y. Cholangiolocellular Carcinoma With "Ductal Plate Malformation" Pattern May Be Characterized by ARID1A Genetic Alterations. *Am J Surg Pathol* 2019; **43**: 352-360 [PMID: 30520820 DOI: 10.1097/PAS.0000000000001201]

41 **Huang YH**, Zhang CZ, Huang QS, Yeong J, Wang F, Yang X, He YF, Zhang XL, Zhang H, Chen SL, Zheng YL, Deng R, Lin CS, Yang MM, Li Y, Jiang C, Kin-Wah Lee T, Ma S, Zeng MS, Yun JP. Clinicopathologic features, tumor immune microenvironment and genomic landscape of Epstein-Barr virus-associated intrahepatic cholangiocarcinoma. *J Hepatol* 2021; **74**: 838-849 [PMID: 33212090 DOI: 10.1016/j.jhep.2020.10.037]

42 **Lin A**, Alpert L, Hart J, Chapman C, Pillai AA. Lymphoepithelioma-Like Carcinomas: A Rare Variant of Cholangiocarcinoma. *Hepatology* 2020; **72**: 353-355 [PMID: 31904867 DOI: 10.1002/hep.31102]

43 **Labgaa I**, Stueck A, Ward SC. Lymphoepithelioma-Like Carcinoma in Liver. *Am J Pathol* 2017; **187**: 1438-1444 [PMID: 28500863 DOI: 10.1016/j.ajpath.2017.02.022]

44 **Hsu HC**, Chen CC, Huang GT, Lee PH. Clonal Epstein-Barr virus associated cholangiocarcinoma with lymphoepithelioma-like component. *Hum Pathol* 1996; **27**: 848-850 [PMID: 8760021 DOI: 10.1016/s0046-8177(96)90460-8]

45 **Mostyka M**, Birch MM, Samstein B, Pittman ME. Hidden Carcinoma: Pitfalls in the Diagnosis of Lymphoepithelioma-Like Cholangiocarcinoma. *Int J Surg Pathol* 2020; **28**: 872-878 [PMID: 32429781 DOI: 10.1177/1066896920921560]

46 **Haas S**, Gütgemann I, Wolff M, Fischer HP. Intrahepatic clear cell cholangiocarcinoma: immunohistochemical aspects in a very rare type of cholangiocarcinoma. *Am J Surg Pathol* 2007; **31**: 902-906 [PMID: 17527078 DOI: 10.1097/PAS.0b013e31802c0c8a]

47 **Tihan T**, Blumgart L, Klimstra DS. Clear cell papillary carcinoma of the liver: an unusual variant of peripheral cholangiocarcinoma. *Hum Pathol* 1998; **29**: 196-200 [PMID: 9490284 DOI: 10.1016/s0046-8177(98)90235-0]

48 **Yamamoto T**, Abe T, Oshita A, Yonehara S, Katamura Y, Matsumoto N, Kobayashi T, Nakahara M, Ohdan H, Noriyuki T. Intrahepatic cholangiocarcinoma with clear cell type following laparoscopic curative surgery. *Surg Case Rep* 2020; **6**: 264 [PMID: 33026548 DOI: 10.1186/s40792-020-01041-2]

49 **Kim DK**, Kim BR, Jeong JS, Baek YH. Analysis of intrahepatic sarcomatoid cholangiocarcinoma: Experience from 11 cases within 17 years. *World J Gastroenterol* 2019; **25**: 608-621 [PMID: 30774275 DOI: 10.3748/wjg.v25.i5.608]

50 **Wang T**, Kong J, Yang X, Shen S, Zhang M, Wang W. Clinical features of sarcomatoid change in patients with intrahepatic cholangiocarcinoma and prognosis after surgical liver resection: A Propensity Score Matching analysis. *J Surg Oncol* 2020; **121**: 524-537 [PMID: 31867746 DOI: 10.1002/jso.25815]

51 **Wang Y**, Ming JL, Ren XY, Qiu L, Zhou LJ, Yang SD, Fang XM. Sarcomatoid intrahepatic cholangiocarcinoma mimicking liver abscess: A case report. *World J Clin Cases* 2020; **8**: 208-216 [PMID: 31970189 DOI: 10.12998/wjcc.v8.i1.208]

52 **Patil PA**, Taddei T, Jain D, Zhang X. HNF-1β is a More Sensitive and Specific Marker Than C-Reactive Protein for Identifying Biliary Differentiation in Primary Hepatic Carcinomas. *Arch Pathol Lab Med* 2021 [PMID: 34086854 DOI: 10.5858/arpa.2020-0725-OA]

53 **Rizvi S**, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013; **145**: 1215-1229 [PMID: 24140396 DOI: 10.1053/j.gastro.2013.10.013]

54 **Hayashi A**, Misumi K, Shibahara J, Arita J, Sakamoto Y, Hasegawa K, Kokudo N, Fukayama M. Distinct Clinicopathologic and Genetic Features of 2 Histologic Subtypes of Intrahepatic Cholangiocarcinoma. *Am J Surg Pathol* 2016; **40**: 1021-1030 [PMID: 27259014 DOI: 10.1097/PAS.0000000000000670]

55 **Liau JY**, Tsai JH, Yuan RH, Chang CN, Lee HJ, Jeng YM. Morphological subclassification of intrahepatic cholangiocarcinoma: etiological, clinicopathological, and molecular features. *Mod Pathol* 2014; **27**: 1163-1173 [PMID: 24406866 DOI: 10.1038/modpathol.2013.241]

56 **Aishima S**, Oda Y. Pathogenesis and classification of intrahepatic cholangiocarcinoma: different characters of perihilar large duct type *vs* peripheral small duct type. *J Hepatobiliary Pancreat Sci* 2015; **22**: 94-100 [PMID: 25181580 DOI: 10.1002/jhbp.154]

57 **Nguyen Canh H**, Takahashi K, Yamamura M, Li Z, Sato Y, Yoshimura K, Kozaka K, Tanaka M, Nakanuma Y, Harada K. Diversity in cell differentiation, histology, phenotype and vasculature of mass-forming intrahepatic cholangiocarcinomas. Histopathology, 2021 [PMID: 34018212 DOI: 10.1111/his.14417]

58 **Fernández Moro C**, Fernandez-Woodbridge A, Alistair D'souza M, Zhang Q, Bozoky B, Vasan SK, Catalano P, Heuchel R, Shtembari S, Del Chiaro M, Danielsson O, Björnstedt M, Löhr JM, Isaksson B, Verbeke C, Bozóky B. Correction: Immunohistochemical Typing of Adenocarcinomas of the Pancreatobiliary System Improves Diagnosis and Prognostic Stratification. *PLoS One* 2017; **12**: e0171283 [PMID: 28125728 DOI: 10.1371/journal.pone.0166067]

59 **Sato Y**, Sasaki M, Harada K, Aishima S, Fukusato T, Ojima H, Kanai Y, Kage M, Nakanuma Y, Tsubouchi H; Hepatolithiasis Subdivision of Intractable Hepatobiliary Diseases Study Group of Japan (Chairman, Hirohito Tsubouchi). Pathological diagnosis of flat epithelial lesions of the biliary tract with emphasis on biliary intraepithelial neoplasia. *J Gastroenterol* 2014; **49**: 64-72 [PMID: 23616173 DOI: 10.1007/s00535-013-0810-5]

60 **Ainechi S**, Lee H. Updates on Precancerous Lesions of the Biliary Tract: Biliary Precancerous Lesion. *Arch Pathol Lab Med* 2016; **140**: 1285-1289 [PMID: 27788047 DOI: 10.5858/arpa.2015-0396-RS]

61 **Busturk O**, Aishima S, Esposito I. Bilairy intraepithelial neoplasia. In: WHO Classification of Tumours Editorial Board, editor. Digestive system tumours. WHO classification of tumours series. 5th edition. Lyon: International Agency for Research on Cancer, 2019: 273-275

62 **Wu TT**, Levy M, Correa AM, Rosen CB, Abraham SC. Biliary intraepithelial neoplasia in patients without chronic biliary disease: analysis of liver explants with alcoholic cirrhosis, hepatitis C infection, and noncirrhotic liver diseases. *Cancer* 2009; **115**: 4564-4575 [PMID: 19670455 DOI: 10.1002/cncr.24471]

63 **Zen Y**, Quaglia A, Heaton N, Rela M, Portmann B. Two distinct pathways of carcinogenesis in primary sclerosing cholangitis. *Histopathology* 2011; **59**: 1100-1110 [PMID: 22175890 DOI: 10.1111/j.1365-2559.2011.04048.x]

64 **Nakanuma Y**, Uesaka K, Okamura Y, Terada T, Fukumura Y, Kakuda Y, Sugino T, Sato Y, Taek JK, Park YN. Reappraisal of pathological features of intraductal papillary neoplasm of bile duct with respect to the type 1 and 2 subclassifications. *Hum Pathol* 2021; **111**: 21-35 [PMID: 33508254 DOI: 10.1016/j.humpath.2021.01.002]

65 **Nakanuma Y**, Uesaka K, Kakuda Y, Sugino T, Kubota K, Furukawa T, Fukumura Y, Isayama H, Terada T. Intraductal Papillary Neoplasm of Bile Duct: Updated Clinicopathological Characteristics and Molecular and Genetic Alterations. *J Clin Med* 2020; **9** [PMID: 33317146 DOI: 10.3390/jcm9123991]

66 **Nakanuma Y**, Busturk O, Esposito I, Klimstra DS, Komuta M, Zen Y. Intraductal Papillary Neoplasm of Bile Duct. In: WHO Classification of Tumours Editorial Board, editor. Digestive system tumours. WHO classification of tumours series. 5th edition. Lyon: International Agency for Research on Cancer, 2019: 279-282

67 **Aoki Y**, Mizuma M, Hata T, Aoki T, Omori Y, Ono Y, Mizukami Y, Unno M, Furukawa T. Intraductal papillary neoplasms of the bile duct consist of two distinct types specifically associated with clinicopathological features and molecular phenotypes. *J Pathol* 2020; **251**: 38-48 [PMID: 32100878 DOI: 10.1002/path.5398]

68 **Nakanuma Y**, Jang KT, Fukushima N, Furukawa T, Hong SM, Kim H, Lee KB, Zen Y, Jang JY, Kubota K. A statement by the Japan-Korea expert pathologists for future clinicopathological and molecular analyses toward consensus building of intraductal papillary neoplasm of the bile duct through several opinions at the present stage. *J Hepatobiliary Pancreat Sci* 2018; **25**: 181-187 [PMID: 29272078 DOI: 10.1002/jhbp.532]

69 **Nakanuma Y**, Uesaka K, Miyayama S, Yamaguchi H, Ohtsuka M. Intraductal neoplasms of the bile duct. A new challenge to biliary tract tumor pathology. *Histol Histopathol* 2017; **32**: 1001-1015 [PMID: 28337739 DOI: 10.14670/HH-11-892]

70 **Akita M**, Hong SM, Sung YN, Kim MJ, Ajiki T, Fukumoto T, Itoh T, Zen Y. Biliary intraductal tubule-forming neoplasm: a whole exome sequencing study of MUC5AC-positive and -negative cases. *Histopathology* 2020; **76**: 1005-1012 [PMID: 32181510 DOI: 10.1111/his.14103]

71 **Katabi N**, Torres J, Klimstra DS. Intraductal tubular neoplasms of the bile ducts. *Am J Surg Pathol* 2012; **36**: 1647-1655

[PMID: 23073323 DOI: 10.1097/PAS.0b013e3182684d4f]

72 **Schlitter AM**, Jang KT, Klöppel G, Saka B, Hong SM, Choi H, Offerhaus GJ, Hruban RH, Zen Y, Konukiewitz B, Regel I, Allgäuer M, Balci S, Basturk O, Reid MD, Esposito I, Adsay V. Intraductal tubulopapillary neoplasms of the bile ducts: clinicopathologic, immunohistochemical, and molecular analysis of 20 cases. *Mod Pathol* 2015; **28**: 1249-1264 [PMID: 26111977 DOI: 10.1038/modpathol.2015.61]

73 **Van Treeck BJ**, Lotfalla M, Czeczok TW, Mounajjed T, Moreira RK, Allende DS, Reid MD, Naini BV, Westerhoff M, Adsay NV, Kerr SE, Rizvi SH, Smoot RL, Liu Y, Davila J, Graham RP. Molecular and Immunohistochemical Analysis of Mucinous Cystic Neoplasm of the Liver. *Am J Clin Pathol* 2020; **154**: 837-847 [PMID: 32880620 DOI: 10.1093/ajcp/aqaa115]

74 **Zen Y**, Pedica F, Patcha VR, Capelli P, Zamboni G, Casaril A, Quaglia A, Nakanuma Y, Heaton N, Portmann B. Mucinous cystic neoplasms of the liver: a clinicopathological study and comparison with intraductal papillary neoplasms of the bile duct. *Mod Pathol* 2011; **24**: 1079-1089 [PMID: 21516077 DOI: 10.1038/modpathol.2011.71]

75 **Busturk O**, Nakanuma Y, Aishima A, Esposito I. Mucinous cystic neoplasms of the liver and biliary systtem. In: WHO Classification of Tumours Editorial Board, editor. Digestive system tumours. WHO classification of tumours series. 5th editon. Lyon: International Agency for Research on Cancer, 2019: 250-253

76 **Nakajima T**, Sugano I, Matsuzaki O, Nagao K, Kondo Y, Miyazaki M, Wada K. Biliary cystadenocarcinoma of the liver. A clinicopathologic and histochemical evaluation of nine cases. *Cancer* 1992; **69**: 2426-2432 [PMID: 1314687 DOI: 10.1002/1097-0142(19920515)69:10<]

77 **Thai E**, Dalla Valle R, Evaristi F, Silini EM. A case of biliary adenofibroma with malignant transformation. *Pathol Res Pract* 2016; **212**: 468-470 [PMID: 26778388 DOI: 10.1016/j.prp.2015.12.015]

78 **Jain D**, Sarode VR, Abdul-Karim FW, Homer R, Robert ME. Evidence for the neoplastic transformation of Von-Meyenburg complexes. *Am J Surg Pathol* 2000; **24**: 1131-1139 [PMID: 10935654 DOI: 10.1097/00000478-200008000-00011]

79 **Song JS**, Lee YJ, Kim KW, Huh J, Jang SJ, Yu E. Cholangiocarcinoma arising in von Meyenburg complexes: report of four cases. *Pathol Int* 2008; **58**: 503-512 [PMID: 18705771 DOI: 10.1111/j.1440-1827.2008.02264.x]

80 **Kongpetch S**, Jusakul A, Ong CK, Lim WK, Rozen SG, Tan P, Teh BT. Pathogenesis of cholangiocarcinoma: From genetics to signalling pathways. *Best Pract Res Clin Gastroenterol* 2015; **29**: 233-244 [PMID: 25966424 DOI: 10.1016/j.bpg.2015.02.002]

81 **Nakamura H**, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. *Nat Genet* 2015; **47**: 1003-1010 [PMID: 26258846 DOI: 10.1038/ng.3375]

82 **Borger DR**, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Iafrate AJ. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012; **17**: 72-79 [PMID: 22180306 DOI: 10.1634/theoncologist.2011-0386]

83 **Saha SK**, Parachoniak CA, Ghanta KS, Fitamant J, Ross KN, Najem MS, Gurumurthy S, Akbay EA, Sia D, Cornella H, Miltiadous O, Walesky C, Deshpande V, Zhu AX, Hezel AF, Yen KE, Straley KS, Travins J, Popovici-Muller J, Gliser C, Ferrone CR, Apte U, Llovet JM, Wong KK, Ramaswamy S, Bardeesy N. Mutant IDH inhibits HNF-4α to block hepatocyte differentiation and promote biliary cancer. *Nature* 2014; **513**: 110-114 [PMID: 25043045 DOI: 10.1038/nature13441]

84 **Personeni N**, Lleo A, Pressiani T, Colapietro F, Openshaw MR, Stavraka C, Pouptsis A, Pinato DJ, Rimassa L. Biliary Tract Cancers: Molecular Heterogeneity and New Treatment Options. *Cancers (Basel)* 2020; **12** [PMID: 33202975 DOI: 10.3390/cancers12113370]

85 **Lowery MA**, Ptashkin R, Jordan E, Berger MF, Zehir A, Capanu M, Kemeny NE, O'Reilly EM, El-Dika I, Jarnagin WR, Harding JJ, D'Angelica MI, Cercek A, Hechtman JF, Solit DB, Schultz N, Hyman DM, Klimstra DS, Saltz LB, Abou-Alfa GK. Comprehensive Molecular Profiling of Intrahepatic and Extrahepatic Cholangiocarcinomas: Potential Targets for Intervention. *Clin Cancer Res* 2018; **24**: 4154-4161 [PMID: 29848569 DOI: 10.1158/1078-0432.CCR-18-0078]

86 **Pedica F**, Grassini G. Pathology and molecular pathology of cholangiocarcinoma. *Hepatoma Res* 2021; **7**: 71 [DOI: 10.20517/2394-5079.2021.89]

87 **Jang S**, Chun SM, Hong SM, Sung CO, Park H, Kang HJ, Kim KP, Lee YJ, Yu E. High throughput molecular profiling reveals differential mutation patterns in intrahepatic cholangiocarcinomas arising in chronic advanced liver diseases. *Mod Pathol* 2014; **27**: 731-739 [PMID: 24186137 DOI: 10.1038/modpathol.2013.194]

88 **Sia D**, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, Peix J, Sole M, Tovar V, Alsinet C, Cornella H, Klotzle B, Fan JB, Cotsoglou C, Thung SN, Fuster J, Waxman S, Garcia-Valdecasas JC, Bruix J, Schwartz ME, Beroukhim R, Mazzaferro V, Llovet JM. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 2013; **144**: 829-840 [PMID: 23295441 DOI: 10.1053/j.gastro.2013.01.001]

89 **Kim YH**, Hong EK, Kong SY, Han SS, Kim SH, Rhee JK, Hwang SK, Park SJ, Kim TM. Two classes of intrahepatic cholangiocarcinoma defined by relative abundance of mutations and copy number alterations. *Oncotarget* 2016; **7**: 23825-23836 [PMID: 27009864 DOI: 10.18632/oncotarget.8183]

90 **Silva VW**, Askan G, Daniel TD, Lowery M, Klimstra DS, Abou-Alfa GK, Shia J. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. *Chin Clin Oncol* 2016; **5**: 62 [PMID: 27829276 DOI: 10.21037/cco.2016.10.04]

91 **Goeppert B**, Roessler S, Renner M, Singer S, Mehrabi A, Vogel MN, Pathil A, Czink E, Köhler B, Springfeld C, Pfeiffenberger J, Rupp C, Weiss KH, Schirmacher P, von Knebel Doeberitz M, Kloor M. Mismatch repair deficiency is a rare but putative therapeutically relevant finding in non-liver fluke associated cholangiocarcinoma. *Br J Cancer* 2019; **120**: 109-114 [PMID: 30377340 DOI: 10.1038/s41416-018-0199-2]

92 **Zhou Y**, Chen Y, Zhang X, Xu Q, Wu Z, Cao X, Shao M, Shu Y, Lv T, Lu C, Xie M, Wen T, Yang J, Shi Y, Bu H. Brahma-Related Gene 1 Inhibition Prevents Liver Fibrosis and Cholangiocarcinoma by Attenuating Progenitor Expansion. *Hepatology* 2021; **74**: 797-815 [PMID: 33650193 DOI: 10.1002/hep.31780]

93 **Dong LQ**, Shi Y, Ma LJ, Yang LX, Wang XY, Zhang S, Wang ZC, Duan M, Zhang Z, Liu LZ, Zheng BH, Ding ZB, Ke

AW, Gao DM, Yuan K, Zhou J, Fan J, Xi R, Gao Q. Spatial and temporal clonal evolution of intrahepatic cholangiocarcinoma. *J Hepatol* 2018; **69**: 89-98 [PMID: 29551704 DOI: 10.1016/j.jhep.2018.02.029]

94 **Montal R**, Sia D, Montironi C, Leow WQ, Esteban-Fabró R, Pinyol R, Torres-Martin M, Bassaganyas L, Moeini A, Peix J, Cabellos L, Maeda M, Villacorta-Martin C, Tabrizian P, Rodriguez-Carunchio L, Castellano G, Sempoux C, Minguez B, Pawlik TM, Labgaa I, Roberts LR, Sole M, Fiel MI, Thung S, Fuster J, Roayaie S, Villanueva A, Schwartz M, Llovet JM. Molecular classification and therapeutic targets in extrahepatic cholangiocarcinoma. *J Hepatol* 2020; **73**: 315-327 [PMID: 32173382 DOI: 10.1016/j.jhep.2020.03.008]

95 **Kim SG**, Chan AO, Wu TT, Issa JP, Hamilton SR, Rashid A. Epigenetic and genetic alterations in duodenal carcinomas are distinct from biliary and ampullary carcinomas. *Gastroenterology* 2003; **124**: 1300-1310 [PMID: 12730870 DOI: 10.1016/s0016-5085(03)00278-6]

96 **Rodrigues PM**, Olaizola P, Paiva NA, Olaizola I, Agirre-Lizaso A, Landa A, Bujanda L, Perugorria MJ, Banales JM. Pathogenesis of Cholangiocarcinoma. *Annu Rev Pathol* 2021; **16**: 433-463 [PMID: 33264573 DOI: 10.1146/annurev-pathol-030220-020455]

97 **Jusakul A**, Cutcutache I, Yong CH, Lim JQ, Huang MN, Padmanabhan N, Nellore V, Kongpetch S, Ng AWT, Ng LM, Choo SP, Myint SS, Thanan R, Nagarajan S, Lim WK, Ng CCY, Boot A, Liu M, Ong CK, Rajasegaran V, Lie S, Lim AST, Lim TH, Tan J, Loh JL, McPherson JR, Khuntikeo N, Bhudhisawasdi V, Yongvanit P, Wongkham S, Totoki Y, Nakamura H, Arai Y, Yamasaki S, Chow PK, Chung AYF, Ooi LLPJ, Lim KH, Dima S, Duda DG, Popescu I, Broet P, Hsieh SY, Yu MC, Scarpa A, Lai J, Luo DX, Carvalho AL, Vettore AL, Rhee H, Park YN, Alexandrov LB, Gordân R, Rozen SG, Shibata T, Pairojkul C, Teh BT, Tan P. Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of Cholangiocarcinoma. *Cancer Discov* 2017; **7**: 1116-1135 [PMID: 28667006 DOI: 10.1158/2159-8290.CD-17-0368]

98 **Morine Y**, Shimada M, Iwahashi S, Utsunomiya T, Imura S, Ikemoto T, Mori H, Hanaoka J, Miyake H. Role of histone deacetylase expression in intrahepatic cholangiocarcinoma. *Surgery* 2012; **151**: 412-419 [PMID: 21982637 DOI: 10.1016/j.surg.2011.07.038]

99 **O'Rourke CJ**, Munoz-Garrido P, Aguayo EL, Andersen JB. Epigenome dysregulation in cholangiocarcinoma. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1423-1434 [PMID: 28645654 DOI: 10.1016/j.bbadis.2017.06.014]

100 **Gradilone SA**, Radtke BN, Bogert PS, Huang BQ, Gajdos GB, LaRusso NF. HDAC6 inhibition restores ciliary expression and decreases tumor growth. *Cancer Res* 2013; **73**: 2259-2270 [PMID: 23370327 DOI: 10.1158/0008-5472.CAN-12-2938]

101 **Mizuguchi Y**, Specht S, Lunz JG 3rd, Isse K, Corbitt N, Takizawa T, Demetris AJ. SPRR2A enhances p53 deacetylation through HDAC1 and down regulates p21 promoter activity. *BMC Mol Biol* 2012; **13**: 20 [PMID: 22731250 DOI: 10.1186/1471-2199-13-20]

102 **Esparza-Baquer A**, Labiano I, Bujanda L, Perugorria MJ, Banales JM. MicroRNAs in cholangiopathies: Potential diagnostic and therapeutic tools. *Clin Res Hepatol Gastroenterol* 2016; **40**: 15-27 [PMID: 26774196 DOI: 10.1016/j.clinre.2015.10.001]

103 **Nakanuma Y**, Sasaki M, Sato Y, Ren X, Ikeda H, Harada K. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. *World J Hepatol* 2009; **1**: 35-42 [PMID: 21160963 DOI: 10.4254/wjh.v1.i1.35]

104 **Sasaki M**, Yamaguchi J, Itatsu K, Ikeda H, Nakanuma Y. Over-expression of polycomb group protein EZH2 relates to decreased expression of p16 INK4a in cholangiocarcinogenesis in hepatolithiasis. *J Pathol* 2008; **215**: 175-183 [PMID: 18393368 DOI: 10.1002/path.2345]

105 **Hsu M**, Sasaki M, Igarashi S, Sato Y, Nakanuma Y. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. *Cancer* 2013; **119**: 1669-1674 [PMID: 23335286 DOI: 10.1002/cncr.27955]

106 **Schlitter AM**, Born D, Bettstetter M, Specht K, Kim-Fuchs C, Riener MO, Jeliazkova P, Sipos B, Siveke JT, Terris B, Zen Y, Schuster T, Höfler H, Perren A, Klöppel G, Esposito I. Intraductal papillary neoplasms of the bile duct: stepwise progression to carcinoma involves common molecular pathways. *Mod Pathol* 2014; **27**: 73-86 [PMID: 23828315 DOI: 10.1038/modpathol.2013.112]

107 **Fujikura K**, Akita M, Ajiki T, Fukumoto T, Itoh T, Zen Y. Recurrent Mutations in APC and CTNNB1 and Activated Wnt/β-catenin Signaling in Intraductal Papillary Neoplasms of the Bile Duct: A Whole Exome Sequencing Study. *Am J Surg Pathol* 2018; **42**: 1674-1685 [PMID: 30212390 DOI: 10.1097/PAS.0000000000001155]

108 **Kuan LL**, Dennison AR, Garcea G. Intraductal Tubulopapillary Neoplasm of the Pancreas and Bile Duct: A Review. *Pancreas* 2020; **49**: 498-502 [PMID: 32282762 DOI: 10.1097/MPA.0000000000001518]

109 **Gross C**, Engleitner T, Lange S, Weber J, Jesinghaus M, Konukiewitz B, Muckenhuber A, Steiger K, Pfarr N, Goeppert B, Keller G, Weichert W, Adsay NV, Klöppel G, Rad R, Esposito I, Schlitter AM. Whole Exome Sequencing of Biliary Tubulopapillary Neoplasms Reveals Common Mutations in Chromatin Remodeling Genes. *Cancers (Basel)* 2021; **13** [PMID: 34205964 DOI: 10.3390/cancers13112742]

110 **Singhi AD**, Nikiforova MN, Chennat J, Papachristou GI, Khalid A, Rabinovitz M, Das R, Sarkaria S, Ayasso MS, Wald AI, Monaco SE, Nalesnik M, Ohori NP, Geller D, Tsung A, Zureikat AH, Zeh H, Marsh JW, Hogg M, Lee K, Bartlett DL, Pingpank JF, Humar A, Bahary N, Dasyam AK, Brand R, Fasanella KE, McGrath K, Slivka A. Integrating next-generation sequencing to endoscopic retrograde cholangiopancreatography (ERCP)-obtained biliary specimens improves the detection and management of patients with malignant bile duct strictures. *Gut* 2020; **69**: 52-61 [PMID: 30971436 DOI: 10.1136/gutjnl-2018-317817]

111 **Poulet G**, Massias J, Taly V. Liquid Biopsy: General Concepts. *Acta Cytol* 2019; **63**: 449-455 [PMID: 31091522 DOI: 10.1159/000499337]

112 **Rompianesi G**, Di Martino M, Gordon-Weeks A, Montalti R, Troisi R. Liquid biopsy in cholangiocarcinoma: Current status and future perspectives. *World J Gastrointest Oncol* 2021; **13**: 332-350 [PMID: 34040697 DOI: 10.4251/wjgo.v13.i5.332]

113 **Yang JD**, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, Zhang X, Hu C, Campion VL, Jen J, Venkatesh SK, Halling KC, Kipp BR, Roberts LR. Circulating tumor cells are associated with poor overall survival in

patients with cholangiocarcinoma. *Hepatology* 2016; **63**: 148-158 [PMID: 26096702 DOI: 10.1002/hep.27944]

114 **Wintachai P**, Lim JQ, Techasen A, Lert-Itthiporn W, Kongpetch S, Loilome W, Chindaprasirt J, Titapun A, Namwat N, Khuntikeo N, Jusakul A. Diagnostic and Prognostic Value of Circulating Cell-Free DNA for Cholangiocarcinoma. *Diagnostics (Basel)* 2021; **11** [PMID: 34070951 DOI: 10.3390/diagnostics11060999]

115 **Bertuccio P**, Malvezzi M, Carioli G, Hashim D, Boffetta P, El-Serag HB, La Vecchia C, Negri E. Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *J Hepatol* 2019; **71**: 104-114 [PMID: 30910538 DOI: 10.1016/j.jhep.2019.03.013]

116 **Brindley PJ**, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, Teh BT, Wongkham S, Gores GJ. Cholangiocarcinoma. *Nat Rev Dis Primers* 2021; **7**: 65 [PMID: 34504109 DOI: 10.1038/s41572-021-00300-2]

117 **Massironi S**, Pilla L, Elvevi A, Longarini R, Rossi RE, Bidoli P, Invernizzi P. New and Emerging Systemic Therapeutic Options for Advanced Cholangiocarcinoma. *Cells* 2020; **9** [PMID: 32168869 DOI: 10.3390/cells9030688]

118 **KLATSKIN G**. ADENOCARCINOMA OF THE HEPATIC DUCT AT ITS BIFURCATION WITHIN THE PORTA HEPATIS. AN UNUAL TUMOR WITH DISTINCTIVE CLINICAL AND PATHOLOGICAL FEATURES. *Am J Med* 1965; **38**: 241-256 [PMID: 14256720 DOI: 10.1016/0002-9343(65)90178-6]

119 **Deoliveira ML**, Schulick RD, Nimura Y, Rosen C, Gores G, Neuhaus P, Clavien PA. New staging system and a registry for perihilar cholangiocarcinoma. *Hepatology* 2011; **53**: 1363-1371 [PMID: 21480336 DOI: 10.1002/hep.24227]

120 **Edge SB**, Byrd DR, Carducci MA, Compton CC, Fritz A, Greene F. AJCC cancer staging manual: Springer New York, 2010

121 **Bismuth H**, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; **215**: 31-38 [PMID: 1309988 DOI: 10.1097/00000658-199201000-00005]

122 **Soares KC**, Kamel I, Cosgrove DP, Herman JM, Pawlik TM. Hilar cholangiocarcinoma: diagnosis, treatment options, and management. *Hepatobiliary Surg Nutr* 2014; **3**: 18-34 [PMID: 24696835 DOI: 10.3978/j.issn.2304-3881.2014.02.05]

123 **Ren B**, Guo Q, Yang Y, Liu L, Wei S, Chen W, Tian Y. A meta-analysis of the efficacy of postoperative adjuvant radiotherapy *vs* no radiotherapy for extrahepatic cholangiocarcinoma and gallbladder carcinoma. *Radiat Oncol* 2020; **15**: 15 [PMID: 31941520 DOI: 10.1186/s13014-020-1459-x]

124 **Sapisochin G**, Javle M, Lerut J, Ohtsuka M, Ghobrial M, Hibi T, Kwan NM, Heimbach J. Liver Transplantation for Cholangiocarcinoma and Mixed Hepatocellular Cholangiocarcinoma: Working Group Report From the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020; **104**: 1125-1130 [PMID: 32217937 DOI: 10.1097/TP.0000000000003212]

125 **Kim BH**, Kim K, Chie EK, Kwon J, Jang JY, Kim SW, Oh DY, Bang YJ. Long-Term Outcome of Distal Cholangiocarcinoma after Pancreaticoduodenectomy Followed by Adjuvant Chemoradiotherapy: A 15-Year Experience in a Single Institution. *Cancer Res Treat* 2017; **49**: 473-483 [PMID: 27554480 DOI: 10.4143/crt.2016.166]

126 **Sarcognato S**, Sacchi D, Fassan M, Fabris L, Cadamuro M, Zanus G, Cataldo I, Covelli C, Capelli P, Furlanetto A, Guido M. Benign biliary neoplasms and biliary tumor precursors. *Pathologica* 2021; **113**: 147-157 [PMID: 34294933 DOI: 10.32074/1591-951X-251]

127 **Sano T**, Shimizu Y, Senda Y, Kinoshita T, Nimura Y. Assessing resectability in cholangiocarcinoma. *Hepat Oncol* 2014; **1**: 39-51 [PMID: 30190940 DOI: 10.2217/hep.13.6]

128 **Aoba T**, Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y, Nagino M. Assessment of nodal status for perihilar cholangiocarcinoma: location, number, or ratio of involved nodes. *Ann Surg* 2013; **257**: 718-725 [PMID: 23407295 DOI: 10.1097/SLA.0b013e3182822277]

129 **Nagino M**, Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y. Evolution of surgical treatment for perihilar cholangiocarcinoma: a single-center 34-year review of 574 consecutive resections. *Ann Surg* 2013; **258**: 129-140 [PMID: 23059502 DOI: 10.1097/SLA.0b013e3182708b57]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 628-645

DOI: 10.4251/wjgo.v14.i3.628

ISSN 1948-5204 (online)

MINIREVIEWS

Clinical significance of molecular subtypes of gastrointestinal tract adenocarcinoma

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gorgisen G, Turkey; Lee SW, South Korea; Yang Y, China

Received: March 10, 2021
Peer-review started: March 10, 2021
First decision: June 4, 2021
Revised: June 4, 2021
Accepted: February 25, 2022
Article in press: February 25, 2022
Published online: March 15, 2022

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Abstract

Adenocarcinomas of the gastrointestinal tract (esophagus, stomach, and colon) represent a heterogeneous group of diseases with distinct etiology, clinical features, treatment approaches, and prognosis. Studies are ongoing to isolate molecular genetic subtypes, perform complete biological characterization of the tumor, determine prognostic groups, and find predictive markers to the effectiveness of therapy. Separate molecular genetic classifications were created for esophageal adenocarcinoma [The Cancer Genome Atlas (TCGA)], stomach cancer (TCGA, Asian Cancer Research Group), and colon cancer (Colorectal Cancer Subtyping Consortium). In 2018, isolation of TCGA molecular genetic subtypes for adenocarcinomas of the gastrointestinal tract (esophagus, stomach, and colon) highlighted the need for further studies and clinical validation of subtyping of gastrointestinal adenocarcinomas. However, this approach has limitations. The

aim of our work was to critically analyze integration of molecular genetic subtyping of gastrointestinal adenocarcinomas in clinical practice.

Key Words: Esophageal adenocarcinoma; Gastric cancer; Colon cancer; Gene sequencing, Gene expression profiling; Molecular subtypes

Core Tip: Here we describe our opinion on molecular genetic subtyping of gastrointestinal adenocarcinomas (esophageal, gastric, and colon adenocarcinomas). The identification of combined molecular and genetic subtypes gave us insights to understanding gastrointestinal adenocarcinoma biology, determining aims for future clinical research, and helping to simplify the implementation of a unified system for subtyping gastrointestinal adenocarcinomas.

Citation: Ignatova EO, Kozlov E, Ivanov M, Mileyko V, Menshikova S, Sun H, Fedyanin M, Tryakin A, Stilidi I. Clinical significance of molecular subtypes of gastrointestinal tract adenocarcinoma. *World J Gastrointest Oncol* 2022; 14(3): 628-645
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/628.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.628

INTRODUCTION

Adenocarcinomas of the gastrointestinal tract represent a heterogeneous group of diseases. Their management depends on localization of the tumor, clinical and morphological characteristics of disease, and tumor biology.

To improve treatment outcomes we actively search for molecular biomarkers that can predict drug effectiveness and foretell the disease course. Molecular genetic typing of gastrointestinal adenocarcinomas is thought to be a promising approach.

Some data show that gastrointestinal tract adenocarcinomas represent several distinguished molecular subtypes that could be associated with different pathogenesis, prognosis, and treatment options. Separate molecular genetic classifications were created for colon cancer (Colorectal Cancer Subtyping Consortium) and gastric cancer (GC) [Asian Cancer Research Group (ACRG)]. In 2018, a pooled analysis of gastrointestinal adenocarcinomas of The Cancer Genome Atlas (TCGA) (esophageal cancer, GC, colon cancer, and rectal cancer) revealed five molecular genetic subtypes based on molecular studies with high-capacity methods.

To date, we have no doubt of the clinical significance of isolating molecular genetic subtypes of gastrointestinal tract adenocarcinomas. The main challenge is to adapt this classification for routine clinical use by defining "surrogate markers" of biological subtypes.

MOLECULAR GENETIC SUBTYPES OF ESOPHAGEAL ADENOCARCINOMA

Esophageal cancer is one of the most aggressive cancers. According to GLOBOCAN, more than 600000 new cases of esophageal cancer were registered in 2020[1]. The main morphological form of esophageal cancer is squamous cell carcinoma (keratinizing or non-keratinizing) (95%). In 5% of cases there is adenocarcinoma of the esophagus (EA) and in rare cases, small cell carcinoma[2]. The distribution of histological tumor subtypes varies widely by country of residence, race, and gender. The pathogenesis of esophageal adenocarcinomas is known to be associated with gastroesophageal reflux disease, Barrett's esophagus, obesity, and smoking[3]. Gastroesophageal reflux is one of the key DNA damaging factors. In a rat esophageal cancer model, reflux induction has been shown to increase mutation rates, mainly C/T and G/A transitions[4].

Drug treatment of esophageal cancer is determined by its histological type as well as the presence of molecular genetic markers such as microsatellite instability (MSI) status, PD-L1 expression, and HER2 expression (in adenocarcinoma). The range of therapeutic options is limited; the most effective drugs for both histological variants are cisplatin, fluoropyrimidines, and taxanes. Oxaliplatin, irinotecan, and trastuzumab (with overexpression/amplification of HER2) are also effective in adenocarcinomas[5]. For high levels of MSI (MSI-H) tumors, pembrolizumab may be prescribed as the second-line therapy. Pembrolizumab could be reasonable in combination with cisplatin and fluorouracil (KEYNOTE-590) as the first-line treatment option in patients with squamous cell carcinoma with a positive PD-L1 combined

positive score (CPS) status ≥ 10. Nivolumab could be reasonable in folinic acid, fluorouracil, and oxaliplatin (FOLFOX) or oxaliplatin and capecitabine combinations (CheckMate 649) as the first-line treatment option in patients with esophageal adenocarcinoma with a positive PD-L1 CPS status of ≥ 5. However, the results of esophageal cancer treatment to date are still unsatisfactory with a 5-year overall survival rate of 20% and a median life expectancy of patients with metastatic cancer of less than a year [6]. Currently, we are actively searching to find new predictive biomarkers for treatment *via* molecular genetic analysis. It would allow us to identify individual subtypes of the disease and therefore a personalized treatment approach.

Dulak *et al*[7] were the first to publish work on advanced molecular genetic analysis of esophageal adenocarcinoma. They studied a spectrum of EA mutations with a pairwise analysis of tumor and normal controls in 149 EA patients *via* full-exome sequencing. However, 15 patients had full-genome sequencing analysis. They described a mutational signature having A>C transversions in AA dinucleotides[7].

In another study of Secrier *et al*[8], they analyzed results of full-genome sequencing of 129 EA samples. They showed that EA was usually prevalent with large rearrangements and extremely high heterogeneity of the tumor as well as a high frequency of mutations and coamplifications of tyrosine kinase receptors. The most common amplifications were ERBB2, EGFR, MET, and FGFRs. They managed to determine mutational signatures and stratify EA into 3 subtypes: the DNA damage repair induced (18%), C>A/T dominant (29%), and mutagenic (53%) subtype.

The DNA damage repair subgroup showed an increase in the frequency of violations of homologous recombination genes. Homologous recombination gene disorders might determine potential sensitivity to platinum-based chemotherapy (CT) and PARP inhibitors as well as sensitivity to radiation therapy[8].

The C>A/T dominant EA subtype was associated with aging, a lower level of duplications, and an increased frequency of interchromosomal translocations. In the C>A/T dominant subgroup there was a higher frequency of ERBB2/MET coamplifications. The use of tyrosine kinase receptor inhibitors[9] may be reasonable in this subtype.

The mutagenic subtype showed a high mutational load and load of neoantigens. These characteristics may mediate sensitivity to immunotherapy. Development of the mutagenic subtype is associated with gastroesophageal reflux. The mutagenic subtype demonstrated sensitivity to WEE1/CHK1 inhibitors [10]. The authors concluded that the use of mutational signatures and subtyping could help in the selection of promising therapeutic options and determine rationale for further research. Limitations for the use of a personalized approach to EA treatment are significant heterogeneity and a high number of coamplifications in tyrosine kinase receptor genes.

Comparative molecular genetic analysis of EA with GC and cancer of the esophageal-gastric junction is of particular clinical interest. In 2017, TCGA project presented a similar analysis. They analyzed 164 EA samples, 359 GC samples, and 36 adenocarcinomas of the esophageal-gastric junction. They found that EA results were consistent with chromosomally instable (CIN) gastric phenotype with its molecular and genetic characteristics and could probably be treated as a single nosology[10] .

EA is considered as the tumor with the most frequent changes in copy number variations[11,12]. The most significant amplifications for both the EA and CIN subtypes of GC are ERBB2, MYC, IKZF3, CDK12, VEGFA, CDK6, FGF3, and FGF4. However, a detailed examination revealed some differences in the molecular genetic characteristics and differences in tumor methylation in accordance with its location[12]. Thus, hypermethylation is more frequent in EA than in the CIN subtype (70% *vs* 30% respectively, $P = 1.0 \times 10^{-8}$). Moreover, the incidence of some genes change depending on the anatomical site. For example, SMARC4 mutations and RUNX1 tumor suppressor deletions are more common for EA, but APC mutations are rare compared to GC. The Wnt/β-catenin pathway seems to play a less important role in EA. In addition, MYC and VEGFA amplifications are more frequent for EA. Thus, there is a significant level of intratumor heterogeneity among EA with obvious properties of the CIN phenotype, like amplification of tyrosine kinase receptors.

In 2018, Guo *et al*[13] conducted a cumulative analysis of EA subtyping using the Gene Expression Omnibus and TCGA databases. When analyzing gene expression profiles of three independent cohorts, they found two molecular genetic subtypes of EA with distinct expression and somatic mutation profiles. They showed that the first subtype (I) shared common molecular expression profiles with GC, and the second one (II) was similar to squamous cell carcinoma. Specific somatic mutations of SMAD4, SOCS4, and SKAP2 were specific for the first subtype[14]. Only 3 patients in the study received CT: 2 patients with type II EA and 1 patient with type I EA. Two patients with subtype II had a complete response to treatment. One patient with subtype I progressed during treatment. Due to the extremely small sample size of patients and insufficient clinical information, it would be reasonable to continue analyzing prognostic and predictive significance of molecular subtype selection.

Molecular genetic analysis of Barrett's esophageal samples is of particular clinical interest and may be useful for understanding the biology of EA. It is known that Barrett's esophagus is a precursor of EA. Barrett's esophagus was shown to be polyclonal and exhibit high mutational properties even in the absence of dysplasia. The genome of Barrett's esophageal tissues is relatively more stable compared to invasive tumors[15]. About 32% of Barrett's esophagus cases perform massive localized chromosomal translocations (chromotripsis), which can result in the activation of oncogenes and inactivation of tumor suppressors mediating rapid development of EA[16]. Genetic changes in EA are usually accompanied

by significant epigenome changes. With the development of high-grade metaplasia and EA there is an additional increase in the methylation of CpG islands[16]. Thus, detection of epigenetic changes specific to EA and Barrett's esophagus can help in the subtyping of patients.

In 2020, there was a study identifying subtypes of Barrett's esophagus and EA based on DNA methylation profiles and integration of transcriptomic and genomic data[17]. They analyzed 150 samples of Barrett's esophagus and 285 samples of EA. They identified four molecular genetic subtypes; each had distinctive biological properties. The first subtype exhibited hypermethylation of DNA (CpG island methylator phenotype-like), high mutational load with numerous mutations in cell cycle genes (CCND1, CCNE1, MYC, CDK6) and tyrosine kinase receptors (GATA4, ERBB2, KRAS). The second one had expression of gene patterns associated with metabolic processes and absence of methylation at specific sites of transcription factors. This subtype was common for Barrett's esophagus (83%). The third subtype had no methylation with gene expression indicating infiltration by immune cells (cytotoxic cells, B cells, mast cells and neutrophils, and tumor-associated fibroblasts), and decreased expression of T helper cells. These patients had a poor prognosis compared to the other subtypes. The fourth subtype presented with hypomethylation of DNA and a high frequency of CCNE1 amplifications. Several preclinical studies demonstrated drug treatment effectiveness depending on the tumor subtype. For instance, irinotecan, a topoisomerase I inhibitor, is known to be effective in the treatment of tumors with a high level of methylation. Given the similarity of the molecular characteristics of CIN GC with EA, the EA I subtype (CpG island methylator phenotype-like) might be sensitive to inhibitors of DNA methyltransferase and topoisomerase I. It was also reported that CDK4/6 inhibitors were effective in EA of all subtypes with CDK2 inhibitors more effective in subtype 4 due to CCNE1 amplification. In addition, organelles with reduced levels of MGMT and CHFR expression were sensitive to temozolomide and taxane drugs[18-20].

Thus, a number of studies by large research groups are devoted to the EA subtyping. Their work aims to isolate molecular genetic subtypes, perform complete biological characterization of the tumor, determine prognostic groups, and find predictive markers to the effectiveness of therapy. The obtained data indicate the need for further research and require additional clinical validation for successful clinical use.

MOLECULAR GENETIC SUBTYPES OF GC

GC has an unfavorable prognosis with a median life expectancy in metastatic patients under a year. Standard molecular genetic diagnosis in metastatic GC includes expression and amplification of HER2/neu, MSI, and PD-L1 expression (CPS). If positive HER2/neu expression status is detected or HER2/neu is amplified, trastuzumab is added for first-line CT. A high level of MSI or positive expression of PD-L1 (CPS > 10) allows the use of pembrolizumab immunotherapy in the second-line setting. Positive expression of PD-L1 (CPS > 1) allows the use of pembrolizumab in subsequent lines of treatment. In addition, in 2020 a randomized phase 3 trial, CheckMate 649, showed a statistically significant ($P < 0.001$) increase in overall survival for combination of nivolumab with oxaliplatin and capecitabine or FOLFOX regimens for the first-line treatment of GC with PD-L1 CPS expression ≥ 5[21].

For the histological classification of GC, we use the World Health Organization classification, which distinguishes some major subtypes like papillary, tubular, and mucinous types[22]. Quite often, histological Lauren classification is used due to its simplicity and easy use. There are several Lauren subtypes according to dominant morphological picture, like intestinal, diffuse, and mixed types of GC [23]. The intestinal type of GC is more common in patients with severe atrophic gastritis and is associated with intestinal metaplasia and presence of persistent *H. pylori* infection. This subtype is more common in men or the elderly and usually has visceral metastasis. Diffuse GC is associated with low differentiation, treatment resistance, and poor prognosis. The diffuse subtype is more common in young women and usually presents with peritoneal dissemination.

There are a series of studies devoted to a predictive role of the GC histological subtypes. In a multicenter study, Messager *et al*[24] retrospectively analyzed the effectiveness of perioperative CT based on platinum and fluorouracil agents on the survival of patients with signet ring cell carcinoma GC. Signet ring cell carcinoma is a diffuse GC subtype according to the Lauren classification. Among 3010 patients receiving treatment in 19 clinics in France from January 1997 to January 2010, 1050 (34.9%) had signet ring cell carcinoma GC; 18.5% (171) of patients received perioperative CT, and 81.5% (753) received surgical treatment alone. With a median follow-up of 31.5 mo, median survival was lower in the signet ring carcinoma group (12.8 *vs* 14.0 mo, $P = 0.043$). Multivariate analysis showed that signet ring carcinoma was an independent factor of poor prognosis [hazard ratio (HR) = 1.4; 95% confidence interval (CI): 1.1-1.9; $P = 0.042$]. This study was retrospective and does not have the necessary power to change clinical practice. In randomized trials of perioperative and adjuvant CT (ACTS1[25], CLASSIC [26], INTERGroup0116[27], FNLCC[28]) there was no preplanned analysis of a patient subgroup with signet ring carcinoma diffuse GC.

In the MAGIC study[29], they compared surgical treatment only with perioperative CT, which included three courses of epirubicin, cisplatin, and 5-fluorouracil (ECF) before the operation and three

courses after the operation. The addition of CT led to a significant increase in the 5-year life expectancy in patients from 23% to 36% (HR 0.75, 95%CI: 0.60 0.93, P = 0.009). After a subgroup analysis there were no differences in the frequency of pathological complete response in the diffuse or intestinal subtype GC according to Lauren.

The FLOT-4 study[30] evaluated two different perioperative CT regimens, the ECF/ECX regimen (epirubicin, cisplatin, fluorouracil or capecitabine) and docetaxel, oxaliplatin, leucovorin, and fluorouracil. The docetaxel, oxaliplatin, leucovorin, and fluorouracil combination benefited over the ECF/ECX regimen, significantly improving the 5-year overall survival rate (48% and 57%, HR = 0.77, P = 0.012). Subgroup analysis demonstrated that docetaxel, oxaliplatin, leucovorin, and fluorouracil efficacy was higher than the ECF/ECX regimen regardless of histological subtype, even in the subgroup of signet ring carcinoma GC.

Survey of experts at the 4th International Conference St. Gallen, dedicated to the treatment of operable GC and esophageal cancer, showed that signet ring carcinoma of diffuse histological subtype was an independent factor of poor prognosis and should be considered as a stratification factor in future studies[31]. The Lauren histological classification has been widely used over the past five decades, but its clinical significance is limited because it does not reflect the full complexity and molecular heterogeneity of the disease. With the use of molecular platforms (NGS, DNA microarrays, RPPA), it became possible to classify GC into molecular subtypes. Various research groups worked to determine molecular subtypes of GC.

The most interesting work in subtyping of GC were the studies of Tan *et al*[32] in 2011 and 2013 (Figure 1)[33], TCGA project in 2014[12], and ACRG study in 2015 (Table 1).

In 2011, Tan *et al*[32] identified GC subtypes *via* analyzing gene expression with a panel of 37 GC cell lines. Gene expression analysis was performed using microarrays (HG-U133 Plus 2.0, Affymetrix). They identified the gene expression signature of 171 genes and identified two GC subtypes with distinct gene expression patterns, namely, the intestinal subtype (G-INT) and the diffuse subtype (G-DIF). These subtypes also were determined when analyzing primary gastric tumors in 270 patients in two independent groups. The G-INT subtype was found to be associated with activation of protein and carbohydrate metabolism (*FUT2*) and cell adhesion (*LGALS4*, *CDH17*) genes. The G-DIF subtype was associated with functional annotations of cell proliferation (*AURKB*) and fatty acid metabolism (*ELOVL5*). In addition, these subtypes had prognostic value. Patients with the G-DIF subtype had poor prognosis compared to patients with G-INT in several cohorts. In addition, 28 samples of cell lines (11 G-INT and 17 G-DIF) showed that G-INT cell lines were more sensitive to 5-fluorouracil and oxaliplatin, whereas G-DIF were more sensitive to cisplatin. Thus, the authors suggested that G-INT and G-DIF subtypes could be used to determine GC prognosis and individualize therapy[33].

Later in 2013, Lei *et al*[33] published a study with another attempt to identify molecular subtypes of GC. They analyzed 248 tumor samples from patients with GC. Three main subtypes of GC were identified: mesenchymal, proliferative, and metabolic. The mesenchymal subtype was named so because of the high activity of the epithelial-mesenchymal transition (EMT) process. EMT is a complex developmental program that allows malignant cells to suppress their epithelial properties, replacing them with mesenchymal ones. These changes allow cells to become mobile and be able to migrate from the primary focus. EMT is associated with metastasis. This subtype has high levels of CDH2 (N-cadherin) expression and low levels of CDH1 (E-cadherin) expression. In addition, cells of the mesenchymal subtype of GC proved to be particularly sensitive to PI3K-AKT-mTOR inhibitors *in vitro*. The proliferative subtype showed a high expression of genes associated with the cell cycle (*E2F*, *MYC*, *RAS*) as well as a high level of mutations in *TP53* and copy number variation loci of *CCNE1*, *MYC*, and *KRAS*. Also, this type showed a decreased relapse-free survival compared to other types. The metabolic subtype showed high expression of genes associated with metabolism, which was also specific to healthy gastric mucosa. Therefore, this subtype is thought to be closer to healthy mucosa because of its molecular and genetic characteristics than the proliferative and mesenchymal types. Moreover, the metabolic subtype has higher sensitivity to 5-fluorouracil than the others[33].

In 2014, TCGA published the most promising and full-scale study, which also classified GC into molecular subtypes. They analyzed samples of 295 patients with GC who had not previously received CT and/or radiation therapy. They used six platforms for analysis: exome sequencing, comparative genomic hybridization, DNA methylation studies, matrix RNA and micro RNA sequencing, and proteomic analysis. They classified GC into four subtypes: Epstein-Barr virus (EBV)-positive tumors, tumors with MSI, tumors with a stable genome (GS), and tumors with CIN[12]. This work is important because it identified molecular and biological subtypes of GC, which determined further research for new therapeutic approaches of treatment[33].

EBV-associated subtype of GC

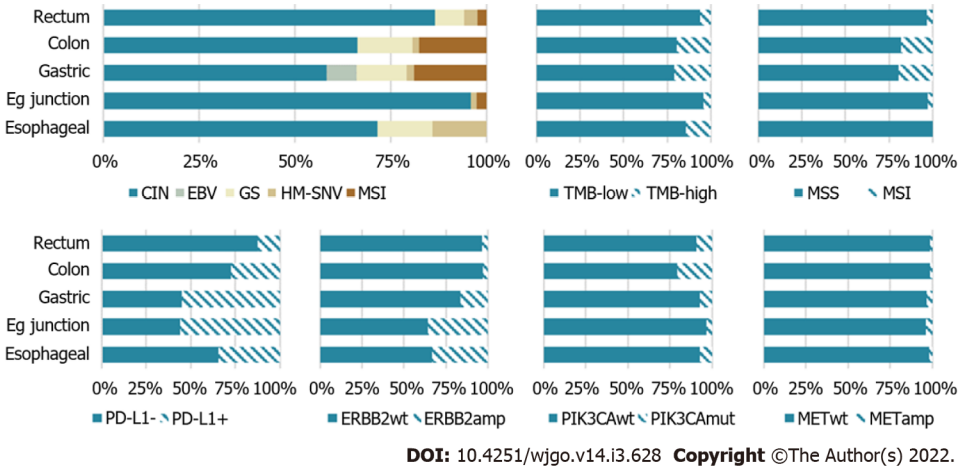
EBV presents in 10% of GC cases worldwide. EBV-associated GC has specific molecular features. TCGA analysis showed that 80% of EBV-positive gastric tumors have *PIK3CA* mutations as well as amplifications of the *JAK2*, *CD274* (*PD-L1*) and *PDCD1LG2* (*PD-L2*) genes. Mutations in *ARID1A* (55%) and *BCOR* (23%) genes are also common, while *TP53* defects are not specific for this subtype of GC[34].

Table 1 Existing molecular classification systems of gastrointestinal tract tumors

Subdividing and data level	Subtype	Prevalence	Defining characteristics
Esophagus subtypes			
Liu *et al*[34] obtained subtypes based on SCNAs, WES, DNA methylation, mRNAseq, microRNAseq, RPPA (TCGA)	EA-CIN	14.1	EA similarity with CIN phenotype of GC. Methylation patterns and gene alterations differ in terms of localization
Guo *et al*[13] determined differences in expression profiles and somatic mutation profiles by using RNA-Seq and exome-Seq data	EA I	40	EA I shares the common expression profiles with GC
	EA II	60	EA II was clustered with esophageal squamous cell carcinomas
Jammula *et al*[17] divided OAC and Barrett's esophagus by integration of WGS and RNA-seq data	Subtype I	28.7	SI: CIMP-like
	Subtype II	27.3	SII: Expression of gene patterns associated with metabolic processes
	Subtype III	22.7	SIII: Immune cell infiltration
	Subtype IV	21.1	SIV: DNA hypomethylation; structural aberrations; CNA
Secrier *et al*[8] received GC subtypes on the basis of mutation signatures obtained from WGS data	DDR-impaired	15	DDR: Enrichment for BRCA signature with prevalent defects in the homologous recombination pathway
	C > A/T dominant	32	C > A/T: Aging imprint
	Mutagenic	53	Mutagenic: The highest mutational load and the highest load of neoantigens
Gastric subtypes			
Tan *et al*[32] obtained subtypes based on gene expression pattern (microarray)	G-INT	58	G-INT: Genes upregulated were related to carbohydrate and protein metabolism (*FUT2*) and cell adhesion (*LGALS4*; *CDH17*)
	G-DIF	42	G-DIF: Cell proliferation (*AURKB*) and fatty acid metabolism (*ELOVL5*) functional annotations were enriched
Lei *et al*[33] compared the patterns of gene expression samples of GC (mRNA, CNAs)	Proliferative	45	Proliferative: High levels of genomic instability; TP53 mutations and DNA hypomethylation
	Metabolic	23	Metabolic: High expression of genes associated with metabolism
	Mesenchymal	31	Mesenchymal: Contain cells with features of cancer stem cells
TCGA obtained subtypes based on SCNAs, WES, DNA methylation, mRNAseq, microRNAseq, RPPA[12]	EBV+	8.8	EBV: Recurrent mutation of PIK3CA; intense hypermethylation; JAK2, CD274, PDCD1LG2 amplification
	MSI	21.7	MSI: Increased frequency of mutations; aberrant epigenetic patterns
	CIN	49.8	CIN: The presence of multiple chromosomal rearrangements; localization mainly in the proximal gastric cancer and EGJ
	GS	19.7	GS: RHOA, CDH1 and ARID1A mutations; CLDN18-ARHGAP6 gene fusion
Cristescu *et al*[55] received GC subtypes based on data of gene expression	MSI-high GC	22.7	MSI-high GC: Mutations in ARID1A, MTOR, KRAS, PIK3CA, ALK, and PTEN. Overexpression of PD-L1; T cell infiltrate
	MSS/EMT GC	15.3	MSS/EMT GC: Loss of CDH1; Loss of cellular adhesion, angiogenesis, motility
	MSS/TP53- GC	35.7	MSS/TP53- GC: Highest prevalence of TP53 and RHOA mutations; APC, ARID1A, KRAS, PIK3CA, and SMAD4 enriched
	MSS/TP53+ GC	26.3	MSS/TP53+ GC: Frequent EBV infection; Frequent mutations in ARID1A, PIK3CA, SMAD4, APC
Colon subtypes			

Guinney *et al*[59] carried out combined molecular genetic analysis of 4151 colon tumor samples from 6 different scientific groups	CMS1	14	CMS1: Hypermutated; microsatellite unstable; strong immune activation
	CMS2	37	CMS2: Epithelial, chromosomally unstable; marked WNT and MYC signaling activation
	CMS3	13	CMS3: Epithelial; evident metabolic dysregulation
	CMS4	23	CMS4: Prominent transforming growth factor β activation; stromal invasion and angiogenesis
Liu *et al*[34] obtained subtypes based on SCNAs, WES, DNA methylation, mRNAseq, microRNAseq, RPPA (TCGA)	MSI	17.5	MSI: MSI tumors with MLH1 methylation were associated with BRAFV600E mutation
	HM-SNV	1.7	HM-SNV: Hotspot mutations in polymerase E
	CIN	66.6	CIMP status is characteristic of CRC with associated mutations in KRAS and TGFβ pathways
	GS	14	GS: Lacking hypermutation and aneuploidy; enriched in DNA hypermethylation and mutations in KRAS, SOX9 and PCBP1

CIMP: CpG island methylator phenotype; CIN: Chromosomally instable; CRC: Colorectal cancer; DDR: DNA damage repair; EA: Esophagus; EBV: Epstein-Barr virus; EGJ: Epigastric junction; GC: Gastric cancer; G-DIF: Gastric diffuse subtype; G-INT: Gastric intestinal subtype; GS: Stable genome; HM-SNV: Hypermutated-single nucleotide variant; MSI: Microsatellite instability; MSS: Microsatellite stability; TCGA: The Cancer Genome Atlas; WGS: Whole genome sequencing.



DOI: 10.4251/wjgo.v14.i3.628

Figure 1 Distribution of major molecular subtypes and the most common predictive biomarker across different tumor types of the gastrointestinal tract. amp: Amplification; CIN: Chromosomally instable; EBV: Epstein-Barr virus; Eg: Epigastric; GS: Stable genome; HM-SNV: Hypermutated-single nucleotide variant; MSI: Microsatellite instability; MSS: Microsatellite stability; TMB: Tumor mutation burden; wt: Wild-type.

PIK3CA mutations usually localize in hot spots: exon 9 (E542K and E545K) and exon 20 (H1047R)[35]. However, for EBV+ GC, the frequency of *PIK3CA* mutations in hot spots is only 28%, and mutations can be observed throughout the nucleotide sequence[39]. Genetic defects in *PIK3CA* may precede EBV infection, which then enhances the activation of the PI3K/Akt/mTOR pathway.

According to TCGA analysis, EBV-associated gastric tumors have a high frequency of DNA hypermethylation. In particular, hypermethylation of the *CDKN2A* gene promoter (*p16INK4a*) was observed in all the studied samples of EBV-positive gastric tumors. Epigenetic inactivation of this gene, along with such oncosuppressors as p14, APC, and TFAP2E, is specific for EBV-positive GC[36].

The EBV+ phenotype showed increased expression of PD-L1 and/or PD-L2 among another four molecular subtypes of GC[37,38]. The phase II study was the first to show a very high response rate to pembrolizumab therapy among patients with metastatic EBV-positive GC and MSI-GC (overall response rates of 100% and 85.7%, respectively). The authors concluded that EBV+ status and MSI-H serve as reliable predictors of response to immunotherapy, along with the high immunohistochemical expression of PD-L1 in the tumor. They proposed to introduce routine determination of EBV status into clinical practice in order to identify patients with gastric adenocarcinomas who could benefit from immunotherapy[39].

GC with MSI

According to various data, from 5% to 37% of cases of GC have an MSI phenotype: 8%-20% in operable GC and 3%-5% in metastatic setting. There was a large meta-analysis of 48 studies with 18000 patients to study clinical and morphological characteristics of MSI-H GC[40]. MSI-H tumor cases included primarily women, the elderly, and the intestinal subtype. The tumor localizes in the body or in the proximal part of stomach. MSI-H tumors present with an absence of lymph node involvement. The meta-analysis showed that MSI-H GC has a favorable prognosis and better survival rates[41].

MSI-positive GC tumors showed hypermethylation of various genes, gene methylation, unpaired DNA base repair system, and a high level of expression of genes regulating mitotic activity[42]. With adjuvant or preoperative CT, the prognosis of patients with MSI-H GC is worse than in patients with microsatellite stability (MSS) tumors[41]. Kim *et al*[42] discovered this phenomenon in a retrospective study.

Other authors confirmed these results later. According to the results of a retrospective analysis of the randomized MAGIC and CLASSIC trials, the rationale for additional CT is questionable in cases of MSI-H GC. Only MSS patients benefited from systemic treatment, whereas MSI-H GC had a favorable prognosis with surgery alone and a poor prognosis with perioperative or adjuvant CT[43,44].

The MSI-GC-01 meta-analysis performed a pooled analysis of MAGIC and CLASSIC studies depending on MSI in the tumor. They showed that CT in patients with MSI-H did not improve the survival rates[45]. In the treatment of metastatic GC, MSI-H subtype allows immunotherapy in the second and subsequent lines of therapy according to the National Comprehensive Cancer Network recommendations[46].

GS GC subtype

The GS subtype of GC accounts for approximately 20% of the total number of GC cases analyzed by TCGA. In addition, TCGA showed that 73% of diffuse type GC cases can be classified as the GS subtype. No copy number variations were found in the GS subtype. However, the mutations of *RHOA*, *CDH1*, and *ARID1A* were detected as well as the chimeric gene *CLDN18-ARHGAP6*[47]. The Rho family of GTPases is known to regulate the dynamics of actomyosin as well as the processes of cell adhesion, proliferation, and survival. The RhoA signaling pathway is associated with invasion and metastasis. TCGA project identified 16 cases of GC with mutations in the *RHOA* gene, which were specific to the GS subtype. The inactivating ARID1A mutation is specific for both GS and EBV subtypes. This protein mediates regulation of cellular processes, such as DNA damage, differentiation, and development. Loss of ARID1A expression significantly correlates with tumor grade as well as with poor prognosis for patients with the GS subtype[48].

CIN subtype of GC

CIN subtype of GC is the most extensive group and accounts for up to 50% of stomach cancer. This subtype shows multiple chromosomal rearrangements, deletions, and translocations. The CIN subtype localizes mainly in the proximal stomach and gastroesophageal junction. It is more common in the intestinal histological type. Its distinctive feature is high frequency of TP53 mutations (in 70% of cases) and activation of tyrosine kinase receptors. Singapore researchers found amplification and coamplification of tyrosine kinase receptors in 40% of stomach cancers. To evaluate the effectiveness of targeted therapy, phase II and III studies were initiated and conducted. The EXPAND[49] and REAL-3[50] studies evaluated anti-EGFR therapy. The MET-gastric study[51] evaluated the effectiveness of MET inhibitor, and the SHINE study evaluated the effectiveness of an anti-FGFR2 agent[52]. However, all these targeted agents were not effective; all studies were negative. However, these studies did not stratify patients according to molecular changes, but they evaluated the entire patient population regardless of biomarker expression. To date, anti-Her2 therapy in patients with high HER2 expression and HER2 amplification is the only successful targeted therapy. The ToGa study proved trastuzumab in combination with CT as a first-line therapy for metastatic HER2-positive GC[53].

To date, we actively validate clinical significance of TCGA classification in GC. Based on TCGA data, Sohn *et al*[54] developed the first prognostic model establishing statistically significant correlations between certain molecular subtypes of GC with patient survival rates and effectiveness of adjuvant CT.

EBV+ GC had the best prognosis in relation to both disease-free survival (P = 0.006) and overall survival (P = 0.004). The worst prognosis was associated with the GS subtype. The other two subtypes (MSI and CIN) had intermediate prognosis in relation to survival rates. They also confirmed that EBV+ GC is more common in men (79%) and at a younger age than the other subtypes (mean age 53, P = 0.01) [54].

Patients with the CIN subtype of GC had the greatest benefit from adjuvant CT showing a significant increase in disease-free survival (HR: 0.39; 95%CI: 0.16-0.94; P = 0.03). On the contrary, there was no statistically significant benefit from adjuvant CT (HR: 0.83; 95%CI: 0.36-1.89; P = 0.65) in patients with GS GC. It was not possible to assess the effectiveness of adjuvant CT in the EBV+ GC subgroup due to the absence of a control group. The authors also developed a single model for assessing risk of relapse after treatment (integrated risk assessment model), which is good predictor of disease-free survival (HR: 1.5; 95%CI: 1.2-1.9; P = 0.001).

Most samples (about 75%) in TGCA represent patients of the Western population, whose clinical course and biological characteristics differ from those of Eastern populations. Data from the ACRG[55] allowed further study of the clinical utility of TCGA classification. ACRG used gene expression data to characterize 300 postoperative GC samples from Korean patients. As in TCGA classification, they identified four subtypes: (1) MSS/EMT; (2) MSI; (3) MSS/p53+; and (4) MSS/p53-[55].

Thus, a lot of data from various researchers showed main four molecular genetic subtypes of stomach cancer. However, it is difficult to implement routine use of this classification system in routine use as multi-omics analysis would be required. Meanwhile, implementation in clinical practice may facilitate translational and retrospective studies, which would enhance understanding of clinical use of such classification systems. Therefore, the next step for the clinical implementation of this classification should be identification of surrogate biomarkers and their validation in further clinical trials.

MOLECULAR GENETIC SUBTYPES OF COLON CANCER

In 2021, the standards of primary molecular genetic diagnostics for metastatic colon cancer included five biomarkers: mutational status of *KRAS*, *NRAS*, and *BRAF* genes, expression and amplification of HER2/neu, and MSI[56,57].

The first four are negative predictors to the effectiveness of anti-EGFR antibodies. *BRAF* gene (V600) mutation and expression of HER2/neu are predictors to the effectiveness of BRAF inhibitors (with anti-EGFR antibodies +/- MEK inhibitors) and anti-HER2 therapy, respectively. MSI is a predictor to the effectiveness of immunotherapy. At the same time, first-line management with targeted agents in wild-type RAS/BRAF tumors depends on clinical factors like localization of the primary tumor. Therefore, it is reasonable to suggest more complex molecular genetic differences between tumors[58,59]. Systematic work continues to determine patients who could benefit from certain targeted agents. Molecular classification of colon cancer implementation to the results of already conducted randomized trials is one option that should be considered first.

In 2015 six different scientific groups that previously proposed different genetic classifiers for colorectal cancer published results of a pooled molecular genetic analysis of 4151 colon tumors. Based on this work, they created a consensus on molecular genetic (expression) subtyping of colon cancer and identified five colon cancer subtypes (CMS1, CMS2, CMS3, CMS4, and unclassified subtype), which are characterized by certain clinical and molecular differences[59].

The immune subtype (CMS1) represents 14% of cases and predominantly describes tumors with a hypermutated phenotype, MSI, tumor infiltration lymphocyte expression, and activated immune cells. More often, such tumors localize in the cecum, colon ascendens, and hepatic flexure. Canonical (CMS2) represents 37% of samples and is characterized by the activity of WNT and MYC signaling pathways. The tumor mainly localizes in colon descendens, sigmoid colon, and rectum. The metabolic (CMS3) subtype represents 13% of cases and is characterized by alterations in the metabolic systems of the cell, *KRAS* gene mutations, low copy number of mutated genes, and it has a CpG island methylator phenotype. The primary tumor predominantly localizes in the sigmoid colon and rectum. Mesenchymal (CMS4) subtype represents 23% of tumors and is characterized by activation of transforming growth factor β (TGF-β), significant stromal response and angiogenesis. Localization of the primary tumor is similar to the specific CMS2 subtype. However, the researchers failed to classify almost every fifth sample.

Initially this classification was not meant to identify differences in disease prognosis. However, when the researchers looked at the survival of patients with different subtypes, they found that resectable tumors with the mesenchymal subtype had the worst prognosis, while the differences between immune, canonical, and metabolic subtypes were not detected [risks ratio (RR): 1.69, $P < 0.001$]. Beyond progression, the situation changed: patients with the immune subtype showed the lowest survival and canonical subtype had the best survival, while patients with the mesenchymal and metabolic subtypes had an intermediate prognosis ($P < 0.001$).

The next step was to use tumor subtypes as predictors to the effectiveness of targeted and CT agents. The FIRE3 study compared folinic acid, fluorouracil, and irinotecan (FOLFIRI) + cetuximab and FOLFIRI + bevacizumab in first-line treatment of patients with metastatic colon cancer with a wild-type *KRAS* gene. The 438 out of 514 patients were classified according to the CMS subtypes. The subtype incidence among tumors with wild-type *KRAS* gene was 14% for CMS1, 37% for CMS2, 15% for CMS3, and 34% for CMS4 subtype. Cetuximab was effective only in the CMS4 group. The distribution by subtype in tumors with wild-type *RAS* genes did not differ significantly and was 12%, 41%, 11%, and 34%, respectively.

When comparing the subtype distribution concerning primary tumor localization (right-sided or left-sided), the differences were 27% *vs* 11% for CMS1 subtype, 28% *vs* 45% for CMS2, 10% *vs* 12% for CMS3, and 35% *vs* 32% for CMS4 subtype. Prognostic differences between subtypes were consistent with the original work of Guinney *et al*[59]. In the group of wild-type *RAS* genes, only the mesenchymal (CMS4) subtype had a significant gain in overall survival in favor of cetuximab (RR = 0.57, 95%CI: 0.38-0.86, $P = 0.008$). Similar trends were in the metabolic (CMS3) subtype group (RR = 0.57, 95%CI: 0.27-1.23, $P =$

0.15). At the same time, the objective responses were the main endpoint in the FIRE3 study. Cetuximab numerically proved to be more effective in all subgroups. However, cetuximab statistically significantly benefited over the combination with bevacizumab in the CMS2 and CMS4 subtypes: 74% *vs* 42% (P = 0.043) and 76% *vs* 55% (P = 0.049), respectively[60].

The CALGB/SWOG 80405 study had a similar design, but it studied other CT regimens (73% of patients were treated with FOLFOX, 27% with FOLFIRI). There were no differences in overall survival between combinations of different targeted agents, even in the wild-type *RAS* genes group[61]. In contrast to the FIRE3 study, they managed to classify tumor subtypes only in half (n = 581) of the patients. In the entire group of patients, the subtypes were CMS1 in 17.90% of cases, CMS2 in 41.65% of cases, CMS3 in 11.70% of cases, and CMS4 in 28.74% of cases. In a comparative analysis of subtype distribution in regard to primary tumor localization (right-sided or left-sided) the differences were 37.34% *vs* 9.01% for CMS1 subtype, 23.42% *vs* 48.26% for CMS2, 11.39% *vs* 12.50% for CMS3, and 27.85% *vs* 30.24% for CMS4 subtype. That did not differ from the similar results in the FIRE3 study. Prognostic differences between subtypes also were consistent with the original work of Guinney *et al*[59]. However, the differences between the metabolic and mesenchymal subtypes were more significant in favor of the latter. In contrast to the results of the FIRE3 study, the CMS2 subtype had an improvement in overall survival, but not progression-free survival, for combinations with cetuximab (RR = 0.62, 95%CI: 0.45-0.86, P = 0.0046). The CMS1 subtype benefited from bevacizumab regimens in regards to overall survival (RR = 2.34, 95%CI: 1.48-3.7, P < 0.001) and progression-free survival (RR = 2.28, 95%CI: 1.47-3.55, P < 0.001). With the immune subtype, tumors with MSI-H had greater benefit from bevacizumab regimens (R = 0.42, P = 0.0091 for overall survival and RR = 0.46, P = 0.0109 for progression-free survival). The MSS CMS1 subtype had no difference between cetuximab and bevacizumab[62]. Researchers partially associate these findings with the tumor microenvironment and in particular the presence of tumor-associated macrophages and their M1/M2 polarization and possible angiogenic immunomodulatory effect of bevacizumab[62].

There are several reasons for the differences in the results of the discussed studies (FIRE3 and CALGB/SWOG 80405). For instance, there were differences in the chemotherapeutic component of the therapy regimen. In the FIRE3 trial all patients were treated with FOLFIRI, while in the CALGB/SWOG 80405 study 73% of patients were prescribed FOLFOX.

Previously, a randomized phase III study, NSABP C-07, examined the efficacy of adding oxaliplatin to leucovorin and fluorouracil in stage II-III colon cancer in an adjuvant setting. They also retrospectively subtyped 67.6% of tumors according to the CMS classification. The CMS2 (canonical) subtype showed a significant benefit from the oxaliplatin addition (RR = 0.61, 95%CI: 0.43-0.87, P = 0.006) but only in patients with enterocytic variant of expression data (CMS2-enterocyte: RR = 0.2, 95%CI: 0.07-0.59, P = 0.003)[63]. All other subgroups had no benefit from the addition of oxaliplatin to leucovorin and fluorouracil. These findings somewhat support the subtyping results of the CALGB/SWOG 80405 study (benefit from the addition of cetuximab in the canonical-CMS2 subtype). However, to ensure integrity of the study, they should have considered including a group of patients treated with cetuximab, leucovorin and fluorouracil (without oxaliplatin).

The ATITG MAX study compared the efficacy of first-line therapy with capecitabine, capecitabine with bevacizumab, and capecitabine with bevacizumab and mitomycin C. They also published results of subtyping according to the CMS classification. Subtype distribution among all patients did not differ significantly from other studies and represented 18% for CMS1, 47% for CMS2, 12% for CMS3, and 23% for CMS4 subtype. Prognostic significance of subtypes also were consistent with the original work of Guinney *et al*[59]. The researchers found that adding bevacizumab to CT significantly increased progression-free survival in CMS1 (RR = 0.83, 95%CI: 0.43-1.62), CMS2 (RR = 0.5, 95%CI: 0.33-0.76), and CMS3 (RR = 0.31, 95%CI: 0.13-0.75) but not in the CMS4 subtype (RR = 1.24, 95%CI: 0.68-2.25)[64].

Therefore, we could conclude that the FIRE3 results might indicate low efficacy of bevacizumab for the CMS4 subtype rather than high efficacy of cetuximab. Although angiogenesis prevails in the CMS4 subtype, it is likely primarily not due to the VEGF-mediated pathway but due to the TGF-β signaling pathway. This angiogenesis type is characterized with co-optation of vessels and vascular mimicry and usually prevails with the mesenchymal component[65,66,67], which explains the ineffectiveness of bevacizumab[68].

A phase III PETACC-8 study confirmed that addition of cetuximab to adjuvant FOLFOX regimen in stage III disease had no benefit. Subtyping with CMS classification revealed 17% of CMS1 tumors, 34% of CMS2 tumors, 4% of CMS3, and 45% CMS4 subtype. The study confirmed poor prognosis of the CMS4 subtype (RR = 1.7, P = 0.021) and revealed that addition of cetuximab worsened patient survival for the CMS1 subtype (P = 0.037)[74]. We could explain these results with fibroblast enrichment of the CMS1 subtype tumors, which decrease cetuximab activity due to the secretion of IL-16A and TGF-β[69, 70]. These results might indicate low efficacy of cetuximab for the CMS1 subtype or bevacizumab high efficacy in the CALGB/SWOG 80405 study. On the other hand, the CMS1 subtype is usually observed in right-sided tumors, which usually have *BRAF* gene mutations[61]. The addition of anti-angiogenic drugs in the first-line or second-line therapy significantly increases the survival of patients with *BRAF* mutations[57,70].

However, subtype heterogeneity for each specific patient and evolution of gene expression upon progression on a particular regimen could result in prevalence of a certain subtype. Thus, we know that progression on oxaliplatin increases epithelial-mesenchymal transition gene expression, which is associated with fibroblast activity of the tumor. These changes determine resistance to anti-EGFR agents and might explain low efficacy of cetuximab in the second-line setting after progression on FOLFOX with bevacizumab[71].

Several groups studied subtype heterogeneity. Piskol *et al*[72] studied samples of 182 primary tumors and 130 metastases and found that the CMS2 and CMS4 subtypes are usually coincidental. However, the CMS1 subtype was somewhat more common for metastases (16.90% *vs* 9.34%), and the CMS3 subtype was less common for metastases (< 1% *vs* 11% in primary tumors). The CMS1 subtype was more typical for metastases in the liver and lungs and the CMS4 subtype for other localizations. This may indicate some tropism of subtypes to metastasize to certain organs. At the same time, the expression data for the CMS2 and CMS4 subtypes were rather consistent between primary tumor and metastases. To study concordance, 71 patient samples were taken. The concordance of the CMS subtypes between primary tumor and metastases was only 60%, primarily due to the transition of the CMS2 and CMS4 subtypes and changes in the expression of epithelial-mesenchymal transition genes. This indicates that results of subtype analysis of the primary tumor could be incorrect in metastatic disease. The researchers found drift and clone selection. In cases with discordance, it was more difficult to identify the subtype. Nevertheless, the authors concluded that specific features of a particular tumor like expression of tumor-specific genes did not change due to disease progression, but the microenvironment did. This might lead to the accuracy of CMS subtype classification[73]. However, Piskol *et al*[72] used a low-cost variant of genetic analysis with tumor material isolated from paraffin blocks, and the original study of Guinney *et al*[59] used fresh frozen samples, but their results were consistent with 85%.

Dunne *et al*[73] examined expression data at different sites of the primary tumor. In particular, when comparing the central part and invasive tumor front, concordance of CMS subtypes was only 38%. The concordance between the central part and lymph node metastases was 29%. Concordance between the invasive front and lymph node metastases was 21%. Concordance between all three zones was 17%. This is likely due to the CMS4 phenotype acquiring in the invasion front and metastasis area, whereas the CMS2 and CMS3 subtypes prevailed in the central zone of the tumor. As in previous work, only 46% of samples were unambiguously classified according to the CMS subtype. The authors also noted that surrounding tumor stroma significantly affects the expression data and therefore the assignment of the sample to the one subtype or another. This is especially true when studying transcription factors using a small sample of biopsy material[73].

In 2018, Laurent-Puig *et al*[74] presented more extensive data on tumor subtyping from the PETACC8 study patients discussed above. The authors noted that it was possible to identify the subtype unambiguously only in 42.6% of samples, while in 57.4% the tumor was treated as the mixed CMS subtype, combining at least up to 20% of two subgroups. The researchers managed to identify 16 variants of tumors with incidence from 2.1%-18.3% when they divided the mixed subgroups in accordance with combination of the largest and the smallest components. Interestingly, the pure metabolic (CMS3) subtype included only tumors with a mutation in the *KRAS* gene. Mixed tumors with the CMS4 component had the worst prognosis in terms of disease-free survival even taking into account clinical factors[74].

To study microenvironment influence on the therapy effectiveness and formation of certain transcriptome subtypes in colon cancer, Becht *et al*[75] created a CMP algorithm that allows determining tumor sample infiltration with various cells of the microenvironment (fibroblasts, macrophages, endotheliocytes, various subgroups of lymphocytes, *etc*.) *via* expression data. This approach was validated immunohistochemically by calculating the cellular composition of the sample. The authors compared the results with different CMS subtypes of colon cancer.

They found that the immune (CMS1) and mesenchymal (CMS4) subtypes were enriched with CD8 T lymphocytes and CD68 macrophages in contrast to the canonical (CMS2) and metabolic (CMS3) subtypes. The mesenchymal (CMS4) subtype proved to have a high density of tumor-associated fibroblasts, myeloid cells, and endothelial cells, which was confirmed with myeloid chemokine expression (CCL2), complement system components, proangiogenic factors (VEGFA, VEGFB, and PDGFC), and immunosuppression molecules (TGFβ1, TGFß3, and PDGFC).

The CMS1 subtype had a high expression of chemoattractants to T lymphocytes (CXCL9, CXCL10, and CXCL16) or molecules involved in tertiary lymphoid structure formation (CXCL13), increased expression of INFγ and IL-15, and high expression of genes encoding PD-1 ligands. Interestingly, the latter was also found in the mesenchymal subtype. The canonical (CMS2) subtype had low presentation of class 1 major histocompatibility complex proteins, and the tumor infiltration by lymphocytes was also low.

There is a strong positive correlation between the number of fibroblasts and myeloid and endothelial cells (in accordance with the CMP algorithm), but there is no such correlation between the fibroblasts and cytotoxic cells. The authors concluded that fibroblasts in the mesenchymal subtype promote angiogenesis, recruitment of proinflammatory cells, and the formation of the immunosuppressive phenotype[76].

Combining data on the tumor expression subtype with characteristics of the microenvironment allowed us to choose investigational therapeutic options. In particular, the combination of antiangiogenic drugs with immune checkpoint inhibitors or with inhibitors of proteins involved in the interaction of cells with extracellular matrix components could be beneficial in the mesenchymal subtype[77]. However, the combination of immune checkpoint inhibitors and bevacizumab failed in metastatic colon cancer without mismatch repair deficiency[78]. Similarly, bevacizumab had no benefit for the CMS4 subtype, although it expressed proangiogenic factors and endothelial cells *via* the CMS algorithm. We could possibly explain this with the fact that this subtype expresses TGF-β, which induces alternative pathways of angiogenesis. Also, tumors resistant to bevacizumab often have an increased expression of TGF-β[79]. We suggest the use of TGF-β inhibitors and immune checkpoint inhibitors in the CMS4 subtype.

The metabolic (CMS3) and canonical (CMS2) subtypes have low tumor lymphocyte infiltration and low expression of type I major histocompatibility complex components, which induces an unsatisfactory antigen-presenting function. Artificial saturation of tumors with lymphocytes in such situations could serve as a possible solution [for example, the use of bispecific antibodies to tumor antigens and lymphocytes (cibisatamab)][80]. Cibisatamab in combination with atezolizumab in a phase I study in patients with metastatic chemorefractory cancer and elevated CEA demonstrated 60% of metabolic responses with positron emission tomography/computed tomography[80]. However, due to heterogeneity and volatility of CEA expression in colon cancer cells, this approach might not prove to be beneficial[81].

Cells expressing CEA, as well as the canonical (CMS2) subtype, have increased activity of the WNT/β-catenin signaling pathway. This raises the rationale of studying inhibitors of this pathway in the corresponding subtype of colon cancer. Such inhibitors, BBI608 (napabucasin) and CGX1321 in combination with pembrolizumab, are thought to be therapy options. CGX1321 studies are still going, but napabucasin has already been studied in the phase II trial in accordance with the subtypes of colon cancer. The efficacy in patients with chemorefractory colon cancer and MSI-H was 50%, and in patients without mismatch repair deficiency it was 10%. In the CMS1 subtype, the objective response was in 1 out of 3 patients. In the CMS2 subtype, the objective response was in 0 out of 6 patients. In the CMS3 subtype, the objective response was in 1 (with polymerase E gene mutation) out of 4 patients. In the CMS4 subtype, the objective response was in 2 out of 6 patients. Therefore, the hypothesis that WNT inhibitors in combination with immune checkpoint inhibitors would be effective in the canonical and metabolic subtypes was not true. At the same time, the efficacy in the CMS4 subtype seemed to be encouraging.

However, the current state of colon cancer subtyping does not seem to be applicable in clinical practice. Nevertheless, CMS subtype classification could serve as a biological basis for searching for new targets. We believe that this approach for selecting targeted therapy should replace dividing patients by the primary tumor localization in the right or left half side of the colon. This seems to become a transitional stage towards real personalized therapy. In order to facilitate implementation of a classification system into clinical practice, further research should be focused on the development of feasible technology of subtyping based on conventional methods used in routine laboratory practice (immunohistochemistry or real time-PCR for example). Surrogate markers of each subtype should be described. Then, it would be possible to develop small panels, which would replace whole-exome or whole-transcriptome analysis.

CONCLUSION

In 2018, TCGA performed a comprehensive full-scale molecular genetic analysis of adenocarcinomas of the esophagus, stomach, and colon concerning their common endodermal origin. This analysis allowed subtyping of gastrointestinal adenocarcinomas and identified five distinct molecular subtypes: (1) EBV-associated GC (EBV+); (2) GC with MSI; (3) GC with CIN; (4) GS GC (GS); and (5) Hypermutated GC with single-nucleotide variants[34]. They initially determined EBV status in the tumor samples of gastrointestinal adenocarcinomas, then they divided EBV-negative gastrointestinal tumors into two groups according to the mutation load: adenocarcinomas with a high mutation load and gastrointestinal adenocarcinomas with a low mutation load. Adenocarcinomas with a high mutational load (hypermutated > 10 mutations per million nucleotides) were further classified into MSI and single nucleotide variant subtypes. They assigned hypermutated tumors with an indel mutation density > 1 mutation per million nucleotides and an indel/single nucleotide variant ratio > 1/150 to the MSI phenotype, while the remaining gastrointestinal adenocarcinomas were assigned to the single nucleotide variant subtype. Adenocarcinomas with low mutational density, in their turn, were divided into two groups depending on somatic copy number alteration presence or absence: tumors with CIN and a GS subtype. The identification of combined molecular and genetic subtypes gave us insights to understanding gastrointestinal adenocarcinomas biology, determining aims for future clinical research, and helping to simplify the implementation of a unified system for subtyping gastrointestinal adenocarcinomas.

However, to date, this approach has a number of limitations. One of these limitations is the significant heterogeneity between the primary tumor and distant metastases as well as evolution in the molecular and genetic properties of the tumor during treatment. One of the decisions may be the possibility of performing subtyping by liquid biopsy, though surrogate markers should be identified, which can be detected using such biological samples.

Full-scale molecular genetic analysis in most of the presented works used fresh frozen tumor tissue samples. The use of such tumor material and its storage is not routine in everyday clinical practice. Research is underway on the use of paraffinized tumor material for molecular typing, in particular for transcriptome analysis.

Another limitation for use of this classification in clinical practice is the significant volume of testing. Identification of surrogate biomarkers of molecular genetic subtypes or creation of small panels to determine these subtypes would accelerate clinical validation and its application in routine practice. Integration of subtyping into clinical studies as stratification factors is promising to assessing their clinical significance. Thus, we are still on the way to achieving successful application of the molecular genetic typing in routine clinical practice.

FOOTNOTES

Author contributions: Ignatova EO and Menshikova S drafted the manuscript; Kozlov E, Mileyko V, Ivanov M, and Sun H performed the literature search; Tryakin A, Fedyanin M and Ivan Stilidi critically reviewed the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

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S-Editor: Wu YXJ
L-Editor: Filipodia
P-Editor: Wu YXJ

REFERENCES

1 **Ferlay J**, Laversanne M, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2020). Global Cancer Observatory: Cancer Tomorrow. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr [DOI: 10.37469/0507-3758-2021-67-6-855]
2 Gonzalez RS. WHO classification. PathologyOutlines.com website. [DOI: 10.1089/glre.2016.201011]
3 **Abdel-Latif MM**, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. *Curr Opin Pharmacol* 2009; **9**: 396-404 [PMID: 19596608 DOI: 10.1016/j.coph.2009.06.010]
4 **Van Hoeck A**, Tjoonk NH, van Boxtel R, Cuppen E. Portrait of a cancer: mutational signature analyses for cancer diagnostics. *BMC Cancer* 2019; **19**: 457 [PMID: 31092228 DOI: 10.1186/s12885-019-5677-2]
5 **Smyth EC**, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P, Cunningham D. Oesophageal cancer. *Nat Rev Dis Primers* 2017; **3**: 17048 [PMID: 28748917 DOI: 10.1038/nrdp.2017.48]
6 **Kauppila JH**, Mattsson F, Brusselaers N, Lagergren J. Prognosis of oesophageal adenocarcinoma and squamous cell carcinoma following surgery and no surgery in a nationwide Swedish cohort study. *BMJ Open* 2018; **8**: e021495 [PMID: 29748347 DOI: 10.1136/bmjopen-2018-021495]
7 **Dulak AM**, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhim R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G, Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet* 2013; **45**: 478-486 [PMID: 23525077 DOI: 10.1038/ng.2591]
8 **Secrier M**, Li X, de Silva N, Eldridge MD, Contino G, Bornschein J, MacRae S, Grehan N, O'Donovan M, Miremadi A, Yang TP, Bower L, Chettouh H, Crawte J, Galeano-Dalmau N, Grabowska A, Saunders J, Underwood T, Waddell N, Barbour AP, Nutzinger B, Achilleos A, Edwards PA, Lynch AG, Tavaré S, Fitzgerald RC; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium. Mutational signatures in esophageal adenocarcinoma define

etiologically distinct subgroups with therapeutic relevance. *Nat Genet* 2016; **48**: 1131-1141 [PMID: 27595477 DOI: 10.1038/ng.3659]

9 **Stewart A**, Thavasu P, de Bono JS, Banerji U. Titration of signalling output: insights into clinical combinations of MEK and AKT inhibitors. *Ann Oncol* 2015; **26**: 1504-1510 [PMID: 25908604 DOI: 10.1093/annonc/mdv188]

10 **Cancer Genome Atlas Research Network**. Analysis Working Group: Asan University; BC Cancer Agency; Brigham and Women's Hospital; Broad Institute; Brown University; Case Western Reserve University; Dana-Farber Cancer Institute; Duke University; Greater Poland Cancer Centre; Harvard Medical School; Institute for Systems Biology; KU Leuven; Mayo Clinic; Memorial Sloan Kettering Cancer Center; National Cancer Institute; Nationwide Children's Hospital; Stanford University; University of Alabama; University of Michigan; University of North Carolina; University of Pittsburgh; University of Rochester; University of Southern California; University of Texas MD Anderson Cancer Center; University of Washington; Van Andel Research Institute; Vanderbilt University; Washington University; Genome Sequencing Center: Broad Institute; Washington University in St. Louis; Genome Characterization Centers: BC Cancer Agency; Broad Institute; Harvard Medical School; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; University of North Carolina; University of Southern California Epigenome Center; University of Texas MD Anderson Cancer Center; Van Andel Research Institute; Genome Data Analysis Centers: Broad Institute; Brown University:; Harvard Medical School; Institute for Systems Biology; Memorial Sloan Kettering Cancer Center; University of California Santa Cruz; University of Texas MD Anderson Cancer Center; Biospecimen Core Resource: International Genomics Consortium; Research Institute at Nationwide Children's Hospital; Tissue Source Sites: Analytic Biologic Services; Asan Medical Center; Asterand Bioscience; Barretos Cancer Hospital; BioreclamationIVT; Botkin Municipal Clinic; Chonnam National University Medical School; Christiana Care Health System; Cureline; Duke University; Emory University; Erasmus University; Indiana University School of Medicine; Institute of Oncology of Moldova; International Genomics Consortium; Invidumed; Israelitisches Krankenhaus Hamburg; Keimyung University School of Medicine; Memorial Sloan Kettering Cancer Center; National Cancer Center Goyang; Ontario Tumour Bank; Peter MacCallum Cancer Centre; Pusan National University Medical School; Ribeirão Preto Medical School; St. Joseph's Hospital &Medical Center; St. Petersburg Academic University; Tayside Tissue Bank; University of Dundee; University of Kansas Medical Center; University of Michigan; University of North Carolina at Chapel Hill; University of Pittsburgh School of Medicine; University of Texas MD Anderson Cancer Center; Disease Working Group: Duke University; Memorial Sloan Kettering Cancer Center; National Cancer Institute; University of Texas MD Anderson Cancer Center; Yonsei University College of Medicine; Data Coordination Center: CSRA Inc; Project Team: National Institutes of Health. Integrated genomic characterization of oesophageal carcinoma. *Nature* 2017; **541**: 169-175 [DOI: 10.17980/2016.252]

11 **Beroukhim R**, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, Cho YJ, Haery L, Greulich H, Reich M, Winckler W, Lawrence MS, Weir BA, Tanaka KE, Chiang DY, Bass AJ, Loo A, Hoffman C, Prensner J, Liefeld T, Gao Q, Yecies D, Signoretti S, Maher E, Kaye FJ, Sasaki H, Tepper JE, Fletcher JA, Tabernero J, Baselga J, Tsao MS, Demichelis F, Rubin MA, Janne PA, Daly MJ, Nucera C, Levine RL, Ebert BL, Gabriel S, Rustgi AK, Antonescu CR, Ladanyi M, Letai A, Garraway LA, Loda M, Beer DG, True LD, Okamoto A, Pomeroy SL, Singer S, Golub TR, Lander ES, Getz G, Sellers WR, Meyerson M. The landscape of somatic copy-number alteration across human cancers. *Nature* 2010; **463**: 899-905 [PMID: 20164920 DOI: 10.1038/nature08822]

12 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

13 **Guo X**, Tang Y, Zhu W. Distinct esophageal adenocarcinoma molecular subtype has subtype-specific gene expression and mutation patterns. *BMC Genomics* 2018; **19**: 769 [PMID: 30355311 DOI: 10.1186/s12864-018-5165-0]

14 **Ross-Innes CS**, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M, Malhotra S, di Pietro M, Ivakhno S, He M, Weaver JMJ, Lynch AG, Kingsbury Z, Ross M, Humphray S, Bentley D, Fitzgerald RC. Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. *Nat Genet* 2015; **47**: 1038-1046 [PMID: 26192915 DOI: 10.1038/ng.3357]

15 **Caspa Gokulan R**, Garcia-Buitrago MT, Zaika AI. From genetics to signaling pathways: molecular pathogenesis of esophageal adenocarcinoma. *Biochim Biophys Acta Rev Cancer* 2019; **1872**: 37-48 [PMID: 31152823 DOI: 10.1016/j.bbcan.2019.05.003]

16 **Islam F**, Tang JC, Gopalan V, Lam AK. Epigenetics: DNA Methylation Analysis in Esophageal Adenocarcinoma. *Methods Mol Biol* 2018; **1756**: 247-256 [PMID: 29600375 DOI: 10.1007/978-1-4939-7734-5_21]

17 **Jammula S**, Katz-Summercorn AC, Li X, Linossi C, Smyth E, Killcoyne S, Biasci D, Subash VV, Abbas S, Blasko A, Devonshire G, Grantham A, Wronowski F, O'Donovan M, Grehan N, Eldridge MD, Tavaré S; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) consortium, Fitzgerald RC. Identification of Subtypes of Barrett's Esophagus and Esophageal Adenocarcinoma Based on DNA Methylation Profiles and Integration of Transcriptome and Genome Data. *Gastroenterology* 2020; **158**: 1682-1697.e1 [PMID: 32032585 DOI: 10.1053/j.gastro.2020.01.044]

18 **Hegi ME**, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352**: 997-1003 [PMID: 15758010 DOI: 10.1056/NEJMoa043331]

19 **Li X**, Francies HE, Secrier M, Perner J, Miremadi A, Galeano-Dalmau N, Barendt WJ, Letchford L, Leyden GM, Goffin EK, Barthorpe A, Lightfoot H, Chen E, Gilbert J, Noorani A, Devonshire G, Bower L, Grantham A, MacRae S, Grehan N, Wedge DC, Fitzgerald RC, Garnett MJ. Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. *Nat Commun* 2018; **9**: 2983 [PMID: 30061675 DOI: 10.1038/s41467-018-05190-9]

20 **Yun T**, Liu Y, Gao D, Linghu E, Brock MV, Yin D, Zhan Q, Herman JG, Guo M. Methylation of CHFR sensitizes esophageal squamous cell cancer to docetaxel and paclitaxel. *Genes Cancer* 2015; **6**: 38-48 [PMID: 25821560 DOI: 10.18632/genesandcancer.46]

21 **Moehler M**, Shitara K, Garrido M, et al: Nivolumab plus chemotherapy vs chemo as first-line treatment for advanced gastric cancer/gastroesophageal junction cancer/esophageal adenocarcinoma: First results of the CheckMate 649 study.

ESMO Virtual Congress 2020. Abstract LBA6_PR. Presented September 21, 2020 [DOI: 10.1016/j.annonc.2020.08.2296]

22 **Lauwers GY**, Carneiro F, Graham DY. Gastric carcinoma. In: Bowman FT, Carneiro F, Hruban RH, eds. Classification of Tumours of the Digestive System. Lyon:IARC; 2010

23 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]

24 **Messager M**, Lefevre JH, Pichot-Delahaye V, Souadka A, Piessen G, Mariette C; FREGAT working group - FRENCH. The impact of perioperative chemotherapy on survival in patients with gastric signet ring cell adenocarcinoma: a multicenter comparative study. *Ann Surg* 2011; **254**: 684-93; discussion 693 [PMID: 22005144 DOI: 10.1097/SLA.0b013e3182352647]

25 **Sasako M**, Sakuramoto S, Katai H, Kinoshita T, Furukawa H, Yamaguchi T, Nashimoto A, Fujii M, Nakajima T, Ohashi Y. Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. *J Clin Oncol* 2011; **29**: 4387-4393 [PMID: 22010012 DOI: 10.1200/JCO.2011.36.5908]

26 **Noh SH**, Park SR, Yang HK, Chung HC, Chung IJ, Kim SW, Kim HH, Choi JH, Kim HK, Yu W, Lee JI, Shin DB, Ji J, Chen JS, Lim Y, Ha S, Bang YJ; CLASSIC trial investigators. Adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): 5-year follow-up of an open-label, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1389-1396 [PMID: 25439693 DOI: 10.1016/S1470-2045(14)70473-5]

27 **Kozak KR**, Moody JS. The survival impact of the intergroup 0116 trial on patients with gastric cancer. *Int J Radiat Oncol Biol Phys* 2008; **72**: 517-521 [PMID: 18249500 DOI: 10.1016/j.ijrobp.2007.12.029]

28 **Ychou M**, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, Ducourtieux M, Bedenne L, Fabre JM, Saint-Aubert B, Genève J, Lasser P, Rougier P. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. *J Clin Oncol* 2011; **29**: 1715-1721 [PMID: 21444866 DOI: 10.1200/JCO.2010.33.0597]

29 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992 DOI: 10.1056/NEJMoa055531]

30 **Al-Batran SE**, Homann N, Pauligk C, Goetze TO, Meiler J, Kasper S, Kopp HG, Mayer F, Haag GM, Luley K, Lindig U, Schmiegel W, Pohl M, Stoehlmacher J, Folprecht G, Probst S, Prasnikar N, Fischbach W, Mahlberg R, Trojan J, Koenigsmann M, Martens UM, Thuss-Patience P, Egger M, Block A, Heinemann V, Illerhaus G, Moehler M, Schenk M, Kullmann F, Behringer DM, Heike M, Pink D, Teschendorf C, Löhr C, Bernhard H, Schuch G, Rethwisch V, von Weikersthal LF, Hartmann JT, Kneba M, Daum S, Schulmann K, Weniger J, Belle S, Gaiser T, Oduncu FS, Güntner M, Hozaeel W, Reichart A, Jäger E, Kraus T, Mönig S, Bechstein WO, Schuler M, Schmalenberg H, Hofheinz RD; FLOT4-AIO Investigators. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. *Lancet* 2019; **393**: 1948-1957 [PMID: 30982686 DOI: 10.1016/S0140-6736(18)32557-1]

31 **Lutz MP**, Zalcberg JR, Ducreux M, Adenis A, Allum W, Aust D, Carneiro F, Grabsch HI, Laurent-Puig P, Lordick F, Möhler M, Mönig S, Obermannova R, Piessen G, Riddell A, Röcken C, Roviello F, Schneider PM, Seewald S, Smyth E, van Cutsem E, Verheij M, Wagner AD, Otto F. The 4th St. Gallen EORTC Gastrointestinal Cancer Conference: Controversial issues in the multimodal primary treatment of gastric, junctional and oesophageal adenocarcinoma. *Eur J Cancer* 2019; **112**: 1-8 [PMID: 30878666 DOI: 10.1016/j.ejca.2019.01.106]

32 **Tan IB**, Ivanova T, Lim KH, Ong CW, Deng N, Lee J, Tan SH, Wu J, Lee MH, Ooi CH, Rha SY, Wong WK, Boussioutas A, Yeoh KG, So J, Yong WP, Tsuburaya A, Grabsch H, Toh HC, Rozen S, Cheong JH, Noh SH, Wan WK, Ajani JA, Lee JS, Tellez MS, Tan P. Intrinsic subtypes of gastric cancer, based on gene expression pattern, predict survival and respond differently to chemotherapy. *Gastroenterology* 2011; **141**: 476-485, 485.e1 [PMID: 21684283 DOI: 10.1053/j.gastro.2011.04.042]

33 **Lei Z**, Tan IB, Das K, Deng N, Zouridis H, Pattison S, Chua C, Feng Z, Guan YK, Ooi CH, Ivanova T, Zhang S, Lee M, Wu J, Ngo A, Manesh S, Tan E, Teh BT, So JB, Goh LK, Boussioutas A, Lim TK, Flotow H, Tan P, Rozen SG. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. *Gastroenterology* 2013; **145**: 554-565 [PMID: 23684942 DOI: 10.1053/j.gastro.2013.05.010]

34 **Liu Y**, Sethi NS, Hinoue T, Schneider BG, Cherniack AD, Sanchez-Vega F, Seoane JA, Farshidfar F, Bowlby R, Islam M, Kim J, Chatila W, Akbani R, Kanchi RS, Rabkin CS, Willis JE, Wang KK, McCall SJ, Mishra L, Ojesina AI, Bullman S, Pedamallu CS, Lazar AJ, Sakai R; Cancer Genome Atlas Research Network, Thorsson V, Bass AJ, Laird PW. Comparative Molecular Analysis of Gastrointestinal Adenocarcinomas. *Cancer Cell* 2018; **33**: 721-735.e8 [PMID: 29622466 DOI: 10.1016/j.ccell.2018.03.010]

35 **Samuels Y**, Waldman T. Oncogenic mutations of PIK3CA in human cancers. *Curr Top Microbiol Immunol* 2010; **347**: 21-41 [PMID: 20535651 DOI: 10.1007/82_2010_68]

36 **Rennekamp AJ**, Lieberman PM. Initiation of Epstein-Barr virus lytic replication requires transcription and the formation of a stable RNA-DNA hybrid molecule at OriLyt. *J Virol* 2011; **85**: 2837-2850 [PMID: 21191028 DOI: 10.1128/JVI.02175-10]

37 **Hoadley KA**, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, Shen R, Taylor AM, Cherniack AD, Thorsson V, Akbani R, Bowlby R, Wong CK, Wiznerowicz M, Sanchez-Vega F, Robertson AG, Schneider BG, Lawrence MS, Noushmehr H, Malta TM; Cancer Genome Atlas Network, Stuart JM, Benz CC, Laird PW. Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* 2018; **173**: 291-304.e6 [PMID: 29625048 DOI: 10.1016/j.cell.2018.03.022]

38 **Pereira MA**, Ramos MFKP, Faraj SF, Dias AR, Yagi OK, Zilberstein B, Cecconello I, Alves VAF, de Mello ES, Ribeiro U Jr. Clinicopathological and prognostic features of Epstein-Barr virus infection, microsatellite instability, and PD-L1 expression in gastric cancer. *J Surg Oncol* 2018; **117**: 829-839 [PMID: 29534305 DOI: 10.1002/jso.25022]

39 **Kim ST**, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, Park YS, Lim HY, Lee H, Choi M, Talasaz A, Kang PS, Cheng J, Loboda A, Lee J, Kang WK. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018; **24**: 1449-1458 [PMID: 30013197 DOI: 10.1038/s41591-018-0101-z]

40 **Polom K**, Marano L, Marrelli D, De Luca R, Roviello G, Savelli V, Tan P, Roviello F. Meta-analysis of microsatellite instability in relation to clinicopathological characteristics and overall survival in gastric cancer. *Br J Surg* 2018; **105**: 159-167 [PMID: 29091259 DOI: 10.1002/bjs.10663]

41 **Sunakawa Y**, Lenz HJ. Molecular classification of gastric adenocarcinoma: translating new insights from the cancer genome atlas research network. *Curr Treat Options Oncol* 2015; **16**: 17 [PMID: 25813036 DOI: 10.1007/s11864-015-0331-y]

42 **Kim SY**, Choi YY, An JY, Shin HB, Jo A, Choi H, Seo SH, Bang HJ, Cheong JH, Hyung WJ, Noh SH. The benefit of microsatellite instability is attenuated by chemotherapy in stage II and stage III gastric cancer: Results from a large cohort with subgroup analyses. *Int J Cancer* 2015; **137**: 819-825 [PMID: 25614197 DOI: 10.1002/ijc.29449]

43 **Smyth EC**, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, Fassan M, Rugge M, Valeri N, Okines A, Hewish M, Allum W, Stenning S, Nankivell M, Langley R, Cunningham D. Mismatch Repair Deficiency, Microsatellite Instability, and Survival: An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial. *JAMA Oncol* 2017; **3**: 1197-1203 [PMID: 28241187 DOI: 10.1001/jamaoncol.2016.6762]

44 **Choi YY**, Kim H, Shin SJ, Kim HY, Lee J, Yang HK, Kim WH, Kim YW, Kook MC, Park YK, Kim HH, Lee HS, Lee KH, Gu MJ, Choi SH, Hong S, Kim JW, Hyung WJ, Noh SH, Cheong JH. Microsatellite Instability and Programmed Cell Death-Ligand 1 Expression in Stage II/III Gastric Cancer: Post Hoc Analysis of the CLASSIC Randomized Controlled study. *Ann Surg* 2019; **270**: 309-316 [PMID: 29727332 DOI: 10.1097/SLA.0000000000002803]

45 **Pietrantonio F**, Raimondi A, Choi Y, Kang W, Langley RE, Kim Y, Kim K, Nankivell M, Perrone F, Kook M, Yeong J An, Grabsch H, Morano F, Cheong J, Noh S, Lee J, Cunningham D, Bartolomeo M, Fucà G, Smyth E. MSI-GC-01: Individual patient data (IPD) meta-analysis of microsatellite instability (MSI) and gastric cancer (GC) from four randomized clinical trials (RCTs). *J Clini Oncol* **37**: 66 [DOI: 10.1200/jco.2019.37.4_suppl.66]

46 National Comprehensive Cancer Network. Gastric cancer (Version 1.2021). Available from: https://www.nccn.org/professionals/physician_gls/pdf/gastric_blocks.pdf [DOI: 10.6004/jnccn.2022.202glup]

47 **Ling Y**, Watanabe Y, Nagahashi M, Shimada Y, Ichikawa H, Wakai T, Okuda S. Genetic profiling for diffuse type and genomically stable subtypes in gastric cancer. *Comput Struct Biotechnol J* 2020; **18**: 3301-3308 [PMID: 33240471 DOI: 10.1016/j.csbj.2020.10.021]

48 **Garattini SK**, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, Negri FV, Berenato R, Ermacora P, Cardellino GG, Giovannoni M, Pella N, Scartozzi M, Antonuzzo L, Silvestris N, Fasola G, Aprile G. Molecular classifications of gastric cancers: Novel insights and possible future applications. *World J Gastrointest Oncol* 2017; **9**: 194-208 [PMID: 28567184 DOI: 10.4251/wjgo.v9.i5.194]

49 **Lordick F**, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M; Arbeitsgemeinschaft Internistische Onkologie and EXPAND Investigators. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 490-499 [PMID: 23594786 DOI: 10.1016/S1470-2045(13)70102-5]

50 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787 DOI: 10.1016/S1470-2045(13)70096-2]

51 **Shah MA**, Bang YJ, Lordick F, Alsina M, Chen M, Hack SP, Bruey JM, Smith D, McCaffery I, Shames DS, Phan S, Cunningham D. Effect of Fluorouracil, Leucovorin, and Oxaliplatin With or Without Onartuzumab in HER2-Negative, MET-Positive Gastroesophageal Adenocarcinoma: The METGastric Randomized Clinical Trial. *JAMA Oncol* 2017; **3**: 620-627 [PMID: 27918764 DOI: 10.1001/jamaoncol.2016.5580]

52 **Van Cutsem E**, Bang YJ, Mansoor W, Petty RD, Chao Y, Cunningham D, Ferry DR, Smith NR, Frewer P, Ratnayake J, Stockman PK, Kilgour E, Landers D. A randomized, open-label study of the efficacy and safety of AZD4547 monotherapy versus paclitaxel for the treatment of advanced gastric adenocarcinoma with FGFR2 polysomy or gene amplification. *Ann Oncol* 2017; **28**: 1316-1324 [PMID: 29177434 DOI: 10.1093/annonc/mdx107]

53 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

54 **Sohn BH**, Hwang JE, Jang HJ, Lee HS, Oh SC, Shim JJ, Lee KW, Kim EH, Yim SY, Lee SH, Cheong JH, Jeong W, Cho JY, Kim J, Chae J, Lee J, Kang WK, Kim S, Noh SH, Ajani JA, Lee JS. Clinical Significance of Four Molecular Subtypes of Gastric Cancer Identified by The Cancer Genome Atlas Project. *Clin Cancer Res* 2017; **23**: 4441-4449 [PMID: 28747339 DOI: 10.1158/1078-0432.CCR-16-2211]

55 **Cristescu R**, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 2015; **21**: 449-456 [PMID: 25894828 DOI: 10.1038/nm.3850]

56 **Fedyanin MY**, Gladkov OA, Gordeev SS, Rykov IV, Tryakin AA. Practical guidelines for drug treatment of colon and rectosigmoid junction cancer. Malignant tumors: Practical Recommendations of RUSSCO 3s2. 2020; 10 [DOI: 10.31917/1802161]

57 National Comprehensive Cancer Network. Colon cancer (Version 2. 2021). Available from: https://www.nccn.org/professionals/physician_gls/pdf/gastric_blocks.pdf [DOI: 10.6004/jnccn.2021.5025]

58 **Dienstmann R**, Salazar R, Tabernero J. Molecular Subtypes and the Evolution of Treatment Decisions in Metastatic Colorectal Cancer. *Am Soc Clin Oncol Educ Book* 2018; **38**: 231-238 [PMID: 30231342 DOI: 10.1200/EDBK_200929]

59 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

60 **Stintzing S**, Wirapati P, Lenz HJ, Neureiter D, Fischer von Weikersthal L, Decker T, Kiani A, Kaiser F, Al-Batran S, Heintges T, Lerchenmüller C, Kahl C, Seipelt G, Kullmann F, Moehler M, Scheithauer W, Held S, Modest DP, Jung A, Kirchner T, Aderka D, Tejpar S, Heinemann V. Consensus molecular subgroups (CMS) of colorectal cancer (CRC) and first-line efficacy of FOLFIRI plus cetuximab or bevacizumab in the FIRE3 (AIO KRK-0306) trial. *Ann Oncol* 2019; **30**: 1796-1803 [PMID: 31868905 DOI: 10.1093/annonc/mdz387]

61 **Lenz HJ**, Ou FS, Venook AP, Hochster HS, Niedzwiecki D, Goldberg RM, Mayer RJ, Bertagnolli MM, Blanke CD, Zemla T, Qu X, Wirapati P, Tejpar S, Innocenti F, Kabbarah O. Impact of Consensus Molecular Subtype on Survival in Patients With Metastatic Colorectal Cancer: Results From CALGB/SWOG 80405 (Alliance). *J Clin Oncol* 2019; **37**: 1876-1885 [PMID: 31042420 DOI: 10.1200/JCO.18.02258]

62 **Peterson TE**, Kirkpatrick ND, Huang Y, Farrar CT, Marijt KA, Kloepper J, Datta M, Amoozgar Z, Seano G, Jung K, Kamoun WS, Vardam T, Snuderl M, Goveia J, Chatterjee S, Batista A, Muzikansky A, Leow CC, Xu L, Batchelor TT, Duda DG, Fukumura D, Jain RK. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci U S A* 2016; **113**: 4470-4475 [PMID: 27044097 DOI: 10.1073/pnas.1525349113]

63 **Song N**, Pogue-Geile KL, Gavin PG, Yothers G, Kim SR, Johnson NL, Lipchik C, Allegra CJ, Petrelli NJ, O'Connell MJ, Wolmark N, Paik S. Clinical Outcome From Oxaliplatin Treatment in Stage II/III Colon Cancer According to Intrinsic Subtypes: Secondary Analysis of NSABP C-07/NRG Oncology Randomized Clinical Trial. *JAMA Oncol* 2016; **2**: 1162-1169 [PMID: 27270348 DOI: 10.1001/jamaoncol.2016.2314]

64 **Mooi JK**, Wirapati P, Asher R, Lee CK, Savas P, Price TJ, Townsend A, Hardingham J, Buchanan D, Williams D, Tejpar S, Mariadason JM, Tebbutt NC. The prognostic impact of consensus molecular subtypes (CMS) and its predictive effects for bevacizumab benefit in metastatic colorectal cancer: molecular analysis of the AGITG MAX clinical trial. *Ann Oncol* 2018; **29**: 2240-2246 [PMID: 30247524 DOI: 10.1093/annonc/mdy410]

65 **Döme B**, Hendrix MJ, Paku S, Tóvári J, Tímár J. Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. *Am J Pathol* 2007; **170**: 1-15 [PMID: 17200177 DOI: 10.2353/ajpath.2007.060302]

66 **Leenders WP**, Küsters B, de Waal RM. Vessel co-option: how tumors obtain blood supply in the absence of sprouting angiogenesis. *Endothelium* 2002; **9**: 83-87 [PMID: 12200959 DOI: 10.1080/10623320212006]

67 **Donnem T**, Hu J, Ferguson M, Adighibe O, Snell C, Harris AL, Gatter KC, Pezzella F. Vessel co-option in primary human tumors and metastases: an obstacle to effective anti-angiogenic treatment? *Cancer Med* 2013; **2**: 427-436 [PMID: 24156015 DOI: 10.1002/cam4.105]

68 **Frentzas S**, Simoneau E, Bridgeman VL, Vermeulen PB, Foo S, Kostaras E, Nathan M, Wotherspoon A, Gao ZH, Shi Y, Van den Eynden G, Daley F, Peckitt C, Tan X, Salman A, Lazaris A, Gazinska P, Berg TJ, Eltahir Z, Ritsma L, Van Rheenen J, Khashper A, Brown G, Nystrom H, Sund M, Van Laere S, Loyer E, Dirix L, Cunningham D, Metrakos P, Reynolds AR. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. *Nat Med* 2016; **22**: 1294-1302 [PMID: 27748747 DOI: 10.1038/nm.4197]

69 **Colangelo T**, Polcaro G, Muccillo L, D'Agostino G, Rosato V, Ziccardi P, Lupo A, Mazzoccoli G, Sabatino L, Colantuoni V. Friend or foe? *Biochim Biophys Acta Rev Cancer* 2017; **1867**: 1-18 [PMID: 27864070 DOI: 10.1016/j.bbcan.2016.11.001]

70 **Fedyanin MY**, Polyanskaya EM, Elsnukaeva HM, Tryakin AA, Pokataev I. A., Ignatova E.O., Sergeev US, Bulanov AA, Tjulandin SA. Is it necessary to add anti-angiogenic therapy to chemotherapy in patients with metastatic colorectal cancer and BRAF mutation? *Malignant tumours* 2020; **10**: 36-44 [DOI: 10.18027/2224-5057-2020-10-2-3]

71 **Bennouna J**, Hiret S, Bertaut A, Bouché O, Deplanque G, Borel C, François E, Conroy T, Ghiringhelli F, des Guetz G, Seitz JF, Artru P, Hebbar M, Stanbury T, Denis MG, Adenis A, Borg C. Continuation of Bevacizumab vs Cetuximab Plus Chemotherapy After First Progression in KRAS Wild-Type Metastatic Colorectal Cancer: The UNICANCER PRODIGE18 Randomized Clinical Trial. *JAMA Oncol* 2019; **5**: 83-90 [PMID: 30422156 DOI: 10.1001/jamaoncol.2018.4465]

72 **Piskol R**, Huw L, Sergin I, Kljin C, Modrusan Z, Kim D, Kljavin N, Tam R, Patel R, Burton J, Penuel E, Qu X, Koeppen H, Sumiyoshi T, de Sauvage F, Lackner MR, de Sousa E Melo F, Kabbarah O. A Clinically Applicable Gene-Expression Classifier Reveals Intrinsic and Extrinsic Contributions to Consensus Molecular Subtypes in Primary and Metastatic Colon Cancer. *Clin Cancer Res* 2019; **25**: 4431-4442 [PMID: 31004000 DOI: 10.1158/1078-0432.CCR-18-3032]

73 **Dunne PD**, Alderdice M, O'Reilly PG, Roddy AC, McCorry AMB, Richman S, Maughan T, McDade SS, Johnston PG, Longley DB, Kay E, McArt DG, Lawler M. Cancer-cell intrinsic gene expression signatures overcome intratumoural heterogeneity bias in colorectal cancer patient classification. *Nat Commun* 2017; **8**: 15657 [PMID: 28561046 DOI: 10.1038/ncomms15657]

74 **Laurent-Puig P**, Marisa L, Ayadi M, Blum Y, Balogoun R, Pilati C, Le Malico K, Lepage C, Emile JF, Salazar R, Aust D, Duval A, Selves J, Guenot D, Milano G, Seitz JF, Taieb J, Boige V, de Reynie`s A. Colon cancer molecular subtype intratumoral heterogeneity and its prognostic impact: An extensive molecular analysis of the PETACC-8. *Ann Oncol* 2018; **29**: viii18 [DOI: 10.1093/annonc/mdy269.058]

75 **Becht E**, de Reyniès A, Giraldo NA, Pilati C, Buttard B, Lacroix L, Selves J, Sautès-Fridman C, Laurent-Puig P, Fridman WH. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. *Clin Cancer Res* 2016; **22**: 4057-4066 [PMID: 26994146 DOI:

10.1158/1078-0432.CCR-15-2879]

76 **Mantovani A**, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436-444 [PMID: 18650914 DOI: 10.1038/nature07205]

77 **Grothey A**, Tabernero J, Arnold D. Fluoropyrimidine and bevacizumab plus or minus atezolizumab as first-line treatment for BRAF wild type metastatic colorectal cancer: Findings from the MODUL trial of biomarker-driven maintenance. ESMO 2018 Congress. Abstract LBA19 [DOI: 10.1093/annonc/mdy424.020]

78 **Itatani Y**, Kawada K, Yamamoto T, Sakai Y. Resistance to Anti-Angiogenic Therapy in Cancer-Alterations to Anti-VEGF Pathway. *Int J Mol Sci* 2018; **19** [PMID: 29670046 DOI: 10.3390/ijms19041232]

79 **Melisi D**, Hollebecque A, Oh DY, Calvo E, Varghese AM, Borazanci EH, Mercade TM, Simionato F, Park JO, Bendell JC, Faivre SJ, Zhao Y, Gueorguieva I, Man M, Estrem S, Benhadji KA, Lanasa M, Guba SC, Garcia-Carbonero R. A phase Ib dose-escalation and cohort-expansion study of safety and activity of the transforming growth factor (TGF) β receptor I kinase inhibitor galunisertib plus the anti-PD-L1 antibody durvalumab in metastatic pancreatic cancer. *J Clin Oncol* 2019; **37**: 4124 [DOI: 10.1200/jco.2019.37.15_suppl.4124]

80 **Tabernero J**, Melero I, Ros W, Argiles G, Marabelle A, Rodriguez-Ruiz ME, et al Phase Ia and Ib studies of the novel carcinoembryonic antigen (CEA) T-cell bispecific (CEA CD3 TCB) antibody as a single agent and in combination with atezolizumab: preliminary efficacy and safety in patients with metastatic colorectal cancer (mCRC). J Clin Oncol 2017; 35: 3002 [DOI: 10.1200/JCO.2017.35.15_suppl.3002]

81 **Gonzalez-Exposito R**, Semiannikova M, Griffiths B, Khan K, Barber LJ, Woolston A, Spain G, von Loga K, Challoner B, Patel R, Ranes M, Swain A, Thomas J, Bryant A, Saffery C, Fotiadis N, Guettler S, Mansfield D, Melcher A, Powles T, Rao S, Watkins D, Chau I, Matthews N, Wallberg F, Starling N, Cunningham D, Gerlinger M. CEA expression heterogeneity and plasticity confer resistance to the CEA-targeting bispecific immunotherapy antibody cibisatamab (CEA-TCB) in patient-derived colorectal cancer organoids. *J Immunother Cancer* 2019; **7**: 101 [PMID: 30982469 DOI: 10.1186/s40425-019-0575-3]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 646-653

DOI: 10.4251/wjgo.v14.i3.646

ISSN 1948-5204 (online)

MINIREVIEWS

Evolving roles of magnifying endoscopy and endoscopic resection for neoplasia in inflammatory bowel diseases

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: El-Shabrawi MH, Panteris V, Truta B

Received: September 1, 2021
Peer-review started: September 1, 2021
First decision: December 4, 2021
Revised: December 6, 2021
Accepted: February 12, 2022
Article in press: February 12, 2022
Published online: March 15, 2022

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Abstract

Magnifying endoscopy is a useful technique to differentiate neoplasia from non-neoplastic lesions. Data regarding the clinical utility of magnifying endoscopy for neoplasia in patients with inflammatory bowel disease (IBD) has been emerging. While Kudo's pit pattern types III-V are findings suggestive of neoplasia in non-IBD patients, these pit patterns are predictive of IBD-associated neoplasia as well. However, active chronic inflammatory processes, particularly regenerative changes, can mimic neoplastic pit patterns and may affect a meticulous evaluation of pit pattern diagnosis in patients with IBD. The clinical evidence regarding the utility of magnifying endoscopy with narrow band imaging or endocytoscopy has also been evolving in regard to the diagnosis of IBD-associated neoplasia. These advanced endoscopic techniques are promising for multiple reasons; not only for making an accurate diagnosis of neoplasia, but also in determining if endoscopic resection is appropriate for such lesions in patients with IBD. In this review, we discuss the diagnostic accuracy and limitations of magnifying endoscopy in assessing IBD-associated neoplasia and examine the feasibility and outcomes of endoscopic resection for these lesions.

Key Words: Magnifying endoscopy; Neoplasia; Ulcerative colitis; Inflammatory bowel disease; Endoscopic resection

Core Tip: Magnifying colonoscopies assessing Kudo's pit patterns or surface/vascular patterns with narrow band imaging are useful techniques to differentiate neoplasia from non-neoplastic lesions. Many investigations have demonstrated the diagnostic utility of magnifying scopes for neoplasia, as well as the feasibility and outcomes of their endoscopic resection in patients with inflammatory bowel disease. We aim to review updated data regarding these important topics.

Citation: Akiyama S, Sakamoto T, Steinberg JM, Saito Y, Tsuchiya K. Evolving roles of magnifying endoscopy and endoscopic resection for neoplasia in inflammatory bowel diseases. *World J Gastrointest Oncol* 2022; 14(3): 646-653
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/646.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.646

INTRODUCTION

Patients with inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer (CRC) compared with general population[1,2]. Surveillance colonoscopy is recommended 8-10 years after the diagnosis of IBD[3]. A forty-year analysis of colonoscopy surveillance program demonstrated that the incidence rate of advanced CRC have decreased while the incidences of early CRC and dysplasia have increased in patients with ulcerative colitis (UC), suggesting that the implementation of surveillance colonoscopy plays a significant role in reducing the likelihood of CRC in IBD patients[4]. During the surveillance colonoscopy for IBD patients, an early detection and an accurate diagnosis of neoplastic lesions are crucial components of the exam that impact further therapeutic plans and might improve patients' prognosis.

High-definition chromoendoscopy or white-light endoscopy with narrow-band imaging (NBI) are the preferred procedures and modalities for the detection of neoplastic lesions in patients with IBD[3]. Magnifying chromoendoscopy with indigo carmine or methylene blue is a useful procedure to assess pit patterns, opening shapes of tumor crypts. Pit patterns can be predictive of the presence of neoplastic lesions and their invasion depth. The classification proposed by Kudo *et al*[5] includes 8 pit pattern types (types I, II, III_L, III_S, IV, $V_I$low-irregularity, $V_I$high-irregularity, and V_N) and can predict pathological diagnosis of colorectal tumors and tumor depths. In general, pit pattern types III-V are considered as neoplastic lesions in non-IBD patients[5]. Furthermore, recent advances in endoscopy now enable narrow-band-imaging (NBI) technology, and magnifying endoscopy with NBI has been widely used to diagnose colorectal neoplasia in non-IBD patients. The Japan NBI expert team (JNET) proposed a classification system based on the vessel and surface patterns of tumors. This JNET classification includes 4 types (Types 1, 2A, 2B, and 3) and types 2-3 are considered as neoplasia[6]. These classifications are essential in determining the indication of endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) to achieve a curative resection of colorectal tumors.

Many investigations have been undertaken to understand the utility of pit patterns or use of NBI to diagnose neoplastic lesions, as well as the feasibility and outcomes of endoscopic resection to remove these lesions in IBD patients. In this review, we summarized updated data regarding these important topics.

UTILITY OF MAGNIFING CHROMOENDOSCOPY FOR IBD

Magnifying chromoendoscopy is a well-validated tool to assess neoplastic lesions in non-IBD patients and data regarding its utility for IBD patients has been emerging. The pit patterns of UC-associated neoplasm are characterized by spare distribution, loss of polarity, irregular pits, size variation, and wide-open or fused pits[7]. Similar to non-IBD patients, neoplasia in IBD patients also show pit pattern types III_L, III_S, IV or V[7,8]. A randomized controlled study including 165 UC patients randomized in a 1:1 ratio to undergo conventional colonoscopy or chromoendoscopy using 0.1% methylene blue demonstrated that pit pattern classification has high sensitivity (93%) and specificity (93%) to differentiate neoplastic lesions from non-neoplastic lesions[9]. A recent multicenter prospective study which assessed the detection rate of dysplasia in IBD patients showed that endoscopic findings of Kudo pit pattern types III-V were predictive of dysplasia (Table 1)[10].

A randomized trial comparing the detection rate of neoplasia between dye spraying colonoscopy and high-definition or electronic virtual chromoendoscopy revealed that Kudo pit pattern type IIo or types III-V were significantly predictive of neoplastic lesions during IBD surveillance colonoscopy (Odds ratio 21.5, 95% confidence interval 8.7-60.1)[11]. Another study using 769 stereomicroscopic pictures (509 neoplastic and 260 non-neoplastic) obtained from surgically resected specimens showed that pit pattern types III-V were significantly associated with the presence of neoplasia (sensitivity 77.4% and specificity

Table 1 Sensitivity and specificity of magnifying chromoendoscopy to diagnose neoplasia in inflammatory bowel disease

	Lesions assessed with MC (*n*)	Pit patterns	Sensitivity	Specificity
Kiesslich *et al*[9], 2003	87	III-V	93%	93%
Carballal *et al*[10], 2018	444	III-V	70%	90%
Shinagawa *et al*[12], 2019	769	III-V	77.4%	89.5%
Aladrén *et al*[13], 2019	709	III-V	36%	94%
Bisschops *et al*[14], 2017	50	III-V	77%	68%
Hata *et al*[15], 2004	35	III-V	100%	57%
Kudo *et al*[26], 2021	103	III-V	97.8%	57.5%
Kudo *et al*[26], 2021	103	V or II-IV with EC irregular-formed nuclei	100%	84.4%

MC: Magnifying colonoscopy; EC: Endocytoscopy.

89.5%)[12]. Further, previous studies also demonstrated that pit pattern types I-II had a high negative predictive value to rule out the diagnosis of neoplasia (Table 1)[13,14]. The findings in all of these studies suggest that magnifying endoscopy assessing Kudo pit patterns may be useful to differentiate between neoplastic lesions and non-neoplastic lesions in IBD patients.

Despite these promising findings, there are several studies that have suggested some limitations of pit patterns in diagnosing colitis-associated neoplasia. A case study assessing histopathological findings of 35 Lesions from UC patients found that pit pattern types III_L and IV can be observed in the neoplastic lesions as well as the surrounding flat mucosa, resulting in the "low specificity" (57%) of pit patterns type III-V to diagnose neoplasia. They suggested that pit patterns type III_L and IV can be observed not only in the neoplastic lesions but also in the "regenerative mucosa" in UC patients[15]. Indeed, histopathological findings of regenerative mucosa can masquerade as dysplastic findings and make the diagnosis of dysplasia equivocal[16]. Several studies have also suggested that the correlation between dysplasia and pit pattern types III-V was low (Table 1)[13,14]. Moreover, Kudo's pit patterns cannot necessarily classify all of the findings which are observed in the neoplastic lesions in UC patients. For instance, a previous observational study showed that "pinecone and villi patterns" were endoscopic signs suggestive of neoplastic lesions in UC patients[12].

These findings suggested that Kudo's pit patterns may have a high sensitivity to rule out neoplasia, but limited utility to accurately diagnose neoplasia in IBD patients due to its low specificity. Given that the regenerative mucosa can present pit patterns type III_L and IV and may decrease its specificity to diagnose neoplasia in IBD, it is suggested that providers must achieve mucosal healing prior to the scopes to overcome this disadvantage.

UTILITY OF MAGNIFYING ENDOSCOPY WITH NBI FOR IBD

With regard to the utility of NBI in IBD patients, a case report initially found that magnifying colonoscopy with NBI can differentiate dysplastic and non-dysplastic lesions, and enabled the detection of dysplastic lesions in a patient with UC[17]. This study revealed that dysplastic lesions have "a stronger capillary vascular pattern" in comparison to normal mucosa[17]. A retrospective single-center study examining 10 flat-type predominant dysplasia in UC patients demonstrated that all lesions can be recognized as "demarcated, red-colored areas with increased vascular densities" on NBI, and such lesions were histologically diagnosed with low- or high-grade dysplasia. This study performed CD34 immunohistostaining to assess intramucosal vessels and found that low-grade dysplasia displayed an increased number of vessels, whereas high-grade dysplasia contained increased/enlarged vessels[18]. Kinoshita *et al*[19] assessed the utility of the Sano magnifying NBI classification (capillary pattern classification) for neoplasia in UC and demonstrated that capillary patterns IIIA (high microvessel density with a lack of uniformity) or IIIB (the presence of an area with nearly avascular or sparse microvascular findings) had a sensitivity of 72.2% and a specificity of 85.7% to diagnose high-grade dysplasia or submucosal deep invasive carcinoma in patients with UC. These data suggest that intramucosal vessels are proliferated in dysplastic lesions in UC patients, and an increased vascular pattern may be a practical sign in detecting such lesions during magnifying colonoscopy with NBI.

A cross-sectional study including 46 UC patients classified the surface structure of neoplasia into honeycomb-like, villous, or tortuous pattern; the detection rate of dysplasia was higher in "tortuous surface pattern" than honeycomb-like or villous patterns[20]. Another observational study assessing both surface and vascular patterns of neoplasia in IBD patients by magnifying colonoscopy with NBI found that "irregular/amorphous surface patterns" were significantly associated with neoplastic lesions

(sensitivity 81.3% and specificity 82.4%), whereas irregular/amorphous vascular patterns showed lower sensitivity (75.0%) and specificity (58.8%) (Table 2)[21]. A systematic review and meta-analysis revealed that the sensitivity and specificity of NBI to discriminate neoplasia from non-neoplastic lesions were 0.64 (95%CI 0.50-0.77) and 0.74 (95%CI 0.69-0.79), respectively[22].

The JNET established a NBI magnifying endoscopic classification based on the vessel and surface patterns of tumors in non-IBD patients[6]. Data regarding its diagnostic utility for neoplastic lesions in IBD patients remain limited. A recent case study including 17 patients with UC-associated neoplasia demonstrated that JNET types 2A, 2B, and 3 were correlated with the pathological diagnosis of low-grade dysplasia/high-grade dysplasia, high-grade dysplasia, and massive submucosal invasion of cancer, respectively[23]. The sensitivity and specificity of JNET type 3 in diagnosing massively invading CRC were 25% and 100%, respectively (Table 2)[23].

Each study assessing the utility of magnifying endoscopy with NBI showed similar sensitivity and specificity for neoplastic lesions in UC, although the sensitivity of JNET type 3 was low. Hence, it is still unclear which of vascular or surface patterns of tumors are important to differentiate neoplasia and non-neoplastic lesions in UC. Given that each study only assessed the small number of UC-associated neoplasia, further investigations with larger sample sizes are warranted to better understand the characteristics of NBI findings of IBD-associated neoplasia and diagnostic accuracy of JNET classification as well as its limitations.

UTILITY OF ENDOCYTOSCOPY FOR IBD

Given the diagnostic limitations of Kudo's pit patterns for IBD-associated neoplasia, Kudo *et al*[5] retrospectively compared the diagnostic utility between pit patterns alone and its combination with an assessment using endocytoscopy, an ultra-magnifying contact microscope which has proven to enable visualization of cell nuclei or microvasculature, and detection of neoplasia in non-IBD patients[24,25]. Their data found that both pit patterns and its combination with endocytoscopy had high sensitivities to diagnose UC-associated neoplasia (97.8% *vs* 100%). In addition, the authors also demonstrated that the specificity was higher in the combined assessment of pit patterns with endocytoscopy compared to pit patterns alone (84.4% *vs* 57.5%) (Table 1)[26]. This study demonstrated that endocytoscopy can enhance the specificity of pit patterns for the diagnosis of neoplasia in IBD patients, suggesting an endocytoscopy-assisted pit pattern assessment may be a novel approach to overcome the limitation of pit patterns alone. However, they also found that the specificity can be affected by active inflammation (Mayo endoscopic subscore 2-3) and that false positive rates were high (16.2% in type II-V pits with positive irregularly-formed nuclei and 10.3% in type V)[26]. Therefore, achieving endoscopic remission is essential for an accurate evaluation of pit-patterns or cellular nuclei using magnifying scopes.

With regard to magnifying colonoscopy with NBI, one case study assessed the utility of an endocytoscopy with NBI to diagnose dysplasia in a patient with UC. The authors demonstrated that an endocytoscopy with NBI identified "surface microvessels of uniform caliber and arrangement" in a reddish lesion (5 mm) in the lower rectum. An artificial intelligence-based system diagnosed it as a neoplasm. Consequently, its pathological diagnosis was high-grade dysplasia[27]. Given that endocytoscopy can increase the specificity of pit patterns for neoplasia in IBD[26], this technique may be useful to improve the diagnostic utility of JNET classification for IBD-associated neoplasia as well.

Figures 1 and 2 show representative findings of a neoplastic lesion in a patient with UC.

ENDOSCOPIC RESECTION FOR NEOPLASTIC LESIONS IN IBD

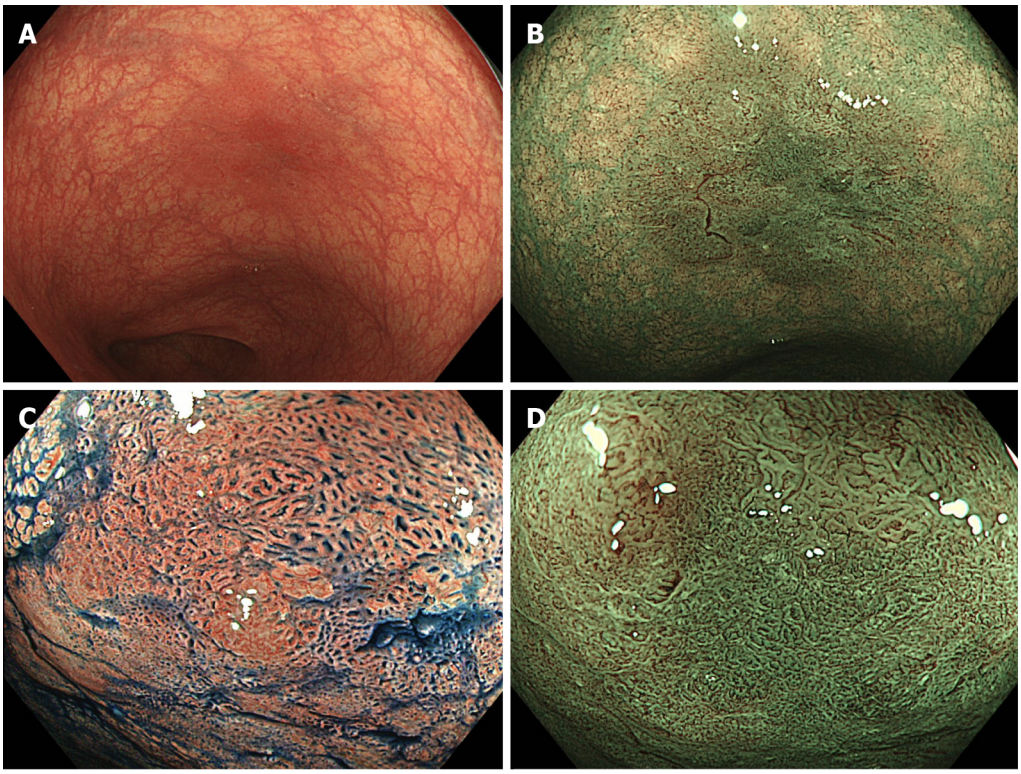
As previously described, magnifying endoscopy is useful to discriminate between dysplasia and non-dysplastic lesions in IBD patients, and determine whether such lesions are completely resectable or not. An endoscopic resection of neoplasia is safe and feasible in most IBD patients. A systematic review and meta-analysis including 1,428 resected colonic lesions in IBD patients showed the pooled incidences of bleeding and perforation after endoscopic resection were 0.022 (95%CI 0.011-0.044) and 0.020 (95%CI 0.009-0.044), respectively[28]. Another meta-analysis revealed that the pooled rates of margin-negative (R0) and *en-bloc* resection rates of non-polypoid dysplasia in IBD patients were 0.70 (95%CI 0.55-0.81) and 0.86 (95%CI 0.65-0.95), respectively[29].

As for an endoscopic technique to remove neoplasia in IBD patients, endoscopic mucosal resection (EMR) has been widely used. However, endoscopic submucosal dissection (ESD) may be a preferred technique in order to remove non-polypoid or larger lesions. Many observational studies published in 2021 have demonstrated the feasibility and safety of ESD for neoplasia in IBD patients[30-35]. Due to the technical difficulty of ESD for IBD-associated neoplasia which are often complicated with submucosal fibrosis, each study subsequently had a small sample size. Thus, further studies with larger sample sizes or analyses with integrated data may be necessary to investigate the feasibility, safety, and long-term outcomes of ESD for neoplasia in IBD patients.

Table 2 Sensitivity and specificity of magnifying colonoscopy with narrow band imaging to diagnose neoplasia in inflammatory bowel disease

	Lesions assessed with MC (*n*)	Patterns	Sensitivity	Specificity
Kinoshita *et al*[19], 2018	25	Sano classification capillary types IIIA or IIIB	72.2%[a]	85.7%[a]
Nishiyama *et al*[21], 2016	33	Irregular/amorphous surface pattern	81.3%	82.4%
Nishiyama *et al*[21], 2016	33	Irregular/avascular vascular pattern	75.0%	58.8%
Kawasaki *et al*[23], 2019	17	JNET type 3	25%[b]	100%[b]

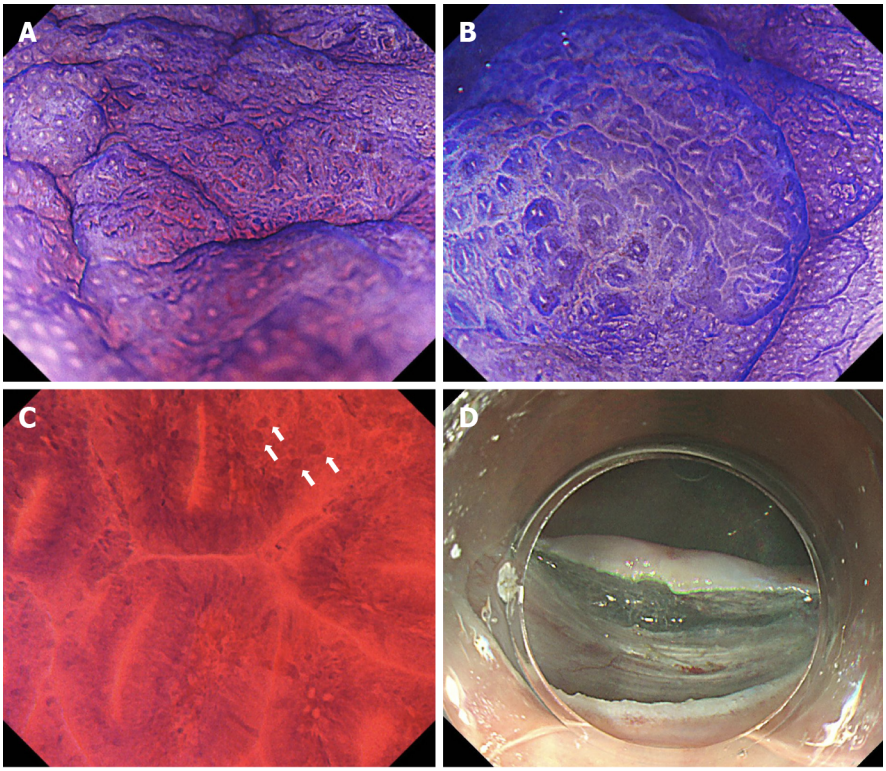
[a]Diagnostic test results for high-grade dysplasia or submucosal deep invasive cancer.
[b]Diagnostic test results for massively invading carcinoma. MC: Magnifying colonoscopy.



DOI: 10.4251/wjgo.v14.i3.646

Figure 1 Endoscopic images of a dysplastic lesion in a patient with ulcerative colitis. A: High-definition colonoscopy with white light shows a tumor recognized by a demarcated, red colored area (Paris classification Type 0-IIa, size 10 mm); B: High-definition colonoscopy with narrow band imaging (NBI); C: Magnifying chromoendoscopy with indigo carmine shows Kudo's pit pattern types III_L and V_I low-irregularity; D: Magnifying colonoscopy with NBI shows an irregular surface pattern with increased irregular vessels (Type 2B of Japan NBI expert team classification).

The SCENIC statement, an international consensus on surveillance and management of neoplasia in IBD patients, recommended a surveillance colonoscopy rather than colectomy after the complete endoscopic resection of "polypoid dysplasia"[36]. On the other hand, given that "non-polypoid lesions" have a greater risk of metachronous dysplasia after the endoscopic resection compared with polypoid lesions, providers must understand the importance of follow-up colonoscopy to survey for other neoplastic lesions. A recent systematic review and meta-analysis showed the pooled risks of CRC and any dysplasia after the endoscopic resection of neoplastic lesions in IBD patients was 2 and 43 per 1,000 person-year of follow-up, respectively, suggesting the requirement of surveillance colonoscopy after the endoscopic resection[28]. Another meta-analysis including 202 IBD patients with non-polypoid dysplasia demonstrated that the pooled incidences of CRC and metachronous dysplasia after the endoscopic resection were 33 and 90 per 1,000 person-year of follow-up, respectively[29], suggesting that the likelihood of metachronous lesions would be higher in non-polypoid lesions compared with other types. These findings emphasize the critical importance of having a discussion regarding the risks and benefits of surveillance colonoscopy and colectomy with patients following endoscopic resection.



DOI: 10.4251/wjgo.v14.i3.646

Figure 2 Endoscopic images of a dysplastic lesion in a patient with ulcerative colitis. A: Magnifying chromoendoscopy with crystal violet shows Kudo's pit pattern type V_I low-irregularity; B: Magnifying chromoendoscopy with crystal violet shows Kudo's pit pattern type III_L; C: Endocytoscopy shows slit glandular lumens with enlarged nuclei (white arrows); D: Endoscopic submucosal dissection was conducted. Pathological report showed well to moderately differentiated adenocarcinoma.

CONCLUSION

Magnifying colonoscopies assessing Kudo's pit patterns or surface/vascular patterns with NBI are practical tools that can be used to aid in the diagnosis of neoplasia in IBD patients. Endoscopic characteristics of neoplasia in IBD patients include Kudo's pit pattern types III-V in magnifying chromoendoscopy or JNET types 2A, 2B, and 3 in magnifying colonoscopy with NBI. Endocytoscopy is a supportive tool which can assist providers in confirming these diagnoses. As compared to non-IBD patients, patients with IBD can have mucosa with active colonic inflammation that may alter the findings on magnifying endoscopy, thus increasing the risk of misdiagnosis of neoplasia. Accordingly, providers should control active mucosal inflammation and achieve mucosal healing in advance to ensure the quality of surveillance colonoscopy with magnifying scopes. Although an endoscopic resection is feasible and safe for IBD-associated neoplasia, it is critically important to take into account the subsequent risk of metachronous neoplasia development, even after the complete endoscopic resection of neoplasia in IBD patients.

FOOTNOTES

Author contributions: Akiyama S designed and performed the research; Akiyama S, Sakamoto T and Saito Y analyzed the data; Akiyama S, Sakamoto T, Steinberg JM and Tsuchiya K wrote the paper.

Conflict-of-interest statement: SA and TS have no relevant disclosures. JMS was on Advisory Board for Pfizer. YS is on Advisory Board for Boston Scientific KT has received grant supports from Takeda Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharmaceutical Corp., and Hitachi Ltd.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

REFERENCES

1 **Fumery M**, Dulai PS, Gupta S, Prokop LJ, Ramamoorthy S, Sandborn WJ, Singh S. Incidence, Risk Factors, and Outcomes of Colorectal Cancer in Patients With Ulcerative Colitis With Low-Grade Dysplasia: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 665-674.e5 [PMID: 27916678 DOI: 10.1016/j.cgh.2016.11.025]
2 **Friedman S**, Rubin PH, Bodian C, Harpaz N, Present DH. Screening and surveillance colonoscopy in chronic Crohn's colitis: results of a surveillance program spanning 25 years. *Clin Gastroenterol Hepatol* 2008; **6**: 993-8; quiz 953 [PMID: 18585966 DOI: 10.1016/j.cgh.2008.03.019]
3 **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]
4 **Choi CH**, Rutter MD, Askari A, Lee GH, Warusavitarne J, Moorghen M, Thomas-Gibson S, Saunders BP, Graham TA, Hart AL. Forty-Year Analysis of Colonoscopic Surveillance Program for Neoplasia in Ulcerative Colitis: An Updated Overview. *Am J Gastroenterol* 2015; **110**: 1022-1034 [PMID: 25823771 DOI: 10.1038/ajg.2015.65]
5 **Kudo S**, Tamura S, Nakajima T, Yamano H, Kusaka H, Watanabe H. Diagnosis of colorectal tumorous lesions by magnifying endoscopy. *Gastrointest Endosc* 1996; **44**: 8-14 [PMID: 8836710 DOI: 10.1016/s0016-5107(96)70222-5]
6 **Sano Y**, Tanaka S, Kudo SE, Saito S, Matsuda T, Wada Y, Fujii T, Ikematsu H, Uraoka T, Kobayashi N, Nakamura H, Hotta K, Horimatsu T, Sakamoto N, Fu KI, Tsuruta O, Kawano H, Kashida H, Takeuchi Y, Machida H, Kusaka T, Yoshida N, Hirata I, Terai T, Yamano HO, Kaneko K, Nakajima T, Sakamoto T, Yamaguchi Y, Tamai N, Nakano N, Hayashi N, Oka S, Iwatate M, Ishikawa H, Murakami Y, Yoshida S, Saito Y. Narrow-band imaging (NBI) magnifying endoscopic classification of colorectal tumors proposed by the Japan NBI Expert Team. *Dig Endosc* 2016; **28**: 526-533 [PMID: 26927367 DOI: 10.1111/den.12644]
7 **Kudo SE**. New frontiers of endoscopy from the large intestine to the small intestine. *Gastrointest Endosc* 2007; **66**: S3-S6 [PMID: 17709026 DOI: 10.1016/j.gie.2007.03.1083]
8 **Sada M**, Igarashi M, Yoshizawa S, Kobayashi K, Katsumata T, Saigenji K, Otani Y, Okayasu I, Mitomi H. Dye spraying and magnifying endoscopy for dysplasia and cancer surveillance in ulcerative colitis. *Dis Colon Rectum* 2004; **47**: 1816-1823 [PMID: 15622573 DOI: 10.1007/s10350-004-0682-0]
9 **Kiesslich R**, Fritsch J, Holtmann M, Koehler HH, Stolte M, Kanzler S, Nafe B, Jung M, Galle PR, Neurath MF. Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* 2003; **124**: 880-888 [PMID: 12671882 DOI: 10.1053/gast.2003.50146]
10 **Carballal S**, Maisterra S, López-Serrano A, Gimeno-García AZ, Vera MI, Marín-Garbriel JC, Díaz-Tasende J, Márquez L, Álvarez MA, Hernández L, De Castro L, Gordillo J, Puig I, Vega P, Bustamante-Balén M, Acevedo J, Peñas B, López-Cerón M, Ricart E, Cuatrecasas M, Jimeno M, Pellisé M; EndoCAR group of the Spanish Gastroenterological Association and Spanish Digestive Endoscopy Society. Real-life chromoendoscopy for neoplasia detection and characterisation in long-standing IBD. *Gut* 2018; **67**: 70-78 [PMID: 27612488 DOI: 10.1136/gutjnl-2016-312332]
11 **Iacucci M**, Kaplan GG, Panaccione R, Akinola O, Lethebe BC, Lowerison M, Leung Y, Novak KL, Seow CH, Urbanski S, Minoo P, Gui X, Ghosh S. A Randomized Trial Comparing High Definition Colonoscopy Alone With High Definition Dye Spraying and Electronic Virtual Chromoendoscopy for Detection of Colonic Neoplastic Lesions During IBD Surveillance Colonoscopy. *Am J Gastroenterol* 2018; **113**: 225-234 [PMID: 29134964 DOI: 10.1038/ajg.2017.417]
12 **Shinagawa T**, Hata K, Morikawa T, Takiyama H, Emoto S, Murono K, Kaneko M, Sasaki K, Nishikawa T, Tanaka T, Kawai K, Fukayama M, Nozawa H. Pine-cone and villi patterns are endoscopic signs suggestive of ulcerative colitis-associated colorectal cancer and dysplasia. *Gastrointest Endosc* 2019; **89**: 565-575.e3 [PMID: 30326231 DOI: 10.1016/j.gie.2018.09.037]
13 **Aladrén BS**, González-Lama Y, García-Alvarado M, Sierra M, Barrio JA, Vicente VP, Hernández L, Velayos B, Garcia LA, Relea L, Suarez P, Atienza R, Vásquez M, Fernández-Salazar L, Muñoz F; GEICYL (group of inflammatory bowel disease of Castilla y León). Even non-experts identify non-dysplastic lesions in inflammatory bowel disease *via* chromoendoscopy: results of a screening program in real-life. *Endosc Int Open* 2019; **7**: E743-E750 [PMID: 31157291 DOI: 10.1055/a-0839-4514]
14 **Bisschops R**, Bessissow T, Dekker E, East JE, Para-Blanco A, Ragunath K, Bhandari P, Rutter M, Schoon E, Wilson A, John JM, Van Steen K, Baert F, Ferrante M. Pit pattern analysis with high-definition chromoendoscopy and narrow-band imaging for optical diagnosis of dysplasia in patients with ulcerative colitis. *Gastrointest Endosc* 2017; **86**: 1100-1106.e1 [PMID: 28986266 DOI: 10.1016/j.gie.2017.09.024]
15 **Hata K**, Watanabe T, Motoi T, Nagawa H. Pitfalls of pit pattern diagnosis in ulcerative colitis-associated dysplasia. *Gastroenterology* 2004; **126**: 374-376 [PMID: 14753219 DOI: 10.1053/j.gastro.2003.05.020]
16 **Riddell RH**, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum Pathol* 1983; **14**: 931-968 [PMID: 6629368 DOI: 10.1016/s0046-8177(83)80175-0]
17 **East JE**, Suzuki N, von Herbay A, Saunders BP. Narrow band imaging with magnification for dysplasia detection and pit

pattern assessment in ulcerative colitis surveillance: a case with multiple dysplasia associated lesions or masses. *Gut* 2006; **55**: 1432-1435 [PMID: 16966701 DOI: 10.1136/gut.2005.087171]

18 **Ikebata A**, Shimoda M, Okabayashi K, Uraoka T, Maehata T, Sugimoto S, Mutaguchi M, Naganuma M, Kameyama K, Yahagi N, Kanai T, Kitagawa Y, Kanai Y, Iwao Y. Demarcated redness associated with increased vascular density/size: a useful marker of flat-type dysplasia in patients with ulcerative colitis. *Endosc Int Open* 2021; **9**: E552-E561 [PMID: 33860072 DOI: 10.1055/a-1352-2709]

19 **Kinoshita S**, Uraoka T, Nishizawa T, Naganuma M, Iwao Y, Ochiai Y, Fujimoto A, Goto O, Shimoda M, Ogata H, Kanai T, Yahagi N. The role of colorectal endoscopic submucosal dissection in patients with ulcerative colitis. *Gastrointest Endosc* 2018; **87**: 1079-1084 [PMID: 29122603 DOI: 10.1016/j.gie.2017.10.035]

20 **Matsumoto T**, Kudo T, Jo Y, Esaki M, Yao T, Iida M. Magnifying colonoscopy with narrow band imaging system for the diagnosis of dysplasia in ulcerative colitis: a pilot study. *Gastrointest Endosc* 2007; **66**: 957-965 [PMID: 17826773 DOI: 10.1016/j.gie.2007.04.014]

21 **Nishiyama S**, Oka S, Tanaka S, Sagami S, Hayashi R, Ueno Y, Arihiro K, Chayama K. Clinical usefulness of narrow band imaging magnifying colonoscopy for assessing ulcerative colitis-associated cancer/dysplasia. *Endosc Int Open* 2016; **4**: E1183-E1187 [PMID: 27853744 DOI: 10.1055/s-0042-116488]

22 **Lv XH**, Wang BL, Cao GW. Narrow Band Imaging for Surveillance in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol* 2019; **53**: 607-615 [PMID: 30096061 DOI: 10.1097/MCG.0000000000001101]

23 **Kawasaki K**, Nakamura S, Esaki M, Kurahara K, Eizuka M, Nuki Y, Kochi S, Fujiwara M, Oshiro Y, Sugai T, Matsumoto T. Clinical usefulness of magnifying colonoscopy for the diagnosis of ulcerative colitis-associated neoplasia. *Dig Endosc* 2019; **31** Suppl 1: 36-42 [PMID: 30994234 DOI: 10.1111/den.13382]

24 **Kudo SE**, Wakamura K, Ikehara N, Mori Y, Inoue H, Hamatani S. Diagnosis of colorectal lesions with a novel endocytoscopic classification - a pilot study. *Endoscopy* 2011; **43**: 869-875 [PMID: 21837586 DOI: 10.1055/s-0030-1256663]

25 **Kudo SE**, Misawa M, Wada Y, Nakamura H, Kataoka S, Maeda Y, Toyoshima N, Hayashi S, Kutsukawa M, Oikawa H, Mori Y, Ogata N, Kudo T, Hisayuki T, Hayashi T, Wakamura K, Miyachi H, Ishida F, Inoue H. Endocytoscopic microvasculature evaluation is a reliable new diagnostic method for colorectal lesions (with video). *Gastrointest Endosc* 2015; **82**: 912-923 [PMID: 26071058 DOI: 10.1016/j.gie.2015.04.039]

26 **Kudo SE**, Maeda Y, Ogata N, Misawa M, Ogawa Y, Takishima K, Ishiyama M, Mochizuki K, Minegishi Y, Ogura Y, Abe M, Okumura T, Matsudaira S, Ishigaki T, Sasanuma S, Mori Y, Kudo T, Hayashi T, Wakamura K, Miyachi H, Baba T, Ishida F, Nemoto T, Hamatani S, Ohtsuka K. Combined endocytoscopy with pit pattern diagnosis in ulcerative colitis-associated neoplasia: Pilot study. *Dig Endosc* 2021 [PMID: 33641190 DOI: 10.1111/den.13964]

27 **Fukunaga S**, Kusaba Y, Ohuchi A, Nagata T, Mitsuyama K, Tsuruta O, Torimura T. Is artificial intelligence a superior diagnostician in ulcerative colitis? *Endoscopy* 2021; **53**: E75-E76 [PMID: 32590852 DOI: 10.1055/a-1195-1986]

28 **Mohan BP**, Khan SR, Chandan S, Kassab LL, Ponnada S, Asokkumar R, Shen B, Iacucci M, Navaneethan U. Endoscopic resection of colon dysplasia in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Gastrointest Endosc* 2021; **93**: 59-67.e10 [PMID: 32592777 DOI: 10.1016/j.gie.2020.06.048]

29 **Chen W**, Zhang YL, Zhao Y, Yang AM, Qian JM, Wu D. Endoscopic resection for non-polypoid dysplasia in inflammatory bowel disease: a systematic review and meta-analysis. *Surg Endosc* 2021; **35**: 1534-1543 [PMID: 33523273 DOI: 10.1007/s00464-020-08225-9]

30 **Manta R**, Zullo A, Telesca DA, Castellani D, Germani U, Reggiani Bonetti L, Conigliaro R, Galloro G. Endoscopic Submucosal Dissection for Visible Dysplasia Treatment in Ulcerative Colitis Patients: Cases Series and Systematic Review of Literature. *J Crohns Colitis* 2021; **15**: 165-168 [PMID: 32710744 DOI: 10.1093/ecco-jcc/jjaa158]

31 **Matsui A**, Hoteya S, Hayasaka J, Yamashita S, Ochiai Y, Suzuki Y, Fukuma Y, Okamura T, Mitsunaga Y, Tanaka M, Nomura K, Dan N, Odagiri H, Kikuchi D. Real-World Experience of Endoscopic Submucosal Dissection for Ulcerative Colitis-Associated Neoplasia. *Inflammatory Intestinal Diseases* 2021; **6**: 70-77 [DOI: 10.1159/000512292]

32 **Matsumoto K**, Oka S, Tanaka S, Tanaka H, Boda K, Yamashita K, Sumimoto K, Ninomiya Y, Arihiro K, Shimamoto F, Chayama K. Long-Term Outcomes after Endoscopic Submucosal Dissection for Ulcerative Colitis-Associated Dysplasia. *Digestion* 2021; **102**: 205-215 [DOI: 10.1159/000503341]

33 **Kasuga K**, Yamada M, Shida D, Tagawa T, Takamaru H, Sekiguchi M, Sakamoto T, Uraoka T, Sekine S, Kanemitsu Y, Saito Y. Treatment outcomes of endoscopic submucosal dissection and surgery for colorectal neoplasms in patients with ulcerative colitis. *United European Gastroenterology Journal* 2021 [DOI: 10.1002/ueg2.12118]

34 **Nishio M**, Hirasawa K, Ozeki Y, Sawada A, Ikeda R, Fukuchi T, Kobayashi R, Makazu M, Sato C, Kunisaki R, Maeda S. An endoscopic treatment strategy for superficial tumors in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2021; **36**: 498-506 [DOI: 10.1111/jgh.15207]

35 **Lightner AL**, Vaidya P, Allende D, Gorgun E. Endoscopic submucosal dissection is safe and feasible, allowing for ongoing surveillance and organ preservation in patients with inflammatory bowel disease. *Colorectal Dis* 2021; **23**: 2100-2107 [PMID: 34021968 DOI: 10.1111/codi.15746]

36 **Laine L**, Kaltenbach T, Barkun A, McQuaid KR, Subramanian V, Soetikno R; SCENIC Guideline Development Panel. SCENIC international consensus statement on surveillance and management of dysplasia in inflammatory bowel disease. *Gastroenterology* 2015; **148**: 639-651.e28 [PMID: 25702852 DOI: 10.1053/j.gastro.2015.01.031]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 654-663

DOI: 10.4251/wjgo.v14.i3.654

ISSN 1948-5204 (online)

MINIREVIEWS

Colorectal cancer carcinogenesis: From bench to bedside

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gao W, Liu Z, Wan X, Xu K, Zhang Y

Received: September 27, 2021
Peer-review started: September 27, 2021
First decision: December 4, 2021
Revised: December 18, 2021
Accepted: February 20, 2022
Article in press: February 20, 2022
Published online: March 15, 2022

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Abstract

Colorectal cancer (CRC) remains one of the main causes of cancer death in developed countries. Yet, it is potentially preventable, by removing the precursor lesions - adenomas or serrated lesions. Several studies proved that this intervention reduces CRC mortality and that the first colonoscopy's results can guide surveillance strategies. More recently, it became clear that several carcinogenesis pathways may lead to sporadic CRC. CRC is a heterogeneous disease, characterized by multiple molecular subtypes. Three main pathways have been implicated in the development of CRC: Chromosomal instability, microsatellite instability, and the "serrated" pathways, with overlapping features between them. This and other molecular and genetic based CRC classifications are known to have clinical implications, spanning from familial risk assessment to therapy choices. The authors review basic science data and provide insight on current implications for the management of patients with CRC.

Key Words: Colorectal cancer; Carcinogenesis pathways; Microsatellite instability; *APC*; *KRAS*; *BRAF*

Core Tip: Colorectal cancer (CRC) is a major cause of cancer death worldwide. It is a heterogeneous entity and its molecular and genetic features have clinical implications. Three main carcinogenesis pathways, with some overlapping features, are now known to lead to CRC: Chromosomal instability, microsatellite instability, and the "serrated" pathways. Their features, namely, microsatellite instability status and *BRAF* or *KRAS* mutation status, among others, have to be studied to assess familial cancer risk and to make adequate therapy choices. Ongoing research will potentially even enlarge basic science's importance for clinical practice.

Citation: Currais P, Rosa I, Claro I. Colorectal cancer carcinogenesis: From bench to bedside. *World J Gastrointest Oncol* 2022; 14(3): 654-663
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/654.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.654

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in the USA and Asia, and the second in Europe[1,2], being one of the leading causes of cancer death worldwide[3]. Sporadic colorectal cancer represents about 70% of all cases and only 5% are related to known hereditary conditions such as Lynch syndrome (LS) and familiar adenomatous polyposis (FAP). The remaining cases have apparent familial predisposition with no identifiable single germline mutations[4].

Adenomas and serrated lesions are the precursors of the vast majority of CRCs and their number/characteristics at baseline screening colonoscopy allow the definition of adequate surveillance programs after polypectomy, with an impact on survival for over more than 10 years[5]. It is reasonable to think that if we understand the molecular mechanisms underlying the appearance of these lesions, we can be even more effective in identifying grades and temporal windows of risk and in designing individualized strategies for preventing and treating CRC.

Colorectal cancer origin

Colonic stem cells (CSC) are now known to be located at the base of the crypt (cells initially identified in 1974 and called "crypt base columnar cells" - CBCC)[6]. In the normal setting, the division of a stem cell does not generate a new stem cell and another cell committed to differentiation; on the contrary, each division usually generates two cells with the same destination, either stemness or differentiation. This is a random phenomenon of "neutral competition"[7], through which certain lineages are lost (when the two daughter cells progress to differentiation, as progenitors in the transit-amplifying zone) and crypts evolve to clonality, constitutionally.

Since 2007, CSC have also been known to be responsible for the generation of the entire CRC population[8,9]. Certain mutations, namely, in the WNT pathway, may confer selective advantage to the stem cells, granting them a greater potential for subsequent clonal progression in the crypt. The WNT pathway is the main responsible for the proliferation and maintenance of stem cells in the colonic epithelium. However, its level of activity is modulated by several factors, such as NF-kB, KRAS, and the NOTCH signaling pathway[10,11]. In the clonal competition process, either an APC loss (with WNT pathway activation) or a KRAS activation (which apparently leads to increased cell division) may confer a selective advantage. In the specific context of inflammatory bowel diseases, the loss of p53 function can also create a selection advantage for the mutated stem cell[10].

Carcinogenesis pathways in colorectal cancer

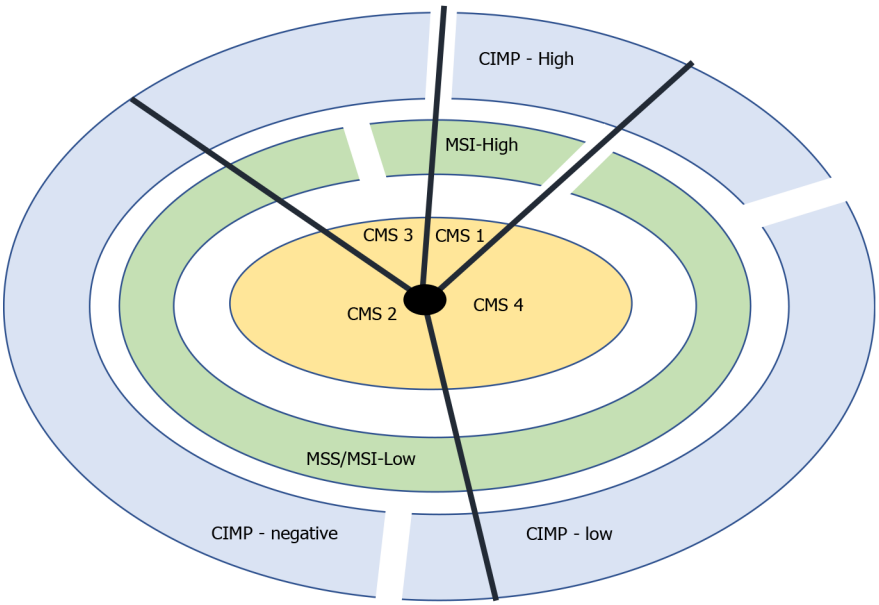
In 1990, Fearon and Vogelstein published an important paper about colorectal carcinogenesis[12]. The authors stated the need for the accumulation of several mutations in oncogenes and/or tumour suppressor genes for the development of a CRC. Although certain sequences of events are more frequent, it is the accumulation of mutations, more than its order, that leads to the biologic properties of the CRC[12,13]. The authors also found that although most tumours have mutations in the same key genes, additional mutations in several other genes occur in highly variable frequencies, which may explain some of the heterogeneity in the biologic properties of tumours found in clinical practice[14].

In fact, the CRC molecular characterization done by the Cancer Genome Atlas Network found an altered WNT signaling pathway in 93% of tumours, but it also described two broadly distinct groups of tumours: The "hypermutated" (more than 12 mutations per 106 bases) and the "non-hypermutated" (less than 12 mutations per 106 bases)[15].

Based on gene expression profiles, a classification system comprising four consensus molecular subtypes (CMS 1-4) was created, each having typical histologic and clinical features[16-19]. In Figure 1, we can see a classification system using the consensus molecular subtypes (CMS 1-4), CIMP (CpG island methylator phenotype), and microsatellite instability (MSI) status. The "non-hypermutated" tumours seem to correspond to group 4 and the "hypermutated" tumours to group 1 in the CRC classification proposed by Jass[20].

The still most widely used classification for CRC origin distinguishes three pathways that, in fact, have some overlapping features: Chromosomal instability (CIN), MSI, and serrated pathways[13].

CIN: CIN is characterized by chromosome changes that include somatic copy number alterations caused by deletions, loss of aneuploidy, insertions, and amplifications. It was recently subdivided into three CMS: CMS 2, epithelial, with marked WNT and MYC activation; CMS 3, epithelial with important metabolic activation; CMS 4, mesenchymal, with TGF-β activation, stromal invasion, and angiogenesis [21]. This pathway is observed in about 65-70% of colorectal tumours and usually associated with



DOI: 10.4251/wjgo.v14.i3.654

Figure 1 Classification system using the consensus molecular subtypes (1-4), CpG island methylator phenotype, and microsatellite instability status. CMS: Consensus molecular subtypes; CIMP: CpG island methylator phenotype; MSI: Microsatellite instability.

karyotypic abnormalities (loss of heterozygosity at chromosome arm 18q in 70% of CRC), with *APC* (in 80% of CIN tumours) and *TP53* (in 60% of CIN tumours) mutations and with *KRAS* activating mutations (in 40% of CRC)[13].

MSI: MSI is characterized by a high frequency of genomic copy number variations, and corresponds to CMS 1 and the hypermutated tumours subgroup. It occurs in the presence of abnormal mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, or PMS2), caused by sporadic epigenetic silencing (most commonly through gene promotor hypermethylation) or constitutional mutations (Lynch syndrome) [21]. This pathway is observed in about 15% of sporadic tumours and in most CRC in LS. The most common cause of the MSI phenotype is somatic - the epigenetic silencing of *MLH1* due to promoter hypermethylation (usually associated with *BRAF* mutation and CIMP-high status, a clear example of pathways' overlap). After the occurrence of MSI, the expression of an inability to correct DNA replication defects, colorectal carcinogenesis progresses more rapidly than through the CIN pathway (1 to 3 years in contrast to 10 years or more)[13].

Serrated pathway: This pathway is characterized by a phenotype of DNA hypermethylation at specific regulatory sites (CpG islands) in the promoter regions of genes - the CIMP[13]. When this hypermethylation affects tumour suppressor genes, it leads to their silencing, promoting carcinogenesis. This pathway is responsible for nearly 15% of CRC and is commonly associated with *BRAF* mutation (usually the first detected event), after which it can follow different routes. It can converge with the MSI pathway, through inactivation of *MMR* genes or it can overlap with the CIN pathway, through *TP53* mutations and WNT or TGFβ signalling pathway activation (resulting in MSS or MSI-L tumours)[13,22].

Other pathways: With the increasing advances in technology, new subgroups of tumours are being identified - an example is the identification of DNA polymerase protein mutations (POLE and POLD1), that led to the description of a new molecular pathway, characterized by a hypermutated phenotype without MSI[13].

Molecular and genetic features in colorectal cancer screening

Currently, the predominant CRC screening tools are fecal occult blood testing (FOBT), endoscopic evaluation (colonoscopy or sigmoidoscopy), and CT colonography. CRC screening has proven to reduce the risk of CRC associated mortality[23]; however, there are multiple limitations regarding test performances, and lack of access or compliance.

Several biomarkers have been investigated for their use in CRC screening, namely, DNA, proteins, and RNA (messenger or micro-RNA). These new non-invasive markers have the potential to improve screening by improving sensitivity, compliance, and accessibility. The detection of these biomarkers in blood, urine, and stool in people with colon polyps or CRC has been assessed and, to date, the most accurate tests are based on stool samples. This is explained by the abundant exfoliation of neoplastic cells from polyps and CRC into the mucocellular layer of the colonic lumen[24]. From all the options,

only DNA-based markers have been used in clinical testing so far.

Multiple stool DNA-based markers have been evaluated but only the Cologuard multitarget stool DNA (MT-sDNA) test has been approved for clinical use - approved for CRC screening in asymptomatic individuals with ages between 50 and 84 years (United States Food and Drug Administration - US FDA). This test detects a combination of gene mutations (*KRAS*), methylated DNA markers, and fecal immunochemical test (FIT) and has demonstrated the best clinical performance of CRC marker screening to date. In a recent study, MT-sDNA test proved to have an overall CRC detection rate similar to colonoscopy and a superior sensitivity (but lower specificity) when compared to FIT, for the detection of advanced adenomas and CRC. However, 10% of patients with positive MT-sDNA have no polyps or CRC when they undergo colonoscopy[24,25]. Overall, models using 3-year screening intervals predict a very high program sensitivity.

Although CRC screening using stool based molecular markers is more and more a reality, there are also multiple promising assays in development regarding plasma molecular markers. For example, plasma detection of methylated *SEPT9* (a gene more frequently methylated in CRC *vs* normal colon tissue) is currently available in China, USA, and Europe for CRC screening. However, these tests have suboptimal sensitivity for screening for colon polyps and early CRC compared to currently available screening tests[24]. It is hypothesized that plasma and urine marker detection may depend on CRC vascular invasion, which would limit the detection of precursor and early lesions.

Circulating tumor cells, circulating tumor DNA, and serum, fecal, and salivary microRNAs and long non-coding RNAs are all potential biomarkers in this emerging area and their role in CRC screening remains to be established[26].

Diagnostic, therapeutic, and prognostic implications of CRC molecular and genetic features

MSI: As previously stated, MSI is a major pathogenic pathway implicated in CRC development. The diagnosis of MSI is usually done by polymerase chain reaction (PCR) analysis of five microsatellite markers (the Bethesda panel) in both tumour and normal tissue - tumours with MSI in two or more of the markers are classified as MSI-High (MSI-H). However, immunohistochemistry (IHC) analysis of MMR protein expression has proven to identify around 95% of MSI-H tumours (in the remaining 5%, mutations that affect protein function but not its antigenicity may have happened). This technique is more widely available and does not require normal tissue samples[13](Figure 2).

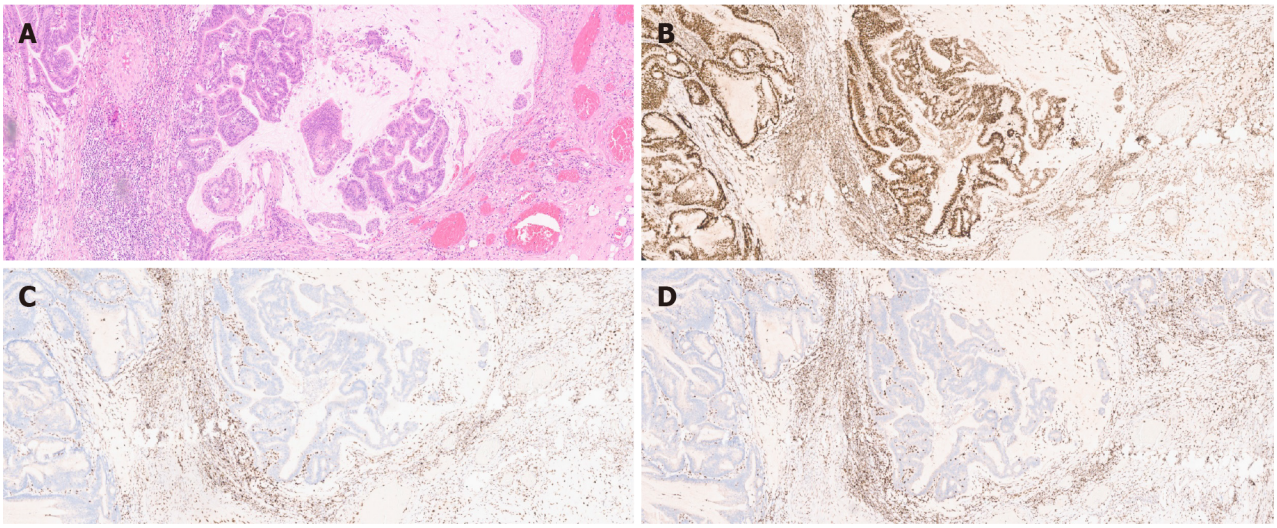
There are multiple implications for the evaluation of MSI status in CRC.

Identifying patients with LS: The MSI status can guide the clinician to better identify patients who will benefit from genetic testing. Studies have shown that almost 17% of all MSI-H tumours happen in LS patients[27], with several of them previously unidentified. Patients with LS may benefit from a more radical surgery due to a higher risk of metachronous cancers and require different surveillance protocols after CRC treatment[13,28,29]. Moreover, the identification of an LS patient may potentially lead to screening and cancer prevention in several other family members. IHC has a significant role in the selection of patients for genetic testing, since the identification of tumours with absent MSH2/MSH6 protein expression should directly lead to referral for genetic testing, while MLH1/PMS2 loss should be followed by testing for *BRAF* V600E mutation and/or gene promoter hypermethylation[21](Figure 3). Since most MSI-H tumours with absent MLH1/PMS2 protein expression are due to somatic changes, genetic testing can be omitted in most of these patients.

Different responses to standard chemotherapy: Several studies indicate that MSI tumours may have a reduced response to 5-FU chemotherapy[13,30-32]. Stage II MSI tumours have a better prognosis than MSS tumours and they probably do not benefit from adjuvant chemotherapy, namely, with 5-FU[13,33]. However, data are more conflicting for stage III tumours, where MSI status does not seem to significantly influence response to 5-FU, especially when oxaliplatin is added to the regimen. Regarding irinotecan, data are scarce, but MSI tumours seem to be sensitive[21].

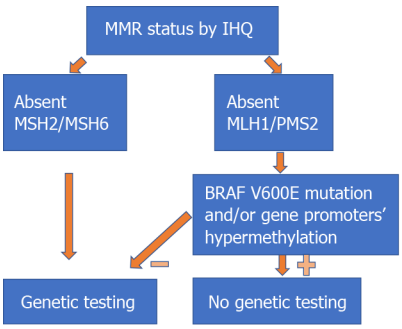
Different responses to immune checkpoint inhibitors: Tumours with MMR deficiency produce several abnormal proteins which seem to elicit antigen-driven immune responses. Perhaps as an adaptative mechanism, these tumours also show increased expression of several immune checkpoints. As a consequence, MSI CRC metastatic tumours have better response and survival patterns when immune checkpoint inhibitors (ICI) are used (as opposed with the disappointing results in non-MSI tumours). Despite a good response to ICI like pembrolizumab and nivolumab, almost 50% of the patients will progress during this therapy and there are no biomarkers available to predict this response. Tumour mutation burden (TMB) is a good predictor of response to ICI and, although they are rare, POLE/D1 mutations can lead to high TMB tumours that are MSS but may still show good response to ICI[22].

Different overall prognosis: MSI tumours are generally considered to have a better prognosis, with less lymph node metastasis and synchronous liver metastasis. However, the prognostic meaning of MSI is modulated by several factors, and their interactions. Tumour stage is an example of this heterogeneity - while stage II MSI cancers have a better prognosis, metastatic MSI CRC globally have a worst prognosis than MSS ones[21,34-37]. However, the grade of the tumour-infiltrating lymphocyte (TIL) response, the *BRAF* mutation status, or the MSI origin (LS *vs* sporadic) all interfere with the impact of MSI on



DOI: 10.4251/wjgo.v14.i3.654

Figure 2 Eosin/hematoxylin staining images of colonic neoplasia (Low power objective - 10 ×). A and B: Maintained expression of MSH2 in colonic neoplasia; C and D: Loss of expression of MLH1 and PMS2.



DOI: 10.4251/wjgo.v14.i3.654

Figure 3 Algorithm for selection of patients for genetic testing (mismatch repair genes). Family history of cancer may indicate genetic testing regardless of immunohistochemistry analysis results. IHQ: Immunohistochemistry; MMR: Mismatch repair.

prognosis[13,22].

BRAF

BRAF encodes a serine-threonine protein kinase that is a regulator of the MAPK pathway. It is an important oncogene that plays a central role in cancer initiation and progression. *BRAF* mutations are strongly associated with MSI and hypermutated tumours in the CMS 1[38,39].

BRAF testing is almost mandatory in the metastatic CRC patient population, since it has both prognostic and therapeutic implications.

Multiple studies reported negative prognostic value of the *BRAF* V600E mutation in patients with stage II, III, or IV CRC[40,41]. Other *BRAF* mutations, much less frequent, are associated with different clinical and histologic characteristics and have a better prognosis. The prognostic meaning of *BRAF* mutations may, again, be modulated by other factors, such as tumour stage or MSI status[42]. In fact, while MSS *BRAF* V600E mutated tumours seem to have the worst prognosis, *BRAF* V600E mutation in MSI tumours may have different meanings at distinct stages[43].

BRAF V600E mutation is known to be associated with intrinsic chemoresistance[43]. Regarding targeted therapy, multiple studies also reported that *BRAF* mutation is associated with cetuximab and panitumumab resistance. Although this association is not as strong as the known influence of the *KRAS* status, and still controversial, it is thought that *BRAF* mutated patients probably do not receive much benefit from being treated with these two drugs[44,45]. Therefore, some authors advocate the triplet FOLFOXIRI in combination with bevacizumab as first-line therapy for stage IV *BRAF* V600E mutated tumours. Due to the low numbers of these tumours in clinical trials, the clinical impact of this strategy remains yet to be demonstrated and the benefit of antiangiogenic drugs like bevacizumab in this subgroup of patients lacks positive results with statistical significance[43].

Finally, several BRAF inhibitors (iBRAF) are now available and have demonstrated important results in several other cancers, starting from melanoma. However, results in CRC were largely disappointing, due to the different carcinogenesis pathways involved. Strategies to overcome these limitations are being developed, mostly by using combinations with standard chemotherapy, targeted agents, and/or immunotherapy.

KRAS status

KRAS protein is a self-inactivating signal. When it binds to a tyrosine kinase receptor such as epidermal growth factor receptor (EGFR), it leads to the activation of the RAS-RAF-MEK kinase pathway. *KRAS* activating mutations lead to oncogenesis. *KRAS* mutations are frequently found in MSS tumours in the CMS 3 CIN subgroup[2]. The assessment of the *KRAS* status is also crucial because it may have prognostic and therapeutic implications.

Some studies associated the *KRAS* mutations with a worse prognosis in the unresectable metastatic setting. However, conflicting results are yet not sufficient to recommend the evaluation of the *KRAS* status for prognostication[46].

The predictive value of the *KRAS* status when choosing therapy in stage IV CRC is, however, undisputed. *KRAS* exon 2 mutation is associated with intrinsic resistance to anti-EGFR antibodies. In *KRAS* exon 2 wild type patients, *KRAS* exons 3 and 4 mutations (as the less common *NRAS* exons 2, 3, and 4 mutations) have also been shown to be associated with intrinsic resistance to anti-EGFR antibodies (cetuximab and panitumumab), and CRC patients with these mutation have a worse overall survival when they receive anti-EGFR antibodies, either as monotherapy or combined with traditional chemotherapy[47,48]

Anti-EGFR antibodies have been used for the treatment of metastatic CRC since 2004. More recently, both cetuximab and panitumumab have been approved as first line treatments for *BRAF*/*KRAS*/*NRAS* wild type patients, with a demonstrated increase in overall survival, response rate, and progression-free survival. However, there are still patients with the above tumour genotype that cannot obtain these benefits or who experience rapid drug resistance and disease progression[47,48].

In *KRAS* wild-type patients, *KRAS* mutant clones frequently emerge and lead to secondary resistance to anti-EGFR therapy[49].

Ongoing studies are investigating the use of targeted agent combinations to overcome both primary and acquired resistance to therapy due to *RAS* mutations.

Also at research stage, Adagrasib, an oral drug that selectively binds and irreversibly inhibits *KRAS* with a specific mutation, has shown promising results in CRC patients in a phase I/II trial[50,51].

Finally, there is also recent evidence showing that metformin may be useful as an antitumor agent in *KRAS* mutated CRC[52].

APC status

The WNT signaling pathway is an important mediator of stem cell activation and is the most commonly dysregulated oncogenic pathway in CRC. *APC*, a crucial element in this pathway, is the most commonly mutated gene in sporadic CRC - this mutation is an early event in 80-85% of cases[53]. So far, however, attempts to use WNT inhibitors as therapy for CRC have failed, mostly due to adverse effects[53].

Recent data has shown that different *APC* mutations can lead to different prognoses in CRC patients [53,54]. For instance, C-terminal *APC* mutation led to a shorter survival, as opposed to N-terminal *APC* mutations - it was suggested that these could be used as prognosis markers (with no therapeutic implications so far)[54]. Research on molecules that target specific types of *APC* mutations is also currently ongoing[55].

TILs

Although highly correlated with dMMR status (MSI-H status), there is evidence that TILs are an independent predictor of outcome in CRC patients[56]. Several lines of data support the fact that the host immunologic response (evaluated by histology) against the tumour is a good prognostic indicator. Elevated lymphocytic reaction to CRC is associated with better oncologic outcomes[57-60]. Extensive lymphocytic infiltration is more common in MSI than MSS tumours. The relation between TIL and MSI status can help us even more to discriminate which CRC patients will have a better prognosis. Based on this premise, a TIL/MMR-based classification was created to distinguish the prognosis of CRC subtypes in patients with stage II and III tumours. TIL-low status identifies a clinically aggressive phenotype despite the MSI status[56]. Although these data seem interesting, there is not enough evidence yet to support the utilization of TIL evaluation or TIL/MMR-status in clinical practice for prognostic stratification.

Liquid biopsy in CRC

The term liquid biopsy refers to the isolation and analysis of tumour-derived material from blood or other bodily fluids[61]. In CRC, potential applications range from diagnosis to therapeutic monitoring. The current limitations in its use for screening have already been discussed.

Regarding prognosis, Diehl *et al*[62] found that cell-free DNA (cfDNA) analysis after surgery for CRC accurately predicted relapse, by identifying patients with otherwise undetectable residual disease. If validated, this information could also be used to select patients for adjuvant chemotherapy.

The utility in therapeutic monitoring has been exemplified in a study by Siravegna *et al*[63], who found that clones with *KRAS* mutations that lead to secondary resistance to anti-EGFR antibodies may lose dominance after therapy withdrawal and that this can be detected by cfDNA analysis, predicting a benefit of reinstitution of anti-EGFR therapy in these patients.

Although several other promising studies are available, liquid biopsy use in CRC still needs standardization of methods and validation in multicentric prospective trials.

CONCLUSION

CRC is a heterogeneous entity and its molecular and genetic subtypes have significant implications, from familial risk assessment to therapeutic choices.

Regarding the most used classification for CRC origin, there are three important oncogenic pathways: CIN, MSI, and serrated pathways. They have different clinical and molecular/genetic characteristics. The MSI status, *BRAF*, *KRAS*, and *APC* mutation status, and the presence of TILs are the most studied tumour features and those more extensively correlated to clinical data. The combination of MSI status and *BRAF* mutation status can be used to help identify patients with SL. However, tumour molecular and genetic analyses are now also known to predict response to chemotherapy or to immune checkpoint inhibitors and to affect prognosis. Finally, DNA-based markers have already undergone clinical testing in the field of CRC screening and were shown to be useful.

Clinicians should be aware of the major known carcinogenesis pathways and most commonly mutated genes, since some clinical implications are already proven and several others are currently under investigation.

FOOTNOTES

Author contributions: Rosa I and Currais P reviewed the literature and wrote the manuscript; Claro I critically reviewed the manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: The authors have no relevant conflicts of interest to declare.

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S-Editor: Wang LL
L-Editor: Wang TQ
P-Editor: Wang LL

REFERENCES

1 **Kasi A**, Handa S, Bhatti S, Umar S, Bansal A, Sun W. Molecular Pathogenesis and Classification of Colorectal Carcinoma. *Curr Colorectal Cancer Rep* 2020; **16**: 97-106 [PMID: 32905465 DOI: 10.1007/s11888-020-00458-z]

2 **Ruffinelli JC**, Santos Vivas C, Sanz-Pamplona R, Moreno V. New advances in the clinical management of RAS and BRAF mutant colorectal cancer patients. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 65-79 [PMID: 32946312 DOI: 10.1080/17474124.2021.1826305]

3 **Ferlay J**, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013; **49**: 1374-1403 [PMID: 23485231 DOI: 10.1016/j.ejca.2012.12.027]

4 **Kastrinos F**, Samadder NJ, Burt RW. Use of Family History and Genetic Testing to Determine Risk of Colorectal Cancer. *Gastroenterology* 2020; **158**: 389-403 [PMID: 31759928 DOI: 10.1053/j.gastro.2019.11.029]

5 **Ballegooijen Van**, Hankey B, Shi W, Bond J. Colonoscopy polipectomy and long term prevention of colorectal death. *N Engl J Med* 2012; **366**: 687-696 [PMID: 22356322 DOI: 10.1056/nejmoa1100370]

6 **Cheng H**. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. IV. Paneth cells. *Am J Anat* 1974; **141**: 521-535 [PMID: 4440634 DOI: 10.1002/aja.1001410406]

7 **Snippert HJ**, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, Clevers H. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 2010; **143**: 134-144 [PMID: 20887898 DOI: 10.1016/j.cell.2010.09.016]

8 **Ricci-Vitiani L**, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111-115 [PMID: 17122771 DOI: 10.1038/nature05384]

9 **O'Brien CA**, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110 [PMID: 17122772 DOI: 10.1038/nature05372]

10 **Vermeulen L**, Morrissey E, van der Heijden M, Nicholson AM, Sottoriva A, Buczacki S, Kemp R, Tavaré S, Winton DJ. Defining stem cell dynamics in models of intestinal tumor initiation. *Science* 2013; **342**: 995-998 [PMID: 24264992 DOI: 10.1126/science.1243148]

11 **Snippert HJ**, Schepers AG, van Es JH, Simons BD, Clevers H. Biased competition between Lgr5 intestinal stem cells driven by oncogenic mutation induces clonal expansion. *EMBO Rep* 2014; **15**: 62-69 [PMID: 24355609 DOI: 10.1002/embr.201337799]

12 **Knudson AG Jr**. Hereditary cancer, oncogenes, and antioncogenes. *Cancer Res* 1985; **45**: 1437-1443 [PMID: 2983882]

13 **Nguyen LH**, Goel A, Chung DC. Pathways of Colorectal Carcinogenesis. *Gastroenterology* 2020; **158**: 291-302 [PMID: 31622622 DOI: 10.1053/j.gastro.2019.08.059]

14 **Dwyer ML**, Colombo A, Bozzi G. Retrieval technique of a PTCA guidewire. *G Ital Cardiol* 1989; **19**: 170-172 [PMID: 2527178]

15 **Cancer Genome Atlas Network**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

16 **De Sousa E Melo F**, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, de Jong JH, de Boer OJ, van Leersum R, Bijlsma MF, Rodermond H, van der Heijden M, van Noesel CJ, Tuynman JB, Dekker E, Markowetz F, Medema JP, Vermeulen L. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013; **19**: 614-618 [PMID: 23584090 DOI: 10.1038/nm.3174]

17 **Sadanandam A**, Lyssiotis CA, Homicsko K, Collisson EA, Gibb WJ, Wullschleger S, Ostos LC, Lannon WA, Grotzinger C, Del Rio M, Lhermitte B, Olshen AB, Wiedenmann B, Cantley LC, Gray JW, Hanahan D. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med* 2013; **19**: 619-625 [PMID: 23584089 DOI: 10.1038/nm.3175]

18 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

19 **Marisa L**, de Reyniès A, Duval A, Selves J, Gaub MP, Vescovo L, Etienne-Grimaldi MC, Schiappa R, Guenot D, Ayadi M, Kirzin S, Chazal M, Fléjou JF, Benchimol D, Berger A, Lagarde A, Pencreach E, Piard F, Elias D, Parc Y, Olschwang S, Milano G, Laurent-Puig P, Boige V. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. *PLoS Med* 2013; **10**: e1001453 [PMID: 23700391 DOI: 10.1371/journal.pmed.1001453]

20 **Jass JR**. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 2007; **50**: 113-130 [PMID: 17204026 DOI: 10.1111/j.1365-2559.2006.02549.x]

21 **Ryan E**, Sheahan K, Creavin B, Mohan HM, Winter DC. The current value of determining the mismatch repair status of colorectal cancer: A rationale for routine testing. *Crit Rev Oncol Hematol* 2017; **116**: 38-57 [PMID: 28693799 DOI: 10.1016/j.critrevonc.2017.05.006]

22 **Cohen R**, Rousseau B, Vidal J, Colle R, Diaz LA Jr, André T. Immune Checkpoint Inhibition in Colorectal Cancer: Microsatellite Instability and Beyond. *Target Oncol* 2020; **15**: 11-24 [PMID: 31786718 DOI: 10.1007/s11523-019-00690-0]

23 **Ahlquist DA**. Molecular detection of colorectal neoplasia. *Gastroenterology* 2010; **138**: 2127-2139 [PMID: 20420950 DOI: 10.1053/j.gastro.2010.01.055]

24 **Dickinson BT**, Kisiel J, Ahlquist DA, Grady WM. Molecular markers for colorectal cancer screening. *Gut* 2015; **64**: 1485-1494 [PMID: 25994221 DOI: 10.1136/gutjnl-2014-308075]

25 **Imperiale TF**, Ransohoff DF, Itzkowitz SH. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014; **371**: 187-188 [PMID: 25006736 DOI: 10.1056/NEJMc1405215]

26 **Vacante M**, Ciuni R, Basile F, Biondi A. The Liquid Biopsy in the Management of Colorectal Cancer: An Overview. *Biomedicines* 2020; **8** [PMID: 32858879 DOI: 10.3390/biomedicines8090308]

27 **Latham A**, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, Middha S, Hechtman J, Zehir A, Dubard-Gault M, Tran C, Stewart C, Sheehan M, Penson A, DeLair D, Yaeger R, Vijai J, Mukherjee S, Galle J, Dickson MA, Janjigian Y, O'Reilly EM, Segal N, Saltz LB, Reidy-Lagunes D, Varghese AM, Bajorin D, Carlo MI, Cadoo K, Walsh MF, Weiser M, Aguilar JG, Klimstra DS, Diaz LA Jr, Baselga J, Zhang L, Ladanyi M, Hyman DM, Solit DB, Robson ME, Taylor BS, Offit K, Berger MF, Stadler ZK. Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. *J Clin Oncol* 2019; **37**: 286-295 [PMID: 30376427 DOI: 10.1200/JCO.18.00283]

28 **Heneghan HM**, Martin ST, Winter DC. Segmental vs extended colectomy in the management of hereditary nonpolyposis colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2015; **17**: 382-389 [PMID: 25510173 DOI: 10.1111/codi.12868]

29 **Järvinen HJ**, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomäki P, De La Chapelle A, Mecklin JP.

Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2000; **118**: 829-834 [PMID: 10784581 DOI: 10.1016/s0016-5085(00)70168-5]

30 **Sargent DJ**, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaniboni A, Seitz JF, Sinicrope F, Gallinger S. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010; **28**: 3219-3226 [PMID: 20498393 DOI: 10.1200/JCO.2009.27.1825]

31 **Hutchins G**, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, Richman S, Chambers P, Seymour M, Kerr D, Gray R, Quirke P. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011; **29**: 1261-1270 [PMID: 21383284 DOI: 10.1200/JCO.2010.30.1366]

32 **Ribic CM**, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247-257 [PMID: 12867608 DOI: 10.1056/NEJMoa022289]

33 **Ota DM**. Colon cancer. *Cancer Treat Res* 1997; **90**: 347-356 [PMID: 9367092 DOI: 10.1007/978-1-4615-6165-1_18]

34 **Sinicrope FA**, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, Bot BM, Tejpar S, Delorenzi M, Goldberg RM, Mahoney M, Sargent DJ, Alberts SR. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology* 2015; **148**: 88-99 [PMID: 25305506 DOI: 10.1053/j.gastro.2014.09.041]

35 **Vasen HF**, Blanco I, Aktan-Collan K, Gopie JP, Alonso A, Aretz S, Bernstein I, Bertario L, Burn J, Capella G, Colas C, Engel C, Frayling IM, Genuardi M, Heinimann K, Hes FJ, Hodgson SV, Karagiannis JA, Lalloo F, Lindblom A, Mecklin JP, Møller P, Myrhoj T, Nagengast FM, Parc Y, Ponz de Leon M, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Sijmons RH, Tejpar S, Thomas HJ, Rahner N, Wijnen JT, Järvinen HJ, Möslein G; Mallorca group. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. *Gut* 2013; **62**: 812-823 [PMID: 23408351 DOI: 10.1136/gutjnl-2012-304356]

36 **Miles A**, McClements PL, Steele RJ, Redeker C, Sevdalis N, Wardle J. The Psychological Impact of a Colorectal Cancer Diagnosis Following a Negative Fecal Occult Blood Test Result. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 1032-1038 [PMID: 25924826 DOI: 10.1158/1055-9965.EPI-15-0004]

37 **Jennings BA**, Loke YK, Skinner J, Keane M, Chu GS, Turner R, Epurescu D, Barrett A, Willis G. Evaluating predictive pharmacogenetic signatures of adverse events in colorectal cancer patients treated with fluoropyrimidines. *PLoS One* 2013; **8**: e78053 [PMID: 24167597 DOI: 10.1371/journal.pone.0078053]

38 **Gonsalves WI**, Mahoney MR, Sargent DJ, Nelson GD, Alberts SR, Sinicrope FA, Goldberg RM, Limburg PJ, Thibodeau SN, Grothey A, Hubbard JM, Chan E, Nair S, Berenberg JL, McWilliams RR; Alliance for Clinical Trials in Oncology. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst* 2014; **106** [PMID: 24925349 DOI: 10.1093/jnci/dju106]

39 **Lipsyc M**, Yaeger R. Impact of somatic mutations on patterns of metastasis in colorectal cancer. *J Gastrointest Oncol* 2015; **6**: 645-649 [PMID: 26697197 DOI: 10.3978/j.issn.2078-6891.2015.045]

40 **Fariña-Sarasqueta A**, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VEPP, Rutten HJT, van den Brule AJC. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol* 2010; **21**: 2396-2402 [PMID: 20501503 DOI: 10.1093/annonc/mdq258]

41 **Morris V**, Overman MJ, Jiang ZQ, Garrett C, Agarwal S, Eng C, Kee B, Fogelman D, Dasari A, Wolff R, Maru D, Kopetz S. Progression-free survival remains poor over sequential lines of systemic therapy in patients with BRAF-mutated colorectal cancer. *Clin Colorectal Cancer* 2014; **13**: 164-171 [PMID: 25069797 DOI: 10.1016/j.clcc.2014.06.001]

42 **Bläker H**, Alwers E, Arnold A, Herpel E, Tagscherer KE, Roth W, Jansen L, Walter V, Kloor M, Chang-Claude J, Brenner H, Hoffmeister M. The Association Between Mutations in BRAF and Colorectal Cancer-Specific Survival Depends on Microsatellite Status and Tumor Stage. *Clin Gastroenterol Hepatol* 2019; **17**: 455-462.e6 [PMID: 29660527 DOI: 10.1016/j.cgh.2018.04.015]

43 **Caputo F**, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, Andrikou K, Cascinu S, Gelsomino F. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. *Int J Mol Sci* 2019; **20** [PMID: 31661924 DOI: 10.3390/ijms20215369]

44 **Rowland A**, Dias MM, Wiese MD, Kichenadasse G, McKinnon RA, Karapetis CS, Sorich MJ. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *Br J Cancer* 2015; **112**: 1888-1894 [PMID: 25989278 DOI: 10.1038/bjc.2015.173]

45 **Pietrantonio F**, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, Cabiddu M, Iacovelli R, Bossi I, Lonati V, Ghilardi M, de Braud F, Barni S. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer* 2015; **51**: 587-594 [PMID: 25673558 DOI: 10.1016/j.ejca.2015.01.054]

46 **Labianca R**, Nordlinger B, Beretta GD, Mosconi S, Mandalà M, Cervantes A, Arnold D; ESMO Guidelines Working Group. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24** Suppl 6: vi64-vi72 [PMID: 24078664 DOI: 10.1093/annonc/mdt354]

47 **Lièvre A**, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Côté JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006; **66**: 3992-3995 [PMID: 16618717 DOI: 10.1158/0008-5472.CAN-06-0191]

48 **De Roock W**, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, Biesmans B, Van Laethem JL, Peeters M, Humblet Y, Van Cutsem E, Tejpar S. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008; **19**: 508-515 [PMID: 17998284 DOI: 10.1093/annonc/mdm496]

49 **Misale S**, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, Bencardino K, Cercek A, Chen CT, Veronese S, Zanon C, Sartore-Bianchi A, Gambacorta M, Gallicchio M, Vakiani E, Boscaro V, Medico E, Weiser M, Siena S, Di Nicolantonio F, Solit D, Bardelli A. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012; **486**: 532-536 [PMID: 22722830 DOI:

10.1038/nature11156]
50 **Jänne P**, Rybkin I, Spira A, Riely G, Papadopoulos K, Sabari J. KRYSTAL-1: Activity and Safety of Adagrasib (MRTX849) in Advanced/ Metastatic Non-Small-Cell Lung Cancer (NSCLC) Harboring KRAS G12C Mutation. *Eur J Cancer* 2020; **138**: S1-2 [DOI: 10.1016/s0959-8049(20)31076-5]
51 **Riely G**, Ou S, Rybkin I, Spira A, Papadopoulos K, Sabari J. KRYSTAL-1: Activity and preliminary pharmacodynamic (PD) analysis of adagrasib (MRTX849) in patients (Pts) with advanced non-small cell lung cancer (NSCLC) harboring KRASG12C mutation. *J Thorac Oncol* 2021; **16**: S751-S752 [DOI: 10.1016/s1556-0864(21)01941-9]
52 **Xie J**, Xia L, Xiang W, He W, Yin H, Wang F, Gao T, Qi W, Yang Z, Yang X, Zhou T, Gao G. Metformin selectively inhibits metastatic colorectal cancer with the *KRAS* mutation by intracellular accumulation through silencing MATE1. *Proc Natl Acad Sci U S A* 2020; **117**: 13012-13022 [PMID: 32444490 DOI: 10.1073/pnas.1918845117]
53 **Zhang L**, Shay JW. Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer. *J Natl Cancer Inst* 2017; **109** [PMID: 28423402 DOI: 10.1093/jnci/djw332]
54 **Mondaca S**, Walch H, Nandakumar S, Chatila WK, Schultz N, Yaeger R. Specific Mutations in APC, but Not Alterations in DNA Damage Response, Associate With Outcomes of Patients With Metastatic Colorectal Cancer. *Gastroenterology* 2020; **159**: 1975-1978.e4 [PMID: 32730818 DOI: 10.1053/j.gastro.2020.07.041]
55 **Wang W**, Zhang L, Morlock L, Williams NS, Shay JW, De Brabander JK. Design and Synthesis of TASIN Analogues Specifically Targeting Colorectal Cancer Cell Lines with Mutant Adenomatous Polyposis Coli (APC). *J Med Chem* 2019; **62**: 5217-5241 [PMID: 31070915 DOI: 10.1021/acs.jmedchem.9b00532]
56 **Williams DS**, Mouradov D, Jorissen RN, Newman MR, Amini E, Nickless DK, Teague JA, Fang CG, Palmieri M, Parsons MJ, Sakthianandeswaren A, Li S, Ward RL, Hawkins NJ, Faragher I, Jones IT, Gibbs P, Sieber OM. Lymphocytic response to tumour and deficient DNA mismatch repair identify subtypes of stage II/III colorectal cancer associated with patient outcomes. *Gut* 2019; **68**: 465-474 [PMID: 29382774 DOI: 10.1136/gutjnl-2017-315664]
57 **Klintrup K**, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, Tuppurainen K, Mäkelä J, Karttunen TJ, Mäkinen MJ. Inflammation and prognosis in colorectal cancer. *Eur J Cancer* 2005; **41**: 2645-2654 [PMID: 16239109 DOI: 10.1016/j.ejca.2005.07.017]
58 **Ogino S**, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, Glickman JN, Ferrone CR, Mino-Kenudson M, Tanaka N, Dranoff G, Giovannucci EL, Fuchs CS. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009; **15**: 6412-6420 [PMID: 19825961 DOI: 10.1158/1078-0432.CCR-09-1438]
59 **Mei Z**, Liu Y, Liu C, Cui A, Liang Z, Wang G, Peng H, Cui L, Li C. Tumour-infiltrating inflammation and prognosis in colorectal cancer: systematic review and meta-analysis. *Br J Cancer* 2014; **110**: 1595-1605 [PMID: 24504370 DOI: 10.1038/bjc.2014.46]
60 **Ropponen KM**, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. *J Pathol* 1997; **182**: 318-324 [PMID: 9349235 DOI: 10.1002/(SICI)1096-9896(199707)182:3<318::AID-PATH862>3.0.CO;2-6]
61 **Corcoran RB**, Chabner BA. Application of Cell-free DNA Analysis to Cancer Treatment. *N Engl J Med* 2018; **379**: 1754-1765 [PMID: 30380390 DOI: 10.1056/NEJMra1706174]
62 **Lindforss U**, Zetterquist H, Papadogiannakis N, Olivecrona H. Persistence of K-ras mutations in plasma after colorectal tumor resection. *Anticancer Res* 2005; **25**: 657-661 [PMID: 15816642]
63 **Siravegna G**, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, Ponzetti A, Cremolini C, Amatu A, Lauricella C, Lamba S, Hobor S, Avallone A, Valtorta E, Rospo G, Medico E, Motta V, Antoniotti C, Tatangelo F, Bellosillo B, Veronese S, Budillon A, Montagut C, Racca P, Marsoni S, Falcone A, Corcoran RB, Di Nicolantonio F, Loupakis F, Siena S, Sartore-Bianchi A, Bardelli A. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med* 2015; **21**: 795-801 [PMID: 26030179 DOI: 10.1038/nm.3870]

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World J Gastrointest Oncol 2022 March 15; 14(3): 664-677

DOI: 10.4251/wjgo.v14.i3.664

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

O6-methylguanine DNA methyltransferase is upregulated in malignant transformation of gastric epithelial cells *via* its gene promoter DNA hypomethylation

Yue-Xia Chen, Lu-Lu He, Xue-Ping Xiang, Jing Shen, Hong-Yan Qi

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Bai G, Batool SN

Received: July 27, 2021
Peer-review started: July 27, 2021
First decision: October 3, 2021
Revised: November 10, 2021
Accepted: February 10, 2022
Article in press: February 10, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

O6-methylguanine-DNA methyltransferase (MGMT) is a suicide enzyme that repairs the mispairing base O6-methyl-guanine induced by environmental and experimental carcinogens. It can transfer the alkyl group to a cysteine residue in its active site and became inactive. The chemical carcinogen N-nitroso compounds (NOCs) can directly bind to the DNA and induce the O6-methylguanine adducts, which is an important cause of gene mutation and tumorigenesis. However, the underlying regulatory mechanism of MGMT involved in NOCs-induced tumorigenesis, especially in the initiation phase, remains largely unclear.

AIM

To investigate the molecular regulatory mechanism of MGMT in NOCs-induced gastric cell malignant transformation and tumorigenesis.

METHODS

We established a gastric epithelial cell malignant transformation model induced

by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) or N-methyl-N-nitroso-urea (MNU) treatment. Cell proliferation, colony formation, soft agar, cell migration, and xenograft assays were used to verify the malignant phenotype. By using quantitative real-time polymerase chain reaction (qPCR) and Western blot analysis, we detected the MGMT expression in malignant transformed cells. We also confirmed the MGMT expression in early stage gastric tumor tissues by qPCR and immunohistochemistry. *MGMT* gene promoter DNA methylation level was analyzed by methylation-specific PCR and bisulfite sequencing PCR. The role of MGMT in cell malignant transformation was analyzed by colony formation and soft agar assays.

RESULTS

We observed a constant increase in MGMT mRNA and protein expression in gastric epithelial cell malignant transformation induced by MNNG or MNU treatment. Moreover, we found a reduction of *MGMT* gene promoter methylation level by methylation-specific PCR and bisulfite sequencing PCR in MNNG/MNU-treated cells. Inhibition of the MGMT expression by O^6-benzylguanine promoted the MNNG/MNU-induced malignant phenotypes. Overexpression of MGMT partially reversed the cell malignant transformation process induced by MNNG/MNU. Clinical gastric tissue analysis showed that MGMT was upregulated in the precancerous lesions and metaplasia tissues, but downregulated in the gastric cancer tissues.

CONCLUSION

Our finding indicated that MGMT upregulation is induced *via* its DNA promoter hypomethylation. The highly expressed MGMT prevents the NOCs-induced cell malignant transformation and tumorigenesis, which suggests a potential novel approach for chemical carcinogenesis intervention by regulating aberrant epigenetic mechanisms.

Key Words: O^6-methylguanine-DNA methyltransferase; DNA methylation; Malignant transformation; Gastric carcinogenesis; Epigenetic regulation

Core Tip: This study revealed a molecular regulatory mechanism of O^6-methylguanine-DNA methyltransferase (*MGMT*) gene upregulation in the early stage of tumor development, and improved the understanding of the dynamic change of MGMT expression in different stages of tumor development, providing a new entry point for further study of the expression mechanism of key regulatory genes in the process of chemical carcinogenesis.

Citation: Chen YX, He LL, Xiang XP, Shen J, Qi HY. O^6-methylguanine DNA methyltransferase is upregulated in malignant transformation of gastric epithelial cells *via* its gene promoter DNA hypomethylation. *World J Gastrointest Oncol* 2022; 14(3): 664-677
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/664.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.664

INTRODUCTION

Gastric cancer (GC) is currently the fifth most frequently diagnosed and the third leading cause of cancer death worldwide with a high prevalence in many Asia countries, particularly in China, Japan, and South Korea[1,2]. Previous studies have reported that epigenetic alterations are widely recognized to be involved in the initiation and progression of gastric tumorigenesis[3,4]. DNA methylation is a common significant epigenetic modification and plays an important role in the development and prognosis of GC[5-8].

O^6-methylguanine-DNA methyltransferase (MGMT) is a suicide enzyme that efficiently removes alkylating lesions at the O^6 position of guanine induced by DNA alkylating agents[9]. Following the transfer of the alkyl group to itself, MGMT becomes inactive and it is ubiquitinated and targeted for proteasomal degradation. MGMT is frequently regulated by epigenetic silencing mediated Fits gene promoter DNA methylation in gliomas[10,11]. The abnormal modifications of histone and aberrant expression of transcriptional activators and repressors, also contribute to the regulation of MGMT expression in different tumors[11]. O^6-methylguanine is a potent mutagenic lesion that leads to base mismatching and double-strand breaks, promoting gene mutagenesis and tumor initiation. MGMT can restore this type of DNA damage and play an important role in maintaining genomic stability[12].

Inhibiting MGMT function can induce G:C to A:T mutation of the onco-suppressors p53 and PTEN to promote human carcinogenesis[13]. In the TCGA database, the probability of point mutation of p53 and PTEN was higher in *MGMT* promoter methylated tissues than in non-methylated tissues of glioma. In colon cancer, lung cancer, and GC, the reduction of MGMT expression induced by DNA methylation in its promoter regions was also observed[14-17]. Yet, MGMT expression can be increased by chemotherapy with alkylating agents, such as temozolomide, which contributes to the chemotherapy resistance[18]. However, the role of MGMT in the early stage of tumorigenesis remains unclear.

The monofunctional alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitroso-urea (MNU) are widely accepted model chemical carcinogens for studying the mechanisms of mutagenesis and carcinogenesis induced by N-nitroso compounds (NOCs). They generate adducts with DNA and protein, such as O^6-methylguanine, which lead to point mutations, chromosomal aberrations, initiation and promotion of various cancer, specially increasing the risk of gastrointestinal cancers[19,20]. Our previously studies showed that MNNG and MNU treatments can stimulate multiple cellular responses, including epigenetic events[21,22]. We revealed a dysregulation of histone modifications and DNA methylation, which contributed to numerous cancer-related gene expression changes promoting cell malignant transformation upon NOCs treatment[21,22]. These findings prompted us to speculate that the abnormal epigenetic regulation could be the critical molecular mechanism of chemical carcinogens-induced gastric carcinogenesis.

In the present study, we investigated the epigenetic changes of MGMT in NOCs-induced human gastric cell malignant transformation. And we demonstrated that DNA methylation level of MGMT promoter was strongly decreased, which resulted the inhibition of MGMT expression, contributing the malignant phenotypes during the cell malignant transformation process.

MATERIALS AND METHODS

Patient samples

A total of 93 clinical gastric tissue samples collected by endoscopic biopsy at the Second Affiliated Hospital of Zhejiang University were used in this study, including 25 cases of gastritis, 18 cases of gastric metaplasia (used as precancerous lesion), 50 pairs of GC and adjacent normal tissues (early stage, 19 cases; advanced stage, 31 cases). The study was approved by the ethics committee of Zhejiang University School of Medicine (No. 2017026). The tissue samples were formalin-fixed and paraffin-embedded for immunohistochemistry or used for mRNA isolation to detect gene expression.

Cell culture

The human gastric normal epithelium cell line GES-1 (Cell Bank of the Chinese Academy of Science, Xiangya, China) was cultured in DMEM (Gibco, Grand Island, NY, United States) supplemented with 10% fetal bovine serum (FBS; Gibco), streptomycin (100 g/mL), and penicillin (100 U/mL) at 37 °C in an atmosphere containing 5% CO_2. And GC cell lines, including AGS, MKN45, SGC7901, KATOIII, and NCI-N87 (Cell Bank of the Chinese Academy of Science, Shanghai, China), were cultured in DMEM or PRIM-1640 supplemented with 10% FBS, streptomycin, and penicillin. The authenticity of cell lines used in this study had been verified by short tandem repeat profiling.

Cell transformation assays

Cells were exposed to MNNG or MNU as described in a previous study to establish the cell transformation model[22]. Briefly, cells were exposed to MNU (TRC, Toronto, Canada) or MNNG (Sigma, St. Louis, MO, United States) for 2 h in serum free medium. Then, the medium was removed and cells were recovered in fresh medium at 37 °C. MNU and MNNG exposure was repeated once a week for 4 wk. After 4 wk of treatment and 4 wk of restoration, characteristics related to malignant phenotype were measured.

Scratch test

Cells were plated in 6-well plates and allowed to reach 90% confluence. The monolayer was scratched with a 10-mL sterile pipette tip. Images of the scratches were taken using an inverted microscope at × 10 magnification at 0, 24, and 48 h of incubation. ImageJ software was used to analyze the percentage of wound closure.

Cell proliferation analysis

Cells (1.2×10^4 cells/well) were seeded in 24-well plates and cultured for 24, 48, 72, 96, and 120 h. Cells were digested by trypsin every 24 h and then re-suspended in fresh medium and counted.

Soft agar and colony formation assays

For soft agar assay, cells (1000 cells/well) were suspended in a culture medium containing 0.4% agarose (A9045-5G) (Sigma) and seeded onto a base layer of 0.7% agar bed in 12-well plates. The culture

medium was changed every 3 d. After 2 wk, colonies were stained with crystal violet and photographed. Colonies ≥ 0.05 mm in diameter were counted.

For colony formation assay, cells (1000 cells/well) were seeded in 6-well plates and cultured. The culture medium was changed every 3 d. After 2 wk, colonies were stained with crystal violet and photographed. Colonies ≥ 0.05 mm in diameter were counted.

Dual-luciferase reporter assay

The *MGMT* promoter sequence (-954/+24) was amplified from the extracted genomic DNA and cloned into pGL3-promoter vector (Promega, Madison, WI, United States). After seeding MNNG/MNU-transformed cells for 24 h, the cells were co-transfected with 0.5 μg of pGL3-*MGMT*-promoter and 0.02 μg of pRL-SV40 renilla luciferase reporter plasmid using X-treme GENE HP (Roche, Basel, Switzerland). Dual-Luciferase Reporter Assay System was used for testing relative luciferase activity after transfection for 24 h (Promega).

DNA methylation specific polymerase chain reaction and bisulfite genomic sequence assay

Total DNA (5×10^6 cells) was isolated from the MNNG/MNU-transformed cells with the Qiagen DNA Isolation Kit. Then, bisulfite conversion was performed with 500 ng of genomic DNA using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, United States). The converted DNA was eluted in 100 mL of nuclease-free water. Methylation specific polymerase chain reaction (PCR) (MSP) analysis was performed in a 25-μL reaction system that consisted of 50 ng of sodium bisulfite-treated DNA, 12.5 μL of 2 × Master Mix (Qiagen, Germany), ddH2O, and 3 μL of isometric mixture of *MGMT* gene methylated and un-methylated primers. *MGMT* methylated and un-methylated primers used are: Forward 5′-TTTCGACGTTCGTAGGTTTTCGC-3′ and reverse, 5′-GCACTCTTCCGAAAACGAAACG-3′; forward, 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′ and reverse, 5′-ACTCCACACTCTTCCAAAAAC AAAACA-3′. Bisulfite genomic sequence (BSP) analysis was performed by Xiangyin Biological Corporation. Bisulfite treatments of the genomic DNA samples were carried out with the Qiagen EpiTect kit according to the manufacturer's instructions, followed by the PCR amplification procedure (30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s; 72 °C for 30 min; and held at 4 °C) using KAPA2G Fast Multiplex Mix and KAPA 2G Robust HS. The PCR products were identified by electrophoresis and gel-purified with the Gel and PCR Clean-up System (Promega). The purified PCR products were inserted into PMD-18T Vector and sequenced by Sanger sequencing.

Chromatin immunoprecipitation assay

For chromatin immunoprecipitation (ChIP) assay, the malignant transformed cells were cross-linked with 1% formaldehyde for 10 min at 37 °C. Then, cells were isolated and lysed for preparation of sheared chromatin. The cell lysates were sonicated for 1 min and repeated ten times at 1-min intervals. After centrifugation at 13000 *g* at 4 °C for 30 min, the cell lysates were diluted with IP buffer and incubated with anti-DNMT1, anti-H3K9Me3, and anti-H3K4Me2 antibodies (CST, Massachusetts, United States) overnight, respectively. For collecting the bound DNA, the coated beads were added in the samples and incubated for 4 h at 4 °C. The beads were collected, washed, and eluted with elution buffer. Then, the bound DNA was extracted with a DNA extraction kit (Qiagen) for quantification by qPCR. Primers used for detecting the binding sites in MGMT promoter are: Forward, 5′-GCCCCTA-GAACGCTTTGC-3′ and reverse, 5′-CAACACCTGGGAGGCACTT-3′.

Immunohistochemistry

Immunohistochemistry was performed using an Envision Detection System (DAKO, Carpinteria, CA, United States) according to the manufacturer's instructions. Mouse monoclonal anti-human MGMT antibody (dilution, 1:150) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States) and used for immunohistochemistry. The staining results were assessed and confirmed by two independent investigators blinded to the clinical data.

qPCR

Total RNA was extracted from cell lines and tissue samples with TRIzol reagent (Invitrogen). For gene expression, mRNA was reverse transcribed using a Prime-Script RT reagent Kit (TaKaRa). qPCR was carried out with SYBR Premix Ex Taq (TaKaRa). Experiments were performed in triplicate and values were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta Ct}$ method for gene expression analysis. The primers used for *MGMT* and *GAPDH* amplification are: Forward, 5′-AACGCTGCCCTTGCTCTATT-3′ and reverse, 5′-AGCTTTCTAGTGTGGACGGC-3′ for *MGMT*; forward, 5′-ATGGGGAAGGTGAAGGTCGGAGT-3′ and reverse, 5′-TGACAAGCTTCCCGTTCTCA GCC-3′ for *GAPDH*.

Immunoblot analysis

Cells were lysed with RIPA buffer, and the total protein was quantified by Bradford assay. Cell lysates (50 mg) were separated on a 10% SDS-PAGE gel and then transferred onto a nitrocellulose membrane (Whatman, Maidstone, United Kingdom). The membrane was blocked with 5% skim milk solution for 2

h and incubated overnight with diluted primary antibody at 4 °C. Then, the membrane was incubated with IRDye 800- or IRDye 680-conjugated secondary antibody (LI-COR Biosciences, Lincoln, NE, United States) and detected with an Odyssey infrared imaging system. Mouse monoclonal anti-human MGMT antibody (dilution, 1:1000) and mouse monoclonal anti-human GADPH antibody (dilution, 1:2000) were purchased from Santa Cruz Biotechnology.

Xenograft assay

Balb/c nude mice (4 wk) were purchased from Shanghai Slac Laboratory Animal Co. LTD. Thirty-six Balb/c nude mice were randomly divided into three groups: Control group, MNNG-induced subclone injected group, and MNU-induced subclone injected group. The mice were subcutaneously injected with 1×10^6 MNNG/MNU-transformed cells (100 μL). Three days after injection, the long diameter (a) and short diameter (b) of the tumors were measured, after which the volume (V) was calculated using the formula $V = 1/2 \times a \times b^2$. Mice were sacrificed, and the tumor tissues were obtained and weighed. The animal experiments were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. The Committee on the Use of Animals of Zhejiang University, China approved the study protocol of our experiments.

Cell transfection and RNA interference

The MGMT protein coding sequences was subcloned into PCDNA3.1 vector. The transformed cells were transfected with the MGMT overexpression plasmid and PCDNA3.1 empty vector (EV), respectively. Then, the proliferative activity of cells was analyzed by colony formation and soft agar assays.

SiRNAs targeting human *MGMT* (GenePharma, Shanghai, China) were transfected into cells using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. A siRNA negative control (siRNA NC) was also used.

Statistical analysis

The two-tailed Student's *t* test and one-way analysis of variance were used for statistical analyses. The data are expressed as the mean ± SD from three separate experiments. $P \leq 0.05$ was considered statistically significant.

RESULTS

MGMT expression is upregulated in early stage GC

To study the role of MGMT in GC development, especially in the early events and tumor initiation, we detected the expression of MGMT in 19 clinical early stage GC tissues. The immunohistochemistry analysis showed that MGMT expression was increased in early stage cancer tissues compared with the normal tissue, though there was an individual difference (Figure 1A). qPCR analysis of endoscopic biopsy samples confirmed the upregulation of *MGMT* mRNA expression in early stage cancer tissues compared with adjacent normal tissues (Figure 1B). Moreover, MGMT expression was also enhanced in the GC cell lines at both the mRNA and protein levels (Figure 1C). Collectively, these results suggest that MGMT expression is upregulated in early stage GC.

MGMT is upregulated in NOCs-induced gastric epithelial cell malignant transformation

To investigate the molecular mechanism of MGMT upregulation, we established a gastric epithelial cell (GES-1) malignant transformation model following MNNG and MNU exposure. MNNG/MNU-treated cells showed an increase of cell proliferation, anchorage-independent growth capability, and colony formation ability, as demonstrated by cell proliferative assay, soft agar assay, and colony formation assays, respectively (Figure 2A-C). We also observed that the cell migration was enhanced upon MNNG/MNU treatment by wound healing assay (Figure 2D). Xenograft assay showed that MNNG/MNU-induced transformed cells demonstrated increased tumor growth (Figure 2E and F), further confirming the malignant phenotypes of NOCs-induced transformed GES-1 cells. Then, we detected the MGMT expression in MNNG/MNU-transformed cells. MGMT expression was persistently increased during the malignant transformation process (Figure 3A and B). But the extent of MGMT upregulation was decreased after removal of MNNG/MNU exposure for 12 wk (data not shown), suggesting that MGMT expression demonstrated a dynamic change in MNNG/MNU-induced cell malignant transformation process. In particular, the augmentation of MGMT expression was negatively correlated with the colony-forming ability of the MNNG/MNU-transformed cell subclones (Figure 3C), but it was positively correlated with the anti-apoptotic effect of malignant transformed cell subclones (Figure 3D).

DNA hypomethylation is responsible for MGMT upregulation in cell malignant transformation

To further investigate the regulatory mechanism underlying the MGMT upregulation upon MNNG/MNU treatment, we constructed a *MGMT* gene promoter luciferase reporter. We did not find an increase of the *MGMT* gene promoter in MNNG/MNU-induced cells by dual-luciferase reporter

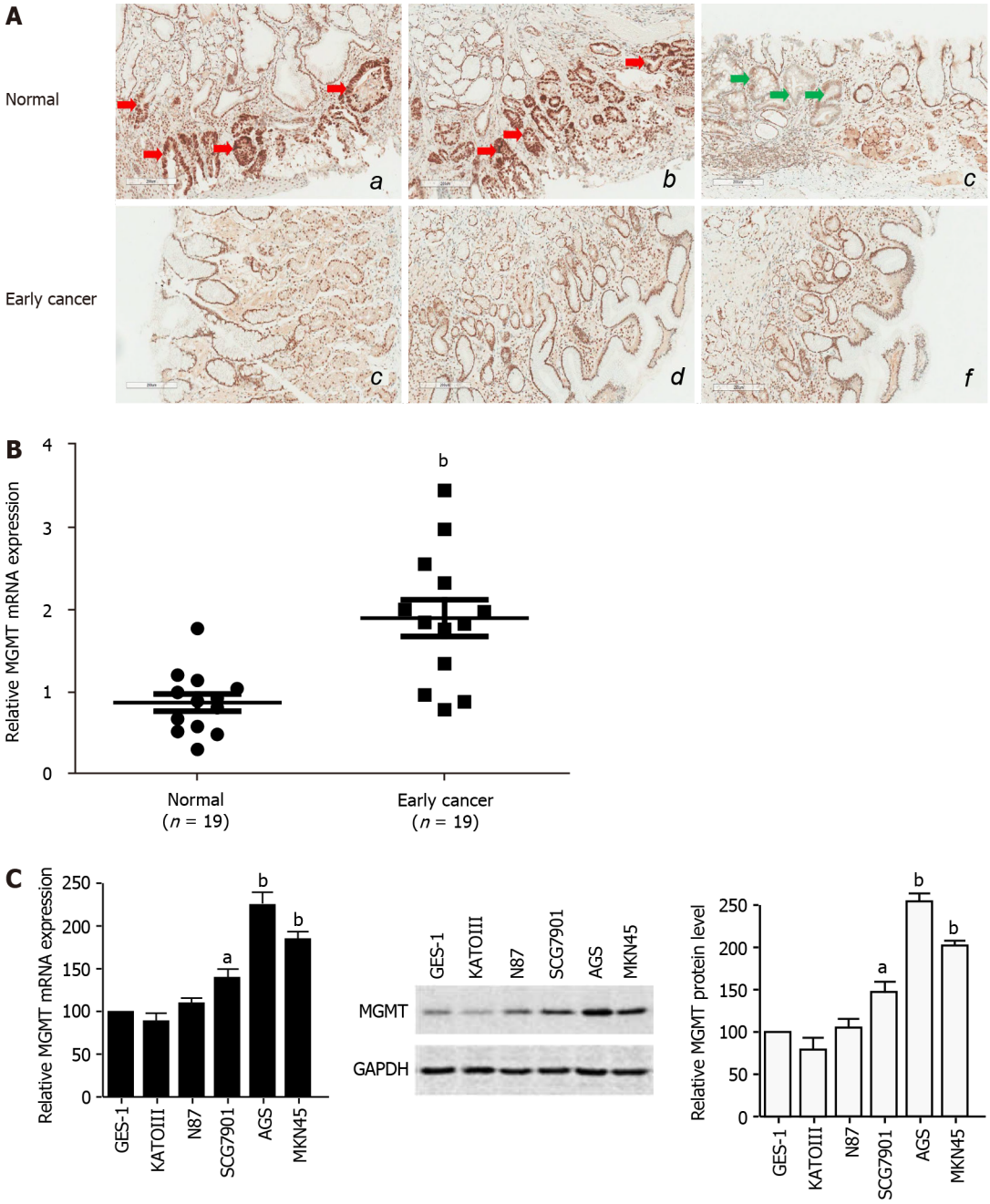

Figure 1 O^6-methylguanine-DNA methyltransferase expression is enhanced in early stage gastric cancer. A: Representative images of immunohistochemistry staining for O^6-methylguanine-DNA methyltransferase (MGMT) in early stage gastric tumor and normal tissues (n = 19); B: The mRNA level of *MGMT* in early stage gastric tumor and adjacent normal tissues (n = 19). The mRNA expression was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH); C: MGMT mRNA and protein expression in normal gastric epithelial cells and cancer cells by quantitative real-time polymerase chain reaction and immunoblot assays. GAPDH was used to normalize MGMT expression. The analyses were repeated three times, and the results are expressed as the mean ± SD. $^aP < 0.05$; $^bP < 0.01$. MG-C: MNNG-induced malignant transformed cell; MU-C: MNU-induced malignant transformed cell; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

assay. We used p53 and JunD as the positive controls, since it was reported that they are the transcriptional activators of the *MGMT* promoter (Figure 4A). It is known that MGMT expression was closely related with its promoter DNA methylation in different cancers. We preformed MSP to detect the DNA methylation level in the *MGMT* promoter, which showed that in MNNG/MNU-transformed subcolones, unmethylated DNA was accumulated, indicating a reduction of DNA methylation level in the *MGMT* gene promoter (Figure 4B). Since it is known that HeLa cells exhibit high expression of MGMT with a low DNA methylation level in the promoter region, they were used as a positive control. M. SssI (CpG methyltransferase) treated cell was used as a negative control. As shown by BSP analysis, MNNG/MNU-transformed subcolones showed few DNA methylation sites compared with the controls, confirming the reduction of DNA methylation level in the *MGMT* gene promoter (Figure 4C). Furthermore, we used 5-aza, a DNMT specific inhibitor, to treat CES-1 cells. After 48 and 72 h, MGMT

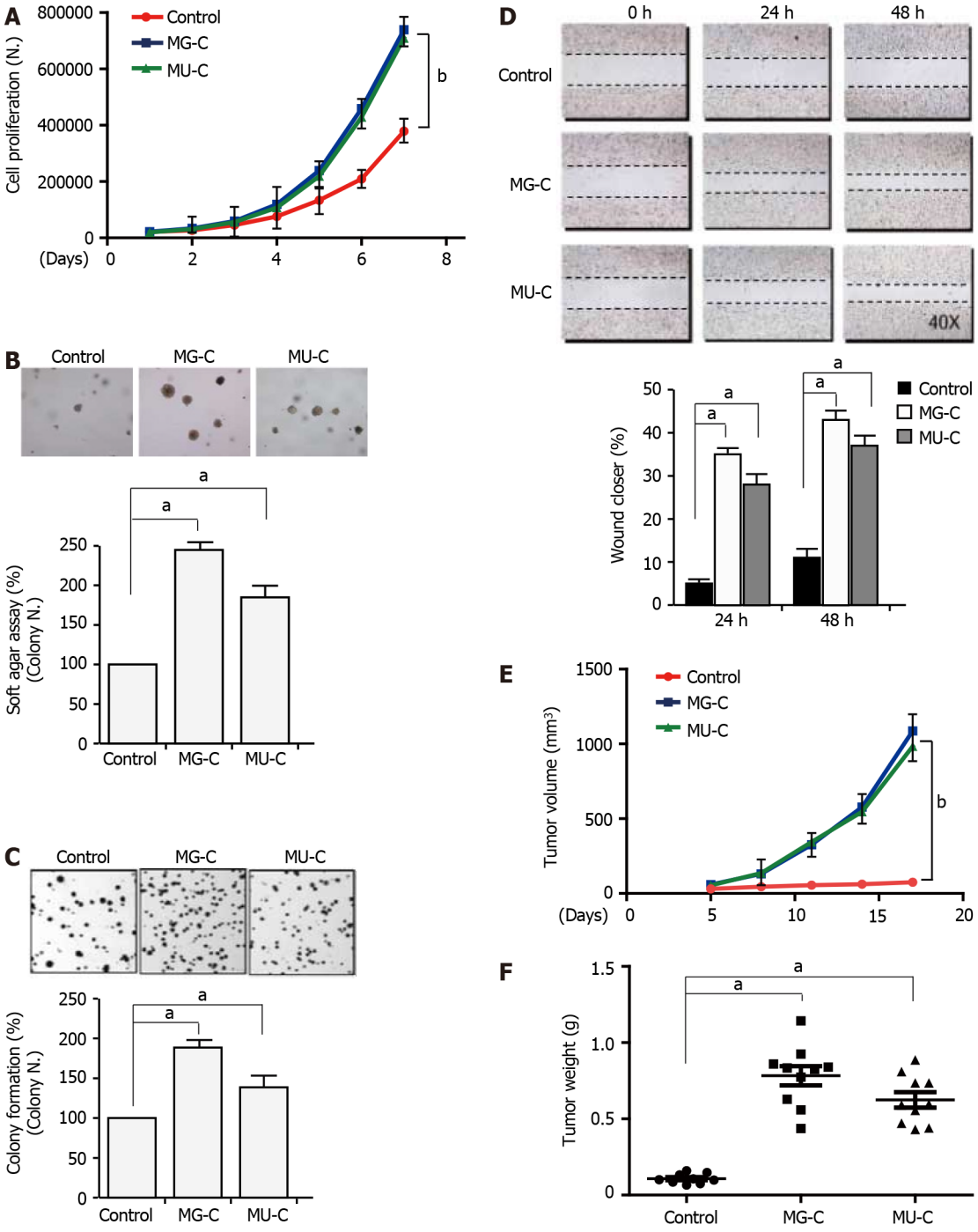

Figure 2 N-nitroso compound treatment induces gastric epithelial cell malignant transformation. A: Cell proliferation monitored by cell counting in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)/N-methyl-N-nitroso-urea (MNU)-treated and control cells; B and C: Cell anchorage-independent growth on soft agar and cell colony formation. Top, representative images; bottom, quantitative results of cell colony *per* field; D: Wound healing assay. Top, representative images of wound healing assay; right, relative percentage of wound closure after treatment; E and F: Tumor growth curve and tumor weight in nude mice injected subcutaneously with the transformed cells induced by MNNG/MNU and control cells. The analyses were repeated three times, and the results are expressed as the mean ± SD. $^{a}P < 0.05$; $^{b}P < 0.01$.

expression was increased upon 5-aza treatment (Supplementary Figure 1). Moreover, based on the CCLE database, we found that MGMT expression was negatively related with DNA methylation levels (Figure 4D), indicating that the DNA methylation level is involved in the upregulation of MGMT.

Next, we preformed ChIP-PCR with anti-DNMT1 and anti-H3K9Met3 and anti-H3K4Met2 (against specific methylation sites) antibodies. H3K9Met3 was known as a transcriptional inhibition signal and H3K4Met2 was reported as a transcriptional activation signal. The results showed that the DMNT1 recruitment was significantly decreased to the promoter region of *MGMT*. We also detected the reduction of H3K9Met3 and the augment of H3K4Met2 located in the promoter region of *MGMT*, as well as a reduction of DNMT1 binding to the *MGMT* promoter (Figure 4E). The results suggested that the upregulation of MGMT expression was dependent on the DNA hypomethylation in its promoter.

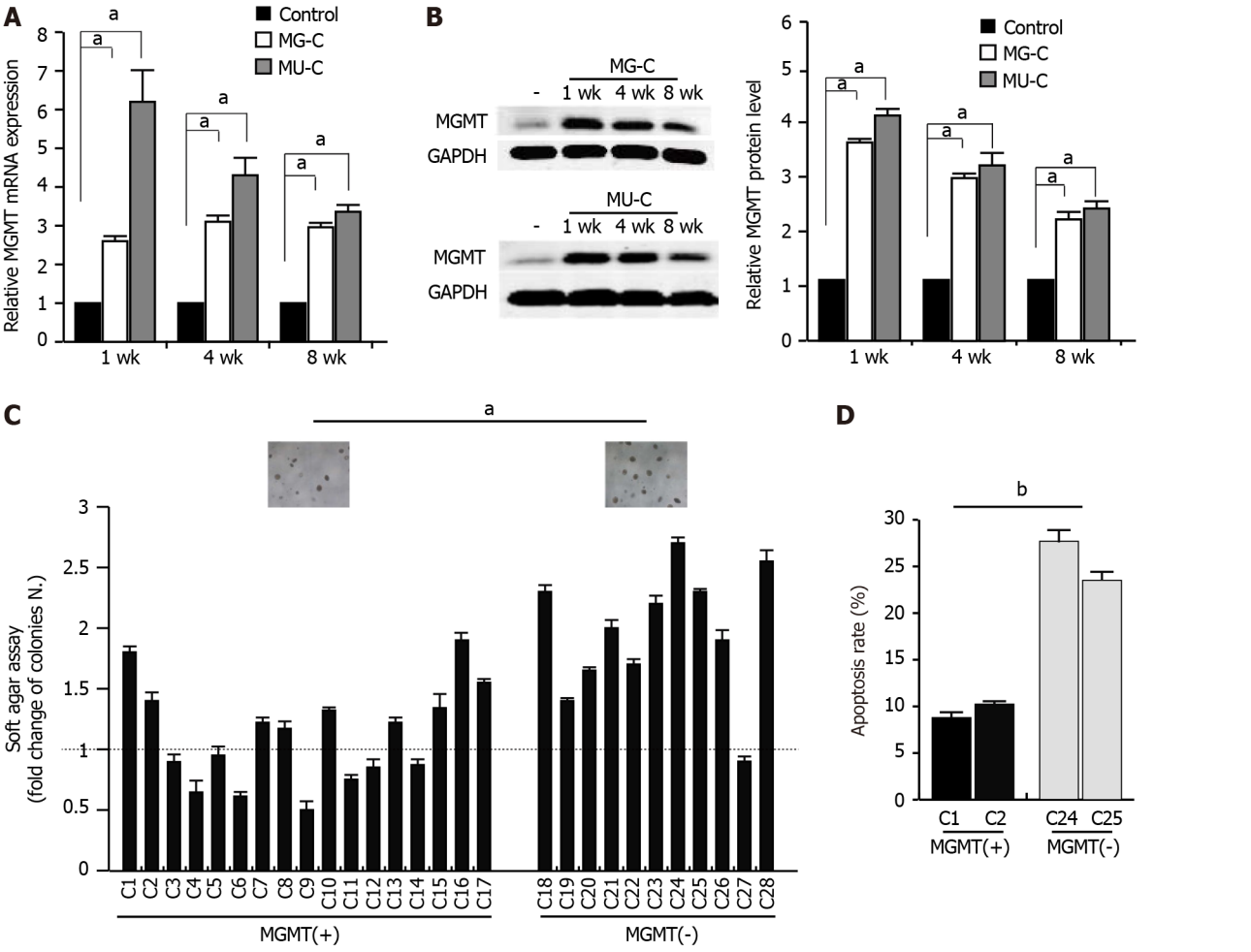

Figure 3 O⁶-methylguanine-DNA methyltransferase is downregulated in N-nitroso compound-induced gastric epithelial cell malignant transformation. A and B: O⁶-methylguanine-DNA methyltransferase (MGMT) mRNA and protein expression in transformed gastric epithelial cells induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)/N-methyl-N-nitroso-urea (MNU) for 1, 4, and 8 wk; C: Cell anchorage-independent growth on soft agar for subcolones of MNNG/MNU-induced cells. C1-28: Different subcolones of MNNG/MNU-induced cells; MGMT(+): MGMT expression is upregulated in these subcolones; MGMT(-): MGMT expression is downregulated or no-changed in these subcolones; D: Apoptosis assay of MNNG/MNU-transformed subcolones after doxycycline treatment. The analyses were repeated three times, and the results are expressed as the mean ± SD. $^{a}P < 0.05$; $^{b}P < 0.01$. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Interestingly, after removal of NOCs exposure for 12 wk, the binding of DNMT1 to the *MGMT* gene promoter returned to the baseline level. The same changes of H3K9Met3 and H3K4Met2 levels in the *MGMT* gene promoter were also observed. These data suggest that the binding of DNMT1 to the *MGMT* gene promoter changes dynamically, which could help us to understand the dynamic changes of MGMT expression regulated by DNA methylation in NOCs-induced malignant transformation.

Inhibition of MGMT contributes to the NOCs-induced cell malignant phenotype

To evaluate the role of MGMT in NOCs-induced cell malignant transformation, we used O⁶-benzylguanine (O⁶-BG), a specific inhibitor of MGMT, to inhibit the activity of MGMT. After treatment with O⁶-BG at different concentrations, we found a reduction of MGMT expression (Supplementary Figure 1). MTT assay showed that treatment with O⁶-BG at low doses did not induce a decrease of cell viability (Supplementary Figure 1). Therefore, we used 2 μM O⁶-BG in the subsequent experiments. O⁶-BG exposure increased the proliferative activity and anchorage-independent growth capability of MNNG/MNU-induced cells (Figure 5A and B). Knock-down of MGMT in MNNG/MNU-transformed cells also resulted in the increase of cell reproductive activity (Figure 5C and D). Moreover, overexpressed MGMT resulted in the decrease of cell proliferative activity in MNNG/MNU-transformed cells (Figure 5E and F). In addition, MGMT was upregulated in precancerous lesions (gastric metaplasia) and early stage GC compared with non-cancerous lesions (gastritis), but the MGMT level was reduced in the advanced GC tissues compared with the precancerous lesion and early tumor tissues (Figure 5G), indicating a protective role of MGMT in GC progression.

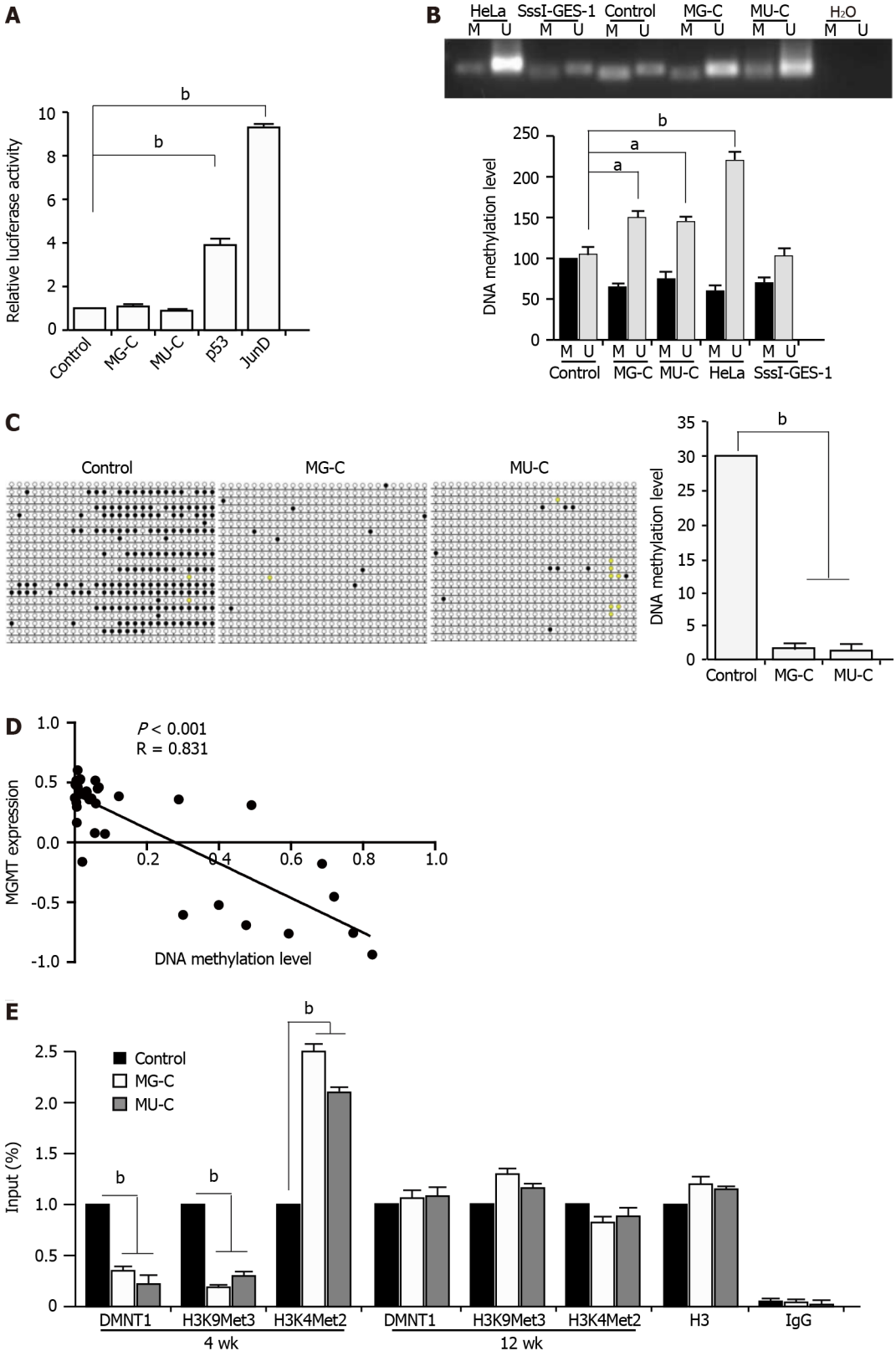

Figure 4 DNA hypomethylation contributes to O^6-methylguanine-DNA methyltransferase upregulation in cell malignant transformation. A: Luciferase reporter assay in control and N-nitroso compound-transformed cells using PGL3-O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter; B and C: Methylation specific polymerase chain reaction and bisulfite genomic sequence analysis of the DNA methylation level of N-methyl-N'-nitro-N-nitrosoguanidine/N-methyl-N-nitroso-urea-induced transformed cells compared with control cells; D: Correlation of MGMT expression and DNA methylation level of *MGMT* promoter based on the CCLE database; E: ChIP assay with anti-DNMT1 and anti-H3K9Me3 and H3K4Me2 antibodies for analyzing the DNMT1 binding to the *MGMT* promoter and the H3K9Me3 and H3K4Me2 levels in the *MGMT* promoter. The analyses were repeated three times, and the results are expressed as the mean ± SD. $^aP < 0.05$; $^bP < 0.01$. M: Methylated; U: Unmethylated; IgG: Immunoglobulin G.

DISCUSSION

By causing DNA damages and activating downstream pathways that promote cancer initiation and development, NOCs can directly induce cell malignant transformation, thus contributing to gastric carcinogenesis[23,24]. The formation of DNA adducts induced by NOCs has been studied in different studies[19,20,25,26]. The present study focused on early events and the molecular mechanisms of *MGMT* gene dysregulation in cell malignant transformation and gastric tumorigenesis following MNNG/MNU exposure. Our data showed persistent upregulation of MGMT expression in gastric epithelial cell malignant transformation induced by NOCs. The reduction of *MGMT* gene promoter DNA methylation level was responsible for the increase of MGMT expression in MNNG/MNU-treated cells. Inhibited MGMT expression promoted the MNNG/MNU-induced malignant phenotype, while overexpression of MGMT partially reversed the cell malignant transformation phenotype, suggesting that stable MGMT upregulation induced by its promoter DNA hypomethylation prevented the NOCs-induced cell malignant transformation and tumorigenesis.

Studies have reported that NOCs can directly act on DNA, mainly cause O^6-methylguanine damage, and subsequently induce DNA mutation and double strand breaks, participating in cancer formation and progression[19,20]. The administration of MNNG can cause the destruction of pyloric mucosal structure and the occurrence of gastric adenocarcinoma in rats[24]. MNNG exposure can also induce the mutation and amplification of oncogenes participating in the occurrence of GC[26]. Moreover, the chromatin-based epigenetics regulation induced by NOCs, especially DNA methylation and histone modifications, has an essential role in cancer biology[27]. In the current study, we demonstrated that *MGMT* gene expression was rapidly increased after MNNG/MNU exposure, and the upregulation was continuously maintained during the early phase of cell malignant transformation. However, the extent of increased MGMT expression level was reduced progressively, leading us to speculate that the dynamic changes of MGMT expression could be involved in different steps of chemical carcinogens-induced gastric cell malignant transformation and tumorigenesis.

MGMT can remove O^6-guanosine alkylation adducts caused by alkylation agents from DNA sequence in one-step reaction that restores the O^6-guanosine residue to itself, consequently forming an inactive form[28]. Hence, the expression level of MGMT is fundamental for accurate DNA repair. It has been known that the transcriptional mechanism and epigenetic regulation are important to regulate the MGMT expression[1,29]. Hypoxia inducible factor 1-α can upregulate the expression level of MGMT and increase the drug resistance of glioma stem cells to temozolomide[30]. In addition, microRNAs can also bind to the 3'-untranslated region of *MGMT*, reduce the stability of the mRNA, and affect protein translation[31,32]. Moreover, *MGMT* gene promoter region lacks TATA box and CAAT box, but has rich GC sequence, which is prone to be methylated and closely related to transcriptional regulation[33]. DNA methylation in the *MGMT* gene has been reported in various human cancers, which can increase the sensitivity to alkylating agents in chemotherapy, influencing the tumor prognosis. However, the high level of gene methylation is usually associated with the low expression of protein level. Inhibition of MGMT protein level decreases its ability of removing O^6-guanosine from the damaged DNA sites, resulting in an increase of mutation frequency and easily leading to the occurrence of tumor[34-36]. We found that MGMT upregulation was regulated by DNA hypomethylation in its gene promoter. And the subclones with high a level of MGMT showed a weak malignant proliferative activity, but with a strong anti-apoptotic effect upon exposure to DNA damage agents. This result suggested a protective effect of MGMT against NOCs-induced cell malignant transformation. Using O^6-BG and by knocking MGMT down with siRNA, we showed an increased malignant proliferative ability of the transformed cells. Overexpressed MGMT decreased this effect, confirming the protected role of MGMT following chemical carcinogen exposure. In particular, the ChIP assay showed that DNMT1 was responsible for the *MGMT* gene promoter methylation. After 12 wk of cell transformation, the MGMT expression level was restored by recovering DNMT1 binding to the *MGMT* promoter region. This result suggested dynamic changes of MGMT expression, which is regulated by DNA methylation. Analysis of clinical gastric tissue samples also confirmed the dynamic changes of MGMT expression in gastric carcinogenesis. Taken together, we hypothesize that MGMT expression shows dynamic changes in gastric tumorigenesis induced by chemical carcinogens. It can be upregulated in the initiation phase for repairing the DNA damage and helping cells survive upon NOCs exposure; but in the progressive stage, it can be restored to the normal level to facilitate GC development. Hence, revealing the molecular mechanism of dynamic regulation of MGMT expression is important to help us understand the role of MGMT in GC formation and progression. However, the exact regulatory mechanisms of the dynamic changes on MGMT expression in different stages of cancer progression need to be further investigated (Supplementary Table 1).

CONCLUSION

In summary, our current study revealed the molecular mechanism of MGMT upregulation mediated by DNA hypomethylation of its gene promoter in NOCs-induced gastric cell malignant transformation,

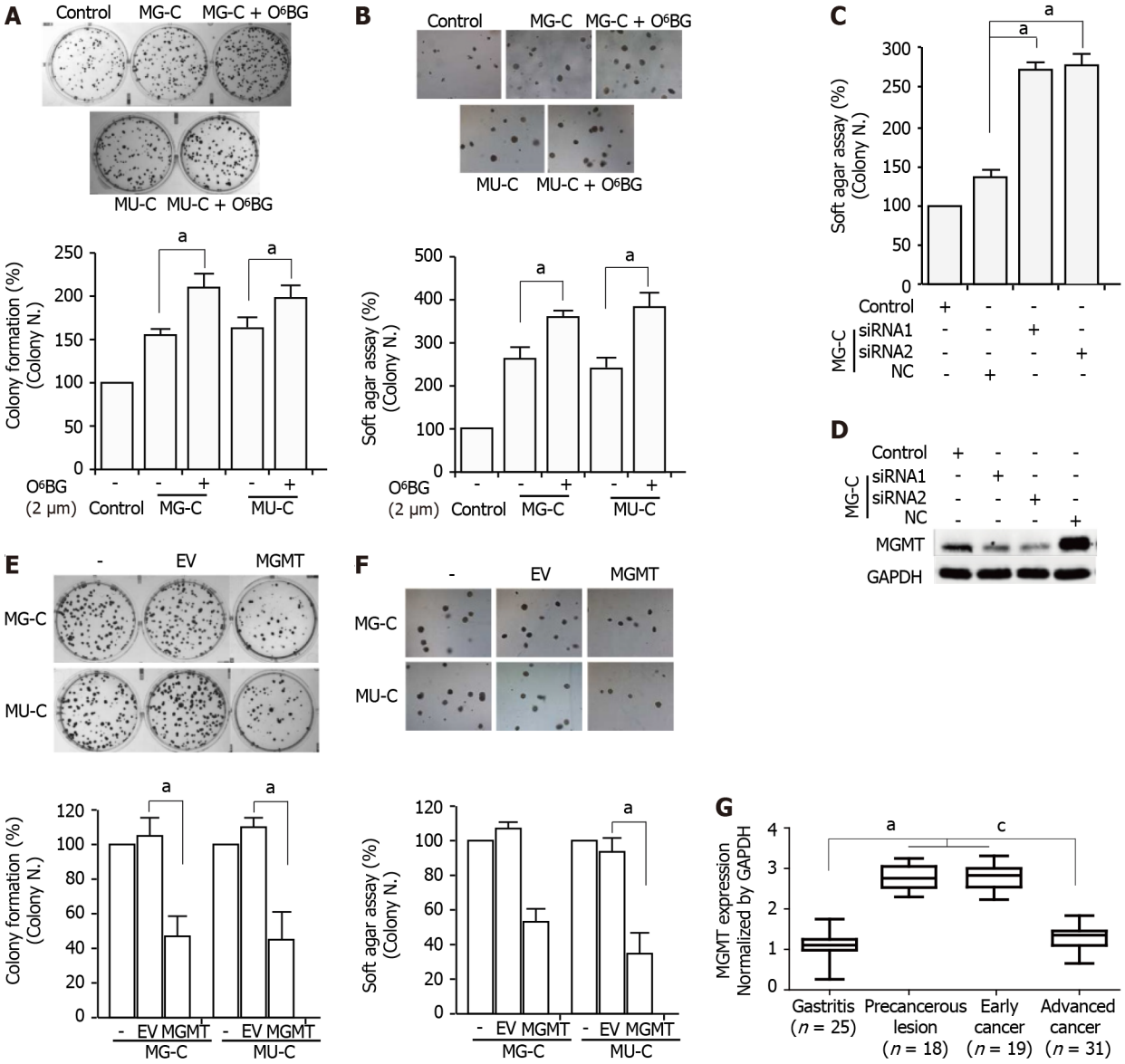

Figure 5 Inhibition of O⁶-methylguanine-DNA methyltransferase contributes to the N-nitroso compound-induced cell malignant phenotype. A and B: Cell anchorage-independent growth on soft agar and cell colony formation of N-methyl-N'-nitro-N-nitrosoguanidine/N-methyl-N-nitroso-urea-induced cells after O⁶-BG treatment; C: Cell anchorage-independent growth on soft agar of cells with O⁶-methylguanine-DNA methyltransferase (MGMT) knock-down; D: Knock-down efficiency of MGMT detected by Western blot; E and F: Cell anchorage-independent growth on soft agar and cell colony formation of MGMT overexpressing cells; G: The mRNA expression of *MGMT* in gastric endoscopic biopsy samples. The analyses were repeated three times, and the results are expressed as the mean ± SD. $^{a,c}P < 0.05$. $^{c}P < 0.05$, precancerous lesion and early cancer *vs* advanced cancer. EV: Empty vector; MGMT: MGMT overexpression; MGMT: O⁶-methylguanine-DNA methyltransferase.

and showed the dual effects of MGMT by regulating its expression level in chemical carcinogen-induced tumorigenesis. Our findings provide a dynamic regulatory mechanism by which MGMT is implicated in cell malignant transformation and tumorigenesis induced by NOCs, and shed new light on MGMT as a potential diagnostic and therapeutic target for gastric carcinogenesis intervention by regulating aberrant epigenetic mechanisms.

ARTICLE HIGHLIGHTS

Research background

O⁶-methylguanine-DNA methyltransferase (MGMT) is a specific enzyme that repairs the mispairing base O⁶-methyl-guanine induced by methylating environmental and experimental carcinogens. The N-nitroso compounds (NOCs) N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitroso-urea (MNU) are monofunctional alkylating agents which can directly bind to the DNA and induce the formation of O⁶-methylguanine adducts to promote gene mutation and tumorigenesis. They are widely

accepted chemical carcinogens for studying the mechanisms of mutagenesis and carcinogenesis induced by NOCs.

Research motivation

The underlying regulatory mechanism of MGMT involved in NOCs-induced tumorigenesis, especially in the initiation phase, remains largely unclear.

Research objectives

To investigate the molecular regulatory mechanism of MGMT in NOCs-induced gastric cell malignant transformation and tumorigenesis.

Research methods

We established a gastric epithelial cell malignant transformation model induced by MNNG or MNU treatment. Cell proliferation, colony formation, soft agar, cell migration, and xenograft assays were used to verify the malignant phenotype. By using quantitative real-time polymerase chain reaction (qPCR) and Western blot analysis, we detected the MGMT expression in malignant transformed cells. We also confirmed the MGMT expression in clinical early stage gastric tumor tissues by qPCR and immunohistochemistry. *MGMT* gene promoter DNA methylation level was analyzed by methylation-specific PCR and bisulfite sequencing PCR. The effect of MGMT in cell malignant transformation was analyzed by colony formation and soft agar assays.

Research results

MGMT expression was upregulated in NOCs-induced gastric cell malignant transformation and in clinical early stage gastric cancer tissues. The upregulation of MGMT was regulated by the hypomethylation of its DNA promoter.

Research conclusions

The upregulation of MGMT expression is mediated by the hypomethylation of its DNA promoter in NOCs-induced gastric cell malignant transformation.

Research perspectives

The findings provide a dynamic regulatory mechanism of MGMT expression in cell malignant transformation and tumorigenesis induced by NOCs, supporting that MGMT might be a potential diagnostic and therapeutic target for gastric carcinogenesis.

FOOTNOTES

Author contributions: Chen YX and Lulu He contributed to the acquisition and analyses of the data; Xiang XP contributed to the collection and immunostaining of clinical samples; Shen J and Qi HY designed the study and made the critical revisions of the manuscript.

Supported by National Natural Science Foundation of China, No. 81472543 and No. 81772919; Zhejiang Provincial Natural Science Foundation of China, No. LY18H160024 and No. LY20H160040.

Institutional review board statement: The study was approved by the ethics committee of Zhejiang University School of Medicine (No. 2017026).

Institutional animal care and use committee statement: The work described herein was approved by the Laboratory Animals Welfare Ethics Review Committee of Zhejiang University (No. ZJU20170522).

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

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S-Editor: Fan JR
L-Editor: Wang TQ
P-Editor: Yuan YY

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]
3 **Padmanabhan N**, Ushijima T, Tan P. How to stomach an epigenetic insult: the gastric cancer epigenome. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 467-478 [PMID: 28513632 DOI: 10.1038/nrgastro.2017.53]
4 **Yamashita S**, Kishino T, Takahashi T, Shimazu T, Charvat H, Kakugawa Y, Nakajima T, Lee YC, Iida N, Maeda M, Hattori N, Takeshima H, Nagano R, Oda I, Tsugane S, Wu MS, Ushijima T. Genetic and epigenetic alterations in normal tissues have differential impacts on cancer risk among tissues. *Proc Natl Acad Sci U S A* 2018; **115**: 1328-1333 [PMID: 29358395 DOI: 10.1073/pnas.1717340115]
5 **Tie J**, Zhang X, Fan D. Epigenetic roles in the malignant transformation of gastric mucosal cells. *Cell Mol Life Sci* 2016; **73**: 4599-4610 [PMID: 27464701 DOI: 10.1007/s00018-016-2308-9]
6 **Klutstein M**, Nejman D, Greenfield R, Cedar H. DNA Methylation in Cancer and Aging. *Cancer Res* 2016; **76**: 3446-3450 [PMID: 27256564 DOI: 10.1158/0008-5472.CAN-15-3278]
7 **Takeshima H**, Ushijima T. Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. *NPJ Precis Oncol* 2019; **3**: 7 [PMID: 30854468 DOI: 10.1038/s41698-019-0079-0]
8 **Zheng SC**, Widschwendter M, Teschendorff AE. Epigenetic drift, epigenetic clocks and cancer risk. *Epigenomics* 2016; **8**: 705-719 [PMID: 27104983 DOI: 10.2217/epi-2015-0017]
9 **Christmann M**, Verbeek B, Roos WP, Kaina B. O(6)-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry. *Biochim Biophys Acta* 2011; **1816**: 179-190 [PMID: 21745538 DOI: 10.1016/j.bbcan.2011.06.002]
10 **Mur P**, Rodríguez de Lope Á, Díaz-Crespo FJ, Hernández-Iglesias T, Ribalta T, Fiaño C, García JF, Rey JA, Mollejo M, Meléndez B. Impact on prognosis of the regional distribution of MGMT methylation with respect to the CpG island methylator phenotype and age in glioma patients. *J Neurooncol* 2015; **122**: 441-450 [PMID: 25682093 DOI: 10.1007/s11060-015-1738-9]
11 **Wick W**, Platten M. Understanding and targeting alkylator resistance in glioblastoma. *Cancer Discov* 2014; **4**: 1120-1122 [PMID: 25274683 DOI: 10.1158/2159-8290.CD-14-0918]
12 **Gerson SL**. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* 2004; **4**: 296-307 [PMID: 15057289 DOI: 10.1038/nrc1319]
13 **Soejima H**, Zhao W, Mukai T. Epigenetic silencing of the MGMT gene in cancer. *Biochem Cell Biol* 2005; **83**: 429-437 [PMID: 16094446 DOI: 10.1139/o05-140]
14 **Fornaro L**, Vivaldi C, Caparello C, Musettini G, Baldini E, Masi G, Falcone A. Pharmacoepigenetics in gastrointestinal tumors: MGMT methylation and beyond. *Front Biosci (Elite Ed)* 2016; **8**: 170-180 [PMID: 26709653]
15 **Alizadeh Naini M**, Kavousipour S, Hasanzarini M, Nasrollah A, Monabati A, Mokarram P. O6-Methyguanine-DNA Methyl Transferase (MGMT) Promoter Methylation in Serum DNA of Iranian Patients with Colorectal Cancer. *Asian Pac J Cancer Prev* 2018; **19**: 1223-1227 [PMID: 29801405 DOI: 10.22034/APJCP.2018.19.5.1223]
16 **Gu C**, Lu J, Cui T, Lu C, Shi H, Xu W, Yuan X, Yang X, Huang Y, Lu M. Association between MGMT promoter methylation and non-small cell lung cancer: a meta-analysis. *PLoS One* 2013; **8**: e72633 [PMID: 24086261 DOI: 10.1371/journal.pone.0072633]
17 **Yousuf A**, Bhat MY, Pandith AA, Afroze D, Khan NP, Alam K, Shah P, Shah MA, Mudassar S. MGMT gene silencing by promoter hypermethylation in gastric cancer in a high incidence area. *Cell Oncol (Dordr)* 2014; **37**: 245-252 [PMID: 25008999 DOI: 10.1007/s13402-014-0179-3]
18 **Oldrini B**, Vaquero-Siguero N, Mu Q, Kroon P, Zhang Y, Galán-Ganga M, Bao Z, Wang Z, Liu H, Sa JK, Zhao J, Kim H, Rodriguez-Perales S, Nam DH, Verhaak RGW, Rabadan R, Jiang T, Wang J, Squatrito M. MGMT genomic rearrangements contribute to chemotherapy resistance in gliomas. *Nat Commun* 2020; **11**: 3883 [PMID: 32753598 DOI: 10.1038/s41467-020-17717-0]
19 **Mirvish SS**. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 1995; **93**: 17-48 [PMID: 7600541 DOI: 10.1016/0304-3835(95)03786-V]
20 **Loh YH**, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am J Clin Nutr* 2011; **93**: 1053-1061 [PMID: 21430112 DOI: 10.3945/ajcn.111.012377]
21 **Chen K**, Zhang S, Ke X, Qi H, Shao J, Shen J. Biphasic reduction of histone H3 phosphorylation in response to N-nitroso compounds induced DNA damage. *Biochim Biophys Acta* 2016; **1860**: 1836-1844 [PMID: 27233451 DOI: 10.1016/j.bbagen.2016.05.028]
22 **Qi H**, Yang Z, Dai C, Wang R, Ke X, Zhang S, Xiang X, Chen K, Li C, Luo J, Shao J, Shen J. STAT3 activates MSK1-

mediated histone H3 phosphorylation to promote NFAT signaling in gastric carcinogenesis. *Oncogenesis* 2020; **9**: 15 [PMID: 32041943 DOI: 10.1038/s41389-020-0195-2]

23 **Higginson J**, DeVita VT Jr. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. *Am Ind Hyg Assoc J* 1980; **41**: A26, A28, A30 passim [PMID: 6998267]

24 **Sugimura T**, Fujimura S. Tumour production in glandular stomach of rat by N-methyl-N'-nitro-N-nitrosoguanidine. *Nature* 1967; **216**: 943-944 [PMID: 6074981 DOI: 10.1038/216943a0]

25 **Kwak H**, Lee M, Cho M. Interrelationship of apoptosis, mutation, and cell proliferation in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced medaka carcinogenesis model. *Aquat Toxicol* 2000; **50**: 317-329 [PMID: 10967394 DOI: 10.1016/s0166-445x(00)00093-x]

26 **Arber N**, Hibshoosh H, Moss SF, Sutter T, Zhang Y, Begg M, Wang S, Weinstein IB, Holt PR. Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. *Gastroenterology* 1996; **110**: 669-674 [PMID: 8608874 DOI: 10.1053/gast.1996.v110.pm8608874]

27 **Baccarelli A**, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr* 2009; **21**: 243-251 [PMID: 19663042 DOI: 10.1097/mop.0b013e32832925cc]

28 **Iyama T**, Wilson DM 3rd. DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair (Amst)* 2013; **12**: 620-636 [PMID: 23684800 DOI: 10.1016/j.dnarep.2013.04.015]

29 **Bocangel D**, Sengupta S, Mitra S, Bhakat KK. p53-Mediated down-regulation of the human DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) *via* interaction with Sp1 transcription factor. *Anticancer Res* 2009; **29**: 3741-3750 [PMID: 19846904]

30 **Pistollato F**, Abbadi S, Rampazzo E, Persano L, Della Puppa A, Frasson C, Sarto E, Scienza R, D'avella D, Basso G. Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. *Stem Cells* 2010; **28**: 851-862 [PMID: 20309962 DOI: 10.1002/stem.415]

31 **Wang J**, Sai K, Chen FR, Chen ZP. miR-181b modulates glioma cell sensitivity to temozolomide by targeting MEK1. *Cancer Chemother Pharmacol* 2013; **72**: 147-158 [PMID: 23645289 DOI: 10.1007/s00280-013-2180-3]

32 **Jesionek-Kupnicka D**, Braun M, Trąbska-Kluch B, Czech J, Szybka M, Szymańska B, Kulczycka-Wojdala D, Bieńkowski M, Kordek R, Zawlik I. MiR-21, miR-34a, miR-125b, miR-181d and miR-648 Levels inversely correlate with MGMT and TP53 expression in primary glioblastoma patients. *Arch Med Sci* 2019; **15**: 504-512 [PMID: 30899304 DOI: 10.5114/aoms.2017.69374]

33 **Malley DS**, Hamoudi RA, Kocialkowski S, Pearson DM, Collins VP, Ichimura K. A distinct region of the MGMT CpG island critical for transcriptional regulation is preferentially methylated in glioblastoma cells and xenografts. *Acta Neuropathol* 2011; **121**: 651-661 [PMID: 21287394 DOI: 10.1007/s00401-011-0803-5]

34 **Butler M**, Pongor L, Su YT, Xi L, Raffeld M, Quezado M, Trepel J, Aldape K, Pommier Y, Wu J. MGMT Status as a Clinical Biomarker in Glioblastoma. *Trends Cancer* 2020; **6**: 380-391 [PMID: 32348734 DOI: 10.1016/j.trecan.2020.02.010]

35 **Yu W**, Zhang L, Wei Q, Shao A. O^6-Methylguanine-DNA Methyltransferase (MGMT): Challenges and New Opportunities in Glioma Chemotherapy. *Front Oncol* 2019; **9**: 1547 [PMID: 32010632 DOI: 10.3389/fonc.2019.01547]

36 **Bouras E**, Karakioulaki M, Bougioukas KI, Aivaliotis M, Tzimagiorgis G, Chourdakis M. Gene promoter methylation and cancer: An umbrella review. *Gene* 2019; **710**: 333-340 [PMID: 31202904 DOI: 10.1016/j.gene.2019.06.023]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 678-689

DOI: 10.4251/wjgo.v14.i3.678

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

RNA-Seq profiling of circular RNAs in human colorectal cancer 5-fluorouracil resistance and potential biomarkers

Pei-Qiu Cheng, Yu-Jing Liu, Sheng-An Zhang, Lu Lu, Wen-Jun Zhou, Dan Hu, Han-Chen Xu, Guang Ji

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Elkady N, Kołat D, Socea B

Received: July 28, 2021
Peer-review started: July 28, 2021
First decision: September 5, 2021
Revised: September 10, 2021
Accepted: January 22, 2022
Article in press: January 22, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

Colorectal cancer (CRC) is a commonly diagnosed cancer of the digestive system worldwide. Although chemotherapeutic agents and targeted therapeutic drugs are currently available for CRC treatment, drug resistance is a problem that cannot be ignored and needs to be solved.

AIM

To explore the relationship between circular RNA (circRNA) and CRC drug resistance. circRNA plays a key role in the occurrence and development of cancers, but its function in the process of drug resistance has not been widely revealed.

METHODS

To explore the role of circRNA in 5-fluorouracil (5-Fu) resistance, we performed the circRNA expression profile in two CRC cell lines and their homologous 5-Fu resistant cells by high-throughput sequencing.

RESULTS

We validated the differentially expressed circRNAs in other two paired CRC cells, confirmed that circ_0002813 and circ_0000236 could have a potential competitive endogenous RNA mechanism and be involved in the formation of 5-Fu resistance. And we combined the sequencing results of mRNA to construct the regulatory network of circRNA-miRNA-mRNA.

CONCLUSION
Our study revealed that circ_0002813 and circ_0000236 may as the biomarkers to predict the occurrence of 5-Fu resistance in CRC.

Key Words: Colorectal cancer; 5-Fluorouracil resistance; Circular RNAs; RNA sequencing; Network prediction; Biomarkers

Core Tip: Therapy resistance has been a culprit for colorectal cancer (CRC) treatment. 5-fluorouracil is a first-line chemotherapeutic agent for CRC, and it is very important to reveal the potential biomarkers and mechanisms of resistance. Circular RNA (circRNA) plays a key role in the occurrence and development of cancers, but its function in the process of drug resistance has not been widely revealed. In this study, through the construction of drug-resistant cell lines and high-throughput sequencing technology, we revealed the changes in the expression of circRNAs during the process of drug resistance, and searched for circRNAs that could predict the occurrence of drug resistance as potential biomarkers.

Citation: Cheng PQ, Liu YJ, Zhang SA, Lu L, Zhou WJ, Hu D, Xu HC, Ji G. RNA-Seq profiling of circular RNAs in human colorectal cancer 5-fluorouracil resistance and potential biomarkers. *World J Gastrointest Oncol* 2022; 14(3): 678-689
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/678.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.678

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and ranks as the second leading cause of cancer-related death[1,2]. In addition, the incidence of CRC has increased in recent years, and the number of patients has increased significantly[3]. Regarding the treatment of CRC, 5-fluorouracil (5-Fu), a fluorinated analogue of uracil, is the basic component and first-line chemotherapeutic agent for CRC[4,5]. Clinically, combination chemotherapy regimens based on 5-Fu, such as FOLFOX (5-Fu, leucovorin, and oxaliplatin) or FOLFIRI (5-Fu, leucovorin and irinotecan), have been shown to increase the survival rate and improve the response rate of patients with CRC; however, the emergence of resistance to 5-Fu was a major bottleneck in treatment[6-8]. Therefore, continuous and in-depth exploration of the 5-Fu resistance mechanism is an essential step to improve the survival benefit of 5-Fu-based therapy for CRC.

The development of 5-Fu resistance involves genetic and epigenetic alterations. In recent years, continuous studies have revealed changes in genes involved in the process of 5-Fu resistance and clarified the regulatory mechanisms of some genes involved in the development of resistance, such as EZH2, FOXM1, and YAP[9-12]. In terms of the role of noncoding RNAs in drug resistance, specific microRNA and lncRNA profiles were identified by RNA sequencing, and mechanisms have also been gradually revealed[13,14].

Circular RNAs (circRNAs), members of the noncoding RNA family, are characterized by covalently closed continuous loop structures without 3′ end poly(A) tails and 5′ end caps[15]. CircRNAs are widely expressed in multiple species and different cell types, and more than 20000 circRNAs have been detected in eukaryotes[16,17]. With the application of high-throughput sequencing, an increasing number of circRNAs have been identified, and at the same time, the functions of circRNAs in diseases have been elucidated, especially in cancers[18,19]. CircRNAs regulate gene expression mainly at the transcriptional and posttranscriptional levels by acting as miRNA sponges or binding to other molecules as their main mechanism of action[20]. Studies have shown important roles for circRNAs in the occurrence and malignant progression of almost all types of cancers[21-23]. However, previous studies were limited to the regulation of circRNAs in the malignant progression of cancers, and the mechanism of circRNAs in chemotherapy resistance has not been clearly studied. Therefore, little is known about the role of circRNA-related competitive endogenous RNA (ceRNA) in 5-Fu resistance in CRC.

In this study, we constructed multiple CRC 5-Fu-resistant cell lines and explored the expression profiles of circRNAs and mRNAs in 5-Fu-resistant cell lines and their parental cell lines using RNA sequencing. After verifying some candidate circRNAs in two additional pairs of cell lines, circRNA-miRNA-mRNA regulatory networks were constructed using Cytoscape software. Our study identified potential circRNAs involved in 5-Fu resistance in CRC and suggested that circRNAs play an important role in the generation of 5-Fu resistance.

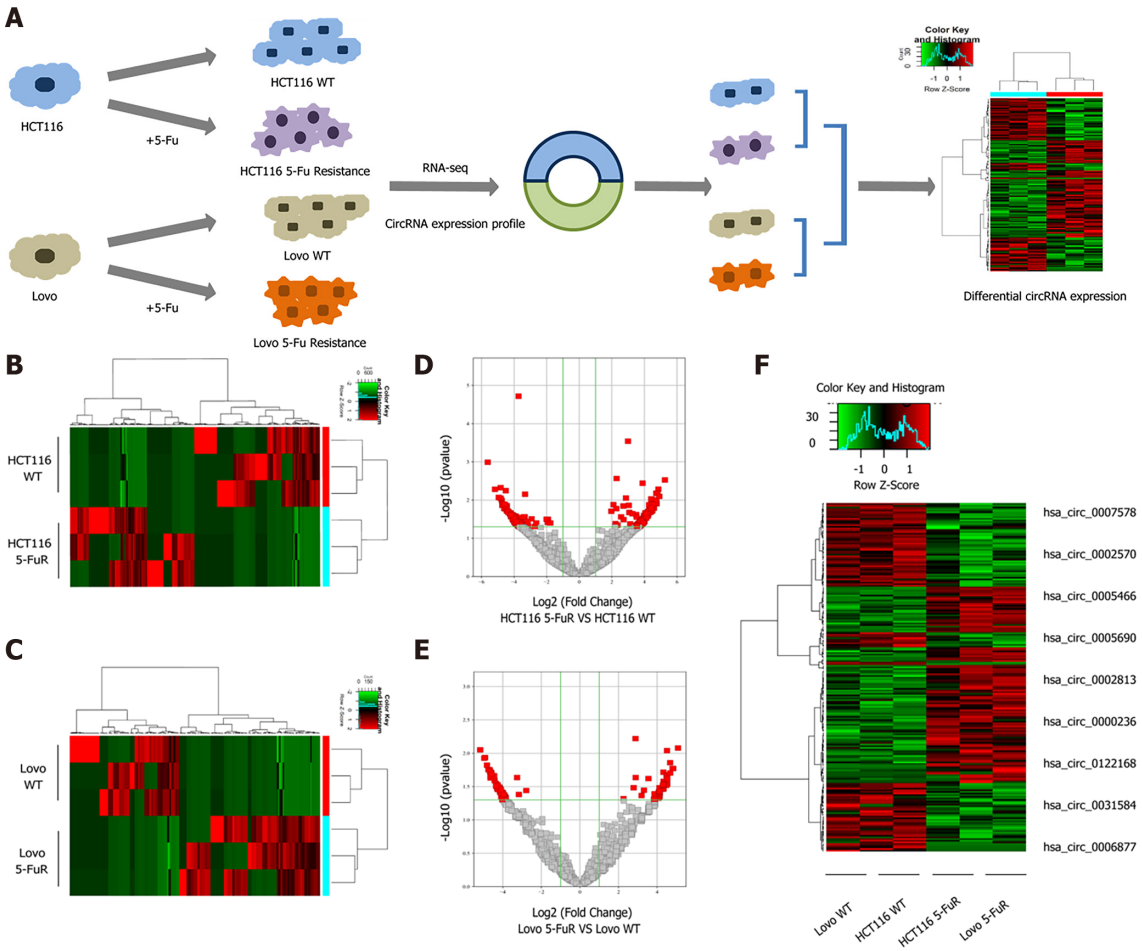

Figure 1 Changes in the expression profiles of significantly differentially expressed mRNAs and circular RNAs in 5-fluorouracil-resistant cells and their parental cells. A: Flow chart of the sequencing analysis; B and C: Clustered heat map indicating differences in circular RNA (circRNA) expression profiling between the HCT116 and HCT116 5-fluorouracil (5-Fu) resistant cell lines and the Lovo and Lovo 5-Fu resistant cell lines; D and E: The volcano plot shows the comparison of the circRNA expression profiles between the parental and 5-Fu resistant HCT116 and Lovo cell lines; F: Clustered heat map indicating differences in circRNA expression profiling in both paired cell lines.

MATERIALS AND METHODS

Cell cultures, and reagents

Human CRC cell lines (HCT116, Lovo, HT29 and SW480) were purchased from the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HCT116 cells were cultured in DMEM (Gibco, Carlsbad, CA, United States), Lovo cells were cultured in F12 medium (Gibco, Beijing, China), HT29 cells were cultured in McCoy's 5A medium (Gibco, Carlsbad, CA, United States), and SW480 cells were cultured in L-15 medium (Gibco, Bleiswijk, Netherlands). All culture media contained 10% foetal bovine serum and 1% penicillin, and these cell lines were maintained in a humidified atmosphere of 5% CO2 at 37 °C. The WST-1 cell proliferation and cytotoxicity detection kit was purchased from Beyotime, China.

Establishment of 5-Fu-resistant cells

The HCT116, Lovo, HT29 and SW480 cells were exposed to an initial 5-Fu concentration of 0.1 μg/mL in medium supplemented with 10% FBS. The surviving population of cells was grown to 80% confluence. 5-Fu-resistant cells were established after sequential treatments with increasing concentrations of 5-Fu (0.2, 0.5, 1, 1.5 and 2 μg/mL). Cells were able to survive at least 3 d of stimulation with 2 μg/mL 5-Fu. Resistance to 5-Fu was confirmed by the WST-1 assay.

RNA sequencing, identification and quantification of circRNA and mRNA

Total RNA was isolated from the cell lines using TRIzol reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Then, we assessed RNA integrity and DNA contamination using electrophoresis on a denaturing agarose gel. High-throughput RNA sequencing was performed by Cloud-Seq Biotech (Shanghai, China). Briefly, rRNAs were removed from total RNA with the NEBNext rRNA Depletion Kit (New England Biolabs, Inc., Massachusetts, United States) according to

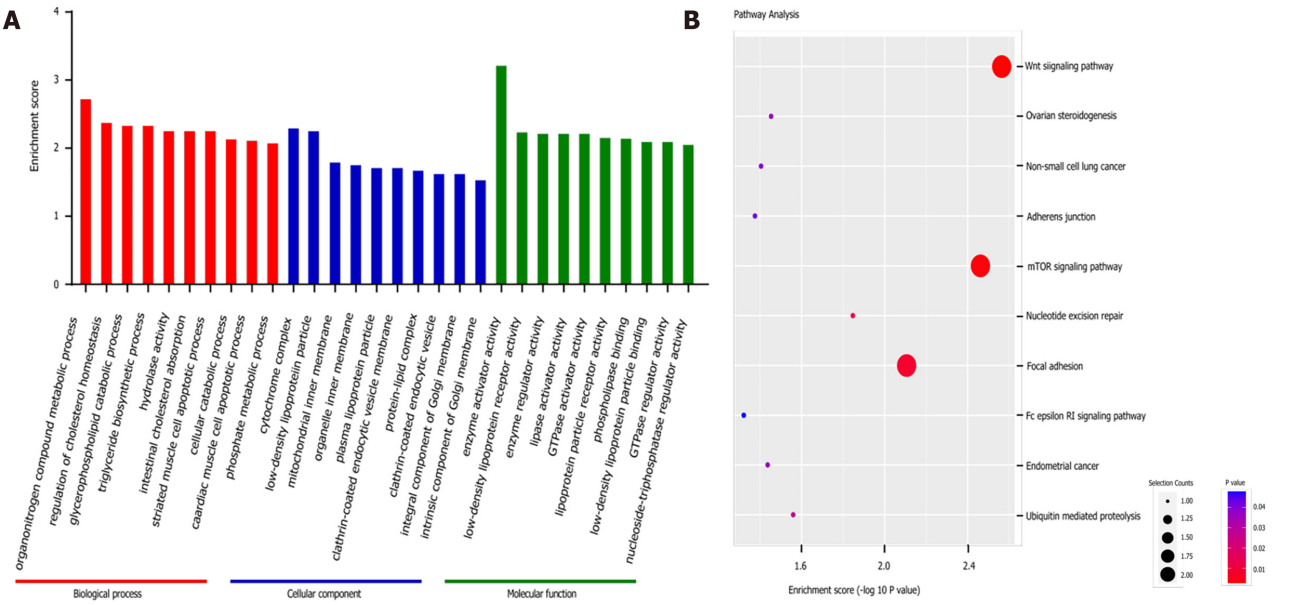

Figure 2 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses based on the sequencing results. A: Top 10 Gene Ontology (GO) terms identified in the GO analysis; B: Top 10 pathways identified in the Kyoto Encyclopedia of Genes and Genomes pathway analysis. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

the manufacturer's instructions. RNA libraries were constructed using the NEBNext® Ultra™ II Directional RNA Library Prep Kit (New England Biolabs, Inc., Massachusetts, United States) according to the manufacturer's instructions. Libraries were controlled for quality and quantified using the BioAnalyzer 2100 system (Agilent Technologies, Inc., United States). Library sequencing was performed on an Illumina NovaSeq 6000 instrument to obtain 150 bp paired end reads. The quality of paired end reads was controlled by Q30. After 3' adaptor trimming and low-quality read removal, cutadapt software (v1.9.3) was used. High-quality trimmed reads were used to analyse circRNAs and mRNAs. CircRNAs: The high-quality reads were aligned to the reference genome/transcriptome with STAR software (v2.5.1b), and circRNAs were detected and identified with DCC software (v0.4.4). EdgeR software (v3.16.5) was used to normalize the data and analyse differentially expressed circRNAs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed on the differentially expressed circRNA-associated genes. For mRNAs, the high-quality reads were aligned to the human reference genome (UCSC hg19) with hisat2 software (v2.0.4). Then, guided by the Ensembl Gene transfer format (GTF) gene annotation file, cuffdiff software (v2.2.1, part of cufflinks) was used to obtain the fragments *per* kilobase *per* million mapped reads (FPKM) as the expression profiles of lncRNAs and mRNAs, and fold changes and *P* values were calculated based on FPKM. Differentially expressed lncRNAs and mRNAs were identified. The target genes of lncRNAs were predicted based on the locations to nearby genes. GO and pathway enrichment analyses were performed on these target genes and the differentially expressed mRNAs.

Analyses of circRNA-miRNA-mRNA interactions in CRC

CircRNA-miRNA interactions were predicted using popular target prediction software programs, including circRNA Interactome and RegRNA. Specific predictions of the target genes of miRNAs were based on the miRanda, miRDB, miRWalk, RNA22 and TargetScan databases. All circRNA-miRNA-mRNA networks were constructed using Cytoscape software.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA, United States) and then reverse-transcribed into cDNAs using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, United States). The cDNA templates were used for quantitative real-time polymerase chain reaction (qPCR) with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, United States) and gene-specific primers, and the results were normalized to β-actin as a control. PCR primers are listed in Supplementary Table 1.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software Inc., CA, United States). Student's t test and one-way ANOVA were used to compare differences between groups, as appropriate. Data are presented as the means ± SD, and $P < 0.05$ was considered statistically significant.

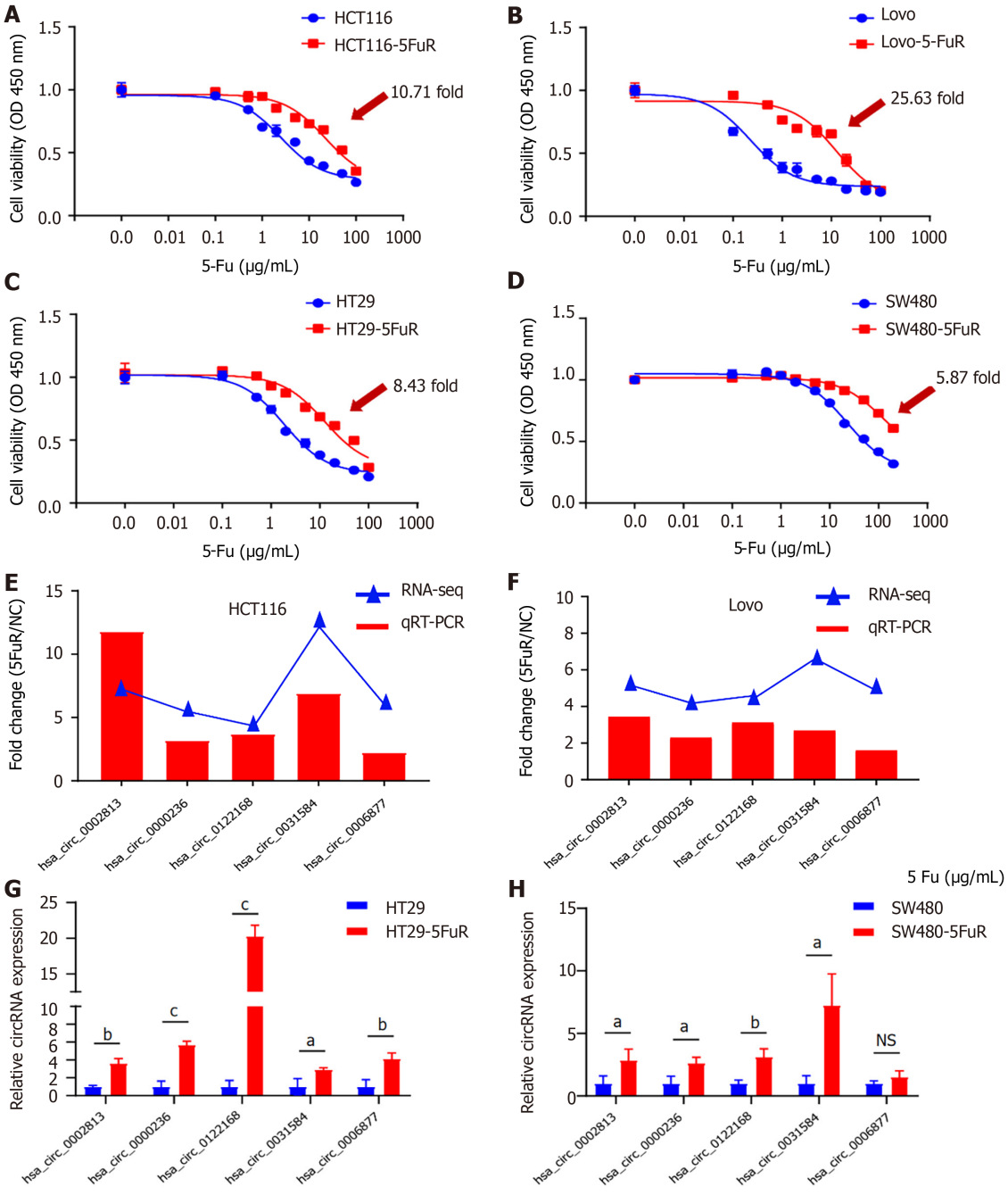

Figure 3 Verification of the sensitivity to 5-fluorouracil and differential circular RNA expression in 4 cell lines. A-D: The inhibition rate of 5-fluorouracil (5-Fu) in these 4 paired cell lines was detected using the WST-1 assay at 48 h; E-H: Relative expression of 5 circular RNAs (hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584, and hsa_circ_0006877) in 4 paired cell lines was measured using quantitative real-time polymerase chain reaction assays. Data are presented as means ± SD. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. NS: No significant difference; 5-Fu: 5-fluorouracil.

RESULTS

Identification of circRNAs expressed in 5-Fu-resistant CRC cell lines using RNA-Seq analyses

We first needed to determine the differentially expressed circRNAs in 5-Fu-resistant cells to explore the roles of circRNAs in the process of 5-Fu resistance in CRC. Secondary sequencing was used to profile circRNA and mRNA expression in two paired CRC 5-Fu resistant cell lines and parental cell lines, with three technical replicates in each group (Figure 1A). A total of 17939 circRNAs were detected in CRC cells, and the list of total circRNA expression profiles. Hierarchical clustering analysis showed significant differences in circRNA expression in the two pairs of cells individually (Figure 1B and C). We also constructed volcano plots (Figure 1D and E) to depict the significantly differentially expressed circRNAs (fold change > 2, and $P < 0.05$); the scatter plot shows the variation in circRNA expression levels (Supplementary Figure 1). After the intersection and merge of the differentially expressed circRNAs in two pairs of CRC cells, only 9 circRNAs showed significant differences in expression,

among which 4 circRNAs were downregulated in 5-Fu-resistant cells and 5 circRNAs were upregulated in 5-Fu-resistant cells (Figure 1F). Sequencing results and analysis suggested that the expression levels of some circRNAs changed during the process of 5-Fu resistance.

GO and KEGG analyses of differentially expressed circRNAs

The GO analysis of differentially expressed circRNAs included three categories: Biological process (BP), cell component (CC), and molecular function (MF). We listed the top ten terms in the BP, CC, and MF categories (Figure 2A). In the KEGG analysis, we also listed the 10 enriched pathways among the upregulated circRNAs, among which "Wnt signalling pathway", "mTOR signalling pathway" and "focal adhesion" were the three most noteworthy pathways identified after combining the selection counts, enrichment scores and *P* values (Figure 2B).

Validation of differentially expressed circRNAs

We focused on and verified the differentially expressed circRNAs obtained from the sequencing results using qRT–PCR in 4 pairs of CRC cell lines: HCT116, Lovo, HT29, and SW480. Before validation, we confirmed the drug resistance of the four types of 5-Fu-resistant cells by performing a WST-1 assay. As shown in Figure 3A-D, after intervention with different concentrations of 5-Fu, the IC50 values of the four 5-Fu-resistant cells increased by 5- to 15-fold compared with their parental cells. Based on the results shown in Figure 1F, we selected the 5 circRNAs that were significantly upregulated in 5-Fu-resistant cells and verified them in these 4 pairs of cell lines. For the HCT116 and Lovo cell lines, the qRT–PCR results were consistent with the results of RNA sequencing, and all 5 circRNAs (hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584, and hsa_circ_0006877) were expressed at high levels in 5-Fu-resistant cells (Figure 3E and F). The results from the HT29 and SW480 cell lines also confirmed that the expression of 4 of 5 circRNAs was significantly increased in 5-Fu-resistant cells, except for hsa_circ_0006877 in SW480 cells, which was not significantly different (Figure 3G and H).

circRNA-miRNA-mRNA network prediction and analyses

Based on the previous verification results, the circRNA-miRNA-mRNA regulatory networks were constructed by prediction and bioinformatics analysis using Cytoscape software for these 5 circRNAs with significantly altered expression levels. As shown in Figure 4, in the prediction network, we chose the top 5 miRNAs that potentially bind to the circRNAs and the 5 most likely target genes of each miRNA. From the prediction network, we clearly see the potential regulatory targets of the 5 circRNAs. This result provided a clear direction for us to further study the specific mechanism of circRNAs in 5-Fu resistance in CRC.

Comparison of mRNA expression profiles in 5-Fu-resistant CRC cell lines

While circRNA sequencing was performed on the two paired CRC 5-Fu-resistant cell lines, mRNA expression levels were also measured (Figure 5A and B and Supplementary Figure 2). Clustering analysis was performed on the differentially expressed mRNAs. In 5-Fu-resistant HCT116 cells, 3247 genes were significantly upregulated, and 267 genes were significantly upregulated in 5-Fu-resistant Lovo cells. An analysis combining the results from the two pairs of cell lines showed that 107 genes were upregulated in the 5-Fu-resistant variants (Figure 5C). The interactions of the 107 genes that were upregulated in both 5-Fu-resistant cell lines with the potential target genes predicted based on the circRNA-miRNA-mRNA regulatory network were analysed. The results focused on two genes, FUT3 and PLAG1, that were expressed at high levels in 5-Fu-resistant cells and predicted to be targets of differentially expressed circRNAs (Figure 5D). Then, we verified the mRNA expression levels of the two genes using a qRT–PCR assay, and the results were consistent with the RNA sequencing results (Figure 5E). The expression levels of both FUT3 and PLAG1 were significantly increased in 5-Fu-resistant cells, consistent with the expression of circRNAs (hsa_circ_0002813 and hsa_circ_0000236) that regulate them in the predicted network. Thus, the regulatory axes composed of circ_0002813-miR-1343-3p-FUT3 and circ_0000236-miR4769-5p-PLAG1 may play an important role in 5-Fu resistance in CRC.

DISCUSSION

With the advent of targeted therapy and immunotherapy, the treatment of CRC has achieved great advances. However, 5-Fu chemotherapy is still the main clinical treatment for CRC[5,24]. The main problem of chemotherapy for CRC is 5-Fu resistance, and 50% of patients with advanced CRC show 5-Fu resistance[10]. Therefore, continuous and thorough investigations of the potential mechanism of 5-Fu resistance are urgently needed. As members of the noncoding RNA family, circRNAs have become the focus of tumour research in recent years. Notably, circRNAs are involved in the malignant processes of a variety of human tumours, such as lung cancer, CRC and breast cancer[21,25,26]. Moreover, the role of circRNAs in chemotherapy resistance in cancers has also been reported. For instance, the circRNA AKT3 upregulates PIK3R1 to enhance cisplatin resistance in gastric cancer by suppressing miR-198[27].

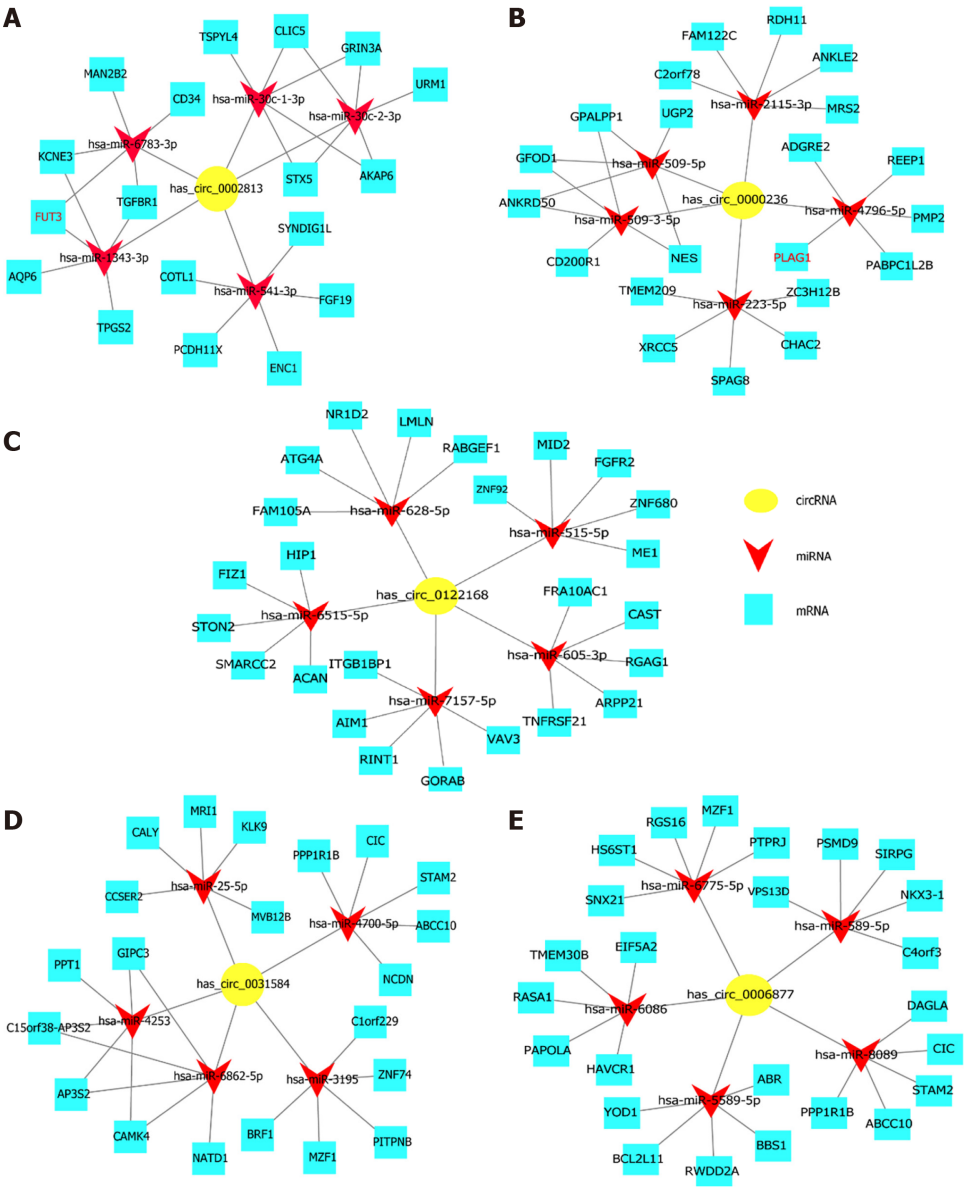

Figure 4 The competing endogenous RNA network. The circRNA-miRNA-mRNA interactions for the 5 circRNAs (hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584, and hsa_circ_0006877) were determined by predictions and bioinformatics analysis using Cytoscape software. A: hsa_circ_0002813; B: hsa_circ_0000236; C: hsa_circ_0122168; D: hsa_circ_0031584; E: hsa_circ_0006877.

Another study showed that circRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1[28].

In this study, we performed high-throughput sequencing to investigate the relationship between circRNAs and 5-Fu resistance, the most common chemotherapeutic drug used to treat CRC. A total of 17939 circRNAs were detected in two 5-Fu-resistant lines and their parental cell lines. We conducted an in-depth analysis of the sequencing results and combined the results of the two paired cell lines and identified 9 circRNAs with significant differences in expression. This study is the first to reveal circRNAs with a potential role in 5-Fu resistance in CRC at the cellular level. Moreover, we postulate that these 9 differentially expressed circRNAs may play a regulatory role in the process of 5-Fu resistance. We focused on 5 circRNAs that were significantly upregulated in 5-Fu-resistant cells and verified them in two paired cell lines selected for sequencing and two other paired cell lines using qRT-PCR assays to further confirm the accuracy of our findings. Four of the 5 circRNAs (hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, and hsa_circ_0031584) were expressed at high levels in the 4 paired cell lines with 5-Fu resistance. Moreover, the remaining circRNA, hsa_circ_0006877, showed significantly higher expression in 5-Fu-resistant variants of all cell lines except SW480 cells. Based on these results, hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584 and hsa_circ_0006877 may play a role in 5-Fu resistance in CRC.

Studies examining the function of circRNAs have indicated that circRNAs function as miRNA sponges and then regulate the expression of their target genes[20]. This function is the key mechanism by which circRNAs participate in various BP[29,30]. Therefore, for these 5 verified circRNAs, we

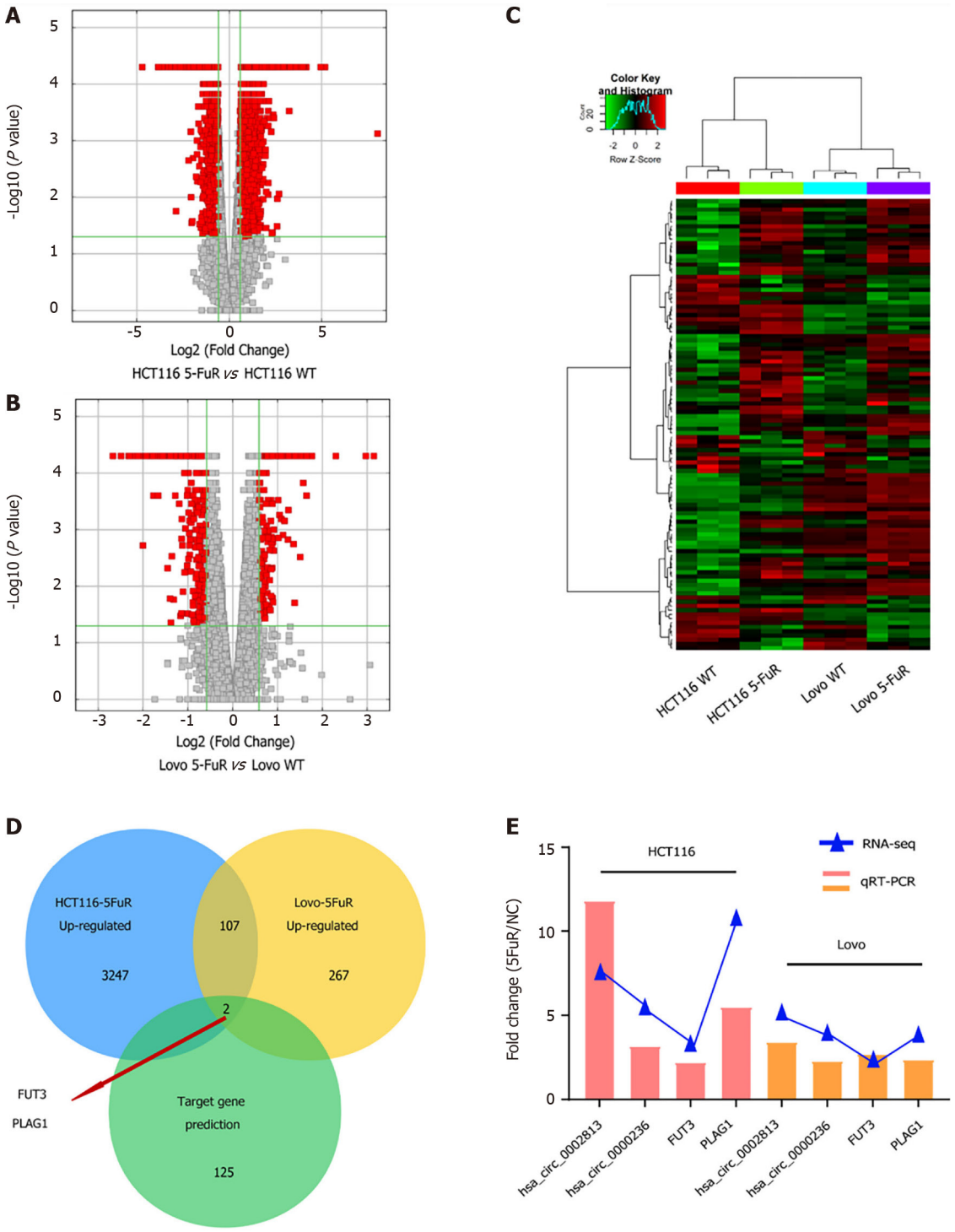

Figure 5 Comparison of mRNA expression profiles in 5-fluorouracil-resistant colorectal cancer cell lines. A and B: The volcano plot shows the comparison of the mRNA expression profiles between the parental and 5-fluorouracil (5-Fu) resistant HCT116 and Lovo cell lines; C: Heatmap of the clustering analysis indicating differences in mRNA expression profiles in both paired cell lines; D: Analysis of interactions between mRNAs that were upregulated in both paired 5-Fu-resistant cell lines and the potential target genes predicted by the circRNA-miRNA-mRNA regulatory network; E: Relative mRNA expression in the 2 paired cell lines was measured using quantitative real-time polymerase chain reaction assays.

predicted the miRNAs that they might sponge and their downstream target genes by analysing different databases to explore the potential regulatory mechanism of circRNAs in drug resistance. According to the 5 circRNAs upregulated in the 5-Fu-resistant cells, we first predicted circRNA-miRNA-mRNA interactions through target prediction software and selected the top 5 interactions to construct the networks. The networks established in our study provided a scientific basis for the subsequent study of the mechanism underlying circRNA function in CRC drug resistance.

In our study, we performed high-throughput sequencing of two paired cell lines, and in addition to including circRNAs, we also measured mRNA expression levels. In the process of exerting their biological functions, circRNAs mostly regulate downstream mRNAs by sponging miRNAs. Therefore,

we analysed the mRNA expression profiles using the sequencing results. The differentially expressed mRNAs involved in drug resistance were comprehensively analysed with the mRNAs in the previously constructed circRNA-miRNA-mRNA regulatory network. We found that two mRNAs (FUT3 and TNS4) that we predicted to be regulated by potential circRNAs showed significantly increased expression in the mRNA sequencing results from the drug-resistant cells. FUT3 is an α-1,3/4 fucosyltransferase that is absorbed by red blood cells and leads to a Lewis phenotype. The biological functions of FUT3 in tumorigenesis and metastasis have been documented in a variety of tumours[31-33]. TNS4, a member of the tensin protein family, is involved in key cellular processes, including cell adhesion, migration, and proliferation[34,35]. Accumulating evidence has suggested that TNS4 may be involved in the pathogenesis of cancers by interacting with miRNAs. For instance, miR-1224-5p inhibits TNS4, subsequently affecting the progression of oesophageal squamous cell carcinoma[36]. According to a recent report, TNS4 was identified as a key effector of cetuximab and a regulator of the oncogenic activity of KRAS mutant CRC[37]. We consistently found that FUT3 and TNS4 were expressed at higher levels in 5-Fu-resistant CRC cells and were target genes in the regulatory network of two significantly differentially expressed circRNAs that we identified. We strongly speculate that the hsa_circ_0002813-miR-541-3p- FUT3 and hsa_circ_0000236- miR-4796-5p-TNS4 axes may be involved in the regulatory mechanisms of drug resistance and may be potential therapeutic targets.

CONCLUSION

In conclusion, we comprehensively analysed the circRNA and mRNA expression profiles in paired 5-Fu-resistant cells. We identified circRNAs and mRNAs that are commonly altered during the development of 5-Fu resistance and suggested that hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584 and hsa_circ_0006877 may play important regulatory roles in the process of 5-Fu resistance in CRC. These circRNAs may represent potential predictive biomarkers and possible therapeutic targets of 5-Fu resistance. Based on our circRNA-related ceRNA networks, two potential regulatory mechanisms have been identified. Our findings may provide new perspectives for understanding the occurrence of 5-Fu resistance in CRC, provide new biomarkers for predicting 5-Fu resistance, and reveal candidate targets for reversing drug resistance.

ARTICLE HIGHLIGHTS

Research background

Therapy resistance has been a culprit for colorectal cancer (CRC) treatment. 5-fluorouracil (5-Fu) is a first-line chemotherapeutic agent for CRC, and it is very important to reveal the potential biomarkers and mechanisms of resistance.

Research motivation

Circular RNA (circRNA) plays a key role in the development and progression of cancer, but its role in the process of drug resistance has not been widely revealed. Therefore, we attempted to explore the relationship between circRNA and CRC drug resistance

Research objectives

Search for circRNAs that can predict the occurrence of CRC 5-Fu resistance, and explore its possibility as potential biomarkers.

Research methods

In this study, through the construction of drug-resistant cell lines and high-throughput sequencing technology, we revealed the changes in the expression of circRNAs during the process of drug resistance, and the potential circRNAs were verified by quantitative real-time polymerase chain reaction.

Research results

We identified circRNAs and mRNAs that are commonly altered in the development of 5-Fu drug resistance and suggested that hsa_circ_0002813, hsa_circ_0000236, hsa_circ_0122168, hsa_circ_0031584 and hsa_circ_0006877 may play an important regulatory role in the process of 5-Fu resistance in CRC. These circRNAs may act as potential predictive biomarkers and possible therapeutic targets of 5-Fu resistan.

Research conclusions

Our findings may offer new perspectives for understanding the occurrence of 5-Fu resistance in CRC,

provide new biomarkers for predicting 5-Fu resistance, and reveal candidate targets for reversing drug resistance.

Research perspectives

Potential circRNA expression differences in drug resistance were sought from the perspective of high-throughput sequencing of paired drug-resistant and parental cell lines.

FOOTNOTES

Author contributions: Cheng PQ and Liu YJ contributed equally to this work, they performed the majority of the experiments and analyzed the data; Lu L and Zhang SA contributed to the analysis of sequencing data and network prediction; Zhou WJ and Hu D coordinated the research; Ji G and Xu HC are co-corresponding authors, and they contributed to the design of the study and editing the manuscript; all authors read, and approved the final manuscript.

Supported by National Natural Science Foundation of China, No. 81874206; Shanghai Rising-Star Program, No. 20QA1409300; and the Program for Young Eastern Scholar at Shanghai Institutions of Higher Learning, No. QD2019034.

Institutional review board statement: This study did not involve a clinical case study.

Conflict-of-interest statement: No conflict of interest.

Data sharing statement: The datasets used during the current study were available from the corresponding authors on reasonable request.

Country/Territory of origin: China

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S-Editor: Fan JR
L-Editor: A
P-Editor: Guo X

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 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
3 **Siegel RL**, Fedewa SA, Anderson WF, Miller KD, Ma J, Rosenberg PS, Jemal A. Colorectal Cancer Incidence Patterns in the United States, 1974-2013. *J Natl Cancer Inst* 2017; **109** [PMID: 28376186 DOI: 10.1093/jnci/djw322]
4 **Dekker E**, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* 2019; **394**: 1467-1480 [PMID: 31631858 DOI: 10.1016/S0140-6736(19)32319-0]
5 **Vodenkova S**, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. *Pharmacol Ther* 2020; **206**: 107447 [PMID: 31756363 DOI: 10.1016/j.pharmthera.2019.107447]
6 **Stintzing S**, Modest DP, Rossius L, Lerch MM, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmüller C, Kahl C, Seipelt G, Kullmann F, Stauch M, Scheithauer W, Held S, Giessen-Jung C, Moehler M, Jagenburg A, Kirchner T, Jung A, Heinemann V; FIRE-3 investigators. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. *Lancet Oncol* 2016; **17**: 1426-1434 [PMID: 27575024 DOI: 10.1016/S1470-2045(16)30269-8]
7 **Van der Jeught K**, Xu HC, Li YJ, Lu XB, Ji G. Drug resistance and new therapies in colorectal cancer. *World J Gastroenterol* 2018; **24**: 3834-3848 [PMID: 30228778 DOI: 10.3748/wjg.v24.i34.3834]

8 **Galluzzi L**, Bravo-San Pedro JM, Levine B, Green DR, Kroemer G. Pharmacological modulation of autophagy: therapeutic potential and persisting obstacles. *Nat Rev Drug Discov* 2017; **16**: 487-511 [PMID: 28529316 DOI: 10.1038/nrd.2017.22]

9 **Wang C**, Li X, Zhang J, Ge Z, Chen H, Hu J. EZH2 contributes to 5-FU resistance in gastric cancer by epigenetically suppressing FBXO32 expression. *Onco Targets Ther* 2018; **11**: 7853-7864 [PMID: 30464532 DOI: 10.2147/OTT.S180131]

10 **Varghese V**, Magnani L, Harada-Shoji N, Mauri F, Szydlo RM, Yao S, Lam EW, Kenny LM. FOXM1 modulates 5-FU resistance in colorectal cancer through regulating TYMS expression. *Sci Rep* 2019; **9**: 1505 [PMID: 30728402 DOI: 10.1038/s41598-018-38017-0]

11 **Intuyod K**, Saavedra-García P, Zona S, Lai CF, Jiramongkol Y, Vaeteewoottacharn K, Pairojkul C, Yao S, Yong JS, Trakansuebkul S, Waraasawapati S, Luvira V, Wongkham S, Pinlaor S, Lam EW. FOXM1 modulates 5-fluorouracil sensitivity in cholangiocarcinoma through thymidylate synthase (TYMS): implications of FOXM1-TYMS axis uncoupling in 5-FU resistance. *Cell Death Dis* 2018; **9**: 1185 [PMID: 30538221 DOI: 10.1038/s41419-018-1235-0]

12 **Huang C**, Chen Z, Yang C, Chen L, Lai C, Zhang Y, Yuan W, Jeong JH. Combinational inhibition of EGFR and YAP reverses 5-Fu resistance in colorectal cancer. *J Cancer* 2020; **11**: 5432-5439 [PMID: 32742490 DOI: 10.7150/jca.44775]

13 **Feng C**, Zhang L, Sun Y, Li X, Zhan L, Lou Y, Wang Y, Liu L, Zhang Y. GDPD5, a target of miR-195-5p, is associated with metastasis and chemoresistance in colorectal cancer. *Biomed Pharmacother* 2018; **101**: 945-952 [PMID: 29635904 DOI: 10.1016/j.biopha.2018.03.028]

14 **Wang X**, Lan Z, He J, Lai Q, Yao X, Li Q, Liu Y, Lai H, Gu C, Yan Q, Fang Y, Zhang Y, Li A, Liu S. LncRNA SNHG6 promotes chemoresistance through ULK1-induced autophagy by sponging miR-26a-5p in colorectal cancer cells. *Cancer Cell Int* 2019; **19**: 234 [PMID: 31516391 DOI: 10.1186/s12935-019-0951-6]

15 **Memczak S**, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013; **495**: 333-338 [PMID: 23446348 DOI: 10.1038/nature11928]

16 **Arnberg AC**, Van Ommen GJ, Grivell LA, Van Bruggen EF, Borst P. Some yeast mitochondrial RNAs are circular. *Cell* 1980; **19**: 313-319 [PMID: 6986989 DOI: 10.1016/0092-8674(80)90505-x]

17 **Fan X**, Zhang X, Wu X, Guo H, Hu Y, Tang F, Huang Y. Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos. *Genome Biol* 2015; **16**: 148 [PMID: 26201400 DOI: 10.1186/s13059-015-0706-1]

18 **Lukiw WJ**. Circular RNA (circRNA) in Alzheimer's disease (AD). *Front Genet* 2013; **4**: 307 [PMID: 24427167 DOI: 10.3389/fgene.2013.00307]

19 **Bachmayr-Heyda A**, Reiner AT, Auer K, Sukhbaatar N, Aust S, Bachleitner-Hofmann T, Mesteri I, Grunt TW, Zeillinger R, Pils D. Correlation of circular RNA abundance with proliferation--exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. *Sci Rep* 2015; **5**: 8057 [PMID: 25624062 DOI: 10.1038/srep08057]

20 **Hansen TB**, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013; **495**: 384-388 [PMID: 23446346 DOI: 10.1038/nature11993]

21 **Vo JN**, Cieslik M, Zhang Y, Shukla S, Xiao L, Wu YM, Dhanasekaran SM, Engelke CG, Cao X, Robinson DR, Nesvizhskii AI, Chinnaiyan AM. The Landscape of Circular RNA in Cancer. *Cell* 2019; **176**: 869-881.e13 [PMID: 30735636 DOI: 10.1016/j.cell.2018.12.021]

22 **Li X**, Ding J, Wang X, Cheng Z, Zhu Q. NUDT21 regulates circRNA cyclization and ceRNA crosstalk in hepatocellular carcinoma. *Oncogene* 2020; **39**: 891-904 [PMID: 31570791 DOI: 10.1038/s41388-019-1030-0]

23 **Xu H**, Wang C, Song H, Xu Y, Ji G. RNA-Seq profiling of circular RNAs in human colorectal Cancer liver metastasis and the potential biomarkers. *Mol Cancer* 2019; **18**: 8 [PMID: 30630466 DOI: 10.1186/s12943-018-0932-8]

24 **Katona BW**, Weiss JM. Chemoprevention of Colorectal Cancer. *Gastroenterology* 2020; **158**: 368-388 [PMID: 31563626 DOI: 10.1053/j.gastro.2019.06.047]

25 **Sang Y**, Chen B, Song X, Li Y, Liang Y, Han D, Zhang N, Zhang H, Liu Y, Chen T, Li C, Wang L, Zhao W, Yang Q. circRNA_0025202 Regulates Tamoxifen Sensitivity and Tumor Progression *via* Regulating the miR-182-5p/FOXO3a Axis in Breast Cancer. *Mol Ther* 2019; **27**: 1638-1652 [PMID: 31153828 DOI: 10.1016/j.ymthe.2019.05.011]

26 **Yang X**, Tian W, Wang S, Ji X, Zhou B. CircRNAs as promising biomarker in diagnostic and prognostic of lung cancer: An updated meta-analysis. *Genomics* 2021; **113**: 387-397 [PMID: 33326833 DOI: 10.1016/j.ygeno.2020.12.013]

27 **Huang X**, Li Z, Zhang Q, Wang W, Li B, Wang L, Xu Z, Zeng A, Zhang X, He Z, Li Q, Sun G, Wang S, Zhang L, Xu H. Circular RNA AKT3 upregulates PIK3R1 to enhance cisplatin resistance in gastric cancer *via* miR-198 suppression. *Mol Cancer* 2019; **18**: 71 [PMID: 30927924 DOI: 10.1186/s12943-019-0969-3]

28 **Xu J**, Ji L, Liang Y, Wan Z, Zheng W, Song X, Gorshkov K, Sun Q, Lin H, Zheng X, Chen J, Jin RA, Liang X, Cai X. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. *Signal Transduct Target Ther* 2020; **5**: 298 [PMID: 33361760 DOI: 10.1038/s41392-020-00375-5]

29 **Chen X**, Chen RX, Wei WS, Li YH, Feng ZH, Tan L, Chen JW, Yuan GJ, Chen SL, Guo SJ, Xiao KH, Liu ZW, Luo JH, Zhou FJ, Xie D. PRMT5 Circular RNA Promotes Metastasis of Urothelial Carcinoma of the Bladder through Sponging miR-30c to Induce Epithelial-Mesenchymal Transition. *Clin Cancer Res* 2018; **24**: 6319-6330 [PMID: 30305293 DOI: 10.1158/1078-0432.CCR-18-1270]

30 **Wu Y**, Xie Z, Chen J, Ni W, Ma Y, Huang K, Wang G, Wang J, Ma J, Shen S, Fan S. Circular RNA circTADA2A promotes osteosarcoma progression and metastasis by sponging miR-203a-3p and regulating CREB3 expression. *Mol Cancer* 2019; **18**: 73 [PMID: 30940151 DOI: 10.1186/s12943-019-1007-1]

31 **Zhan L**, Chen L, Chen Z. Knockdown of FUT3 disrupts the proliferation, migration, tumorigenesis and TGF-β induced EMT in pancreatic cancer cells. *Oncol Lett* 2018; **16**: 924-930 [PMID: 29963165 DOI: 10.3892/ol.2018.8738]

32 **He C**, Li A, Lai Q, Ding J, Yan Q, Liu S, Li Q. The DDX39B/FUT3/TGFβR-I axis promotes tumor metastasis and EMT in colorectal cancer. *Cell Death Dis* 2021; **12**: 74 [PMID: 33436563 DOI: 10.1038/s41419-020-03360-6]

33 **Gao HF**, Wang QY, Zhang K, Chen LY, Cheng CS, Chen H, Meng ZQ, Zhou SM, Chen Z. Overexpressed N-fucosylation on the cell surface driven by FUT3, 5, and 6 promotes cell motilities in metastatic pancreatic cancer cell lines. *Biochem Biophys Res Commun* 2019; **511**: 482-489 [PMID: 30808544 DOI: 10.1016/j.bbrc.2019.02.092]

34 **Kwon SH**, Nedvetsky PI, Mostov KE. Transcriptional profiling identifies TNS4 function in epithelial tubulogenesis. *Curr*

Biol 2011; **21**: 161-166 [PMID: 21236678 DOI: 10.1016/j.cub.2010.12.037]

35 **Seo EY**, Jin SP, Kim YK, Lee H, Han S, Lee DH, Chung JH. Integrin-β4-TNS4-Focal Adhesion Kinase Signaling Mediates Keratinocyte Proliferation in Human Skin. *J Invest Dermatol* 2017; **137**: 763-766 [PMID: 27840236 DOI: 10.1016/j.jid.2016.10.039]

36 **Shi ZZ**, Wang WJ, Chen YX, Fan ZW, Xie XF, Yang LY, Chang C, Cai Y, Hao JJ, Wang MR, Bai J. The miR-1224-5p/TNS4/EGFR axis inhibits tumour progression in oesophageal squamous cell carcinoma. *Cell Death Dis* 2020; **11**: 597 [PMID: 32732965 DOI: 10.1038/s41419-020-02801-6]

37 **Kim S**, Kim N, Kang K, Kim W, Won J, Cho J. Whole Transcriptome Analysis Identifies TNS4 as a Key Effector of Cetuximab and a Regulator of the Oncogenic Activity of KRAS Mutant Colorectal Cancer Cell Lines. *Cells* 2019; **8** [PMID: 31409052 DOI: 10.3390/cells8080878]

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World J Gastrointest Oncol 2022 March 15; 14(3): 690-702

DOI: 10.4251/wjgo.v14.i3.690

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Cost-effective low-coverage whole-genome sequencing assay for the risk stratification of gastric cancer

Li-Ping Ye, Xin-Li Mao, Xian-Bin Zhou, Yi Wang, Shi-Wen Xu, Sai-Qin He, Zi-Liang Qian, Xiao-Gang Zhang, Li-Juan Zhai, Jin-Bang Peng, Bin-Bin Gu, Xiu-Xiu Jin, Ya-Qi Song, Shao-Wei Li

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Socea B

Received: August 10, 2021
Peer-review started: August 10, 2021
First decision: December 4, 2021
Revised: December 25, 2021
Accepted: February 22, 2022
Article in press: February 22, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

Gastric cancer (GC), a multifactorial disease, is caused by pathogens, such as *Helicobacter pylori* (*H. pylori*) and Epstein-Barr virus (EBV), and genetic

components.

AIM
To investigate microbiomes and host genome instability by cost-effective, low-coverage whole-genome sequencing, as biomarkers for GC subtyping.

METHODS
Samples from 40 GC patients were collected from Taizhou Hospital, Zhejiang Province, affiliated with Wenzhou Medical University. DNA from the samples was subjected to low-coverage whole-genome sequencing with a median genome coverage of 1.86 × (range: 1.03 × to 3.17 ×) by Illumina × 10, followed by copy number analyses using a customized bioinformatics workflow ultrasensitive chromosomal aneuploidy detector.

RESULTS
Of the 40 GC samples, 20 (50%) were found to be enriched with microbiomes. EBV DNA was detected in 5 GC patients (12.5%). *H. pylori* DNA was found in 15 (37.5%) patients. The other 20 (50%) patients were found to have relatively higher genomic instability. Copy number amplifications of the oncogenes, *ERBB2* and *KRAS*, were found in 9 (22.5%) and 7 (17.5%) of the GC samples, respectively. EBV enrichment was found to be associated with tumors in the gastric cardia and fundus. *H. pylori* enrichment was found to be associated with tumors in the pylorus and antrum. Tumors with elevated genomic instability showed no localization and could be observed in any location. Additionally, *H. pylori*-enriched GC was found to be associated with the Borrmann type II/III and gastritis history. EBV-enriched GC was not associated with gastritis. No statistically significant correlation was observed between genomic instability and gastritis. Furthermore, these three different molecular subtypes showed distinct survival outcomes (P = 0.019). EBV-positive tumors had the best prognosis, whereas patients with high genomic instability (CIN+) showed the worst survival. Patients with *H. pylori* infection showed intermediate prognosis compared with the other two subtypes.

CONCLUSION
Thus, using low-coverage whole-genome sequencing, GC can be classified into three categories based on disease etiology; this classification may prove useful for GC diagnosis and precision medicine.

Key Words: Gastric cancer; Whole-genome sequencing; *Helicobacter pylori* infections; Epstein-Barr virus infections; Genetic components; Precision medicine

Core Tip: This study investigated the microbiomes and host genome instability *via* cost-effective low-coverage whole-genome sequencing, to establish the findings for consideration in the development of a biomarker for gastric cancer (GC) subtyping. We believe that our study makes a significant contribution to the literature because it identified three different GC subtypes in the Chinese population, and these were related to different tumorigenesis mechanisms, chronic Epstein-Barr virus infection, *Helicobacter pylori* infections, and chromosomal instabilities. This discovery may therefore provide guidance for conducting future studies to realize GC treatment and prevention.

Citation: Ye LP, Mao XL, Zhou XB, Wang Y, Xu SW, He SQ, Qian ZL, Zhang XG, Zhai LJ, Peng JB, Gu BB, Jin XX, Song YQ, Li SW. Cost-effective low-coverage whole-genome sequencing assay for the risk stratification of gastric cancer. *World J Gastrointest Oncol* 2022; 14(3): 690-702
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/690.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.690

INTRODUCTION

Gastric cancer (GC) is the fourth leading cause of cancer-related deaths worldwide, with an estimated 768793 deaths in 2020, according to the GLOBOCAN of the International Agency for research on Cancer [1]. Most GCs are adenocarcinomas with considerable heterogeneity. According to Lauren's criteria, GC is classified into three subtypes: intestinal, diffuse, and mixed[2]. World Health Organization (WHO)

has divided GC into four subtypes-papillary, tubular, mucinous and poorly cohesive carcinomas[3]. However, these traditional morphology-based classification systems have limited clinical utility due to the molecular heterogeneity of GC. Therefore, it is necessary to develop a robust GC molecular classification to guide clinical practice, determine prognosis, or predict the treatment response.

Infection with microorganisms plays an important role in the development of GC. In contrast to other tumor types, gastric carcinogenesis is closely related to infectious pathogens. Among them, *Helicobacter pylori* (*H. pylori*) infection is one of the risk factors for GC, responsible for almost 90% of all noncardia GC[4]. A relationship between *H. pylori* and GC has been discovered and is characterized as a stepwise inflammatory process that eventually leads to malignancy[5]. In addition to pathogenic bacteria, viral infections significantly contribute to gastric carcinogenesis. Epstein-Barr virus (EBV) is the most characterized gastric oncogenic virus[6]. In a comprehensive molecular analysis of GC conducted by The Cancer Genome Atlas (TCGA), EBV-positive tumors were classified as a distinct subtype with statistical significance ($P = 1.5 \times 10^{-18}$), and ~9% of GC patients were EBV-positive[7]. Although these pathogens infect more than half of the world's population, fortunately, only a small fraction of those infected develop GC, indicating the complexity that drives gastric tumorigenesis[8].

In addition to microbial infection, alterations in genomic stability also play a key role as drivers of GC. Among these, chromosomal instability (CIN) is one of the most common types of genetic changes, which is usually described as somatic copy number aberrations (SCNAs) accompanied by focal amplification of oncogenes or deletion of tumor suppressor genes[9]. According to information presented in TCGA database, GC can be divided into two distinct subtypes based on the presence or absence of SCNAs[7]. Our previous studies demonstrated that CIN was a valuable prognosis factor in GC using array-based comparative genomic hybridization. Two distinct subtypes of GC were identified, high CIN and low CIN, with distinguished gene expression signatures and different survival outcomes of patients[10,11].

However, these methods are more expensive and sophisticated, limiting their application in clinical practice. Low-coverage whole-genome sequencing (LC-WGS) was first developed as a simple, cost-effective, and reliable technology to identify SCNAs in tumors in 2014[12]. Therefore, the aim of the present study was to develop a robust and cost-effective molecular classification method for GC using LC-WGS to identify candidate drivers of gastric tumorigenesis and to provide a roadmap for gastric risk stratification and targeted therapy trials.

MATERIALS AND METHODS

Patient characteristics and ethical statement

Samples from 40 GC patients were collected and the deadline for the follow-up was May 2021. The study was reviewed and approved by the Institutional Ethics Committee of Taizhou Hospital of Zhejiang Province (Approval No. K20201205), and informed consent was obtained from the patients prior to specimen collection (Table 1).

DNA extraction

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples using the Qiagen nucleic acid kits (69504).

LC-WGS

For LC-WGS, libraries were prepared using the Kapa Hyper Prep kit (Roche, CA, United States) with custom adapters (IDT, CA, United States) starting with 50 to 1000 ng of DNA input (median, 471 ng), which was used for low-pass WGS. The 22 Libraries were pooled and sequenced using the 150-base paired-end runs over 1× lane on a HiSeq X10 system (Illumina, CA, United States). Segment copy numbers were derived using a customized workflow ultrasensitive chromosomal aneuploidy detector (UCAD). If the median absolute deviation of the copy ratio (log ratio) between the adjacent bins of the whole-genome was greater than 0.38, indicating poor-quality sequence data, the sample was excluded.

Reads were mapped to the EBV reference genome (gi|82503188). Matches with no more than 1 mismatch were counted as EBV reads. The same approach was applied for *H. pylori* (gi|261838873). Samples with more than 4 EBV reads were marked as EBV-positive tumor samples. Samples with more than 4 *H. pylori* reads were marked as *H. pylori*-positive tumor samples.

GC pathology

Specimens from 40 gastric patients were found to have pathological characteristics of GC. The prepared slides were then sent to pathologists for analysis following the standard protocol. The pathology test results were recorded as tumor type and tumor grade.

Statistical analyses

The Illumina X10 system was used for DNA extraction and analysis. At least 10 M paired reads were

Table 1 Clinical features of the 40 study patients diagnosed with gastric cancer

	EBV+	*H. Pylori*+	ERBB2+	KRAS+	*P* value
Age (yr), mean ± SD	62.2 ± 6.4				No significance
Sex, *n*					No significance
Male	3	13	8	5	
Female	2	2	1	2	
Tumor location, *n*					*P* = 0.013 (*H. Pylori* associated with antrum)
Cardia/fundus	5	2	3	1	
Polyrus/antrum	0	13	5	6	
Other	0	0	1	0	
Borrmann, *n*					*P* = 0.013 (*H. Pylori* associated with ulcerative)
Type I	1	1	2	2	
Type II	1	7	4	1	
Type III	1	7	2	2	
Type IV	2	0	1	1	
Gastritis, *n*					No significance
Yes	0	4	2	2	
No	5	11	7	5	
Vascular invasion, *n*					No significance
Yes	2	9	1	2	
No	3	6	8	5	

H. Pylori: *Helicobacter pylori*.

collected for each sample. The reads were mapped to the human reference genome hg19. The genomic coverage was then counted using the software package mpileup[13]. Then, the average coverage for each 200 k bin was calculated, and the Z-score for each bin was normalized using the following formula-1:

$$\text{Coverage}_{\text{normalized}} = \frac{\text{coverege}_{\text{raw}}\text{-mean (coverage}_{\text{controls,raw}})}{\text{stdev (coverage}_{\text{controls,raw}})}$$

Then, using the circular binary segmentation algorithm from the R package DNACopy[14], significant genomic breakpoints and copy number changes in the genomic segments were found.

We used the R package "DNACopy" to analyze the copy number changes. A P value of < 0.05 was considered to denote a statistically significant binary segmentation. The absolute segment value was used for further analysis. The sensitivity and specificity of UCAD were estimated by receiver operating characteristic curves. For categorical variables, the chi-square test was employed. All statistical analyses were performed using SPSS17.0 (IBM, Foster City, CA, United States).

The associations between the clinicopathological UCAD screening positivity and clinicopathological parameters were analyzed by the proportional trend test[15]. Data were reported as means and standard deviations, medians and interquartile ranges, and hazard ratios or odds ratios with 95%CIs, as appropriate. The missing data were removed from the analyses. All analyses were performed using R software, version 3.4.3 (R Foundation for Statistical Computing, GNU project https://www.r-project.org/). The anonymized data and R code used in the statistical analysis will be made available on request.

RESULTS

Patient characterization

In total, 40 FFPE samples were collected. All samples passed QC and were included in this study (Table 1).

Cancer genome of GC

In Figure 1, we summarize the genome-wide copy number variations observed. Interestingly, it was found that chromosomal-arm imbalance was caused by chromosomal breakpoints on the centromeres (Figure 1A for averaged data plot). There were 70 genomic segments with statistically significant copy number changes (details in Table 2). Frequent chromosomal changes in GC include 1p-, 1q+, 3q+, 4-, 5p+, 7p+, 8q+, 9p-, 13+, 17p-, 17q+, and 20q+. The focal events include amplification of 17q12, which contains *ERBB2* (chr17:37, 844167-37886679, hg19). Z-scores (formula-1) are listed in Table 2.

CIN scores were summarized by the formula, CIN = sum ($L_{chr} \times Z_{chr}$), where L_{chr} is the length of the chromosome segment and Z_{chr} is the Z score of the segment. By using the CIN score cutoff value of 20, 19 (47.5%) patients had low CIN scores. The other 21 (52.5%) patients had elevated CIN scores (Figure 2).

Three groups of GC

Forty GC tissues were analyzed, and twenty (50% of the total) GC samples were found to be enriched with microbiomes. As shown in Figure 2, the samples of patients with abundant EBV DNA showed less abundance of *H. pylori* DNA (Figure 2, top), which may suggest that EBV and *H. pylori* are different drivers of GC tumorigenesis. As a control, the random distribution of *Escherichia coli* DNA may suggest less contribution of this microbiome to GC tumorigenesis. Furthermore, patients with low EBV and *H. pylori* DNA showed high CIN scores (Figure 2 bottom).

EBV DNA was detected in 5 GC patients (12.5%). *H. pylori* DNA was found in 15 (37.5%) patients. The other 20 (50%) patients were found to have relatively higher genomic instability. The copy number amplifications of the oncogenes, *ERBB2* and *KRAS,* were found in 9 (22.5%) and 7 (17.5%) GC samples, respectively.

EBV-positive GC showed a relatively stable genome (Figure 1B). The patients with positive *H. pylori* statuses showed an unstable genome (Figure 1C), where chr5p amplifications are frequently located in *TERT* (5p15.33). The other patients with *H. pylori*- and EBV-negative GC were also characterized by an unstable genome, where *ERBB2* amplifications were significantly enriched (Figure 1D).

Molecular subtypes correlated with tumor locations and gastritis history

As shown in Table 1, EBV enrichment was found to be associated with tumors in the gastric cardia and fundus (P = 0.013). *H. pylori* enrichment was found to be associated with tumors in the pylorus and antrum. Tumors with elevated genomic instability could be found in any location (Figure 3).

Additionally, *H. pylori*-enriched GC was found to be associated with Borrmann type II/III and gastritis history (P = 0.013). The EBV-enriched GC was not associated with gastritis. There was no statistically significant correlation between genomic instability and gastritis.

Overall survival associated with CIN and Borrmann type

Furthermore, we analyzed the overall survival (OS) of different molecular groups. As shown in Figure 4, patients with different molecular subtypes show distinct prognoses (long rank test, P = 0.019). EBV-positive tumors showed the best OS, with a median OS of ~2800 d. CIN+ patients were found to have the worst survival, with a median OS less than 500 d. Patients with *H. pylori* infections also showed worse survival than those infected with EBV but tended to be better than CIN+ patients. Moreover, the OS was investigated among four Borrmann types of GC, and no significant difference was found (log-rank test, P = 0.078) (Figure 5).

DISCUSSION

GC has a high incidence and fatality rate in China. It shows high histopathological and molecular heterogeneity[16]. The disease involves multiple genes with different genetic events occurring at different stages. The heredity of GC, individual differences, and the complexity of molecular mechanisms necessitates its characterization by gene groups or cluster[7]. Molecular subtyping of GC involves the screening of the genes or protein markers related to tumorigenesis, diagnosis, and prognosis. The currently studied genes and protein markers include oncogenes, tumor suppressor genes, intercellular adhesion molecules, growth factors, and certain hormone receptors[17]. Although most of them show poor sensitivity, specificity, or reliability, a few have been recognized as effective biomarkers. Gastric adenocarcinoma, the most common type of GC, shows a remarkable heterogeneity among different patients with a high mortality due to the tumor's innate aggressiveness. Despite recent advances in diagnosis and treatment, the 5-year OS remains poor. Moreover, gastrointestinal stroma tumor is a rare but highly curable cancer and has a satisfactory prognosis with a 5-year OS ranging from 60%-85%[18,19]. Notably, multimodal complications associated with radical gastrectomy during perioperative period should be addressed. Anastomotic fistula, one of the main surgical complications that raises the risk of local recurrence and worsens the overall prognosis, has been reported to be positively correlated with the neutrophil/lymphocyte ratio[20]. The traditional morphology-based subtyping systems include the Lauren classification (intestinal, diffuse, and mixed) and WHO-based classification

Table 2 Copy number variation segments identified in gastric cancer patients

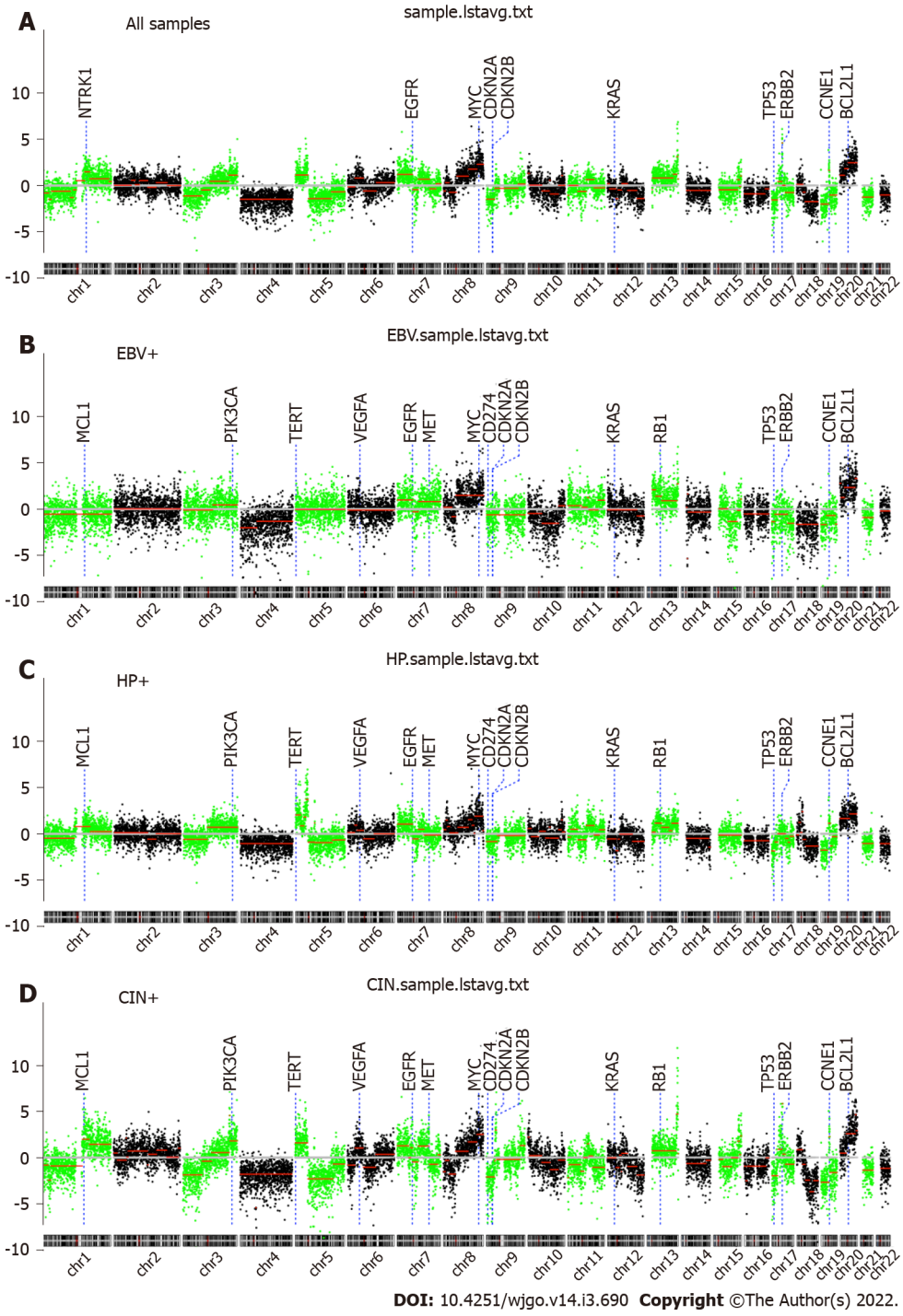
Chrom	Loc.start	Loc.end	Seg.mean	Log*P*	Key genes
Chr04	0	190800000	-1.63	-100.00	*FHIT*
Chr05	45800000	131000000	-1.49	-100.00	
Chr13	19000000	110000000	0.87	-88.38	
Chr18	30800000	77800000	-1.86	-86.67	
Chr03	0	66200000	-1.23	-80.98	
Chr07	0	55600000	1.28	-75.03	*EGFR*
Chr20	30200000	62800000	2.54	-74.06	
Chr16	0	76200000	-0.93	-67.73	
Chr08	90600000	122000000	1.84	-59.87	
Chr05	0	45600000	1.29	-56.55	*TERT*
Chr01	170000000	240000000	0.78	-55.86	
Chr21	9400000	47800000	-1.33	-54.61	
Chr09	0	29800000	-1.56	-50.52	*CDKN2A*
Chr08	47800000	90400000	1.02	-49.62	
Chr08	122200000	146000000	2.42	-49.24	*MYC*
Chr01	29600000	98400000	-0.69	-47.14	
Chr19	0	28800000	-2.03	-43.15	
Chr10	50800000	111400000	-0.77	-41.27	
Chr03	165800000	197800000	1.19	-40.36	
Chr22	16000000	51000000	-1.11	-38.57	
Chr14	19000000	107000000	-0.57	-37.54	
Chr12	107600000	133600000	-1.44	-36.53	
Chr15	20000000	88200000	-0.60	-34.61	
Chr01	151200000	169800000	1.47	-32.21	
Chr17	0	21800000	-1.75	-31.78	*TP53*
Chr03	96200000	165600000	0.48	-30.10	
Chr19	32200000	58800000	-1.15	-29.94	
Chr07	76400000	117800000	0.66	-28.48	
Chr05	131200000	180600000	-0.70	-28.10	
Chr17	49200000	80800000	-0.85	-26.77	
Chr06	26000000	57000000	0.80	-25.75	
Chr12	73400000	107400000	-0.64	-23.98	
Chr06	57200000	104200000	-0.63	-22.49	
Chr20	0	17000000	1.09	-22.19	
Chr20	17200000	30000000	1.73	-18.89	
Chr01	98600000	109600000	-1.31	-18.38	
Chr11	67000000	96000000	0.58	-17.22	
Chr08	20200000	47600000	-0.82	-16.59	
Chr03	66400000	96000000	-0.54	-16.41	
Chr01	19800000	29400000	-1.60	-15.48	
Chr02	92200000	122200000	0.51	-14.89	

Chr11	37000000	66800000	-0.69	-14.39
Chr02	153200000	193600000	0.37	-14.32
Chr09	30000000	115400000	-0.36	-13.72
Chr01	0	19600000	-0.77	-12.44
Chr10	200000	5600000	1.32	-10.20
Chr02	62400000	76000000	0.58	-9.30
Chr12	7400000	31200000	-0.40	-9.25
Chr13	110200000	113800000	3.69	-8.57
Chr06	104400000	170800000	0.24	-8.31
hr18	23000000	30600000	-0.85	-7.70
Chr12	31400000	38200000	-1.65	-7.02
Chr15	88400000	95200000	0.80	-6.84
Chr16	76400000	90000000	-0.45	-6.61
Chr12	52200000	58200000	0.72	-6.00
Chr19	29000000	32000000	1.50	-6.00
Chr08	0	6200000	-0.65	-5.82
Chr10	123000000	126800000	1.09	-5.72
Chr11	96200000	134800000	-0.26	-5.51
Chr17	37600000	39600000	4.54	-5.50
Chr02	122400000	153000000	-0.28	-5.38
Chr07	55800000	76200000	-0.63	-5.35
Chr15	99200000	102200000	1.24	-5.04
Chr11	0	4000000	-1.16	-4.92
Chr12	38400000	52000000	-0.46	-4.85
Chr01	109800000	119800000	-0.44	-4.03
Chr01	120000000	151000000	0.52	-4.01
Chr07	118000000	158800000	-0.21	-3.77
Chr17	22000000	37400000	0.44	-3.48
Chr08	6400000	20000000	0.27	-3.01

(papillary, tubular, mucinous, and poorly cohesive). For the prediction of lymph node metastasis risk, a modified WHO classification can be used to distinguish GCs into the differentiated and undifferentiated types[21]. However, the dysregulation of oncogenes and suppressors owing to multiple genetic and epigenetic alterations has been shown in several studies to be a significant driver of tumorigenesis[22]. The current morphology-based clinical classification of GC can neither convey the molecular heterogeneity of GC nor can it guide clinical practice in predicting the prognosis or treatment response of patients with advanced GC. Although subclassification by molecular testing may add complexity to the classification, it is essential to identify specific GC subtypes based on the molecular and genetic features for the precise and selective targeting of anticancer therapies[23].

A recent publication by TCGA project proposed a molecular classification of GC, which divided it into four subtypes[7]: (1) EBV-positive type, characterized by frequent *PIK3CA* mutations; DNA hypermethylation; and *JAK2*, *CD274*, and *PDCD1LG2* amplification; (2) Microsatellite unstable type, which has a high mutation rate, including the activation of gene mutations that encode oncogene signaling pathway proteins; (3) The genome stable type, which mostly occurs in the diffuse histology and is caused by *RHOA* mutation or THO family GTPase activation protein gene fusion phenomenon; and (4) CIN type, which has an aneuploid chromosome and the receptor tyrosine kinase, which is amplified in situ.

In the present study, we identified three GC subtypes through WGS: (1) EBV-positive GC; (2) *H. pylori*-positive GC; and (3) CIN type GC. Exclusivity was observed among the three subtypes, indicating different modes of tumorigenesis among the subtypes. The three subtypes showed a different genetic



DOI: 10.4251/wjgo.v14.i3.690

Figure 1 The chromosomal landscape. A-D: The whole-genome overview for all (A), EBV-positive (B), *Helicobacter pylori*-positive (C), and chromosomal unstable (D) gastric cancers. Cancer-relevant genes are also indicated. CIN: Chromosomal instability; HP: *Helicobacter pylori*; EBV: Epstein-Barr virus.

pattern. The CIN group was enriched in *ERBB2*amplification, and the *H. pylori* group was enriched in *H. pylori* DNzA and 5p (*TERT*) copy number gains. The distinct genetic patterns may suggest a different treatment approach for each GC subtype, which may require further research. In addition, patients with different molecular subtypes showed distinct prognoses by long rank test (P = 0.019), in which CIN+ patients were found to have the worst survival with a median OS less than 500 d. However, no significant difference of OS was found among the Borrmann types (P = 0.078), a classic GC classification widely used currently. It may indicate that the molecular subtypes in our study have advantages in guiding the prognosis of patients with GC. Nonetheless, owing to the limited sample sizes in this study, additional clinical evidence is needed to support this argument.

Further analyses showed that *H. pylori* positive tumors were associated with gastritis history, which may suggest chronic infections. *H. pylori* colonization causes chronic inflammation as well as a significant increase in the possibility of developing GC[24]. Currently, a persistent *H. pylori* infection is the strongest risk factor for the development of GC. Once *H. pylori* colonizes the gastric epithelium, it

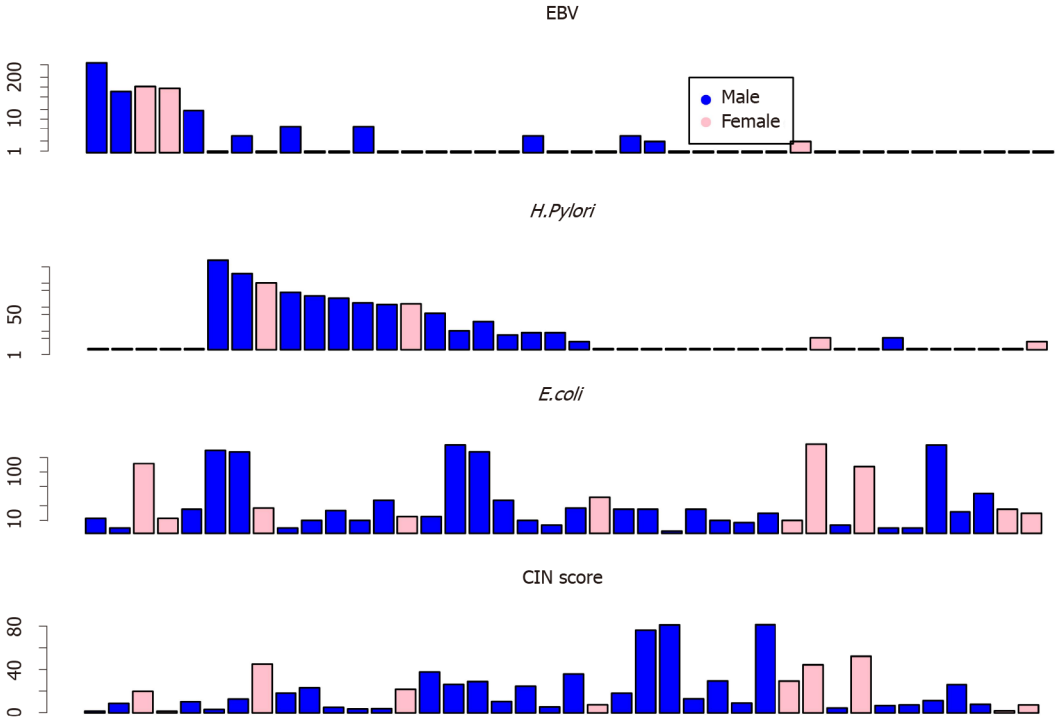


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Figure 2 The bar plot of microbiome read counts and chromosomal instability scores. Read counts of Epstein-Barr virus (top), *Helicobacter pylori* (second row), and *Escherichia coli* (third row) are shown in the bar plots. Chromosomal instability scores for each sample are shown at the bottom. CIN: Chromosomal instability; *H. pylori*: *Helicobacter pylori*; EBV: Epstein-Barr virus; *E. coli*: *Escherichia coli*.

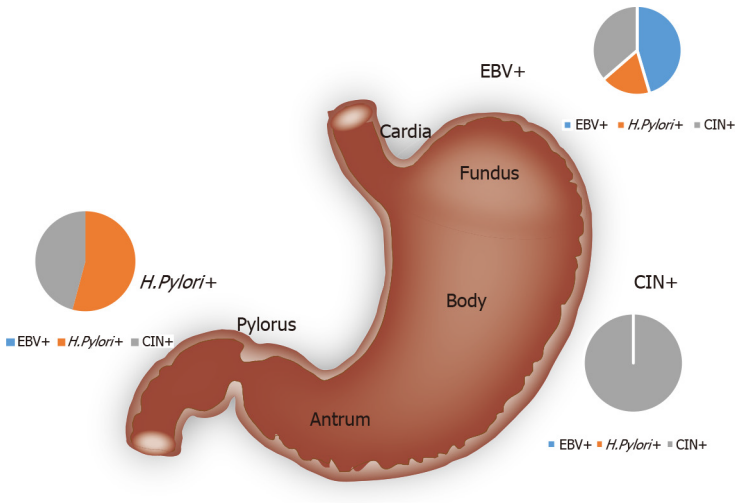

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Figure 3 The gastric cancer subtypes. Epstein-Barr virus enrichment is associated with tumors in the gastric cardia and fundus. *Helicobacter pylori* enrichment associated with tumors in the pylorus and antrum. Tumors with elevated genomic instability can be observed in any location. CIN: Chromosomal instability; *H. pylori*: *Helicobacter pylori*; EBV: Epstein-Barr virus.

may persist for the host lifetime, which may increase the risk of developing GC[25]. Since *H. pylori* inhabits the gastric epithelium of half of the population and has been linked to 38% of the cases of gastric adenocarcinoma included in this study, it is critical to further understand the interrelationship between the host and microbial factors to reduce the risk of gastric adenocarcinoma, which necessitates further studies in this direction.

Approximately, 40% of GCs are characterized by high CIN. Among them, *ERBB2* amplifications were frequently found in this study. HER2, also known as ERBB2, belongs to the ERBB family of proteins, including EGFR (or HER1), HER3, and HER4. Trastuzumab is a humanized monoclonal antibody that binds to HER2 specifically and inhibits its homodimerization and phosphorylation, which results in the inhibition of the proliferation of HER2-overexpressing tumor cells.

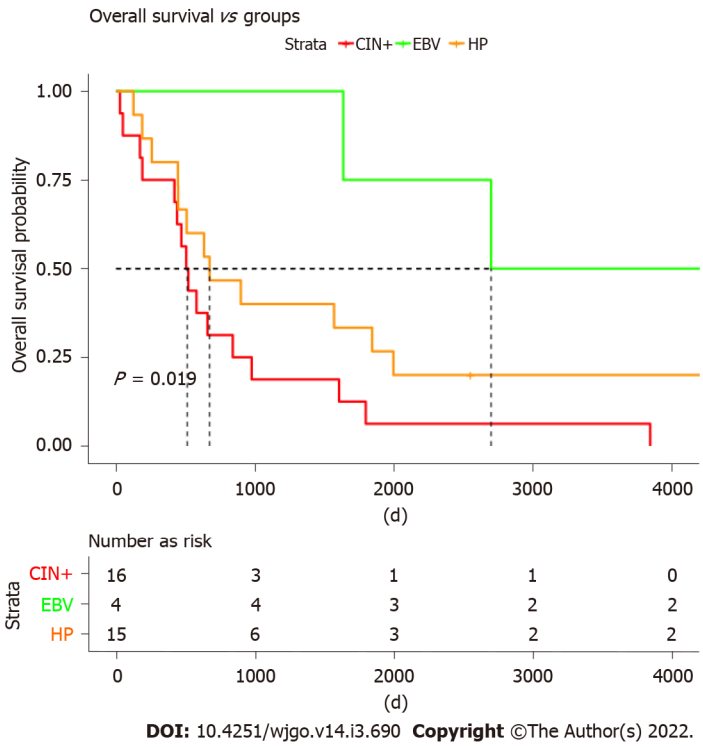

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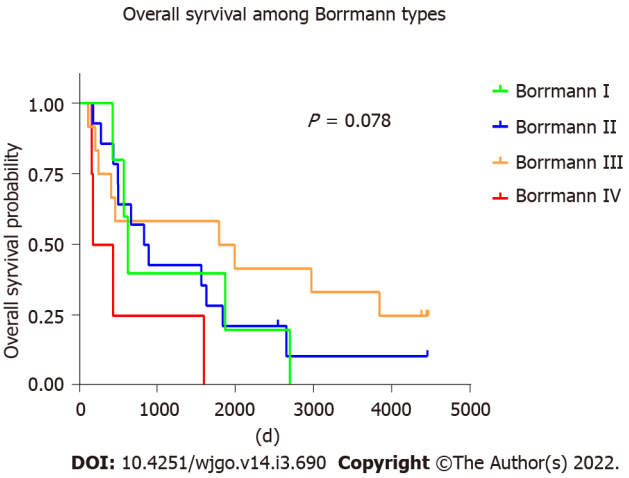
Figure 4 High chromosomal instability associated with worse overall survival. Epstein-Barr virus-positive tumors present with the best overall survival; chromosomal instability (CIN)+ patients presented with the worst survival. Patients with *Helicobacter pylori* infections also showed worse survival as compared to Epstein-Barr virus ones but tended to be better than CIN+ patients. CIN: Chromosomal instability; HP: *Helicobacter pylori*; EBV: Epstein-Barr virus.



DOI: 10.4251/wjgo.v14.i3.690

Figure 5 Overall survival among Borrman types of gastric cancer.

In the present study, approximately 60% of the GC cases were linked to microbiomes, including chronic EBV and *H. pylori* infections. Animal studies have also shown that *H. pylori* eradication treatment at the early stage has considerable potential to reduce the incidence of *H. pylori*-associated GC. Early clinical evidence has shown that *H. pylori* eradication may help prevent the progression of gastric precancerous lesions in some cases. Additionally, *H. pylori* eradication might be the most efficient method for preventing GC. The current clinical data in humans support the idea that the removal of *H. pylori* leads to a reduced risk of developing GC. It is even more useful in patients without intestinal metaplasia or atrophic gastritis[26]. However, the mechanism through which *H. pylori* induces tumorigenesis requires further investigation.

EBV can be found in the vast majority of the general population (at least 90%). However, typically, EBV causes a silent infection in the patient and does not lead to clinically positive symptoms[27]. In some adolescents and young individuals, EBV infection usually leads to infectious mononucleosis with fever, fatigue, headache, lymphadenopathy, sore throat, hepatomegaly, and rash. EBV can also cause B-cell lymphomas, Hodgkin's disease, GC, and nasopharyngeal carcinoma[28]. Hence, targeting EBV may

be an approach to prevent EBV-related GC. However, the latent EBV load in healthy individuals becomes generally stable over time, maintaining a "set point"[29]. Currently, there exists no efficient treatment regimen for the complete clearance of EBV infection.

In the present study, we subtyped GC into four groups using a cost-effective LC-WGS assay. The subtypes showed exclusive genetic features similar to each other, which may suggest different carcinogenesis processes and clinical outcomes of GC. We further analyzed the prognosis of the groups, and CIN+ groups showed poor prognosis. Moreover, EBV-positive patients showed better prognosis than that of negative patients, with median OS around 2800 d. The different clinical outcomes may help clinicians with differential treatment decisions; for example, adjuvant treatment might be recommended for CIN+ patients due to poor prognosis expectations.

The cost of this UCAD assay of LC-WGS is estimated to be ~$ 50 per patient. With the rapid reductions in next-generation sequencing costs, the UCAD assay is expected to become much more cost-effective in the near future. Conventionally, multiple assays, including *H. Pylori*, EBV, and copy number variation assays (most of these assays use the FISH technique, such as HER2 FISH) are performed separately. This leads to a high cost burden for GC patients. Secondly, due to the utility of the WGS technique, the UCAD assay captures not only human DNA but also microbiome DNA, which makes it a more informative technique for GC subtyping than other methods. Collectively, the new technique may help guide GC precision therapy in a cost-effective manner.

This study has a few limitations. The most important limitation of the present study is the limited number of patients recruited. Although statistically significant findings were reported, such as OS for each subtype, the conclusions should be confirmed by increasing the patient numbers. In addition, we only studied the copy number variations and microbiomes (EBV, *H. Pylori*) as GC subtyping biomarkers. Other molecular changes, including methylation and oncogene single nucleotide variations, were not included in this study. In future studies, the potential molecular subtyping markers in addition to the CNV and microbiome markers must be investigated.

CONCLUSION

In the present study, we identified three different GC subtypes associated with different tumorigenesis mechanisms in the Chinese population-chronic EBV infection, *H. pylori* infection, and CIN. Additionally, there were significant differences in the survival outcomes of patients among the three molecular subtypes. Therefore, these findings may beinstructive for future research on the treatment and prevention of GC.

ARTICLE HIGHLIGHTS

Research background

These findings from our research may be instructive for future research on the treatment and prevention of gastric cancer (GC).

Research motivation

Thus, using low-coverage whole-genome sequencing, GC can be classified into three categories based on disease etiology; this classification may prove useful for GC diagnosis and precision medicine.

Research objectives

Epstein-Barr virus (EBV) enrichment was found to be associated with tumors in the gastric cardia and fundus. *Helicobacter pylori* (*H. pylori*) enrichment was found to be associated with tumors in the pylorus and antrum.

Research methods

DNA from the 40 GC patients were subjected to low-coverage whole-genome sequencing by Illumina × 10, followed by copy number analyses using a customized bioinformatics workflow ultrasensitive chromosomal aneuploidy detector. EBV-positive tumors had the best prognosis, whereas patients with higher genomic instability showed the worst survival.

Research results

To investigate biomarkers for GC sub-typing by cost-effective, low-coverage whole-genome sequencing.

Research conclusions

To search for new biomarkers of GC subtypes.

Research perspectives

GC, a multifactorial disease, is caused by pathogens like *H. pylori* or EBV and by genetic components.

ACKNOWLEDGEMENTS

We wish to acknowledge Professor. Tao-Hsin Tung and Dr. Mei-Xian Zhang, Evidence-based Medicine Center, Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University, Linhai 317000, Zhejiang Province, China.

FOOTNOTES

Author contributions: Zhou XB, Wang Y, He SQ, Zhang XG, Zhai LJ, Peng JB, Gu BB, Jin XX, Song YQ, and Ye LP participated in the design of the study and performed the statistical analysis; Mao XL, Xu SW, Qian ZL, and Li SW drafted the manuscript. All authors read and approved the final manuscript.

Supported by Program of Taizhou Science and Technology Grant, No. 20ywb29; Medical Health Science and Technology Project of Zhejiang Province, No. 2021PY083; Key Technology Research and Development Program of Zhejiang Province, No. 2019C03040; Open Project Program of Key Laboratory of Minimally Invasive Techniques & Rapid Rehabilitation of Digestive System Tumor of Zhejiang Province, No. 21SZDSYS01 and 21SZDSYS09; and Major Research Program of Taizhou Enze Medical Center Grant, No. 19EZZDA2.

Institutional review board statement: The study was reviewed and approved by the the Institutional Ethics Committee of Taizhou Hospital of Zhejiang Province (Approval No. K20201205).

Conflict-of-interest statement: Author Qian ZL was employed by the company Suzhou Hongyuan Biotech Inc., and Zhang XG and Zhai LJ were employed by the company Catcher Bio Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at li_shaowei81@hotmai.com.

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S-Editor: Zhang H
L-Editor: A
P-Editor: Zhang H

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 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]
3 **Assarzadegan N**, Montgomery E. What is New in the 2019 World Health Organization (WHO) Classification of Tumors of the Digestive System: Review of Selected Updates on Neuroendocrine Neoplasms, Appendiceal Tumors, and Molecular Testing. *Arch Pathol Lab Med* 2021; **145**: 664-677 [PMID: 32233993 DOI: 10.5858/arpa.2019-0665-RA]
4 **Plummer M**, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to Helicobacter

pylori. *Int J Cancer* 2015; **136**: 487-490 [PMID: 24889903 DOI: 10.1002/ijc.28999]
5 **Moss SF**. The Clinical Evidence Linking *Helicobacter pylori* to Gastric Cancer. *Cell Mol Gastroenterol Hepatol* 2017; **3**: 183-191 [PMID: 28275685 DOI: 10.1016/j.jcmgh.2016.12.001]
6 **de Martel C**, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012; **13**: 607-615 [PMID: 22575588 DOI: 10.1016/S1470-2045(12)70137-7]
7 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
8 **Selgrad M**, Malfertheiner P, Fini L, Goel A, Boland CR, Ricciardiello L. The role of viral and bacterial pathogens in gastrointestinal cancer. *J Cell Physiol* 2008; **216**: 378-388 [PMID: 18338378 DOI: 10.1002/jcp.21427]
9 **Ottini L**, Falchetti M, Lupi R, Rizzolo P, Agnese V, Colucci G, Bazan V, Russo A. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol* 2006; **17** Suppl 7: vii97-vi102 [PMID: 16760303 DOI: 10.1093/annonc/mdl960]
10 **Wang X**, Liu Y, Shao D, Qian Z, Dong Z, Sun Y, Xing X, Cheng X, Du H, Hu Y, Li Y, Li L, Dong B, Li Z, Wu A, Wu X, Bu Z, Zong X, Zhu G, Ji Q, Wen XZ, Zhang LH, Ji JF. Recurrent amplification of MYC and TNFRSF11B in 8q24 is associated with poor survival in patients with gastric cancer. *Gastric Cancer* 2016; **19**: 116-127 [PMID: 25618371 DOI: 10.1007/s10120-015-0467-2]
11 **Qian Z**, Zhu G, Tang L, Wang M, Zhang L, Fu J, Huang C, Fan S, Sun Y, Lv J, Dong H, Gao B, Su X, Yu D, Zang J, Zhang X, Ji J, Ji Q. Whole genome gene copy number profiling of gastric cancer identifies PAK1 and KRAS gene amplification as therapy targets. *Genes Chromosomes Cancer* 2014; **53**: 883-894 [PMID: 24935174 DOI: 10.1002/gcc.22196]
12 **Scheinin I**, Sie D, Bengtsson H, van de Wiel MA, Olshen AB, van Thuijl HF, van Essen HF, Eijk PP, Rustenburg F, Meijer GA, Reijneveld JC, Wesseling P, Pinkel D, Albertson DG, Ylstra B. DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly. *Genome Res* 2014; **24**: 2022-2032 [PMID: 25236618 DOI: 10.1101/gr.175141.114]
13 **Camargo MC**, Murphy G, Koriyama C, Pfeiffer RM, Kim WH, Herrera-Goepfert R, Corvalan AH, Carrascal E, Abdirad A, Anwar M, Hao Z, Kattoor J, Yoshiwara-Wakabayashi E, Eizuru Y, Rabkin CS, Akiba S. Determinants of Epstein-Barr virus-positive gastric cancer: an international pooled analysis. *Br J Cancer* 2011; **105**: 38-43 [PMID: 21654677 DOI: 10.1038/bjc.2011.215]
14 **Yan M**, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. *Cancer Metastasis Rev* 2015; **34**: 157-164 [PMID: 25712293 DOI: 10.1007/s10555-015-9552-6]
15 **Wilson EB**. Probable Inference, the Law of Succession, and Statistical Inference. *J Am Stat Assoc* 1927; **22**: 209-212
16 **GBD 2017 Stomach Cancer Collaborators**. The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 42-54 [PMID: 31648970 DOI: 10.1016/S2468-1253(19)30328-0]
17 **Zhou C**, Zhong X, Song Y, Shi J, Wu Z, Guo Z, Sun J, Wang Z. Prognostic Biomarkers for Gastric Cancer: An Umbrella Review of the Evidence. *Front Oncol* 2019; **9**: 1321 [PMID: 31850212 DOI: 10.3389/fonc.2019.01321]
18 **Ceausu M**, Socea B, Ciobotaru VP, Constantin VD, Enache S, Enache V, Bancu A, Socea LI, Șerban D, Predescu D, Smarandache CG, Ceausu Z. A multidisciplinary approach in the diagnostic challenge of GIST. *Exp Ther Med* 2021; **22**: 1063 [PMID: 34434277 DOI: 10.3892/etm.2021.10497]
19 **Call J**, Walentas CD, Eickhoff JC, Scherzer N. Survival of gastrointestinal stromal tumor patients in the imatinib era: life raft group observational registry. *BMC Cancer* 2012; **12**: 90 [PMID: 22429770 DOI: 10.1186/1471-2407-12-90]
20 **Radulescu D**, Baleanu VD, Padureanu V, Radulescu PM, Bordu S, Patrascu S, Socea B, Bacalbasa N, Surlin MV, Georgescu I, Georgescu EF. Neutrophil/Lymphocyte Ratio as Predictor of Anastomotic Leak after Gastric Cancer Surgery. *Diagnostics (Basel)* 2020; **10** [PMID: 33050137 DOI: 10.3390/diagnostics10100799]
21 **Wang Q**, Liu G, Hu C. Molecular Classification of Gastric Adenocarcinoma. *Gastroenterology Res* 2019; **12**: 275-282 [PMID: 31803306 DOI: 10.14740/gr1187]
22 **Cheng Y**, He C, Wang M, Ma X, Mo F, Yang S, Han J, Wei X. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther* 2019; **4**: 62 [PMID: 33323938]
23 **Locke WJ**, Guanzon D, Ma C, Liew YJ, Duesing KR, Fung KYC, Ross JP. DNA Methylation Cancer Biomarkers: Translation to the Clinic. *Front Genet* 2019; **10**: 1150 [PMID: 31803237 DOI: 10.3389/fgene.2019.01150]
24 **Salama NR**, Hartung ML, Müller A. Life in the human stomach: persistence strategies of the bacterial pathogen Helicobacter pylori. *Nat Rev Microbiol* 2013; **11**: 385-399 [PMID: 23652324 DOI: 10.1038/nrmicro3016]
25 **Wroblewski LE**, Peek RM Jr, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev* 2010; **23**: 713-739 [PMID: 20930071 DOI: 10.1128/CMR.00011-10]
26 **Cheung TK**, Wong BC. Treatment of Helicobacter pylori and prevention of gastric cancer. *J Dig Dis* 2008; **9**: 8-13 [PMID: 18251788 DOI: 10.1111/j.1443-9573.2007.00315.x]
27 **Cohen JI**. Optimal treatment for chronic active Epstein-Barr virus disease. *Pediatr Transplant* 2009; **13**: 393-396 [PMID: 19032417 DOI: 10.1111/j.1399-3046.2008.01095.x]
28 **Kimura H**, Cohen JI. Chronic Active Epstein-Barr Virus Disease. *Front Immunol* 2017; **8**: 1867 [PMID: 29375552 DOI: 10.3389/fimmu.2017.01867]
29 **Hatton OL**, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between Epstein-Barr virus and B lymphocytes: implications for infection, immunity, and disease. *Immunol Res* 2014; **58**: 268-276 [PMID: 24619311 DOI: 10.1007/s12026-014-8496-1]

World Journal of Gastrointestinal Oncology

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World J Gastrointest Oncol 2022 March 15; 14(3): 703-715

DOI: 10.4251/wjgo.v14.i3.703

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Computed tomography-based radiomic to predict resectability in locally advanced pancreatic cancer treated with chemotherapy and radiotherapy

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: He YQ, Kamiyama T

Received: May 17, 2021
Peer-review started: May 17, 2021
First decision: July 14, 2021
Revised: August 6, 2021
Accepted: February 11, 2022
Article in press: February 11, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

Surgical resection after neoadjuvant treatment is the main driver for improved survival in locally advanced pancreatic cancer (LAPC). However, the diagnostic performance of computed tomography (CT) imaging to evaluate the residual tumour burden at restaging after neoadjuvant therapy is low due to the difficulty in distinguishing neoplastic tissue from fibrous scar or inflammation. In this context, radiomics has gained popularity over conventional imaging as a complementary clinical tool capable of providing additional, unprecedented information regarding the intratumor heterogeneity and the residual neoplastic tissue, potentially serving in the therapeutic decision-making process.

AIM

To assess the capability of radiomic features to predict surgical resection in LAPC treated with neoadjuvant chemotherapy and radiotherapy.

METHODS

Patients with LAPC treated with intensive chemotherapy followed by ablative radiation therapy were retrospectively reviewed. One thousand six hundred and fifty-five radiomic features were extracted from planning CT inside the gross tumour volume. Both extracted features and clinical data contribute to create and

validate the predictive model of resectability status. Patients were repeatedly divided into training and validation sets. The discriminating performance of each model, obtained applying a LASSO regression analysis, was assessed with the area under the receiver operating characteristic curve (AUC). The validated model was applied to the entire dataset to obtain the most significant features.

RESULTS

Seventy-one patients were included in the analysis. Median age was 65 years and 57.8% of patients were male. All patients underwent induction chemotherapy followed by ablative radiotherapy, and 19 (26.8%) ultimately received surgical resection. After the first step of variable selections, a predictive model of resectability was developed with a median AUC for training and validation sets of 0.862 (95%CI: 0.792-0.921) and 0.853 (95%CI: 0.706-0.960), respectively. The validated model was applied to the entire dataset and 4 features were selected to build the model with predictive performance as measured using AUC of 0.944 (95%CI: 0.892-0.996).

CONCLUSION

The present radiomic model could help predict resectability in LAPC after neoadjuvant chemotherapy and radiotherapy, potentially integrating clinical and morphological parameters in predicting surgical resection.

Key Words: Computed tomography; Radiomics; Predictive model; Resectability; Locally advanced pancreatic cancer; Radiation oncology

Core Tip: The present study proposes a computed tomography (CT)-based radiomics model to predict resectability in locally advanced pancreatic cancer (LAPC) treated with intensive chemotherapy followed by ablative radiation therapy. The model was built, tested, and validated in a homogeneous cohort of LAPC patients, using clinical data and radiomic features extracted from the simulation-CT, and showed a reliable performance to predict surgical resection. If further confirmed, the results of this study may allow integrating radiomic information into the pool of clinical and morphological parameters to consider when a LAPC patient is candidate for surgical exploration after neoadjuvant therapy.

Citation: Rossi G, Altabella L, Simoni N, Benetti G, Rossi R, Venezia M, Paiella S, Malleo G, Salvia R, Guariglia S, Bassi C, Cavedon C, Mazzarotto R. Computed tomography-based radiomic to predict resectability in locally advanced pancreatic cancer treated with chemotherapy and radiotherapy. *World J Gastrointest Oncol* 2022; 14(3): 703-715

URL: https://www.wjgnet.com/1948-5204/full/v14/i3/703.htm

DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.703

INTRODUCTION

Pancreatic cancer (PC) is an aggressive disease, with increasing incidence and mortality rates, and a 5-year survival of less than 10%[1,2]. At the time of diagnosis, roughly one-third of patients present with locally advanced PC (LAPC), typically due to extensive involvement of peripancreatic vessels (*e.g.*, celiac axis, superior mesenteric artery and vein, portal vein, common hepatic artery), that precludes surgical resection[3]. Nowadays, multiagent chemotherapy regimens, including gemcitabine plus nanoparticle albumin-bound (nab)-paclitaxel and 5-fluorouracil, leucovorin, irinotecan, plus oxaliplatin (FOLFIRINOX), represent the standard of care for LAPC, able to significantly improve survival compared to mono-chemotherapy schedules[4-6]. In parallel, the integration of dose-escalated radiation therapy (RT) approaches to intensive chemotherapy regimens have suggested the possibility to maximize the benefits of the oncological treatment[7,8]. Technological innovations, such as the use of stereotactic techniques, advanced organ motion management solutions, and accurate image guidance before treatment delivery, have allowed to safely deliver ablative doses to LAPC while sparing the surrounding critical organs at risk (OARs)[9,10]. In addition, different series reported that a multimodal approach, including systemic induction therapy followed by (chemo-) radiotherapy, might represent an effective therapeutic option for LAPC, potentially improving the oncological outcome and the probability of surgical resection, at the price of acceptable postoperative complication rates[11-14]. Thus, there is a strong need to determine the resectability of LAPC for treatment decision-making.

After neoadjuvant treatment, the therapeutic decision whether to perform surgical exploration is typically based on a multidisciplinary and multiparametric evaluation that includes patients- and tumour-specific features. Cross-sectional imaging at restaging is essential to rule out disease progression that would contraindicate surgery and drive the surgical strategy if radical resectability is considered feasible. Indeed, several studies have reported that computed tomography (CT) misestimates the resectability of LAPC after neoadjuvant treatment[15-17]. After RT, CT cannot discriminate between post-therapy fibrosis, locoregional oedema, inflammatory changes, and viable tumour, thus underestimating the histological response[18-20].

Radiomics is gaining more and more popularity for the possibility of decoding crucial information underneath medical imaging. Unlike conventional CT image analysis, radiomics can use imaging data for the high-throughput extraction of large numbers of quantitative features, able to offer additional information related to tumour phenotype, its heterogeneity, and microenvironment, as well as post-treatment changes[21]. Additionally, this information can be used to create accurate, reliable, and efficient predictive models throughout the application of machine learning algorithms[22]. To date, only a few studies have investigated the application of the radiomics framework to CT imaging to obtain potential biomarkers predictive and prognostic for treatment response and survival in PC treated with chemotherapy and radiotherapy[23-27]. More recently, the possibility of using CT radiomic biomarkers as predictors of resectability in oesophageal cancer[28] and thymic malignancies[29] has been investigated.

This study aimed to explore, for the first time, whether a CT-based radiomic model could assess LAPC patients' resectability after neoadjuvant treatment, including induction chemotherapy followed by ablative RT.

MATERIALS AND METHODS

Study design

Clinical, radiological, laboratory, surgical, and pathology data of LAPC patients receiving intensive induction chemotherapy followed by ablative RT from January 2017 to April 2020 were prospectively collected and retrospectively evaluated. The Institutional Review Board (IRB) approved the prospective collection of patient data (PAD-R n.1101 CESC). The National Comprehensive Cancer Network (NCCN) classification was used to define LAPC[30]. As described in the original published study[14], inclusion criteria for Risk Adapted Ablative Radiation Therapy (RAdAR) were: Histologically-proven pancreatic ductal adenocarcinoma, ECOG performance status < 2, at least 3 mo of chemotherapy (with gemcitabine/nab-paclitaxel or FOLFIRINOX), biochemical response, and absence of disease progression at restaging CT-scan after induction chemotherapy.

RT protocol and surgery

Details of the radiation treatment have previously been reported[14]. Briefly, the RAdAR approach consisted of anatomy- and simultaneous integrated boost (SIB)-based dose prescription strategy. If anatomically and dosimetrically feasible, the first treatment choice was stereotactic ablative RT (SAbR). However, in the following cases, the (hypo-) fractionated ablative radiotherapy (HART) schedule was adopted: Tumour 6 cm in greatest dimension, nodal spread of disease that could not be included in the SAbR target volume, tumour adhesion/infiltration of the stomach or duodenum, and/or impossibility to achieve SAbR planning objectives (*e.g.*, non-respect of OARs dose constraints).

Following induction chemotherapy, RT was delivered with SAbR, administering 30 Gy in 5 fractions to the tumour volume (PTVt) and 50 Gy SIB to the vascular involvement, or with HART prescribing 50.4 Gy in 28 fractions to the PTVt, with a vascular SIB of 78.4 Gy. Thus, an ablative biologically effective dose (BED_{10} = 100 Gy) within the tumour was prescribed for both SAbR and HART. The RAdAR was delivered using RapidArc® Technology (Varian Medical Systems, Palo Alto, CA, United States) or TomoTherapy® System (Accuray, Sunnyvale, CA, United States). Daily on-line volumetric image-guided radiotherapy (cone beam or megavoltage CT) was performed before each treatment fraction. After restaging, a multidisciplinary team re-evaluated the patient and, in the absence of tumour progression, re-considered surgery if radical resection was deemed feasible; if not, patients were candidate for follow-up.

Image acquisitions and tumour segmentation

For simulation CT, patients were immobilized in the supine position with arms over the head. After a scan without contrast, a tri-phase contrast-enhanced simulation CT scan was carried out, including an arterial, a pancreatic parenchymal, and a portal venous phase, using a 64-row scanner (Brilliance 64; Philips, Eindhoven, The Netherlands). A minimum 3-h fasting period was required for all patients before simulation CT. For contrast-enhanced imaging, a weight-based amount of iodinated nonionic contrast agent was used, with an automatic power injector at a flow rate of 2–3.5 mL/s. A bolus-tracking technique at standardized time was used for contrast-enhanced phases. CT images were acquired with a 64 mm × 0.625–1.25 mm collimation, with a reconstruction thickness of 2 mm.

Texture analysis was performed using contrast-free simulation CT imaging. Tumour segmentation was performed using a software for medical image processing (MIM software; Mim Software Inc., United States). The volume of interest (VOI) corresponded to the gross tumour volume (GTV) and was defined by a radiation oncologist with experience in PC and validated by a second radiation oncologist (Figure 1). The segmentation excluded vessels, biliary stent, calcifications, fiducial markers, or any other potential source of artifact from the GTV (VOI) (Figure 1).

Radiomic feature extraction

Radiomic features were extracted from the VOI using PyRadiomics v3.0[31]. Firstly, the VOI was resampled with an isotropic voxel of 2 mm × 2 mm × 2 mm, and HU were binned considering a width of 5 HU. The extracted features are defined according to Imaging Biomarker Standardized Initiative (IBSI)[32] and include first order statistics, shaped based both 2D and 3D, Gray Level Co-occurrence Matrix, Gray Level Run Length Matrix, Gray Level Size Zone Matrix, Neighbouring Gray Tone Difference Matrix, and Grey Level Dependence Matrix, as well as filtered features (logarithm exponential, gradient, LBP3D, and wavelet). A total of 1655 radiomic features were considered.

Statistical analysis

Statistical analysis of clinical (tumour location and size, cancer antigen 19-9 (CA19-9) value at diagnosis and after chemotherapy, clinical stage, chemotherapy regimen and radiation approach) and radiomic data was implemented in R (v3.6.3). For the analysis, patients surgically explored (*e.g.* exploratory laparotomy after RT) but not resected were integrated into the non-resected group. The complete workflow of statistical analysis included a first step of variable selection and a training/validation step to find the model that better predicted the outcome. Subsequently the validated model was applied to the whole dataset.

Multivariate analysis was performed firstly on the training set that included 70% of the whole database. Clinical data and radiomic features were tested for their capability to predict surgical resection using Wilcoxon rank-sum test. Correlated features were identified using Pearson correlation considering a threshold of 0.9 and were removed for further analysis. *P* values of the remaining variables were corrected for multiple comparisons considering Bonferroni correction (*P* corrected < 0.01). Multivariate LASSO regression analysis was performed to select relevant variables and build the model. The optimal lambda parameter was chosen as an average of the regularization parameter obtained from 50 times repetition of the 5-fold cross-validation process.

The model was then tested on the validation dataset, and corresponding area under the receiver operating characteristic curve (AUC) and their confidence intervals were computed. To improve the statistical significance and robustness of our analysis, all the steps were repeated 100 times. More precisely, among these repeated analyses, the solution that presents the median AUC was chosen as representative, and corresponding confidence intervals were computed throughout a bootstrap. The already tested model was re-trained on the whole dataset to obtain robust selected variables, and AUC was computed. To assess the predictive capability of our model, this was finally applied to the entire cohort to predict the surgical resection status and compute the OS for predicted surgery *vs* no predicted surgery patients. The log-rank test was used to assess statistical significance for survival curves.

RESULTS

Study population

Seventy-one LAPC patients were included in the analysis. Baseline characteristics are outlined in Table 1. The median age was 65 years [interquartile range (IQR) 57-69], and 57.8% of patients were male. The median period of induction chemotherapy was 6 mo (IQR 6-6 mo). SAbR was used in 59 (83.1%) patients and HART in the remaining 12 (16.9%). All patients completed the prescribed treatment. Thirty-two patients (45.1%) underwent exploratory laparotomy after RT, and 19 (26.8%) patients ultimately received surgical resection, with a resection/exploration ratio of 59.4%. Postoperative 90-d mortality was nil. The median follow-up for the analysis was 15.0 mo (IQR 11.2-20.2 mo). Overall survival (OS) curves and their confidence intervals, estimated by Kaplan–Meier method as a function of surgical resection (resected *vs* non-resected patients; $P < 0.001$), are shown in Figure 2A.

Prediction model for resectability

Median AUC for training and validation sets were 0.862 (95%CI: 0.792-0.921) and 0.853 (95%CI: 0.706-0.960), respectively. Box plots in Figure 3 summarized the AUC distributions for both datasets for all the 100 repetitions and the ROC curve of the median solution both for train and validation test. Among the 100 repetitions of the training process, the clinical variables were rarely selected from LASSO. On average, 98% of the selected variables were radiomic features, indicating a higher predictive power of radiomic data with respect to clinical data.

Table 1 Baseline characteristics and treatment details

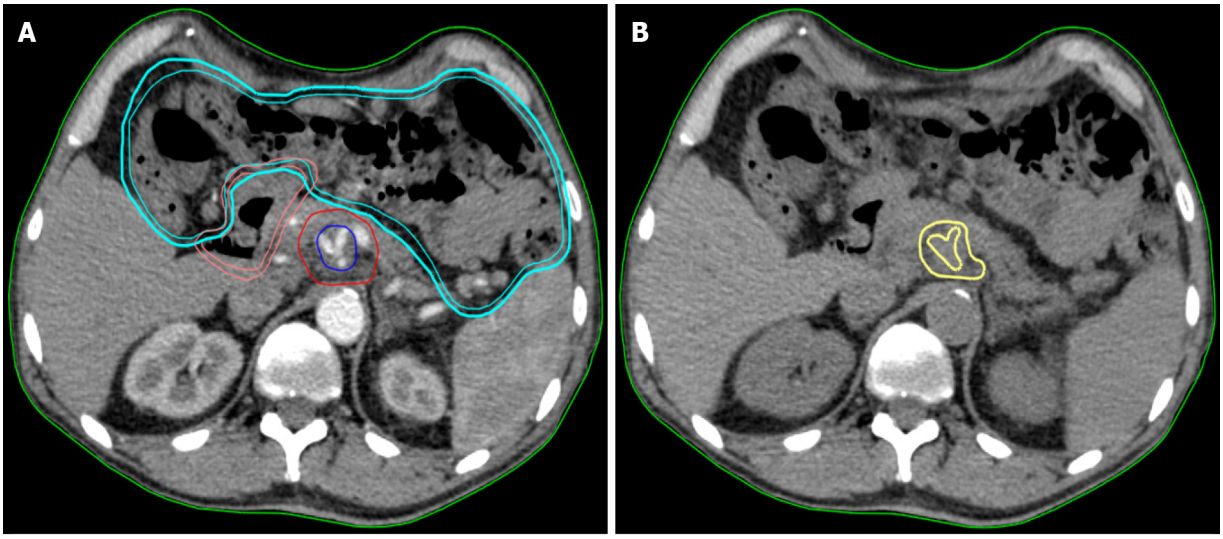
Characteristic	Value
No. of patients	71
Age, yr, median (IQR)	64.8 (57.1-69.6)
Sex, male, *n* (%)	41 (57.8)
ECOG, 0, *n* (%)	61 (85.9)
Primary tumour location, *n* (%)	
Head	44 (62.0)
Body	25 (35.2)
Neck	2 (2.8)
Tumour size (mm), median (IQR)	40 (30-45)
Biliary stent, present, *n* (%)	25 (35.2)
CA19-9 (U/mL) at diagnosis	951 (± 1134)
CA19-9 (U/mL) after chemotherapy (before RAdAR)	106 (± 160)
Clinical T stage[1], *n* (%)	
T2	6 (8.5)
T3	11 (15.5)
T4	54 (76.0)
Clinical N stage[1], *n* (%)	
N0	34 (47.9)
N1	37 (52.1)
Pre-RAdAR chemotherapy regimen, *n* (%)	
FOLFIRINOX	30 (42.3)
Gemcitabine + nab-paclitaxel	41 (57.7)
RAdAR approach, *n* (%)	
SAbR	59 (83.2)
HART	12 (16.9)
Delivery technique, *n* (%)	
RapidArc® Technology	55 (77.5)
TomoTherapy® System	16 (22.5)
Resected patients[2], *n* (%)	19 (26.8)
R status[3], *n* (%)	
R0	12 (63.2)
R1	7 (36.8)

[1]*Per* the AJCC staging system, eighth edition.
[2]Exploratory laparotomy in 32 patients (45.1%), resection/exploration ratio 59.4%.
[3]Among resected patients, *n* = 19.
CA: Celiac artery; CHA: Common hepatic artery; FOLFIRINOX: Fluorouracil, leucovorin, oxaliplatin; HART: Hypofractionated ablative radiotherapy; IQR: Interquartile range; PV: Portal vein; SMA: Superior mesenteric artery; SAbR: Stereotactic ablative radiation therapy; SMV: Superior mesenteric vein.

Applying the validated model on the entire dataset, 4 features were selected from the LASSO regression. Figure 4 depicts the LASSO variable selection process and the AUC as a function of the lambda parameter. Four variables survived to LASSO regression in correspondence with the best lambda value (dotted line in Figure 4). Lambda was chosen as the value at one standard deviation after the value that maximises AUC. The selected features, the *P* value from the Wilcoxon test, and their adjusted *P*-value for Bonferroni correction are reported in Table 2. In addition, the slope of LASSO regression coefficients for each variable is shown. Starting from the selected variables, the correlation

Table 2 Selected features, *P* value from Wilcoxon test, adjust *P* value for Bonferroni correction, and LASSO regression coefficients (for each variable)

Variable name	*P* value Wilcoxon	*P* value corrected	LASSO slope
lbp.3D.m1_glrlm_LongRunLowGrayLevelEmphasis	1.01E-09	4.82E-07	-0.146
wavelet.LLL_glcm_Imc2	1.17E-06	5.61E-04	0.039
lbp.3D.m2_glszm_GrayLevelNonUniformityNormalized	4.01E-06	1.92E-03	0.113
exponential_glrlm_RunLengthNonUniformityNormalized	4.02E-06	1.92E-03	0.056



DOI: 10.4251/wjgo.v14.i3.703

Figure 1 Texture analysis performed using contrast-free simulation computed tomography imaging. A: Target volumes delineation in a stereotactic ablative radiation therapy case. The high-dose planning target volume (blue) encompasses the tumour-vessel interface (TVI, encasement of celiac axis) inside the tumour planning target volume (PTVt, red). The prescription dose is 30 Gy and 50 Gy in 5 fractions, with simultaneous integrated boost, to PTVt and TVI, respectively. The following organ at risk are shown: duodenum (pink) and bowel (cyan); B: The gross tumour volume (GTV) of the pancreatic lesion without vessels (yellow) is shown in the same axial computed tomography (CT)-simulation image without contrast. This is the final CT used for analysis.

matrix between these features and all other features with at least one Pearson correlation higher than 0.9 are reported in Figure 5, as a reference for external validation.

AUC obtained for the model applied on the entire dataset is 0.944 (95%CI: 0.892-0.996) (Figure 6). OS curves and their confidence intervals, estimated by Kaplan–Meier method as a function of surgical resection (resected *vs* non-resected patients) as predicted by the model, are shown in Figure 2B ($P < 0.001$).

DISCUSSION

To the best of our knowledge, the present study is the first to propose a CT-based radiomics model to predict resectability in LAPC treated with intensive chemotherapy followed by ablative RT. The model was built, tested, and validated in a homogeneous cohort of LAPC patients, using clinical data and radiomic features extracted from the simulation-CT, and showed a reliable performance to predict surgical resection.

Surgical resection after neoadjuvant treatment is the main driver for improved survival in LAPC[11]. However, the diagnostic performance of CT imaging to evaluate the residual tumour burden at restaging after neoadjuvant therapy is low, due to the difficulty in distinguishing neoplastic tissue from fibrous scar or inflammation[33]. In this context, radiomics has gained popularity over conventional imaging as a complementary clinical tool capable of providing additional, unprecedented information regarding the intratumor heterogeneity and the residual neoplastic tissue, potentially serving in the therapeutic decision-making process.

In the present study, we identified four potential CT-based radiomic features (lbp.3D.m1_glrlm_LRLGE; wavelet.LLL_glcm_Imc2; lbp.3D.m^2_glszm_GLLN; exponential_glrlm_RLNN) to build a CT radiomic model, which could be useful in predicting resectability in LAPC patients undergoing

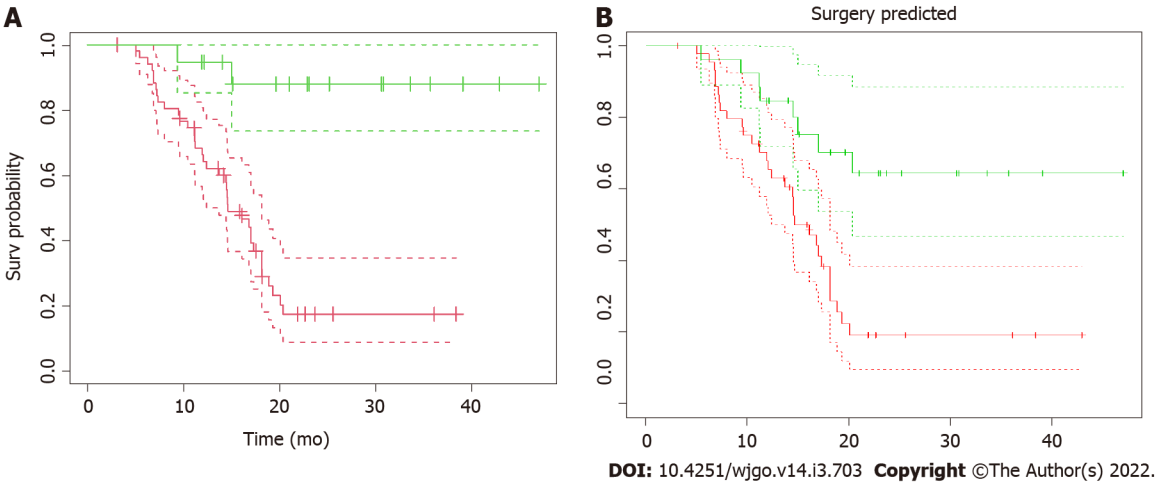


Figure 2 Kaplan–Meier overall survival. A: Kaplan–Meier overall survival (OS) as a function of surgical resection. Patients were stratified according to resectability status and OS curves from radiotherapy for resected (green line) *vs* non-resected (red line) and their confidence intervals (dotted lines) were plotted. The median OS from radiation therapy for resected patients has not been reached, compared to 14.6 mo (CI: 12.4-18.1 mo) of non-resected patients ($P < 0.001$); B: Kaplan–Meier OS as a function of surgical resection as predicted by the model. The "predicted" OS curves from radiotherapy for resected (green line) *vs* non-resected (red line) patients and their confidence intervals (dotted lines) showed here were obtained applying the model to the dataset to predict the resectability status. The two curves are significantly different ($P < 0.001$).

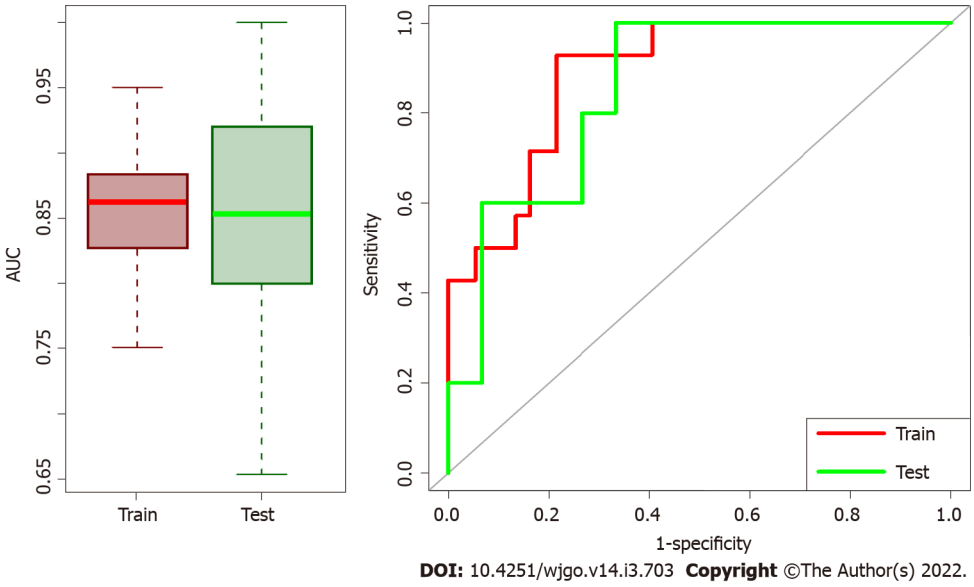
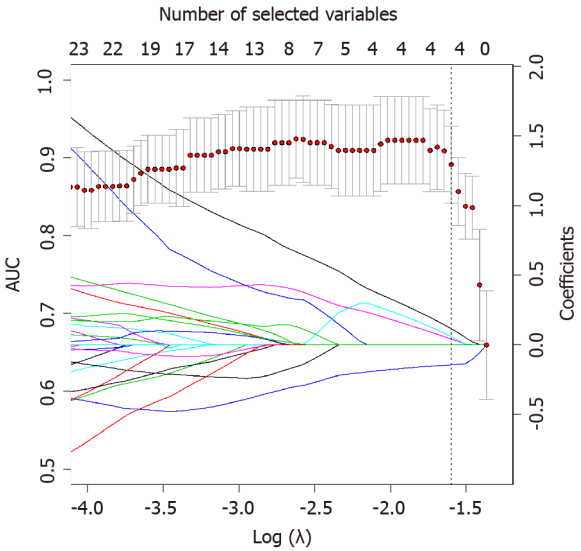


Figure 3 Box plots of area under the receiver operating characteristic curve distributions and receiver operating characteristic curves for test/train datasets. The first part of training model and validation was repeated 100 times and Box plots of area under the receiver operating characteristic (ROC) curve (AUC) distributions for test/train datasets are represented on the left side. Median AUC both for train and validation sets are higher than 0.85 meaning that the model has high performance in both datasets. On the right the ROC curves of the median solution among the repetitions both for train and validation test are shown.

neoadjuvant therapy. Notably, the AUC obtained for the model was 0.94 if applied to the entire dataset. Radiomics has already been used in other contexts of surgical oncology, predicting resectability in oesophageal[28] and thymic cancers[29].

An important consideration of the current study is that clinical data was not significant to separate resected from not-resected patients and did not contribute to the building of the predictive model. This means that radiomic represents a fundamental tool to decode information that cannot be obtained from the direct observation of CT images or other clinical data alone[34]. Notably, the "real" OS curves (Figure 2A) and "predicted" OS curves obtained applying the validated model to the entire database without considering the information of resectability status (Figure 2B) are comparable. This is an indirect validation of the model that can independently predict before RT whether the patient can be a candidate for surgical resection, leading to comparable OS curves. This finding is particularly relevant from a clinical point of view. Indeed, although recent retrospective series have suggested that RT can complement induction chemotherapy and improve LAPC resectability[11-13], the role of (chemo-) RT



DOI: 10.4251/wjgo.v14.i3.703

Figure 4 LASSO regression coefficients and area under the receiver operating characteristic curve as function of lambda parameter. LASSO regression coefficients for each variable (represented by continuous lines, each line is a variable that are imposed to reach zero by LASSO regression as lambda increases) and area under the receiver operating characteristic curve (AUC) as function of Log(λ) are superimposed. Dotted line represents the best lambda parameters that corresponds to the value at one standard deviation after the lambda that maximize AUC. As it is possible to notice, the best lambda value corresponds to four variables selected by LASSO.

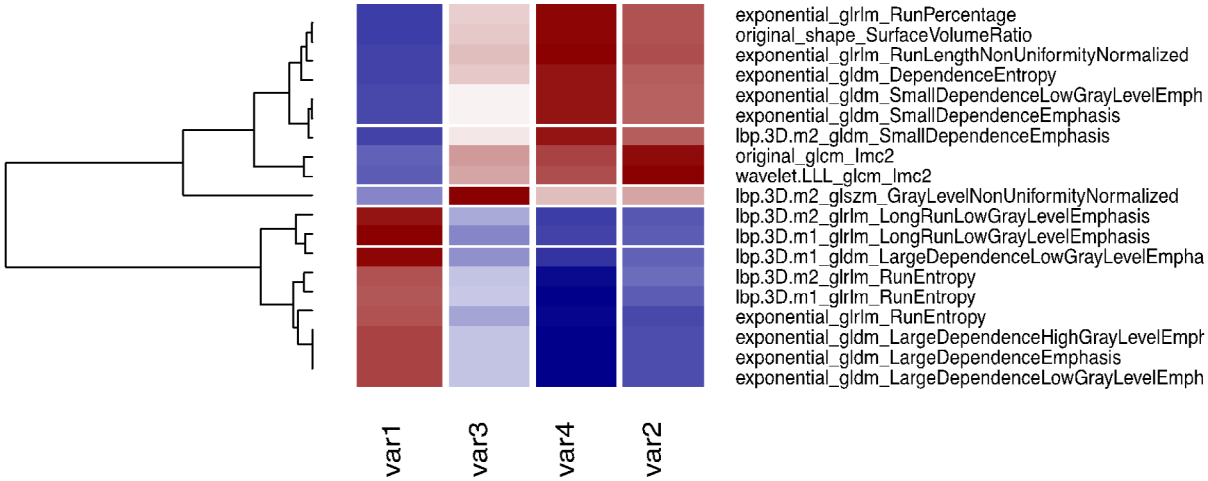


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Figure 5 Correlation plot of the selected variables and other significant features. The four selected variables (named var1, var2, var3 and var4 for simplicity, please refers to the end of this caption for the correspondence with variable names), are correlated (r > 0.9) with other variables that are rejected for further analysis. Here we report the correlated variables as reference for further studies that could find significant features correlated with our variables. Var1: Lbp.3D.m1_glrlm_LongRunLowGrayLevelEmphasis; Var2: Wavelet.LLL_glcm_Imc2; Var3: Lbp.3D.m^2_glszm_GrayLevelNonUniformityNormalized; Var4: Exponential_glrlm_RunLengthNonUniformityNormalized.

after systemic therapy in LAPC is still controversial[35].

The potential to predict surgical resection of LAPC, especially in patients still considered unresectable after induction chemotherapy, could drive to a more aggressive effort of conversion to surgery by the means of RT. For this purpose, the use of simulation CT for features' extraction appears particularly appropriate to add homogeneity, repeatability, robustness, and simplicity to the model under evaluation. The use of free-contrast planning CT scans was previously reported as a basis of the analysis to derive the textural features related to survival in LAPC treated with SAbR. Analysing data of 100 patients, the authors found a significant association between a clinical-radiomic signature and survival of LAPC in both training and validation sets (P = 0.01 and 0.05 and concordance index 0.73 and 0.75, respectively)[25].

The analysis of the present study has some significant strengths. First, a three-step machine learning method was implemented. More precisely, the model was trained on a training subset of patients finding a robust AUC (median value: 0.86); subsequently, this model was validated and confirmed on a

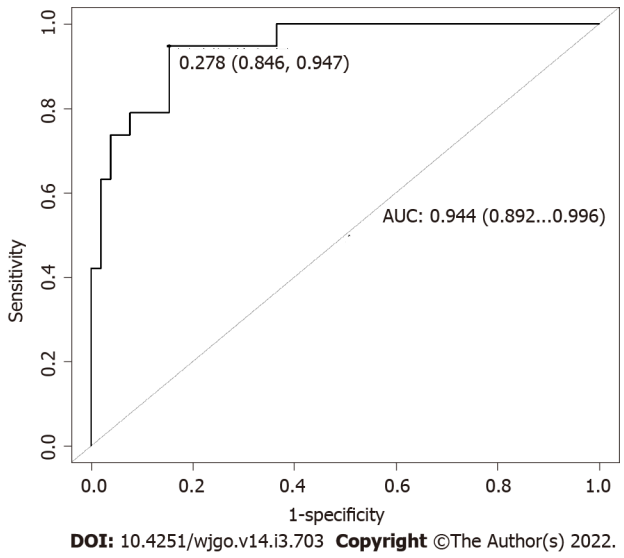

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Figure 6 Area under the receiver operating characteristic curve obtained for the model applied on the entire dataset. To test the performance of our model in the entire dataset, area under the receiver operating characteristic curve obtained applying the model to the entire dataset was computed and represented here. AUC: Area under the receiver operating characteristic curve.

smaller subset (30% of the total patients), confirming high AUC values (median value: 0.85). Ultimately, it was re-applied on the whole database to extract the more significant features that contribute to the prediction, obtaining a high AUC value (0.94). Second, the statistical significance of all the steps gives robust and reliable predictions. Indeed, radiomic analysis and machine learning implementation are prone to lead to several errors and unreproducible results[36]. To give strengths and add reproducibility to the analysis, all results were cross-validated or obtained throughout multiple repetitions and corrected for multiple testing.

In the analysis, all the features correlated with the most significant ones were rejected to maintain only those variables that explained more variance. All the significant features correlated with at least one of the selected features are shown in Figure 5. The names of correlated variables are provided, allowing other centres to reproduce the presented results. Further studies could find significant variables different to those reported in our study, but correlated with them, indicating they have the same underlying radiomic information. Lastly, the possibility to extract valuable information on planning CT without additional diagnostic methodologies adds simplicity and the possibility to apply this in the clinical practice.

A further frontier of the application of radiomics, not evaluated in the present study, is to analyse the change in radiomic features during or after treatment (the so-called delta radiomics)[37,38]. The application of delta radiomic might further improve the ability of radiomics to provide predictive models for oncological outcomes and instruct clinical decisions. Furthermore, another potential application of radiomics in oncology is to realize prognostic and predictive models of the tumour pathological response (TRG) to neoadjuvant treatment[39-41]. This information could lead to better identification of patients who may or may not benefit from preoperative approach, in order to allow for more effective treatment personalization.

This study had several limitations. First, the design was observational, with a retrospective analysis and a relatively small sample size. Second, the results may have been biased by the patient selection process since indications to chemotherapy, radiotherapy, and surgery were defined on a case-by-case basis by the Institutional board. The lack of external validation represents a third limit. An external multi-institutional validation may have been preferable. Finally, due to the limited number of events in the resected group, we were unable to perform an analysis on the impact of resection status (R0 *vs* R1) on survival.

CONCLUSION

The present CT-radiomic model demonstrated a reliable performance to predict resectability in LAPC treated with induction chemotherapy followed by ablative RT. Radiomic information may complement clinical and morphological parameters in predicting surgical resectability. If further confirmed, the results of this study may allow integrating radiomic information into the pool of clinical and biochemical data to consider when a LAPC patient is candidate for surgical exploration after neoadjuvant therapy.

ARTICLE HIGHLIGHTS

Research background

Radiomics is emerging as a promising tool in oncology, potentially improving, through the development of predictive and prognostic models, the therapeutic decision-making process. To date, however, few data are available regarding the use of radiomics in pancreatic cancer (PC). Since computed tomography (CT) misestimate the resectability of locally advanced PC (LAPC) after neoadjuvant treatment, the role of radiomics could be decisive to integrate traditional morphological parameters in predicting surgical resection.

Research motivation

To explore the potential role of CT-radiomic features to integrate clinical and morphological data to predict surgical resection in LAPC treated with neoadjuvant chemotherapy and radiotherapy.

Research objectives

To create and validate a predictive model to predict LAPC resectability, throughout the application of machine learning algorithms to planning CT-radiomic features.

Research methods

A total of 1655 radiomic features were extracted from planning CT inside the gross tumour volume. Resectability status predictive model was build starting from these radiomic features and clinical data. A first step of variable selection and a training/validation step to find the model that better predicted the outcome was adopted. Subsequently, the validated model was applied to the whole dataset. The discriminating performance of each model was assessed with the area under the receiver operating characteristic curve (AUC).

Research results

Seventy-one LAPC patients were included in the analysis. After neoadjuvant chemotherapy and radiotherapy, 19 (26.8%) patients underwent surgical resection. The training and validation steps resulted in a predictive model of resectability with a median AUC of 0.862 (95%CI: 0.792-0.921) and 0.853 (95%CI: 0.706-0.960), respectively. This model applied to the entire dataset allowed to select 4 radiomic features that predict the respectability status with an AUC of 0.944 (95%CI: 0.892-0.996). No clinical data contributed to the predictive model.

Research conclusions

The present radiomic model could help predict resectability in LAPC treated with neoadjuvant therapy, suggesting a promising role in the context of a complex long-course downstaging and a challenging indication to surgery.

Research perspectives

The analysis of the change of radiomic features during or after treatment (delta radiomics) and the correlation with tumour response (*e.g.*, tumour regression grade) represent another intriguing application of radiomics that needs further exploration.

FOOTNOTES

Author contributions: Rossi G, Altabella L, and Simoni N designed the research; Rossi G, Benetti G, Rossi R, Venezia M, Paiella S, and Malleo G collected data; Rossi G and Simoni N analysed clinical and radiation data; Altabella L and Benetti G performed the radiomic features extraction, machine learning algorithm implementation, and statistical analysis; Rossi G, Altabella L and Simoni N wrote the manuscript; Benetti G, Rossi R, Venezia M, Paiella S, Malleo G, Salvia R, Guariglia S, Bassi C, Cavedon C, and Mazzarotto R reviewed the manuscript; All authors approved the final version of the manuscript.

Institutional review board statement: The Institutional Review Board (IRB) approved the prospective collection of patient data, No. PAD-R n.1101 CESC.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: Italy

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S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Fan JR

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7-30 [PMID: 28055103 DOI: 10.3322/caac.21387]
2 **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]
3 **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]
4 **Maggino L**, Malleo G, Marchegiani G, Viviani E, Nessi C, Ciprani D, Esposito A, Landoni L, Casetti L, Tuveri M, Paiella S, Casciani F, Sereni E, Binco A, Bonamini D, Secchettin E, Auriemma A, Merz V, Simionato F, Zecchetto C, D'Onofrio M, Melisi D, Bassi C, Salvia R. Outcomes of Primary Chemotherapy for Borderline Resectable and Locally Advanced Pancreatic Ductal Adenocarcinoma. *JAMA Surg* 2019; **154**: 932-942 [PMID: 31339530 DOI: 10.1001/jamasurg.2019.2277]
5 **Suker M**, Beumer BR, Sadot E, Marthey L, Faris JE, Mellon EA, El-Rayes BF, Wang-Gillam A, Lacy J, Hosein PJ, Moorcraft SY, Conroy T, Hohla F, Allen P, Taieb J, Hong TS, Shridhar R, Chau I, van Eijck CH, Koerkamp BG. FOLFIRINOX for locally advanced pancreatic cancer: a systematic review and patient-level meta-analysis. *Lancet Oncol* 2016; **17**: 801-810 [PMID: 27160474 DOI: 10.1016/S1470-2045(16)00172-8]
6 **Reni M**, Zanon S, Balzano G, Nobile S, Pircher CC, Chiaravalli M, Passoni P, Arcidiacono PG, Nicoletti R, Crippa S, Slim N, Doglioni C, Falconi M, Gianni L. Selecting patients for resection after primary chemotherapy for non-metastatic pancreatic adenocarcinoma. *Ann Oncol* 2017; **28**: 2786-2792 [PMID: 28945895 DOI: 10.1093/annonc/mdx495]
7 **Chung SY**, Chang JS, Lee BM, Kim KH, Lee KJ, Seong J. Dose escalation in locally advanced pancreatic cancer patients receiving chemoradiotherapy. *Radiother Oncol* 2017; **123**: 438-445 [PMID: 28464997 DOI: 10.1016/j.radonc.2017.04.010]
8 **Krishnan S**, Chadha AS, Suh Y, Chen HC, Rao A, Das P, Minsky BD, Mahmood U, Delclos ME, Sawakuchi GO, Beddar S, Katz MH, Fleming JB, Javle MM, Varadhachary GR, Wolff RA, Crane CH. Focal Radiation Therapy Dose Escalation Improves Overall Survival in Locally Advanced Pancreatic Cancer Patients Receiving Induction Chemotherapy and Consolidative Chemoradiation. *Int J Radiat Oncol Biol Phys* 2016; **94**: 755-765 [PMID: 26972648 DOI: 10.1016/j.ijrobp.2015.12.003]
9 **Toesca DAS**, Ahmed F, Kashyap M, Baclay JRM, von Eyben R, Pollom EL, Koong AC, Chang DT. Intensified systemic therapy and stereotactic ablative radiotherapy dose for patients with unresectable pancreatic adenocarcinoma. *Radiother Oncol* 2020; **152**: 63-69 [PMID: 32763253 DOI: 10.1016/j.radonc.2020.07.053]
10 **Reyngold M**, O'Reilly EM, Varghese AM, Fiasconaro M, Zinovoy M, Romesser PB, Wu A, Hajj C, Cuaron JJ, Tuli R, Hilal L, Khalil D, Park W, Yorke ED, Zhang Z, Yu KH, Crane CH. Association of Ablative Radiation Therapy With Survival Among Patients With Inoperable Pancreatic Cancer. *JAMA Oncol* 2021; **7**: 735-738 [PMID: 33704353 DOI: 10.1001/jamaoncol.2021.0057]
11 **Gemenetzis G**, Groot VP, Blair AB, Laheru DA, Zheng L, Narang AK, Fishman EK, Hruban RH, Yu J, Burkhart RA, Cameron JL, Weiss MJ, Wolfgang CL, He J. Survival in Locally Advanced Pancreatic Cancer After Neoadjuvant Therapy and Surgical Resection. *Ann Surg* 2019; **270**: 340-347 [PMID: 29596120 DOI: 10.1097/SLA.0000000000002753]
12 **Murphy JE**, Wo JY, Ryan DP, Clark JW, Jiang W, Yeap BY, Drapek LC, Ly L, Baglini CV, Blaszkowsky LS, Ferrone CR, Parikh AR, Weekes CD, Nipp RD, Kwak EL, Allen JN, Corcoran RB, Ting DT, Faris JE, Zhu AX, Goyal L, Berger DL, Qadan M, Lillemoe KD, Talele N, Jain RK, DeLaney TF, Duda DG, Boucher Y, Fernández-Del Castillo C, Hong TS. Total Neoadjuvant Therapy With FOLFIRINOX in Combination With Losartan Followed by Chemoradiotherapy for Locally Advanced Pancreatic Cancer: A Phase 2 Clinical Trial. *JAMA Oncol* 2019; **5**: 1020-1027 [PMID: 31145418 DOI: 10.1001/jamaoncol.2019.0892]
13 **Simoni N**, Micera R, Paiella S, Guariglia S, Zivelonghi E, Malleo G, Rossi G, Addari L, Giuliani T, Pollini T, Cavedon C, Salvia R, Milella M, Bassi C, Mazzarotto R. Hypofractionated Stereotactic Body Radiation Therapy With Simultaneous Integrated Boost and Simultaneous Integrated Protection in Pancreatic Ductal Adenocarcinoma. *Clin Oncol (R Coll Radiol)* 2021; **33**: e31-e38 [PMID: 32682686 DOI: 10.1016/j.clon.2020.06.019]

14 **Rossi G**, Simoni N, Paiella S, Rossi R, Venezia M, Micera R, Malleo G, Salvia R, Giuliani T, Di Gioia A, Auriemma A, Milella M, Guariglia S, Cavedon C, Bassi C, Mazzarotto R. Risk Adapted Ablative Radiotherapy After Intensive Chemotherapy for Locally Advanced Pancreatic Cancer. *Front Oncol* 2021; **11**: 662205 [PMID: 33959509 DOI: 10.3389/fonc.2021.662205]

15 **Jang JK**, Byun JH, Kang JH, Son JH, Kim JH, Lee SS, Kim HJ, Yoo C, Kim KP, Hong SM, Seo DW, Kim SC, Lee MG. CT-determined resectability of borderline resectable and unresectable pancreatic adenocarcinoma following FOLFIRINOX therapy. *Eur Radiol* 2021; **31**: 813-823 [PMID: 32845389 DOI: 10.1007/s00330-020-07188-8]

16 **Ferrone CR**, Marchegiani G, Hong TS, Ryan DP, Deshpande V, McDonnell EI, Sabbatino F, Santos DD, Allen JN, Blaszkowsky LS, Clark JW, Faris JE, Goyal L, Kwak EL, Murphy JE, Ting DT, Wo JY, Zhu AX, Warshaw AL, Lillemoe KD, Fernández-del Castillo C. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. *Ann Surg* 2015; **261**: 12-17 [PMID: 25599322 DOI: 10.1097/SLA.0000000000000867]

17 **Wagner M**, Antunes C, Pietrasz D, Cassinotto C, Zappa M, Sa Cunha A, Lucidarme O, Bachet JB. CT evaluation after neoadjuvant FOLFIRINOX chemotherapy for borderline and locally advanced pancreatic adenocarcinoma. *Eur Radiol* 2017; **27**: 3104-3116 [PMID: 27896469 DOI: 10.1007/s00330-016-4632-8]

18 **Kim YE**, Park MS, Hong HS, Kang CM, Choi JY, Lim JS, Lee WJ, Kim MJ, Kim KW. Effects of neoadjuvant combined chemotherapy and radiation therapy on the CT evaluation of resectability and staging in patients with pancreatic head cancer. *Radiology* 2009; **250**: 758-765 [PMID: 19164113 DOI: 10.1148/radiol.2502080501]

19 **Cassinotto C**, Cortade J, Belleannée G, Lapuyade B, Terrebonne E, Vendrely V, Laurent C, Sa-Cunha A. An evaluation of the accuracy of CT when determining resectability of pancreatic head adenocarcinoma after neoadjuvant treatment. *Eur J Radiol* 2013; **82**: 589-593 [PMID: 23287712 DOI: 10.1016/j.ejrad.2012.12.002]

20 **Cassinotto C**, Mouries A, Lafourcade JP, Terrebonne E, Belleannée G, Blanc JF, Lapuyade B, Vendrely V, Laurent C, Chiche L, Wagner T, Sa-Cunha A, Gaye D, Trillaud H, Laurent F, Montaudon M. Locally advanced pancreatic adenocarcinoma: reassessment of response with CT after neoadjuvant chemotherapy and radiation therapy. *Radiology* 2014; **273**: 108-116 [PMID: 24960211 DOI: 10.1148/radiol.14132914]

21 **Lambin P**, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, Sanduleanu S, Larue RTHM, Even AJG, Jochems A, van Wijk Y, Woodruff H, van Soest J, Lustberg T, Roelofs E, van Elmpt W, Dekker A, Mottaghy FM, Wildberger JE, Walsh S. Radiomics: the bridge between medical imaging and personalized medicine. *Nat Rev Clin Oncol* 2017; **14**: 749-762 [PMID: 28975929 DOI: 10.1038/nrclinonc.2017.141]

22 **Giraud P**, Giraud P, Gasnier A, El Ayachy R, Kreps S, Foy JP, Durdux C, Huguet F, Burgun A, Bibault JE. Radiomics and Machine Learning for Radiotherapy in Head and Neck Cancers. *Front Oncol* 2019; **9**: 174 [PMID: 30972291 DOI: 10.3389/fonc.2019.00174]

23 **Ciaravino V**, Cardobi N, DE Robertis R, Capelli P, Melisi D, Simionato F, Marchegiani G, Salvia R, D'Onofrio M. CT Texture Analysis of Ductal Adenocarcinoma Downstaged After Chemotherapy. *Anticancer Res* 2018; **38**: 4889-4895 [PMID: 30061265 DOI: 10.21873/anticanres.12803]

24 **Borhani AA**, Dewan R, Furlan A, Seiser N, Zureikat AH, Singhi AD, Boone B, Bahary N, Hogg ME, Lotze M, Zeh HJ III, Tublin ME. Assessment of Response to Neoadjuvant Therapy Using CT Texture Analysis in Patients With Resectable and Borderline Resectable Pancreatic Ductal Adenocarcinoma. *AJR Am J Roentgenol* 2020; **214**: 362-369 [PMID: 31799875 DOI: 10.2214/AJR.19.21152]

25 **Cozzi L**, Comito T, Fogliata A, Franzese C, Franceschini D, Bonifacio C, Tozzi A, Di Brina L, Clerici E, Tomatis S, Reggiori G, Lobefalo F, Stravato A, Mancosu P, Zerbi A, Sollini M, Kirienko M, Chiti A, Scorsetti M. Computed tomography based radiomic signature as predictive of survival and local control after stereotactic body radiation therapy in pancreatic carcinoma. *PLoS One* 2019; **14**: e0210758 [PMID: 30657785 DOI: 10.1371/journal.pone.0210758]

26 **Sandrasegaran K**, Lin Y, Asare-Sawiri M, Taiyini T, Tann M. CT texture analysis of pancreatic cancer. *Eur Radiol* 2019; **29**: 1067-1073 [PMID: 30116961 DOI: 10.1007/s00330-018-5662-1]

27 **Parr E**, Du Q, Zhang C, Lin C, Kamal A, McAlister J, Liang X, Bavitz K, Rux G, Hollingsworth M, Baine M, Zheng D. Radiomics-Based Outcome Prediction for Pancreatic Cancer Following Stereotactic Body Radiotherapy. *Cancers (Basel)* 2020; **12** [PMID: 32344538 DOI: 10.3390/cancers12041051]

28 **Ou J**, Li R, Zeng R, Wu CQ, Chen Y, Chen TW, Zhang XM, Wu L, Jiang Y, Yang JQ, Cao JM, Tang S, Tang MJ, Hu J. CT radiomic features for predicting resectability of oesophageal squamous cell carcinoma as given by feature analysis: a case control study. *Cancer Imaging* 2019; **19**: 66 [PMID: 31619297 DOI: 10.1186/s40644-019-0254-0]

29 **Batista Araujo-Filho JA**, Mayoral M, Zheng J, Tan KS, Gibbs P, Shepherd AF, Rimner A, Simone CB 2nd, Riely G, Huang J, Ginsberg MS. CT Radiomic Features for Predicting Resectability and TNM Staging in Thymic Epithelial Tumors. *Ann Thorac Surg* 2021 [PMID: 33844992 DOI: 10.1016/j.athoracsur.2021.03.084]

30 **National Comprehensive Cancer Network**. Pancreatic Adenocarcinoma Version 2. 2015. NCCN Guidelines. (2015). [cited 10 April 2021]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

31 **van Griethuysen JJM**, Fedorov A, Parmar C, Hosny A, Aucoin N, Narayan V, Beets-Tan RGH, Fillion-Robin JC, Pieper S, Aerts HJWL. Computational Radiomics System to Decode the Radiographic Phenotype. *Cancer Res* 2017; **77**: e104-e107 [PMID: 29092951 DOI: 10.1158/0008-5472.CAN-17-0339]

32 **Zwanenburg A**, Vallières M, Abdalah MA, Aerts HJWL, Andrearczyk V, Apte A, Ashrafinia S, Bakas S, Beukinga RJ, Boellaard R, Bogowicz M, Boldrini L, Buvat I, Cook GJR, Davatzikos C, Depeursinge A, Desseroit MC, Dinapoli N, Dinh CV, Echegaray S, El Naqa I, Fedorov AY, Gatta R, Gillies RJ, Goh V, Götz M, Guckenberger M, Ha SM, Hatt M, Isensee F, Lambin P, Leger S, Leijenaar RTH, Lenkowicz J, Lippert F, Losnegård A, Maier-Hein KH, Morin O, Müller H, Napel S, Nioche C, Orlhac F, Pati S, Pfaehler EAG, Rahmim A, Rao AUK, Scherer J, Siddique MM, Sijtsema NM, Socarras Fernandez J, Spezi E, Steenbakkers RJHM, Tanadini-Lang S, Thorwarth D, Troost EGC, Upadhaya T, Valentini V, van Dijk LV, van Griethuysen J, van Velden FHP, Whybra P, Richter C, Löck S. The Image Biomarker Standardization Initiative: Standardized Quantitative Radiomics for High-Throughput Image-based Phenotyping. *Radiology* 2020; **295**: 328-338 [PMID: 32154773 DOI: 10.1148/radiol.2020191145]

33 **Cassinotto C**, Sa-Cunha A, Trillaud H. Radiological evaluation of response to neoadjuvant treatment in pancreatic cancer.

Diagn Interv Imaging 2016; **97**: 1225-1232 [PMID: 27692675 DOI: 10.1016/j.diii.2016.07.011]

34 **Limkin EJ**, Sun R, Dercle L, Zacharaki EI, Robert C, Reuzé S, Schernberg A, Paragios N, Deutsch E, Ferté C. Promises and challenges for the implementation of computational medical imaging (radiomics) in oncology. *Ann Oncol* 2017; **28**: 1191-1206 [PMID: 28168275 DOI: 10.1093/annonc/mdx034]

35 **Hammel P**, Huguet F, van Laethem JL, Goldstein D, Glimelius B, Artru P, Borbath I, Bouché O, Shannon J, André T, Mineur L, Chibaudel B, Bonnetain F, Louvet C; LAP07 Trial Group. Effect of Chemoradiotherapy *vs* Chemotherapy on Survival in Patients With Locally Advanced Pancreatic Cancer Controlled After 4 Months of Gemcitabine With or Without Erlotinib: The LAP07 Randomized Clinical Trial. *JAMA* 2016; **315**: 1844-1853 [PMID: 27139057 DOI: 10.1001/jama.2016.4324]

36 **Rizzo S**, Botta F, Raimondi S, Origgi D, Fanciullo C, Morganti AG, Bellomi M. Radiomics: the facts and the challenges of image analysis. *Eur Radiol Exp* 2018; **2**: 36 [PMID: 30426318 DOI: 10.1186/s41747-018-0068-z]

37 **Nasief H**, Zheng C, Schott D, Hall W, Tsai S, Erickson B, Allen Li X. A machine learning based delta-radiomics process for early prediction of treatment response of pancreatic cancer. *NPJ Precis Oncol* 2019; **3**: 25 [PMID: 31602401 DOI: 10.1038/s41698-019-0096-z]

38 **Cusumano D**, Boldrini L, Yadav P, Casà C, Lee SL, Romano A, Piras A, Chiloiro G, Placidi L, Catucci F, Votta C, Mattiucci GC, Indovina L, Gambacorta MA, Bassetti M, Valentini V. Delta Radiomics Analysis for Local Control Prediction in Pancreatic Cancer Patients Treated Using Magnetic Resonance Guided Radiotherapy. *Diagnostics (Basel)* 2021; **11** [PMID: 33466307 DOI: 10.3390/diagnostics11010072]

39 **Li ZY**, Wang XD, Li M, Liu XJ, Ye Z, Song B, Yuan F, Yuan Y, Xia CC, Zhang X, Li Q. Multi-modal radiomics model to predict treatment response to neoadjuvant chemotherapy for locally advanced rectal cancer. *World J Gastroenterol* 2020; **26**: 2388-2402 [PMID: 32476800 DOI: 10.3748/wjg.v26.i19.2388]

40 **Yuan Z**, Frazer M, Zhang GG, Latifi K, Moros EG, Feygelman V, Felder S, Sanchez J, Dessureault S, Imanirad I, Kim RD, Harrison LB, Hoffe SE, Frakes JM. CT-based radiomic features to predict pathological response in rectal cancer: A retrospective cohort study. *J Med Imaging Radiat Oncol* 2020; **64**: 444-449 [PMID: 32386109 DOI: 10.1111/1754-9485.13044]

41 **Yang Z**, He B, Zhuang X, Gao X, Wang D, Li M, Lin Z, Luo R. CT-based radiomic signatures for prediction of pathologic complete response in esophageal squamous cell carcinoma after neoadjuvant chemoradiotherapy. *J Radiat Res* 2019; **60**: 538-545 [PMID: 31111948 DOI: 10.1093/jrr/rrz027]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 716-723

DOI: 10.4251/wjgo.v14.i3.716

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Pancreatic head *vs* pancreatic body/tail cancer: Are they different?

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Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Luyer MDP, Reshkin SJ, Xu K

Received: September 29, 2021
Peer-review started: September 29, 2021
First decision: November 7, 2021
Revised: November 16, 2021
Accepted: January 27, 2022
Article in press: January 27, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

The impact of pancreatic tumor location on patient survival has been studied in large national data-based analyses which yielded controversial results.

AIM

To explore if pancreatic head cancer (PHC) and pancreatic body/tail cancer (PBTC) have different overall survival (OS), molecular signature and response to chemotherapy.

METHODS

We retrospectively queried patient records from July 2016 to June 2020 in our institution. Patient demographics, cancer stage on diagnosis, tumor location, somatic mutations, treatment, and survival are recorded and analyzed. A test is considered statistically significant if the *P* value was < 0.05.

RESULTS

We reviewed 101 patients with complete records, among which 67 (66.34%) were PHC and 34 (33.66%) were PBTC. More PHC were diagnosed at younger age [61.49 *vs* 68.97, *P* = 0.010], earlier stages (*P* = 0.006) and underwent surgical resection (*P* = 0.025). There were no significant differences among all mutations and pathways studied except for TP53 mutations (37.0% in PHC *vs* 70.0% in PBTC, *P* = 0.03). OS was not statistically different between PHC and PBTC (*P* = 0.636) in the overall population and in subgroups according to surgical resection status or stages. In terms of response to chemotherapy, chemotherapy regimens (FOLFIRINOX-based *vs* gemcitabine-based) didn't impact disease free interval in

those who had surgical resection in either PHC (P = 0.546) or PBTC (P = 0.654), or the duration of response to first line palliative treatment in those with advanced disease in PHC (P = 0.915) or PBTC (P = 0.524).

CONCLUSION

Even though PHC and PBTC have similar poor OS and response to chemotherapy, the different presentations and molecular profiles indicate they are different diseases. Utilization of molecular profiling to develop targeted therapy for individualization of treatment is needed.

Key Words: Pancreatic cancer; Tumor location; Molecular profiling; Survival; Response to chemotherapy

Core Tip: The study is a retrospective study of the impact of pancreatic cancer location on survival, molecular profiling and response to chemotherapy among patients who were diagnosed with pancreatic cancer in our institution. Even though there was no significant difference in survival or response to chemotherapy between pancreatic head and pancreatic body/tail cancer, we did observe a trend of long-term survival in stage I/II pancreatic tail patients who underwent surgical resection. TP53 mutations were significantly more in pancreatic body/tail cancer than that in pancreatic head cancer and we propose that gemcitabine-based chemotherapy should be considered in those patients.

Citation: Sun K, Mylavarapu C, Crenshaw A, Zhang Y, Hsu E, Xu J, Niravath M, Jones SL, Ordonez A, Abdelrahim M. Pancreatic head *vs* pancreatic body/tail cancer: Are they different? *World J Gastrointest Oncol* 2022; 14(3): 716-723

URL: https://www.wjgnet.com/1948-5204/full/v14/i3/716.htm

DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.716

INTRODUCTION

Pancreatic cancer is the fourth-leading cause of cancer deaths in the United States. Even though the survival rates have improved slightly over the past four decades, the outcome of pancreatic cancer is still dismal. Anatomically pancreatic cancer can be divided into pancreatic head cancer (PHC) and pancreatic body/tail cancer (PBTC). The lower part of head and uncinate process of pancreas has different embryological origins from the rest of the pancreas[1]. This embryological difference leads to significant differences in cell composition, blood supply, lymphatic and venous drainage and innervations between the head and body/tail of pancreas.

Pancreatic tumor location impacts patient presentation and survival, which has been shown in large data-based analyses, even though with conflicting results. 49%-77.5% pancreatic cancers are PHC[2-4], which tend to present at earlier stages than PBTC. Historically, survival of PBTC cancer is believed to be worse than PHC; and PBTC is considered as an independent poor prognostic risk factor. However, PBTC was found to have much better survival over PHC (20% *vs* 9%) when the tumor is localized[2].

Genetic analyses of pancreatic cancer have suggested that PHC and PBTC are different tumors. Advanced technology including whole genome sequencing and RNA sequencing further classified pancreatic cancer into four subtypes: Classical, squamous, ADEX and immunogenic[5]. The squamous subtype is characterized by genes highly expressed in the C2-squamous-like class of tumors (*e.g.*, lung and head and neck cancer)[6]. PBTC is found to have more squamous subtypes[7,8].

Pancreatic squamous subtype shares similar molecular abnormalities with lung squamous subtype, which include loss of TP53, RB1, CDKN2A and PIK3CA, NOTCH1, NFE2L2, KDM6A and EP300 mutations. Squamous cell lung cancer was found to be more sensitive to platinum and gemcitabine combination therapy[9]. When the combination therapy was tested in advanced pancreatic cancer, there was an improvement in overall survival, disease free survival and response rate, even though not statistically significant[10]. Given that PBTC had more squamous subtype, it is possible that PBTC is more responsive to gemcitabine-based treatment.

We hypothesize that PHC and PBTC are distinct diseases based on their embryological origins and current genetic profiling evidence. To further explore the impact of tumor location on molecular profiling, survival, and response to chemotherapy, we retrospectively reviewed patients with pancreatic cancer in our institution.

MATERIALS AND METHODS

The study was approved by the institutional review boards. Informed consent was waived given the retrospective nature of the study. The patient data was queried from Epic electronic medical record system of Houston Methodist Hospital. Patients who had a diagnosis of pancreatic cancer from July 2016 through June 2020 were included. Patient demographics, tumor location, pathology, staging, molecular profiles, treatment history and survival were collected retrospectively. Molecular profiles were performed through a multiplatform approach including next gene sequencing (NGS) and RNA sequencing by commercially available testing from Caris Life Sciences (Phoenix, AZ), FoundationOne (Cambridge, MA), Guardant360 (Redwood City, CA), Tempus (Chicago, IL) and NeoGenomics (Fort Meyers, FL), and in house 50 gene or 70 gene panel that was developed and validated in our institution. Panels of gene mutations are available on each company's website. Duration of response is defined as the duration of having complete response, partial response or stable disease. Overall survival is defined as the time from pancreatic cancer diagnosis to the date of death or date of last follow-up. Disease free interval is defined as the time from definitive treatment to the date of disease recurrence. Patients who had response and survival data were included in the survival and response analysis. Patients who had molecular profiling data were included in the tumor location analysis. Those who didn't have either records were excluded from the study.

Mean and standard deviation were calculated for the continuous variables and frequency and percentage [*n* (%)] were calculated for those categorical variables. T-test was used to compare the mean of a continuous variables between the 2 groups of pancreatic cancer and the Fisher's exact test was used to find the association between a patient's characteristic and the pancreatic cancer's groups. Kaplan Meier curves and Log-rank test were used to compare the survival time between the two location groups of pancreatic cancer. Stata/MP 16.1 for Windows was used to analyze the data. A test was considered statistically significant if the P value was < 0.05.

RESULTS

Patients

From July, 2016 to June, 2020, a total of 500 records was retrieved and 101 patients with complete medical records were included in the analysis. 67 patients had PHC and 34 patients had PBTC. Compared to patients with PHC, patients with PBTC are older at diagnosis (68.97 *vs* 61.49, $P = 0.01$), diagnosed at more advanced stage ($P = 0.006$) and are less likely to undergo surgical resection ($P = 0.025$) (Table 1). There is no significant difference in gender, ethnicity between patients with PHC and patients with PBTC ($P = 0.10$ and 0.53 respectively).

Survival and tumor location

In the total population, the OS between PHC and PBTC was not statistically different ($P = 0.64$, Figure 1A). Patients who underwent surgical resection had better OS than patients who did not ($P < 0.001$, Figure 1B), with a median OS of 2.05 years (interquartile range, 1.21 to 2.86) and 1.00 year (interquartile range, 0.77 to 1.70) respectively. There were no differences in survival between PHC and PBTC in those who underwent surgical resection, those who didn't undergo surgical resection, or those who had Stage IV disease on presentation. In the subgroup of patients who had stage I and II disease, there were 3 patients with PBTC and those were long-term survivors. However, due to small number of patients, no definite conclusion can be made.

Molecular profiling and tumor location

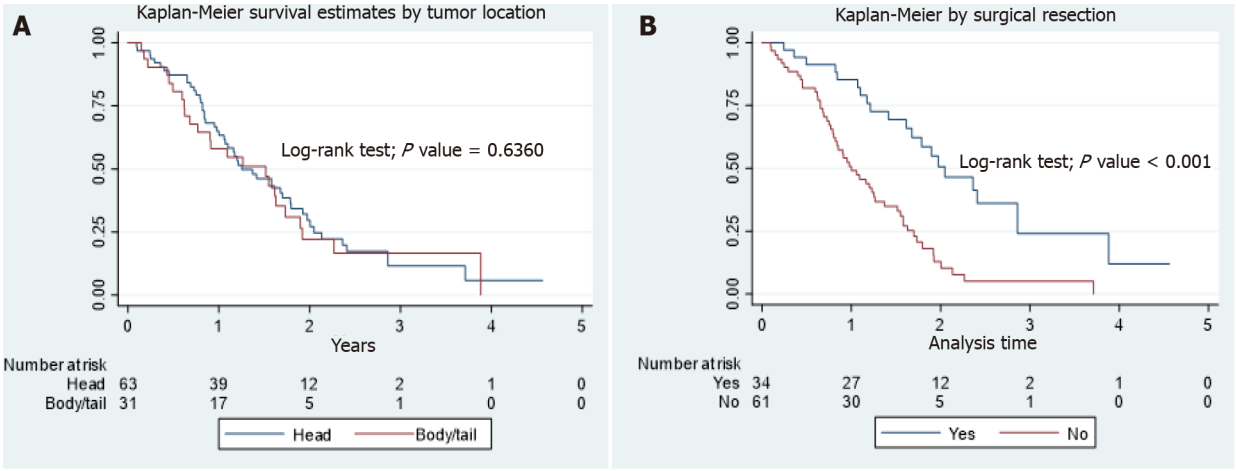
A total of 66 patients (46 PHC and 20 PBTC) who had complete medical records and molecular profiling were reviewed. 20/66 (30.3%) had molecular testing performed on biopsy specimen, 24/66 (36.4%) on peripheral blood, 14/66 (21.2%) on surgical resection specimen and the remaining on samples with unknown sources. Rates of pathogenic mutations were recorded and compared between PHC and PBTC (Figure 2). PHC and PBTC have similar tumor mutation numbers ($P = 0.79$). The most common mutations were KRAS mutations (63.6% in total, 65.2% in PHC *vs* 60.6% in PBTC, $P = 0.78$), TP53 mutations (47.0% in total, 37.0% in PHC *vs* 70.0% in PBTC, $P = 0.03$), SMAD mutations (12.1% in total, 15.2% in PHC *vs* 5.0% in PBTC, $P = 0.42$) and CDKN2a/b mutations (19.7% in total, 19.6% in PHC *vs* 20.2% in PBTC, $P = 1.00$). Only TP53 mutations were significantly different. 12.1%% of the mutations were involved in cell cycle pathway (15.2% in PHC *vs* 5%% in PBTC, $P = 0.47$), 34.9% in MAPK pathway (34.8% in PHC *vs* 35.0% in PBTC, $P = 0.99$) and 15.1% in DNA repair pathway (17.4% in PHC *vs* 10.0% in PBTC, $P = 0.71$). There were no differences in the mutations involved in these pathways.

Response to chemotherapy and tumor location

In patients who underwent resection, there were no statistical differences in disease free interval between patients with PHC or PBTC who received FOLFIRINOX based chemotherapy and those who

Table 1 Patient characteristics by location

	Total	Head	Body/tail	*P* value
	n = 101, %	*n* = 67, %	*n* = 34, %	
Age at diagnosis	63.90 (13.03)	61.49 (13.96)	68.97 (9.12)	**0.010**
Gender				0.095
Female	51 (50.50)	38 (56.72)	13 (38.24)	
Male	50 (49.50)	29 (43.28)	21 (61.76)	
Ethnicity				0.53
Caucasian	76 (75.25)	48 (71.64)	28 (82.35)	
Black	11 (10.89)	8 (11.94)	3 (8.82)	
Other	14 (13.86)	11 (16.42)	3 (8.82)	
Pathologic initial stage				**0.006**
I	5 (4.95)	3 (4.48)	2 (5.88)	
II	19 (18.81)	18 (26.87)	1 (2.94)	
III	17 (16.83)	13 (19.40)	4 (11.76)	
IV	51 (50.50)	28 (41.79)	23 (67.65)	
Missing	9 (8.91)	5 (7.46)	4 (11.76)	
Surgical resection				**0.025**
Yes	34 (33.66)	28 (41.79)	6 (17.65)	
No	67 (66.34)	39 (58.21)	28 (82.35)	



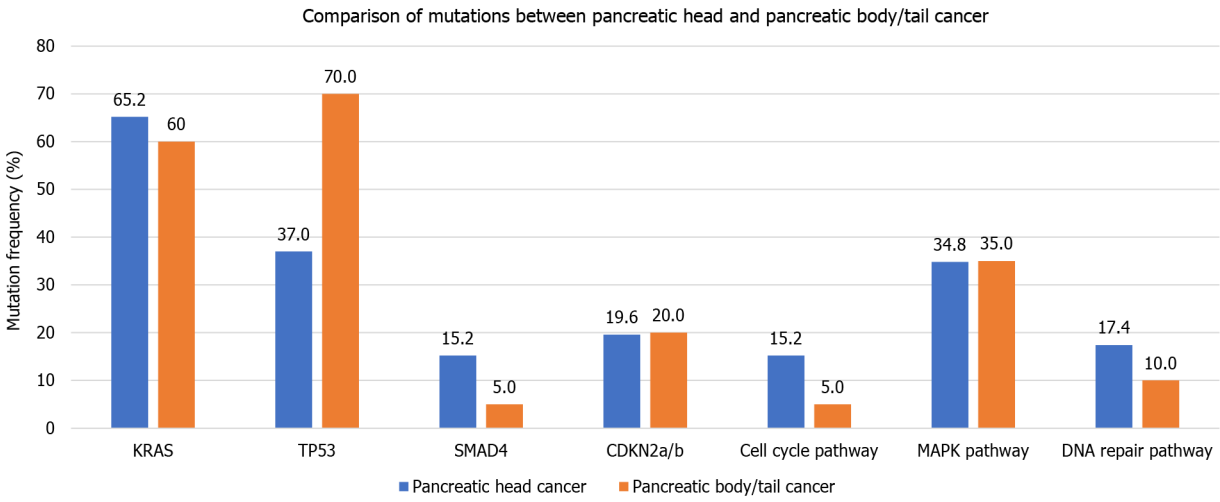
DOI: 10.4251/wjgo.v14.i3.716

Figure 1 Survival and tumor location. A: Kaplan-Meier curve of overall survival in pancreatic head cancer and pancreatic body/tail cancer in the total population; B: Kaplan-Meier curve of overall survival in patients who underwent surgical resection versus those who did not.

received gemcitabine-based chemotherapy (*P* values are 0.55 and 0.65 respectively, Figure 3A and B). In patients with metastatic disease, there were no statistical differences in duration of response to first line palliative chemo between patients with PHC or PBTC who received FOLFIRINOX-based chemotherapy and those who received gemcitabine-based chemotherapy (*P* values are 0.91 and 0.52 respectively, Figure 3C and D).

DISCUSSION

In this retrospective study, patients with PHC and PBTC were retrieved and the relationships of tumor locations with molecular profiling, overall survival, or response to chemotherapy were explored. Our



DOI: 10.4251/wjgo.v14.i3.716

Figure 2 Comparison of mutations between pancreatic head and pancreatic body/tail cancer.

study showed that patients with PBTC tend to present at later stages and are less likely to undergo resection, consistent with previous studies[11-13]. PBTC are older at diagnosis in our study, which could have explained the lower resection rate.

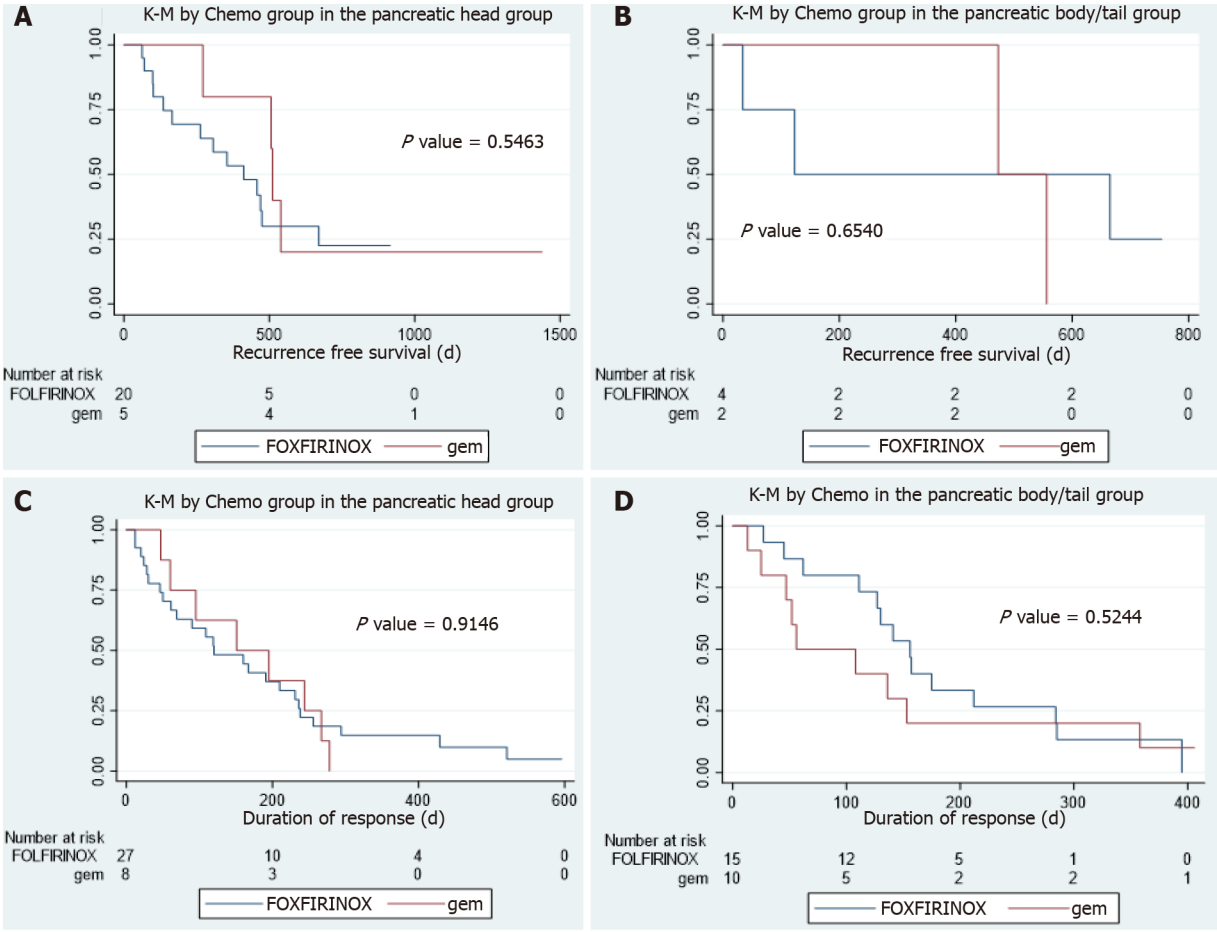
The impact of tumor location on survival has been a controversy. Several population-based studies[2,3,11,14,15] reported contradictory survival of PBTC and PHC in the overall population; however, better survival in PBTC is seen in early stage patients, especially stage I and II. Winer *et al*[14] examined the relationship of survival and tumor location in patients who had tumor resection and found that even though PHC were more of early stage at presentation and more likely to be resected, they tended to have higher grade, more positive lymph nodes and worse overall survival. The survival advantage of PBTC from this study was further supported by a single center study[16] which examined survival in matched stage II PBTC and PHC. Only one single center study[12] reported worse outcome for PBTC patients who had resection and among those patients with Stage I disease, the survival seemed to be better in PBTC however not statistically significant. Even though our study didn't show a survival difference in PHC and PBTC, long-term survival was seen in three patients with Stage I/II PBTC who underwent resection. The findings from previous studies and our study suggest that resected early stage PBTC have better survival than those with PHC.

The four most common mutations in our study were KRAS, TP53, SMAD and CDKN2a/b mutations, which is consistent with previous reports[13,17]. Among these most common mutations, only TP53 mutations were found to be significantly higher in PBTC in our study. Even though the frequency of SMAD mutations was higher in PHC (15.5%) compared to PBTC (5%), this result was not statistically significant because the total number of SMAD mutations observed was only 12.12% in our study. The lower frequencies of those mutations detected in our study could be explained by NGS being performed on insufficient tissues, as majority of samples were biopsy specimens or peripheral blood (66.7%). TP53 mutation is enriched in pancreatic squamous cell type[5] that is similar to lung squamous subtype, and might be similarly more sensitive to gemcitabine therapy. TP53 was also found to predict sensitivity to gemcitabine-based adjuvant therapy in a survival and mutational analysis from CONKOO-001 study [17]. Based on these, our finding of more TP53 mutations in PBTC suggest gemcitabine-based adjuvant therapy to be considered in PBTC, especially in those who can't tolerate FOLFIRINOX therapy.

To our knowledge, our study is the first study that looked at the impact of tumor location on response to different chemotherapy regimens. Neither PHC or PBTC responded differently to FOLFIRINOX based or gemcitabine-based chemotherapies, suggesting a universal poor prognosis of pancreatic cancer regardless of tumor location when compared based on chemotherapy response. However, differential response to targeted therapy or immunotherapy is yet to be explored given the different distribution of pancreatic cancer subtype like immunogenic.

Limitations

Our study was a retrospective study in a single institution and a relatively small number of patients was retrieved. Due to the retrospective nature, the NGS platforms utilized, and the depths of sequencing were not uniform. This added another layer of bias in data interpretation. However, this reflects the real-world experience in many community and academic cancer centers that often rely heavily on commercial NGS platforms. Even though there is a trend to suggest better survival in early stage PBTC patients after resection, there were few patients and events in this subgroup and no definitive conclusion can be made.



DOI: 10.4251/wjgo.v14.i3.716

Figure 3 Response to chemotherapy and tumor location. A: Kaplan Meier curve of recurrence free interval in patients with pancreatic head cancer who had resection and received FOLFIRINOX-based therapy versus gemcitabine based therapy (P = 0.5463); B: Kaplan Meier curve of recurrence free interval in patients with pancreatic body/tail cancer who had resection and received FOLFIRINOX-based therapy versus gemcitabine-based therapy (P = 0.6540); C: Kaplan Meier curve of response duration in patients with metastatic pancreatic head cancer who received FOLFIRINOX-based therapy versus gemcitabine-based therapy (P = 0.9146); D: Kaplan Meier curve of response duration in patients with metastatic pancreatic body/tail cancer who received FOLFIRINOX-based therapy versus gemcitabine-based therapy (P = 0.5244).

CONCLUSION

There is no difference in OS between PHC and PBTC but the long-term survival observed in early stage PBTC after resection suggests better survival in this subgroup of patients. PBTC has significantly more mutations involved in TP53 mutations and its predictive role in gemcitabine sensitivity should be explored in future studies.

ARTICLE HIGHLIGHTS

Research background

Pancreatic head and pancreatic body/tail have different embryological origins. Tumors arising at different locations of pancreas might carry different mutations and respond differently to chemotherapy.

Research motivation

To better define pancreatic cancer and search for precision oncological targets that yield better outcomes.

Research objectives

To study the relationships of pancreatic cancer location with molecular profiling, response to chemotherapy and survival.

Research methods

This is a single institution retrospective study that retrieved patients who carry a diagnosis of pancreatic cancer from July 2016 to June 2020. Patient demographics and molecular profiling information were reviewed and the relationship between tumor location and molecular profiling, response to chemotherapy and survival were analyzed.

Research results

Pancreatic head cancer and pancreatic body/tail cancer (PBTC) have different presentations but similar overall survival and response to chemotherapy. PBTC have significantly more TP53 mutations.

Research conclusions

Given that TP53 mutations predict gemcitabine sensitivity, gemcitabine containing chemotherapy should be considered for PBTC as first line.

Research perspectives

A larger and prospective study should be performed to explore the role of gemcitabine in PBTC.

FOOTNOTES

Author contributions: Sun K and Abdelrahim M designed the study; Sun K, Mylavarapu C, Crenshaw A and Zhang Y performed chart review; Hsu E, Xu JQ and Ordonez A analyzed the data; Niravath M and Jones SL performed bioinformatics and retrieved patient records; Sun K and Abdelrahim M wrote the manuscript; all authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Houston Methodist Hospital Institutional Review Board, Approval No. MOD00002232.

Informed consent statement: Informed consent from patients was waived.

Conflict-of-interest statement: There is no conflict-of-interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at ksun2@houstonmethodist.org.

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S-Editor: Wu YXJ
L-Editor: A
P-Editor: Wu YXJ

REFERENCES

1 **Bastidas-Ponce A**, Scheibner K, Lickert H, Bakhti M. Cellular and molecular mechanisms coordinating pancreas development. *Development* 2017; **144**: 2873-2888 [PMID: 28811309 DOI: 10.1242/dev.140756]

2 **Lau MK**, Davila JA, Shaib YH. Incidence and survival of pancreatic head and body and tail cancers: a population-based study in the United States. *Pancreas* 2010; **39**: 458-462 [PMID: 19924019 DOI: 10.1097/MPA.0b013e3181bd6489]

3 **Matsuno S**, Egawa S, Fukuyama S, Motoi F, Sunamura M, Isaji S, Imaizumi T, Okada S, Kato H, Suda K, Nakao A, Hiraoka T, Hosotani R, Takeda K. Pancreatic Cancer Registry in Japan: 20 years of experience. *Pancreas* 2004; **28**: 219-230 [PMID: 15084961 DOI: 10.1097/00006676-200404000-00002]

4 **Saad AM**, Turk T, Al-Husseini MJ, Abdel-Rahman O. Trends in pancreatic adenocarcinoma incidence and mortality in the United States in the last four decades; a SEER-based study. *BMC Cancer* 2018; **18**: 688 [PMID: 29940910 DOI: 10.1186/s12885-018-4610-4]

5 **Bailey P**, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM; Australian Pancreatic Cancer Genome Initiative, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; **531**: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

6 **Hoadley KA**, Yau C, Wolf DM, Cherniack AD, Tamborero D, Ng S, Leiserson MDM, Niu B, McLellan MD, Uzunangelov V, Zhang J, Kandoth C, Akbani R, Shen H, Omberg L, Chu A, Margolin AA, Van't Veer LJ, Lopez-Bigas N, Laird PW, Raphael BJ, Ding L, Robertson AG, Byers LA, Mills GB, Weinstein JN, Van Waes C, Chen Z, Collisson EA; Cancer Genome Atlas Research Network, Benz CC, Perou CM, Stuart JM. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* 2014; **158**: 929-944 [PMID: 25109877 DOI: 10.1016/j.cell.2014.06.049]

7 **Birnbaum DJ**, Bertucci F, Finetti P, Birnbaum D, Mamessier E. Head and Body/Tail Pancreatic Carcinomas Are Not the Same Tumors. *Cancers (Basel)* 2019; **11** [PMID: 30965637 DOI: 10.3390/cancers11040497]

8 **Dreyer SB**, Jamieson NB, Upstill-Goddard R, Bailey PJ, McKay CJ; Australian Pancreatic Cancer Genome Initiative, Biankin AV, Chang DK. Defining the molecular pathology of pancreatic body and tail adenocarcinoma. *Br J Surg* 2018; **105**: e183-e191 [PMID: 29341146 DOI: 10.1002/bjs.10772]

9 **Scagliotti GV**, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, Serwatowski P, Gatzemeier U, Digumarti R, Zukin M, Lee JS, Mellemgaard A, Park K, Patil S, Rolski J, Goksel T, de Marinis F, Simms L, Sugarman KP, Gandara D. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008; **26**: 3543-3551 [PMID: 18506025 DOI: 10.1200/JCO.2007.15.0375]

10 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/jco.2005.05.1490]

11 **van Erning FN**, Mackay TM, van der Geest LGM, Groot Koerkamp B, van Laarhoven HWM, Bonsing BA, Wilmink JW, van Santvoort HC, de Vos-Geelen J, van Eijck CHJ, Busch OR, Lemmens VE, Besselink MG; Dutch Pancreatic Cancer Group. Association of the location of pancreatic ductal adenocarcinoma (head, body, tail) with tumor stage, treatment, and survival: a population-based analysis. *Acta Oncol* 2018; **57**: 1655-1662 [PMID: 30264642 DOI: 10.1080/0284186X.2018.1518593]

12 **Sheng W**, Dong M, Wang G, Shi X, Gao W, Wang K, Song H, Shi G, Tan X. The diversity between curatively resected pancreatic head and body-tail cancers based on the 8th edition of AJCC staging system: a multicenter cohort study. *BMC Cancer* 2019; **19**: 981 [PMID: 31640615 DOI: 10.1186/s12885-019-6178-z]

13 **Zhang X**, Feng S, Wang Q, Huang H, Chen R, Xie Q, Zhang W, Wang A, Zhang S, Wang L, Yao M, Ling Q. Comparative genomic analysis of head and body/tail of pancreatic ductal adenocarcinoma at early and late stages. *J Cell Mol Med* 2021; **25**: 1750-1758 [PMID: 33452856 DOI: 10.1111/jcmm.16281]

14 **Winer LK**, Dhar VK, Wima K, Morris MC, Lee TC, Shah SA, Ahmad SA, Patel SH. The Impact of Tumor Location on Resection and Survival for Pancreatic Ductal Adenocarcinoma. *J Surg Res* 2019; **239**: 60-66 [PMID: 30802706 DOI: 10.1016/j.jss.2019.01.061]

15 **Zheng Z**, Wang M, Tan C, Chen Y, Ping J, Wang R, Liu X. Disparities in survival by stage after surgery between pancreatic head and body/tail in patients with nonmetastatic pancreatic cancer. *PLoS One* 2019; **14**: e0226726 [PMID: 31856205 DOI: 10.1371/journal.pone.0226726]

16 **Ling Q**, Xu X, Ye P, Xie H, Gao F, Hu Q, Liu Z, Wei X, Röder C, Trauzold A, Kalthoff H, Zheng S. The prognostic relevance of primary tumor location in patients undergoing resection for pancreatic ductal adenocarcinoma. *Oncotarget* 2017; **8**: 15159-15167 [PMID: 28122349 DOI: 10.18632/oncotarget.14768]

17 **Sinn M**, Sinn BV, Treue D, Keilholz U, Damm F, Schmuck R, Lohneis P, Klauschen F, Striefler JK, Bahra M, Bläker H, Bischoff S, Pelzer U, Oettle H, Riess H, Budczies J, Denkert C. TP53 Mutations Predict Sensitivity to Adjuvant Gemcitabine in Patients with Pancreatic Ductal Adenocarcinoma: Next-Generation Sequencing Results from the CONKO-001 Trial. *Clin Cancer Res* 2020; **26**: 3732-3739 [PMID: 32234756 DOI: 10.1158/1078-0432.CCR-19-3034]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 724-733

DOI: 10.4251/wjgo.v14.i3.724

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Clinical efficacy and prognostic risk factors of endoscopic radiofrequency ablation for gastric low-grade intraepithelial neoplasia

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kim E, United States; Snyder M, United States

Received: November 1, 2021
Peer-review started: November 1, 2021
First decision: December 2, 2021
Revised: December 15, 2021
Accepted: February 27, 2022
Article in press: February 27, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

The use of radiofrequency ablation (RFA) has been reported in the treatment of gastric low-grade intraepithelial neoplasia (LGIN). However, its efficacy and prognostic risk factors have not been well analyzed.

AIM

To explore the efficacy and prognostic risk factors of RFA for gastric LGIN in a large, long-term follow-up clinical study.

METHODS

The clinical data of 271 consecutive cases from 198 patients who received RFA for treatment of gastric LGIN at the Chinese PLA General Hospital from October 2014 to October 2020 were reviewed in this retrospective study. Data on operative parameters, complications, and follow-up outcomes including curative rates were recorded and analyzed.

RESULTS

The curative rates of endoscopic RFA for gastric LGIN at 3 mo, 6 mo, and 1-5 years after the operation were 93.3%, 92.8%, 91.5%, 90.3%, 88.5%, 85.7%, and 83.3%, respectively. Multivariate analyses revealed that *Helicobacter pylori* (*H. pylori*) infection and disease duration > 1 year had a significant effect on the curative rate ($P < 0.001$ and $P = 0.013$, respectively). None of patients had bleeding, perforation, infection, or other serious complications after RFA, and the main discomfort was postoperative abdominal pain.

CONCLUSION

RFA was safe and effective for gastric LGIN during long-term follow-up. *H. pylori* infection and disease course > 1 year may be the main risk factors for relapse of LGIN after RFA.

Key Words: Endoscopic radiofrequency ablation; Gastric low-grade intraepithelial neoplasia; Clinical efficacy; Prognostic risk factors

Core Tip: This is a retrospective study to explore the efficacy and prognostic risk factors of radiofrequency ablation (RFA) for gastric low-grade intraepithelial neoplasia (LGIN). The curative rates of endoscopic RFA for gastric LGIN at 3 mo, 6 mo, and 1-5 years after the operation were 93.3%, 92.8%, 91.5%, 90.3%, 88.5%, 85.7%, and 83.3%, respectively. Multivariate analyses revealed that *Helicobacter pylori* infection and disease duration > 1 year had a significant effect on the curative rate. No serious complications occurred after RFA in all 198 patients. RFA was safe and effective for gastric LGIN during long-term follow-up.

Citation: Wang NJ, Chai NL, Tang XW, Li LS, Zhang WG, Linghu EQ. Clinical efficacy and prognostic risk factors of endoscopic radiofrequency ablation for gastric low-grade intraepithelial neoplasia. *World J Gastrointest Oncol* 2022; 14(3): 724-733
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/724.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.724

INTRODUCTION

Gastric cancer is a commonly occurring cancer, with morbidity and mortality ranking second among all malignant tumors[1]. In 2000, the World Health Organization (WHO) introduced the concept of intraepithelial neoplasia in the new classification of digestive system tumors[2]. This classification divides gastric mucosal intraepithelial neoplasia into low-grade intraepithelial neoplasia (LGIN) and high-grade intraepithelial neoplasia (HGIN) according to the degree of cellular and structural atypia. Currently, the consensus has been reached on the treatment of HGIN[3,4]. For LGIN, relevant studies[5,6] have shown that it can still develop into gastric cancer; thus, some guidelines advocate endoscopic therapy for long-term gastric LGIN[7-9].

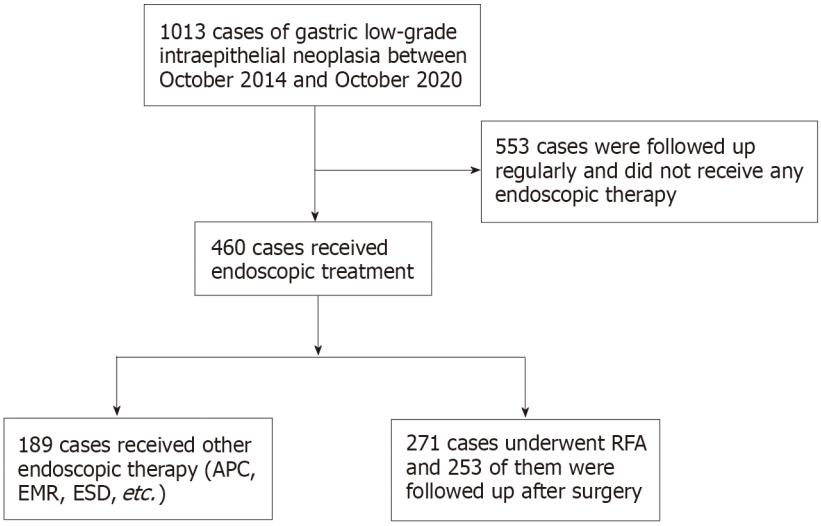
At present, endoscopic treatment of gastric LGIN mainly includes two methods: resection therapy and damage therapy. Although resection therapy, such as endoscopic mucosal resection and endoscopic submucosal dissection (ESD), has shown to be effective in treating LGIN, the operation is difficult, the treatment cost is high, postoperative management is complex, and there is still the possibility of serious complications[10]. Radiofrequency ablation (RFA), as a kind of damage therapy, has been preliminarily reported in some small clinical studies for treatment of gastric LGIN[11-13], which has the advantages of simple operation, lower risk, lower cost and rapid recovery. However, its efficacy and especially the prognostic risk factors are still not fully understood. The aim of this research was to further explore the efficacy and prognostic risk factors of RFA for gastric LGIN in a large clinical sample study.

MATERIALS AND METHODS

Study population and data source

The records of 271 consecutive lesions from 198 patients who received RFA to treat gastric LGIN at the Chinese PLA General Hospital between October 2014 and October 2020 were reviewed for this retrospective study. The detailed flowchart of the study is shown in Figure 1. All the patients provided written informed consent for the procedure. The clinicopathological characteristics and treatment outcomes were retrospectively reviewed using our medical digital engineering database system (Medcare, Qingdao, Shandong Province, China). The study was reviewed and approved by the Ethics Committee of the Chinese PLA General Hospital.

The inclusion criteria were: (1) Macroscopic types of lesion defined as type IIa (superficially elevated), type IIb (flat), and type IIc (superficially depressed), according to the Paris classification[14]; and (2) preoperative biopsy confirmed the lesion as LGIN using the WHO standards before treatment[2]. The exclusion criteria were: (1) Patients with severe systemic disease or advanced chronic liver disease and a history of gastric surgery; (2) a lesion in which HGIN or early gastric cancer (EGC) was found in the biopsy specimen before treatment; and (3) patients with coagulation dysfunction or those unable to



DOI: 10.4251/wjgo.v14.i3.724

Figure 1 Flowchart of the patients enrolled in this study. RFA: Radiofrequency ablation; APC: Argon plasma coagulation; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection.

comply with follow-up requirements.

RFA techniques

A gastroscope (GIF-Q260J; Olympus Medical, Tokyo, Japan) and the BARRX System (Covidien GI Solutions, Sunnyvale, CA, United States) were used for RFA. A disposable injector (NM-200L-0425; Olympus, Tokyo, Japan) with a normal saline solution was used for submucosal injections. The accessory of the BARRX System (Covidien TTS-1100, 60RFA Conduit 909300) was used for lesion damage. Hemostatic forceps (FD-410 LR; Olympus) and EZ Clip (HX-610-135) were used to prevent hemorrhage and perforation. Other equipment and accessories included a high-frequency generator (ICC-200; ERBE Elektromedizin, Tübingen, Germany) and an argon plasma coagulation unit (APC 300; ERBE) for RFA.

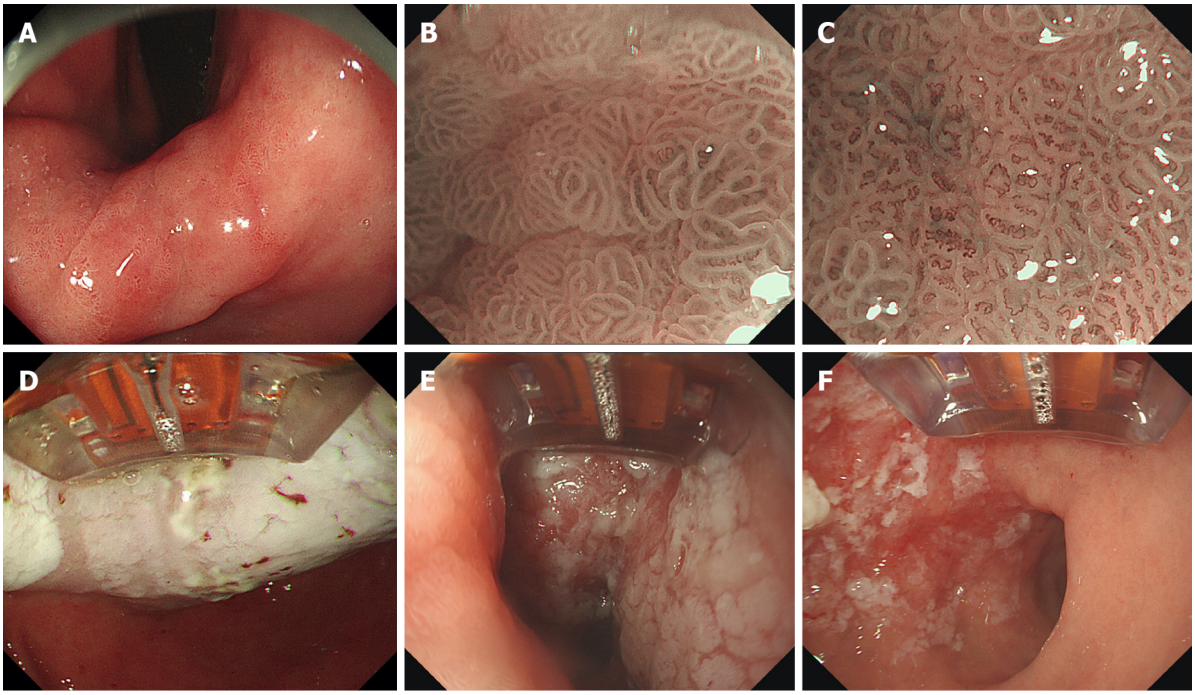
The specific procedures for RFA were as follows. After the lesions were found by routine upper gastrointestinal endoscopy, the lesions were further observed using magnifying endoscopy (ME) combined with narrow-band imaging (NBI) to determine the size and range. Subsequently, with endoscopic assistance, the RFA electrode was attached to the lesions. We set the power output for RFA as 57 W and the energy density as 15 J/cm^2. After ablation, the surface of the lesions showed white coagulation and necrosis. The ablation was repeated three times for each lesion to ensure that the lesion was completely ablated. Before the next ablation, the coagulated necrotic tissue on the surface was removed, which was accomplished with the aid of RFA electrodes. Moreover, there was also a possibility of administering a submucosal injection to the lesion, which is easier to operate. Other details of the RFA procedures were described in our previous study[13].

The RFA procedure, which was performed by three experienced GI endoscopists (Linghu EQ, Chai NL and Wang NJ), is shown in Figure 2. Hemorrhage after ESD was defined as symptomatic bleeding with the need for emergency endoscopy. Perforation was diagnosed by endoscopy or by the presence of free air on abdominal computed tomography. Postoperative abdominal pain was evaluated by Wong-Baker FACES Pain Rating Scale[9,15]. Hemorrhage, perforation, and postoperative abdominal pain were the variables recorded and analyzed as complications to evaluate the safety of the procedure. We recommend ME-NBI and targeted biopsies for histological prediction before RFA to treat gastric LGIN. Additionally, along with ME-NBI, it is necessary to combine various endoscopic techniques, including endoscopic ultrasound and chromoendoscopy, in some difficult cases before RFA.

Additional treatments and follow-up

Each patient fasted for 4-6 h after surgery. After that, a liquid or semiliquid diet was administered, followed by gradual transition to a normal diet. At the same time, patients needed oral proton pump inhibitor (PPI) and mucosal protectant for 1 mo after surgery. In addition, we explained the Wong-Baker FACES Pain Rating Scale to each patient and provided them with a form used for self-recording their daily pain score in the first month after RFA. The form was returned 3 mo after the patient came back to our hospital for the first review.

The curative effect was determined by the pathological results of the biopsy from the original treatment area when patients came back to the hospital for a review after surgery. The specific time of gastroscopy follow-up was 3 mo, 6 mo, and 1-5 years after the operation. The evaluation criteria were: (1) Disappearance of LGIN in the original treatment area indicated by pathological biopsy was



DOI: 10.4251/wjgo.v14.i3.724

Figure 2 The radiofrequency ablation procedure of gastric low-grade intraepithelial neoplasia. A: White-light imaging of the lesion (reversed view); B: Magnifying endoscopy with narrow-band imaging of the lesion (weak magnification); C: Magnifying endoscopy with narrow-band imaging of the lesion (strong magnification); D: After ablation, the surface of the lesions showed white coagulation and necrosis; E: Radiofrequency ablation for the same area was repeated three times; F: After scraping off the necrotic mucosal tissue on the surface.

considered as curative effect; (2) biopsy of the area of the original treatment that still indicated LGIN was considered as relapse; (3) pathological result of biopsy in the nontherapeutic area indicating LGIN was considered as recurrence; and (4) pathological result of biopsy of the original treatment area indicating HGIN or cancer was considered as disease progression.

We judged the safety of the operation by monitoring the occurrence of complications such as perioperative bleeding and perforation and the time and degree of postoperative abdominal pain in all patients.

Statistical analysis

Data analysis was performed using SPSS Statistics for Windows, version 24.0 (IBM, Armonk, NY, United States). Measurement data are expressed as mean value ± SD, whereas numerical data are described by frequency and percentage and were compared by χ^2 or Fisher's exact test. The measurement data were analyzed by *t*-test and one-way analysis of variance or rank-sum test according to whether the data conformed to a normal distribution. Survival curves were drawn with the Kaplan–Meier method, and intragroup comparisons were made with a log-rank test. Univariate survival analysis was performed with the Cox proportional hazards model, where the variables with $P < 0.10$ were included in Cox multivariate survival analysis. The hazard ratio and its 95% confidence interval were used to express the relative risk, and the relationship of each variate with the recurrence-free and overall survival of patients was analyzed. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of patients

Among all 271 cases that underwent RFA therapy, 253 completed postoperative follow-up. The basic characteristics and endoscopic features of all 253 cases are summarized in Table 1. This study included 167 men, aged 22-84 years (mean 58.51 years), and 86 women, aged 43-78 years (mean 58.23 years). Most of the lesions were located in the antrum of the stomach - pylorus area, while no lesions were ulcerated. All cases underwent RFA in a day ward or outpatient setting and did not require hospitalization.

Therapeutic efficacy and long-term outcomes of RFA

The data of all 253 cases that received RFA and completed follow-up are shown in Table 2. All 253 cases were followed up for 3 mo after surgery, and the curative, relapse, recurrence and progression rates

Table 1 The basic characteristics and endoscopic features of all 253 cases

	Curative	Relapse	Total	*P* value
Patients, *n*	188 (74.3)	65 (25.7)	253	
Age, mean ± SD (yr)	58.51 ± 10.53	58.23 ± 8.33	58.43 ± 9.99	0.83
Sex, *n* (%)				0.24
Male	128 (68.1)	39 (60.0)	167 (66.0)	
Female	60 (31.9)	26 (40.0)	86 (34.0)	
Location of lesions, *n* (%)				0.08
Gastric fundus - cardia area	5 (2.7)	1 (1.5)	6 (2.4)	
Gastric body	19 (10.1)	5 (7.7)	24 (9.5)	
Angle of stomach	32 (17.0)	21 (32.3)	53 (20.9)	
Antrum of the stomach - pylorus area	132 (70.2)	38 (58.5)	170 (67.2)	
Ulceration	0	0	0	
Helicobacter pylori infection, *n* (%)				< 0.001
Yes	8 (4.3)	37 (56.9)	45 (17.8)	
No	180 (95.7)	28 (43.1)	208 (82.2)	
Atrophy, *n* (%)				< 0.001
Yes	49 (26.1)	39 (60.0)	88 (34.8)	
No	139 (73.9)	26 (40.0)	165 (65.2)	
A course of disease, *n* (%)				< 0.001
< 1 yr	138 (73.4)	14 (21.5)	152 (60.1)	
> 1 yr	50 (26.6)	51 (78.5)	101 (39.9)	

Table 2 The data of all 253 cases that received radiofrequency ablation and completed follow-ups

Follow-up period	*n*	Curative, *n* (%)	Relapse, *n* (%)	Recurrence, *n* (%)	Progression, *n* (%)
3 mo	253	236 (93.3)	17 (6.7)	18 (7.1)	2 (0.8)
6 mo	208	193 (92.8)	15 (7.2)	11 (5.3)	0
1 yr	141	129 (91.5)	12 (8.5)	14 (9.9)	0
2 yr	93	84 (90.3)	9 (9.7)	8 (8.6)	1 (1.1)
3 yr	61	54 (88.5)	7 (11.5)	6 (9.8)	2 (3.3)
4 yr	28	24 (85.7)	4 (14.3)	4 (14.3)	0
5 yr	6	5 (83.3)	1 (16.7)	1 (16.7)	0

were 93.3%, 6.7%, 7.1% and 0.8%, respectively. During the 6-mo follow-up, there were no cases of progression, and the curative, relapse and recurrence rates were 92.8%, 7.2% and 5.3%, respectively. Similarly, there were also no cases that had progressed at 1-year follow-up, and the curative, relapse and recurrence rates were 91.5%, 8.5% and 9.9%, respectively. The 2-year curative rate of RFA was 90.3%, and the relapse rate, recurrence rate, and progression rate were 9.7%, 8.6%, and 1.1%, respectively. Moreover, 3 years of postoperative follow-up were completed in 61 cases, and the curative, relapse, recurrence and progression rates were 88.5%, 11.5%, 9.8% and 3.3%, respectively. Among all cases, 28 completed the 4-year follow-up, and the curative, relapse and recurrence rates were 85.7%, 14.3% and 14.3%, respectively, with no cases of progression. Only six cases completed the 5-year follow-up, and the curative, relapse and recurrence rates were 83.3%, 16.7% and 16.7%, respectively, and there were also no cases of progression.

Among the five cases with progression in postoperative follow-up, four were pathologically indicated as HGIN, and one was highly differentiated adenocarcinoma. Three cases with HGIN received additional ESD, while one highly differentiated adenocarcinoma case and one case with HGIN were treated with additional surgery. All of these cases achieved curative resection with no recurrence or

local lymph node metastasis during follow-up. In addition, some of the relapse and recurrent cases were treated with RFA again, while others chose to remain under observation. So far, there are still a few cases of LGIN that did not disappear, none of which progressed to HGIN or EGC.

Complications

No bleeding, perforation, infection or other serious complications occurred in any of 253 cases. Regarding postoperative abdominal pain, 136 cases showed varying degrees of pain (from grade A to C), with an incidence of 53.8% (136/253). The first day to 12 d after RFA was the main time period for pain occurrence. There were 126 cases with abdominal pain in the curative group and 10 cases in the relapse group, with no significant difference between them (126/236 *vs* 10/17, $P = 0.815$).

Analysis of prognostic risk factors

The univariate and multivariate analyses of the outcomes of LGIN after RFA are shown in Table 3. According to results of univariate analysis, sex, age and location of the lesion had no significant effect on the prognosis of LGIN after RFA, with P values of 0.43, 0.89 and 0.29, respectively, while patients with *Helicobacter pylori* (*H. pylori*) infection, atrophic gastritis and disease course > 1 year were more likely to relapse after the procedure ($P < 0.001$). Subsequently, we included three variables, *H. pylori* infection, atrophic gastritis, and disease course > 1 year in multivariate analysis, which showed that the two factors of *H. pylori* infection and disease course > 1 year might be the main risk factors leading to relapse of LGIN after RFA ($P < 0.001$ and $P = 0.013$, respectively) (Figure 3).

DISCUSSION

The working principle of RFA is to cause the movement of charged particles in tissues to generate heat through the action of high-frequency alternating current, so as to make the water inside and outside cells evaporate, dry, shrink and fall off, resulting in aseptic necrosis. The power output and energy density of each RFA is rated and does not increase with the duration of operation. RFA is easy to perform and can be completed if the endoscopic physician has the ability to operate the gastroscope. At the same time, there is no bleeding, perforation, infection or other serious complications after RFA. All the above advantages show that RFA has good clinical development prospects.

In our study, the curative rates of endoscopic RFA for gastric LGIN at 3 and 6 mo, and 1-5 years after the operation were 93.3%, 92.8%, 91.5%, 90.3%, 88.5%, 85.7% and 83.3%, respectively. Both the short-term and long-term efficacy were satisfactory. However, these results showed that gastric LGIN still relapsed in some cases after RFA. Therefore, we included multiple variables for univariate and multivariate analyses to try to find risk factors affecting prognosis. The results of the multivariate analysis suggested that *H. pylori* infection and disease duration > 1 year may be the risk factors for disease relapse, while age, sex, and location of the lesion were not related to disease relapse. Univariate analysis indicated atrophic gastritis as one of the possible risk factors for relapse after RFA; however, the results of multivariate analysis were not fully consistent; thus, the accuracy of this conclusion needs to be further investigated. Alternatively, the longer course of the disease and infection of *H. pylori* may change the overall state and microenvironment of the gastric mucosa to some extent, which may be one of the possible reasons for the recurrence of LGIN. This is similar to the results of some previous studies [16,17], because the presence of *H. pylori* makes the mucosa more prone to intestinal metaplasia, and the probability of intraepithelial neoplasia in atrophic and intestinal metaplasia is higher than that in normal mucosa. At the same time, we noted that about two thirds of the lesions were concentrated in the gastric antrum, which may be related to the early occurrence of mucosal atrophy in the gastric antrum and its susceptibility to *H. pylori*. This also supports our conclusions.

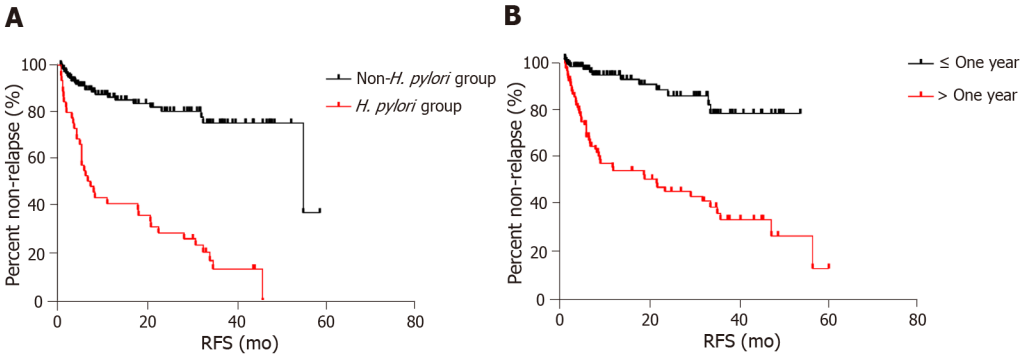
As for the risk factors of LGIN progressing to HGIN or EGC, some studies have reported that it may be related to lesion size > 1 cm, various changes of the lesion surface such as erythema, nodules, erosion and ulceration, and obvious depression of the lesion[18,19]. By reviewing five cases that progressed to HGIN or EGC in the present study, we found that they were all > 1 cm in size. Moreover, four of them showed erythematous nodular changes on the surface, while the other case showed obvious erosion on the surface. All these factors were reflected in the aforementioned studies. We have added ESD or surgical procedures for all five of these cases, which achieved short-term cure, and the long-term prognosis is still being followed up.

Our diagnosis of LGIN was mainly based on preoperative endoscopic biopsy pathology. However, the pathological diagnosis based on endoscopic biopsy is not completely consistent with the real nature of the lesion[20]. Some small and early cancers may exist in the deep mucosa, which exceeds a depth of 200 μm, that can be seen by ME, resulting in diagnostic deviation. However, after RFA treatment, these cancers in the deep mucosa are more likely to be detected by re-examination. The above two reasons may have some influence on the efficacy of RFA for gastric LGIN. Therefore, prospective studies based on a unified pathological definition pathology are needed to verify the reported findings[21].

Table 3 Univariate and multivariate analysis of demographic and clinical features covariates for relapse

	Univariate		Multivariate	
	***P* value**	**HR (95%CI)**	***P* value**	**HR (95%CI)**
Sex	0.43	0.817 (0.494-1.351)		
Age	0.89	0.998 (0.974-1.023)		
Location	0.29			
Gastric body		0.674 (0.092-4.919)		
Angle of stomach		1.242 (0.487-3.167)		
Antrum of the stomach - pylorus area		1.644 (0.963-2.808)		
H. pylori infection	< 0.001	6.053 (3.679-9.957)	< 0.001	2.662 (1.225-5.788)
Atrophy	< 0.001	2.136 (1.024-4.453)	0.597	1.170 (0.653-2.097)
Course of disease	< 0.001	5.482 (3.029-9.919)	0.013	2.662 (1.225-5.788)

H. pylori: *Helicobacter pylori*.



DOI: 10.4251/wjgo.v14.i3.724 **Copyright** ©The Author(s) 2022.

Figure 3 Relapse-free survival curves. A: Non-*Helicobacter pylori* (*H. pylori*) infection *vs H. pylori* infection ($P < 0.001$); B: Disease course less than 1 year *vs* disease course over 1 year ($P = 0.013$). RFS: Relapse-free survival.

In terms of complications, more than half of the cases had abdominal pain (136/253) after RFA. The grade of pain was mainly graded as A or B (123/136), with a few grade C (13/136). The pain was tolerated by all patients and gradually relieved with oral PPI and mucosal protectant. Based on the principle of RFA, we considered that such postoperative pain was associated with the local mucosal injury caused by RFA. A small comparative study has shown that submucosal injection can have a protective role in the treatment of mucosal damage and effectively relieve postoperative pain[22], which needs to be confirmed in subsequent large comparative studies.

In this study, we only included LGIN lesions that were macroscopic type 0-II as type 0-I and 0-III were at risk of progressing to HGIN or EGC. Flat lesions were also more conducive to the effective adhesion of the RFA electrode so as to fully achieve the therapeutic effect. Therefore, after taking the above factors into consideration, we chose such inclusion criteria.

During RFA, the electrode should be closely attached to the mucosal surface so that the energy can be fully transmitted. Before the next ablation, the necrotic mucosal tissue on the lesion surface after the previous ablation should be fully removed to avoid reduction of energy conduction, so as to ensure the ablation effect[13]. The above steps can be completed by rotating the endoscope, inhalation, and aeration.

RFA has the following advantages. First, in addition to the satisfactory efficacy and safety demonstrated in our study, the procedure is simple and easy to learn, generally taking 10-20 min to complete. Second, RFA has low cost, which can reduce the economic burden of patients. Third, patients can eat on the day after surgery, without the need for prophylactic antibiotics, which is conducive to recovery. Last but not least, RFA can be performed on an outpatient basis without requiring hospitalization, which is important for saving medical resources. Therefore, based on the above advantages, the future clinical application and popularization of RFA is worth exploring.

The present study had several limitations. First, it was a single-center retrospective study, and future multicenter comparative and randomized trials are needed to confirm our findings. Second, the number of patients with follow-up > 3 years was small, which may affect our prediction of the long-term prognosis of LGIN after RFA. Finally, more cases are needed to evaluate the difference in pain grades between the submucosal and the nonsubmucosal injection groups. Also, future prospective studies based on a unified pathological definition are warranted.

CONCLUSION

RFA is a safe and effective treatment strategy for gastric LGIN, which is worthy of clinical application and promotion. *H. pylori* infection and disease course > 1 year may be the main risk factors leading to relapse of LGIN after RFA. For relapsing and recurrent cases, secondary RFA therapy may be considered.

ARTICLE HIGHLIGHTS

Research background

The efficacy and prognostic risk factors of radiofrequency ablation (RFA) for gastric low-grade intraepithelial neoplasia (LGIN) have not been well analyzed.

Research motivation

We look forward to promoting the use of RFA ablation for gastric LGIN in the future.

Research objectives

To explore the efficacy and prognostic risk factors of RFA for gastric LGIN.

Research methods

The large sample clinical data of RFA for gastric LGIN were reviewed in this retrospective study. Data on operative parameters, complications, and follow-up outcomes including curative rates were recorded and analyzed.

Research results

The near- and long-term efficiency of RFA is satisfactory. Multivariate analyses revealed that *Helicobacter pylori* (*H. pylori*) infection and disease duration > 1 year had a significant effect on the curative rate. None of patients had bleeding, perforation, infection, or other serious complications after RFA, and the main discomfort was postoperative abdominal pain.

Research conclusions

RFA is a safe and effective treatment strategy for gastric LGIN, which is worthy of clinical application and promotion. *H. pylori* infection and disease course > 1 year may be the main risk factors leading to relapse of LGIN after RFA.

Research perspectives

We look forward to conducting a multicenter prospective controlled study in the future to further confirm the efficacy of RFA in the treatment of gastric LGIN.

FOOTNOTES

Author contributions: Wang NJ and Chai NL are co-first authors and contributed equally to this work; Wang NJ and Chai NL designed and performed the research and wrote the paper; Tang XW designed the research and supervised the report; Li LS designed the research and contributed to the analysis; Zhang WG supervised the report; Linghu EQ designed the research and provided clinical advice.

Supported by National Key R&D Program of China, No. 2016YFC1303601.

Institutional review board statement: The Medical Ethics Committee of the PLA General Hospital agreed to exempt this study from medical ethics review.

Informed consent statement: All patients have signed informed consent forms.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

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S-Editor: Yan JP
L-Editor: A
P-Editor: Yan JP

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
2 **Watterson JD**, Mahoney JE, Futter NG, Gaffield J. Iatrogenic ureteric injuries: approaches to etiology and management. *Can J Surg* 1998; **41**: 379-382 [PMID: 9793505 DOI: 10.1046/j.1365-2168.1998.00956.x]
3 **Ono H**, Yao K, Fujishiro M, Oda I, Nimura S, Yahagi N, Iishi H, Oka M, Ajioka Y, Ichinose M, Matsui T. Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer. *Dig Endosc* 2016; **28**: 3-15 [PMID: 26234303 DOI: 10.1111/den.12518]
4 **Expert group of Beijing Municipal Science and Technology Commission major project "Study on the treatment standard of early gastric cancer"**. Expert consensus on endoscopic standardized resection of early gastric cancer (2018, Beijing). *Zhonghua Xiaohua Neijing Zazhi* 2019; **36**: 381-392 [DOI: 10.3760/cma.j.issn.1007-5232.2019.06.001]
5 **Hasuike N**, Ono H, Boku N, Mizusawa J, Takizawa K, Fukuda H, Oda I, Doyama H, Kaneko K, Hori S, Iishi H, Kurokawa Y, Muto M; Gastrointestinal Endoscopy Group of Japan Clinical Oncology Group (JCOG-GIESG). A non-randomized confirmatory trial of an expanded indication for endoscopic submucosal dissection for intestinal-type gastric cancer (cT1a): the Japan Clinical Oncology Group study (JCOG0607). *Gastric Cancer* 2018; **21**: 114-123 [PMID: 28224238 DOI: 10.1007/s10120-017-0704-y]
6 **Wu B**, Linghu E, Yang J. The clinical pathology and prognosis of gastric mucosal low-grade intraepithelial neoplasia. *Jiefangjun Yixueyuan Xuebao* 2011; **32**: 598-600
7 **ASGE Standards of Practice Committee**, Evans JA, Chandrasekhara V, Chathadi KV, Decker GA, Early DS, Fisher DA, Foley K, Hwang JH, Jue TL, Lightdale JR, Pasha SF, Sharaf R, Shergill AK, Cash BD, DeWitt JM. The role of endoscopy in the management of premalignant and malignant conditions of the stomach. *Gastrointest Endosc* 2015; **82**: 1-8 [PMID: 25935705 DOI: 10.1016/j.gie.2015.03.1967]
8 **Goddard AF**, Badreldin R, Pritchard DM, Walker MM, Warren B; British Society of Gastroenterology. The management of gastric polyps. *Gut* 2010; **59**: 1270-1276 [PMID: 20675692 DOI: 10.1136/gut.2009.182089]
9 **Division of Digestive Endoscopy of Beijing Medical Association**. Expert consensus on standardized diagnosis and treatment of gastric low-grade intraepithelial neoplasia (2019, Beijing). *Zhonghua Weichang Neijing Dianzi Zazhi* 2019; **6**: 49-56 [DOI: 10.3877/cma.j.issn.2095-7157.2019.02.001]
10 **Kim SY**, Sung JK, Moon HS, Kim KS, Jung IS, Yoon BY, Kim BH, Ko KH, Jeong HY. Is endoscopic mucosal resection a sufficient treatment for low-grade gastric epithelial dysplasia? *Gut Liver* 2012; **6**: 446-451 [PMID: 23170148 DOI: 10.5009/gnl.2012.6.4.446]
11 **Baldaque-Silva F**, Cardoso H, Lopes J, Carneiro F, Macedo G. Radiofrequency ablation for the treatment of gastric dysplasia: a pilot experience in three patients. *Eur J Gastroenterol Hepatol* 2013; **25**: 863-868 [PMID: 23510961 DOI: 10.1097/MEG.0b013e32835f8fa5]
12 **Leung WK**, Tong DK, Leung SY, Chan FS, Tong TS, Ho RS, Chu KM, Law SY. Treatment of Gastric Metaplasia or Dysplasia by Endoscopic Radiofrequency Ablation: A Pilot Study. *Hepatogastroenterology* 2015; **62**: 748-751 [PMID: 26897966]
13 **Linghu E**, Feng J, Ma X. Clinical study of endoscopic radiofrequency ablation for treatment of low-grade gastroesophageal intraepithelial neoplasia. *Zhonghua Weichang Neijing Dianzi Zazhi* 2015; **2**: 14-17 [DOI: 10.3877/cma.j.issn.2095-7157.2015.01.004]
14 The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest Endosc* 2003; **58**: S3-43 [PMID: 14652541 DOI: 10.1016/s0016-5107(03)02159-x]
15 **Lawson SL**, Hogg MM, Moore CG, Anderson WE, Osipoff PS, Runyon MS, Reynolds SL. Pediatric Pain Assessment in the Emergency Department: Patient and Caregiver Agreement Using the Wong-Baker FACES and the Faces Pain Scale-Revised. *Pediatr Emerg Care* 2021; 37: e950-e954 [PMID: 31335787 DOI: 10.1097/PEC.0000000000001837]
16 **Sung JK**. Diagnosis and management of gastric dysplasia. *Korean J Intern Med* 2016; **31**: 201-209 [PMID: 26932397 DOI:

10.3904/kjim.2016.021]
17 **Liu Y**, Cai Y, Chen S, Gou Y, Wang Q, Zhang M, Wang Y, Hong H, Zhang K. Analysis of Risk Factors of Gastric Low-Grade Intraepithelial Neoplasia in Asymptomatic Subjects Undergoing Physical Examination. *Gastroenterol Res Pract* 2020; **2020**: 7907195 [PMID: 32256566 DOI: 10.1155/2020/7907195]
18 **Kim JW**, Jang JY. Optimal management of biopsy-proven low-grade gastric dysplasia. *World J Gastrointest Endosc* 2015; **7**: 396-402 [PMID: 25901219 DOI: 10.4253/wjge.v7.i4.396]
19 **Kang DH**, Choi CW, Kim HW, Park SB, Kim SJ, Nam HS, Ryu DG. Predictors of upstage diagnosis after endoscopic resection of gastric low-grade dysplasia. *Surg Endosc* 2018; **32**: 2732-2738 [PMID: 29214514 DOI: 10.1007/s00464-017-5971-5]
20 **Lim H**, Jung HY, Park YS, Na HK, Ahn JY, Choi JY, Lee JH, Kim MY, Choi KS, Kim DH, Choi KD, Song HJ, Lee GH, Kim JH. Discrepancy between endoscopic forceps biopsy and endoscopic resection in gastric epithelial neoplasia. *Surg Endosc* 2014; **28**: 1256-1262 [PMID: 24310738 DOI: 10.1007/s00464-013-3316-6]
21 **Kato M**. Diagnosis and therapies for gastric non-invasive neoplasia. *World J Gastroenterol* 2015; **21**: 12513-12518 [PMID: 26640329 DOI: 10.3748/wjg.v21.i44.12513]
22 **Niu XT**. The protective effects of submucosal injection for gastric low-grade intraepithelial neoplasia treatment. *Nankai Daxue* 2016; 1-50

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2022 March 15; 14(3): 734-745

DOI: 10.4251/wjgo.v14.i3.734

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

Association of *Blastocystis hominis* with colorectal cancer: A systematic review of *in vitro* and *in vivo* evidences

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Farouk S

Received: May 17, 2021
Peer-review started: May 17, 2021
First decision: July 4, 2021
Revised: July 16, 2021
Accepted: February 22, 2022
Article in press: February 22, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

Recently, there have been several findings that showed intestinal colonisation of *Blastocystis hominis* (*Blastocystis*) as a risk factor to the worsening of colorectal cancer (CRC). However, studies have shown controversial results in the pathogenicity of *Blastocystis*.

AIM

To review systematically the evidence available on the association between CRC and *Blastocystis* and the prevalence of *Blastocystis* in CRC patients and to investigate cytopathic and immunological effects of *Blastocystis* in *in vitro* and *in vivo* studies.

METHODS

PRISMA guidelines were utilised in conducting this systematic review. Original

articles published before February 2, 2020 were included. PubMed, Science Direct, Scopus and Google scholar databases were searched. Manual searching was carried out to find articles missed during the online search.

RESULTS

Out of 12 studies selected for this systematic review, seven studies confirmed the prevalence of *Blastocystis* and found it to be between 2%-28% in CRC patients, whereby subtype 1 and subtype 3 were predominantly seen. A total of four studies employing *in vitro* human colorectal carcinoma cell line study models showed significant cytopathic and immunological effects of *Blastocystis*. In addition, one *in vivo* experimental animal model study showed that there was a significant effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

CONCLUSION

Blastocystis is a commonly identified microorganism in CRC patients. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage. Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its relationship with other intestinal bacteria.

Key Words: *Blastocystis hominis*; Colorectal cancer; Cytopathic effect; Immunological effect

Core Tip: Certain gut microorganisms are known to be important factors associated with initiation and development of colorectal cancer (CRC). However, data on the roles of parasites are vague and restricted. *Blastocystis hominis* (*Blastocystis*) is one of the most commonly recovered microorganisms in faecal specimens, and its widespread presence is found in CRC patients. This systematic review aims to quantify the studies published so far that revealed the association of *Blastocystis* and CRC. We sought to identify the prevalence of *Blastocystis* and its subtypes among CRC patients, *in vitro* studies using *Blastocystis* antigen and *in vivo* studies using animal models.

Citation: Kumarasamy V, Atroosh WM, Anbazhagan D, Abdalla MMI, Azzani M. Association of *Blastocystis hominis* with colorectal cancer: A systematic review of *in vitro* and *in vivo* evidences. *World J Gastrointest Oncol* 2022; 14(3): 734-745

URL: https://www.wjgnet.com/1948-5204/full/v14/i3/734.htm

DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.734

INTRODUCTION

Blastocystis hominis (*Blastocystis*) is one of the most commonly recovered microorganisms in faecal specimens, and its widespread presence is found in colorectal cancer (CRC) patients[1]. Its distribution is known to be prevalent in both rural and urban areas[2]. This microorganism has been in discussion since early 1900s[3,4]; however, the taxonomic position of *Blastocystis* remains unanswered. *Blastocystis* treatment is often difficult due to its drug resistance and the failure of the host defenses to counter the infection[5]. Previous studies suggest that *Blastocystis* is a common and diverse element of microbiota in human host, as it has been highly prevalent in healthy individuals[6-8]. *Blastocystis* is commonly found in both patients with gastrointestinal symptoms and in healthy people widely across the world. More recently, researchers consider *Blastocystis* as an emerging zoonotic disease, and its pathogenic potential in human is somewhat controversial[9]. Although accumulating data suggest that *Blastocystis* is a pathogen, the pathogenic role in humans is still a matter of debate.

It is suggested that around 20% of cancer reported worldwide could have been due to infectious agents[10,11]. Viruses such as hepatitis B virus, human papilloma virus and Epstein-Barr virus have been associated with carcinogenesis. Various other bacteria also have been described previously to exacerbate cancer[12]. There are numerous epidemiological evidences that strongly support the fact that parasites can be a factor of various malignant tumours[13], but it is challenging to validate this relationship. Previously, a review article highlighted the correlation of various protozoan parasites including *Blastocystis* with carcinogenesis[13]. In addition, there was a case report in India that demonstrated a possible association of subtype 3 *Blastocystis* in the worsening of CRC[14].

There are a few other systematic reviews on the interventional studies done on *Blastocystis*, but we did not find any systematic reviews on the association between *Blastocystis* and CRC. Therefore, this systematic review aimed to (1) identify prevalence of *Blastocystis* in CRC patients; (2) review *in vitro* colorectal carcinoma cell line study models on the cytopathic and immunological effects of *Blastocystis* antigens; and (3) review an *in vivo* experimental animal model study to investigate the effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

MATERIALS AND METHODS

PRISMA guidelines were utilised in conducting this review[15].

Eligibility criteria

Original articles that reported the prevalence and association of *Blastocystis* subtypes with CRC patients, *in vitro* studies using *Blastocystis* antigen and an *in vivo* study using an animal model published before February 2, 2020 were included.

Exclusion

Articles that reported the association of *Blastocystis* with cancer in general without specific findings on its association with CRC, reviews papers, conference proceedings and case reports were excluded from this review.

Search

PubMed, Science Direct, Scopus and Google scholar databases were searched.

The various keywords used were: *Blastocystis* infection and CRC and their MESH terms and synonyms. Manual searching through reference lists of included journal articles was done to find the missed studies during online search.

Study selection

We identified 872 papers in the initial screening (Figure 1).

Identification

First, two authors developed a search strategy with different key words and their synonyms. All articles were moved to the Endnote X7 software (Clarivate Analytics, Philadelphia, PA, United States), and 146 duplicate papers were removed. Two authors independently went through the titles and abstracts of the remaining 726 papers. Subsequently, a total of 22 papers were retained for full text review. The eligibility of retained papers was evaluated by two other authors. Authors checked the reference lists of all included articles for any relevant studies that met this systematic review inclusion criteria and had not been found during the database searches.

After full review of the 22 papers, 12 were selected. All authors have completed data extraction and agreed eligibility of included papers (Figure 1).

Quality assessment

For *in vivo* studies, the quality of the studies was assessed using Quasi experimental appraisal tool[16], which contained nine questions. For *in vitro* studies, checklist of Systematic Review Center for Laboratory Animal Experimentation's Risk of Bias tool for assessing risk of bias as quality assessment was used[17]. The quality of all included studies was acceptable.

Data extraction

The data extracted included author-year, country, sampling, setting, methods, results and study conclusion (Table 1).

RESULTS

Prevalence of Blastocystis in CRC patients

Based on the seven reviewed articles, the prevalence of *Blastocystis sp.* in CRC patients were found to be between 2.8%-46.7%, whereby subtype 1 and subtype 3 were predominantly isolated.

A study by Esteghamati *et al*[35] evaluated the prevalence of *Blastocystis* in 85 cancer patients, including 39 CRC and 46 cancers outside gastrointestinal tract (COGT). In this study, *Blastocystis* was identified in 11/39 (28.2%) among CRC group. Another study in China by Zhang *et al*[18] showed the prevalence of *Blastocystis* in 4 among 49 (8.1%) patients with CRC. In another study conducted in Saudi Arabia, the prevalence of *Blastocystis* among CRC patients was 29.7%[19]. Subtype I was the

Table 1 Data extraction table

No.	Ref.	Country	Sampling	Setting	Method Please note: (1) Main outcomes assessed; (2) If the protocol is published; and (3) If risk of bias is reported	Main results	Conclusion
Prevalence studies							
1	Esteghamati *et al*[35], 2019	Tehran, Iran	Study design: cross-sectional. Study duration: July 2016 and November of 2017. Sample size (190): 80 patients (Primary Immunodeficiency), 85 (cancer patients) and 25 (organ transplant recipients)	3 hospitals in in Tehran, Iran	The aim of this study to determine the prevalence of intestinal parasites in 3 different groups of patients referred to 3 hospitals. Method used for parasite identification: Conventional methods, nested PCR and amplification of the *18S rRNA* gene	The prevalence of *Blastocystis* hominis among CRC patient was 13/39 (28.2%)	The prevalence of *Blastocystis hominis* was found high in cancer patients, especially CRC patients
2	Zhang *et al* [18], 2017	China	Sample size: 381 faecal specimens were collected from cancer patients including CRC. Study duration: 2016 to 2017	Tumor Hospitalof Harbin Medical University	The aim of this study to determine the prevalence and genotypes/subtypes-*Blastocystis* in CP and analysed for the *Blastocystis* by PCR amplifying and sequencing	Prevalence of *Blastocystis* was 4 (8.1%) among CRC patient	*Blastocystis* subtype 1 and 3 have been identified in humans and animals
3	Mohamed *et al* [19], 2017	Saudi Arabia	Total sample size: 218. Two groups of participants: (1) CP (138) of which 74 had CRC and 46 had cancers outside gastrointestinal tract; and (2) NCP (80). Exclusion criteria: (1) Patient started chemotherapy regime; and (2) Receiving any anti-parasitic medication. Study duration: 2013-2015	King Abdulla Medical city (KAMC), Makkah	Case control study design: Aim, to determine the prevalence of *Blastocystis* among CRC patients compared to patients who had cancers outside gastrointestinal tract and control group. Obtained *Blastocystis* isolates were grouped into 2 categories (A and C), then subtyped into 3 various subtypes; subtype-I, subtype-II and subtype-V	Prevalence of *Blastocystis* among CRC = 22 (29%). *Blastocystis* infection frequency was significantly different between CP group and NC group. There was a higher probability of *Blastocystis sp*. among CP. Subtype I was the common subtype among CRC patients (54.5%). Interestingly, an association risk between *Blastocystis* subtype 1 with a greater risk of association in CRC group	The study revealed a probable association between subtype 1 of *Blastocystis* and CRC
4	Toychiev *et al* [20], 2018	Uzbekistan	A total sample of 400 participants, two groups of participants: (1) 200 CRC patients; and (2) 200 of Tashkent residents (without any gastrointestinal tract complaints). Exclusion criteria: (1) patient had problems with stool sample collection; and (2) received any treatment 2–3 wk before the study. Study duration: 2015-2017	Research Institute of Epidemiology, Microbiology and Infectious Diseases and the Research Center of Oncology, Tashkent, Uzbekistan, during the period	Study design: Prospective cohort: Prevalence of some parasites including *Blastocystis sp*. in CRC patients before and after surgery and chemotherapy compared to control group. Methods: "3 stool samples for parasitological examination were taken at 2-d intervals during CRC diagnosis before and after surgery and chemotherapy"	A significantly higher prevalence of protozoa was found in CRC patients than in control population "the prevalence of *Blastocystis* in CRC patients is 4 times as high as in the control population. The overall prevalence of *Blastocystis sp*. was 2.8% and was higher than the other protozoa"	Data revealed a potential role for *Blastocystis sp*. in CRC pathogenesis
5	Kumarasamy *et al*[21], 2014	Malaysia	Sample size: 425 patients who go through diagnostic colonoscopy. faecal samples and colonic washouts were obtained from 221 control patients and 204 patients	University of Malaya Medical Centre	To determine the *Blastocystis* genotype present by comparing the prevalence using colonic washouts and faecal samples PCR and standard stool culture. Both techniques were used to detect *Blastocystis* from control and	The prevalence of *Blastocystis* was 15.29% (65/425). "Colonic washouts and faecal samples showed 12.24% (n = 52) and 5.65% (n = 24) of *Blastocystis* infection respectively". A total of 43 individuals were positive for *Blastocystis* in CRC patients and	*Blastocystis sp*. is common in CRC patients. Subtype 3 is the most common genotype in the infected individuals

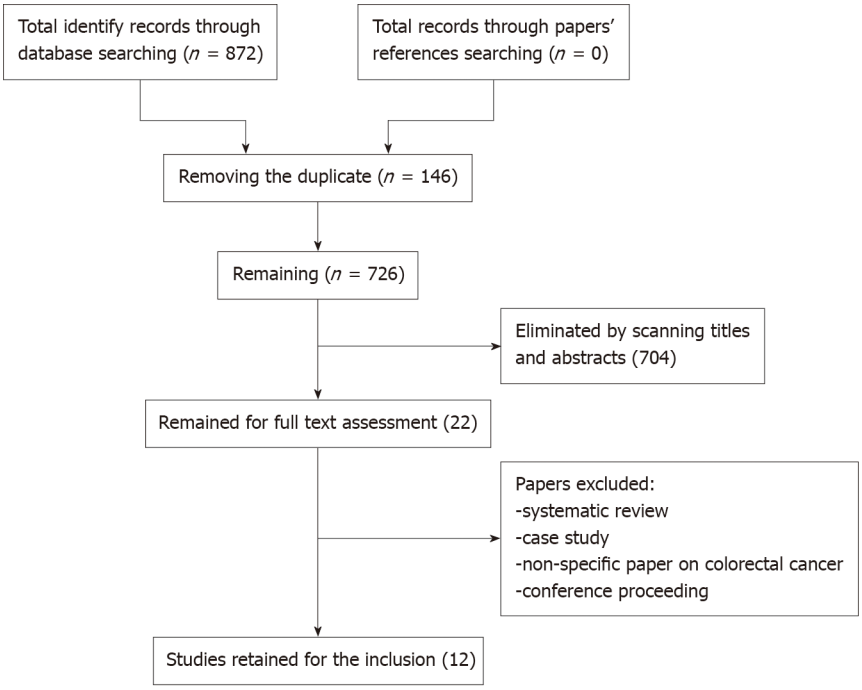
			with CRC. Study duration: 2010 and 2012		patients with CRC.	was significantly higher compared to control group. Subtype 3 was predominant compared to other subtypes. It was significantly higher in CRC group as compared with control group	
6	Chandramathi *et al*[1], 2012	Malaysia	Stool samples were obtained from 46 and 15 breast cancer and CRC patients, respectively	Department of Parasitology, Faculty of Medicine, University of Malaya	Aim: To investigate whether intestinal parasites can be an opportunistic infection in breast cancer and CRC patients who are undergoing chemotherapy treatment. Molecular detection of microsporidia species was done using a PCR technique. The presence of *Blastocystis hominis* was further confirmed by culturing stool samples	This study found that 7 out of 15 CRC patients were positive *Blastocystis* in various chemotherapy cycles accounting for 46.7%	*Blastocystis hominis* and microsporidia could appear as opportunistic infections during chemotherapy treatment of CP. This infection may diminish the efficacy of chemotherapy treatments and consequently advance the progression of cancer
7	Majeed *et al* [22], 2019	Iraq	116 faecal specimens with *Blastocystis* and *Helicobacter pylori* infection, 15 biopsy specimens from CRC patients	Middle Technical University/Baghdad 1st Feb 2018-15th June 2018	Faecal specimens were screened for *Blastocystis* and *Helicobacter pylori*. Direct DNA sequencing was done to evaluate mutations in CRC-associated molecular pathways	Prevalence of *Blastocystis* infection statistically insignificant in various age groups. Prevalence of *Blastocystis* infection was more in females [females 29 (46.9 %), males 22(43.1%)]. Prevalence of mixed infection (*Blastocystis* and *Helicobacter pylori*) was 27 (23.32%)	Prevalence of *Blastocystis* infection was more in females. *KRAS* and *TP53* gene mutation was observed in the CRC patients with mixed infection (*Blastocystis* and *H. pylori*)
In vitro studies							
8	Chandramathi *et al*[7], 2010	Malaysia	*In vitro* study model. PBMCs were isolated from blood collected from healthy persons. Solubilised antigen of *Blastocystis* isolate was obtained from a human subject. Human colorectal carcinoma cell line, HCT116, was used	University of Malaya, Kuala Lumpur	Effect solubilised antigen of *Blastocystis* on the HCT116 proliferation quantified. Gene expressions of certain genes in HCT116 and PBMCs evaluated *via* real-time reverse transcription PCR. PBMCs were isolated from blood using Histopaque technique. Cell proliferations were measured using MTT assay	Increased number of PBMCs/ HCT116 cells observed with *Blastocystis* antigen. *IFN-γ* and *TNF-α* were downregulated and *IL-6*, *IL-8* and *NF-κB*, *p53* were upregulated in the PBMCs treated with the antigen. *IFN-γ* was downregulated and *IL-6* and *NF-κB* was upregulated in HCT116 cells	Solubilised antigen of *Blastocystis* could facilitate increased number of PBMCs/ HCT116 cell and has the ability to downregulate immune cell responses
9	Chan *et al*[23], 2012	Malaysia	*In vitro* study model. Solubilised antigen of *Blastocystis* isolate was obtained from symptomatic and asymptomatic human subject. HCT116 was used	University of Malaya, Kuala Lumpur	Effects of solubilised antigen of *Blastocystis* isolate was obtained from symptomatic and asymptomatic human subject on HCT116. Gene expressions of certain genes in HCT116 and PBMCs evaluated *via* real-time reverse transcription PCR	Increased number of HCT116 cells observed with symptomatic *Blastocystis* antigen. Th2 cytokines/ *CTSB* were upregulated in HCT116. *NF-κB* was observed upregulated in HCT116 exposed to symptomatic *Blastocystis* antigen	Solubilized antigen *of Blastocystis* from symptomatic individual was more virulent than that in asymptomatic. Higher inflammatory reaction and increased proliferation of cancer cells was observed
10	Kumarasamy *et al*[24], 2013	Malaysia,	*In vitro* study model using HCT116 treated with solubilised *Blastocystis* antigen from 5 *Blastocystis* subtypes	University Malaya research Lab	*In vitro* study. HCT116 treated with solubilised antigen from *Blastocystis*. Following Assays: Proliferation of the cell line, HCT116 on exposure to different *Blastocystis* subtypes; Gene expression profile of apoptotic genes like *p53* and *CTSB*; Transcription factor gene expression profile	*Blastocystis* subtypes (5) increased the proliferation of HCT116, especially subtype 3. *Blastocystis* antigen caused the upregulation of Th2 and Th1 cytokines, and downregulation of *IFN-γ* and *p53* in HCT116 cells. *Blastocystis* antigen caused a higher stimulation of gene expression of *CTSB* and *TGF-β* genes	Infection with *Blastocystis* caused exacerbation of existing colon cancer cells. The effect may be due to weakening of the cellular immune response and dysregulation of *IFN-γ* and *p53* expression. Infection with *Blastocystis* subtype 3 has a higher pathogenic potential

11	Ahmed *et al* [25], 2019	Cairo, Egypt	Seven *Blastocystis* isolates were from stools specimen from patients with early diagnosed CRC (Oncology and Surgery and Colonoscopy unit) of a Hospital in Egypt. The different groups were: Group I (GI), 12 isolates from infected non-CRC; Group II (GII), 6 from infected symptomatic patients and Group III (GIII), 6 from infected non-symptomatic carriers	Department of Parasitology lab, Faculty of Medicine, Ain Shams University, Cairo, Egypt	Aim: To investigate some phenotypic characters like the surface ultrastructure, protein profiles and protease activity of *Blastocystis* from three different clinical groups. Techniques performed: Scanning electron microscopy to study morphology of the organism; SDS-PAGE to analyse the *Blastocystis* protein profiles and their protease activities	Observations: All CRC *Blastocystis* isolates showed a very rough intensely folded surface when compared to less rough and smooth surface of isolates from symptomatic and asymptomatic and non-CRC isolates; SDS-PAGE showed presence of 2 protein bands of 230 and 32 KDa in 42.9% of *Blastocystis* CRC isolates and these proteins were absent in Non-CRC isolates. When the protease activity of the parasite was tested, no significant difference existed between isolates of the three groups	There was significant difference in the surface structure and the protein profiles between different clinical isolates of *Blastocystis*. Differences indicate that it may be: (1) secondary to the altered gut environment in the presence of CRC or (2) indicators of a different pathogenic potential of the parasite in inducing malignancy
In Vivo studies							
12	Kumarasamy *et al*[26], 2017	Malaysia	Different specimens collected: Blood, urine, faecal samples and gastrointestinal tract sections from 24 male Wistar rats. Age of the rats: 3 wk. Weight of each rat: Average of 65 g/rat	University Malaya research Lab	*In vivo* experimental study. Aim: To investigate the effect of infection with *Blastocystis* cyst on exacerbation of carcinogenesis. Twenty-four rats divided into different groups for the study (4 groups, 6 rats each): Control group, AOM group, group inoculated with *Blastocystis* cyst, the group inoculated with *Blastocystis* cyst and AOM injection. Body weights recorded once a week. Rat faecal samples screened for presence of *Blastocystis* post-inoculation. Histopathological assessment of the rat colon for aberrant crypts. Urine and blood samples assessed for oxidative stress	Observations: lower body weight showed by *Blastocystis* infected rats than rats infected with *Blastocystis* and injected with AOM ($P < 0.05$). Stools from AOM-rats with *Blastocystis* infection were softer and watery compared to the AOM-rats without *Blastocystis* infection. *Blastocystis* was present in the stool of all infected rats from Day 3 to 7 post-inoculation. All the rats injected with AOM developed numerous abnormal, hyperplastic colonic crypts. Co-administration of *Blastocystis* cyst showed a 1.6-fold increase in the number of crypts when compared with control rats treated with AOM only. Two of the co-*Blastocystis* infected AOM-rats were found to have adenomas. Major dysplasia and presence of hyperplastic aberrant crypts were observed in rats injected with AOM and co-infected with *Blastocystis*	*Blastocystis* infection considerably enhanced the AOM-induced carcinogenesis because of the oxidative damage of the intestinal epithelium

AOM: Azoxymethane; CP: Cancer patients; CRC: Colorectal cancer; CTSB: Cathepsin B; IFN: Interferon; IL: Interleukin; NCP: Non cancer patients; NF-kB: Nuclear factor kappa B; PBMCs: Peripheral blood mononuclear cells; PCR: Polymerase chain reaction; SDS-PAGE: Sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

predominant (54.5%) among CRC patients, while subtype II was predominant (43.7%) among COGT patients[19]. Higher prevalence of intestinal helminths and protozoa was observed in CRC patients than in the control population in a study conducted in Uzbekistan. The prevalence of *Blastocystis* in CRC patients was four times higher than that in the control population. The overall prevalence of *Blastocystis* (2.8%) was significantly higher than the other protozoa[20]. In a study by Kumarasamy *et al*[21] in Malaysia, among 221 control patients and 204 CRC patients with colorectal malignancies, the overall prevalence of *Blastocystis* infection was 15.29% (65/425). A total of 43 (21.08%) samples were positive for *Blastocystis* infection in CRC patients and was significantly higher compared to normal individuals (n = 22, 9.95%, $P < 0.01$). Subtype 3 was present at higher levels compared to other subtypes detected in both groups and was significantly higher in CRC patients as compared with control patients[21].

Another study was designed to investigate the emergence of *Blastocystis* and Microsporidia infections in breast and CRC patients undergoing chemotherapy treatment. This study found that 7 out of 15 CRC



DOI: 10.4251/wjgo.v14.i3.734

Figure 1 Search strategy.

patients were positive *Blastocystis* in various chemotherapy cycles, accounting for 46.7%. However, the researchers did not mention whether the isolate was from the same patients in different cycles[1]. In a study carried out in Iraq, stool samples from 116 patients with *Blastocystis* and *H. pylori* infections were investigated. Fifteen tissue samples of CRC were taken from 15 suspected patients out of 116 infected cases, and it was shown that the infection with *Blastocystis* and *H. pylori* was associated with pathological gene mutation in the CRC patients[22].

In vitro colorectal carcinoma cell line studies on the cytopathic and immunological effects of Blastocystis antigen

Three of the reviewed articles used *in vitro* study models and observed considerable cytopathic and immunological effects induced by the solubilised antigen of *Blastocystis* to the human colorectal carcinoma cell line[7,23,24]. These findings speculated that *Blastocystis* infection may enhance the proliferation, invasiveness and metastatic properties of CRC cells. One study investigated some phenotypic characteristics of *Blastocystis* isolated from CRC patients[25].

The three *in vitro* model studies used the human colorectal carcinoma cell line HCT116 and the *Blastocystis* isolated from a human subject[7,23,24]. One of the studies demonstrated the cytopathic effect of *Blastocystis* antigen on peripheral blood mononuclear cells. This study findings showed increased cell proliferations in *Blastocystis* antigen-stimulated HCT116 cell-lines, which suggested that the infection by *Blastocystis* may facilitate the growth of colon cancer cells[7]. Another *in vitro* study showed that the five subtypes of *Blastocystis* significantly increased the proliferation of HCT116, especially subtype 3. *Blastocystis* antigen caused the upregulation of T helper (Th)2 and Th1 cytokine gene expressions, and downregulation of interferon gamma and *p53* gene expressions in HCT116 cells. In addition, *Blastocystis* antigen caused a significantly higher stimulation of Cathepsin B (CTSB) and Transforming growth factor beta (TGF-β) gene expression, which indicates the pathogenic potential of this protozoan[24]. Another study showed an increase in cell proliferation in HCT116 cells inoculated with the symptomatic *Blastocystis* antigen. Gene expression studies carried out in this research also showed a significant upregulation of Th2 cytokines, which indicates the parasites' potential in weakening the cellular immune response[23]. HCT116 cells exposed to symptomatic and asymptomatic *Blastocystis* antigen caused a significant upregulation of *CTSB*, which led to the postulation that the *Blastocystis* antigen may enhance the invasive and metastasis properties of CRC[23].

Another study sought to investigate some phenotypic characteristics such as the surface ultrastructure, protein profiles and protease activity of *Blastocystis* isolated from three different clinical groups: CRC patients, non-CRC symptomatic and asymptomatic infected persons. This study showed the presence of two protein bands of 230 and 32 KDa in 42.9% of *Blastocystis* CRC isolates with their complete absence from non-CRC isolates. There was no significant difference in the protease activity of the protein among isolates of the three groups, CRC and Non-CRC *Blastocystis* isolates[25].

In vivo experimental animal model to investigate the effects of infection with Blastocystis on exacerbation of colorectal carcinogenesis

An animal model study compared the effects of *Blastocystis* infected rats and rats infected with *Blastocystis* co-administered with Azoxymethane (AOM), a potent carcinogen. This finding showed that the co-administration of *Blastocystis* cyst resulted in a 1.6-fold increase in the number of colonic crypts when compared with control rats treated with AOM only. Two of the co-*Blastocystis* infected AOM rats were found to have adenomas. Major dysplasia and the presence of hyperplastic aberrant crypts were also observed in rats injected with AOM and co-infected with *Blastocystis*[26].

DISCUSSION

Blastocystis is one of the most common gut microorganisms found in healthy individuals[27]. Besides being associated with a healthy gut microbiota[27,28], *Blastocystis* infection is also known to be opportunistic in immunocompromised patients[29]. CRC is the third most common cancer diagnosed worldwide and one of the major causes of cancer-associated fatality[30]. The reason for high mortality is due to the asymptomatic progression of the disease that usually results in late diagnosis[31,32]. Certain gut microorganisms are known to be one of the important factors that had been associated with initiation and development of CRC[33]. However, data on the roles of parasites are vague and restricted. Various findings have been reported regarding the association between *Blastocystis* among CRC patients, whereby positive association was shown in all the studies[1,23,24]. Therefore, the aim of this systematic review was to quantify the studies published so far that revealed the direct association of *Blastocystis* and CRC. This paper outlines the results of a systematic review to evaluate the prevalence of *Blastocystis* in CRC patients, *in vitro* studies using *Blastocystis* antigen and an *in vivo* study using animal models.

Out of the data extracted from 12 studies relevant to this topic, all the studies showed positive association between *Blastocystis* and CRC. Prevalence studies, *in vitro* investigations and *in vivo* studies were used to evaluate the pathogenicity of *Blastocystis* with CRC.

The global prevalence of *Blastocystis* infection ranged from 1.5%-20% in developed countries, which was much less than that in developing countries, which was 30%-50%[34]. Based on our review, the prevalence of *Blastocystis* infection in CRC patients ranged between 2.8%-46.7%. It has been widely reported in the world, in developing countries such as Iran, China, Saudi Arabia, Uzbekistan and Malaysia[18-20,24,35]. The first demonstration of *Blastocystis* infection in Iran was reported in 11 CRC patients in Tehran province[35]. In Malaysia, a total of 43 samples were positive for *Blastocystis* from 204 CRC patients[21]. This study utilised colonic washout in addition to stool sample to recover the parasites. Subtype 3 *Blastocystis* was detected predominantly as compared to other subtypes[21]. Some of these findings highlighted the high prevalence of certain subtypes of *Blastocystis* among these patients [19,21]. A previous study showed that subtype 1 was the most common genotype identified (54.5%) among CRC patients[19]. DNA was extracted from *Blastocystis* cultures *via* conventional method and subtyped using multiplex polymerase chain reaction (PCR) with restriction fragment length polymorphism and sequence-tagged site primers-based PCR. In another study, subtype 3 was predominant compared to other subtypes found in both CRC patients and healthy individuals[21]. Subtype 3 is speculated as the most pathogenic subtype in symptomatic individuals[36]. Some researchers have attributed subtype 3 to be more pathogenic compared to other subtypes[37,38]. *Blastocystis* was initially screened *via in vitro* culture and conventional PCR using stool samples and colonic washouts. The presence of *Blastocystis* infection in CRC patients could be contributed by various reasons including health status. For instance, positive cases were more likely in patients with gastrointestinal symptoms compared to healthy individuals[18]. Besides, *Blastocystis* was also identified in higher frequency in immunosuppressed CRC patients who were undergoing chemotherapy treatment[1,18].

A total of three *in vitro* studies were carried out using colorectal carcinoma cell line models to study cytopathic and immunological effects of *Blastocystis* antigen[7,23,24]. Some of the research studies suggested that solubilised antigen of *Blastocystis* could facilitate the exacerbation of CRC cells, HCT116 [7,23]. In another study by Kumarasamy *et al*[24], *Blastocystis* subtype 3 stimulated significantly higher *CTSB* and *TGF-β* gene expression in HCT116, which indicates the pathogenic potential of this protozoan. Result of *in vitro* studies that were performed in Malaysia were similar[7,23,24].

Blastocystis is commonly found in both patients with gastrointestinal symptoms and in healthy people widely across the world. More recently, researchers consider *Blastocystis* as an emerging zoonotic disease, and its pathogenic potential in human is unclear[9]. The pathogenic potential of *Blastocystis* was widely debated, as they are found in both symptomatic and asymptomatic patients. The significant expression of nuclear factor kappa light chain enhancer of activated B cells was observed in HCT116 exposed to *Blastocystis* antigen isolated from individuals with gastrointestinal symptoms, but such observations were not found when the colon cells treated with *Blastocystis* antigen isolated from asymptomatic individuals. This finding shows the potential pathogenicity of symptomatic *Blastocystis* in CRC patients[23]. Similarly, HCT116 cells exposed to symptomatic and asymptomatic *Blastocystis*

antigen caused a significant upregulation of *CTSB*. These gene expression findings lead to a postulation that the *Blastocystis* antigen may enhance the invasive and metastasis properties of CRC[23]. Besides, proliferation of HCT116 when exposed with *Blastocystis* antigen could be a result of higher levels of interleukin (IL)-6 and IL-8 expression[23].

Another study revealed that solubilised antigen isolated from subtype 2 and 3 isolates introduced to colon cancer cells showed significant IL-8 and IL-6 expression[24]. The production of inflammatory cytokines such as IL-8 together with reactive oxygen species could contribute to the pathogenesis of cancer[38]. In a few studies conducted previously, IL-6 expression was associated with proliferation of colon carcinoma[39,40]. Besides that, subtype 3 *Blastocystis* also triggered positive expression of *CTSB* in cancer cells. A previous study showed that *CTSB* expression is significant in CRC patients[41].

Only one study was conducted to investigate the *in vivo* effect of *Blastocystis* in Wistar rats. In parallel with *in vitro* studies, an *in vivo* study showed similar findings on the possible role of *Blastocystis* to exacerbate CRC. The results demonstrated that *Blastocystis* may cause damage to the intestinal mucosal layer and result in increased crypts formation. Furthermore, an increased oxidative stress was also observed in these rats. There have been numerous animal models of human CRC and animal model of tumour carried out *via* quantification of aberrant crypt foci[42,43]. This allows the study of gut microbiome and its role in pathogenesis. In humans, there are many potential pathogenic and non-pathogenic gut microbial infections, and various animal models have been used for such studies. Aberrant crypt foci are known as putative precancerous lesions of the colon in both animal models and humans[44,45]. Even though various studies associating *Blastocystis* and CRC were carried out *via in vitro* model using colon cancer cells, this study utilised animal model to bridge between *in vitro* findings in the laboratory and studies in humans. As such, this extensive *in vivo* study showed that *Blastocystis* had a major impact on normal intestinal function in Wistar rats resulting in damage to the intestinal mucosal layer and inducing oxidative stress, which caused increase in crypts formation in AOM-treated rat models. The study establishes that *Blastocystis* is a pathogen, and there is a need to screen cancer patients for harbouring this parasite.

This systematic review has some limitations. According to these investigations, a greater prevalence of *Blastocystis* was found in CRC patients, but the question whether increased prevalence of *Blastocystis* could be linked with increased high risk to CRC is unclear. The studies discussed in this review did not highlight the association of *Blastocystis* according to cancer stages, and it was unclear if *Blastocystis* itself could result in the initiation of malignancy as *Blastocystis* acquisition alone is insufficient for cancer development. Even though strong association between *Blastocystis* and CRC is apparent, some questions remain unanswered. Therefore, we propose future studies should focus on the pathogenicity of *Blastocystis* in various stages of CRC by concentrating on the molecular pathways involved in tumorigenesis.

CONCLUSION

In conclusion, according to various recent studies, *Blastocystis* is one of the most commonly identified microorganisms in CRC patients, whereby subtype 1 and subtype 3 were predominantly isolated. It is apparent in most cases that the prevalence is higher in developing countries compared to developed countries. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage. An *in vivo* study well-established that *Blastocystis* infections resulted in tissue damage from host inflammatory responses that may predispose the host towards neoplasm exacerbation. Upregulation of gene expression responsible for proinflammatory cytokines and downregulation of apoptotic genes was observed in *in vitro* studies. Through continued research in *Blastocystis* and CRC, we may discover new findings as well as develop new effective means of prevention. Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its association with other intestinal bacteria. In addition, future *in vivo* studies should evaluate exposure to various subtypes of *Blastocystis*.

ARTICLE HIGHLIGHTS

Research background

Intestinal colonisation of *Blastocystis hominis* (*Blastocystis*) as a risk factor to the worsening of colorectal cancer (CRC).

Research motivation

There has been an increase in the prevalence of *Blastocystis* in CRC patients. Besides, various *in vitro* and *in vivo* studies have highlighted *Blastocystis* as an important risk factor for the worsening of CRC.

Research objectives
To perform a systematic review on all evidence on the association between CRC and *Blastocystis*.

Research methods
A systematic review of the literature was performed by searching PubMed, Science Direct, Scopus and Google scholar databases up to February 2020.

Research results
Out of 12 studies selected for this systematic review, seven studies have confirmed the prevalence of *Blastocystis*. A total of four studies employing *in vitro* human colorectal carcinoma cell line study models showed significant cytopathic and immunological effects of *Blastocystis*. One *in vivo* experimental animal model study showed that there was a significant effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

Research conclusions
Blastocystis is a commonly identified microorganisms in CRC patients. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage.

Research perspectives
Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its association with other intestinal diseases.

FOOTNOTES

Author contributions: Azzani M designed the research, performed the literature search and extracted the data; Kumarasamy V wrote the discussion and extracted the data; Atroosh WM wrote the methodology and extracted the data; Anbazhagan D wrote the results and extracted the data; Abdalla M wrote the introduction and extracted the data; All authors read and approved the final manuscript.

Conflict-of-interest statement: Dr. Azzani has nothing to disclose.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country/Territory of origin: Malaysia

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S-Editor: Chang KL
L-Editor: Filipodia
P-Editor: Chang KL

REFERENCES

1 **Chandramathi S**, Suresh K, Anita ZB, Kuppusamy UR. Infections of Blastocystis hominis and microsporidia in cancer patients: are they opportunistic? *Trans R Soc Trop Med Hyg* 2012; **106**: 267-269 [PMID: 22340948 DOI: 10.1016/j.trstmh.2011.12.008]
2 **Aksoy U**, Akisü C, Bayram-Delibaş S, Ozkoç S, Sahin S, Usluca S. Demographic status and prevalence of intestinal parasitic infections in schoolchildren in Izmir, Turkey. *Turk J Pediatr* 2007; **49**: 278-282 [PMID: 17990581]
3 **Brumpt E**. *Blastocystis hominis* n. sp. et formes voisines. *Bull Soc Pathol* 1912; **5**: 725-730
4 **Low GC**. Two Chronic Amoebic Dysentery Carriers treated by Emetine, with some Remarks on the Treatment of Lamblia, Blastocystis and E. *coli* Infections. *J Trop Med Hyg* 1916; **19**: 29-34
5 **Roberts T**, Ellis J, Harkness J, Marriott D, Stark D. Treatment failure in patients with chronic Blastocystis infection. *J Med Microbiol* 2014; **63**: 252-257 [PMID: 24243286 DOI: 10.1099/jmm.0.065508-0]

6 **Borralho PM**, Kren BT, Castro RE, da Silva IB, Steer CJ, Rodrigues CM. MicroRNA-143 reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human colorectal cancer cells. *FEBS J* 2009; **276**: 6689-6700 [PMID: 19843160 DOI: 10.1111/j.1742-4658.2009.07383.x]

7 **Chandramathi S**, Suresh K, Kuppusamy UR. Solubilized antigen of Blastocystis hominis facilitates the growth of human colorectal cancer cells, HCT116. *Parasitol Res* 2010; **106**: 941-945 [PMID: 20165878 DOI: 10.1007/s00436-010-1764-7]

8 **Cirioni O**, Giacometti A, Drenaggi D, Ancarani F, Scalise G. Prevalence and clinical relevance of Blastocystis hominis in diverse patient cohorts. *Eur J Epidemiol* 1999; **15**: 389-393 [PMID: 10414382 DOI: 10.1023/a:1007551218671]

9 **Basak S**, Rajurkar MN, Mallick SK. Detection of Blastocystis hominis: a controversial human pathogen. *Parasitol Res* 2014; **113**: 261-265 [PMID: 24169810 DOI: 10.1007/s00436-013-3652-4]

10 **Bouvard V**, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V; WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part B: biological agents. *Lancet Oncol* 2009; **10**: 321-322 [PMID: 19350698 DOI: 10.1016/s1470-2045(09)70096-8]

11 **zur Hausen H**. Streptococcus bovis: causal or incidental involvement in cancer of the colon? *Int J Cancer* 2006; **119**: xi-xii [PMID: 16947772 DOI: 10.1002/ijc.22314]

12 **Lax AJ**. Opinion: Bacterial toxins and cancer--a case to answer? *Nat Rev Microbiol* 2005; **3**: 343-349 [PMID: 15806096 DOI: 10.1038/nrmicro1130]

13 **El-Gayar EK**, Mahmoud MM. Do protozoa play a role in carcinogenesis? *Parasitol United J* 2014; **7**: 80 [DOI: 10.4103/1687-7942.149553]

14 **Padukone S**, Mandal J, Parija SC. Severe *Blastocystis* subtype 3 infection in a patient with colorectal cancer. *Trop Parasitol* 2017; **7**: 122-124 [PMID: 29114493 DOI: 10.4103/tp.TP_87_15]

15 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

16 **(JBI) TJBI**. Critical Appraisal Tools. [cited 1 January 2021]. Available from: http://joannabriggs.org/research/critical-appraisal-tools.html

17 **Hooijmans CR**, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; **14**: 43 [PMID: 24667063 DOI: 10.1186/1471-2288-14-43]

18 **Zhang W**, Ren G, Zhao W, Yang Z, Shen Y, Sun Y, Liu A, Cao J. Genotyping of *Enterocytozoon bieneusi* and Subtyping of *Blastocystis* in Cancer Patients: Relationship to Diarrhea and Assessment of Zoonotic Transmission. *Front Microbiol* 2017; **8**: 1835 [PMID: 28983297 DOI: 10.3389/fmicb.2017.01835]

19 **Mohamed AM**, Ahmed MA, Ahmed SA, Al-Semany SA, Alghamdi SS, Zaglool DA. Predominance and association risk of *Blastocystis hominis* subtype I in colorectal cancer: a case control study. *Infect Agent Cancer* 2017; **12**: 21 [PMID: 28413436 DOI: 10.1186/s13027-017-0131-z]

20 **Toychiev A**, Abdujapparov S, Imamov A, Navruzov B, Davis N, Badalova N, Osipova S. Intestinal helminths and protozoan infections in patients with colorectal cancer: prevalence and possible association with cancer pathogenesis. *Parasitol Res* 2018; **117**: 3715-3723 [PMID: 30220046 DOI: 10.1007/s00436-018-6070-9]

21 **Kumarasamy V**, Roslani AC, Rani KU, Kumar Govind S. Advantage of using colonic washouts for Blastocystis detection in colorectal cancer patients. *Parasit Vectors* 2014; **7**: 162 [PMID: 24708637 DOI: 10.1186/1756-3305-7-162]

22 **Majeed GH**, Mohammed NS, Muhsen SS. The synergistic effect of Blastocystis hominis and H. pylori in Iraqi colorectal cancer patients. *J. Pharm. Sci. & Res* 2019; **11**: 523-526

23 **Chan KH**, Chandramathi S, Suresh K, Chua KH, Kuppusamy UR. Effects of symptomatic and asymptomatic isolates of Blastocystis hominis on colorectal cancer cell line, HCT116. *Parasitol Res* 2012; **110**: 2475-2480 [PMID: 22278727 DOI: 10.1007/s00436-011-2788-3]

24 **Kumarasamy V**, Kuppusamy UR, Samudi C, Kumar S. Blastocystis sp. subtype 3 triggers higher proliferation of human colorectal cancer cells, HCT116. *Parasitol Res* 2013; **112**: 3551-3555 [PMID: 23933809 DOI: 10.1007/s00436-013-3538-5]

25 **Ahmed MM**, Habib FSM, Saad GA, El Naggar HM. Surface ultrastructure, protein profile and zymography of *Blastocystis* species isolated from patients with colorectal carcinoma. *J Parasit Dis* 2019; **43**: 294-303 [PMID: 31263336 DOI: 10.1007/s12639-019-01092-9]

26 **Kumarasamy V**, Kuppusamy UR, Jayalakshmi P, Samudi C, Ragavan ND, Kumar S. Exacerbation of colon carcinogenesis by Blastocystis sp. *PLoS One* 2017; **12**: e0183097 [PMID: 28859095 DOI: 10.1371/journal.pone.0183097]

27 **Scanlan PD**, Stensvold CR, Rajilić-Stojanović M, Heilig HG, De Vos WM, O'Toole PW, Cotter PD. The microbial eukaryote Blastocystis is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiol Ecol* 2014; **90**: 326-330 [PMID: 25077936 DOI: 10.1111/1574-6941.12396]

28 **Audebert C**, Even G, Cian A; Blastocystis Investigation Group, Loywick A, Merlin S, Viscogliosi E, Chabé M. Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota. *Sci Rep* 2016; **6**: 25255 [PMID: 27147260 DOI: 10.1038/srep25255]

29 **Bednarska M**, Jankowska I, Pawelas A, Piwczyńska K, Bajer A, Wolska-Kuśnierz B, Wielopolska M, Welc-Falęciak R. Prevalence of Cryptosporidium, Blastocystis, and other opportunistic infections in patients with primary and acquired immunodeficiency. *Parasitol Res* 2018; **117**: 2869-2879 [PMID: 29946765 DOI: 10.1007/s00436-018-5976-6]

30 **Favoriti P**, Carbone G, Greco M, Pirozzi F, Pirozzi RE, Corcione F. Worldwide burden of colorectal cancer: a review. *Updates Surg* 2016; **68**: 7-11 [PMID: 27067591 DOI: 10.1007/s13304-016-0359-y]

31 **O'Connell JB**, Maggard MA, Liu JH, Etzioni DA, Ko CY. Are survival rates different for young and older patients with rectal cancer? *Dis Colon Rectum* 2004; **47**: 2064-2069 [PMID: 15657655 DOI: 10.1007/s10350-004-0738-1]

32 **Siegel RL**, Ward EM, Jemal A. Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992-2008. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 411-416 [PMID: 22219318 DOI: 10.1158/1055-9965.EPI-11-1020]

33 **Zhu Q**, Gao R, Wu W, Qin H. The role of gut microbiota in the pathogenesis of colorectal cancer. *Tumour Biol* 2013; **34**: 1285-1300 [PMID: 23397545 DOI: 10.1007/s13277-013-0684-4]

34 **Su FH**, Chu FY, Li CY, Tang HF, Lin YS, Peng YJ, Su YM, Lee SD. Blastocystis hominis infection in long-term care facilities in Taiwan: prevalence and associated clinical factors. *Parasitol Res* 2009; **105**: 1007-1013 [PMID: 19488784 DOI: 10.1007/s00436-009-1509-7]

35 **Esteghamati A**, Khanaliha K, Bokharaei-Salim F, Sayyahfar S, Ghaderipour M. Prevalence of Intestinal Parasitic Infection in Cancer, Organ Transplant and Primary Immunodeficiency Patients in Tehran, Iran. *Asian Pac J Cancer Prev* 2019; **20**: 495-501 [PMID: 30803212 DOI: 10.31557/APJCP.2019.20.2.495]

36 **Souppart L**, Sanciu G, Cian A, Wawrzyniak I, Delbac F, Capron M, Dei-Cas E, Boorom K, Delhaes L, Viscogliosi E. Molecular epidemiology of human Blastocystis isolates in France. *Parasitol Res* 2009; **105**: 413-421 [PMID: 19290540 DOI: 10.1007/s00436-009-1398-9]

37 **Ragavan ND**, Govind SK, Chye TT, Mahadeva S. Phenotypic variation in Blastocystis sp. ST3. *Parasit Vectors* 2014; **7**: 404 [PMID: 25174569 DOI: 10.1186/1756-3305-7-404]

38 **Rajamanikam A**, Hooi HS, Kudva M, Samudi C, Kumar S. Resistance towards metronidazole in Blastocystis sp.: A pathogenic consequence. *PLoS One* 2019; **14**: e0212542 [PMID: 30794628 DOI: 10.1371/journal.pone.0212542]

39 **Chung YC**, Chang YF. Serum interleukin-6 Levels reflect the disease status of colorectal cancer. *J Surg Oncol* 2003; **83**: 222-226 [PMID: 12884234 DOI: 10.1002/jso.10269]

40 **Galizia G**, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, De Vita F. Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. *Clin Immunol* 2002; **102**: 169-178 [PMID: 11846459 DOI: 10.1006/clim.2001.5163]

41 **Herszényi L**, Farinati F, Cardin R, István G, Molnár LD, Hritz I, De Paoli M, Plebani M, Tulassay Z. Tumor marker utility and prognostic relevance of cathepsin B, cathepsin L, urokinase-type plasminogen activator, plasminogen activator inhibitor type-1, CEA and CA 19-9 in colorectal cancer. *BMC Cancer* 2008; **8**: 194 [PMID: 18616803 DOI: 10.1186/1471-2407-8-194]

42 **Kim J**, Ng J, Arozulllah A, Ewing R, Llor X, Carroll RE, Benya RV. Aberrant crypt focus size predicts distal polyp histopathology. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1155-1162 [PMID: 18483337 DOI: 10.1158/1055-9965.EPI-07-2731]

43 **Reddy BS**. Studies with the azoxymethane-rat preclinical model for assessing colon tumor development and chemoprevention. *Environ Mol Mutagen* 2004; **44**: 26-35 [PMID: 15199544 DOI: 10.1002/em.20026]

44 **Bird RP**. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett* 1987; **37**: 147-151 [PMID: 3677050 DOI: 10.1016/0304-3835(87)90157-1]

45 **Roncucci L**, Stamp D, Medline A, Cullen JB, Bruce WR. Identification and quantification of aberrant crypt foci and microadenomas in the human colon. *Hum Pathol* 1991; **22**: 287-294 [PMID: 1706308 DOI: 10.1016/0046-8177(91)90163-j]

World Journal of Gastrointestinal Oncology

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World J Gastrointest Oncol 2022 March 15; 14(3): 746-747

DOI: 10.4251/wjgo.v14.i3.746

ISSN 1948-5204 (online)

LETTER TO THE EDITOR

Re: Association between intestinal neoplasms and celiac disease - beyond celiac disease and more

Kenji Okumura

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Makovicky P, Rostami-Nejad M

Received: September 26, 2021
Peer-review started: September 26, 2021
First decision: December 4, 2021
Revised: December 17, 2021
Accepted: February 23, 2022
Article in press: February 23, 2022
Published online: March 15, 2022

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Abstract

The association between celiac disease and enteropathy-associated T cell lymphoma has been known. The pathogenesis of the development of malignant neoplasms remains limited. In addition to celiac disease, we believe that other underlying mechanisms contribute to the developing malignant neoplasms.

Key Words: Celiac disease; Entropathy-associated T cell lymphoma; c-MYC; JAK-STAT

Core Tip: The pathogenesis of enteropathy-associated T cell lymphoma (EATL) remains limited. This letter suggests oncogene mutations were reported and would be pertinent to develop malignant neoplasms in EATL.

Citation: Okumura K. Re: Association between intestinal neoplasms and celiac disease - beyond celiac disease and more. *World J Gastrointest Oncol* 2022; 14(3): 746-747
URL: https://www.wjgnet.com/1948-5204/full/v14/i3/746.htm
DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.746

TO THE EDITOR

I read with great interest the paper by Wang *et al*[1] in the issue 13 of World Journal of Gastrointestinal Oncology, a review article regarding the association between intestinal neoplasms and celiac disease. The authors showed that the total risk of small bowel cancer (SBC) and enteropathy-associated T cell lymphoma (EATL) increased in celiac disease (CD) patients. I have agreed with the authors opinions and they mainly mentioned EATL type I, which is associated with CD. The pathogenesis of EATL

remains limited, however, as the authors mentioned in the manuscript that CD disrupts cell-level regulation and chronic intestinal inflammation, which leads to the proliferation of intestinal intraepithelial lymphocytes. The presence of chronic inflammation leads to increase the turnover of cell cycle and contribute to the development of neoplasm due to gene mutation in oncogenes or tumor suppressor genes in EATL.

We previously showed that c-myc mutation was seen in EATL type 2[2]. Our findings support that gene mutation is one of the factors developing malignant neoplasm in the absence of celiac disease. JAK/STAT3 signaling pathway was also reported as the main drivers of CD associated lymphomagenesis[3]. JAK/STAT pathway regulates MYC expression[4], which lead to proliferation of malignant cells.

CD is one of the significant gastrointestinal diseases and increases the risk of malignant neoplasms. In addition to CD, we believe that other underlying mechanisms contribute to the developing malignant neoplasms[3]. We believe that these facts would be a helpful to understand CD and EATL and these findings are highly pertinent and provide a context that helps understand those reported by Wang *et al* [1].

FOOTNOTES

Author contributions: Okumura K performed writing the paper.

Conflict-of-interest statement: The authors have declared no conflicts of interest.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

REFERENCES

1 **Wang M**, Yu M, Kong WJ, Cui M, Gao F. Association between intestinal neoplasms and celiac disease: A review. *World J Gastrointest Oncol* 2021; **13**: 1017-1028 [PMID: 34616509 DOI: 10.4251/wjgo.v13.i9.1017]

2 **Okumura K**, Ikebe M, Shimokama T, Takeshita M, Kinjo N, Sugimachi K, Higashi H. An unusual enteropathy-associated T-cell lymphoma with MYC translocation arising in a Japanese patient: A case report. *World J Gastroenterol* 2012; **18**: 2434-2437 [DOI: 10.3748/wjg.v18.i19.2434]

3 **Cording S**, Lhermitte L, Malamut G, Berrabah S, Trinquand A, Guegan N, Villarese P, Kaltenbach S, Meresse B, Khater S, Dussiot M, Bras M, Cheminant M, Tesson B, Bole-Feysot C, Bruneau J, Molina TJ, Sibon D, Macintyre E, Hermine O, Cellier C, Asnafi V, Cerf-Bensussan N; CELAC network. Oncogenetic landscape of lymphomagenesis in coeliac disease. *Gut* 2021 [PMID: 33579790 DOI: 10.1136/gutjnl-2020-322935]

4 **Huang L**, Liu D, Wang N, Ling S, Tang Y, Wu J, Hao L, Luo H, Hu X, Sheng L, Zhu L, Wang D, Luo Y, Shang Z, Xiao M, Mao X, Zhou K, Cao L, Dong L, Zheng X, Sui P, He J, Mo S, Yan J, Ao Q, Qiu L, Zhou H, Liu Q, Zhang H, Li J, Jin J, Fu L, Zhao W, Chen J, Du X, Qing G, Liu H, Liu X, Huang G, Ma D, Zhou J, Wang QF. Integrated genomic analysis identifies deregulated JAK/STAT-MYC-biosynthesis axis in aggressive NK-cell leukemia. *Cell Res* 2018; **28**: 172-186 [PMID: 29148541 DOI: 10.1038/cr.2017.146]

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