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WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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EDITORIAL

Molecular mechanisms underlying roles of long non-coding RNA small nucleolar RNA host gene 16 in digestive system cancers

Ting-Fang Yang, Xin-Rui Li, Mo-Wei Kong

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Abstract

This editorial reviews the molecular mechanisms underlying the roles of the long non-coding RNA (lncRNA) small nucleolar RNA host gene 16 (*SNHG16*) in digestive system cancers based on two recent studies on lncRNAs in digestive system tumors. The first study, by Zhao *et al*, explored how hBD-1 affects colon cancer, *via* the lncRNA *TCONS_00014506*, by inhibiting mTOR and promoting autophagy. The second one, by Li *et al*, identified the lncRNA prion protein testis specific (*PRNT*) as a factor in oxaliplatin resistance by sponging ZNF184 to regulate HIPK2 and influence colorectal cancer progression and chemoresistance, suggesting *PRNT* as a potential therapeutic target for colorectal cancer. Both of these two articles discuss the mechanisms by which lncRNAs contribute to the development and progression of digestive system cancers. As a recent research hotspot, *SNHG16* is a typical lncRNA that has been extensively studied for its association with digestive system cancers. The prevailing hypothesis is that *SNHG16* participates in the development and progression of digestive system tumors by acting as a competing endogenous RNA, interacting with other proteins, regulating various genes, and affecting downstream target molecules. This review systematically examines the recently reported biological functions, related molecular mechanisms, and potential clinical significance of *SNHG16* in various digestive system cancers, and explores the relationship between *SNHG16* and digestive system cancers. The findings suggest that *SNHG16* may serve as a potential biomarker and therapeutic target for human digestive system cancers.

Key Words: Digestive system cancers; Long non-coding RNAs; Small nucleolar RNA host gene 16; Colon cancer

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Core Tip: The long non-coding RNA (lncRNA) small nucleolar RNA host gene 16 (*SNHG16*) plays a significant role in the development and progression of various digestive system cancers, including esophageal, liver, pancreatic, gastric, and colorectal cancers. It is involved in processes such as cell proliferation, migration, invasion, apoptosis, and chemoresistance. *SNHG16* acts as a competing endogenous RNA, interacting with microRNAs and regulating target genes, and is associated with a poor prognosis in digestive system cancers. Its expression is influenced by transcription factors and its polymorphisms are linked to cancer susceptibility. *SNHG16* has potential as a biomarker and therapeutic target for digestive system cancers.

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INTRODUCTION

This editorial reviews the molecular mechanisms underlying the roles of the long non-coding RNA (lncRNA) small nucleolar RNA host gene 16 (*SNHG16*) in digestive system cancers based on two recent studies on long lncRNAs in colorectal cancer (CRC), one is by Zhao *et al*[1] and the other by Li *et al*[2]. The study of Zhao *et al*[1] linked hBD-1 to mTOR pathway regulation and autophagy *via* the lncRNA *TCONS_00014506*, highlighting the potential of hBD-1 in cancer cell destruction. The study of Li *et al*[2] identified the lncRNA prion protein testis specific (*PRNT*) as a regulator of oxaliplatin resistance, showing its upregulation in resistant CRC cells and its role in HIPK2 expression, suggesting PRNT as a therapeutic target for CRC treatment. Both of these two articles discuss the mechanisms by which lncRNAs contribute to the development and progression of digestive system tumors.

Digestive system tumors remain a major cause of global mortality, with increasing incidence and mortality rates[1,2]. In 2018, there were approximately 18.1 million new cancer cases and 9.6 million cancer-related deaths worldwide, with digestive system tumors accounting for a significant proportion[3]. Despite significant advances in understanding the potential molecular mechanisms of digestive system tumors[4-7] and substantial progress in their treatment[8,9], the recurrence and mortality rates of digestive system tumors remain dismal[1]. Therefore, it is crucial to seek novel effective biomarkers and therapeutic targets for these tumors.

Recent studies have shown that non-coding RNAs (ncRNAs) play a role in the development of digestive system tumors [10]. ncRNAs are generally divided into two categories based on their length: Small ncRNAs with less than 200 nucleotides (nts) and long ncRNAs (lncRNAs) with more than 200 nts[11,12]. MicroRNAs (miRNAs) are small ncRNAs with a length of 20 to 25 nts, which have been proven to negatively regulate the expression of specific key genes and participate in various aspects of cell biology[13,14]. In recent years, lncRNAs have attracted increasing attention from scholars worldwide. Although lncRNAs represent transcripts without protein-coding potential, they can regulate gene expression at multiple levels, including epigenetic, transcriptional, and post-transcriptional regulation[15-17]. Increasing evidence indicates that many lncRNAs display abnormal expression levels in digestive system tumors[18]. The dysregulation of lncRNAs is often involved in cell events related to tumors, including growth, programmed cell death, metastasis, and stem cell characteristics[19]. The lncRNA *SNHG16* contains 2435 nts and is located on chromosome 17q25.1[20]. *SNHG16* has two splice variants, the long form Nbla10727 (2186 nts) and the short form Nbla12061 (2087 nts)[21]. *SNHG16* was initially described as a strong carcinogenic factor leading to poor prognosis in patients with neuroblastoma [20]. Subsequent studies show that *SNHG16* plays a role in the development and progression of various digestive system tumors, and recent research has made breakthroughs in this field[10-12]. However, there is still a lack of research summarizing the molecular mechanisms of *SNHG16* in the development and progression of digestive system tumors.

This paper aims to systematically review the recent findings on the biological functions, related mechanisms, and potential clinical significance of *SNHG16* in various digestive system cancers, and to explore the close relationship between *SNHG16* and digestive system cancers.

MOLECULAR REGULATORY MECHANISMS OF LNCRNAS AND *SNHG16*

LncRNAs are single-stranded RNA molecules transcribed by RNA polymerase II, with a length of more than 200 nts and lacking protein-coding ability[22]. Overwhelming evidence from numerous studies indicates that lncRNAs play a crucial role in the development, proliferation, migration, and prognosis of various cancers by regulating a series of biological processes, such as interacting with target genes at the transcriptional level, regulating histone modifications and chromatin remodeling, and interacting with miRNAs of approximately 22 nts in length (also known as competing endogenous RNAs [ceRNAs])[22-25]. For example, the lncRNA BC069792 acts as a ceRNA to sponge miR-658 and miR-4739, increasing the expression of the target gene *KCNQ4*, leading to AKT phosphorylation, and thus inhibiting the prolif-

eration and invasion of breast cancer[24,25].

SNHG16 is a member of the SNHG family, located on human chromosome 17q25.1, and consists of four exons. Initially, it was identified as a potent carcinogenic factor that promotes the progression of neuroblastoma[26]. Therefore, *SNHG16* is also known as non-coding RNA expressed in aggressive neuroblastoma. Subsequent studies further revealed the widespread involvement of *SNHG16* in the complex molecular regulatory networks in different human cancers[27-29]. For instance, by regulating the miR-32-5p/SPN axis, the silencing of *SNHG16* inhibits the proliferation and radioresistance of nasopharyngeal carcinoma cells[28]. *SNHG16* may act as an oncogene by binding and recruiting EZH2 to the *p21* promoter, silencing the expression of p21, thereby promoting cell proliferation and reducing apoptosis in bladder cancer cells[29]. *SNHG16* plays a key role in the progression, distant metastasis, and prognosis of ovarian cancer by increasing the expression of MMP9[30]. In addition, in oral squamous cell carcinoma, the expression of *SNHG16* is regulated by a transcription factor called c-Myc, which recruits histone acetyltransferases and induces the clearance of RNA polymerase II[31]. These functions indicate that *SNHG16* plays an important role in the progression, invasion, and carcinogenesis of human cancers through various molecular mechanisms.

MECHANISMS OF *SNHG16* IN DIGESTIVE SYSTEM TUMORS

SNHG16 and esophageal squamous cell carcinoma

Esophageal cancer (EC) is one of the major cancer types worldwide, ranking 7th in incidence (3.1%, 604100 new cases) and 6th in mortality (5.5%, 544076 deaths) among all cancers[2]. There are differences in the incidence and mortality rates of EC across geographic regions[2]. For example, due to economic underdevelopment and dietary habits, the burden of EC in East Asia is heavier, with esophageal squamous cell carcinoma (ESCC) being the predominant histological type[2]. Studies have shown that *SNHG16* is upregulated in EC and closely related to tumor stage, lymph node metastasis, and clinical stage. Silencing of *SNHG16* inhibits the proliferation and invasion of EC-1 and Eca-109 cells by reducing the expression of β-catenin, cyclin D1, and c-Myc proteins, and promotes apoptosis[32]. This study also showed that *SNHG16* is upregulated in ESCC tissue and cell lines, and disrupting *SNHG16* expression promotes apoptosis and inhibits epithelial-mesenchymal transition (EMT) through the miR-140-5p/ZEB1 axis. Another study found that increased expression of *SNHG16* also promotes the growth and metastasis of ESCC, and is related to tumor differentiation and T stage, with the mechanism being that *SNHG16* can bind and recruit EIF4A3 to regulate the expression of RhoU by enhancing the stability of *RhoU* mRNA[33]. These results indicate that the upregulation of *SNHG16* is closely related to the development of ESCC, and *SNHG16* is expected to serve as a marker for ESCC, providing new clues for its clinical treatment and the development of related drugs.

SNHG16 and hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, accounting for 4.7% of all new cancer cases (906000 cases) and 8.3% of cancer-related deaths (830000 cases), ranking sixth and third among all malignancies, respectively[2]. In most studies, *SNHG16* is considered an oncogene for HCC, and the upregulation of *SNHG16* expression is closely related to the malignant prognosis and tumor stage of HCC. The expression of *SNHG16* in late-stage HCC is significantly higher than that in early-stage HCC[34,35]. Additionally, high expression of *SNHG16* is also associated with tumor size, TNM stage, and vascular invasion in HCC patients[36]. *SNHG16*, as a ceRNA, targets STAT3 and GALNT1 through miR-4500 and miR-let-7b-5p in Huh-7 and HUVEC cells, respectively, promoting the proliferation, metastasis, and angiogenesis of Huh-7 cells and enhancing vascular formation in HUVEC cells[34,36] (Figure 1).

Other studies have shown that downregulating *SNHG16* expression affects the *SNHG16*/miR-195, *SNHG16*/miR-17-5p/P62, and *SNHG16*/miR-302a-3p/FGF19 axes, inhibiting the proliferation, migration, and invasion of HepG2 and Hep3B cells[34,37-39] (Figure 2).

The overexpression of *SNHG16* affects the G2/M transition of HCC cells by sponging miR-let-7b-5p[40]. *SNHG16* is overexpressed in sorafenib-resistant HCC tumor tissues and cells, enhancing the resistance of HCC cells to sorafenib[36]. Conversely, when the expression of *SNHG16* is inhibited, the resistance to sorafenib disappears[41]. Assarzadegan *et al*[42] proposed that *SNHG16* enhances the autophaic function of HCC cells to protect HCC from sorafenib resistance through the miR-23b-3p/EGR1 axis. Moreover, *SNHG16* exosomes can be engulfed by microglia, and through the miR-942-3p/MMP9 axis, they mediate microglia to promote the metastasis of HCC cells[43]. In addition, Hu *et al*[44] found that the overexpression of *SNHG16* promotes the expression of TRAF6 by sponging miR-605-3p, activating NF-κB and thus promoting the development of HCC. Conversely, activated NF-κB can enhance the activity of the *SNHG16* promoter, forming a positive *SNHG16*-NF-κB feedback loop, further exacerbating HCC.

Studies have shown that *SNHG16* regulates a large lncRNA-miRNA-mRNA network in HCC and is closely related to immune cell infiltration, the release of immune regulatory factors, and the expression of chemokines in tumor tissues[45-47]. In addition, researchers have pointed out that the progression of HCC promoted by UBE4B and SEMA3F is regulated by its upstream *SNHG16*/miR-22-3p and *SNHG16*/Let-7c-5p axes[47,48]. Furthermore, Liu *et al*[49] revealed that *SNHG16* can serve as a potential biomarker for poor prognosis in HCC patients. In summary, *SNHG16* is upregulated in HCC and promotes the development of HCC. However, a recent paper presented the opposite view, suggesting that compared to normal liver tissue, the expression of *SNHG16* is reduced in HCC, and the overexpression of *SNHG16* reduces the proliferation of Hep3B and Huh-7 cells by sponging miR-93, inhibiting the development and chemoresistance of HCC[50]. Further research is needed to address the above-mentioned inconsistent findings.

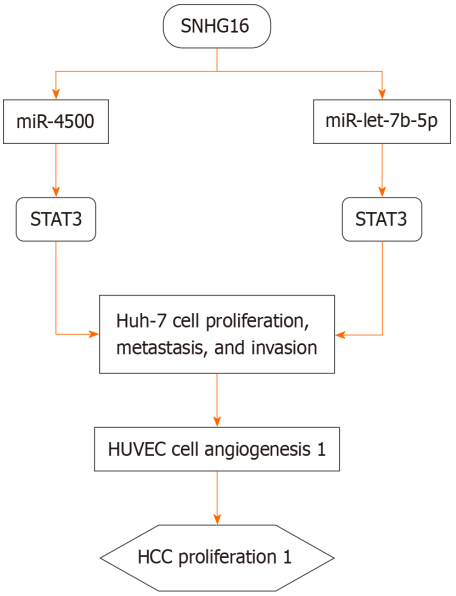

Figure 1 Small nucleolar RNA host gene 16 promotes tumor vascular proliferation. SNHG16: Small nucleolar RNA host gene 16; HCC: Hepatocellular carcinoma.

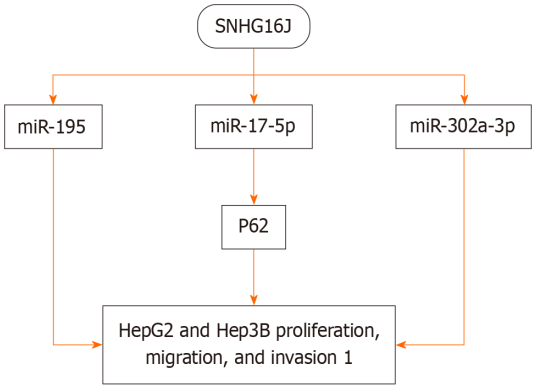

Figure 2 Downregulation of small nucleolar RNA host gene 16 inhibits proliferation, migration, and invasion of HepG2 and Hep3B cells *via* multiple mechanisms. SNHG16: Small nucleolar RNA host gene 16.

SNHG16 and pancreatic cancer

Pancreatic cancer (PC) is one of the most severe malignant tumors in the digestive system. Due to its poor prognosis, the number of PC-related deaths (466000 cases) is almost equivalent to the number of cases (496000 cases), making it the seventh leading cause of cancer death in both genders[2]. Similar to EC, the incidence of PC in high human development index (HDI) countries is 4 to 5 times higher than that in low HDI countries[2]. Studies have shown that *SNHG16* expression is regulated in PC tissue, and the overexpression of *SNHG16* is closely related to patient survival rate, cancer cell differentiation, TNM stage, and lymph node invasion[37]. Altering the expression of *SNHG16* can inhibit the proliferation, migration, invasion, and tumor-forming ability of AsPC-1 cells through miR-218-5p[51], and suppress lipogenesis in AsPC-1 and PANC-1 cells through the miR-195/SREBP2 axis[52]. The overexpression of *SNHG16* is closely related to the resistance of PC cells to gemcitabine. *SNHG16* can interact with EZH2, inhibiting the expression of Smad4 by EZH2 binding to the *Smad4* promoter[53]. The downregulation of SMAD4 reduces its ability to inhibit AKT phosphorylation, thus promoting the resistance of PC cells to gemcitabine[54]. Collectively, all these lines of evidence suggest that *SNHG16* may play a key role in the development of PC and can even be regarded as a marker for poor prognosis in PC.

SNHG16 and gastric cancer

Gastric cancer (GC) remains a significant malignancy worldwide, being the fifth most common malignant tumor (5.6%, over 1000000 new cases) and the fourth leading cause of cancer-related deaths (7.7%, about 769000 deaths)[2]. GC has different characteristics in different parts of the world. The highest age-standardized incidence rate was observed in East Asia, followed by Central and Eastern Europe[2]. Many studies have shown that the expression of *SNHG16* is significantly related to the depth of invasion, lymph node metastasis, TNM stage, histological differentiation, and expression of PTBP1 in GC[55,56]. Inhibiting the expression of *SNHG16* can significantly inhibit GC cell migration and invasion, and

cause cell arrest in the G1 phase[42,43]. In addition, inhibiting the expression of *SNHG16* can reduce the expression of c-Myc and affect the formation of p27/cyclin D1/CDK6, p53/cyclin E1, and cyclin A2/CDK2 complexes[55]. Many GC patients develop resistance to 5-fluorouracil (5-Fu), which is more susceptible to damage than the original GC cells. In GC, blocking the *SNHG16*/miR-506-3p-PTBP1 axis can effectively limit the growth of 5-Fu-resistant GC cell-derived xenograft tumors under 5-Fu treatment[56,57]. PTBP1 stabilizes glycolytic mRNAs by directly binding to the 3′-untranslated region, while *SNHG16* promotes EMT by downregulating the WNT signaling pathway and inhibiting the expression of DKK3[58]. In addition, *SNHG16* activated by CTCF can regulate the proliferation, migration, invasion, and apoptosis of gastrointestinal stromal tumor cells through the miR-128-3p/CASC3 axis[59]. Another study also showed that *SNHG16* can mediate the upregulation of JAK2 and STAT3 by sponging miR-135a, affecting the proliferation, invasion, and apoptosis of GC cells, and *SNHG16* may be regulated by p-STAT3 directly or indirectly[60]. In summary, *SNHG16* is closely related to the occurrence and development of GC and may become a potential marker for poor prognosis in GC.

SNHG16 and CRC

In 2020, there were over 1.9 million new cases of CRC worldwide, with 935000 deaths, ranking third in incidence after breast cancer in women and lung cancer, and second in mortality, close to lung cancer[2]. Increasing evidence indicates that the expression level of *SNHG16* is positively correlated with advanced TNM stage, distant metastasis, and shortened overall survival time in CRC[61-63]. *SNHG16* is mainly present in the cytoplasm, suggesting that *SNHG16* functions as a ceRNA to regulate the activity of multiple miRNAs and target genes. Li *et al*[62] revealed that *SNHG16* is associated with the malignancy and poor prognosis of CRC by sponging miR-200a-3p. Tan *et al*[64] demonstrated that *SNHG16* promotes the proliferation of CRC cells by upregulating its target gene *ABCB1* through interaction with miR-214-3p. It has also been found that *SNHG16* promotes the progression of CRC by activating the expression of USP22 *via* sponging miR-132-3p[65,66]. Ke *et al*[67] discovered that *SNHG16* supports the growth of colon cancer cells by targeting the miR-302a-2p/AKT axis. Chen *et al*[47] showed that the expression level of *SNHG16* in cancerous tissue is higher than that in matched normal tissue and is positively correlated with CRC grade. Moreover, *SNHG16* promotes the proliferation, migration, and EMT of CRC cells *via* the miR-124-3p/MCP-1 axis[48]. Bioinformatics analysis also led to the same conclusion, indicating that *SNHG16* plays an important role in CRC[50,53]. Recent studies have further established the close relationship between *SNHG16* and autophagy in CRC[68,69].

The expression of *SNHG16* is also activated by other proteins such as c-Myc. Christensen *et al*[70] found that in CRC, the expression of *SNHG16* is determined by Wnt-regulated transcription factors, and the inhibition of β-catenin reduces the expression of *SNHG16* and c-Myc. Additionally, the inhibition or overexpression of c-Myc can respectively decrease or increase the expression of *SNHG16*. In another study, Xiang *et al*[71] for the first time discovered a positive feedback loop of *SNHG16*-YAP1/TEAD1 in CRC cells. *SNHG16*, as a ceRNA, physically binds to miR-195-5p, further regulating the expression of YAP1 and promoting tumor progression. YAP1 binds to TEAD1 to form a YAP1/TEAD1 complex, which in turn binds to two sites on the *SNHG16* promoter, activating the transcription of *SNHG16*[71]. The mechanism of *SNHG16*'s involvement in CRC is shown in Figure 3.

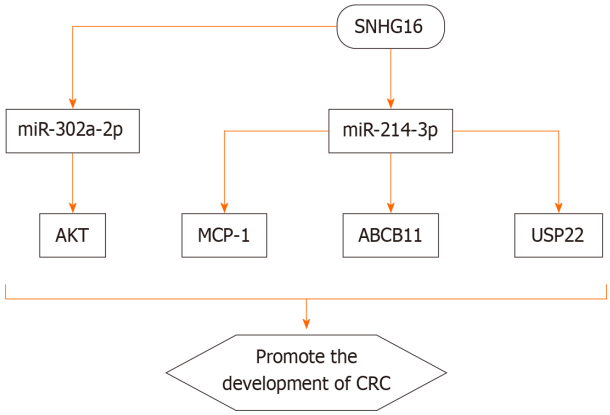

Figure 3 Mechanism of small nucleolar RNA host gene 16's involvement in colorectal cancer. SNHG16: Small nucleolar RNA host gene 16; CRC: Colorectal cancer.

The polymorphisms of *SNHG16* are significantly associated with susceptibility to CRC. The A>G variant at the rs7353 locus of the *SNHG16* gene is associated with a reduced susceptibility to CRC. However, the G>A variant at the rs8038 locus and the A>G and G>A variants at the rs15278 locus may increase the susceptibility to CRC[72].

CONCLUSION

An increasing number of studies indicate that the occurrence of tumors is caused by a combination of genetic and

environmental factors. The focus of this editorial is on the molecular mechanisms underlying the roles of the lncRNA *SNHG16* in the development and progression of digestive system cancers (Figure 3). Existing data suggest that *SNHG16* is closely related to the proliferation, migration, invasion, apoptosis, and poor prognosis of EC, HCC, PC, GC, and CRC. Moreover, *SNHG16* is positively correlated with clinical stage and lymph node metastasis of digestive system cancers. In terms of mechanisms of action of *SNHG16* in digestive system cancers, there are mainly four aspects: (1) Many transcription factors, including CTCF, c-Myc, NF-κB, STAT3, and TEAD1, are positively correlated with *SNHG16*; (2) *SNHG16* directly controls the expression of downstream target genes such as *DKK3*; (3) *SNHG16* can bind and recruit EIF4A3 to regulate the expression of RhoU by enhancing the stability of *RhoU* mRNA, and *SNHG16* can also bind to EZH2 and recruit EZH2 to the promoter of *Smad4*, thereby inhibiting the expression of Smad4; and (4) *SNHG16* can compete with miRNAs, regulate the expression of downstream target genes, and activate different signaling pathways. However, these studies are only preliminary, and the expression levels of *SNHG16* in body fluids and chemical stability have not yet been clarified. In addition, the clinical application of *SNHG16* as a biomarker in digestive system cancers needs further research in the future.

FOOTNOTES

Author contributions: Yang TF and Li XR contributed equally to this work as co-first authors; Kong MW provided crucial suggestions and guidance for manuscript writing; Yang TF wrote the manuscript; Li XR reviewed and revised the manuscript; and all authors read and approved the final manuscript.

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EDITORIAL

Navigating the complex landscape of crawling-type gastric adenocarcinomas: Insights and implications for clinical practice

Hai-Bo Yu, Ke-Feng Jia, Xing-Fen Wang, Bao-Yu Li, Qi Xin

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Abstract

In this editorial, we comment on an article by Xu *et al*. This article describes a case of crawling-type gastric adenocarcinoma (CRA) distinguished by its rare occurrence and diagnostic complexity. We reviewed the detailed case-report findings showcasing clinical, pathological, and molecular characteristics of CRA that shed light on its elusive nature and challenges for early detection and treatment. This case underscored the significance of advanced diagnostic tools such as endoscopic submucosal dissection. Emphasis was placed on the molecular peculiarities of CRA, including the higher mutation rates of genes such as *TP53* and *RHOA* and the notable absence of *HER2* amplification, differentiating it from more conventional forms of gastric adenocarcinoma. In this editorial, we advocate for a multidisciplinary approach to effectively manage this rare subtype and highlight the necessity for precision in both diagnostic and therapeutic strategies. Moreover, a heightened awareness urging the adoption of advanced diagnostic techniques and collaborative approaches is necessary among clinicians and researchers. We aim to contribute to the ongoing discourse in gastrointestinal oncology, emphasizing the importance of recognizing and addressing the complexities associated with rare cancer subtypes such as CRA.

Key Words: Crawling-type gastric adenocarcinoma; Diagnosis; Pathology; Endoscopy;

Treatment

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Core Tip: Crawling-type gastric adenocarcinoma (CRA) is characterized by its elusive presentation and propensity for lateral expansion within the mucosal layer, posing significant challenges to early detection. This study emphasizes the need for a multidisciplinary approach to achieve accurate diagnosis and effective treatment. The findings reveal distinct molecular features of CRA including the notably increased mutation frequencies in the *TP53* and *RHOA* genes, and the absence of *HER2* amplification. These characteristics highlight the critical need for precision in diagnostic and therapeutic modalities. The objective is to augment clinical awareness, thereby facilitating prompt identification and efficient management of this subtype of gastric cancer.

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INTRODUCTION

In the dynamic field of gastrointestinal oncology, enhancing patient outcomes depends on identification and a thorough understanding of rare cancer subtypes. This expertise is crucial for advancing clinical practice[1]. A recent case report on crawling-type gastric adenocarcinoma (CRA) presented in the *World Journal of Gastrointestinal Oncology* exemplifies this crucial endeavor[2]. CRA is a rare subtype of gastric cancer (GC) with an elusive presentation that presents diagnostic challenges and offers profound insights into the complexities of the pathology, endoscopy, and management of GC[3,4].

In 1999, Endoh *et al*[5] described eight cases of well-differentiated gastric adenocarcinoma resembling intestinal metaplasia. These cases were distinguished by mild cytological atypia and structurally complex glands with characteristics including branching, tortuous formations, anastomoses, and a plexiform arrangement, in addition to an intestinal immunophenotype. Subsequently, Okamoto *et al*[6] referred to these gastric adenocarcinomas as the "crawling type," alternatively known as "shaking-hands carcinoma," a term not widely recognized in the medical community. CRA is distinguished by its propensity for lateral spread within the mucosal layer and is characterized by low-grade cellular atypia. Its deceptive presentation, which mimics benign conditions, underscores the challenges faced in early diagnoses using conventional diagnostic methods. This case, emblematic of the elusive presentation and diagnostic challenges, highlights the necessity for precision in diagnostic and therapeutic strategies, thus enhancing clinical knowledge of the diverse pathology of GC in the medical community.

CASE PRESENTATION

This report meticulously described the case from patient presentation to diagnosis and treatment, providing valuable lessons at each step. The initial lack of symptoms, a common scenario in early GC, and the subsequent findings from routine examinations illustrated the silent character of this disease and the serendipitous nature of its detection. The narrative further explored the diagnostic enigma posed by this cancer subtype, highlighting the critical role of endoscopic submucosal dissection (ESD) as a definitive diagnostic tool, not just a therapeutic intervention.

This case emphasized the necessity for a multidisciplinary approach to gastrointestinal oncology combining the varied expertise of endoscopists, pathologists, surgeons, and oncologists to navigate the complexities of such rare cancers. Discussions on the use of various diagnostic techniques including narrow-band imaging (NBI), chromoendoscopy, and immunohistochemical markers illustrated the evolving landscape of GC diagnosis and the requirement for a magnitude of clinical suspicion and skill.

Moreover, the successful outcome in this case, with no evidence of recurrence at the one-year follow-up, served as a testament to the potential for a favorable prognosis when CRA is identified early and managed effectively. These findings highlighted the importance of early detection and the role of advanced endoscopic techniques in achieving diagnostic and therapeutic success.

PATHOLOGICAL DIAGNOSIS

CRA, a notable subtype of moderately differentiated adenocarcinoma, is increasingly being recognized for its distinct clinicopathological and molecular features. Unlike typical moderately differentiated adenocarcinomas that progress

through hyperdifferentiation, CRAs originate directly from moderately differentiated adenocarcinomas. This subtype often occurs simultaneously with a pronounced inflammatory backdrop and low-grade cellular heterogeneity closely mimicking intestinal epithelial hyperplasia, leading to frequent misdiagnoses as benign or indeterminate lesions.

CLINICO-PATHOLOGICAL CHARACTERISTICS

Clinically, CRA is characterized by low-grade cellular atypia and pronounced structural anomalies[7]. Cells exhibit minimal dysplasia resembling intestinal metaplasia, or mildly atypical epithelial cells against a background rich in inflammatory cells. Structurally, these adenocarcinomas display irregular proliferation zones, with glands merging in a pattern marked by branching, anastomosis, and dilation. These features result in cystic glandular dilatation with sparse eosinophilic cells, exfoliation, and sharp angles[8]. The unique merging pattern of glands, described as resembling the letters "WHYX,"[5,9] serves as a critical diagnostic tool. Glandular basement membranes exhibit discontinuity and roughness blending into the stroma with protruding "tentacles", occasionally accompanied by undifferentiated elements such as signet ring cells[6] and eventually evolving into a diffuse form. Immunohistochemistry (IHC) assays showing variable expression of protein markers encoded by the *MUC2, MUC5AC, MUC6* genes, and the Ki-67 protein may provide insights into the cellular makeup and proliferative index of tumors. *MUC2* is typically associated with intestinal-type GC s and is rarely expressed in diffuse-type cancers, including CRA. The lack of *MUC2* expression in CRA can help differentiate it from intestinal-type gastric adenocarcinoma, aiding in accurate subtype identification. *MUC5AC* is commonly expressed in the foveolar epithelium of the stomach and is frequently observed in both intestinal and diffuse types of gastric adenocarcinomas, including CRA. Its presence can help confirm the gastric origin of the tumor. *MUC6* is expressed in the deep gastric glands and variably present in gastric adenocarcinomas. In CRA, *MUC6* expression can further characterize tumor differentiation status. The Ki-67 Labeling index provides information on the proliferative activity of the tumor. Higher Ki-67 indices in CRA can indicate aggressive tumor behavior and help gauge the tumor growth rate.

The IHC results contribute to prognostic information. Loss of E-cadherin protein expression is a hallmark of CRA, particularly in diffuse-type and signet ring cell carcinomas. This loss helps identify tumors with a high invasive potential and diffuse growth pattern. While HER2 protein overexpression is less common in CRA compared to intestinal-type gastric adenocarcinoma, its detection can aid in identifying subgroups that may benefit from targeted therapies such as trastuzumab.

MOLECULAR PATHOLOGICAL CHARACTERISTICS

The *TP53* mutation rate in CRAs is significantly higher than that in conventional adenocarcinomas, and predominantly features deletion mutations. Consequently, IHC for p53 shows a negative expression. Moreover, the c.529_546 deletion mutation in the *TP53* gene is notably absent in the conventional types[10]. *TP53* is a tumor suppressor gene, and its mutation leads to the loss of cell cycle regulation, allowing for uncontrolled cell proliferation. In CRA, *TP53* mutations are indicative of genomic instability and are often associated with high-grade tumors. The presence of mutations in *TP53* generally correlate with a more aggressive disease course and poorer overall survival. Identifying *TP53* mutations can help stratify patients into different risk categories and tailor treatment strategies accordingly.

RHOA mutations are significantly more prevalent in CRA than in conventional tubular adenocarcinoma, similar to the pattern observed in diffuse-type GC[8]. A higher incidence of *RHOA* mutations and *CLDN18-ARHGAP* gene fusions has been found in CRA, mirroring the genetic phenotype of diffuse-type GC (poorly cohesive carcinoma)[11]. *RHOA* is involved in regulating the cytoskeleton, cell motility, and epithelial-mesenchymal transition. Mutations in *RHOA* disrupt these processes, leading to enhanced invasiveness and metastasis in CRA. *RHOA* mutations are specific to diffuse-type GCs and can serve as a molecular marker for this subtype. They are associated with distinctive histopathological features and worse prognosis, emphasizing the need for targeted therapeutic approaches and rigorous follow-up. (4) CRA shows non-amplification of HER2[8].

ENDOSCOPIC DIAGNOSIS

CRA is predominantly found in the middle third of the stomach, particularly in the transitional zones. Furthermore, CRA can be categorized into two types: Superficial depression (0-IIc) or superficial flatness (0-IIb). The boundaries of CRA can sometimes appear indistinct. This characteristic feature is likely due to the "creeping" infiltration of the CRA tumor glands into the proliferative zone of the epithelium, where they are partially covered by the non-tumorous pit epithelium. Traditional white-light endoscopy may not adequately reveal the distinct features of the tumor, often presenting as flat or slightly depressed lesions with an indistinct border[6] and closely mimicking benign gastric conditions such as gastritis or intestinal metaplasia[3]. NBI, magnifying endoscopy, and chromoendoscopy enhance the visualization of mucosal patterns and vascular architecture, which are indicative of neoplastic changes[12]. NBI enhances the contrast between neoplastic and non-neoplastic tissues, revealing irregular microvascular patterns and mucosal structures possibly indicating the presence of this cancer subtype[13]. Chromoendoscopy, which involves the application of special dyes, further delineates subtle mucosal abnormalities, aiding in the identification and targeted biopsy of suspicious areas.

The specificity of endoscopic findings for CRA is relatively low due to its resemblance to benign conditions and other types of GC. The irregular and subtle appearance can lead to misdiagnosis or underdiagnosis, especially in the early stages. Sensitivity for detecting CRA is also a challenge. Standard white-light endoscopy may miss CRA due to its flat and infiltrative nature. Sensitivity improves with the use of enhanced imaging techniques. Studies have shown that NBI and chromoendoscopy can significantly increase the detection rates of early GCs, including CRA, by highlighting abnormal mucosal structures and vascular patterns that are not visible with conventional endoscopy. While benign conditions like gastritis and intestinal metaplasia appear similar to CRA, they lack the irregular, invasive margins characteristic of CRA. In contrast, intestinal-type gastric adenocarcinomas present as raised, ulcerative, or polypoid lesions with well-defined edges. Endoscopic ultrasonography aids differentiation by providing detailed images of submucosal invasion depth.

The propensity of CRA for lateral spread, rather than deeper penetration into the gastric layers, necessitates a high degree of vigilance and expertise from endoscopists. Therefore, special attention must be paid to accurately determining tumor margins. Given the potential difficulty in endoscopically identifying CRA margins, the possibility of CRA should be considered when superficial depressed or flat lesions are detected in the middle third of the stomach, necessitating a careful boundary assessment.

TREATMENT

The borders of intramucosal CRA are often poorly defined because of the lack of contrast from the surrounding non-neoplastic mucosa. This feature of CRA often causes failure of complete resection after ESD[14]. CRA treatment is complicated by the high rate of margin positivity, which is reported to be approximately 30%, even after meticulous examination. This requires a careful and tailored treatment approach that emphasizes the need for complete tumor resection with clear margins to minimize the risk of recurrence. ESD has been employed in selected cases of CRA in which the tumor is confined to the mucosa or superficial submucosa and has well-defined margins. However, the high risk of incomplete resection necessitates close postoperative surveillance and, in some cases, additional surgical intervention. For more advanced cases or when endoscopic resection is not feasible, surgical resection remains the cornerstone of treatment, often accompanied by lymph node dissection to address potential metastatic spread.

FOLLOW-UP STRATEGY

The follow-up strategy for CRA patients is crucial for early detection of recurrence and management of any long-term treatment-related complications. A structured follow-up protocol typically includes regular clinical assessments, imaging studies, and endoscopic evaluations. (1) Clinical assessments: Patients are seen every 3-6 months for the first two years following surgery, and every 6-12 months thereafter. During these visits, a thorough physical examination and review of symptoms are conducted to identify any signs of recurrence or metastasis; (2) Imaging studies: Routine imaging, such as computed tomography scans or positron emission tomography scans, are performed at scheduled intervals, usually every 6-12 months in the first two years and annually thereafter. These studies help monitor for any anatomical changes indicative of recurrence; (3) Endoscopic evaluations: Regular endoscopic surveillance is essential for detecting local recurrence, particularly given the submucosal and diffuse-growth patterns characteristic of CRA. Endoscopies are typically performed every 6-12 months in the initial follow-up period and then annually; and (4) Laboratory tests: Periodic blood tests including tumor markers such as carcinoembryonic antigen and carbohydrate antigen 19-9, are also part of the follow-up protocol. These markers can provide early indications of tumor recurrence.

ADJUVANT TREATMENTS AND RATIONALE FOR FOLLOW-UP DURATION

Adjuvant treatments are tailored based on individual patient factors and tumor response to initial therapy. The rationale for follow-up duration and frequency is informed by the aggressive nature of CRA, its potential for late recurrences, and the need for ongoing monitoring to manage any long-term effects of treatment. (1) Adjuvant chemotherapy: Given the high risk of recurrence in CRA, adjuvant chemotherapy is critical. The selected regimens aim to eliminate microscopic residual diseases that might not be addressed by surgery alone; and (2) Radiotherapy: While not routinely used for all patients, radiotherapy may be considered in cases where there is a high risk of local recurrence or where surgical margins are close.

The primary goal of the follow-up strategy is to balance early detection of recurrence with patient quality of life, minimizing unnecessary interventions while ensuring timely identification and treatment of any recurrence or metastasis.

CRA CONTRASTS WITH COMMON-TYPE GC

Endoscopic appearance

The endoscopic features of CRA are distinct when compared with those of more common types of gastric adenocarcinoma. CRA is characterized by its unique "crawling" growth pattern, which is typically observed as a flat or slightly

elevated lesion with an irregular shape and poorly defined margins[15]. The surface may appear granular or nodular, often mimicking benign conditions such as gastritis or intestinal metaplasia. Therefore, distinguishing CRA from non-malignant lesions solely based on endoscopic appearance is challenging. In contrast, more common forms of gastric adenocarcinoma, such as tubular or papillary adenocarcinomas, usually present as raised, ulcerative, or polypoid lesions. These types often exhibit well-defined edges and a more obvious mass effect that is more readily identifiable during endoscopy. The differences in endoscopic appearance are significant for early detection and accurate diagnosis, emphasizing the need for heightened awareness and careful examination when CRA is suspected.

Biological behavior

The biological behavior of CRA also differs notably from other gastric adenocarcinomas. CRA is known for its aggressive infiltration into the submucosa and muscularis propria, often extending laterally over a broad area of the stomach wall [16]. This lateral spread can lead to significant submucosal invasion without forming a prominent mass, complicating detection and staging. This behavior contrasts with the vertical invasion pattern commonly observed in other types of gastric adenocarcinoma, where the tumor tends to penetrate deeper layers more directly.

Moreover, CRA often exhibits a diffuse growth pattern with signet ring cells, a hallmark of diffuse-type GC (Lauren classification). This histological feature is associated with a poorer prognosis and a higher likelihood of peritoneal dissemination and lymph node metastasis when compared with those of intestinal-type GCs, which typically display glandular structures and a more cohesive growth pattern.

CONCLUSION

The CRA case presented in this issue serves as a compelling reminder to clinicians of the diversity of GC and the challenges that are posed by rare subtypes. It advocates increased awareness, improved diagnostic accuracy, and the adoption of multidisciplinary care models to enhance outcomes for patients with this and other rare forms of GC. As we continue to advance our understanding and refine our approaches to gastrointestinal oncology, this case serves as an inspiration for continued research, collaboration, and innovation in the effort to control GC.

FOOTNOTES

Author contributions: Yu HB contributed to data collection; Wang XF evaluated the images; Xin Q contributed to the pathological analysis; Yu HB, Jia KF, and Li BY contributed to revision of the manuscript; All authors agreed to publication of the manuscript.

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EDITORIAL

Present and prospect of transarterial chemoembolization combined with tyrosine kinase inhibitor and PD-1 inhibitor for unresectable hepatocellular carcinoma

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Abstract

In this editorial, we comment on the article (*World J Gastrointest Oncol* 2024; 16: 1236-1247), which is a retrospective study of transarterial chemoembolization (TACE) combined with multi-targeted tyrosine kinase inhibitor (TKI) and programmed cell death protein-1 (PD-1) inhibitor for the treatment of unresectable hepatocellular carcinoma (HCC). Herein, we focus specifically on the mechanisms of this triple therapy, administration sequence and selection of each medication, and implications for future clinical trials. Based on the interaction mechanisms between medications, the triple therapy of TACE + TKI + PD-1 is proposed to complement the deficiency of each monotherapy, and achieve synergistic antitumor effects. Although this triple therapy has been evaluated by several retrospective trials, it is still controversial whether the triple therapy achieves better clinical benefits, due to the flawed study design and heterogeneity in medications. In addition, the administration sequence, which may greatly affect the clinical benefit, needs to be fully considered at clinical decision-making for obtaining better prognosis. We hope that this editorial could contribute to the

design and optimization of future trials.

Key Words: Transarterial chemoembolization; Multi-targeted tyrosine kinase inhibitor; Programmed cell death protein-1 inhibitor; Unresectable hepatocellular carcinoma; Mechanism

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Core Tip: This editorial focuses on the mechanisms for combining transarterial chemoembolization with multi-targeted tyrosine kinase inhibitor and programmed cell death protein-1 inhibitor for unresectable hepatocellular carcinoma, administration sequence and selection of each medication, and implications for future clinical trials. Despite several retrospective trials have evaluated the efficacy and safety of this triple therapy, the flawed study design and heterogeneity in medications still arise controversial concerns on the results. Especially, the administration sequence between each medication varied across trials, which could greatly affect the clinical benefit. So, the administration sequence needs to be fully considered in future trials based on the interaction mechanisms between each medication.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common and lethal form of liver cancer[1]. Different treatment modalities have been developed for HCC at different stages. Based primarily on the Barcelona Clinic Liver Cancer (BCLC) staging system, liver transplantation/hepatectomy/local ablation are preferred options for early-stage HCC, transarterial chemoembolization (TACE) is recommended for intermediate-stage HCC, and systemic therapy is the mainstay for advanced HCC[2,3]. Due to the latent property, most HCC is not diagnosed until it is unresectable, making TACE and systemic therapy the only feasible options for most patients.

In practice, TACE is frequently performed across all disease stages, not only HCC at BCLC-B stage[4]. Moreover, systemic therapy is recommended for HCC at BCLC-B stage with extensive bilobar liver involvement and BCLC-C stage. Sorafenib, a multi-targeted tyrosine kinase inhibitor (TKI), became the first effective systemic agent for advanced HCC in 2008[5]. Afterwards, other TKIs, such as lenvatinib and regorafenib, were demonstrated effective for advanced HCC[6,7]. Recently, immune checkpoint inhibitors (ICIs) have shown robust efficacy in the first-line or second-line settings, especially programmed cell death protein-1 (PD-1) inhibitors[8,9]. Indeed, these therapy options have exhibited clinical benefit to patients with unresectable HCC, however, the efficacy of mono-therapy with TACE, TKI or PD-1 inhibitor remains unsatisfactory. Thus, combination treatment exerting synergistic antitumor effects is a promising strategy for achieving improved clinical outcomes[10]. For instance, TACE plus TKI have been evaluated for unresectable HCC by many trials[11], as well as TKI in combination with PD-1 inhibitor[12]. Furthermore, as a potential combination scheme, the triple therapy consisting of TACE + TKI + PD-1 inhibitor has been increasingly evaluated for unresectable HCC.

MECHANISMS OF TACE + TKI + PD-1 INHIBITOR FOR HCC

TACE deprives tumor cells of nutrient supply and concentrates chemotherapeutic agents at tumour site, thus inducing necrosis and apoptosis of tumor cells. Whereas, it hardly guarantees a complete tumour death, meanwhile triggers the deterioration liver function[13]. In addition, TACE aggravates hypoxia in tumor tissues, thus enhancing the expression of hypoxia inducible factor-1α, which in turn upregulates the expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). These increased growth factors consequently promote tumor angiogenesis, which takes major responsibility for tumor regrowth and extra-hepatic metastasis[14,15]. Naturally, then, this offers a clue that inhibition of VEGF/PDGF receptors may block the effects of proangiogenic factors.

TKIs can inhibit a number of serine/threonine and tyrosine kinases, such as VEGF and PDGF receptor, thereby exerting both anti-angiogenic and direct antitumour effects[14,16]. Therefore, the combination of TACE with TKI is designed to counteract the proangiogenic effect of TACE, meanwhile exert synergistic anti-tumor effects arisen from different action mechanisms[11]. On the one hand, all TKIs with proven efficacy in HCC inhibit VEGF signaling pathway, while anti-VEGF therapy can induce tumuor hypoxia[17]. To sum up, both TACE and TKI can lead to hypoxia in tumor. Hypoxia supports immunosuppression to aid tumor immune escape, especially by upregulating immune checkpoint molecules[18], for instance, up-regulated PD-L1 expression induced by sorafenib[19]. Therefore, there is a rationale for combining TKI with ICI.

In recent years, the advent of ICIs, including the PD-1 and programmed death-ligand 1 (PD-L1), have gradually shifted the direction of research to immunotherapy. Especially, the combination of atezolizumab and bevacizumab have recently outperformed sorafenib as the first-line treatment[20]. ICIs can prevent the recognition escape of tumor cells and reactivate immune responses in the tumor microenvironment, thereby enable T cells to identify and kill tumor cells. So, ICIs can antagonize the immunosuppressive effects caused by TKIs. In turn, TKIs have also been found to enhance the antitumor sensitivity of PD-1 inhibitors[21]. Indeed, TKI in combination with PD-1 inhibitor have been demonstrated to improve anti-tumor efficacy, such as prolonged overall survival (OS)[12]. Additional, TACE-induced necrosis increase the release of tumor antigens, which may further increase the efficacy of immunotherapy[22]. Overall, the combination treatment scheme consisting of TACE, TKI and PD-1 inhibitor is proposed with expectation to complement the deficiency of each monotherapy, and achieve synergistic antitumor effects.

ADMINISTRATION SEQUENCE AND SELECTION OF EACH MEDICATION

Only in recent years, the triple therapy of TACE + TKI + PD-1 inhibitor has been evaluated for its efficacy and safety in unresectable HCC by clinical trials (Table 1). In the study by Qin *et al*[23], TACE + TKI + PD-1 was found to significantly extended progression-free survival (PFS) and increased disease control rate (DCR) as compared to TACE + PD-1, but no significant difference in OS and objective response rate (ORR). For treatment procedure, several chemotherapeutic agents were used in TACE. Concurrent sorafenib and PD-1 inhibitor (sintilimab or camrelizumab) were administered on day 4 after the initial TACE, and then sorafenib at a 4–7-day interval before and after each subsequent TACE. By contrast, in the study of Chen *et al*[24], pembrolizumab and lenvatinib were administered before initiating TACE in which only pharmorubicin was used as the chemotherapy drug. Patients received triple therapy showed significantly longer OS and PFS than that in duplex group (TACE + TKI). Subsequently, Wang *et al*[25] reported a retrospective trial indicating that triple therapy was superior to TKI + PD-1 regarding OS, PFS, ORR and DCR. The treatment procedure differed from the above two trials. Patients received TKI (lenvatinib) after initial TACE, then PD-1 (pembrolizumab, camrelizumab, or sintilimab) within 7 days of initial TKI. The latest retrospective trial by Ma *et al*[26], reported the encouraging efficacy of triple therapy in patients, especially the longest OS (26.43 months) among mentioned trials. In this trial, TACE was initiated before the administration of lenvatinib or PD-1 inhibitors.

In summary, most of the above trials have shown encouraging results of the triple therapy in unresectable HCC, such as extended PFS and OS. The administration sequence of TACE + TKI + PD-1 varied across trials, which may affect the outcomes. Likewise, the selection of each medication was also flexible, especially the diverse PD-1 inhibitors. However, the limitations existed in them may impair the robustness. For instance, all these trials are retrospective, and the sample sizes are small. Therefore, it is still controversial whether triple therapy achieves a better prognosis for patients, meanwhile the randomized controlled trials on large populations are of requisite.

IMPLICATIONS FOR FUTURE CLINICAL TRIALS

TKI administration is scheduled to suppress tumour angiogenesis induced by TACE, thus administration timing is a key factor affecting efficacy. Since it has been reported that serum VEGF reaches maximum concentration on day 1 after TACE[14], immediate administration after TACE or even pretreatment of TKI could contribute to favorable clinical outcomes[11]. Moreover, TACE can increase the release of tumor antigens, thereby contributing to tumor-specific immune response. So, it may favor that scheduling the administration of PD-1 inhibitors closing to TACE to make the most of an immune support environment induced by TACE. Therefore, the administration sequence between TACE, TKI and PD-1 inhibitor is an important variable affecting the clinical benefit, which needs to be fully considered in future trials.

Most trials are single-center studies and limited by the relatively small sample size. Future studies should be multicentered and conducted on large populations. In addition, the varied kinds of each medication may affect the consistency of treatment regimens. The agents used in each therapy should be consistent or well balanced, and subgroup analysis should be conducted if needed in future trials. Although most trials are double-arm studies, the control arms across trials are differed, *i.e.*, TACE + TKI, TACE + PD-1, TKI + PD-1. The control arm should be carefully designed in future trials since it contributes significantly to the results.

CONCLUSION

This work introduces the interaction mechanisms of TACE + TKI + PD-1 inhibitor, reviews the administration sequence and selection of each medication across trials, and discusses the implications for future clinical trials. We hope that this editorial could contribute to the design and optimization of future trials.

Table 1 Characteristics of clinical trials evaluating the transarterial chemoembolization combined with multi-targeted tyrosine kinase inhibitor and programmed cell death protein-1 inhibitor for unresectable hepatocellular carcinoma

Ref.	Study design	Sample size	Hepatocellular carcinoma stage	TACE	TKI	PD-1	Administration sequence	Outcomes
Qin *et al* [23], 2022	Retrospective, double-arm	25 (TACE + TKI + PD-1) *vs* 41 (TACE + PD-1)	Advanced	Pirarubicin, epirubicin, loplatin, raltitrexed	Sorafenib	Sintilimab or camrelizumab	TKI+PD-1 after initial TACE, TKI before and after each subsequent TACE *vs* PD-1 after initial TACE	OS: 21.63 months *vs* 16.43 months, P = 0.103; PFS: 7.63 months *vs* 2.9 months, P = 0.034; ORR: 59.09% *vs* 50%, P = 0.761; DCR: 95.45% *vs* 72.72%, P = 0.095
Chen *et al* [24], 2022	Retrospective, double-arm	70 (TACE + TKI + PD-1) *vs* 72 (TACE + TKI)	Unresectable	Pharmorubicin	Lenvatinib	Pembrolizumab	TKI+PD-1 before initial TACE *vs* TKI before initial TACE	OS: 18.1 months *vs* 14.1 months, P = 0.004; PFS: 9.2 months *vs* 5.5 months, P = 0.006
Wang *et al* [25], 2023	Retrospective, double-arm	46 (TACE + TKI + PD-1) *vs* 59 (TKI+PD-1)	Unresectable	Epirubicin, raltitrexed, oxaliplatin	Lenvatinib	Pembrolizumab, camrelizumab, or sintilimab	TKI after initial TACE, then PD-1 within 7 days of initial MKI *vs* TKI+PD-1	OS: 20.5 months *vs* 12.6 months, P = 0.015; PFS: 10.2 months *vs* 7.4 months, P = 0.035; ORR: 54.3% *vs* 25.4%, P = 0.002; DCR: 82.6% *vs* 64.4%, P = 0.038
Ma *et al* [26], 2024	Retrospective, single-arm	102 (TACE + TKI + PD-1)	Unresectable	Epirubicin, oxaliplatin, 5-fluorouracil, calcium folinate	Lenvatinib	Sintilimab, nivolumab, camrelizumab, pembrolizumab, toripalimab	TKI+PD-1 after initial TACE	OS: 26.43 months; PFS: 10.07 months; ORR: 61.76%; DCR: 81.37%
Dong *et al* [11], 2023	Retrospective, double-arm	228 (TACE + MKI + PD-1) *vs* 228 (TACE)	Unresectable	NA	Sorafenib, lenvatinib, donafenib, regorafenib, apatinib, anlotinib, bevacizumab	Atezolizumab, pembrolizumab, nivolumab, camrelizumab, sintilimab, tislelizumab, toripalimab	PD-1 at least 3 days before or after TACE, TKI within two weeks before or after TACE	OS: 19.2 months *vs* 15.7 months, P = 0.037; PFS: 9.5 months *vs* 8.0 months, P = 0.015; ORR: 60.1% *vs* 32.0%, P < 0.001

DCR: Disease control rate; TACE: Transarterial chemoembolization; TKI: Tyrosine kinase inhibitor; PD-1: Programmed cell death protein-1; MKI: Multikinase inhibitors; OS: Overall survival; ORR: Objective response rate; PFS: Progression-free survival.

FOOTNOTES

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EDITORIAL

Unveiling the clinicopathological enigma of crawling-type gastric adenocarcinoma

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Abstract

In this editorial we comment on the article by Xu *et al*. Gastric adenocarcinoma (GA) is a malignancy which arises from the gastric mucosa and encompasses heterogenous tumors with varying characteristics. There are two main classifications: Lauren's and the World Health Organization distinguishing the diverse types of GA depending on clinical, genetic, morphological and epidemiological features. "Crawling-type" adenocarcinoma (CRA) is a subtype characterized by irregularly fused glands with low-grade cellular atypia. Moreover, CRA represents differentiated tumor cells resembling intestinal metaplasia which results in misdiagnosis. The diagnosis is of utmost importance, as well as the subclassification and thorough pathological assessment. With regard to the symptoms of GA, these depend on the stage of the disease. Diagnostic methods play a crucial role in assessing the extent of the tumor and the stage of the disease. Nevertheless, early detection of CRA remains challenging due to its histological features. In summary, CRA is a distinct type of GA with particular clinicopathological and histological characteristics. Despite its significance, it not distinguished as a subtype, resulting in diagnostic challenges. Diagnosis is based on careful observation and thorough biopsy analysis, indicating the importance of comprehensive pathological assessment.

Key Words: Crawling-type adenocarcinoma; Clinicopathological characteristics; Histological features; Gastric adenocarcinoma; Diagnosis; Treatment

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Core Tip: Crawling-type adenocarcinoma has specific features, such as irregularly fused glands and low-grade cellular atypia, resembling intestinal metaplasia. Given this, precise attention to its histological characteristics is crucial when diagnosing "crawling-type" adenocarcinoma (CRA). Early detection of CRA is challenging which leads to the importance of the detection of molecular markers and thorough biopsy analysis for accurate classification and clinical management strategies.

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INTRODUCTION

In this editorial, we comment on the article published on the *World J Gastrointest Oncol*. Gastric adenocarcinoma (GA) is a malignant neoplasm located in the epithelium, which emerges from the gastric mucosa and is characterized by differences in the glands. It represents a biologically diverse group of malignant neoplasms in terms of morphology, histogenesis, molecular features and etiology[1]. Moreover, GA is defined by low-grade cellular atypia and irregular glandular anastomosis. It is important to note that GA is the third cause of cancer-related deaths and the fifth most common cancer[2]. While there has been a decline in its incidence and mortality, in some regions, gastric cancer (GC) prevalence remains high, particularly in Asia[3,4]. Intestinal histology is more common among Caucasians, whereas gastric tumors located in the cardia are less frequent in Africa and Latin America[4]. Risk factors for GC include both nonmodifiable factors such as sex, age, genetics and race as well as controllable factors like *Helicobacter pylori* infection, lifestyle, diet and exposure to certain chemicals or viruses[5]. Early detection and surveillance are crucial due to the challenging nature of diagnosing certain variants, particularly those presenting as superficial depressed or flat type tumors in the stomach. Treatment typically involves surgical interventions, including endoscopic mucosal resection or various forms of gastrectomy, depending on the location and stage of the tumor. Chemotherapy, either alone or in combination with surgery, is a common treatment approach, aimed at improving patient outcomes, particularly for those diagnosed at advanced stages[3].

There are two commonly used morphological classifications; the Lauren's and the World Health Organization (WHO). More specifically, the Lauren classification categorizes GA into diffuse type (33%), intestinal type (53%) and intermediate type (14%)[6,7]. This classification depends on the different clinical features, morphology, genetics, epidemiology, and expansion ability[8]. The diffuse type involves poorly cohesive single cells without gland formation, whereas the intestinal type has glandular and tubular components[8]. The WHO subdivides GAs into papillary, micropapillary, tubular, parietal cell, mucinous, mixed type, mucoepidermoid, hepatoid adenocarcinoma, poorly cohesive (including signet-ring cell carcinoma), Paneth cell and medullary carcinoma[2,7].

Gastric "crawling-type" adenocarcinoma (CRA) is a subtype of GA that constitutes a specific histological pattern and has specific clinicopathological features[9]. More specifically, it is characterized by irregularly fused glands with low-grade cellular atypia that spreads in the mucosa[10,11]. Furthermore, it is described by its growth in branching, tortuous, distending, spiky, abortive, and anastomosing patterns with glandular outgrowth. Additionally, the histological findings of CRA are well-differentiated tumor cells that mimic intestinal metaplasia, and it is cytologically low-grade. The lesions of this distinct type appear as ill-demarcated depressed lesions and the tumor glands are mainly in the middle third of the stomach with occasional signet-ring cells[10,12]. Another important histological diagnostic characteristic is that the shape of CRA glands recreate the letters W, H, Y and X[13]. Importantly, many cases of CRA are often misdiagnosed because the cells show minimal cellular atypia with extension into the epithelial proliferative zone but with sparing of the mucosal surface[10]. Given the subtle cytological abnormalities, tumor glands may resemble "intestinal metaplasia", a benign condition. Consequently, discriminating this form of GC from non-neoplastic lesions like intestinal metaplasia can be a huge challenge for both endoscopic and histological assessments. It is of outmost importance to remain vigilant for this variant, especially when encountering superficial depressed or flat type tumors in the midsection of the stomach[3]. In this Editorial we aimed to elucidate the clinicopathological features and histological characteristics of CRA. The uniqueness of the current investigation is based in its comprehensive exploration of CRA. What sets this investigation apart is its meticulous examination of the clinical and histological characteristics of CRA, emphasizing its subtle cytological abnormalities that contribute to frequent misdiagnosis. Moreover, the study emphasizes the necessity of utilizing various diagnostic modalities, contributes to our understanding of GC pathology and highlights the need for observation and biopsies for accurate diagnosis.

CLINICOPATHOLOGICAL AND HISTOLOGICAL CHARACTERISTICS OF CRAWLING-TYPE ADENOCARCINOMA

Diagnosis of GA is of foremost importance for staging and treatment determination. Initially, the subclassification of malignancies may indicate the treatment of the disease as cases with intestinal-type GA are prone to overexpression of human epidermal growth factor receptor-2 (HER2). Additionally, the pathologic report must involve the tumor grade and invasion as these are necessary for staging of the disease. Moreover, newly diagnosed patients should undergo universal testing for microsatellite instability by polymerase chain reaction/next-generation sequencing or mismatch repair (MMR) deficiency by immunohistochemistry. Finally, the tumor diameter and depth of invasion, the lymphovascular invasion and the mucosal and deep margin status should be included in the pathology report of endoscopic mucosal resection[14].

Studies have shown that crawling-type adenocarcinoma is distinguished by low-grade nuclear atypia and a morphology that mimics intestinal metaplasia with a laterally spreading pattern. The glands of this subtype are included in the characteristics of extremely well-differentiated adenocarcinomas of the stomach[15]. It is of utmost importance to note that not only the CRA tumor is superficial flat or a depressed type but also the margin is often indistinct[15,16]. On histological examination, the irregularly fused glands with architectural features similar to branching are the most important diagnostic key[15].

A study conducted by Okamoto *et al*[11] evaluated 25 crawling type GC (CTACs) consisting of 16 intramucosal and 9 submucosal invasive cancers. The results showed that CTACs were more often located in the middle third of the stomach. Histologically, all CTACs displayed cystic dilated glands and 16 lesions exhibited focal signet-ring cells. Invasive areas of the submucosal CTACs were characterized by poorly differentiated adenocarcinoma with an infiltrative growth pattern and abundant stroma[11].

Most importantly, the stage and the topography of GA are related to the clinical presentation of the disease[1]. The majority of people with early GC are asymptomatic but they are successfully diagnosed by screening programs, resulting in a better survival rate[1]. A study examined a total of 51 lesions classified as CRA and 126 categorized as conventional differentiated adenocarcinoma (CDA) were identified. There were notable disparities in tumor location frequency between CRA (62.8%) and CDA (36.5%). CRAs were situated in the middle third of the stomach compared to CDAs. Depressed type tumors were more prevalent in CRA (72.5%) than in CDA (36.5%)[1]. Immunohistochemistry findings such as β-catenin nuclear expression was notably less common in CRAs (2%) compared to CDAs (30.3%). Similarly, the occurrence of loss of MLH-1 expression was lower in CRAs (3.9%) compared to CDAs (15.1%). The frequency of TP53 mutations was notably higher in CRAs (37.3%) compared to CDAs (7.9%)[1]. On the contrary, physical examination may not be of such significance in the early stage of the disease[17]. Notably, symptoms such as abdominal mass, dyspepsia, epigastric pain, weight loss, emesis, nausea, dysphagia, and gastrointestinal hemorrhage are frequent at advanced disease stages[1,17,18].

CRA is frequently misdiagnosed as a benign non-neoplastic lesion, such as intestinal metaplasia, by pathologists. Furthermore, CRA is related to particular histological features and clinicopathological characteristics, but it is not included as a distinct histological subtype in the WHO classification[9].

Fujita *et al*[9] in their study compared the clinicopathologic and molecular characteristics of CRA and those of CDA by examining 51 lesions from patients with CRA and 126 lesions from patients with CDA[9]. It was observed that CRAs were located more in the middle third of the stomach than CDAs. Also, the CRA tumors were depressed type and larger than the CDA tumors. Additionally, the frequency of tumors with a mixed differentiated and poorly differentiated adenocarcinoma component was statistically higher for CRA than CDA[9]. Moreover, in this study it was observed that the loss of MLH-1, a MMR protein, and nuclear accumulation of β-catenin were not often found in CRA compared with CDA. Finally, it was shown that TP53 mutation was related to CRA pathogenesis, and that the presence of multiple allelic imbalances was linked to early carcinogenesis of CRA[9].

Another study conducted by Woo *et al*[10], involved 94 patients with CRA, 72 patients with CDA and 71 patients with poorly cohesive adenocarcinoma (PCA)[10]. It was observed that the rate of younger patients with CRA was greater compared with CDA. In addition, the size of the tumors was larger in CRA than in CDA. Furthermore, this study showed a nonsignificant difference in tumor location among CRA patients and CDA patients[10]. Also, CRAs and PCAs did not have loss of expression of the MMR proteins and showed negativity for HER2. In addition, MET overexpression was observed in CRA at a rate 4.4%, in PCA at a rate 2.8% and 19.4% in CDA. Also, EGFR overexpression in CRAs was 36.5%, in CDAs was 31.4% and in PCAs was 31%. Additionally, diffuse, and strong positive or complete loss of p53 expression was noted in 12.4% of CRA, 8.5% of PCA and 62.55% in CDA. Lastly, a minor component of poorly differentiated adenocarcinoma was observed in 25.5% of CRA cases[10]. The study by Joshi and Badgwell[18], compared the clinicopathological characteristics of CRA and CDA patients. CRAs tended to occur in younger patients compared to CDAs, the tumor sizes ranged from 0.1-8.4 cm, with CRAs generally larger than CDAs[14]. Among the 237 cases evaluated, all CRA cases displayed either negativity (89.8%) or weak positivity (10.2%)[18].

The gold standard method for GC diagnosis is endoscopic examinations of the gastric mucosa and biopsy of suspicious lesions[1,15,17]. In addition, endoscopic ultrasound (EUS) plays a significant role in the diagnosis and staging of the tumor as it can assess the depth of the tumor, and identify suspected metastasis[17,18]. Also, laparoscopy is particularly important in the peritoneal staging of GA as it allows for biopsy of the lesions and visualization of the peritoneal surface [17]. Another method of evaluating indeterminate lesions is fluorodeoxyglucose-positron emission tomography (FDG-PET)/computed tomography[18]. Diagnosis of CRA is difficult in the early stage due to low-grade nuclear atypia and the results of endoscopic resection can be incomplete with positive lateral margins[15].

CONCLUSION

In conclusion, CRA is a subtype of GA with specific clinicopathological and histological characteristics. Despite these features, it is not categorized in the WHO classification as a distinct histological subtype. Notably, it is difficult to diagnose at an early stage due to misinterpretation of the tumor with other types of GA. The complexities surrounding the diagnosis and management of CRA underscore the necessity for a thorough understanding of its clinicopathological and histological characteristics. Its subtle cytological abnormalities often lead to misdiagnosis, posing significant challenges for both endoscopic and histological assessments. Moreover, studies comparing CRA with CDA have shed light on key differences in tumor location, size and molecular characteristics, emphasizing the importance of accurate diagnosis and subclassification for tailored treatment strategies. Diagnostic modalities such as endoscopic examinations, EUS, laparoscopy, and FDG-PET/CT scans play vital roles in the evaluation and staging of GA, yet early detection of CRA remains challenging due to its low-grade nuclear atypia. Thorough observation and biopsies of all mucosal layers are of great importance as these are the key to accurate diagnosis of CRA. Continued research efforts aimed at elucidating the mechanisms and diagnostic approaches are essential for improving outcomes in patients with this subtype of GC.

FOOTNOTES

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EDITORIAL

Practical hints for the diagnosis of mixed neuroendocrine-non-neuroendocrine neoplasms of the digestive system

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Abstract

In this editorial, a comment on the article by Díaz-López *et al* published in the recent issue of the 2024 is provided. We focus on the practical implications critical for providing a correct and complete diagnosis of mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) in the gastrointestinal system. The diagnosis of MiNEN begins with the recognition of neuroendocrine features in one component of a biphasic tumor. The non-neuroendocrine counterpart can be virtually represented by any neoplastic type, even though the most frequent histologies are glandular and squamous. However, qualification of the neuroendocrine component requires histological and immunohistochemical confirmation. Neuroendocrine tumors are characterized by a peculiar architectural organization and bland nuclei with granular "salt and pepper" chromatin. Although neuroendocrine carcinomas have multiple and variable presentations, they typically show a solid or organoid architecture. The histological aspect needs to be confirmed by immunohistochemistry, and a diagnosis is confirmed whenever the expression of keratin and neuroendocrine markers is observed. Once both histopathological and immunohistochemical features of neuroendocrine neoplasms are identified, it is important to consider the three major pitfalls of MiNEN diagnostics: (1) Entrapment of neuroendocrine non-neoplastic cells within the tumor mass; (2) Differential diagnosis with amphicrine neoplasms; and (3) Differential diagnosis of tumors that partially express neuroendocrine markers. According to the current guidelines for diagnosing digestive MiNEN, each component must represent at least 30% of the entire neoplastic mass. Although the high-grade histopathological subtype frequently determines disease prognosis, both components can significantly affect prognosis. Thus, if one of the components, either neuroendocrine or non-neuroendocrine, does not fulfill the volumetric criteria, the guidelines still encourage reporting it. These strict criteria are essential for correctly recognizing and characterizing digestive MiNENs. This task is essential

because it has prognostic relevance and substantial potential value for guiding further studies in this field. In the future, systematic analyses should be performed to validate or reconsider the current 30% cutoff value.

Key Words: Mixed neuroendocrine-non-neuroendocrine neoplasm; Digestive system; Neuroendocrine neoplasm; Immunohistochemistry

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Core Tip: Mixed neuroendocrine-non-neuroendocrine neoplasms are a heterogeneous group of neoplastic diseases that share histological and immunohistochemical features. The most important factor is the uncertain presence of a neuroendocrine component. The presence of entrapped neuroendocrine cells, differential diagnosis of amphicrine neoplasms, and neuroendocrine expression in non-neuroendocrine carcinoma can lead to misdiagnosis. Current guidelines require the fulfillment of volumetric criteria, but the prognostic relevance of the current cutoff remains to be proven.

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INTRODUCTION

Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNENs) are mixed neoplasms consisting of two components, one neuroendocrine and the other frequently showing an epithelial nature, with features that overlap with those of their *pure* counterparts in the same region[1]. Neuroendocrine neoplasms represent a particular and heterogeneous group of malignancies that share histological and immunohistochemical profiles and present some site-based specificities[1]. It is crucial to identify some practical features that support the diagnosis of MiNENs in routine clinical practice. These were appropriately presented and discussed by Díaz-López *et al*[2] in their recent manuscript, however some considerations should be added.

MiNENs refers to a diagnostic category rather than a specific diagnosis. It refers to a neoplastic lesion with two recognizable components, one of which must present neuroendocrine differentiation[1,3], which must be assessed through an examination[1,3]. The typical histomorphology of neuroendocrine differentiation [well-differentiated neuroendocrine tumors (NETs)] includes an architecture showing organoid, nested, ribbon-like, or trabecular growth patterns; neoplastic cells with eosinophilic cytoplasm; and monomorphic nuclei with "salt and pepper" chromatin. A few mitoses and the absence of necrosis are typical features[1-6]. Conversely, high-grade neuroendocrine carcinomas typically harbor two different cellular morphologies (small cell and large cell features), sometimes within the same tumor mass. Small-cell neuroendocrine carcinoma has a distinct histomorphology characterized by a solid and/or sheet-like growth pattern, with atypical round or spindle cells that present scant cytoplasm, hyperchromatic nuclei, inconspicuous nucleoli, and nuclear molding. As suggested by the name, the cell size is small and typically does not exceed three times the dimensions of a resting lymphocyte. Large-cell neuroendocrine carcinomas differ in their larger cell size and different growth patterns; they are often nest-like with peripheral palisading tumor cells and usually exhibit eosinophilic cytoplasm, polymorphous nuclei, prominent nucleoli, and vesicular chromatin[1,3,7-11].

Neuroendocrine differentiation must be confirmed by immunohistochemical analysis. The most sensitive markers are synaptophysin, insulinoma-associated protein 1, and chromogranin A, which sometimes present diminished intensity in poorly differentiated neuroendocrine neoplasia (NEN)[1,3,12,13]. Such markers are beneficial and have reliable staining patterns in a biopsy setting[14]. The epithelial origin of neoplastic cells should also be proven by staining for keratin to rule out the presence of paragangliomas[1]. To complete the final pathology report of NETs, a study of proliferative activity using the Ki-67 index (MIB-1 clone) must be conducted. The mitotic count is now considered poorly reproducible and, thus, is less meaningful than Ki-67 for diagnosis of NENs of the digestive system[1,3]. Ki-67 can be estimated using digital pathology-based tools to improve standardization, even in a biopsy setting[15].

One could ask why immunohistochemical analysis of neuroendocrine differentiation is not widely performed, considering that NENs have a heterogeneous appearance that could be misinterpreted. Immunohistochemical analysis has low specificity in unselected cases, with a risk of overdiagnosis of neuroendocrine neoplasms[11,12,16]. The three main examples of this phenomenon are as follows: (1) Since neuroendocrine cells are typically present in different tissues, normal neuroendocrine cells can be misinterpreted as part of a neoplastic process. A classic example is pancreatic insulae entrapped in pancreatic ductal adenocarcinoma or enterochromaffin-like cells intermingled with neoplastic elements in gastric cancer[17,18]; (2) The second possible misinterpretation in the neuroendocrine context is amphicrine neoplasia. An amphicrine tumor is defined as a neoplastic process in which tumor cells present both exocrine and neuroendocrine phenotypes[19-21]. An example is appendiceal goblet cell adenocarcinoma, in which the cells are both mucinous and positive for neuroendocrine markers[22,23]. Immunohistochemical, genetic, and transcriptome analyses revealed that this histological type significantly differs from adenocarcinoma, neuroendocrine neoplasms, and MiNENs in the same site.

Therefore, special attention should be given to identifying this complex entity[24]; and (3) The last example in this context is neoplasms showing aberrant or partial expression of neuroendocrine markers. For example, a solid pseudopapillary neoplasm of the pancreas is a well-known entity that can express synaptophysin and CD56; however, it is not listed as NEN[25-28]. Another example is colorectal adenocarcinoma with synaptophysin expression, a subgroup of *BRAF*-mutated colorectal adenocarcinomas that does not fulfill the histological criteria for NEN diagnosis[29]. Differentiation of this subgroup is important due to its poor prognosis, as confirmed by other investigators[30].

A PRACTICAL DIAGNOSTIC ALGORITHM FOR MiNENs

One of the most important topics in the digestive MiNEN landscape is identification of the correct diagnostic algorithm (Figure 1), as highlighted by Díaz-López *et al*[2]. The first step is to histologically identify a biphasic neoplasm with at least one component that presents both epithelial aspects and a neuroendocrine phenotype[1,3]. The second step concerns the immunohistochemical characterization of both neoplastic components using different staining, including: (1) Keratins to support the epithelial origin[31]; (2) Synaptophysin, chromogranin A, and preferably also INSM-1. In addition, CD56 is recognized as a neuroendocrine marker; nevertheless, the aforementioned markers are used more often for immunohistochemical staining because of their major sensitivity and specificity[1,3,32]; (3) Ki-67 (MIB-1 clone) is also used to investigate the proliferation index of the neoplasm[5,13,33,34]; and (4) Other potentially useful markers are used to identify the site of origin, such as CDX-2 for small bowel NENs[12,31], SATB2 for large bowel NENs and Merkel cell carcinomas[35], and Islet-1 for pancreatic neoplasms[3]. The neuroendocrine component may present challenging features that could hamper the immediate distinction between NET G3 and NEC. In those cases, immunohistochemical assessment of Rb and SSTR2, which are typically lost in NECs and retained in G3 NETs, and p53, which is more frequently altered in NECs than in G3 NETs, is helpful[4,36,37]. Moreover, pancreatic G3 NETs are enriched in *DAXX/ATRX* mutations, corresponding to a lack of immunohistochemical expression of the homonymous protein, while the expression of these proteins is conserved in NECs[37-40]. In addition, the alternative lengthening of telomeres, ALT, a vital biological mechanism important in pancreatic NETs, is never activated in NEC[39,41]. The presence of necrosis favors the diagnosis of poorly differentiated carcinomas, but it is not listed among the grading parameters of tumors in the gastrointestinal and pancreaticobiliary tracts[1,3]. The diagnosis of a MiNENs is supported whenever neuroendocrine marker expression is identified in one component of the biphasic mass. Notably, NEC can develop a glandular phenotype, but the diagnosis of MiNEN cannot be made in these patients because of the diffuse staining of neuroendocrine markers[42]. The last consideration is tumor staging for MiNEN, which is classified as a site-specific non-neuroendocrine component according to the AJCC/UICC TNM classification[43]. This information is crucial for stratifying patients according to prognosis and guiding clinical management.

PROGNOSIS AND FUTURE PERSPECTIVE OF MiNENs

The recognition of MiNENs is a critical task because there is much evidence that the two components do not behave like their isolated counterparts[41,44,45]. Generally, the high-grade histological subtype guides the prognosis of the disease; however, both components can progress and metastasize, significantly impacting the clinical history of patients with MiNENs[46].

In most districts, there is no minimum percentage of the two tumor components for establishing a diagnosis of MiNEN. For the gastrointestinal tract, the discussion is less straightforward, and the guidelines indicate that the smaller component must occupy at least 30% of the entire tumor mass[47]. Although this cutoff was arbitrarily determined when there was limited knowledge about these lesions, it has been maintained over time[2,48]. Systematic studies have not yet investigated the possibility of changing the cutoff value. Recent findings suggest that the presence of a high-grade component significantly impacts prognosis, even in cases where the high-grade component represents less than 30% of the entire mass[49]. For gastrointestinal tumors that do not satisfy the current diagnostic criteria for MiNENs, reports of the presence of smaller components (< 30%), both neuroendocrine and non-neuroendocrine, are highly recommended[46,49].

CONCLUSION

Although first described in 1924[50], MiNENs remain poorly understood. This is partly due to the rarity of these lesions and the different clinical behaviors of MiNENs at distinct sites. In addition, the misdiagnosis or underrecognition of this rare entity could have affected its identification and the overall literature on mixed neoplasms. In this editorial, inspired by the elegant manuscript by Díaz-López *et al*[2], some controversial lesions that are not real MiNENs have been highlighted, including: (1) Tumors with (scattered) neuroendocrine non-neoplastic cells; (2) Amphicrine neoplasms; and (3) Adenocarcinomas that partially or aberrantly express neuroendocrine markers.

Current guidelines/diagnostic criteria for MiNENs require that the smallest tumor component comprises at least 30% of the entire neoplastic mass. Nevertheless, smaller components (< 30%) should be reported since they can affect prognosis and may represent an additional point of inspiration for further research on MiNEN.

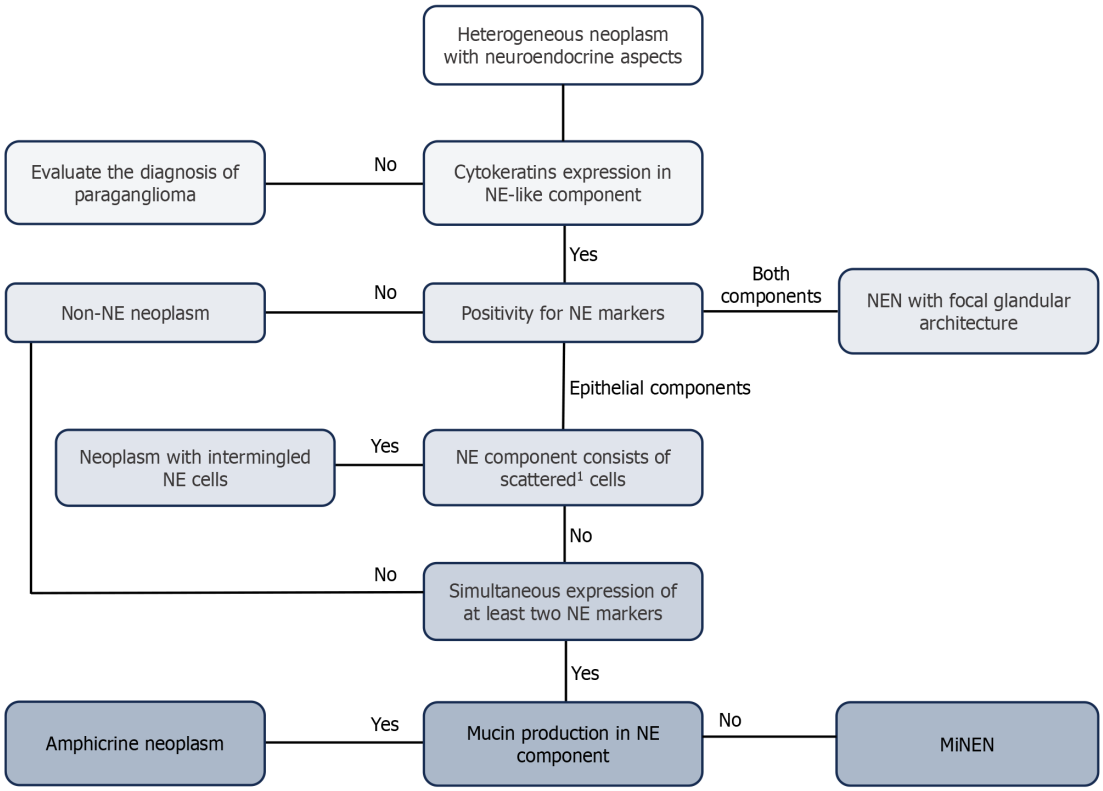

Figure 1 Diagnostic algorithm for the distinction of mixed neuroendocrine and non-neuroendocrine neoplasm. Mixed neuroendocrine and non-neuroendocrine neoplasm needs to be distinguished from neoplasms with focal neuroendocrine aspects like paraganglioma, non-neuroendocrine neoplasm with single neuroendocrine marker expression, neoplasm with interspersed neuroendocrine cells, or amphicrine neoplasm. [1]The definition of scattered cells includes, but is not exclusive of, other appearances like sheets, ribbons, micronodules, or insulae. MiNEN: Mixed neuroendocrine and non-neuroendocrine neoplasm; NE: Neuroendocrine; NEN: Neuroendocrine neoplasm.

FOOTNOTES

Author contributions: Mattiolo P contributed to this paper, designed the overall concept and outline of the manuscript, provided the discussion and design of the manuscript, and wrote the manuscript.

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EDITORIAL

Endoscopic diagnosis and management of gallbladder carcinoma in minimally invasive era: New needs, new models

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Abstract

Gallbladder cancer (GBC) is a rare and lethal malignancy; however, it represents the most common type of biliary tract cancer. Patients with GBC are often diagnosed at an advanced stage, thus, unfortunately, losing the opportunity for curative surgical intervention. This situation leads to lower quality of life and higher mortality rates. In recent years, the rapid development of endoscopic equipment and techniques has provided new avenues and possibilities for the early and minimally invasive diagnosis and treatment of GBC. This editorial comments on the article by Pavlidis *et al*. Building upon their work, we explore the new needs and corresponding models for managing GBC from the endoscopic diagnosis and treatment perspective.

Key Words: Endoscopy; Diagnosis and treatment; Gallbladder carcinoma; Minimal invasive; New need; New model

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Core Tip: Gallbladder cancer (GBC) poses a significant challenge due to its low rates of early diagnosis and high mortality. The evolving endoscopic technologies in the minimally invasive era present new possibilities for the early diagnosis and treatment of GBC, making them highly research-worthy and promising for application. The promotion and application of advanced endoscopic equipment and techniques, such as endoscopic ultrasound, magnifying endoscopy, choledochoscopy, confocal laser endomicroscopy, and natural orifice transluminal endoscopic surgery technology, are expected to offer new management models for the global demand in GBC diagnosis and treatment.

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INTRODUCTION

Despite being relatively rare, gallbladder cancer (GBC) is the most common malignancy of the biliary tract and is globally recognized for its poor prognosis[1,2]. Recent studies have found relatively higher GBC mortality rates in countries with medium human development index and high-income Asia-Pacific regions[1,3]. Additionally, there has been a rising trend in GBC mortality after an initial decline in some high-income countries[4].

Although regional and demographic variations in GBC are pronounced, the global burden of this cancer is expected to rise considerably in the next two decades, a trend that merits serious attention and contemplation[3,5]. The poor treatment outcomes for GBC patients are not mainly due to inadequate surgical techniques or the absence of therapeutic modalities such as chemotherapy, immunotherapy, targeted therapy, and radiotherapy. The primary issue lies in the low rate of early diagnosis of GBC, which results in futile attempts at various treatment modalities at an advanced cancer stage. From this perspective, the most cost-effective approach to improving GBC patients' five-year survival rate and quality of life (QoL) would be early diagnosis followed by subsequent minimally invasive treatment. Advanced endoscopic equipment and techniques with rapid developments in recent years, such as endoscopic ultrasound (EUS), magnifying endoscopy, choledochoscopy, confocal laser endomicroscopy (CLE), and natural orifice transluminal endoscopic surgery (NOTES) technology, offer new possibilities for achieving the abovementioned objectives[6]. We are very interested in the review by Pavlidis *et al*[7] published in a recent issue of *World Journal of Gastrointestinal Oncology*. In the ever-evolving landscape of digestive oncology, this enlightening review reiterates the emerging trends in diagnosing and treating GBC. We thank Pavlidis *et al*[7] for their review, which has raised attention to new trends in diagnosing and treating GBC.

ENDOSCOPIC DIAGNOSIS AND MANAGEMENT

Reference Citation Analysis (*RCA*, https://www.referencecitationanalysis.com/) is a unique artificial intelligence system for citation evaluation of biomedical literature. RCA has been employed to analyze previous studies of GBC's endoscopic diagnosis and management to April 2024. Published research in this field primarily focuses on early endoscopic diagnosis and its comparison with traditional examinations, palliative endoscopic treatment for advanced-stage patients, and minimally invasive endoscopic surgery for gallbladder diseases.

First, early diagnosis of GBC is paramount for improving patients' far-from-ideal five-year survival rates and QoL. However, preoperative diagnosis of neoplastic gallbladder polyps and gallbladder wall thickening remains a challenge, and the application value of a 1 cm threshold as a surgical indication for cholecystectomy in gallbladder polyps has also been questioned[8]. Despite the availability of various diagnostic methods for GBC, such as abdominal ultrasound, computed tomography, magnetic resonance imaging, positron emission tomography computed tomography, or 18F-FDG positron emission tomography-magnetic resonance imaging, it is regrettable that these methods have not significantly improved the early detection rate of GBC. Compared to these methods, as Pavlidis *et al*[7] mentioned, endoscopic techniques enable pathological diagnosis of GBC. Moreover, current endoscopic techniques allow visualization of lesions inside the gallbladder, facilitating targeted biopsies such as EUS-guided fine needle aspiration/fine needle biopsy to obtain cancer cells for pathological confirmation, thereby definitively diagnosing GBC and improving accuracy[9,10]. Recent research has also demonstrated the safety and diagnostic efficacy of transpapillary biopsies, NOTES biopsies, and EUS-guided fine needle aspiration/fine needle biopsy of gallbladder lesions[11]. The use of advanced imaging techniques like EUS, magnifying endoscopy, chromoendoscopy, and CLE, coupled with targeted biopsies within the gallbladder inner wall, facilitates the management of high-risk gallbladder polyps, eliminating high-risk factors and attaining primary prevention goals for GBC[12,13].

Second, palliative endoscopic treatment for patients with advanced GBC is one of the earliest areas involved in GBC endoscopic therapy. The previous endoscopic treatments for advanced GBC patients primarily aimed to improve biliary obstruction through endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangioscopy and alleviate gastric outlet obstruction *via* endoscopic dilation or gastrointestinal stenting. With the advent of EUS, SpyGlass™ Direct Visualization system, lumen-apposing metal stents, and other novel endoscopic equipment, the specific procedures and success rates of the above operations have been optimized[14]. Moreover, innovative and effective endoscopic diagnostic and therapeutic methods have emerged, including EUS-guided gastroenterostomy, EUS-guided celiac plexus neurolysis, photodynamic therapy, radiofrequency ablation, and intraluminal brachytherapy[15,16]. These advances contribute to more minimally invasive and effective treatment goals for patients with advanced GBC, such as pain relief, suppressing tumor progression, and improving gastrointestinal and biliary obstructions.

Third, advancing endoscopic equipment and techniques have offered expanded possibilities for minimally invasive endoscopic surgery for gallbladder diseases. Current endoscopic methods have been able to achieve a series of medical objectives in the diagnosis and treatment of acute cholecystitis, gallbladder stones, gallbladder polyps, and gallbladder

tumors, such as targeted biopsy for definitive diagnosis, removal of stones or polyps, and alleviation of obstruction or pain symptoms[12,17]. Treating gallbladder inflammation, polyps, and stones through endoscopic procedures helps prevent or reduce the occurrence of GBC. Moreover, a systematic review found that patients with T1a GBC had a 5-year survival rate of up to 100% after cholecystectomy alone[18]. From this perspective, endoscopic cholecystectomy *via* NOTES could theoretically be an effective and minimally invasive option for early-stage GBC (Tis and T1a) patients. Even more, implementing a whole-process management approach that encompasses early endoscopic detection of GBC (Tis and T1a) in high-risk patients, minimally invasive endoscopic surgical removal of the gallbladder, and endoscopic postoperative surveillance may establish a novel model for the entire management of these early-stage GBC patients using endoscopic techniques.

NEW NEEDS AND NEW MODELS

"Prevention is always better than cure", this adage also holds for GBC. In the current landscape of diverse and often conflicting health information, accessing accurate and beneficial cancer prevention information has become an urgent need for people worldwide, especially those at high risk of GBC. Public health initiatives aimed at educating people about risk factors associated with GBC, such as obesity, gallstones, chronic inflammation of the gallbladder, and factors contributing to increased mortality rates like the consumption of red meat, can help reduce the incidence and improve the prognosis for GBC patients[19-22]. Moreover, promoting regular check-ups, particularly in high-risk regions and populations, can lead to early detection and better outcomes.

In the context of increasing health consciousness and elevated expectations for medical efficacy, there is a significant and growing demand for a comprehensive and integrated suite of medical services, particularly in regions with a high prevalence of GBC. These services should encompass GBC risk prediction, differential diagnoses for suspicious lesions, precise preoperative staging, minimally invasive intervention options, and comprehensive postoperative surveillance, all geared towards the early detection and diagnosis of this relentless killer to enhance treatment success and prolong survival[19]. The advantages of endoscopy in direct visualization and targeted biopsy of early-staged GBC, eradicating high-risk factors, removal of early-stage lesions, and high-quality postoperative surveillance are indispensable in meeting these needs. For patients with advanced GBC, palliative care is crucial for improving their QoL. Pain management, symptom control, and psychological support are vital components of palliative care. Further integrating and standardizing applicable palliative treatments can substantially improve patients' sense of well-being during and after therapy [15,23,24].

Meanwhile, the complexity of GBC necessitates a collaborative effort among various medical disciplines. A multidisciplinary team of gastroenterologists, surgeons, endoscopists, oncologists, radiologists, pathologists, and support staff can provide comprehensive medical care that addresses the entire spectrum of GBC management[25,26]. These professionals can ensure that GBC patients receive the most appropriate and effective treatment plans based on the latest research and individualized needs. Another fascinating development in the field is the potential integration of artificial intelligence technologies and machine learning methods into GBC practice. These advanced technologies have seen rapid progress in recent years and promise to improve early diagnosis and prognosis prediction in GBC patients[27,28]. The multidisciplinary team may more accurately identify and characterize lesions, predict disease progression, and develop personalized treatment plans using artificial intelligence and machine learning.

To achieve early diagnosis of GBC for optimal treatment outcomes, our endoscopy center recommends incorporating EUS examination of the gallbladder inner wall as a quality control indicator for high-risk GBC patients. We also advocate using EUS-guided elastography, contrast-enhanced EUS, trans-papillary biopsy, and NOTES biopsy when necessary. Additionally, we recommend regular endoscopic follow-up, including EUS, for post-cholecystectomy GBC patients to improve the quality of postoperative monitoring. Our explorations may provide valuable insights for endoscopic practices in GBC patients, and we expect to share our work with colleagues worldwide once we have gathered sufficient data.

CONCLUSION

In conclusion, the endoscopic diagnosis and management of GBC have seen significant advancements in recent years, with a growing emphasis on early detection and minimally invasive treatment options. With the ongoing advancements in endoscopic equipment and techniques, further research and exploration are likely to lead to more minimally invasive and high-quality approaches to meeting the medical needs of GBC patients.

FOOTNOTES

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REVIEW

Advances in the diagnosis and treatment of MET-variant digestive tract tumors

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Abstract

The receptor tyrosine kinase encoded by the *MET* gene plays an important role in various cellular processes such as growth, survival, migration and angiogenesis, and its abnormal activation is closely related to the occurrence and development of various tumors. This article reviews the recent advances in diagnosis and treatment of MET-variant digestive tract tumors. In terms of diagnosis, the application of next-generation sequencing technology and liquid biopsy technology makes the detection of MET variants more accurate and efficient, providing a reliable basis for individualized treatment. In terms of treatment, MET inhibitors such as crizotinib and cabotinib have shown good efficacy in clinical trials. In addition, the combination of immunotherapy and MET inhibitors also demonstrated potential synergies, further improving the therapeutic effect. However, the complexity and heterogeneity of drug resistance mechanisms are still one of the difficulties in current research. In the future, it is necessary to further deepen the understanding of the mechanism of MET variation and explore

new combination treatment strategies to improve the overall survival rate and quality of life of patients. The diagnosis and treatment of MET-variant digestive tract tumors are moving towards precision and individualization, and have broad application prospects.

Key Words: Digestive tract neoplasms; Interstitial epithelial transfer factor; Targeted therapy; MET variant; Survival prognosis

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Core Tip: Our study reviews the progress in the use of MET variation in the diagnosis and treatment of digestive tract tumors and discusses the molecular mechanism of MET variation and its role in the genesis and development of digestive tract tumors. The analysis focused on the potential of MET variants as diagnostic markers and therapeutic targets, covering the latest research results and clinical trial data for MET-based targeted therapies. This paper also summarizes the current challenges and future research directions, aiming to provide new ideas and references for improving the diagnostic accuracy and treatment effectiveness of MET-variant digestive tract tumors.

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INTRODUCTION

Gastrointestinal tumors account for more than 50% of the global incidence and mortality of malignant tumors[1-4]. In addition to conventional surgery, chemotherapy and radiotherapy for the treatment of digestive tract tumors, precise targeted therapy guided by molecular typing has also achieved precise effects in clinical practice[5-8]. In addition to classical RAS, human epidermal growth factor receptor 2 (HER2) and other molecular targets, rare or rare mutation targets such as MET, ROS1, ALK, NTRK and other related drugs have been put on the market one after another and have achieved significant efficacy, becoming one of the current research hotspots in this field[9-12]. This article reviews recent progress in the diagnosis and treatment of MET mutations in patients with digestive tract tumors and explores the application prospects of anti-MET targeted therapy in the field of the digestive tract[13-16].

MET GENE AND ITS EPIDEMIOLOGICAL CHARACTERISTICS

c-MET is a member of the receptor tyrosine kinase family that is expressed mainly in epithelial cells and is structurally divided into an extracellular region, a transmembrane helical domain and an intracellular region, and the extracellular SEMA domain is a key region for ligand binding[17-20]. The hepatocyte growth factor (HGF) synthesized and secreted by mesenchymal cells is the only known ligand of MET[21-24]. HGF and MET receptors bind specifically to the extracellular domain, and the conformation of the MET protein changes to activate the PTK domain of the intracellular tyrosine protein kinase[25-28]. Thus, two phosphorylation sites, Try1234 and Try1235, in the intracellular kinase active region of MET receptors recruit and phosphorylate a variety of effector proteins after phosphorylation[29-32]. Many previous studies have confirmed that the c-MET signaling pathway plays an important role in tumor proliferation, invasion, metastasis, angiogenesis, tumor treatment tolerance, *etc.*, and is abnormally expressed in a variety of digestive tract tumors[33-36] (Figure 1). Therefore, targeted MET therapy may become a new choice for the specific molecular classification of digestive tract tumors[37-40].

The detection rate of *MET* gene variation in patients with solid tumors in China is low, which conforms to the category of rare mutations[41-44]. Large-scale second-generation sequencing (NGS) analysis revealed *MET* gene abnormalities in 10445 cancer patients in China; the incidence of MET amplification was 0.9% (141/10445), and that of exon 14 jump mutations was 0.7%[45-48]. MET amplification is most common in hepatocellular carcinoma (1.7%), gastric cancer (1.3%), and non-small cell lung cancer (NSCLC) (0.7%), whereas MET exon 14 jump mutations are most common in NSCLC (0.5%), hepatocellular carcinoma (0.3%), and colorectal cancer (0.2%)[49-52]. The incidence of MET fusion mutations is even lower[53-56]. A retrospective study in China revealed that the detection rate was only 0.15%, and the heterogeneity of fusion partner genes was very strong[57-60]. National and international guidelines currently identify MET14 exon jump mutations and increased *MET* gene copy number as potential targeted therapeutic molecular features of NSCLC[61-64]. In recent years, major breakthroughs have been made in the precision treatment of digestive tract tumors, including clinical studies on the treatment of rare mutations, including HER2 and MET, which have also achieved satisfactory survival benefits[65-68].

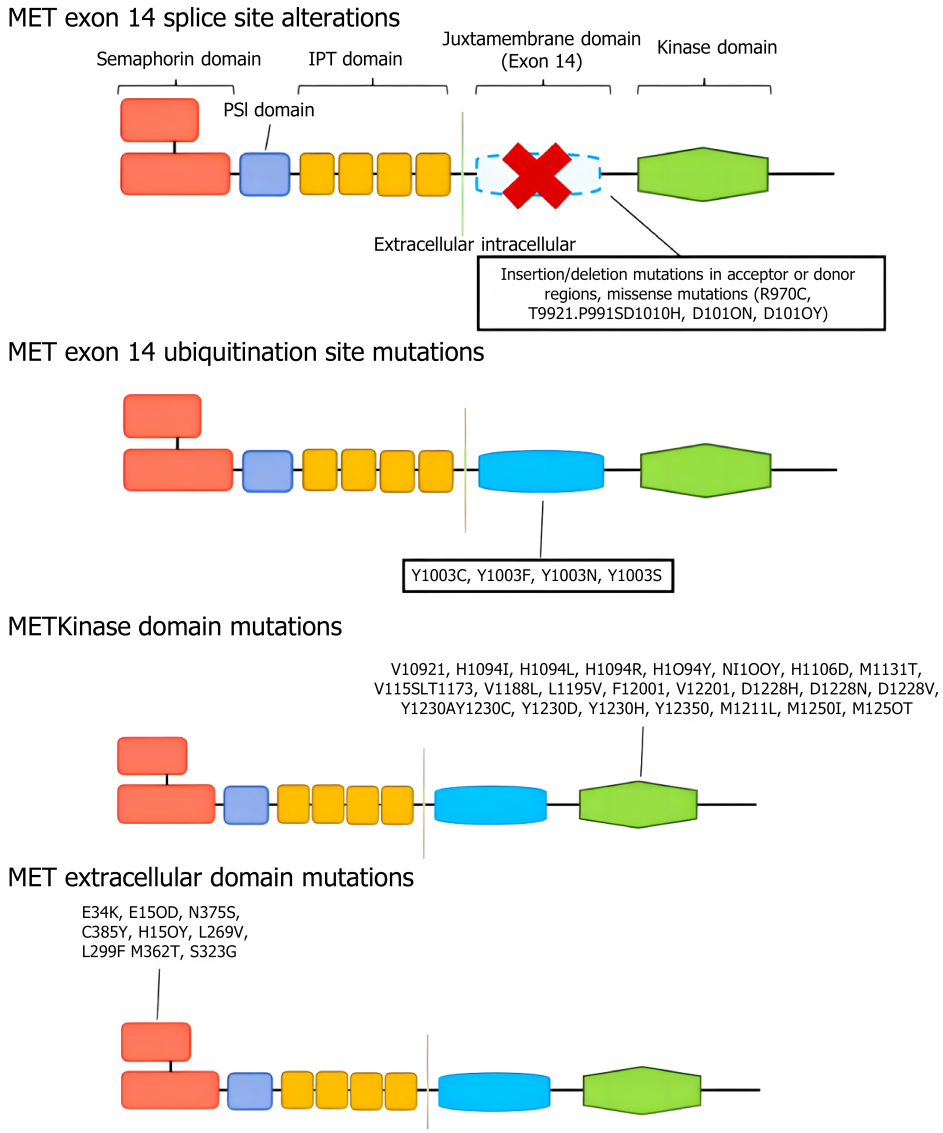

Figure 1 MET mutations. MET mutations include point mutations, insertion mutations, amplification and other types. Common mutation sites are mainly concentrated in tyrosine kinase domains (such as Exon 14 hops) and extracellular domains, which lead to abnormal activation of MET signaling pathway.

Forms of MET variation and its detection

MET variation mainly refers to *MET* gene mutations (mainly MET exon 14 jump mutations), MET gene amplification and MET protein overexpression[69-72]. MET mutations were first identified in patients with hereditary papillary renal cell carcinoma with germline mutations V1092I, H1094R/Y, M1131T, V1188L, V1220I, M1250T, and D1228H/N/V[73-76]. Mutations in the MET kinase domain increase kinase activity, leading to phenotypic transformation or the formation of tumor foci[77-80]. When exon 14 jumps, the Y1003 and c-CblE3 ubiquitin ligase binding sites are missing, resulting in reduced ubiquitination, increased MET stability and continued activation, and the gene eventually becomes a carcinogenic agent[81]. The incidence of MET14 exon spikes in the general population ranges from 0.9% to 4.0%, but the incidence of MET14 exon spikes in lung sarcomatoid carcinoma is greater, ranging from 5% to 32%[82-85]. At present, the detection methods for *MET* gene mutations mainly include NGS and reverse transcription polymerase chain reaction[86-89]. MET amplification is a duplication of a gene or a gene in a region that is not replicated on chromosome 7, resulting in increased MET expression and the activation of downstream signaling pathways independent of ligands. Notably, a gene copy number increase refers to chromosome 7 polysomy and *MET* gene amplification[90-93]. Gene amplification occurs in a variety of tumors, including lung cancer, gastric cancer, bowel cancer, and liver cancer[94-97] (Figure 2). The incidence of primary amplification is approximately 2%-10%, and the prognosis is poor[98-101]. Fluorescence *in situ* hybridization is the current standard method for detecting MET amplification and can detect local amplification and multisoma; real-time fluorescence quantitative polymerase chain reaction and NGS can also be used for detecting MET amplification[102-106]. In different studies, there were differences in the threshold of MET amplification, and MET/CEP7 was reported to have low expression between 1.8 and 2.2, intermediate expression between 2.2 and 5.0, and high expression > 5[107-111]. In general, the greater the degree of MET amplification and the lower the proportion of other driver mutations are, the stronger the driver[112-116]. MET polysoma was defined as MET polysoma when the gene copy number was ≥ 5 but the MET/CEP7 ratio was < 2[117-120]. The increased copy number of the *MET* gene caused by

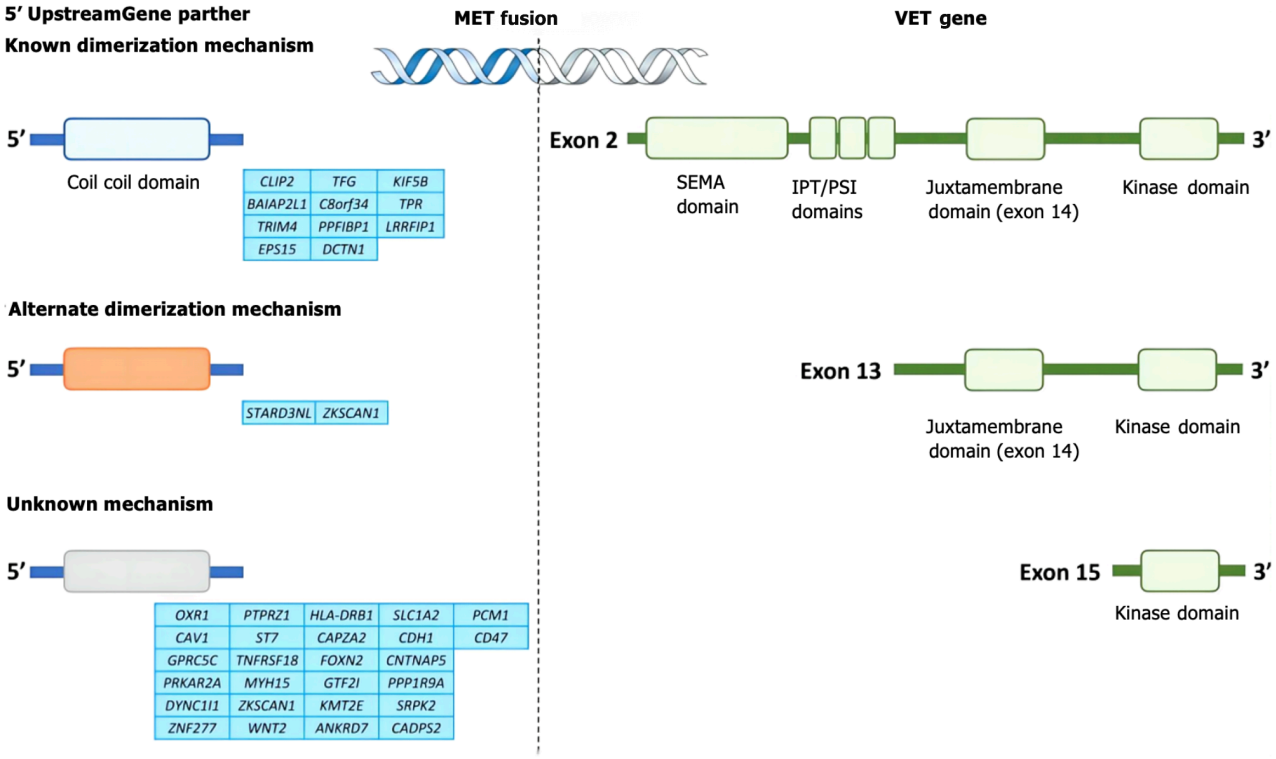

Figure 2 MET fusion. MET fusion refers to the gene fusion between *MET* gene and other genes. Common fusion partner genes include *TPR*, *KIF5B*, *etc*. Fusion mutations usually occur in the tyrosine kinase domain of MET, resulting in its continuous activation.

polysomy cannot be identified as a driver of gene variation[121-124] (Figure 3). MET protein overexpression may be present regardless of whether the *MET* gene is abnormal. MET can induce cancer cell proliferation, reduce apoptosis and promote metastasis under hypoxia and/or inflammation[125-128]. Thus, tumors may rely on MET signaling even in the absence of genomic drivers such as MET amplification, mutation, or fusion[129-131]. The incidence of MET protein overexpression is greater than those of MET exon 14 jump mutation and *MET* gene amplification, which are associated with poor patient prognosis. MET protein expression can be evaluated *via* immunohistochemistry[132]. We believe that the definition and criteria for MET variation in gastrointestinal tumors can be compared with those for NSCLC, and routine screening for rare mutations, including MET, should be conducted[133-137].

EFFECT OF MET VARIATION ON THE PROGNOSIS AND TREATMENT OF DIGESTIVE TRACT TUMORS

The incidence of MET amplification is approximately 5% to 8% in gastric cancer patients and approximately 15% in diffuse gastric cancer patients[138-140]. MET protein overexpression is highly common in enteric gastric cancer (63% overexpression rate; previous reports vary and may be related to different detection methods). Met-amplified gastric cancer is characterized by low differentiation of tumor cells, easy peritoneal metastasis and malignant lymphangitis[141]. Moreover, a previous study revealed that the MET status of approximately 35% of gastric cancer patients changes with the course of treatment, requiring dynamic detection[142-145]. MET amplification is often accompanied by HER2 overexpression in gastric cancer, and co-expression of MET and HER2 can synergistically enhance tumor invasion, invasion and metastasis, which is an important factor for poor prognosis (Figure 4). According to a retrospective analysis of 233 patients, survival was 24.6 months in the non-MET-amplified group and 9.3 months in the MET-amplified group [hazard ratio (HR) = 1.6, 95% confidence interval (CI): 1.0-2.5, P = 0.049], suggesting shorter survival and a worse prognosis in patients with MET-amplified gastric cancer[146-149]. Abnormal activation of the MET signaling pathway in liver cancer is due mainly to ligand binding, which leads to its overexpression, but its high expression rate is due to differences in detection methods (the subjective influence of immunohistochemistry is relatively large), resulting in a large variation in its incidence rate (25.4%-61.2%)[150]. A meta-analysis evaluating the prognostic value of MET overexpression in 1408 patients who underwent liver resection revealed that progression-free survival (PFS) and overall survival (OS) were significantly worse in patients with high MET expression than in those with low MET expression (HR = 1.26, P = 0.03; HR = 1.16, P = 0.01), suggesting that MET overexpression may be a poor prognostic indicator of liver cancer[151-154]. There are also clinical data showing that patients with high MET expression in liver cancer are more likely to develop intrahepatic metastasis[155-158]. The high expression of MET in patients with early colorectal cancer is related to the depth of intestinal wall lymph node invasion and regional lymph node metastasis, and some studies have shown that the high expression of MET is related to clinical characteristics such as tumor invasion and liver metastasis. Some scholars have analyzed the changes in gene expression in the ctDNA of advanced colorectal cancer patients who previously

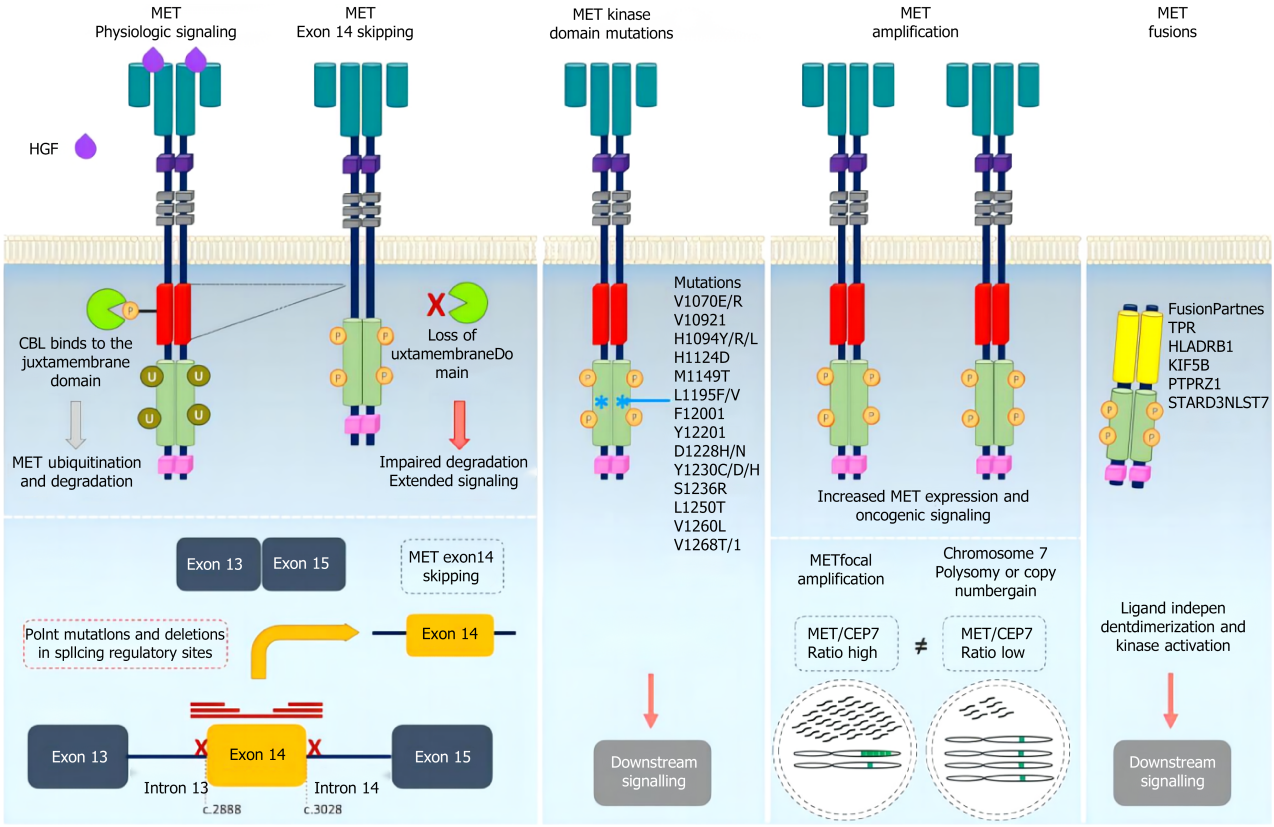

Figure 3 Mechanisms of MET oncogenic activation. The mechanism of MET carcinogenic activation mainly includes gene mutation, gene amplification and fusion, *etc*. Through abnormal activation of MET receptor tyrosine kinase, downstream signaling pathways such as RAS/mitogen-activated protein kinase, phosphoinositide 3-kinase/protein kinase B and Janus kinase/signal transducer and activator of transcription are triggered to promote cell proliferation, migration and anti-apoptosis. HGF: Hepatocyte growth factor.

received anti-epidermal growth factor receptor (EGFR) monoclonal antibody therapy and reported that, after treatment with anti-EGFR monoantibodies, especially in resistant patients, the number of copies of the *MET* gene significantly increased compared with that in patients who were not exposed to EGFR monoantibodies[159-162]. MET amplification is considered to be one of the main mechanisms of retroline resistance to EGFR monoantibodies in metastatic colorectal cancer[163-166] (Figure 5).

Research progress on MET variation in digestive tract tumors

MET inhibitors include highly selective tyrosine kinase inhibitors (TKIs) (sevotinib, tepotinib, camatinib, *etc*.), pantargeted TKIs (crizotinib), and monoclonal antibodies (TKIs, *etc*.)[163]. Preclinical studies suggest that the MET inhibitor sevotinib has a dose-dependent killing effect on MET-amplified gastric cancer, and this inhibition is more significant when it is combined with chemotherapy[164-167]. The antitumor efficacy of sevoflurane has been preliminarily demonstrated in a human tumor xenotransplantation (PDX) model of gastric cancer with MET overexpression [168-170]. The VIKTORY clinical trial explored the efficacy of single-agent sevotinib in patients with MET-amplified gastric cancer, and the results revealed that the objective response rate of single-agent sevotinib reached 50%; 10 patients achieved a partial response, and 1 patient achieved a complete response, among which the second-line PFS duration was 4-6 months, and the efficacy was obvious[171-174]. Subgroup analysis revealed that patients with a MET copy number > 10 had a better response rate to sevoflurane, and the MET copy number was strongly associated with PFS duration[175-178]. Crizotinib has also been used to treat advanced gastric cancer patients with MET amplification and liver metastasis who achieved complete remission of liver lesions after 2 months of treatment and a PFS duration of up to 20 months[179, 180]. In addition to the effects of sevotinib, the effects of other MET inhibitors, including monoclonal antibodies, have also been explored in patients with MET-variant gastric cancer[148-151] (Figure 6). In general, the efficacy of specific TKIs is greater than that of monoclonal antibodies. However, at present, these are small sample size studies, and more high-level clinical studies are needed to demonstrate its efficacy and safety[181-184].

DISCUSSION

MET is a therapeutic target for liver cancer, but few monoclonal antibodies and HGF inhibitors have been developed, and related clinical studies have focused on MET TKIs[185-188]. As a highly selective MET TKI, tivantinib significantly improved PFS and OS (2.7 months *vs* 1.4 months and 7.2 months *vs* 3.8 months, respectively) compared with placebo in a

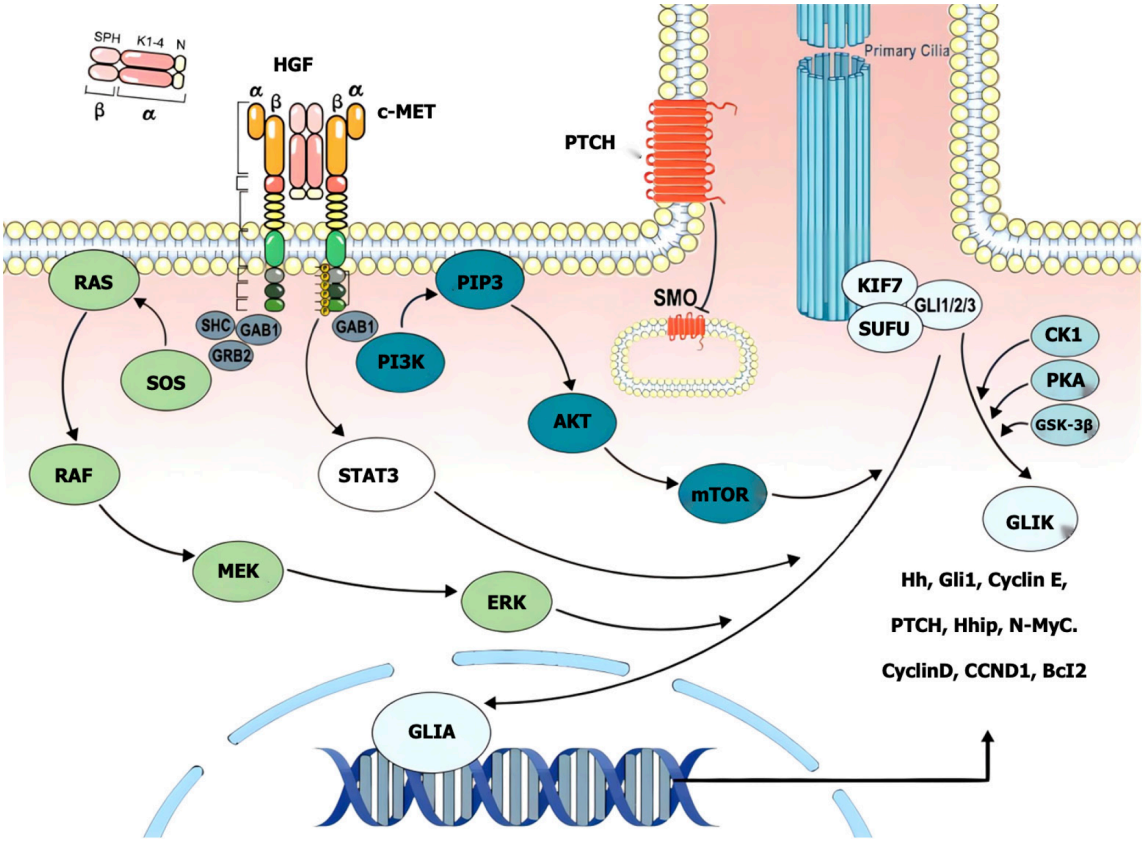

Figure 4 The hepatocyte growth factor/c-Met axis activates the Hedgehog pathway through downstream signaling. The hepatocyte growth factor (HGF)/c-Met axis activates the Hedgehog pathway through its downstream signaling pathway. After binding to the c-Met receptor, hepatocyte growth factor activates the extracellular signal-regulated kinase 1/2 and protein kinase B signaling pathways, further promoting the activation of GLI transcription factors, and thereby initiating the transcription activity of the Hedgehog pathway. HGF: Hepatocyte growth factor; Hh: Hedgehog; SPH: Secreted protein, acidic and rich in cysteine; MEK: Mitogen-activated protein/extracellular signal-regulated kinase; ERK: Extracellular signal-regulated kinase; STAT3: Signal transducer and activator of transcription 3; SHC: Src homology 2 domain containing; GAB: Grb2-associated binder; PI3K: Phosphoinositide 3-kinase; KIF7: Kinesin family member 7; GLI: GLI family zinc finger transcription factor; PKA: Protein kinase A; GSK-3β: Glycogen synthase kinase 3 β; AKT: Protein kinase B; mTOR: Mammalian target of rapamycin; GLIA: Glioma-associated oncogene homolog; Bcl: B-cell lymphoma; CCND: Cyclin D.

phase II clinical study of second-line MET TKIs for highly expressed MET liver cancer[189-191]. Therefore, screening the right people or finding new combinations may be effective[192,193]. Therefore, screening the right people or finding new combinations might be useful. Preclinical studies have shown that hypoxia induced by inhibition of the vascular endothelial growth factor signaling pathway induces hypoxia-inducible factor-1 nuclear aggregation, leading to increased MET expression[194]. CELESTIAL clinical studies have shown that cabotinib is a nonselective multitarget inhibitor with anti-MET and angiogenic effects and is effective in patients with liver cancer who have failed sorafenib treatment and received more than 2 systems, with initial results indicating improved OS in patients[195-198]. Although there are no large-scale clinical studies demonstrating the efficacy and safety of MET inhibitors in the field of liver cancer, MET inhibitors may be a promising choice for liver cancer patients with specific molecular types, such as those with high MET expression[199-202].

Crizotinib, camatinib, and tevantinib have been used as selective MET inhibitors in preclinical and in-clinical trials for the treatment of MET-variant colorectal cancer[203-205]. Scholars have reported that crizotinib can effectively improve the sensitivity of cetuximab-resistant cell lines to radiotherapy both *in vivo* and *in vitro*[206]. A clinical study of MET-positive metastatic colorectal cancer (NCT02205398) revealed that the combination of carmatinib and cetuximab in four patients with EGFR-resistant MET-positive colorectal cancer achieved a partial response and was well tolerated. However, tivantinib in combination with cetuximab failed to significantly improve PFS in patients with colorectal cancer, and adverse effects persisted. The antitumor activities of SU11274, PHA665752, nororitin and other MET inhibitors have been confirmed in preclinical studies of colorectal cancer, but relevant clinical studies are lacking. The effectiveness of MET inhibitors in colon cancer patients with MET variants still needs to be further explored.

CONCLUSION

Through a systematic review of the progress in the diagnosis and treatment of MET-variant digestive tract tumors, we found that the molecular mechanisms of MET-variant digestive tract tumors are complex and diverse and show specificity for different tumor types. Therapeutic strategies targeting MET, including small molecule inhibitors and

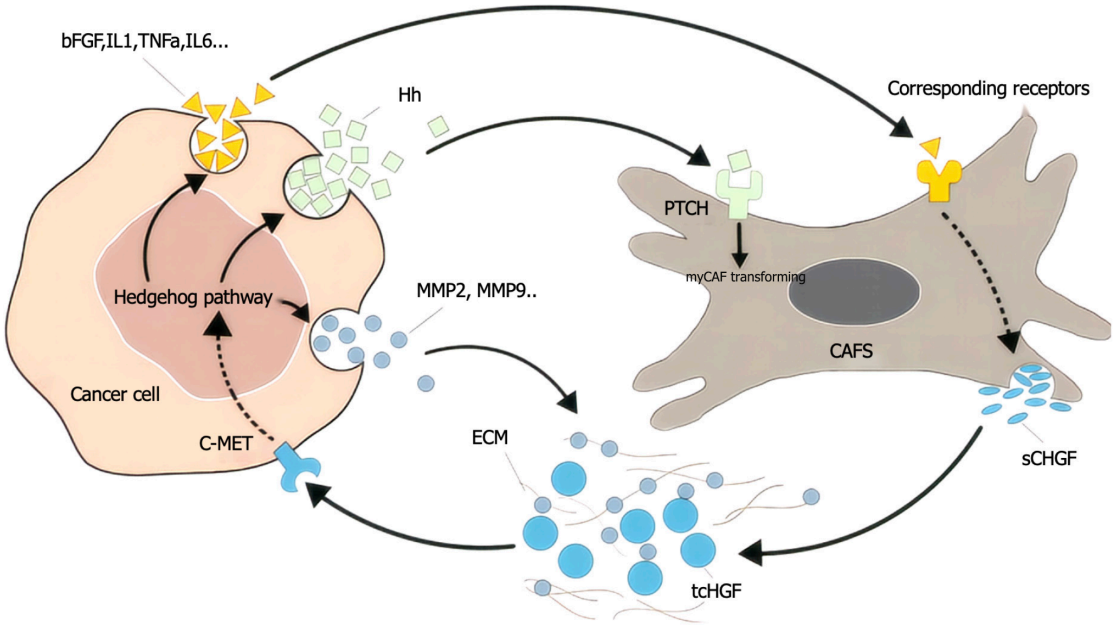

Figure 5 Hepatocyte growth factor and Hedgehog loops between cancer cells and cancer-associated fibroblasts. Hepatocyte growth factor (HGF) and Hedgehog (Hh) pathways form a feedback loop between cancer cells and cancer-associated fibroblasts. Cancer-associated fibroblasts secrete hepatocyte growth factor, activate c-Met receptors in cancer cells, trigger downstream signaling pathways, and thus enhance the proliferation, migration, and invasion of cancer cells. bFGF: Basic fibroblast growth factor; IL: Interleukin; TNF: Tumor necrosis factor; Hh: Hedgehog; MMP: Matrix metalloproteinase; ECM: Extracellular matrix; tcHGF: Truncated hepatocyte growth factor; CAFS: Cancer-associated fibroblasts; sCHGF: Soluble hepatocyte growth factor.

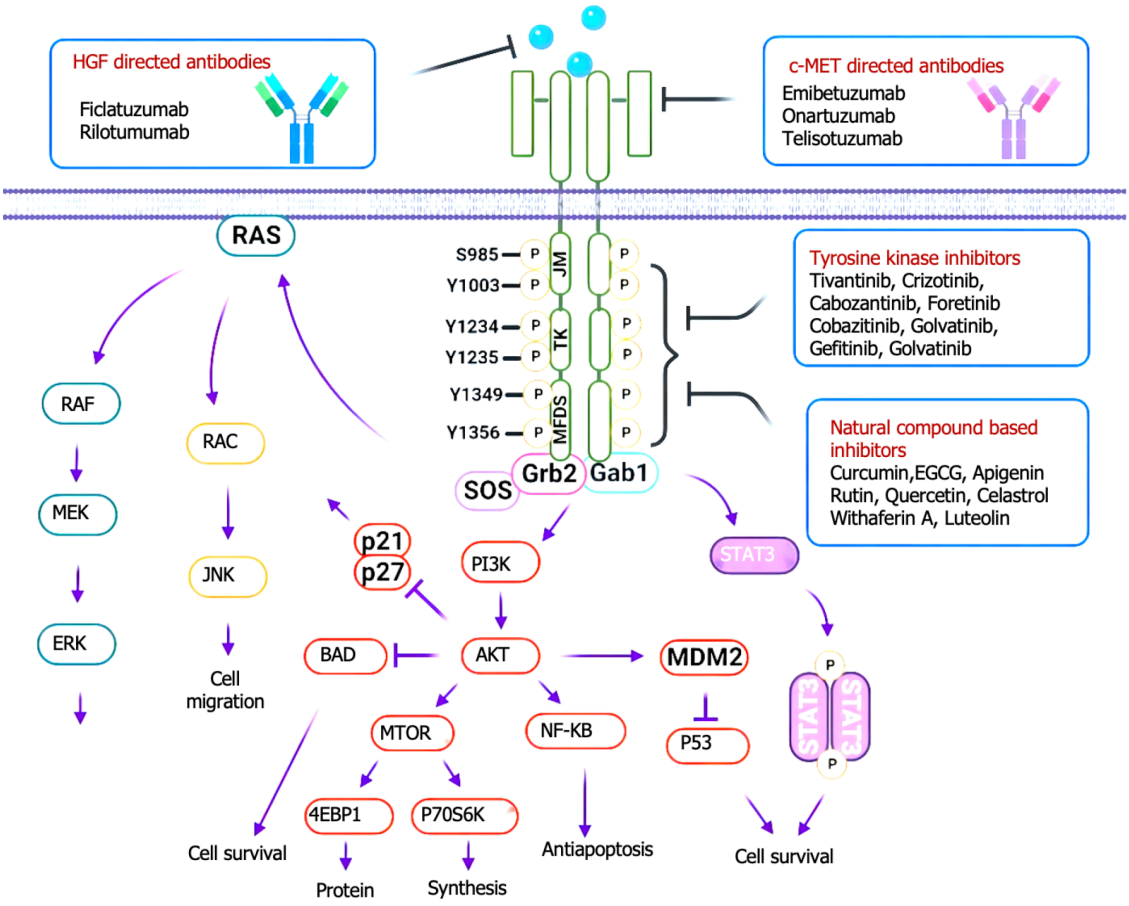

Figure 6 The c-MET pathway relays downstream signals to activate multiple oncogenic events to promote cell proliferation, migration, protein synthesis, and antiapoptosis. The c-MET pathway transmits downstream signals by activating its receptor tyrosine kinase, triggering multiple carcinogenic events. This pathway activates signaling pathways such as RAS/mitogen-activated protein kinase, phosphoinositide 3-kinase/protein kinase B, and

promotes cell proliferation, migration, protein synthesis, and anti-apoptosis. HGF: Hepatocyte growth factor; MEK: Mitogen-activated protein/extracellular signal-regulated kinase; ERK: Extracellular signal-regulated kinase; RAC: Ras-related C3 botulinum toxin substrate; JNK: Jun N-terminal kinase; BAD: Bcl-2-associated death promoter; mTOR: Mechanistic target of rapamycin; Grb2: Growth factor receptor-bound protein 2; Gab1: Grb2-associated binder 1; STAT3: Signal transducer and activator of transcription 3; MDM2: Mouse double minute 2; PI3K: Phosphoinositide 3-kinase; AKT: Protein kinase B; NF-κB: Nuclear factor kappa B; EGCG: Epigallocatechin gallate.

immunotherapy, have shown significant clinical efficacy. However, drug resistance and individual variability remain major challenges. Future research should focus on precision medicine and optimize individualized treatment plans by integrating multilevel data such as genomic and transcriptomic data, thereby improving patient survival and quality of life.

FOOTNOTES

Author contributions: Zhang C and Dong HK wrote the manuscript, they contributed equally to this study, and they are the co-first authors of this manuscript; Gao JM, Zeng QQ, and Qiu JT collected the data; Wang JJ guided the study. All authors reviewed, edited, and approved the final manuscript and revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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REVIEW

Effect of colorectal cancer stem cells on the development and metastasis of colorectal cancer

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Abstract

The relevant mechanism of tumor-associated macrophages (TAMs) in the treatment of colorectal cancer patients with immune checkpoint inhibitors (ICIs) is discussed, and the application prospects of TAMs in reversing the treatment tolerance of ICIs are discussed to provide a reference for related studies. As a class of drugs widely used in clinical tumor immunotherapy, ICIs can act on regulatory molecules on cells that play an inhibitory role - immune checkpoints - and kill tumors in the form of an immune response by activating a variety of immune cells in the immune system. The sensitivity of patients with different types of colorectal cancer to ICI treatment varies greatly. The phenotype and function of TAMs in the colorectal cancer microenvironment are closely related to the efficacy of ICIs. ICIs can regulate the phenotypic function of TAMs, and TAMs can also affect the tolerance of colorectal cancer to ICI therapy. TAMs play an important role in ICI resistance, and making full use of this target as a therapeutic strategy is expected to improve the immunotherapy efficacy and prognosis of patients with colorectal cancer.

Key Words: Colorectal cancer; Colorectal cancer stem cells; Tumor metastasis; Tumor immune microenvironment; Review

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Core Tip: The role of colorectal cancer stem cells in the tumor immune microenvironment, the development and metastasis of colorectal cancer. This paper focuses on how colorectal cancer stem cells affect the tumor immune microenvironment through immune escape, immunosuppression and microenvironment remodeling, and analyzes their key functions in tumor progression and metastasis. In addition, this paper also summarizes potential therapeutic strategies for colorectal cancer stem cells, aiming to inhibit their cancer-promoting effects and provide new targets and ideas for the treatment of colorectal cancer.

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INTRODUCTION

Colorectal cancer is one of the most common types of cancer worldwide, accounting for approximately 10% of all new cancers and 8.5% of all cancer deaths[1-5]. The incidence of colorectal cancer tends to increase with age, with patients under the age of 40 accounting for 2%-8% of all colorectal cancer patients[6-10]. At present, the treatment of colorectal cancer is mainly based on the combination of surgical treatment, radiotherapy and chemotherapy[11-14]. The vast majority of early-stage patients can be cured by surgery, but only a small proportion of patients with metastatic colorectal cancer can be cured by surgery, and the 5-year survival rate is very low[15-20]. The recurrence of colorectal cancer occurs mostly in the form of metastasis, which is mainly caused by residual tumor cells that spread to distant organs before surgery[21-26]. However, current systemic therapy (systemic chemotherapy) does not eliminate latent residual tumor cells and has no therapeutic effect on the growth of metastases, providing patients with a survival advantage of only a few months[27-30]. Metastasis occurs when cancer spreads to distant organs and forms metastases, mainly involving the liver and lungs, in patients with colorectal cancer. To metastasize, cancer cells need to invade surrounding tissue, survive in circulation, colonize distant organs, and eventually regain their ability to grow, but metastasis is an inefficient process, and most cancer cells lose their ability to grow during metastasis[31-35]. Since 2016, scholars have gradually discovered related factors affecting colorectal cancer metastasis: Tumor regeneration in distant organs is closely related to the acquisition of stem cell-like phenotypes by cancer cells. Metastatic tumor stem cells have a variety of phenotypes and biological behaviors, and tumors mainly rely on interactions with the microenvironment to migrate, survive in circulation, and regenerate in distant organs[36-38]. This article reviews the effects of colorectal cancer stem cells and the microenvironment on colorectal cancer metastasis.

OVERVIEW OF COLORECTAL CANCER

Normal colon epithelial cells are constantly renewed, and the crypt cells at the base of the colon mucosal glands are rapidly proliferating and differentiating intestinal stem cells, which can maintain the steady regeneration of epithelial cells[39-44]. Signaling pathways regulating intestinal stem cell renewal and proliferation, including the Wnt, epidermal growth factor receptor/mitogen-activated protein kinase and Notch signaling pathways, have been continuously identified, and the transforming growth factor (TGF)-β signaling pathway induces cell cycle arrest[45-48]. The high proliferation state of intestinal stem cells increases the possibility of mutation in the DNA replication stage, and environmental factors such as lifestyle, diet and microorganisms also have a great influence on the transformation of epithelial cells. In patients with colorectal cancer, the most common genetic change is inactivation of the tumor suppressor gene *APC*, which activates the Wnt signaling pathway to maintain a continuous stem cell proliferation state in the initial stage of tumor development, resulting in benign epithelial cell proliferation, namely, adenoma[49-52]. Genetic experiments conducted in mouse models confirmed the hypothesis that mutations in the *APC* gene in intestinal stem cells are the origin of intestinal polyps[53-55]. However, a small percentage of primitive accumulations become aggressive through other mutations, which are mainly mediated by three signaling pathways: (1) The mitogen-activated protein kinase pathway. By activating mutations in the *KRAS*, *BRAF*, or *PIK3CA* genes, cancer cells acquire the ability to undergo autonomous mitosis and proliferate; (2) The p53 pathway is activated and inactivated by mutated p53 proteins, resulting in genomic instability; and (3) TGF-β pathway. Commonly, TGFBR2, SMAD4, SMAD2, or SMAD3 are functionally deficient and inactivated, thus preventing the inhibitory effect of high TGF-β levels in the tumor microenvironment[56-60]. The acquisition of these mutations is a slow process. Due to chromosome instability and defects in the DNA mismatch repair system, tumors often accumulate hundreds or even thousands of genetic variants, some of which cause new biological behaviors in

cancer cells and increase their aggressiveness; moreover, some of the effects of these mutations are still unknown, and these factors work together to make the treatment of colorectal cancer difficult[61-66].

As noted above, mutations that promote the development of colorectal cancer affect signaling pathways that regulate the behavior of intestinal stem cells, allowing cancer cells to divide and grow autonomously, which is unregulated by intestinal stem cell signaling[67-70]. It has been hypothesized that mutations acquired in the mitogen-activated protein kinase pathway, p53 pathway, and TGF-β pathway promote the growth of tumor cells in unfavorable environments[71-74]. Although these mutations promote tumor development, metastasis is still uncommon, suggesting that other factors are involved in limiting tumor spread[75-77]. Most colorectal cancers are aggressive at diagnosis, which gives shed cancer cells a chance to enter the circulatory system for months or longer, and when disseminated colorectal cancer cells enter the portal circulation, they are transported to the hepatic sinuses within minutes and into the liver parenchyma when the blood vessels open[78-80]. In the case of lung metastasis, colorectal cancer cells first enter the systemic circulation and then infiltrate the lung parenchyma, while the ability of colorectal cancer cells to infiltrate other organs (the brain) is unknown[81-84]. Studies have shown that the limiting factor in tumor metastasis is the ability of circulating tumor cells to colonize distant organs[85-89]. Most tumor cells that survive in the circulatory system and infiltrate distant organs die for reasons that are unclear and may be related to the recognition and killing of tumor cells by the innate and adaptive immune systems, while tumor cells that survive and adapt to the new environment can metastasize[90-94]. However, not all cancer cells that successfully spread to distant locations can proliferate in a new environment, and they often remain dormant in distant organs for months to years before regaining their ability to proliferate.

EFFECT OF COLORECTAL CANCER STEM CELLS ON COLORECTAL CANCER METASTASIS

Hierarchical structure of tumor cells: The concept of tumor stem cells

Tumors that first appear in the blood are a class of cells with self-renewal ability and unlimited differentiation potential [95-100]. Tumor stem cells differentiate into tumor cells with phenotypes different from those of the original cells in a suitable environment, thus leading to the formation of tumor metastases[101-106]. Some scholars have proposed that colorectal cancer is caused by groups of cells with different tumorigenic potentials, called tumor-initiating cells[107-109]. Studies have shown that tumor-initiating cells (also known as colorectal cancer stem cells), located at the top of the tumor cell hierarchy, can self-renew and have the potential to proliferate and differentiate over a long period of time, causing tumors when injected into mice[110-115]. The balance between stem cell pluripotency and cell differentiation in colorectal cancer relies on signaling pathways that regulate normal intestinal stem cells, many of which are supplied by tumor stromal cells, and the presence of other stroma-derived cytokines and growth factors at invasion sites further promotes the self-renewal of colorectal cancer stem cells[116-118]. Because colorectal cancer stem cells have the ability to self-renew and initiate tumors, they may represent (or give rise to) so-called metastatic stem cells, the cells of origin of metastasis. Studies have shown that only cells with long-term self-renewal capabilities are likely to metastasize[119-121].

Most colorectal cancers are a relatively disordered mix of stem cells and differentiated cells, and distant metastases form metastases with the same properties[122-128]. Although colorectal cancer stem cells present in tumor glands are tumorigenic when isolated and xenografted in mice, under natural conditions, they are only capable of metastasizing when they first undergo phenotypic changes that enable them to migrate and excrete, a process that occurs when cancer cells exchange substances with neighboring tissues and tumor stroma.

The metastasis of dormant and slowly proliferating tumor cells usually occurs

The incubation period of colorectal cancer cells is up to five years after the incubation period, which is the result of disseminated tumor cells remaining dormant[129-131]. Currently, the standard chemotherapy for removing these surviving tumor cells is standard chemotherapy, but chemotherapy causes limited damage to these surviving tumor cells because chemotherapy usually targets rapidly proliferating tumor cells, while dormant and slowly proliferating tumor cells are largely resistant to chemotherapy. Dormant tumor cells develop a special state of response to signals that induce cell death, thus protecting them from the immune system[132-136]. The latency of metastasis may be caused by two mechanisms: Population dormancy, that is, the equilibrium state of tumor cell proliferation and death, resulting in the non-expansion of micrometastases; this is a state in which cells are stationary or temporarily mitotic[137-140]. Understanding these mechanisms gives physicians the opportunity to cure patients by eliminating remaining lesions (or inhibiting their growth) before significant metastases occur. Although the origin, characterization, and regulation of the dormant cell population in colorectal cancer are unknown, these findings may provide directions for the study of the diversity of normal intestinal epithelial cells.

Genetic changes in tumor cells

Due to genomic instability, tumors acquire hundreds of genetic and epigenetic changes that give tumor cells a different phenotype, which can lead to clonal expansion, an evolutionary phenomenon that is the basis for tumors to adapt to different environments, colonize distant areas, and resist treatment[141-145]. Conceptually, phenotypic heterogeneity due to tumor cell grade and clonal diversity may have some correlation, with colorectal cancer stem cells representing clonally selected units that mutate in cells that are more differentiated but have a lower chance of being selected due to the population's shorter lifespan[146-148]. Although clonal diversity has been associated with treatment resistance and an enhanced ability to metastasize, genetic sequencing of primary colorectal cancer and metastases did not reveal specific genetic mutations associated with tumor spread, and similarities between primary tumors and metastases suggest that nongenetic factors may play a special role in this process (Figure 1).

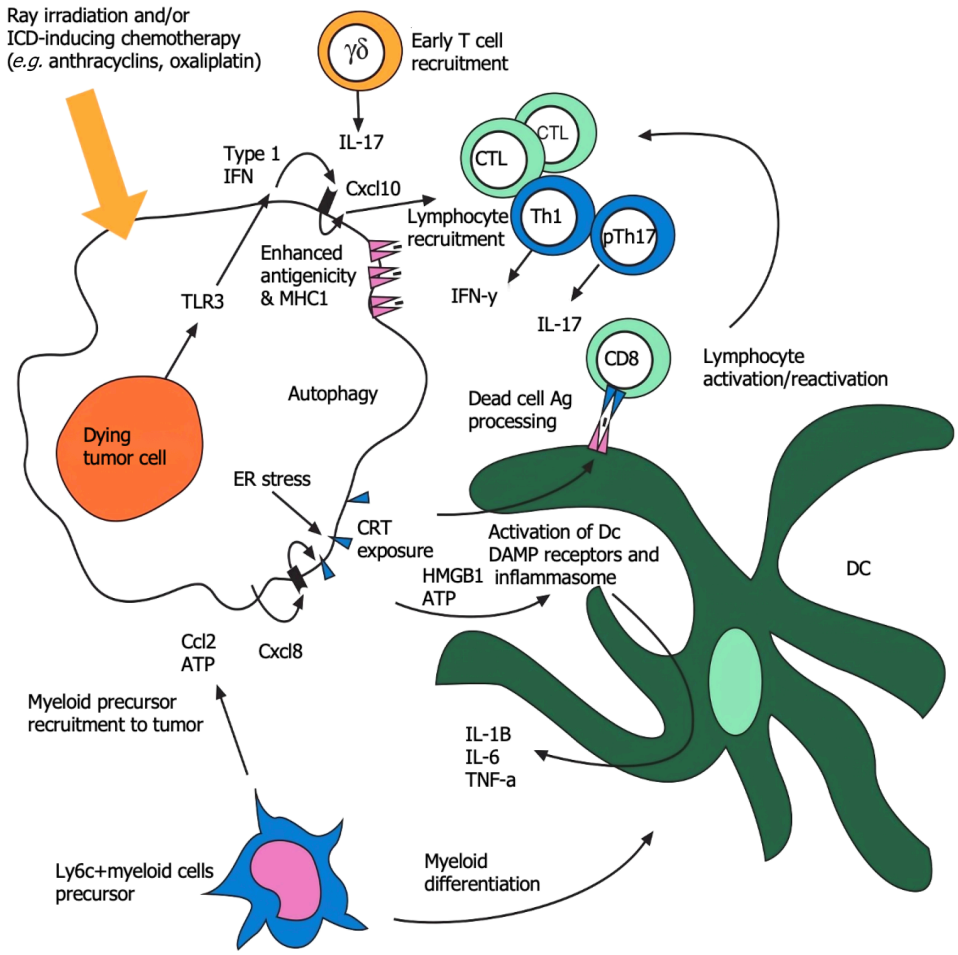

Figure 1 Immunogenic chemotherapy and radiotherapy to restore the tumor microenvironment. ICD: International Classification of Diseases; IL: Interleukin; TFN: Tumor necrosis factor; CXCL: Chemokine (C-X-C motif) ligand; TLR3: Toll-like receptor 3; MHC1: Major histocompatibility complex class I; ER: Endoplasmic reticulum; CRT: Calreticulin; CCL: Chemokine (C-C motif) ligand; HMGB: High mobility group box; DC: Dendritic cell; DAMP: Damage-associated molecular pattern; TNF: Tumor necrosis factor.

EFFECT OF THE MICROENVIRONMENT ON COLORECTAL CANCER METASTASIS

Colorectal cancer epithelial mesenchymal heterogeneity brings new ideas to the diagnosis and treatment of colorectal cancer patients, and intratumoral heterogeneity also includes many other cell types that penetrate the tumor, collectively known as the stroma or tumor microenvironment[149-151]. The function of the stroma is related to all steps of cancer progression and metastasis. During the process of cancer progression, cancer cells exchange substances with the stroma and develop together[152-154]. Conceptually, transformed cancer cells strongly change the properties and composition of the matrix, and these changes form a suitable microenvironment for cancer cell growth, providing protection for cancer cells[155]. Therefore, an increasing number of studies have focused on the characteristics of specific stromal cell populations related to tumor malignancy and metastatic colonization (Figure 2).

Cancer-associated fibroblasts in the microenvironment

Cancer-associated fibroblasts are a group of highly homologous activated fibroblasts with special phenotypes in the tumor stroma that can be isolated from different parts of the tumor. Cancer-associated fibroblasts differ from normal fibroblasts in that they activate the myofibroblast phenotype and promote the expression of alpha smooth muscle actin, fibroblast-specific protein-4, and fibroblast-activating protein, which are specific markers for the recognition of cancer-associated fibroblasts[156-158]. Cancer-associated fibroblasts provide a range of cytokines to colorectal cancer cells that promote cancer cell survival and tumor development[159-162]. In addition, gene expression signatures obtained from cancer-associated fibroblasts may be associated with poor prognosis in patients with colorectal cancer[163-165]. Therefore, cancer-associated fibroblasts are an important cell population in the tumor microenvironment that provides a suitable environment for cancer progression (Figure 3).

Stromal environment

The stromal environment surrounding tumor cells has a growing network of blood vessels that provide nutrients and oxygen for tumor cell growth[166-168]. Growth factors secreted by tumor cells stimulate the proliferation and differentiation of endothelial cells, thus promoting the formation of blood vessels, while tumor-related angiogenesis leads to

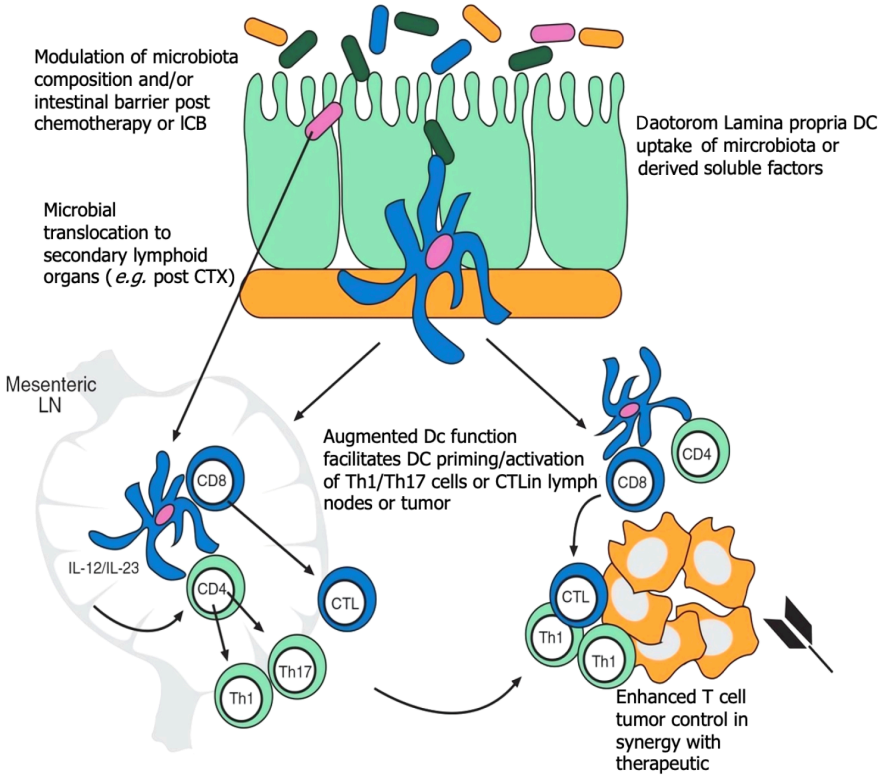

Figure 2 The microbiota can augment dendritic cell function and thus contribute to anticancer immunity. ICB: Immune checkpoint blockade; CTX: Cyclophosphamide; LN: Lymph node; IL: Interleukin; Th: T helper cell; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell.

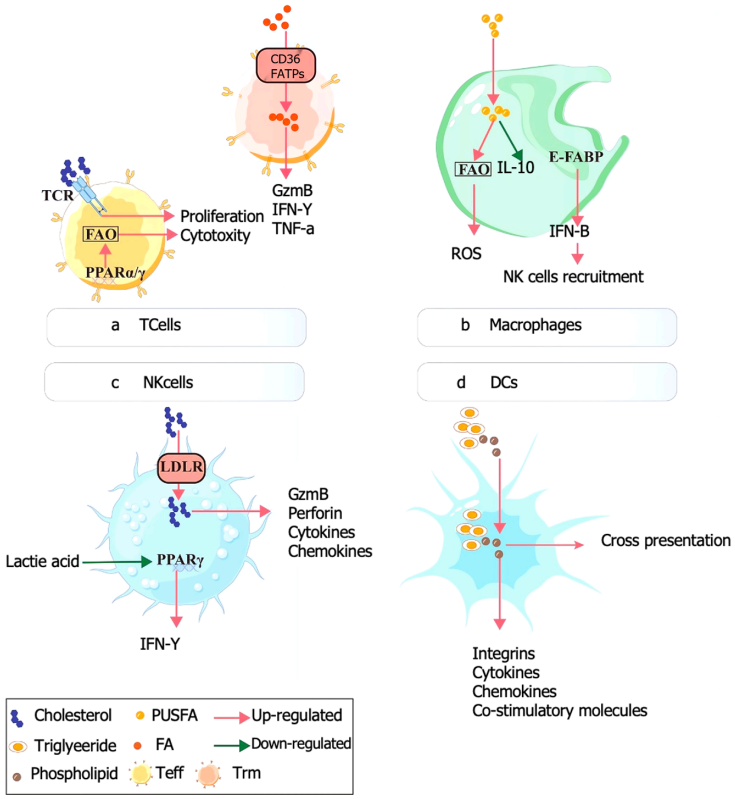

Figure 3 Lipid metabolism in the antitumor immune response. FATPs: Fatty acid transport proteins; TCR: T cell receptor; FAO: Fatty acid oxidation; IFN: Interferon; TNF: Tumor necrosis factor; IL: Interleukin; E-FABP: Epidermal fatty acid binding protein; NK: Natural killer; ROS: Reactive oxygen species; LDLR: Low-density lipoprotein receptor; PPAR: Peroxisome proliferator-activated receptor; PUSFA: Polyunsaturated fatty acids; FA: Fatty acid.

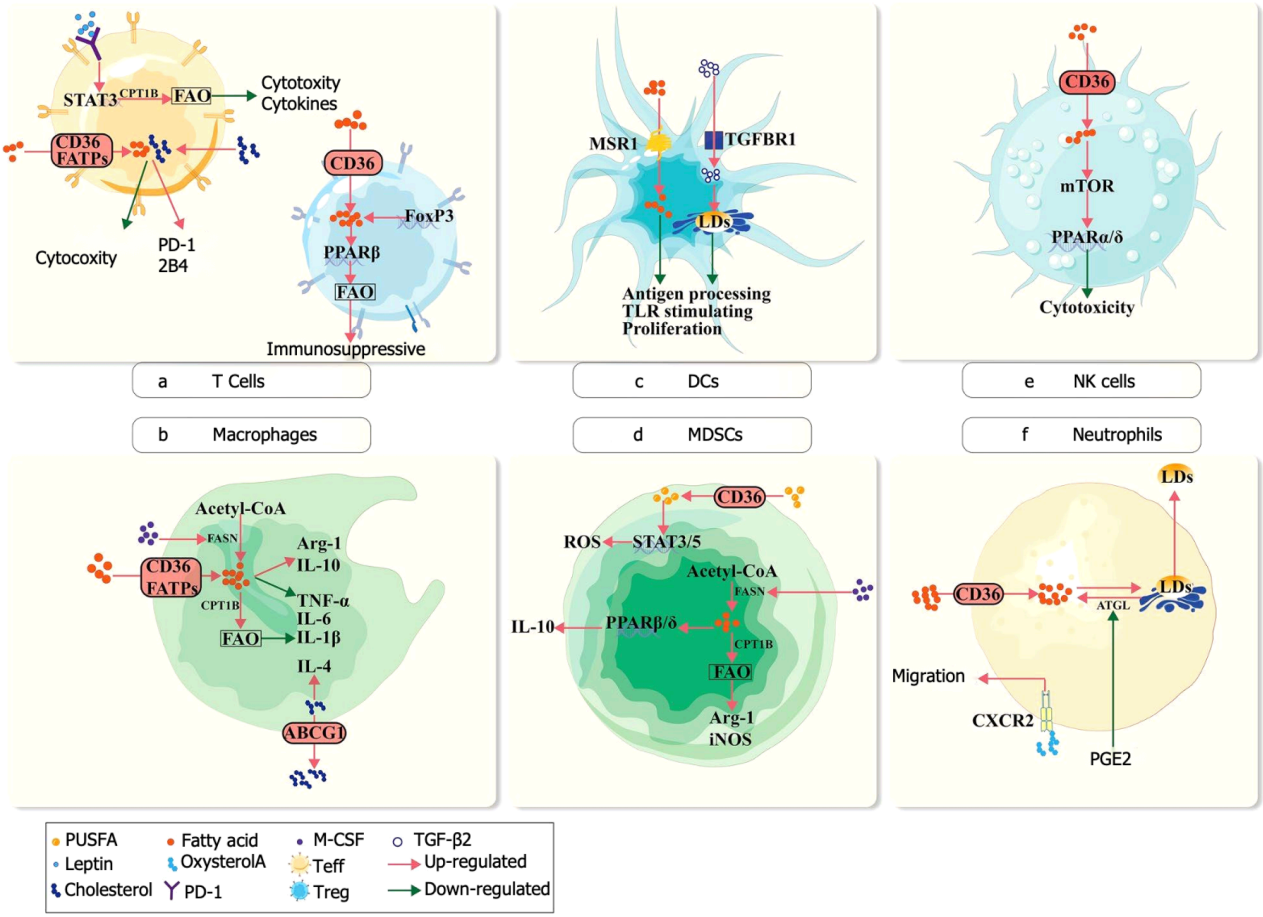

Figure 4 Lipid metabolism in protumor immune responses. STAT3: Signal transducer and activator of transcription 3; CPT1B: Carnitine palmitoyltransferase 1B; FAO: Fatty acid oxidation; PD-1: Programmed cell death protein 1; PPAR: Peroxisome proliferator-activated receptor; MSR1: Macrophage scavenger receptor 1; TGFBR1: Transforming growth factor beta receptor 1; TLR: Toll-like receptor; mTOR: Mechanistic target of rapamycin; DC: Dendritic cell; MDSC: Myeloid-derived suppressor cell; NK: Natural killer; FATP: Fatty acid transport protein; Arg: Arginine; IL: Interleukin; TNF: Tumor necrosis factor; ABCG1: ATP-binding cassette sub-family G member 1; ROS: Reactive oxygen species; iNOS: Inducible nitric oxide synthase; CXCR: C-X-C chemokine receptor; ATGL: Adipose triglyceride lipase; PGE2: Prostaglandin E2; PUSFA: Polyunsaturated fatty acids; M-CSF: Macrophage colony-stimulating factor; TGF: Transforming growth factor; Treg: Regulatory T cell.

vascular abnormalities, which often manifest as network disorders, excessive branching, reduced coverage and leakage of surrounding cells[169-171]. Vascular abnormalities are associated with metastasis and poor prognosis in patients with colorectal cancer. Vascular endothelial growth factor (VEGF) is a key regulator of the proliferation of most human tumor endothelial cells and can induce the activation of mitogen-activated protein kinase/extracellular signal-regulating kinase signaling pathways[172]. VEGF expression in tumors is associated with the invasion of cancer cells, increased vascular density and metastasis[173-176]. The combination of bevacizumab, monoclonal antibodies against VEGF, and angiogenesis inhibitors with chemotherapy has been shown to improve survival in patients with stage IV colorectal cancer (Figure 4).

SA100A6 protein

SA100A6 is a calcium-ion binding protein and a member of the SA100 protein family[177-183]. At present, the SA100A4 protein family is known to indirectly promote tumor cell metastasis through the tumor microenvironment, and SA100A4 can promote monocyte polarization to the M2 type, thus promoting tumor metastasis. Studies have shown that SA100A6 can promote the progression and metastasis of colorectal cancer, but the underlying mechanism is not yet clear[184-190].

Interleukin-33

Interleukins are cytokines whose molecular structure and biological function are largely clear, and they play important regulatory roles[191-195]. It plays multiple roles in the activation and proliferation of immune cells and the regulation of the immune response[196-200]. Interleukin-33 is a cytokine secreted by endothelial and epithelial cells that can activate the mitogen-activated protein kinase signaling pathway and plays an important role in tumor angiogenesis in colorectal cancer[200-204]. Recent studies have shown that interleukin-33 plays a regulatory role in the occurrence, development and metastasis of tumors, and its abnormal expression may be related to the clinical characteristics of patients with tumors[205-209]. Interleukin-33 secreted by tumor cells significantly increases the generation of new blood vessels by increasing the proliferation, migration and differentiation of endothelial cells into blood vessels, thus increasing the

metastasis and spread of colorectal cancer cells to the liver[210-212]. Blocking the signaling pathway mediated by interleukin-33 can inhibit angiogenesis and reduce the occurrence of tumors[213-215].

CONCLUSION

The effects of colorectal cancer stem cells and tumor microenvironment regulation on the progression and metastasis of colorectal cancer, as well as the formulation, optimization and application of treatments, are highly important. The theory of colorectal cancer stem cells explains why colorectal cancer is difficult to cure. Although the ability of colorectal cancer stem cells to cure colorectal cancer can be eradicated from the root, research on the use of these cells is still in its infancy, and further research is needed to determine the optimal treatment for colorectal cancer. The tumor microenvironment is closely related to the aggressiveness of the tumor, so the study of the tumor microenvironment is highly important for the prevention and treatment of tumor metastasis. Tumor heterogeneity plays a role in the generation and maintenance of drug resistance and metastasis ability of colorectal cancer stem cells, especially colonized metastasis supported by the tumor microenvironment. Treatments that target colorectal cancer stem cells and the microenvironment can prevent distant metastases in early-stage colorectal cancer patients who are at risk for distant metastases. In the future, targeted therapy targeting colorectal cancer stem cells and the microenvironment of cancer cells combined with radical surgery and systemic chemotherapy may provide a new direction for the treatment of colorectal cancer patients.

FOOTNOTES

Author contributions: Deng RZ wrote the manuscript; Zheng X, Lu ZL, Yuan M, Meng QC, and Wu T collected the data; Tian Y guided the study. All authors reviewed, edited, and approved the final manuscript and revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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MINIREVIEWS

Current clinical trials on gastric cancer surgery in China

Shun Zhang, Ren-Hao Hu, Xi-Mao Cui, Chun Song, Xiao-Hua Jiang

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Abstract

Gastric cancer (GC) is the leading diagnosed malignancy worldwide, especially in China. Radical surgery is the cornerstone of GC treatment. We reviewed previous clinical trials and aimed to provide an update on the factors related to the surgical treatment of GC. The number of registered clinical trials in the field of GC surgery is rapidly increasing. With the development and popularization of endoscopic, laparoscopic, and robotic techniques, GC surgery has gradually entered a new era of precise minimally invasive surgery. Postoperative quality of life has become a major issue in addition to surgical oncological safety. Although great progress has been made in clinical research on GC in China, there are still deficiencies. Many studies enrolled large numbers of patients, but the research data were not of high quality. The characteristics of GC in China include a high incidence, large population, and large proportion of patients with advanced GC, which provides sufficient reason for studying this disease. There is still a need for well-designed, large, randomized clinical trials to improve our knowledge of the surgical treatment of GC.

Key Words: Gastric cancer; Clinical trial; Surgery; China; Minimally invasive surgery; Quality of life

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Core Tip: Gastric cancer (GC) is the leading common malignancy in China. This review summarizes the study design and the clinical or translational research focus to explore the state-of-the-art and development trends in Chinese GC cancer surgery studies.

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INTRODUCTION

The incidence of gastric cancer (GC) has decreased overall, but it is still a significant cancer with high mortality rates globally. Radical surgery is the primary treatment option for completely eradicating GC and forms the basis of GC treatment. Over the years, the evolution of GC surgical treatment has witnessed a transition in the scope of surgery: "from small to large, and then from large to small"[1]. This evolution in surgical approaches reflects advancements in medical practices and the desire for more effective and less invasive treatments.

China accounts for almost 24% of new cases and 30% of cancer-related deaths worldwide[2]. Unfortunately, most patients present with advanced disease because GC is largely asymptomatic in its early stages. Many developments in surgical techniques aim to improve long-term survival while minimizing postoperative complications. Understanding which of these approaches are optimal for patients should be based on robust evidence from well-designed trials. Clinical trials are the gold standard for evaluating the safety and efficacy of therapeutics and generating evidence-based knowledge in the field of medicine. In recent years, the number of clinical trials initiated in China has increased rapidly. Several high-level clinical studies published by Chinese investigators have fulfilled clinical guidance or served as opinion-change references[3]. Clinical trials, which are strictly governed health research investigations, facilitate the transition of medical advancements into everyday clinical applications. These trials present an opportunity for individuals to access cutting-edge treatments and supply essential data for enhancing medical care[4]. Clinical trials, especially randomized clinical trials, can reliably evaluate the true effects of different surgical interventions.

Data on the characteristics of clinical trials about GC surgery in China and how they have changed over time are scarce. Analyzing clinical trials can illuminate important trends over time. Here, we review the content and status of clinical trials for GC surgery in China.

DATA SOURCE

The National Library of Medicine operates ClinicalTrials.gov, a platform that offers details on ongoing clinical research projects across over 200 nations. To find all registered clinical trials involving patients with GC who had surgical procedures (using any technique) as part of their treatment up to April 4, 2022, the ClinicalTrials.gov database was accessed. The criteria for inclusion required "gastric cancer" to be listed as a Medical Subject Headings condition term, along with at least one of the following terms appearing in the keywords or titles: "surgery," "surgical," "gastrectomy," "laparoscopic," "robot," or "resection." This search was confined to randomized trials aimed primarily at treatment, with procedures or devices classified according to the type of intervention. Trials in which the main purpose was related to basic science, diagnostics, health services, prevention, or supportive care were excluded. Subsequently, studies were initially selected through a review of titles and abstracts, followed by a comprehensive review of full texts to confirm that the inclusion criteria were consistently applied.

The number of studies originating from China (271 [31.5%]) ranked first, followed by the United States (151 [17.6%]) and Korea (123 [14.3%]). We excluded trials that did not involve patients who underwent surgery for GC and that were not conducted in mainland China. Finally, 83 studies were further reviewed.

GENERAL CONDITIONS

From 2008 to April 2022, a total of 83 GC-related clinical trials were conducted in mainland China, of which 2 were Phase 1 (2.4%), 14 were Phase 2 (16.9%), 2 were Phase 2/3 (2.4%), 14 were Phase 3 (16.9%), 42 were not applicable (50.6%), and the last 9 (10.8%) fell into other categories (Figure 1). From 2008 to 2016, the number of trials conducted increased annually. Since 2014, the number of GC surgery-related clinical trials has increased significantly. Conversely, in 2018, the increase began to slow down. Thirty-two research institutes were responsible for 83 clinical trials, with the most active research institute being Fujian Medical University Union Hospital (15 cases). The majority of the 66 trials included were single-center studies (79.5%), and a large proportion of the clinical trials (89.2%) were interventional studies. Nine protocols were published, and 22 studies presented 28 results.

Regarding research content, surgeries more commonly involved laparoscopic gastrectomy (LG) than open gastrectomy (OG) in 16 trials, robotic gastrectomy (RG) than LG in 12 trials, function-preserving gastrectomy (FPG) and endoscopic treatment in 4 trials, digestive tract reconstruction after gastrectomy in 16 trials, the extent of surgical resection in 14 trials, image-guided minimally invasive treatment in 6 trials, and other methods (*e.g.*, enhanced recovery after surgery, reduced-port surgery, three-dimensional or ultra-high-definition laparoscopic surgery) in 15 trials. The major clinical research interests related to Chinese GC surgery are summarized in Table 1.

Table 1 Topics of major clinical research interests in gastric cancer surgery in China

Group	Subgroup	Research interests
CLASS	CLASS-01	LDG for AGC
	CLASS-02	LTG for EGC
	CLASS-03	LDG for AGC after NACT
	CLASS-04	Laparoscopic spleen-preserving No. 10 LN dissections for AGC
	CLASS-07	LTG for AGC
	CLASS-08	TLTG *vs* LATG
	CLASS-10	Laparoscopic *vs* open lower mediastinal lymphadenectomy for EGJ cancer
	CLASS-11	IGC in LG for AGC
	CKLASS01	TLDG *vs* LADG
Laparoscopic gastrectomy	LG	LG for AGC
	LDG	LDG for AGC (especially in the elderly patients)
	LTG	LTG with spleen-preserving No. 10 LN dissections for AGC
Robotic gastrectomy	RTG	RTG for EGC, AGC
	RDG	RDG for AGC
	TRG	TRG for GC
	Chinese RG	Chinese MIS robot system for GC
Reconstruction	DG	Uncut R-Y, Modified delta-shaped gastroduodenostomy, Modified BII + Braun
	PG	Double tract reconstruction, gastric tube reconstruction, Cheng's giraffe reconstruction
	TG	Functional jejunal interposition, R-Y pouch, Intracorporeal or extracorporeal esophagojejunostomy
The extent of surgical resection	Extended surgery	Bursectomy, type of omentectomy, complete mesogastrium excision, D2+ lymphadenectomy, D2 plus para-aortic nodal dissection, 14v LN dissection
	Limited surgery	D1 lymphadenectomy for elderly patients in AGC, role of No. 10 LN dissections, length of the proximal resection margin for Adenocarcinomas of the EGJ
Image-guided MIS		Method of ICG injection
		Carbon nanoparticles
Functional preservation and organ preservation		PPG or LAPPG, PG, partial gastrectomy
		ESD for EGC, ESD plus laparoscopic regional LN dissection for EGC
ERAS		NACT for AGC patients in ERAS programs
		ERAS in LDG
Reduced port surgery		1-3 ports LG
		Single-incision plus one-port LG
Others		Laparoscopic staging
		3D LTG plus spleen-preserving No. 10 LN dissections for AGC
		4K laparoscopic surgery
		LTG for remnant GC
		Closure of the mesenteric defects
		Intraoperative leak testing in gastrectomy

	Ultrasonic scalpel *vs* monopolar electrocautery for DG
	Extensive intraoperative peritoneal lavage

AGC: Advanced gastric cancer; CLASS: Chinese laparoscopic gastrointestinal surgery study; DG: Distal gastrectomy; EGC: Early gastric cancer; EGJ: Esophagogastric junction; ERAS: Enhanced recovery after surgery; ESD: Endoscopic submucosal dissection; GC: Gastric cancer; IGC: Indocyanine green; LADG: Laparoscopic-assisted distal gastrectomy; LAPPG: Laparoscopic-assisted preserving distal gastrectomy; LDG: Laparoscopic distal gastrectomy; LG: Laparoscopic gastrectomy; LN: Lymph node; LTG: Laparoscopic total gastrectomy; MIS: Minimally invasive surgery; NACT: Neoadjuvant chemotherapy; PG: Proximal gastrectomy; PPG: Preserving distal gastrectomy; RDG: Robotic distal gastrectomy; RG: Robotic gastrectomy; RTG: Robotic total gastrectomy; TG: Total gastrectomy; TLDG: Totally laparoscopic distal gastrectomy; TLTG: Totally laparoscopic total gastrectomy; TRG: Totally robotic gastrectomy.

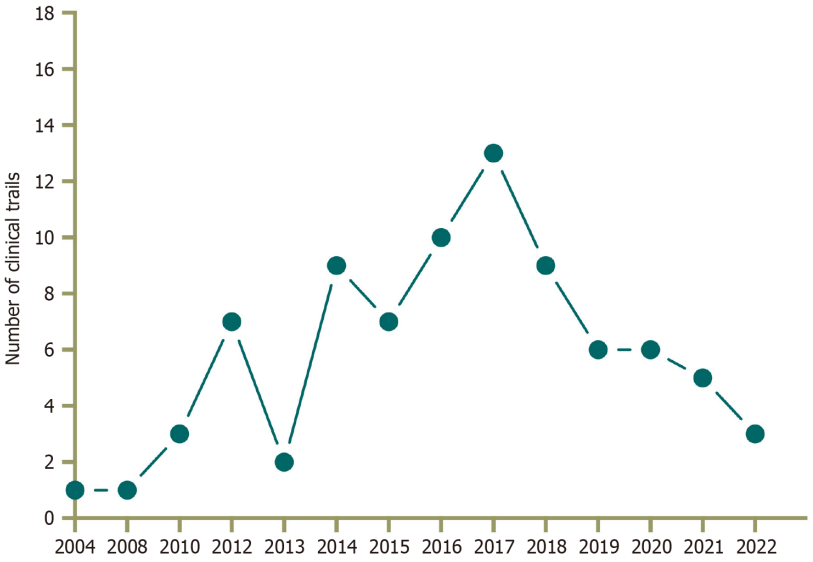

Figure 1 Initial registration date and development trends.

Chinese laparoscopic gastrointestinal surgery study group

Since its establishment in 2010, the Chinese laparoscopic gastrointestinal surgery (CLASS) group has been devoted to enhancing the quality of life (QoL) of patients through the use of minimally invasive surgery[5]. Prior to commencing numerous prospective trials, the multicenter database was created by integrating the current datasets from the CLASS group members[6].

At that time, the CLASS group performed the largest multicenter retrospective clinical study of laparoscopic advanced GC (AGC) in China. Data from 1184 consecutive patients between February 2003 and December 2009 were collected from the CLASS database and retrospectively analyzed. Postoperative morbidity and mortality after laparoscopic-assisted gastrectomy (LAG) and D2 dissection in AGC patients were 10.1% and 0.1%, respectively. LAG for locally AGC has been proven safe and technically viable, and it may also provide satisfactory short-term oncologic results[7]. Notably, elderly patients with resectable GC should not be denied the possible advantages of LAG, as long as their comorbid conditions are thoroughly considered[8]. Since then, the importance of multicenter clinical studies has been recognized, and high-quality clinical trials for laparoscopic GC surgery have been gradually progressing in China.

The CLASS-01 trial, which was conducted at 14 centers in China between 2012 and 2014, included 1056 patients with clinical stage T2 to T4a GC without bulky nodes or distant metastases. Earlier results were published in 2016 in the Journal of Clinical Oncology[9]. Both morbidity after surgery (15.2% for LAG compared with 12.9% for OG; $P = 0.285$) and mortality rates (0.4% for LAG compared with 0% for OG; $P = 0.249$) were found to be similar. These findings suggest that proficient surgeons are able to safely conduct D2 LAG for AGC. The final follow-up of the CLASS-01 trial was December 2017. Three-year survival results published in 2019 in The Journal of the American Medical Association[10] revealed no significant difference in 3-year disease-free survival (DFS; laparoscopic distal gastrectomy [LDG] 76.5% *vs* open DG [ODG] 77.8%) or overall survival (OS; LDG 83.1% *vs* ODG 85.2%). Similar to long-term survival with laparoscopy, OG was released in 2022[11]. The OS was 73% in the laparoscopic group and 76% in the open group. For patients with stage I tumors, the 5-year survival rate was 90% with laparoscopy and 89% with open surgery. For stage II and III tumors, the rates were 79% *vs* 85% and 59% *vs* 60%. None of these differences were significant after adjusting for age, sex, body mass index, performance status, comorbidities, tumor size, histologic features, or chemotherapy. The CLASS-01 trial concluded that experienced surgeons performing D2 LDG at high-volume specialized institutions achieved comparable long-term survival outcomes to ODG for patients with locally AGC.

Based on the experience of the CLASS-01 trial, the CLASS group has designed and launched several multicenter, prospective, high-quality clinical trials concerning the clinical problems of gastrointestinal surgery. In January 2017, the CLASS group launched the CLASS-02 trial to compare the safety of laparoscopic total gastrectomy (LTG) for clinical stage

I GC with that of conventional open TG (OTG)[12]. The JCOG1401[13] and KLASS03[14] trials, which reported the safety of laparoscopic assisted TG (LATG) for patients with clinical stage I proximal GC, were single-arm trials. Single-arm studies, while valuable in certain contexts, do not consistently provide an accurate representation of real-world outcomes. By contrast, the findings from prospective randomized controlled trials (RCTs) offer a higher level of credibility and are generally more persuasive. This CLASS-02 trial was the first prospective randomized two-arm multicenter, open-label, noninferiority RCT to determine the safety of LTG compared with OTG. From January 2017 to September 2018, a total of 227 patients were enrolled in this clinical trial. Its short-term results were published in 2020 in *JAMA Oncology*[15]. The overall incidence and death rates were 19.1% for the LTG group and 20.2% for the OTG group, with no significant difference observed. Similarly, the overall complication rate post-surgery (17.4% *vs* 18.1%) and mortality rate (0% *vs* 1%) were not significantly different between the LTG and OTG groups. Findings from the CLASS-02 trial indicated that LTG can be performed by seasoned surgeons with the same safety level as that for OTG in clinical stage I GC. This research establishes a baseline for subsequent oncological safety studies of LTG in early-stage GC and, more extensively, for AGC.

The safety and efficacy of the laparoscopic procedure in patients who have undergone neoadjuvant chemotherapy remain uncertain. There is a critical question in clinical practice regarding whether reduced surgical trauma is associated with improved postoperative safety, successful completion of chemotherapy, and enhanced survival benefits. To address this, the CLASS-03 trial was conducted from April 2015 to November 2017, aiming to investigate the safety and efficacy of the laparoscopic procedure for these patients (NCT02404753)[16]. The findings revealed that the overall postoperative complication rate was significantly lower in patients who underwent LDG than in those who underwent ODG, with rates of 20% and 46%, respectively (P = 0.007). Additionally, patients in the laparoscopic group experienced less postoperative pain and achieved better completion rates of adjuvant chemotherapy (adjusted odds ratio, 4.39; 95% confidence interval [CI]: 1.63–11.80; P = 0.003)[17]. These short-term results indicate that patients with locally AGC may benefit from LDG because of reduced postoperative complications and improved tolerance to adjuvant chemotherapy.

The rate of No. 10 lymph node (LN) metastasis is high in advanced proximal GC (APGC; 9.8%–27.9%)[18] and is still necessary for a portion of proximal GC[19]. In the past, splenectomy was conducted concurrently to ensure effective No. 10 LN dissection at the splenic hilum. However, various prospective RCTs have indicated that splenectomy should not be routinely performed due to elevated morbidity and mortality rates[20,21]. Because of the unique and intricate anatomy of the spleen, spleen-preserving splenic hilar lymphadenectomy poses challenges, especially during open surgery[22]. As a result, there is an urgent need is to establish an optimal and secure method for laparoscopic spleen-preserving No. 10 LN dissection[23]. CLASS-04 is a single-arm clinical trial conducted in 19 centers in China that enrolled 251 patients. The aim was to provide solid evidence supporting the use of laparoscopic spleen-preserving TG (LSTG) in advanced upper third GC[24]. The mean counts of No. 10 LN dissections and metastases were 2.4 and 0.1, respectively. The overall rate of postoperative complications was 13.6% (33/242). The rates of major complications and mortality were 3.3% (8/242) and 0.4% (1/242), respectively. The 3-year OS was 79.1% (95%CI: 74.0%-84.2%), and the 3-year DFS was 73.1% (95%CI: 67.4%-78.8%). Patients with No. 10 LN metastasis might experience poorer survival outcomes and are more susceptible to recurrence (42.1% *vs* 20.7%; P = 0.03)[25]. The short- and long-term findings of the CLASS-04 trial demonstrated that LSTG was both safe and efficient when performed by highly skilled surgeons. This approach could be considered for patients requiring splenic hilar LN dissection. Nonetheless, this study lacks information on the reproducibility, oncologic outcomes, and benefits of such endeavors.

Total LDG (TLDG) has several advantages over laparoscopy-assisted DG (LADG), including smaller wounds[26] and easy anastomosis, especially for obese patients. However, evidence is still lacking concerning whether the QoL of patients undergoing TLDG is superior to that of patients undergoing LADG. Therefore, the Korean laparoendoscopic gastrointestinal surgery study (KLASS) group and the CLASS group jointly launched the CKLASS-01 trial for the first time. This CKLASS-01 trial, activated in January 2018, is a prospective, multicenter RCT that compared the QoL of TLDG and LADG patients[27]. The CKLASS-01 trial is a successful attempt of the CLASS group to explore the transnational clinical research cooperation mode of minimally invasive surgery for GC.

After the successful CLASS-01 to CLASS-04 trials, the trials from the CLASS-05 to CLASS-11 series advanced in succession. The CLASS-05 investigated cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy and chemotherapy for patients with GC with peritoneal metastasis (NCT03023436). The CLASS-06 trial focused on the surgical safety of laparoscopic surgery for gastrointestinal stromal tumors in an unfavorable location. Based on previous CLASS-02 results of LTG for early GC (EGC), the prospective CLASS-07 trial is a multicenter RCT (NCT04710758) that aims to evaluate long-term outcomes in patients with locally AGC following either LTG or OTG. Additionally, the CLASS-08 trial seeks to compare the immediate surgical safety and postoperative QoL between total LTG and laparoscopy-assisted TG (NCT04351321). The CLASS-09 trial investigated the application of enhanced recovery after surgery in laparoscopic GC surgery. The CLASS-10 trial aims to provide standard technical details of laparoscopic mediastinal LN dissection and explore the potential clinical effects for Siewert type II/III adenocarcinoma of the esophagogastric junction (NCT04443478). The safety, efficacy, and feasibility of indocyanine green (ICG) near-infrared imaging tracing in guiding laparoscopic D2 LN dissection for GC were investigated in the CLASS-11 trial (NCT04593615).

The successful implementation of the CLASS series trails has created a new standard in the clinical research of minimally invasive gastrointestinal surgery in China. The CLASS group has cooperated with domestic counterparts, developed guidelines and consensuses[28] based on previous research results, and standardized and established a complete set of standard systems for laparoscopic preoperative evaluation, surgical indications, treatment principles, surgical quality control, oncological efficacy evaluation, and tissue specimen treatment for GC.

RG compared with laparoscopic surgery for GC

While laparoscopy is currently the primary technique in the realm of modern surgical procedures, minimally invasive surgery has been steadily progressing towards robotic-assisted procedures. Early studies conducted in Korea and Japan

compared the advantages of RG to LG, with findings suggesting that RG does not show any inferiority[29-31]. Since the first report on RG for GC in 2010[32], several retrospective studies from China have shown that RG is associated with less blood loss[33-36], a shorter hospital stay, more harvested LNs[34-37], and similar long-term oncological outcomes compared with those of LG[33-36,38]. The disadvantages of RG, such as longer operation times[33,35,37,38] and higher total costs[33,34,38], have also been investigated.

However, the findings from a prospective comparative study involving multiple centers revealed that LG is not inferior to RG in terms of immediate surgical results[39]. Additionally, some studies have reported that there were no disparities in long-term cancer-related outcomes between LG and RG. Despite the potential technical advantages of the robotic system over laparoscopy, there was no improvement in cancer-related outcomes following gastrectomy[40]. Criticism of robotic surgery in gastrectomy has focused on the perceived absence of advantages in relation to the cost of the procedure. Consequently, there is an urgent need for additional extensive studies, particularly RCTs, to evaluate the accuracy of these findings.

There were 13 registered clinical trials from China, among which 7 were RCTs including 2 multicenter studies. Eight trials investigated robotic *vs* LG for DG, proximal gastrectomy (PG) and TG in early to advanced stages (NCT03273920, NCT03524300, NCT03313700, NCT03500471, NCT03524287, NCT03447106, and NCT05235932). One trial compared vagus nerve-preserving robotic-assisted DG (RADG) and conventional RADG for AGC (NCT02806661). One trial is concerned with the learning curve for robot-assisted gastrectomy in GC (NCT03940417), and the other trial is investigating the Chinese domestic surgical robot for GC (NCT02752698).

To date, the results of only two prospective studies comparing RG and LG have been published. One RCT (FUGES-011, NCT03313700) enrolled 300 patients with clinical stages T1--4a and N0/+ disease between September 2017 and January 2020. The findings indicated that the robotic group experienced quicker postoperative recovery ($P < 0.05$), a greater number of dissected LNs (17.6 ± 5.8 *vs* 15.8 ± 6.6; $P = 0.018$), and decreased postoperative morbidity (9.2% *vs* 17.6%; $P = 0.039$) compared with those of the laparoscopic group. Moreover, patients in the robotic DG (RDG) group started adjuvant chemotherapy sooner (median [interquartile range] postoperative days: 28 [24-32] *vs* 32 [26-42]; $P = 0.003$)[41]. Another prospective single-arm study (FUGES-014, NCT03524287) conducted at the same institution reported similar favorable surgical outcomes, including the number of LNs retrieved and intraoperative blood loss, in the robotic TG group compared with those in the LTG group[42].

Laparoscopic surgery compared with open surgery for the treatment of GC

Numerous multicenter RCTs conducted in Asia (including KLASS-01, KLASS-02, JCOG0912, JLSSG0901, and CLASS-01) have established that LDG results in improved short-term and long-term outcomes compared with those of open surgery. In addition to the CLASS series trials, eight clinical trials from China have been registered in Clinical Trials. All the studies were RCTs.

Initially, Southwest Hospital, registered in China, conducted an RCT to evaluate the effectiveness of LAG in treating patients with local AGC who underwent proximal, DG, or TG (NCT01043835). From January 2010 to June 2012, a total of 328 patients with preoperative cT2-4aN0-3M0 GC participated in the study. Only 8% of the patients enrolled were in the early stage. Conversely, the percentage of patients with advanced-stage disease exceeded that reported in the CLASS-01 trials. Short-term findings indicated that there was no significant disparity in morbidity rates between the LAG (11.72%) and OG groups (14.38%)[43]. The research confirmed that LAG offered improved short-term results, aligning with that of the prospective CLASS-01 investigations. Notably, there have been no previous prospective RCTs investigating LATG for AGC treatment. The trial included 61 (37.65%) individuals who underwent D2 LATGs. The study reported an overall complication rate of 14.75% in the LATG group, which was within acceptable limits and comparable to OTG outcomes. Nevertheless, limitations included the small patient sample size and low complication rates, potentially impacting the reliability of the subgroup analysis for TG. The long-term outcomes of the trial published in 2019 revealed no significant differences in 5-year OS (LAG 49% *vs* OG 50.7%) or DFS (LDG 47.2% *vs* ODG 49.6%)[44]. Similar short-term results from the Beijing Cancer Hospital (Beijing, China) RCT support the same conclusions[45].

Individuals residing in the northern regions of China exhibit a higher prevalence of obesity than their counterparts in the southern areas, primarily attributed to economic disparities, climate variations, and regional distinctions[46]. The presence of obesity plays a significant role in impacting the outcomes of surgical procedures[47]. In contrast to the CLASS-01 study, which encompassed a nationwide RCT, the SWEET trial was specifically designed to assess the comparability of D2 LADG with ODG concerning the safety of operations for locally AGC in northern China (NCT02464215)[48]. The overall incidence of postoperative complications in the SWEET trial (LADG *vs* ODG: 13.1% *vs* 17.7%; $P = 0.174$) mirrored the findings of the CLASS-01 study (LDG *vs* ODG: 15.2% *vs* 12.9%; $P = 0.285$). For the conversion rate from LADG to ODG, the results were also comparable between the two trials (6.3% *vs* 6.4%). Moreover, the mean number of retrieved LNs was comparable in the SWEET trial (LADG *vs* ODG: 29.5 ± 10.4 *vs* 31.4 ± 12.3; $P = 0.083$), which was slightly lower than that reported in the CLASS-01 trial (LDG *vs* ODG: 36.1 ± 16.7 *vs* 36.9 ± 16.1; $P = 0.738$).

Guo *et al*[49,50] from the Chinese PLA General Hospital conducted a single-center RCT to compare the short-term surgical outcomes between laparoscopic spleen-preserving splenic hilar lymphadenectomy (LSPL) and open spleen-preserving splenic hilar lymphadenectomy (OSPL; NCT02980861). Unlike the CLASS-04 trial, which focused primarily on the laparoscopic technique for spleen-preserving No. 10 LN dissection, this trial is the first to evaluate the value of laparoscopy specifically for No. 10 LN dissection. The study enrolled a total of 222 patients, with 114 patients assigned to the LSPL group and 108 to the OSPL group. The results revealed no significant difference between the two groups in terms of the number of harvested LNs ($P = 0.669$), including No. 10 LNs, with averages of 2.1 ± 1.4 in the LSPL group compared with 2.3 ± 1.2 in the OSPL group ($P = 0.713$). Moreover, there was no statistically significant difference in the operative time ($P = 0.152$) or postoperative complication rates (18.3% in the LSPL group *vs* 16.1% in the OSPL group; $P = 0.331$). However, one notable finding was that the LSPL group experienced significantly less blood loss than the OSPL

group, indicating an advantage of the laparoscopic technique. While the assessment of long-term survival outcomes is still ongoing, this trial demonstrated that laparoscopy could offer significant benefits over open surgery in terms of surgical outcomes. Moreover, the radical effects of laparoscopy were found to be noninferior to those of open surgery.

China has experienced a gradual shift towards an aging population. The increasing elderly population has raised significant concerns. Compared with younger individuals, elderly patients typically require more stringent control over surgical injury management. Therefore, local AGC has prioritized research on surgical safety and effectiveness for elderly patients. To date, only one prospective cohort study from Japan revealed that LG can be performed safely in elderly patients and can shorten the length of the postoperative hospital stay[51]. All other currently available evidence supports that the benefits of the laparoscopic approach for elderly patients, such as less trauma and lower systemic morbidities, is from observational studies[52]. There are two ongoing prospective single-center RCTs from China evaluating the safety and efficacy of LG compared with those of OG for AGC in elderly patients (NCT03564834[53]; NCT02246153[54]).

FPG and endoscopic treatment

For patients with EGC, increasing postoperative QoL without sacrificing long-term survival is receiving increasing attention. When aiming to maintain a patient's QoL after surgery, FPG, including techniques such as PG and pylorus-preserving gastrectomy, can be considered. Beijing Cancer Hospital is performing multicenter RCTs to evaluate the safety and effectiveness of FPG, including pylorus-preserving DG (PPG), PG, and wedge gastrectomy, for T1 and T2 GC patients (NCT03874871).

In the treatment of middle stomach EGC, either PPG or DG can be conducted. Compared with DGs, PPG has been recognized for its functional advantages, such as nutritional benefits and a reduced risk of dumping syndrome, bile reflux, and gallstone formation[55,56]. Nonetheless, PPG may pose potential risks to oncologic safety because fewer or incompletely dissected LNs are needed compared with that for DGs[57,58]. To date, no RCTs have compared the perioperative outcomes and long-term nutritional status between laparoscopic-assisted PPG (LAPPG) and LADG. One ongoing prospective RCT (NCT02936193) comparing LAPPG to LADG was performed at Shanghai Renji Hospital (Shanghai, China) based on the leading experience of LAPPG in China[56].

Many EGCs with relatively low LN metastasis can be curatively treated with endoscopy. Several retrospective cohort studies have explored the topic of endoscopic submucosal dissection (ESD) *vs* surgery. Findings from these studies demonstrated that, compared with surgery, ESD resulted in shorter procedure times, quicker recovery, and fewer early and severe complications. Despite these benefits, the ESD group also exhibited a higher recurrence rate[59]. No RCTs have compared the long-term outcomes of ESD and surgery for EGC treatment. Peking University Third Hospital (Beijing, China) designed one RCT to compare the long- and short-term outcomes between ESD and surgery in the treatment of EGC (NCT03857737). The trial enrolled 300 patients.

On the other hand, for some EGC patients with independent risk factors for LN metastasis, such as submucosal tumor invasion, lymphovascular invasion, undifferentiated type, and size larger than 2 cm, standard gastrectomy with LN dissection is usually recommended even if the gastric lesions can be completely removed *via* endoscopy[19]. Among the new possibilities, the combination of ESD with laparoscopic LN dissection (LLND), the so-called hybrid laparoscopic approach, represents one of the most interesting procedures for EGC patients with a potential risk of LN metastasis[60,61]. The hybrid approach was considered to improve patients' postoperative QoL. One RCT was conducted at Beijing Friendship Hospital (Beijing, China) to determine whether ESD combined with LLND for EGC can improve long-term outcomes (NCT02325999).

Digestive tract reconstruction after gastrectomy

Reconstruction of the digestive tract is a crucial skill in laparoscopic procedures. Nonetheless, there is presently no unanimous agreement on the optimal selection process for the different techniques. There are 16 registered clinical trials from China, among which 14 are RCTs, including one multicenter study.

Billroth-I, Billroth-II, Roux-en-Y, and uncut Roux-en-Y reconstructions are possible for DGs. Delta-shaped gastroduodenostomy (DSG), a method of intracorporeal Billroth-I anastomosis using only endoscopic linear staplers, was first reported in 2002[62]. Huang *et al*[63] simplified the technique of DSG and reported the safety and advantages of their modified DSG. After that, they performed one prospective RCT that compared modified DSG with the Billroth-I for DGs to explore the clinical application value and long-term oncology results (NCT02289183). Diabetes mellitus is a challenging health issue linked to a greater likelihood of cardiovascular events and diseases. Due to its antidiabetic properties, Roux-en-Y gastric bypass is commonly utilized in bariatric and metabolic surgery. These findings suggest that Roux-en-Y reconstruction during GC surgery could lead to improved glucose metabolism compared with that associated with the Billroth-I and Billroth-II procedures; however, there is still a lack of strong evidence. Two RCTs were designed to separately compare different reconstruction methods in patients with GC comorbid with type 2 diabetes (NCT01528059; NCT01637350). To date, researchers have not published these results.

The Uncut Roux-en-Y gastrojejunostomy modifies the Billroth-II procedure with Braun anastomosis by incorporating a jejunal occlusion to prevent Roux-Stasis Syndrome (RSS)[64]. The number of trials concerning uncut Roux-en-Y anastomosis ranked first among all the registered trials about digestive tract reconstruction. One single-arm RCT evaluated whether the QoL after uncut Roux-en-Y was superior to that after traditional BII + Braun reconstruction (NCT03624725). To analyze the advantages and disadvantages of the Uncut Roux-en-Y and Billroth-II reconstructions, the First Hospital of Jilin University (Jilin, China) performed the first RCT enrolling 158 patients between February 2015 and February 2016. The short-term surgical results revealed that stomach pH values in uncut Roux-en-Y grafts were lower than 7 during the postoperative period and presented a lower incidence of biliary reflux and alkaline gastritis after 1 year of follow-up (NCT02694081)[65]. The lower incidence of bile reflux gastritis and RSS was also confirmed by a multicenter RCT enrolling 124 patients from January 2017 to May 2018[66]. Another ongoing RCT by the Sixth Affiliated Hospital,

Sun Yat-sen University, is designed to compare uncut Roux-en-Y with Billroth-II in terms of postoperative nutritional status (NCT02763878)[67]. Two prospective RCTs comparing Uncut Roux-en-Y with conventional Roux-en-Y (NCT03349398; NCT02644148) have also been conducted to investigate the incidence of RSS and QoL.

Several different reconstruction techniques are possible after PG, such as gastric tube reconstruction, jejunal interposition, and jejunal pouch interposition, *via* laparoscopic or minilaparotomy. Cheng *et al*[68] from Zhejiang Cancer Hospital created a new reconstruction named Cheng's GIRAFFE reconstruction. This technique combines the advantages of gastric tube reconstruction with rebuilding the His angle and fundus of the stomach[68,69]. They performed one prospective RCT to investigate the efficacy and safety of PG combined with GIRAFE anastomosis for early adenocarcinoma of the esophagogastric junction (NCT04657848).

There is no agreement on the optimal reconstruction technique following TG. Roux-en-Y anastomosis between the esophagus and jejunum is a straightforward choice for reconstructing the gastrointestinal system[70]. Roux-en-Y OE can be performed *via* an intracorporeal linear staple and an extracorporeal circular staple. OrVil™ (Covidien, Mansfield, MA, United States) utilizes a transorally inserted anvil along with a circular stapler for conducting intracorporeal esophagojejunostomy[71]. Based on their experience with intracorporeal Roux-en-Y anastomosis *via* an OrVil, Lu *et al*[71] designed a prospective RCT to explore the safety of intracorporeal esophagojejunostomy using a transorally inserted anvil (OrVil) compared with extracorporeal circular anastomosis (NCT02085031).

However, some surgeons have noted that the emptying time can be extremely fast, which increases the incidence of dumping syndrome following Roux-en-Y anastomosis. A lack of a stomach can significantly affect a patient's nutritional status, leading to a reduced QoL[72]. To create a substitute for the stomach, the remaining part of the intestine might be reconstructed into a reservoir, with or without maintaining passage through the duodenum. Functional jejunal interposition (FJI) entails PG reconstruction that includes a reservoir and retains transduodenal passage[73,74]. In one prospective, multicenter RCT, 113 patients were enrolled to compare the nutritional status between those who underwent FJI and those who underwent Roux-en-Y after TG (NCT01996059). After a follow-up period of 12 months, food intake per meal (P = 0.021), the prognostic nutritional index (P = 0.015), weight loss (P = 0.019), and Gastrointestinal Symptom Rating Scale score (P = 0.015) were significantly lower in the FJI group than in the Roux-en-Y group. The results of this trial revealed that the FJI procedure is not superior to Roux-en-Y anastomosis after TG[75]. Roux-en-Y + jejunal pouch anastomosis is another newly designed method that can significantly increase volume to improve postoperative QoL. The Western China Gastric Cancer Collaboration (WCGCC) performed its first RCT to compare the postoperative QoL after traditional Roux-en-Y and Roux-en-Y + jejunal pouch anastomosis for TG (WCGCC-1202, NCT02110628), which began in 2015, and this study is now in the recruitment period[76].

Extent of surgical resection

Given the elevated frequencies of morbidities and recurrence after D2 gastrectomy, minimizing surgical risks and enhancing the long-term survival of GC patients presents an ongoing challenge for surgical professionals. Xie *et al*[77] and Cao *et al*[78] described the novel notion of integrating D2 lymphadenectomy with total mesogastrium excision (D2 + CME) as a mesentery-based approach in GC surgery. Over the period spanning from September 2014 to June 2018, Gong and colleagues executed a prospective RCT (NCT01978444) to compare D2+ CME with standard D2 dissection in the context of GC management[79]. A cohort of 486 patients receiving DG were randomly allocated to either the D2 arm or the D2 + CME arm[80]. Initial findings revealed that D2 + CME was associated with improvements in surgical outcomes, such as reduced intraoperative blood loss, augmented LN retrieval (34 ± 16 *vs* 27 ± 13; P < 0.0001), and decreased postoperative flatus duration. The total procedural time was greater for D2 + CME than for D2. The primary endpoint, 3-year DFS, is undergoing evaluation during the ongoing follow-up phase. Notably, the implementation of D2 + CME was limited by its proponent, whereas seven other surgeons carried out D2 procedures, a factor that could introduce potential bias stemming from varying levels of personal experience.

Splenic hilar LN (No. 10) dissection is still controversial for patients in whom APGC does not invade the greater curvature. Huang's team performed a prospective RCT to evaluate (FUGES-002, NCT02333721) the benefit of LTG combined with spleen-preserving splenic hilar lymphadenectomy (LSTG) for APGC not invading the greater curvature and the characteristics of No. 10 LN metastasis. Between January 2015 and December 2018, 536 patients were enrolled and randomized to receive either LSTG (D2 + No. 10 group) or conventional LTG (D2 group). There were no significant differences in intraoperative or postoperative morbidity between the two groups. The 3-year DFS was not significantly different (70.3% *vs* 64.3%). However, individuals in whom APGC was positioned behind the gastric wall might gain advantages from No. 10 LN dissection. Stratification analysis revealed that those with advanced posterior GC in the D2 + No. 10 group had superior 3-year DFS (92.9% compared with 39.3%; P < 0.001) and OS (92.9% compared with 42.9%; P < 0.001)[81].

The central controversies include whether bursectomy can reduce local recurrence, improve OS, and increase postoperative complications. Theoretically, both the omentum and bursa omentalis should be resected to prevent peritoneal metastasis. Guangdong Provincial Hospital of Traditional Chinese Medicine published two RCTs to explore the short- and long-term outcomes of LTG with bursectomy (NCT02969148, NCT03117283). However, the sample size of these trials was small. Moreover, the results of a large-scale multi-institutional randomized trial (JCOG 1001) from Japan released in 2019 indicated that bursectomy did not provide a survival advantage over nonbursectomy[82]. Nevertheless, it remains unclear whether omentectomy should be performed. Some meta-analyses have shown that, compared with total omentectomy in GC surgery, partial omentectomy has noninferior oncological outcomes and comparable safety outcomes[83]. Recently, a randomized Phase 2 trial (the TOP-G trial) comparing omentectomy and omentum preservation for GC in Japan reported short-term outcomes, which indicated that the operative risks were generally similar between the two groups[84]. Moreover, Tianjin Medical University Cancer Institute and Hospital (Tianjin, China) successfully conducted single-center (NCT04108494) and multicenter (NCT04843215) prospective RCTs to prove the non-inferiority of

omentum preservation over omentectomy in patients with T3–T4a GC. The NCT04843215 trial from China is large scale, and the estimated number of enrolled participants is 950. It is believed that with the publication of the results in the future, more favorable evidence can be provided.

D2 gastrectomy is commonly accepted as the main treatment for AGC. However, there is ongoing debate regarding the scope of LN dissection depending on tumor stage, tumor location, and patient condition. Presently, the disputed regions beyond the typical D2 range include stations No. 13, No. 14v, and No. 16a2/b1 LN[85]. As stated in the Japanese GC treatment guidelines, No. 13 LNs are considered regional LNs in GC patients with duodenal invasion. Optimizing for D2 plus No. 13 lymphadenectomy could be a viable choice in curative gastrectomy for tumors with duodenal involvement. Although 14v is not part of standard D2 lymphadenectomy, it is still classified as a regional gastric LN, and conducting D2+ No. 14v lymphadenectomy could benefit patients with cancer spread to the No. 6 LN[19]. Nevertheless, the guidelines do not address the importance of dissecting No. 8p, 12b, or 12p LNs in cases of duodenal invasion. Due to the lack of RCTs investigating the survival advantages of 14v dissection, no definitive conclusions can be drawn[86]. To further shed light on the necessity of No. 14v node dissection for localized AGC, Tianjin Medical University Cancer Institute and Hospital conducted a multicenter prospective RCT to analyze the potential influence of No. 14v node dissection on the long-term survival outcomes of GC patients (NCT02272894). Zhejiang Cancer Hospital (Zhejing, China) performed another RCT to compare D2 and D2 plus (D2 + 8p, 12b, 13, 14v) radical surgery for distal AGC (NCT01996059). Between February 2013 and September 2015, individuals diagnosed with GC infiltrating the pylorus were included in this research. In regard to radical gastrectomy for GC with pyloric invasion, dissection of the No. 13 and No. 14v LNs resulted in a survival benefit over a 3-year period. Notably, the No. 8p LNs did not show the same survival benefit. For cT3 GC patients with pyloric invasion, it may be more advantageous to dissect the No. 12b and No. 12p LNs[87].

JCOG9501 reported that D2 lymphadenectomy plus para-aortic nodal dissection (PAND) did not improve the prognosis of patients with curable GC[88]. Some patients with limited para-aortic LN (PALN) metastasis may benefit from curative D2 plus PAND[88]. The First Affiliated Hospital of Sun Yat-Sen University (Guangdong, China) reviewed their database and reported that D2 lymphadenectomy plus PAND improved the prognosis of T4 tumors and tumors with LN metastasis at the second station[89]. They conducted one multicenter RCT to explore the long-term effect of D2 lymphadenectomy plus PAND for the treatment of GC (NCT02423278).

Despite improvements in surgical methods and patient management, age remains a major factor in the risk of postoperative complications and death. There is ongoing debate regarding the possibility of elderly individuals with GC undergoing less invasive procedures with decreased LN removal. In a study conducted by Huang and colleagues, an RCT was conducted to assess the safety of laparoscopic D1 lymphadenectomy and determine its impact on survival rates for older patients diagnosed with AGC (NCT03290209).

Image-guided minimally invasive treatment

Recently, different tracers and dyes have been utilized in clinical settings to monitor LN drainage from initial tumors. Due to the effective use of ICG fluorescence imaging technology in laparoscopic instruments, the exploration of ICG fluorescence imaging-guided minimally invasive therapy in individuals with GC has emerged as a novel focus[90]. The existing injection methods include the submucosal approach and subserosal approach. There is a lack of RCTs evaluating the safety, efficacy, feasibility, and method of ICG-guided laparoscopic D2 lymphadenectomy. A Phase 3 parallel open-label RCT (FUGES-012, NCT03050879) took place at Fujian Medical University Union Hospital. Between November 2018 and July 2019, a total of 258 patients were enrolled in the study. The number of LNs retrieved in the ICG group (50.5 ± 15.9) was notably greater than that in the non-ICG group (42.0 ± 10.3). The LN retrieval rate was significantly lower in the ICG group (31.8% *vs* 57.4%; $P < 0.001$). The postoperative recovery process was not significantly different, with a similar incidence (15.5% *vs* 16.3%, $P = 0.86$) and severity of complications within 30 days postsurgery[91]. The researchers indicated that the study included all potential tumor sites, and the unevenly matched tumor location may be a limitation in the trial. Next, they designed another RCT to evaluate ICG in LDG for EGC (FUGES-023, NCT04973475). The same team continued to explore the optimal ICG injection method for LN tracing during LG. The FUGES-019 trial showed that ICG administered by subserosal injection during surgery imposed a lower economic and mental burden on patients and had similar surgical results to those of the preoperative submucosal injection (FUGES-019, NCT04219332)[92].

Carbon nanoparticles (CNPs) are another type of tracer used in laparoscopic surgery. Two RCTs explored the efficacy and safety of CNPs compared with (NCT05229874) or without those of ICG (NCT02123407) during LG.

Thirty patients were enrolled in the NCT02123407 trial conducted by Beijng Cancer Hospital. The mean number of harvested LNs was greater in the CNP group than in the noncoloring tracer group (38.33 *vs* 28.27; $P = 0.041$). A smaller diameter of LNs was recorded in the CNP group (3.32 mm *vs* 4.30 mm; $P = 0.023$)[93].

DISCUSSION

The quantity and quality of clinical research on minimally invasive surgery for GC have increased annually. The use of traditional minimally invasive laparoscopic surgery for treating GC has reached a plateau, and various improved new technologies have gradually emerged. Although great progress has been made in clinical research on GC in China, deficiencies still exist. There are more single-center retrospective studies than multi-center prospective studies and more repetitive research than innovative research. Many studies enrolled large numbers of patients, but the research data were not of high quality. However, it should also be noted that Chinese clinical research has distinct advantages and great development opportunities. The large population, high incidence and large proportion of AGC cases are characteristic of GC in China, which provides favorable conditions for studying this disease.

This study had some limitations. First, our review focused solely on the GC surgery trials listed on Clinical Trials.com, which may have led to the omission of some trials that are registered in domestic clinical trial registries in China. Second, the way outcomes are reported in GC surgery trials lacks consistency, showing significant variability in terms of definitions, measurement instruments, and timing of assessments. Consequently, this hinders efficient data synthesis.

CONCLUSION

This review highlighted the advancement and increasing adoption of endoscopic, laparoscopic, and robotic methods, marking a transition towards more accurate and less invasive surgical alternatives for the treatment of GC. Additionally, the results emphasized the increasing focus on improving the postoperative QoL of patients, suggesting a comprehensive treatment strategy that extends beyond mere surgical oncological safety. However, there is still a need for well-designed, large randomized clinical trials to improve our knowledge of the surgical treatment of GC.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Pattern of colorectal surgery and long-term survival: 10-year experience from a single center

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Abstract

BACKGROUND

The incidence of colorectal cancer (CRC) has increased in recent decades, and ranks fourth among males and third among females in China. Surgical resection remains the most important treatment modality for curative intent in CRC. Several studies found that surgeon volumes and specialization appeared to be associated with improved overall survival (OS). Moreover, numerous reports have suggested that specialization and minimally invasive surgery have gained increased popularity in CRC surgery. However, few studies have specifically examined the role and long-term survival of all stage CRC in a real-world study.

AIM

To evaluate the effect of surgeon specialization on survival changes and minimally invasive surgery utilization in a real world study.

METHODS

A retrospective analysis on the association between surgeon specialization and OS between 2008 and 2013 in Zhongshan Hospital CRC database was performed. Standard demographic, clinicopathologic, surgical and follow-up data were obtained from the CRC database. Surgeon specialty was categorized as colorectal surgeon (CS) and general surgeon (GS). CRC patients who underwent primary surgical resection were enrolled.

RESULTS

A total of 5141 CRC patients who underwent primary surgical resection between 2008 and 2013 were evaluated, 1748 (34.0%) of these by CS. The percentage of minimally invasive procedures in the CS group showed an increasing trend. There was no benefit associated with surgeon specialization for stage I, II and IV patients. Surgeon specialization exhibited a significant association with OS solely among stage III patients, with 5-year OS rates of 76% and 67% for the CS and GS

groups, respectively ($P < 0.01$). Further analyses found that surgeon specialization was significantly associated with survival only in stage III rectal patients, and the 5-year OS rate in the CS group and GS group was 80% and 67%, respectively ($P < 0.01$).

CONCLUSION
Surgeon specialization is associated with improved OS after primary surgery in stage III rectal patients. An appropriate surgical technique, perioperative program and adjuvant therapy may contribute to survival benefit in these patients.

Key Words: Colorectal surgery; Minimally invasive surgery; Primary location; Overall survival; Tumor stage; Follow-up

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Core Tip: This investigation is the largest real-world study comparing colorectal cancer surgery patterns and overall survival (OS) in a Chinese tertiary university hospital. A total of 5141 colorectal cancer patients who underwent primary surgical resection between 2008 and 2013 were evaluated, 1748 (34.0%) of these by a colorectal surgeon (CS). The percentage of minimally invasive procedures in the CS group showed an increasing trend. We found that surgeon specialization is associated with improved OS after primary surgery in stage III rectal patients. An appropriate surgical technique, perioperative program and adjuvant therapy may contribute to survival benefit in these patients.

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INTRODUCTION

The incidence of colorectal cancer (CRC) has increased in recent decades, and ranks fourth among males and third among females in China[1]. Despite the emergence of a variety of effective chemotherapy and targeted drugs for CRC, surgical resection remains the most important treatment modality for curative intent. Furthermore, the effectiveness of surgical care depends heavily on the experience of the surgical team. Numerous reports have suggested that higher hospital and surgeon volumes are associated with improved survival outcomes[2,3]. In addition, several studies also found that surgeon specialization appeared to be associated with improved overall survival (OS)[4-6].

Since first introduced in 1991, laparoscopic colon resection can significantly reduce surgical trauma, promote recovery, and provide equivalent long-term oncological outcome, compared with open surgery[7-9]. Therefore, laparoscopic colon resection has been recommended as the standard procedure in national and international guidelines. However, adoption of the laparoscopic approach for rectal cancer still remains controversial, as quality data on long-term survival is limited [10-13]. Newly emerged in this century, robotic surgery is generally easier to learn, improves the probability of autonomic nerve preservation, and produces similar perioperative outcomes in oncologic procedures to conventional laparoscopic surgery[14-16].

Taken together, specialization and minimally invasive surgery have gained increased popularity in CRC surgery. However, few studies have specifically examined the role and long-term survival of all stage CRC in a real-world study. Thus, the purpose of this retrospective study was to evaluate and compare the effect of surgeon specialization on survival changes and minimally invasive surgery utilization.

MATERIALS AND METHODS

Patients and methods

We obtained data of all patients diagnosed with colon or rectal adenocarcinoma who underwent surgical resection of CRC between 2008 and 2013 from Zhongshan Hospital CRC database. All patients in the CRC Database provided written informed consent. This retrospective study was reviewed by the institutional review board of Zhongshan Hospital, Fudan University.

Standard demographic, clinicopathologic, surgical and follow-up data were collected for each patient from the CRC database, and, when necessary, from patient records. The primary outcome was OS, and our primary interest was the association between surgeon specialization and OS. Prior to analysis, we also defined patient (age and gender), tumor (location and TNM stage) and surgical approach (minimally invasive surgery) characteristics, which may be associated with OS.

The surgeons were categorized as colorectal surgeons (CSs) and general surgeons (GSs) based on the subspecialty of the surgeon. The CS performed more than 120 colorectal resections each year, and colorectal cases comprised more than 80% of their surgical caseload. Mortality was defined as death occurring within 30 days after colorectal surgery. Survival time was calculated from the date of colorectal surgery to death or until December 31, 2018. We censored observations of patients who were alive at the end of follow-up. The median follow-up time was 60.0 months.

Statistical analysis

Categorical data were compared using the chi-square test, and continuous data with the independent-samples *t*-test. Survival rates were calculated using the life-table method, and compared with Kaplan-Meier survival curves and log-rank tests. To investigate OS, we compared survival between patients undergoing surgery by CS to those by GS using the log-rank test. A P value < 0.05 was considered statistically significant. All statistical calculations were performed using SPSS software, version 16.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

We identified 5141 CRC patients who underwent primary surgical resection between 2008 and 2013. Of these, 1748 patients (34.0%) had colorectal resection performed by a CS. The patient demographics and tumor characteristics between the CS group and GS group are shown in Table 1. There were more male patients, and the median age was approximately 61 years. Not unexpectedly, nearly half of the patients had rectal cancer, and the vast majority of patients were TNM stage II and III. There were no significant differences in terms of gender, age, primary cancer location or TNM stage of primary cancer between the two groups. The postoperative 30-day mortality was 0.3% (6/1748) in the CS group, and 0.5% (18/3393) in the GS group, with no significant difference.

In the early period (2008-2010), the proportion of minimally invasive procedures in the CS group (8.0%) was fewer than that in the GS group (12.5%). However, in the late period (2011-2013), this showed an increasing trend in the CS group and reached 32.3%, compared to 27.7% in the GS group (Table 2). This was mainly due to the widespread use of robotic surgery in CRC.

Survival in the CS and GS groups varied by TNM stage. In stage I CRC patients, 5-year OS was 94% following surgery by a CS, and 94% following surgery by a GS (P = 0.30). A lower significant difference was noted in stage II patients where 5-year OS rates in the CS and GS groups were 88% and 87%, respectively (P = 0.33). The 5-year OS rate of stage IV patients in the CS group was 35%, greater than 30% observed in the GS group; however, the difference was not significant (P = 0.98). There was no benefit associated with surgeon specialization for stage I, II and IV patients. Surgeon specialization exhibited a significant association with OS only in stage III patients, with 5-year OS rates of 76% and 67% in the CS and GS groups, respectively (P < 0.01; Figure 1).

Additional analyses were conducted to assess the variation in the aforementioned survival advantage among stage III CRC patients. In stage III right-sided colon cancer patients, the 5-year OS rate was 68% in the CS group and 64% in the GS group (P = 0.29). In stage III left-sided colon cancer patients, there was no significant survival benefit associated with CS (75% *vs* 68% in the GS group, P = 0.27). Surgeon specialization was significantly associated with survival only in stage III rectal patients, and the 5-year OS rate in the CS group and GS group was 80% and 67%, respectively (P < 0.01; Figure 2). Surgeon specialization was associated with a 43% reduction in the risk of death in stage III rectal cancer patients (HR = 0.57, 95%CI: 0.42-0.76). The P value regarding the interaction between surgeon specialization and tumor location for OS was 0.01.

Finally, we performed analyses of surgical and perioperative management elements in all stage III rectal cancer patients (Table 3). In this cohort, few patients received neoadjuvant radiotherapy or radiochemotherapy, and almost one in five underwent minimally invasive rectal surgery. The surgeons used the perioperative enhanced recovery after surgery (ERAS) program in 215 patients (74.9%) of the GS group, and none in the CS group. Two hundred and three patients (70.7%) in the GS group and 352 (61.9%) in CS group had more than 12 lymph nodes removed. After primary surgery, more patients received adjuvant chemotherapy or radiochemotherapy in the GS group (89.2% *vs* 64.7%).

DISCUSSION

This study examined a university hospital cohort of over 5000 CRC patients who underwent CRC resection by CSs or GSs. Several studies have demonstrated that increased specialization significantly contributed to lower perioperative mortality rates and the observed improvement in longer-term survival following CRC surgery[17-19]. However, to the best of our knowledge, this represents the initial large-scale study investigating stage-dependent differences in survival associated with specialization in a single university hospital in China. It was demonstrated that those patients who underwent CRC resections performed by CSs had better survival.

In our analysis, we observed no association between surgeon specialization and survival for stage I or II CRC. However, a national study, which only enrolled patients aged between 66 and 99 years, found that CSs seemed to confer a notable survival advantage in patients diagnosed with stage II rectal cancer, which is different to our findings[5]. The authors attributed the observed survival benefit to technical proficiency with total mesenteric excision, multidisciplinary treatment decisions and guideline-adherent surveillance. The above study data were from the SEER Medicare files, where nearly half of which were from non-teaching hospitals, while our study was conducted at a single university hospital.

Table 1 Patient demographics, tumor characteristics and surgeon specialization

	CS group (*n* = 1748)	GS group (*n* = 3393)	*P* value
Male: Female	1045:703	2029:1364	> 0.05
Median age (years)	62.0 (23-87)	61.0 (17-90)	> 0.05
Primary cancer location			> 0.05
Right-sided colon, *n* (%)	466 (26.7)	882 (26.0)	
Left-sided colon, *n* (%)	472 (27.0)	971 (28.6)	
Rectum, *n* (%)	791 (45.3)	1495 (44.1)	
Multiple, *n* (%)	19 (1.1)	45 (1.3)	
TNM stage, *n* (%)			> 0.05
In situ	41 (2.3)	91 (2.7)	
I	259 (14.8)	626 (18.4)	
II	590 (33.8)	1199 (35.3)	
III	579 (33.1)	1199 (35.3)	
IV	279 (16.0)	278 (8.2)	

CS: Colorectal surgeon; GS: General surgeon.

Table 2 Surgical procedures in different periods, *n* (%)

	CS group	GS group
2008-2010 (the early period)		
Laparoscopic	54 (7.3)	166 (12.5)
Robotic	5 (0.7)	0
Open	676 (92.0)	1160 (87.5)
Total	735	1326
2011-2013 (the late period)		
Laparoscopic	159 (15.7)	572 (27.7)
Robotic	168 (16.6)	0
Open	686 (67.7)	1495 (72.3)
Total	1013	2067

CS: Colorectal surgeon; GS: General surgeon.

GSs at our center have also been standardly well trained and experienced; thus, we consider that surgeon specialization does not affect survival in relatively early stage II CRC based on our observations. Therefore, it is not surprising that given the inherently good prognosis for stage I and II CRC, external surgeon specialization may be less likely to significantly impact survival.

Survival benefit was associated with surgeon specialization in stage III CRC, and especially in stage III rectal cancer. Those advanced CRC patients were recommended to undergo radical resection and adjuvant chemotherapy or radiochemotherapy. Total mesorectal excision can reduce local recurrence rates and improve survival, and has been widely used as the new gold standard for rectal cancer[20]. Recently, Japanese D3 resection and European complete mesocolic excision with central vascular ligation have both been performed with superior oncological outcomes[21,22]. CSs may be more familiar with these surgical techniques, and the proportion of rectal cases who had more than 12 lymph nodes removed in the CS group was greater than that in the GS group.

Although the percentage of minimally invasive procedures in the CS group was lower than that in the GS group, there was an obvious increasing trend in the late period, due to the introduction of robotic surgery. Thus, the switch from open to minimally invasive surgery may start late, but develops quickly especially for experienced CSs. Patients can achieve improved short-term outcomes and recover easily after minimally invasive CRC surgery.

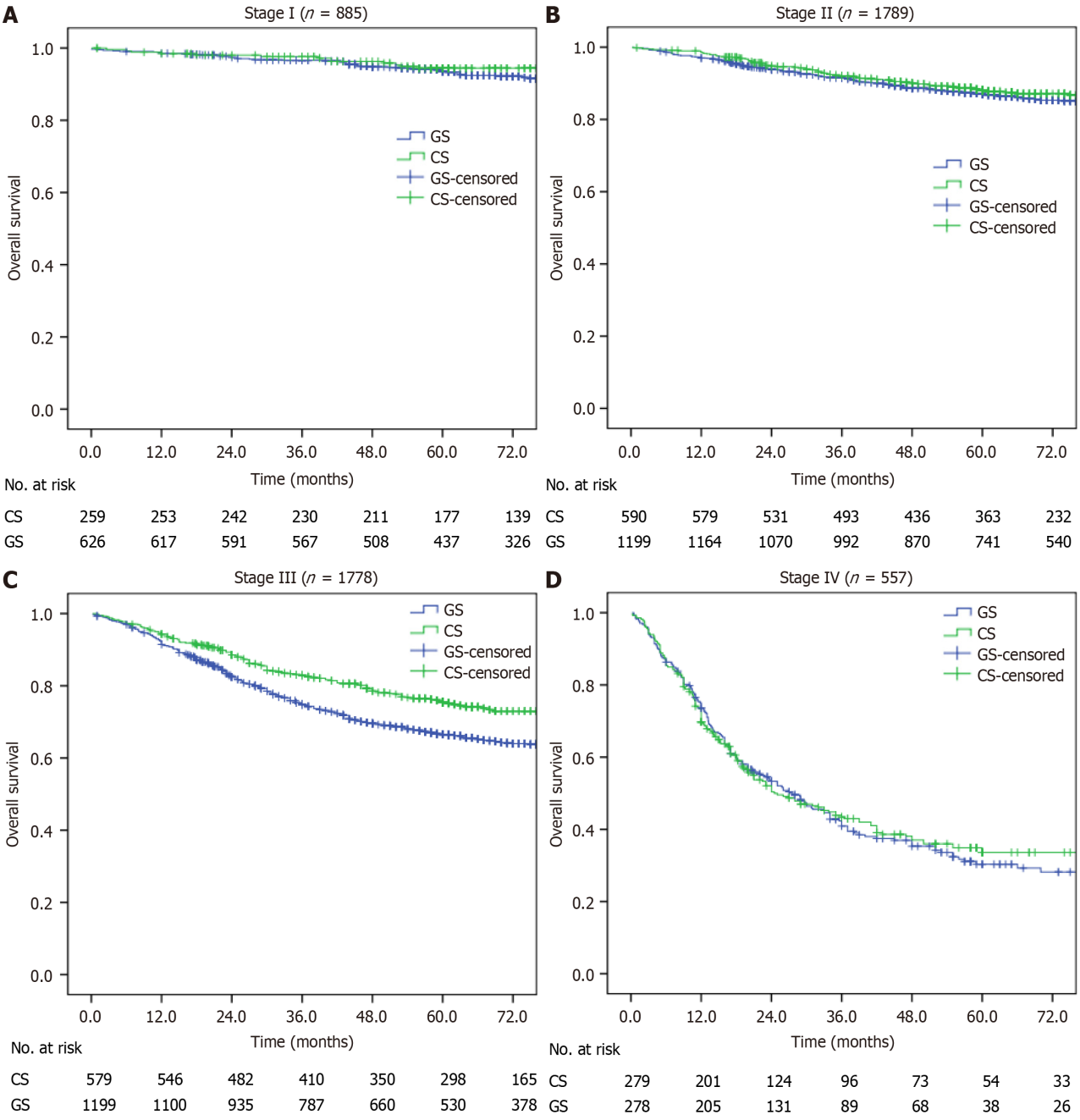

Figure 1 Overall survival of different stage colorectal cancer patients after primary resection. A: In stage I colorectal cancer (CRC) patients, overall survival (OS) was not significantly different between the colorectal surgeon (CS) and general surgeon (GS) groups ($P = 0.30$); B: In stage II CRC patients, a less significant difference was noted between the two groups ($P = 0.33$); C: In stage III CRC patients, OS in the CS group was better than that in the GS group ($P < 0.01$); D: In stage IV CRC patients, differences in OS between the CS and GS groups were not significant ($P = 0.98$). CS: Colorectal surgeon; GS: General surgeon.

ERAS, a multimodal perioperative treatment pathway of evidence-based care items, can attenuate surgical stress response and accelerate postoperative recovery[23,24]. When first introduced in China, CSs widely adopted the perioperative ERAS program [25]. Together, minimally invasive surgery combined with ERAS multimodal management has been shown to be the best perioperative strategy[26,27]. Therefore, patients can recover quickly and receive adjuvant chemotherapy earlier. A meta-analysis demonstrated that a 4-week increase in time to adjuvant chemotherapy was associated with a significant decrease in both OS and disease-free survival[28]. CSs may also appropriately pay more attention to chemotherapeutic decision making and sequence therapy compliance, and more stage III rectal patients received adjuvant chemotherapy or radiochemotherapy in the GS group after primary surgery. Therefore, our results highlight the fact that professional surgical technique, optimal perioperative care and standardized adjuvant therapy may primarily dictate survival of stage III rectal cancer.

Patients with stage IV metastatic CRC are recommended to undergo multidisciplinary team (MDT) treatment in China [29]. CSs are more inclined to implement MDT treatment than GSs. Recent significant improvements in outcome of patients with metastatic CRC seem to be associated with the sequential increase in the use of hepatic resection in selected patients and advancements in medical therapy[30,31]. CSs frequently offer intense comprehensive treatment for initially

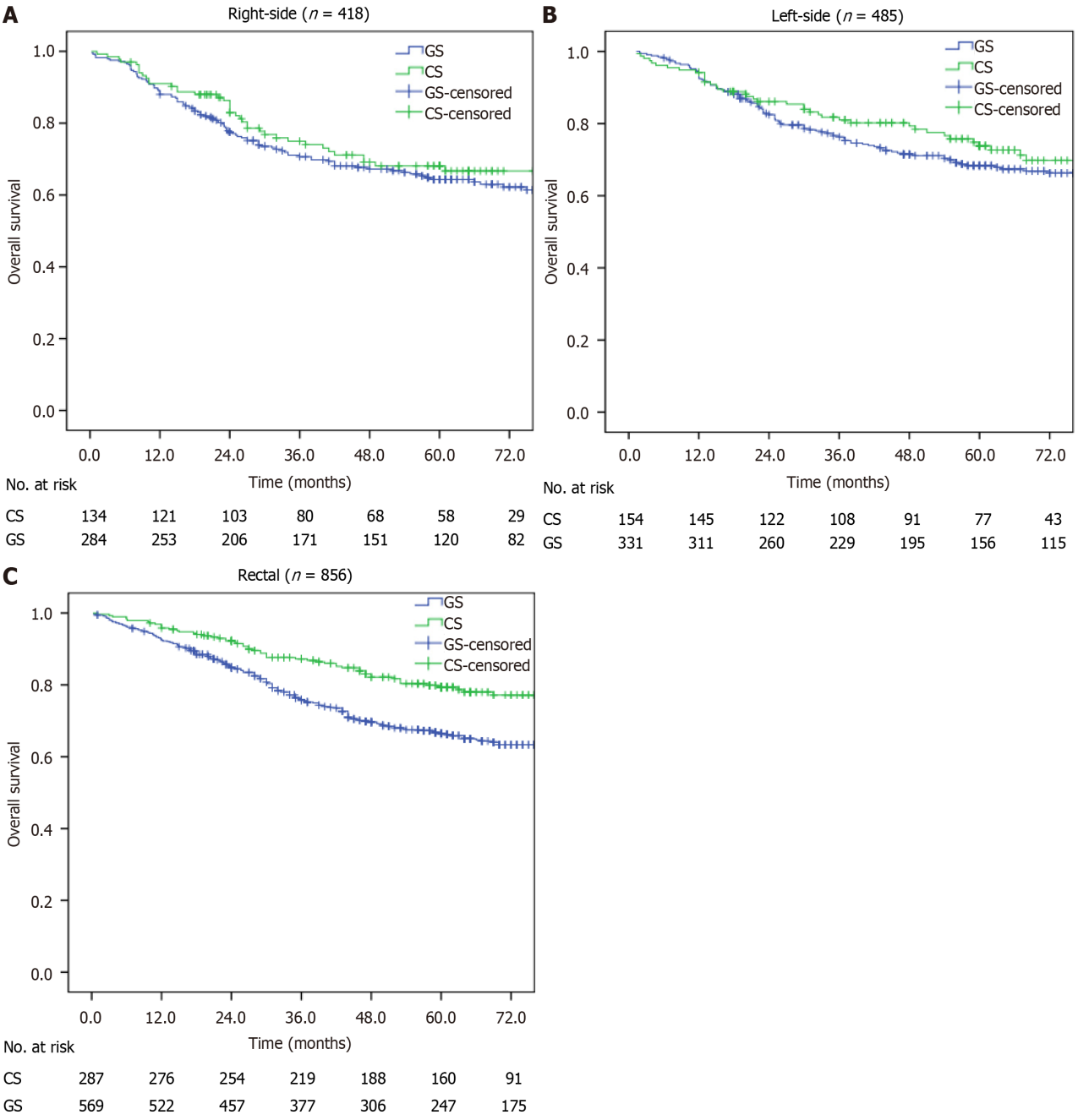

Figure 2 Overall survival after primary resection of patients with different locations of stage III colorectal cancer. A: In stage III right-sided colon cancer patients, overall survival (OS) was not significantly different between the colorectal surgeon (CS) and general surgeon (GS) groups (P = 0.29); B: In stage III left-sided colon cancer patients, a less significant difference was noted between the two groups (P = 0.27); C: In stage III rectal cancer patients, OS in the CS group was better than that in the GS group (P < 0.01). CS: Colorectal surgeon; GS: General surgeon.

unresectable patients under the guidance of MDT, and some convert to resectable and undergo conversion hepatectomy, with comparable survival to primary hepatectomy[32,33].

In this single center study, we demonstrated that colorectal specialization is linked to OS only in patients with stage III rectal cancer, but not in those with stage I, II or IV CRC, or stage III colon cancer. Our results highlight that the survival advantage for stage III rectal cancer is likely to represent a combination of improved technical proficiency, perioperative treatment pathway and adjuvant therapy execution.

The limitation of our study is that it is a retrospective study. We collected all the data from our large database, and errors in misregistration and omission could have occurred despite quality control. Moreover, we can only speculate on the reasons for the observed association between colorectal specialization and OS, and cannot determine causality for the demonstrated associations. Another potential limitation lies in the number of participants in the study, where local variations among a substantial number of diverse surgeons may impede generalizability.

Table 3 Surgical and perioperative management in stage III rectal cancer patients, *n* (%)

	CS group (*n* = 287)	GS group (*n* = 569)
Neoadjuvant radiotherapy/radiochemotherapy		
Yes	6 (2.1)	14 (2.5)
No	281 (97.9)	555 (97.5)
Surgical procedures		
Minimally invasive	47 (16.4)	149 (26.2)
Open	240 (83.6)	420 (73.8)
Perioperative ERAS treatment		
Yes	215 (74.9)	0
No	72 (25.1)	569 (100.0)
Number of lymph nodes removed		
< 12	84 (29.3)	217 (38.1)
≥ 12	203 (70.7)	352 (61.9)
Adjuvant chemotherapy/radiochemotherapy		
Yes	256 (89.2)	368 (64.7)
No	31 (10.8)	201 (35.3)

CS: Colorectal surgeon; GS: General surgeon.

CONCLUSION

The clinical implications of our findings in a real-world study suggest that surgeon specialization may play a crucial role in the survival of patients with stage III rectal cancer. The potential benefit of surgeon specialization for patients with stage I, II, and IV CRC, as well as stage III colon cancer, appears to be less definitive.

FOOTNOTES

Author contributions: Zhu DX, Xu JM conceptualized the study; Ren L, Xu JM provided study material or patients; Zhu DX, Chen M, Xu DH curated the data; Zhu DX, Chen M, Xu DH performed formal analysis; Xu JM acquired funding; Zhu DX, Chen M, Xu DH, Xu PP conducted the investigation; Zhu DX, Chen M, Xu DH developed the methodology; Qi Lin, Ren L, Xu JM administered the project; Zhu DX developed the software and performed visualization; Xu JM supervised and validated the study; Zhu DX, Chen M, Xu JM wrote and revised the manuscript; all authors accessed and verified the study data.

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Informed consent statement: All patients in the study provided informed consent.

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Data sharing statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Retrospective Study

Drug-eluting beads chemoembolization combined with programmed cell death 1 inhibitor and lenvatinib for large hepatocellular carcinoma

Hui Yang, Guang-Ping Qiu, Jie Liu, Tie-Quan Yang

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Abstract

BACKGROUND

The combination of transarterial chemoembolization (TACE), lenvatinib, and programmed cell death 1 (PD-1) inhibitor has been widely used in the treatment of advanced hepatocellular carcinoma (HCC) and has achieved promising results. However, there are few studies comparing whether drug-eluting beads TACE (D-TACE) can bring more survival benefits to patients with large HCC compared to conventional TACE (C-TACE) in this triplet therapy.

AIM

To compare the efficacy and adverse events (AEs) of triple therapy comprising D-TACE, PD-1 inhibitors, and lenvatinib (D-TACE-P-L) and C-TACE, PD-1 inhibitors, and lenvatinib (C-TACE-P-L) in patients with large HCC (maximum diameter ≥ 5 cm), and analyze the prognostic factors.

METHODS

Following a comprehensive review of our hospital's medical records, this retrospective study included 104 patients: 50 received D-TACE-P-L, and 54 received C-TACE-P-L. We employed Kaplan-Meier estimation to assess the median progression-free survival (PFS) between the two groups, utilized Cox multivariate regression analysis to identify prognostic factors, and applied the χ^2 test to evaluate AEs.

RESULTS

The objective response rate (ORR) and median PFS were significantly higher in the D-TACE-P-L group compared to the C-TACE-P-L group (ORR: 66.0% *vs* 44.4%, $P = 0.027$; median PFS: 6.8 months *vs* 5.0 months, $P = 0.041$). Cox regression analysis identified treatment option, portal vein tumor thrombus, and hepatic vein invasion as protective factors for PFS. AEs were comparable between the two

groups.

CONCLUSION

D-TACE-P-L may have significantly better PFS and ORR for large HCC, while exhibiting similar AEs to C-TACE-P-L.

Key Words: Large hepatocellular carcinoma; Conventional transarterial chemoembolization; Drug-eluting beads transarterial chemoembolization; Programmed cell death 1 inhibitor; Lenvatinib

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Core Tip: A retrospective analysis encompassing 104 patients diagnosed with large hepatocellular carcinoma (≥ 5 cm), focused on comparing the efficacy and safety of two treatment modalities, which were the triple combination therapy of drug-eluting beads transarterial chemoembolization (D-TACE), programmed cell death 1 inhibitor, and lenvatinib (D-TACE-P-L) and the triple therapy consisting of conventional TACE, programmed cell death protein 1 inhibitor, and lenvatinib. Progression-free survival, tumor response, and adverse events were compared between the two groups, and the findings revealed that D-TACE-P-L demonstrated significantly superior median progression-free survival and objective response rate, while maintaining comparable toxicity profiles. Based on these outcomes, this study proposed that the D-TACE-P-L therapy served as a preferential treatment option for individuals suffering from large hepatocellular carcinoma.

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INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the sixth most prevalent cancer worldwide and is associated with a poor prognosis. Notably, Chinese patients constitute approximately half of the global HCC caseload[1,2]. Due to insidious onset and rapid progression, large lesions, defined as having a maximum diameter exceeding 5 cm, are frequently diagnosed at an advanced stage, rendering them ineligible for surgical resection[3].

Currently, transarterial chemoembolization (TACE) is a widely adopted technique for managing unresectable HCC (uHCC)[4]. However, conventional TACE (C-TACE), which employs lipiodol mixed with chemotherapeutic agents, demonstrates limited efficacy in treating large HCC lesions. The objective response rate (ORR) ranges from 16% to 29%, while overall survival (OS) is restricted to between 6.5 and 9.9 months[5-7]. In contrast, drug-eluting beads TACE (D-TACE) offers sustained drug release alongside persistent embolization, potentially enhancing treatment outcomes for HCC[8]. For large or massive HCC tumors, D-TACE has been shown to achieve higher ORR rates, longer progression-free survival (PFS), and fewer adverse reactions compared to C-TACE[9,10]. Nevertheless, monotherapy with TACE remains inadequate regarding effectiveness; local recurrence rates at three months, six months, and one year are reported to be 18.6%, 33.4%, and 61.8%, respectively[11]. The TACE procedure induces a hypoxic environment within residual tumor tissue that stimulates increased production of vascular endothelial growth factor, leading to neovascularization - a significant contributor to tumor recurrence post-TACE. Furthermore, TACE does not effectively counteract immune evasion by tumor cells.

In recent years, substantial advancements have been made in systemic therapies for HCC encompassing tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors. TKIs have demonstrated efficacy in inhibiting tumor vessel proliferation while improving median OS among patients with HCC; thus, sorafenib and lenvatinib are recommended as first-line TKIs for uHCC management[12,13]. Immunotherapy has the potential to effectively inhibit the immune evasion mechanisms employed by tumor cells. Although immunotherapy alone has not exhibited superior efficacy relative to traditional treatments for HCC on its own merit, numerous studies indicate that combining TKIs with programmed cell death 1 (PD-1) inhibitors yields improved outcomes for patients suffering from this malignancy[14-18].

Therapies that inhibit angiogenesis, such as antibodies directed against vascular endothelial growth factor or TKIs, may postpone the revascularization and recurrence of tumors following TACE. PD-1 inhibitors can limit immune evasion, thereby enhancing the immune response to kill tumor cells. Due to their synergistic effect, combined therapy with TACE, PD-1 inhibitors, and lenvatinib has been proven to have better efficacy in treating uHCC[13,19]. However, the efficacy of combined therapy with C-TACE, PD-1 inhibitors, and lenvatinib (C-TACE-P-L) for treating large HCC remains unsatisfactory. D-TACE combined with PD-1 inhibitor and lenvatinib (D-TACE-P-L) may be better than C-TACE-P-L. Current available clinical studies on the utilization of D-TACE-P-L for large HCC are scarce, reflecting the need for further investigations and clinical trials to fully assess its potential benefits and risks in this specific patient population. Consequently, this retrospective study aimed to evaluate and compare the efficacy and safety of these two therapies in patients with large HCC.

MATERIALS AND METHODS

Patient criteria

Our research strictly adhered to the ethical principles outlined in the Declaration of Helsinki. The ethics committee of Ningbo No. 2 Hospital conducted a thorough review of our study and granted its approval. Since this study was conducted retrospectively, the ethics committee waived the necessity for informed consent. Furthermore, data were collected and analyzed anonymously to ensure participant privacy and confidentiality. Data were obtained from a cohort of patients with large HCC who received either D-TACE-P-L or C-TACE-P-L between May 1, 2019, and December 1, 2022. The inclusion criteria for our study included: (1) Age between 18-75 years; (2) Diagnosis of HCC confirmed by pathological examination; (3) Treatment with either D-TACE-P-L or C-TACE-P-L; and (4) Presence of at least one measurable lesion that exhibited arterial enhancement, with the largest lesion exceeding 5 cm in diameter. Patients meeting any of the following exclusion criteria were excluded: (1) Eastern Cooperative Oncology Group Performance Status score > 1; (2) Receipt of other anticancer treatments; (3) Concurrent Child-Pugh grade C status; (4) Presence of other malignancies; or (5) Incomplete medical records or information. Magnetic resonance imaging (MRI), or contrast-enhanced computed tomography (CT), was performed within one week prior to initial treatment, along with all necessary laboratory tests completed within three days.

Treatment

All patients received intravenous injections of PD-1 inhibitors, including tislelizumab (200 mg), sintilimab (200 mg), toripalimab (240 mg), and camrelizumab (200 mg) every three weeks. Additionally, they were administered lenvatinib orally at a standard dose of 8 mg for those weighing less than 60 kg or 12 mg for those weighing 60 kg or more, or an individualized dose as appropriate. Furthermore, TACE procedures were performed every one to two months if enhanced CT or MRI indicated significant arterial blood supply to the tumor; PD-1 inhibitors and lenvatinib were withheld three days prior to and following TACE. Digital subtraction angiography was utilized to identify arteries supplying blood to the tumor during TACE. Subsequently, a microcatheter was inserted into these arteries based on patient preference for either D-TACE or C-TACE. Drug-eluting beads or iodized oil containing doxorubicin (20 mg in C-TACE and 50 mg in D-TACE) were then slowly injected through the microcatheters for embolization. The efficacy of embolization was assessed *via* digital subtraction angiography, with the procedure concluded upon achieving satisfactory results.

Evaluation criteria

According to the mRECIST criteria, lesions were classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD)[20]. The primary objectives were to assess the median PFS, ORR, disease control rate (DCR), and prognostic factors of PFS. The ORR was defined as the incidence of CR and PR. The DCR was defined as the incidence of CR, PR, or SD. PFS was defined as the duration from the first TACE session to the occurrence of PD, death, or the last day of follow-up. Additionally, we aimed to evaluate adverse events (AEs) as secondary outcomes. AEs were evaluated and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4.0).

Follow-up

Patients were monitored at intervals of 1 to 3 months following their initial TACE. During each follow-up visit, a comprehensive evaluation was performed, which included hematological and biochemical tests as well as contrast-enhanced CT or MRI scans. If the lesion was classified as PD or if the patient could not tolerate treatment, the original therapy was discontinued, and alternative appropriate treatment options were considered for these patients. The follow-up endpoint was established as May 1, 2024.

Statistical analyses

Categorical variables are presented as percentages and analyzed using the χ^2 test. Continuous variables are reported as means ± standard deviations and compared with the Student's *t*-test. The median PFS between the two groups was assessed using Kaplan-Meier estimation. We assessed clinical characteristics through Cox univariate analysis. The items that exhibited statistical significance ($P < 0.1$) were assessed again using Cox multivariate regression to uncover prognostic factors of PFS ($P < 0.05$). Other statistically significant differences were defined as those for which $P < 0.05$. All the statistical analyses were performed using SPSS Statistics version 24.3.

RESULTS

Patient characteristics

After reviewing the medical records of our hospital, we identified 178 patients with large HCC who received either D-TACE-P-L or C-TACE-P-L, meeting the inclusion criteria. A total of 74 patients were excluded based on the predefined exclusion criteria, as illustrated in Figure 1. Ultimately, our study included 104 patients: 50 in the D-TACE-P-L group and 54 in the C-TACE-P-L group. There were four categories of PD-1 inhibitors utilized: Tislelizumab for 14 patients (13.5%), sintilimab for 17 patients (16.3%), camrelizumab for 45 patients (43.3%), and toripalimab for 17 patients (26.9%). No statistically significant differences were observed in baseline characteristics (Table 1).

Table 1 Baseline characteristics of the patients

Characteristic	D-TACE-P-L group (*n* = 50)	C-TACE-P-L group (*n* = 54)	*P* value
Sex, *n* (%)			0.564
Male	42 (84.0)	43 (79.6)	
Female	8 (16.0)	11 (20.4)	
Age (years), mean ± SD	60.8 ± 9.2	62.2 ± 9.4	0.443
ECOG PS, *n* (%)			0.252
0	14 (28.0)	10 (18.5)	
1	36 (72.0)	44 (81.5)	
Child-Pugh class, *n* (%)			0.561
A	26 (52.0)	25 (46.3)	
B	24 (48.0)	29 (53.7)	
BCLC, *n* (%)			0.556
B	14 (28.0)	18 (33.3)	
C	36 (72.0)	36 (66.7)	
AFP, *n* (%)			0.727
≤ 400 ng/mL	23 (46.0)	23 (42.6)	
> 400 ng/mL	27 (54.0)	31 (57.4)	
Number of tumors, *n* (%)			0.392
≤ 3	31 (62)	29 (53.7)	
> 3	19 (38)	25 (46.3)	
Largest tumor size (mm), mean ± SD	96.3 ± 27.7	91.0 ± 36.7	0.324
PVTT, *n* (%)			0.873
No	23 (46.0)	24 (44.4)	
Yes	27 (54.0)	30 (55.6)	
Hepatic vein invasion, *n* (%)			0.656
No	38 (76.0)	43 (20.4)	
Yes	12 (24.0)	11 (79.6)	
Extrahepatic metastasis, *n* (%)			0.661
No	41 (82.0)	46 (85.2)	
Yes	9 (18.0)	8 (14.8)	
Number of TACE, mean ± SD	2.46 ± 1.0	2.56 ± 1.1	0.640

C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; ECOG PS: Eastern Cooperative Oncology Group Performance Status; BCLC: Barcelona Clinic Liver Cancer; AFP: Alpha-fetoprotein; PVTT: Portal vein tumor thrombus; TACE: Transarterial chemoembolization.

Tumor response

The tumor response and DCR were comparable between the two groups, with no significant differences observed (P = 0.113, P = 0.143; Table 2). However, a significant difference in ORR was noted between the groups (66.0% *vs* 44.4%, P = 0.027). In the D-TACE-P-L group, the percentages of CRs and PRs were 18.0% and 48.0%, whereas in the C-TACE-P-L group, these percentages were 7.4% and 37.0%. No significant differences were found among the various PD-1 inhibitor subgroups (P = 0.927; Table 3).

PFS and analysis of its prognostic factors

The D-TACE-P-L group exhibited a superior median PFS of 6.8 months [95% confidence interval (CI): 4.45-9.15] compared to that of the C-TACE-P-L group, which had a median PFS of 5.0 months (95%CI: 4.314-5.753). The hazard ratio

Table 2 Tumor response, *n* (%)

Tumor response	D-TACE-P-L	C-TACE-P-L	*P* value
CR	9 (18.0)	4 (7.4)	0.113
PR	24 (48.0)	20 (37.0)	
SD	12 (24.0)	19 (35.2)	
PD	5 (10.0)	11 (20.4)	
ORR (CR + PR)	33 (66.0)	24 (44.4)	0.027
DCR (CR + PR + SD)	45 (90.0)	43 (81.0)	0.143

C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; ORR: Objective response rate; DCR: Disease control rate.

Table 3 Tumor response of different programmed cell death 1 inhibitor groups, *n* (%)

PD-1 inhibitor	CR	PR	SD	PD	*P* value
Tislelizumab	2 (14.3)	5 (35.7)	4 (28.6)	3 (24.1)	0.927
Sintilimab	1 (5.9)	10 (58.8)	5 (29.4)	1 (5.9)	
Camrelizumab	6 (13.3)	17 (37.8)	14 (31.1)	8 (17.8)	
Toripalimab	4 (14.3)	12 (42.9)	8 (28.5)	4 (14.3)	

PD-1: Programmed cell death 1; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

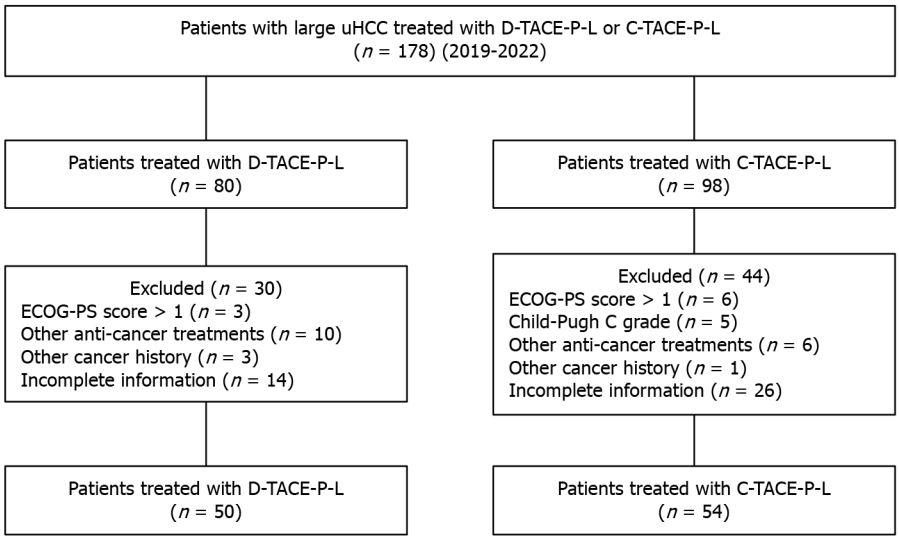

Figure 1 Selection criteria. uHCC: Unresectable hepatocellular carcinoma; C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; ECOG-PS: Eastern Cooperative Oncology Group Performance Status.

(HR) was 1.422, with a 95%CI of 0.961-2.104, indicating a statistically significant difference (Figure 2, $P = 0.041$). Cox regression analysis (as detailed in Table 4) revealed the following prognostic factors for PFS: Portal vein tumor thrombus (PVTT) (No/Yes, HR = 1.670; 95%CI: 1.120-2.491; $P = 0.012$), hepatic vein invasion (No/Yes, HR = 1.807; 95%CI: 1.105-2.956; $P = 0.018$), and treatment option (D-TACE-P-L/C-D-TACE-P-L, HR = 1.536; 95%CI: 1.028-2.293; $P = 0.036$).

AEs

We summarized AEs in both groups (Table 5) and found that the most common AEs were fatigue (48.0% *vs* 37.0%), anorexia and nausea (52.0% *vs* 55.6%), rash (38% *vs* 46.3%), fever (92.0% *vs* 89.9%), and abdominal pain (68.0% *vs* 61.1%). The percentages of grade 3 AEs ranged from 0% to 12%, while no grade 4 or grade 5 AEs were observed. No statistically

Table 4 Univariate and multivariate analyses of risk factors for progression-free survival

Factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Sex						
Male/female	1.145	0.692-49	0.599			
Age (years)	1.007	0.987-1.027	0.495			
ECOG PS						
0/1	1.246	0.781-1.988	0.357			
Child-Pugh class						
A/B	1.301	0.881-1.921	0.185			
AFP (ng/mL)						
≤ 400/> 400	1.215	0.821-1.798	0.329			
Number of tumors						
≤ 3/> 3	1.276	0.857-1.900	0.229			
Largest tumor size (mm)	1.006	0.998-1.013	0.139			
PVTT						
No/yes	1.590	1.073-2.358	0.021	1.670	1.120-2.491	0.012
Hepatic vein invasion						
No/yes	1.621	1.012-2.596	0.044	1.807	1.105-2.956	0.018
Extrahepatic metastasis						
No/yes	1.778	1.038-3.044	0.036	1.554	0.900-2.686	0.114
Treatment option						
D-TACE-P-L/C-TACE-P-L	1.422	0.961-2.104	0.078	1.536	1.028-2.293	0.036

C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; ECOG PS: Eastern Cooperative Oncology Group Performance Status; AFP: Alpha-fetoprotein; PVTT: Portal vein tumor thrombus; HR: Hazard ratio; CI: Confidence interval.

significant differences emerged in either the occurrence or severity of any AEs between the two groups, indicating comparable safety profiles overall; symptomatic treatment and dose reduction proved effective in mitigating these AEs.

DISCUSSION

Iodized oil used in C-TACE consists of droplets of variable size that are prone to being washed away by blood flow, resulting in reperfusion of tumor blood vessels. In contrast, drug-eluting beads maintain a consistent size and can permanently occlude the artery supplying blood to the target tumor. Furthermore, D-TACE allows for sustained release of therapeutic agents directly into the tumor vasculature while minimizing systemic exposure. D-TACE has demonstrated efficacy and significantly reduces the incidence of AEs[21,22]. However, there remains no consensus on whether D-TACE is superior to C-TACE. Three randomized controlled trials reported varying outcomes[22-24]. Nevertheless, several studies have suggested that D-TACE may be more effective for treating large HCC[25-27]. For instance, a study conducted by Li *et al*[27] included patients with Barcelona Clinic Liver Cancer stage A/B and evaluated their response to therapy using mRECIST, revealing an ORR of 81.5% for D-TACE compared to 49.4% for C-TACE[27]. Additionally, research by Zhao *et al*[25] indicated median tumor diameters of 12.2 cm and 8.1 cm ($P < 0.005$) in the D-TACE group and C-TACE group, respectively; furthermore, both CRs and ORRs at one and three months were higher in the D-TACE group than those observed in the C-TACE group. These findings provide a foundation for our study aimed at verifying that D-TACE remains superior to C-TACE among patients with large uHCC when combined with lenvatinib and PD-1 inhibitors.

The combination therapy of TACE, lenvatinib, and PD-1 inhibitors is employed globally for the treatment of uHCC. PD-1 inhibitors function by disrupting signals that inhibit the immune system's attack on tumors, thereby enhancing the immune response against cancerous cells[17,28]. The clinical efficacy of PD-1 inhibitors can be further augmented by reducing tumor blood supply and releasing tumor-specific antigens through TACE[29,30]. However, TACE induces a

Table 5 Treatment-related adverse events in the two groups, *n* (%)

Adverse events	D-TACE-P-L (*n* = 50)		C-TACE-P-L (*n* = 54)		*P* value
	Any grade	Grade 3	Any grade	Grade 3	
Diarrhea	9 (18.0)	2 (4.0)	7 (13.0)	1 (1.8)	0.717
Hand-foot syndrome	12 (24.0)	2 (4.0)	15 (27.8)	2 (3.7)	0.882
Hypertension	13 (26.0)	1 (2.0)	14 (26.0)	0 (0.0)	0.572
Fatigue	24 (48.0)	4 (8.0)	20 (37.0)	3 (5.6)	0.522
Anorexia and nausea	26 (52.0)	4 (8.0)	30 (55.6)	5 (9.3)	0.928
Rash	19 (38.0)	1 (2.0)	25 (46.3)	3 (5.6)	0.518
Thyroid dysfunction	6 (12.0)	0 (0.0)	10 (18.5)	1 (1.8)	0.485
Hyperbilirubinemia	11 (22.0)	2 (4.0)	9 (16.7)	1 (1.8)	0.738
Fever	46 (92.0)	6 (12.0)	48 (89.9)	3 (5.6)	0.462
Abdominal pain	34 (68.0)	6 (12.0)	33 (61.1)	2 (3.7)	0.336

C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor.

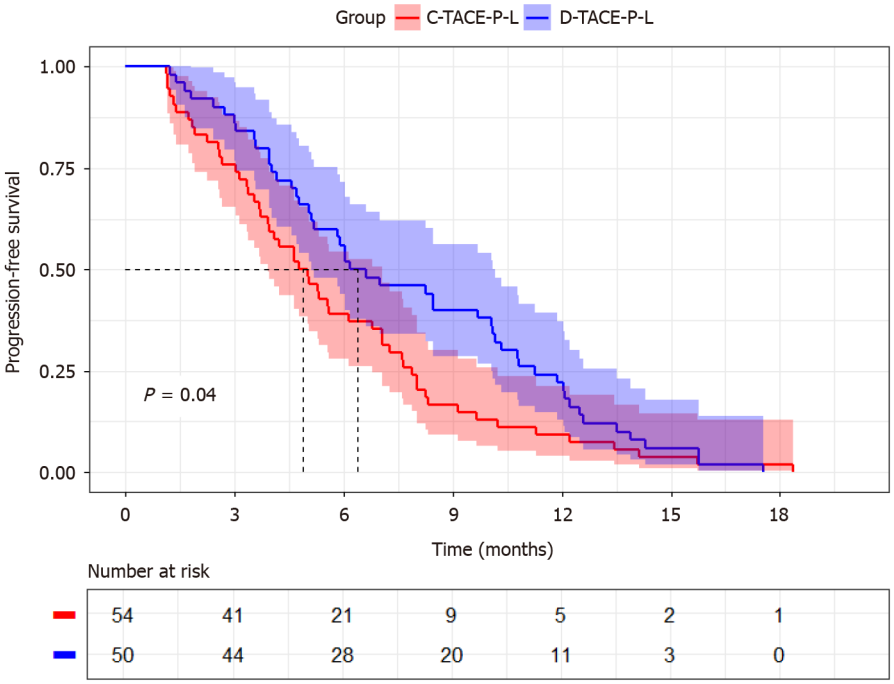

Figure 2 Kaplan-Meier curves of progression-free survival. C-TACE-P-L: Conventional transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor; D-TACE-P-L: Drug-eluting beads transarterial chemoembolization combined with lenvatinib plus programmed cell death 1 inhibitor.

hypoxic microenvironment that may lead to tumor angiogenesis, recurrence, and metastasis. Fortunately, lenvatinib has been shown to inhibit angiogenesis and counteract tumor immunosuppressive mechanisms, thus improving clinical outcomes[31]. Due to the synergistic effects of this combination therapy involving TACE, lenvatinib, and PD-1 inhibitors, patients with uHCC demonstrate improved tumor responses and survival rates. Previous research has indicated that D-TACE enhances the infiltration of immune cells in tumor tissues, whereas C-TACE decreases it[32]. It is anticipated that incorporating immune checkpoint inhibitors will enhance the therapeutic efficacy of D-TACE and potentially extend OS and PFS. Our study found that D-TACE-P-L significantly improved median PFS from 5.0 months to 6.8 months compared with C-TACE-P-L (P = 0.041). However, due to the short follow-up time, more than 80% of the patients are still alive. It was not feasible to assess differences in OS adequately at this time point. Additionally, while there was a statistically significant difference in ORR, no such difference was observed in DCR. More than 90% of patients experienced PD during follow-up; therefore, this duration was sufficient for evaluating ORR and DCR effectively. A larger sample size may provide more robust validation regarding differences in ORR and DCR between groups.

Overall, our experimental results align well with theoretical predictions outlined previously. However similar studies are scarce within existing literature focused on triple therapy effectiveness for treating large uHCC where tumor diameter did not serve as an inclusion criterion or prognostic factor for PFS among treatment options like D-TACE *vs* C-TACE[33,34]. However, in one study, patients received either D-TACE or C-TACE in addition to camrelizumab[35]. The average tumor diameter (9.4 cm) in this study was significantly greater than that in other studies. The study suggested that D-TACE-C was superior to C-TACE-C in median PFS (10.0 months *vs* 3.0 months, $P = 0.017$). D-TACE-C shares similarities with D-TACE-P-L in mechanism, and enrolled participants with relatively large tumors. Thus, this similar result provides potentially relevant evidence for our outcomes. Nevertheless, further research remains essential.

Cox multivariate regression analysis identified PVTT, hepatic vein invasion, and treatment modality as independent prognostic factors for PFS. Numerous studies have corroborated that both PVTT and hepatic vein invasion serve as independent prognostic indicators for PFS, aligning with the findings of our study[33-35]. The presence of PVTT and hepatic vein invasion signifies a more advanced disease state, which is associated with poorer PFS outcomes. Furthermore, the treatment modality independently influenced prognosis, consistent with our hypothesis and reinforcing our conclusions.

Although D-TACE reduces the distribution of drugs to non-target regions, our study found no significant difference in the incidence of AEs. Similar findings have been reported in related studies[35,36]. In the investigation conducted by Ren *et al*[35], AEs included renal cell carcinoma embolization syndrome, rash, asthenia, anemia, and hypothyroidism; *P* values between the two groups were 0.111, 0.535, 0.484, 0.639, and 0.552, respectively, indicating no statistical significance.

However, several limitations must be acknowledged in this study. First, the retrospective design implies that treatment decisions were made by both physicians and patients, which may lead to selection bias. Additionally, the sample size was comparatively limited, highlighting the need for larger randomized controlled trials to provide more robust evidence. Finally, due to the short follow-up duration, we were unable to ascertain median OS, leaving uncertainty regarding whether D-TACE-P-L confers a superior OS compared to C-TACE-P-L.

CONCLUSION

D-TACE-P-L may have significantly better PFS and ORR for large HCC, while exhibiting similar AEs compared to C-TACE-P-L.

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FOOTNOTES

Author contributions: Yang H contributed to the conceptualization, data curation, and writing - original draft; Qiu GP and Liu J participated in the investigation of this study; Qiu GP, Liu J, and Yang TQ took part in the writing - review & editing; Qiu GP contributed to the resources of this manuscript; Liu J was involved in the investigation of this study; Yang TQ contributed to the methodology and visualization.

Institutional review board statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Ningbo No. 2 Hospital (approval No: YJ-NBEY-KY-2024-004-01; date of approval: 2024-01-16).

Informed consent statement: The need for patient consent was waived due to the retrospective nature of the study which was approved by the Ethics Committee of Ningbo No. 2 Hospital.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data underlying the findings of this study are accessible upon request to the corresponding author, owing to privacy and ethical considerations that preclude their public dissemination.

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ORIGINAL ARTICLE

Retrospective Study

Effect of endoscopic submucosal dissection on gastrointestinal function and nutritional status in patients with early gastric cancer

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Abstract

BACKGROUND

Gastric cancer (GC) endangers the survival and prognosis of patients worldwide. Improving the prognosis of patients with early GC (EGC) is crucial to prolong their survival time.

AIM

To analyze the effects of endoscopic submucosal dissection (ESD) on gastrointestinal function and nutritional status in patients with EGC.

METHODS

Eighty patients with EGC between January 2021 and January 2024 were divided according to different surgical protocol into following two groups: 42 patients who underwent ESD in the ESD group and 38 patients treated with endoscopic mucosal resection (EMR) in the EMR group. Two groups were compared in the operative indices, lesion resection rate, postoperative recovery of gastrointestinal function, nutritional status, and incidence of surgical complications.

RESULTS

The overall resection rate of the lesion in the ESD group was higher. The operative bleeding volume and operation time were higher and gastrointestinal ventilation time was shorter in the ESD group than those in the EMR group ($P < 0.05$). The nutritional statuses of the two groups decreased after operation; however, the

levels of albumin, prealbumin, hemoglobin, and transferrin were higher in the ESD group than in the EMR group ($P < 0.05$). The post-operative pepsinogen (PG) I level in the ESD group was higher than that in the EMR group, and the PG II level was lower than that in the EMR group ($P < 0.05$). The incidence of postoperative complications was compared between the two groups ($P > 0.05$).

CONCLUSION
ESD can promote the immediate recovery of patient's postoperative gastrointestinal function, improve their nutritional level, and signifies its application in patients with EGC.

Key Words: Endoscopic submucosal dissection; Endoscopic mucosal resection; Early gastric cancer

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Core Tip: Endoscopic submucosal dissection (ESD) application in patients with early gastric cancer (EGC) can significantly promote the recovery of gastrointestinal function, improve nutritional status, and reduce complications. This study confirmed the effectiveness of ESD in promoting the recovery of gastrointestinal function by observing the changes in gastrointestinal function and nutritional status in patients with EGC.

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INTRODUCTION

Dietary habit changes and excessive life pressure is causing an increase in the annual incidence of gastric cancer (GC). According to relevant surveys[1], the incidence of GC ranks second after lung cancer among all malignant tumors, with the highest incidence in East Asia, accounting for 50%. The development of diagnostic and treatment technologies has significantly increased the screening rate for early GC (EGC). EGC refers to the tumor that is only confined to the gastric mucosa or the lower layer, without lymph node metastasis, and a stable patient condition, which can be directly cured by surgery[2]. The extensive trauma and various complications associated with traditional radical gastrectomy significantly reduced the quality of life of patients after radical gastrectomy. In recent years, endoscopic surgery, which retain the structural and functional integrity of the patient's stomach, has gradually replaced traditional surgery as an important treatment method for EGC. Studies have confirmed that the long-term outcomes of endoscopic surgery for patients with EGC are comparable to those of traditional laparotomy[3,4]. With the advantages of less trauma, fewer complications, and shorter time, endoscopic surgery has been widely used for EGC. The rapid development of digestive endoscopy in China and the enhancement of health awareness has significantly increased the rates of early detection, diagnosis, and treatment of GC. Radical resection can completely remove tumor lesions with a low residual tumor rate for patients with EGC; however, it destroys the gastric structure of patients, affects gastric function, and cannot completely improve the quality of life of patients with EGC[5]. Recently, endoscopic surgery has become an important research direction in EGC treatment, with a low impact on gastric structure and function during the operation and a high tumor resection rate, which has been favored by most patients and physicians. The selection of endoscopic surgery has become the focus of clinical research on the classification of endoscopic surgery for GC into submucosal dissection and mucosal resection. Since the endoscopic submucosal dissection (ESD) was developed based on mucosal resection and starts late in clinical application, its surgical effect is still under study. Therefore, we analyzed the therapeutic effect of ESD and its impact on gastrointestinal function and nutritional status in patients with EGC.

MATERIALS AND METHODS

General data

Eighty patients with EGC between January 2021 and January 2024 were selected and divided into two groups according to different surgical protocol. The ESD group had 42 cases (22 males and 20 females), with age ranging from 35 to 70 (52.81 ± 5.68) years, and tumor diameter from 1.0 to 4.5 (2.48 ± 0.68) cm. Clinical TNM staging: 31 cases in stage Ia and 11 cases in stage Ib; Tumor sites: Gastric antrum 15 cases, gastric fundus 18 cases, gastric horn 9 cases. Undifferentiated and differentiated cases were 18 and 24, respectively. The endoscopic mucosal resection (EMR) group included 38 cases (20 males and 18 females), with age ranging from 32 to 70 (53.18 ± 6.01) years, and tumor diameter from 1.5 to 4.8 (2.51 ± 0.72) cm. Regarding clinical TNM staging, 30 cases were included in stage Ia and 8 cases in stage Ib; concerning tumor sites, 12 cases involved gastric antrum, 4 cases in gastric fundus, and 11 cases in gastric horn; 15 cases were undifferentiated and

23 cases were differentiated. No comparable difference was found in basic data between the two groups ($P > 0.05$).

Inclusion criteria: (1) The patient was diagnosed with EGC by gastrointestinal endoscopy and pathology[6] and the TNM stage was stage I; (2) No neoadjuvant chemotherapy was performed before surgery; (3) Patients underwent gastric surgery for the first time; and (4) The clinical data of the patients were complete.

Exclusion criteria: (1) Lymph node and organ metastases; (2) TNM stage II; (3) Anemia and malnutrition; (4) Autoimmune diseases, coagulation disorders, and organ dysfunction; (5) Preoperative history of anti-tumor treatment; and (6) Incomplete research data.

Methods

The patients in both groups underwent gastrointestinal endoscopic surgery under general anesthesia. After placing the patient in the lateral decubitus position, the lesion site was explored using the gastrointestinal endoscope and enlarged using the indigo carmine staining method or narrow-band imaging technology of the endoscope to identify the junction between the lesion and normal mucosa. Argon ion coagulation was used to mark the resection range, and one marking point was set at every interval of 2.0 mm - 3.0 mm. Submucosal injection was administered around the marked sites, and a mixture of epinephrine and indigo carmine was prepared at a ratio of 1:10000. At the end of the injection, the lesion was elevated, the basal eminence of the lesion was obvious, and it could be operated upon when it exceeded the normal surrounding mucosa by 5.0 mm - 8.0 mm.

In the ESD group, a HOOK or IT knife was used to annularly cut the lesion mucosa from the edge along the marked points and peel off the submucosa. Submucosal injection was intermittently administered during peeling to keep the lesion mucosa in a raised state, and ensure that the peeling layer was in the submucosa. Larger blood vessels were clamped and bleeding was controlled with hemostatic forceps, and the lesion was separated completely using an argon-ion scalpel. No active bleeding was detected, the bare blood vessels were treated with electrocoagulation for hemostasis, and the wound surface was sutured. Dissected lesion specimens were sent for inspection.

In the EMR group, after the lesion was elevated, a transparent cap with a high-frequency snare was placed at the front end of the endoscope. After the lesion site was attracted so that the marked tissue completely entered the transparent cap, the snare was tightened and the lesion tissue was cut by electric coagulation. If a one-time resection was difficult, multiple resections of the lesion were performed, and the wound surface was effectively treated after resection. Both groups underwent routine fasting, anti-infection therapy, and nutritional support after surgery.

Outcome indicators

Surgical effect: The diagnosis and treatment of GC were determined by referring to the guidelines for the diagnosis and treatment of endoscopic GC[7]. Lump resection: The lesion was removed as a lump during the endoscopic examination, and a single specimen was obtained. Curative resection: Lumpectomy without risk of lymph node metastasis; basal lesion residual: The lesion site and tumor lesion within 1 cm of the edge of the lesion were resected within six months after the operation.

Surgery-related indicators included operation time, bleeding volume, gastrointestinal ventilation time, and length of hospital stay.

Nutritional conditions: Collect 5 mL of venous blood of patients, and the levels of albumin (ALB), prealbumin (PA), hemoglobin (Hb), and transferrin (TRF) were measured using a Hitachi 7600 automatic biochemical analyzer.

Gastrointestinal hormones: Collect 3 mL of venous blood of patients and centrifuged for 10 minutes at 3000 rpm. After collecting the serum, the levels of pepsinogen (PG) I and PG II were detected using ELISA.

Complications: Surgical complications, such as mucosal perforation, anastomotic bleeding, and gastroparesis, were observed in both groups.

Statistical analysis

SPSS 26.0 statistical software was used for data analysis. The measurement data conformed to the normal distribution (mean ± SD) representation, and the inter-group and intra-group data were subjected to independent and paired sample *t* tests. Count data rate (%) was calculated using χ^2 tests; $P < 0.05$ indicated the statistical significance.

RESULTS

Surgical effect

The overall resection rate of lesions after surgery was higher in the ESD group than in the EMR group ($P < 0.05$; Table 1).

Surgical conditions

The bleeding volume and operation time were higher in the ESD group than in the EMR group; however, the gastrointestinal ventilation time was shorter in the ESD group than in the EMR group ($P < 0.05$). The difference in the length of hospital stay between the two groups was not significant ($P > 0.05$; Table 2).

Nutrition status

The difference in nutritional status between the two groups before surgery was not significant ($P > 0.05$). Nutritional status decreased in both groups after surgery but was higher in the ESD group than in the EMR group ($P < 0.05$; Table 3).

Table 1 Surgical effect, *n* (%)

Group	Lump resection	Curative resection	Basal lesion residual
ESD group (n = 42)	40 (95.24)	2 (4.76)	0
EMR group (n = 38)	28 (73.68)	8 (21.05)	2 (5.26)
χ^2	7.269	3.466	0.622
P value	0.007	0.063	0.430

ESD: Endoscopic submucosal dissection; EMR: Endoscopic mucosal resection.

Table 2 Surgical conditions, mean ± SD

Group	Operation time (minute)	Intraoperative bleeding volume (mL)	Gastrointestinal ventilation time (hour)	Length of stay (day)
ESD group (n = 42)	62.83 ± 8.48	28.48 ± 5.28	25.85 ± 5.48	12.83 ± 4.28
EMR group (n = 38)	51.36 ± 7.15	22.52 ± 4.19	36.76 ± 7.15	13.28 ± 4.51
t value	6.504	5.553	7.701	0.458
P value	< 0.001	< 0.001	< 0.001	0.648

ESD: Endoscopic submucosal dissection; EMR: Endoscopic mucosal resection.

Table 3 Nutrition status, mean ± SD

Group	ALB (g/L)		PA (mg/L)		Hb (g/L)		TRF (g/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ESD group (n = 42)	47.86 ± 4.28	42.28 ± 3.67[a]	328.84 ± 35.26	302.15 ± 24.78[a]	118.20 ± 8.87	108.24 ± 7.48[a]	2.38 ± 0.82	1.90 ± 0.47[a]
EMR group (n = 38)	48.04 ± 4.36	35.86 ± 4.15[a]	331.05 ± 37.82	278.53 ± 25.69[a]	120.49 ± 9.36	98.89 ± 7.27[a]	2.40 ± 0.85	1.67 ± 0.41[a]
t value	0.186	7.343	0.270	4.184	1.123	5.658	0.107	2.321
P value	0.853	< 0.001	0.788	< 0.001	0.265	< 0.001	0.915	0.023

[a]P < 0.05 compared with that in the same group before operation.
ESD: Endoscopic submucosal dissection; EMR: Endoscopic mucosal resection; ALB: Albumin; PA: Prealbumin; TRF: Transferrin.

Gastrointestinal hormones

The difference in the index expression between the two groups before surgery was not significant (P > 0.05). PG I levels increased and PG II levels decreased in both groups after surgery. However, the PG I level in the ESD group was higher than that in the EMR group, and the PG II levels were lower in the ESD group than those in the EMR group (P < 0.05; Table 4).

Complications

The incidence rate of ESD group was 4.76%. The incidence rate of EMR group was 10.53%. These differences were not significant (χ^2 = 0.305, P = 0.581).

DISCUSSION

This study showed that the overall resection rate of the lesion in the ESD group was higher. The surgical bleeding volume and operative time were higher and gastrointestinal ventilation time was shorter in the ESD group than those in the EMR group (P < 0.05). The results indicated that ESD required a long operation time and resulted in copious bleeding; however, it had a high resection rate of lesions, a low residual rate of basal lesions, and a short recovery time for postoperative gastrointestinal function. Meng *et al*[8] confirmed that the complete resection rate of the lesion in patients with heterogeneous EGC treated with ESD was higher than that in patients undergoing EMR. Yang *et al*[9] reported that

Table 4 Gastrointestinal hormones, mean ± SD (μg/L)

Group	PG I		PG II	
	Pre	Post	Pre	Post
ESD group (n = 42)	65.82 ± 6.89	82.48 ± 6.48[a]	25.04 ± 2.48	15.28 ± 3.15[a]
EMR group (n = 38)	66.27 ± 7.15	73.89 ± 6.35[a]	24.76 ± 2.51	18.96 ± 3.37[a]
t value	0.287	5.978	0.501	5.048
P value	0.775	< 0.001	0.618	< 0.001

[a]P < 0.05 compared with that in the same group before operation.

ESD: Endoscopic submucosal dissection; EMR: Endoscopic mucosal resection; PG: Pepsinogen.

the recurrence rate of undifferentiated EGC after ESD was lower than that after traditional surgery. Several studies have confirmed the therapeutic effect of ESD. EMR can inhibit lesion metastasis by removing the local mucosal tissue. The operation is relatively simple, and the operation time is shorter with less bleeding. However, the requirement of repeated submucosal injections in ESD increases the operation time and bleeding volume to a certain extent[10]. However, the mucosa is stripped without removing the mucosal tissue, which preserves the relatively complete gastric mucosal tissue of the patient. This method has a relatively low impact on the overall structure and function of the patient's stomach and is conducive to the immediate recovery of gastrointestinal function of the patient after surgery[11]. Simultaneously, the lesion could not be included once for mucosal resection owing to the influence of the transparent cap or snare. The lesion could not be removed at once for lesions with volume ≥ 2 cm, and it requires multiple removal, resulting in lesion residue. However, when using mucosal stripping, the lesion tissue can be stripped simultaneously and gradually separated into the submucosa, reducing tumor residue and achieving curative resection of tumor lesions[12].

Most cases of EGC display no specific symptoms; however, they experience upper abdominal discomfort, dyspepsia and other symptoms and no weight loss, vomiting, anemia, malnutrition, or other manifestations. Although we found that the nutritional status of the two groups decreased after surgery, the levels of ALB, PA, Hb, and TRF were higher in the ESD group than in the EMR group (P < 0.05). The results showed that gastrointestinal endoscopic surgery led to a decreased nutritional level in patients, but the use of ESD had little effect on the nutritional level of patients. Mucosal stripping could strip the mucosal tissue of the lesion without damaging the surrounding tissues around the lesion. The normal physiological structure of the stomach can be preserved to some extent, which promotes the recovery of gastrointestinal motility and improves gastrointestinal digestion and absorption function of patients. Simultaneously, it can reduce the impact of the operation on the intestinal mucosa, maintain intestinal barrier function, and maintain gastrointestinal physiological function, thereby reducing the impact of the operation on the nutritional status of patients and maintaining a high nutritional level after surgery[13].

PG I and PG II are important indicators of gastric mucosal function. PG I is secreted by the mucous neck cells and main cells through the gastric fundus glands, whereas PG II is secreted by glands throughout the gastric mucosal layer. When the gastric mucosa becomes cancerous, the secretion of PG by cells is affected, leading to changes in serum PG levels. When the gastric mucosa shrinks and gastric acid secretion is reduced, the secretory function of the main cells of the gastric fundus is affected and PG I levels are reduced. However, after tumor cells destroy the gastric wall function, they can promote PG II entry into the peripheral blood and increase the level of PG II[14]. This study showed that the PG I level after surgery in the ESD group was higher, and that the PG II level in the ESD group was lower (P < 0.05). This indicates that ESD can promote the recovery of gastric mucosal function. As ESD can completely strip the tumor tissue and avoid tumor residue, the damage of the tumor cells to the gastric mucosa layer can be avoided, and the secretion function of PG can be improved. EMR can also remove tumor lesions, but the complete resection rate is low, and the damage to the gastric mucosa layer of patients is more obvious, so the effect of pepsin is not as good as that of ESD. The difference in the incidence of postoperative complications between the two groups was not significant. The complications between the two surgeries probably did not differ owing to the limitations of the small sample size and short observation time, which require further clinical exploration.

ESD has a strong therapeutic effect on EGC; however, there are significant indications. The main complications of ESD include perforations and hemorrhages. According to a survey by Odagiri *et al*[15], the bleeding rate after ESD was 4.9%, and the incidence of perforation during the operation was 1%-5.2%. Therefore, it is necessary to avoid excessive surgery and repeated electrocoagulation during surgery. Moreover, gastrointestinal decompression should be performed after surgery to avoid the occurrence of postoperative perforation. Simultaneously, appropriate medications should be administered postoperatively to avoid postoperative hemorrhage.

In summary, ESD is superior to EMR for treating EGC and has the advantages of good outcomes and rapid recovery. However, this was a single-center study and all participants were from the same hospital, which may have led to biased results. In future, we will conduct multicenter studies with different populations to validate these results.

CONCLUSION

Treatment of EGC with ESD can promote the immediate recovery of gastrointestinal function after surgery and improve the nutritional status of patients. It has a low impact on the gastric mucosa and is therefore worthy of clinical application. However, this study had certain limitations. This was a retrospective study with a small sample size and did not observe long-term prognosis of the patients, which biased the results. Therefore, prospective randomized controlled trials with larger sample sizes and long-term follow-up studies are needed to provide a reference for the surgical treatment of EGC. The effects of different surgical protocols on the psychological state of patients can be considered to explore the influence of different surgical schemes on patients' anxiety levels.

FOOTNOTES

Author contributions: Xu QD and Wu ZY designed the study; Zhang HW, Gao XM, Li YG, and Wu ZY contributed to the analysis of the manuscript; Xu QD and Liu H involved in the data and writing of this article; all authors have read and approved the final manuscript. Xu QD and Liu H contributed equally to this work as co-first authors. They jointly participated in the designing and planning of the research, proposed innovative research ideas and methods, laid the foundation for the smooth progress of the research. They played a crucial role in data collection and organization, rigorously screened and organized patient data to ensure accuracy and completeness. They jointly undertook the important tasks of data analysis and interpretation. Meaningful research conclusions were drawn through in-depth exploration and comprehensive judgment, and core viewpoints and important arguments were contributed during the paper writing process.

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ORIGINAL ARTICLE

Retrospective Study

Comparison of clinical features of patients with or without severe gastrointestinal complications in aggressive gastrointestinal lymphomas

Xiao-Hong Liu, Gong Chen, De-Dong Cao, Hui Liu, Xiao-Kang Ke, Yu-Gang Hu, Wei Tan, Dong Ke, Xi-Ming Xu

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Abstract

BACKGROUND

Aggressive primary gastrointestinal non-Hodgkin lymphoma (PGINHL) is an uncommon and heterogeneous group of lymphoid malignancies, that differs from indolent lymphoma and has a high incidence of severe gastrointestinal complications (GICs).

AIM

To investigate and compare the clinicopathological characteristics, treatments and outcomes in the GICs and No-GICs group with aggressive PGINHL.

METHODS

This retrospective analysis was performed on aggressive PGINHL patients

between January 2013 and December 2021 at our hospital. The independent influence factors of GICs were obtained by univariate and multivariate Logistic regression analysis, the selected variables significantly related to GICs were selected as the final predictors to construct nomogram. Kaplan-Meier curves further analyzed the survival of patients in GICs and No-GICs groups. Survival analysis of GICs group was performed using Cox regression.

RESULTS

We focused on 124 aggressive PGINHL cases, which had a relatively high incidence 48.4% (60/124 cases) of GICs, the most common histological type in GICs group was diffuse large B-cell lymphoma (DLBCL) (n = 49, 81.7%). In the GICs group, small intestine was the most common anatomic site of lesion (43.3%), followed by large intestine (31.7%), and then stomach and esophagus (25.0%). Multivariate Logistic regression analysis showed that the independent risk factors for GICs were the small intestine [odd ratio (OR) = 3.33; 95% confidence interval (CI): 1.47-9.41; P = 0.009), aggressive B-cell (OR = 0.09; 95%CI: 0.01-0.83; P = 0.033), maximum tumor diameter (OR = 1.25; 95%CI: 1.07-1.47; P = 0.005), invaded deep serous layer (OR = 3.38; 95%CI: 1.24-9.19; P = 0.017). We developed a nomogram to predict risk of GICs in aggressive PGINHL patients based on independent risk factors. The value of area under curve calculated by receiver operating characteristic curve was 0.815, and calibration curve and decision curve analysis further indicated that the prediction effect was superior. The majority of patients with GICs were given combination therapy (chemotherapy combined with surgery or radiation). Event-free survival and overall survival in GICs group were no worse than those in the No-GICs group.

CONCLUSION

The complication rate of GICs in patients with aggressive PGINHL was relatively high, particularly in PGI-DLBCL. The independent risk factors for GICs were the small intestine, PGI-TNKL, bulky tumor, and depth of invasion. A combination treatment, involving surgery, improved survival in the GICs group.

Key Words: Primary gastrointestinal; Aggressive; Non-Hodgkin lymphoma; Gastrointestinal complication; Risk factor

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Core Tip: The characteristics of aggressive primary gastrointestinal non-Hodgkin lymphoma (PGINHL) with or without severe gastrointestinal complications (GICs) were explored in this study. The relationship between GICs and clinicopathological features of aggressive PGINHL patients, such as primary site, histological type, tumor size, depth of gastrointestinal invasion and other factors, was investigated by statistical analysis. The aim of this study was to summarize the risk factors and treatment strategies for aggressive PGINHL patients with or without GICs and to provide evidence-based evidence for clinical decision-making and individualized treatment strategies.

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INTRODUCTION

Primary extra-nodal lymphoma was defined as a lymphoma whose primary site was not the lymph node but originated in tissues and organs other than the lymph node[1]. The digestive system organs had relatively rich lymphatic tissue, especially the gastrointestinal tract lymphatic drainage area, so the incidence of lymphoma invading the gastrointestinal tract was relatively high. Primary gastrointestinal lymphomas were rare compared to epithelial cancers, accounting for only 1% of gastrointestinal tumors. The most commonly involved organ was the stomach, followed by the small and large intestine, while the esophagus, appendix, and rectum were rare[2]. Primary gastrointestinal lymphoma was classified into B cell and nature killer (NK)/T cell lymphoma. It also was divided into aggressive and indolent lymphoma according to the course of the disease. Indolent B-cell lymphoma such as mucosal associated lymphoid tissue (MALT) and follicular cell lymphoma (FL) had little invasive damage to the gastrointestinal tract[1,2]. The indolent T-cell and NK-cell lymphomas usually had non-destructive infiltration. Among aggressive primary gastrointestinal non-Hodgkin lymphoma (PGINHL), diffuse large B-cell lymphoma (DLBCL) was the most common pathological subtype (47.4%)[3]. Primary gastrointestinal T/NK cell lymphomas (PGI-TNKL) were also aggressive entities consisting of various subtypes with distinct clinicopathological features and prognoses[4-6]. Aggressive gastrointestinal lymphoma had a more invasive growth and a worse prognosis than indolent lymphoma. Due to aggressive lymphoma occurred in the gastrointestinal tract, aggressive behaviors such as severe gastrointestinal complications (GICs)-gastrointestinal bleeding (GIB),

gastrointestinal obstruction (GIO), and gastrointestinal perforation (GIP) were also common at the first visit, especially after chemotherapy, GIP can lead to a very high mortality rate[7-9]. Special attention should be paid to the high risk of GICs in PGINHL patients, necessitating a multidisciplinary discussion and even emergency surgical intervention.

Early identification of GICs risk factors may be beneficial to early treatment and improve the prognosis of aggressive PGINHL patients[1]. A retrospective study suggested that GICs occurred in 56.8% of 148 PGI-DLBCL patients[7]. DLBCL was the most common type of aggressive PGINHL. T-cell lymphomas were uncommon, estimated to account for 4% to 6% of GI tract lymphomas[10]. A relatively large cohort study of PGI-TNKL was performed in 38 patients from South Korea[11]. Aggressive PGI-TNKL mainly referred to as intestinal T-cell lymphoma (ITCL)[1,10]. ITCLs comprised two main entities: Enteropathy-associated T-cell lymphoma (EATL) and monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), plus ITCL, not specifically (NOS)[5], they were both aggressive in behavior but differ in their clinicpathological features[11]. Aggressive extranodal natural killer/T-cell lymphoma (NKTCL) was more common in Asia, gastrointestinal tract also was involved[12]. However, no studies had systematically summarized the occurrence of GICs in aggressive gastrointestinal lymphomas.

At present, there were few prospective studies on treatments. How to identify high-risk GICs in aggressive PGINHL patients, improving the therapeutic effect and overall survival time was still under discussion. In this study, we analyzed aggressive PGINHL patients in our cancer center in the past 10 years. We were the first to analyze the GICs group *vs* the No-GICs group in aggressive PGINHL, to find risk factors that predicted GICs, and establish a prediction nomogram, which was conducive to the early identification of clinicians. At the same time, we analyzed the treatment characteristics and prognosis of aggressive PGINHL with GICs, and attempted to provide evidence-based basis for treatment.

MATERIALS AND METHODS

Patient Selection and evaluation criterion

The patients included inpatient and outpatient lymphoma patients at our hospital from January 2013 to December 2021, and followed up until December 2022. Follow-up methods included in-patient review, outpatient review, and telephone follow-up. Primary gastrointestinal lymphoma was defined as predominant lesions in the alimentary tract with or without regional lymph node involvement[13,14]. All cases were diagnosed according to the World Health Organization diagnostic criteria[1]. In our cancer center, the flow chart of the selected aggressive gastrointestinal lymphomas was shown in Figure 1. The aggressive PGI-B-cell lymphomas were DLBCL and Burkitt's lymphoma (BL). ITCL patients accounted for the majority including aggressive PGI-TNKL, specifically EATL, MEITL, ITCL-NOS, and NKTCL. The exclusion criteria were defined as follows: Indolent primary PGINHL, pathological diagnosis inconsistent, infected with human immunodeficiency virus, younger than 18 years.

The clinical data of all patients at the time of initial diagnosis were collected in our cancer center, including age, gender, disease stage, eastern cooperative oncology group (ECOG) score, blood lactate dehydrogenase (LDH), hepatitis virus, Epstein-Barr virus (EBV) DNA, primary site, immunohistochemical markers, tumor size, endoscopy or endoscopic ultrasonography, imaging, bone marrow examinations, treatment methods, and outcomes. Ann Arbor staging was still the main clinical staging, referring to the modified Lugano staging[15]. This retrospective research was approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University (No. WDRY2021-KS024) and conducted in accordance with the declaration of Helsinki.

Pathological immunophenotypic markers

Diagnosis confirmation depended on the pathology of endoscopic biopsy or surgical specimens. The subtype of each lesion was determined using immunohistochemical analysis. According to the expression of cluster of differentiation (CD) 10, multiple myeloma oncogene 1, and human B-cell lymphoma 6 in Han's classification, patients were classified as germinal center B cells (GCB) and non-GCB. Double expression was defined as *MYC* expression ≥ 40% and *Bcl-2* expression ≥ 50%[16]. *MYC* gene rearrangement and lesion positivity for EBV-encoded small RNA were assayed by in situ hybridization.

Macroscopy/endoscopy and imaging findings

Macroscopic findings regarding morphology and size were reviewed by expert endoscopists and pathologists based on the endoscopic findings and/or surgically resected specimens. Lesion morphology was classified as polypoid, ulcerative, diffuse nodular, or diffuse-infiltrating type in some literatures[14,17-20]. In our study, we divided these types into neoplasm, ulceration and other types. The neoplasm consisted of polypoid or mass without surface ulceration. Ulceration included diffuse ulceration or focal/elevated ulceration. Slightly thickened or inconspicuous luminal walls were classified as others. For later statistical analyses, the depth of tumor invasion was classified as inside or outside the serosal layer based on endoscopic ultrasonography or postoperative pathological findings in our cases.

The radiologist's first impression of imaging was classified as normal, non-specific inflammation, cancer, or lymphomain some literature[14]. In our study, imaging findings were divided into two categories: Neoplastic lesions or others (normal, non-specific inflammation). 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) and maximum standardized uptake value could not be obtained before diagnosis because most patients in the GICs group were diagnosed after acute abdomen surgery.

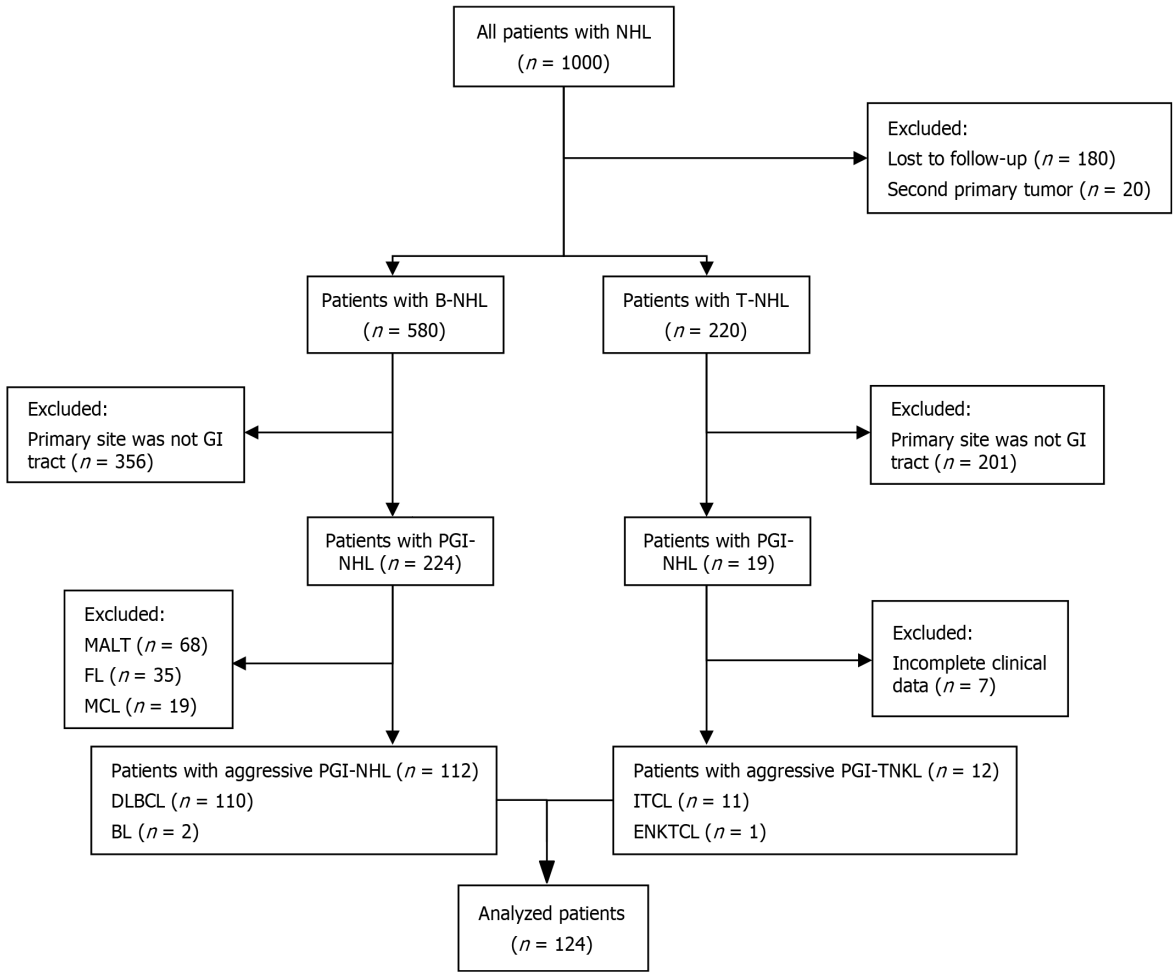

Figure 1 Screening flowchart for aggressive gastrointestinal non-Hodgkin lymphoma. NHL: Non-Hodgkin lymphoma; PGI: Primary gastrointestinal; GI: Gastrointestinal; MALT: Mucosal associated lymphoid tissue; FL: Follicular cell lymphoma; MCL: Mantle cell lymphoma; DLBCL: Diffuse large B-cell lymphoma; BL: Burkitt's lymphoma; PGI-TNKL: Primary gastrointestinal T/NK cell lymphomas; ITCL: Intestinal T-cell lymphoma; ENKTCL: Extranodal natural killer T cell lymphoma.

Evaluation outcome criteria

GICs were confirmed by clinical symptoms, laboratory tests, imaging, and /or surgery. GIO, including partial and complete obstruction, was considered to be the absence of passage of flatus or feces[21]. GIP was defined as the presence of free air under the diaphragm on abdominal imaging, or intestinal perforation during laparotomy[22]. GIB was defined as overt bleeding (hematemesis, bloody stools) and drop in hemoglobin of ≥ 2 g/dL[23]. All the above GICs were confirmed using imaging or surgery. During the course of the disease, it was possible to develop two or more complications. Patients with GICs required surgery, we also included those who suffered from GICs but could not undergo surgery due to their poor general condition.

Revised response criteria, known as the 2014 Lugano criteria, was divided into imaging remission and metabolic remission using FDG-PET scans[15]. The response was categorized as complete remission, partial remission, stable disease and relapsed disease or progressive disease. Event-free survival (EFS) was defined as the time from the diagnosis to disease progression, recurrence, death or last follow-up. The overall survival (OS) duration was measured from the time of diagnosis to the time of death or last follow-up.

Statistical analysis

After data collection, frequency (%) was used as a descriptive statistic. Quantitative variables were expressed as mean ± SD or median (quartile range). The differences in clinical parameters were compared using χ^2 test or Fisher's exact for categorical variables, or one-way analysis of variance for continuous variables. P values < 0.05 at both sides were considered statistically significant. The GICs risk nomogram was constructed with the most significant factors associated with GICs using Logistic regression analysis. The receiver operating characteristic (ROC) curve was used to evaluate the accuracy of nomogram in predicting the risk of GICs. Then, calibration curves were generated for the comparison between the actual outcomes and nomogram-predicted outcomes. Finally, decision curve analysis (DCA) was conducted by measuring the net benefits for a group of threshold probabilities to measure clinical utility[24]. EFS and OS were evaluated using Kaplan Meier analysis with a log rank comparison. Cox regression univariate and multivariate analyses were used to assess predictors of survival. Statistical analyses were performed using statistical product and service

solutions (SPSS) software (version 25.0) for Windows (SPSS Inc., Chicago, IL, United States), R software (version 3.3.0) (http://www.r-project.org/).

RESULTS

Clinicopathologic characteristics of GICs group with aggressive PGINHL

Among approximately 224 PGINHL cases diagnosed from 2013 to 2021, more than 60 indolent gastrointestinal lymphomas (MALT, FL) were excluded, ten cases of mantle cell lymphoma (MCL) with small B-cell lymphoma were also excluded, leaving 124 patients with aggressive PGINHL. Among them, aggressive PGI-B cell lymphomas accounted for 90.3% (110 cases of DLBCL and 2 cases of BL), aggressive PGI-TNKLs only accounted for 9.7% (11 cases of ITCL and 1 case of NKTCL). The final follow-up period was December 2022 (Figure 1).

In our study of 124 cases of aggressive PGINHL, GICs occurred in 48.4% (60/124), including 36 cases of GIO, 9 cases of GIP, 12 cases of GIB, and 3 cases of GIO and GIB. In the subgroup that combined GICs, the median age was 61 years (range: 28 to 89 years), and the male to female ratio was 1.73:1. The most common histological type in the GICs group was DLBCL (n = 49, 81.7%), followed by ITCL (n = 10, 16.7%), NKTCL (n = 1, 1.6%). The most frequently affected anatomic location in GICs group was the small intestine (43.3%), followed by large intestine (31.7%), stomach and esophagus (25.0%), and tumor maximum diameter was 7.03 cm ± 3.7 cm. Compared with the No-GICs group (8/37 cases), the GIC group had a significantly higher proportion of serosal layer and outside (29/37 cases). No matter in No-GICs group or GICs group, in terms of the macroscopic appearance of gastrointestinal tract growth (through endoscopy or surgery), lymphoma was mainly neoplasm, followed by an ulcerative type. In terms of imaging findings, radiologists often considered neoplastic lesions, but they could not further indicate cancer or lymphoma (Table 1). Supplementary Figure 1 showed an endoscopic picture of inflammatory changes in a patient with primary small intestinal TNKL, and another endoscopic picture of a large ulcer in a patient with primary ileocecal junction DLBCL was shown in Supplementary Figure 2.

Risk factors associated with GICs in aggressive PGINHL patients

Results of univariate analysis [odds ratio (OR); 95% confidence interval (CI); P value] were as follows: Primary site (small intestine OR = 6.55; 95%CI: 2.61-16.40; P < 0.001; colon OR = 5.60; 95%CI: 2.15-14.61; P < 0.001), aggressive B cell lymphomas (OR = 0.09; 95%CI: 0.01-0.83; P = 0.033), max tumor diameter (OR = 1.30; 95%CI: 1.13-1.48; P < 0.001), invasive depth (serosal layer and outside OR = 6.55; 95%CI: 2.67-16.06; P < 0.001), ECOG ≥ 2 (OR = 3.35; 95%CI: 1.02-11.05; P = 0.047), and double expression (OR = 0.40; 95%CI: 0.16-0.97; P = 0.044) were risk factors for GICs.

Multivariate analysis revealed that primary site (small intestine OR = 3.33; 95%CI: 1.47-9.41; P = 0.009; colon OR = 2.50; 95%CI: 1.17-7.15; P = 0.023), aggressive B cell lymphomas (OR = 0.09; 95%CI: 0.01-0.83; P = 0.033), max tumor diameter (OR = 1.25; 95%CI: 1.07-1.47; P = 0.005), invasive depth (serosal layer and outside OR = 3.38; 95%CI: 1.24-9.19; P = 0.017) were independent indicators for GICs (Table 2).

Development of GICs risk predictive nomogram

Based on univariate and multivariate analyses, four parameters (primary tumor site, histopathological type, depth of tumor invasion, and max tumor diameter) were identified as independent risk factors. Hence, based on these four significant variables, a nomogram was created to predict risk of GICs in aggressive PGINHL patients (Figure 2). Add up the corresponding scores for each item and find the corresponding percentage on the GICs risk score.

Then, DCA showed that if the threshold probability was over 0.5, the nomogram for GICs prediction added more benefit than all or none, indicating that our nomogram provided a better clinical net benefit (Figure 3). As depicted in Figure 4, ROC analysis revealed that the nomogram exhibited favorable predictive performance for GICs. ROC curves were generated to further evaluate the predictive performance (area under curve = 0.815). Additionally, the calibration curves for the GICs probability exhibited optimal agreement (Figure 5).

Treatment characteristics and prognosis of patients with GICs in aggressive PGINHL

Patients with aggressive PGINHL in the GICs group were treated differently from those in the No-GICs group (Table 3). In the former group, emergency surgery was performed because of GICs accounting for 88.3% in the GICs group, while in the No-GICs group, 3 patients underwent surgery because the tumors were not detected by endoscopy but were highly suspected by imaging. The rate of chemotherapy (71.7%) and radiotherapy (8.3%) in the GICs group were lower than those in the No-GICs group, probably because some patients could not tolerate chemotherapy because of poor ECOG for GICs, some patients improved after surgical resection of bulky masses and did not require radiotherapy. In conclusion, the patients with GICs were mainly given combination therapy (chemotherapy combined with surgery or radiotherapy). EFS and OS of GICs group were close to those in the No-GICs group (P > 0.05). At the end of follow-up, 26 patients (43.3%) died in the GIC group, and 20 patients (31.3%) died in the No-GIC group, there was no significant difference between the two groups (P > 0.05) (Figure 6).

Analysis of prognostic factors in patients with aggressive PGINHL with GICs: Univariate analysis suggested that histopathology, LDH, stage, ECOG, non-GCB, chemotherapy combined with surgery or radiotherapy were related to OS (P < 0.05). Multivariate analysis suggested that histopathology, LDH, stage, ECOG, chemotherapy combined with surgery or radiotherapy were independent factors (P < 0.001, P = 0.005, P = 0.007, P < 0.001, P < 0.001 respectively) (Table 4).

Table 1 Clinicopathologic characteristics of 124 cases of aggressive primary gastrointestinal non-Hodgkin lymphoma, *n* (%)

Characteristics	No-GICs (*n* = 64)	GICs (*n* = 60)	*P* value
Age, mean ± SD	59.8 ± 12.5	60.0 ± 14.2	0.932
≥ 60	32 (50.0)	34 (56.7)	0.573
< 60	32 (50.0)	26 (43.3)	
Gender			0.427
Male	35 (54.7)	38 (63.3)	
Female	29 (45.3)	22 (36.7)	
Stage			0.875
I/II	29 (45.3)	29 (48.3)	
III/IV	35 (54.7)	31 (51.7)	
LDH			0.406
Elevated	27 (42.2)	20 (33.3)	
Normal	37 (57.8)	40 (66.7)	
Virus			0.876
Yes	8 (12.5)	6 (10.0)	
No	56 (87.5)	54 (90.0)	
ECOG			0.139
0-1	59 (92.2)	49 (41.7)	
≥ 2	5 (7.8)	11 (18.3)	
Depth			< 0.001
Inside serous membrane	54 (87.5)	31 (51.7)	
Serosal layer and outside	8 (12.5)	29 (48.3)	
Diameter, mean ± SD	4.65 ± 2.4	7.03 ± 3.7	< 0.001
Primary site			< 0.001
Esophagus or stomach	40 (62.5)	15 (25.0)	
Small intestine	12 (18.8)	26 (43.3)	
Colon	12 (18.8)	19 (31.7)	
Histopathology			< 0.001
DLBCL + BL	63 (98.4)	49 (81.7)	
ITCL + NKTCL	1 (1.6)	11 (18.3)	
N-GCB	39 (60.9)	43 (71.7)	0.284
CD5 +	13 (20.3)	7 (11.7)	0.287
CD30 +	5 (7.8)	7 (11.7)	0.673
Double expression	18 (28.1)	9 (15.0)	0.121
Ki 67 ≥ 70%	39 (60.9)	40 (66.7)	0.634
Macroscopic findings			0.858
Neoplasm	29 (45.3)	30 (50.0)	
Ulceration	31 (48.4)	27 (45.0)	
Others	4 (6.2)	3 (5.0)	
Imaging findings			0.205
Neoplasticlesions	53 (82.8)	43 (71.7)	
Others	11 (17.2)	17 (28.3)	

PGINHL: Primary gastrointestinal non-Hodgkin lymphomas; GICs: Gastrointestinal complications; LDH: Lactate dehydrogenase, ECOG: Eastern cooperative oncology group; DLBCL: Diffuse large B-cell lymphoma; BL: Burkitt lymphoma; ITCL: Intestinal T-cell lymphoma; NKTCL: Natural killer/T-cell lymphoma; N-GCB: Non-germinal center B cells; CD: Cluster of differentiation; Ki: Kiel proliferation index.

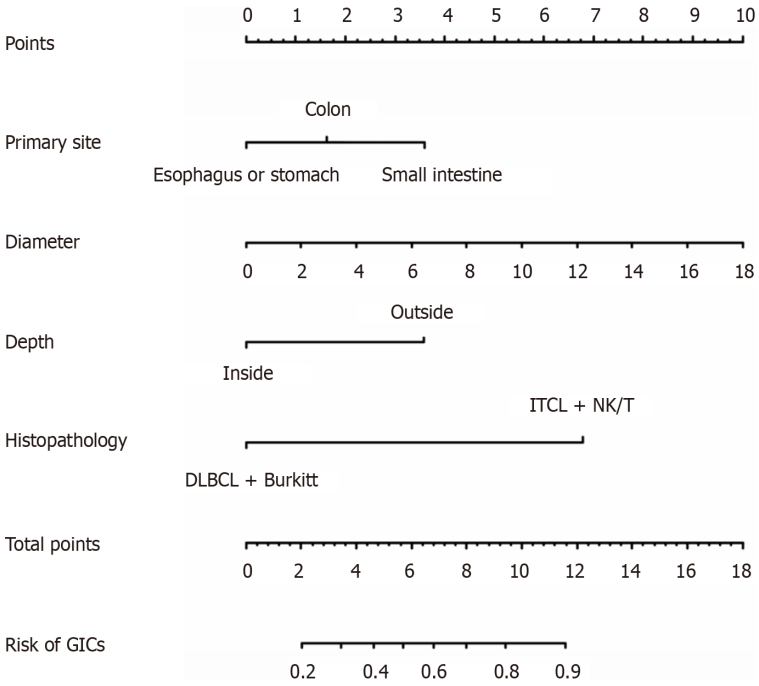

Figure 2 Nomogram for predicting the risk of gastrointestinal complications in aggressive gastrointestinal non-Hodgkin lymphoma. GICs: Gastrointestinal complications; ITCL: Intestinal T-cell lymphoma; NK: Nature killer; DLBCL: Diffuse large B-cell lymphoma.

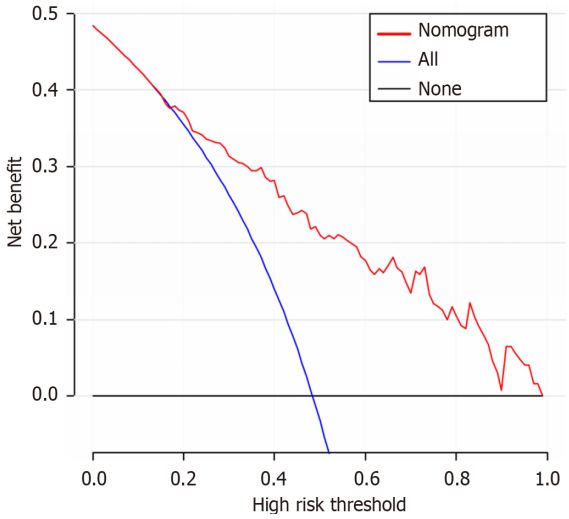

Figure 3 Decision curves analysis for the risk nomogram of gastrointestinal complications in aggressive gastrointestinal non-Hodgkin lymphoma.

DISCUSSION

Primary gastrointestinal lymphoma can occur in any part of the digestive tract, most commonly in the stomach, followed by the small intestine, colon and rectum, rarely in the esophagus[25,26]. The most common pathological type of PGINHL was DLBCL, followed by MALT, MCL, rare types included ITCL, BL, NKTCL, *etc.*[27]. More attention should be paid to aggressive gastrointestinal lymphomas such as DLBCL, BL, ITCL, and NKTCL. Owing to the rapid progression of the aggressive lymphomas, the enlargement and invasive characteristics of the mass were easy to cause GICs, the management of GICs remained a clinical challenge[22]. In our study of aggressive gastrointestinal lymphoma cohort, GICs occurred in 48.4% of patients. The most common histological type in GICs group was DLBCL (81.7%). The most

Table 2 Univariate and multivariate analyses of factors associated with gastrointestinal complications in aggressive Primary gastrointestinal non-Hodgkin lymphomas patients

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age				
≥ 60	1.21 (0.60-2.45)	0.597		
< 60	Ref.	-		
Gender				
Male	1.48 (0.72-3.03)	0.289		
Female	Ref.	-		
Primary site				
Esophagus or stomach	Ref.	-	Ref.	
Small intestine	6.55 (2.61-16.40)	< 0.001	3.33 (1.47-9.41)	0.009
Colon	5.60 (2.15-14.61)	< 0.001	2.50 (1.17-7.15)	0.023
Histopathology				
DLBCL + BL	0.08 (0.01-0.63)	0.017	0.09 (0.01-0.83)	0.033
ITCL + NK/T	Ref.	-	Ref.	-
Max tumor diameter	1.30 (1.13-1.48)	< 0.001	1.25 (1.07-1.47)	0.005
Invasive depth				
Inside serous membrane	Ref.	-	Ref.	
Serosal layer and outside	6.55 (2.67-16.06)	< 0.001	3.38 (1.24-9.19)	0.017
LDH				
Elevated	0.67 (0.33-1.40)	0.287		
Normal	Ref.	-		
Virus				
Yes	0.70 (0.23-2.14)	0.529		
No	Ref.	-		
Stage				
I/II	Ref.	-		
III/IV	0.82 (0.40-1.66)	0.581		
ECOG				
0-1	Ref.	-	Ref.	-
≥ 2	3.35 (1.02-11.05)	0.047	1.02 (0.24-4.39)	0.974
N-GCB	0.62 (0.29-1.31)	0.207		
CD5 +	0.46 (0.17-1.25)	0.128		
CD30 +	1.40 (0.42-4.68)	0.584		
Double expression				
Yes	0.40 (0.16-0.97)	0.044	0.51 (0.17-1.52)	0.227
No	Ref.	-	Ref.	-
Ki 67 ≥ 70%				
Yes	1.13 (0.54-2.25)	0.747		
No	Ref.	-		
Macroscopic findings				

Neoplasm	0.59 (0.12-2.88)	0.515
Ulceration	0.69 (0.33-1.42)	0.311
Others	Ref.	-
Imaging findings		
Neoplasticlesions	0.49 (0.21-1.17)	0.109
Others	Ref.	-

LDH: Lactate dehydrogenase, ECOG: Eastern cooperative oncology group; DLBCL: Diffuse large B-cell lymphoma; BL: Burkitt lymphoma; ITCL: Intestinal T-cell lymphoma; NKTCL: Natural killer/T-cell lymphoma; N-GCB: Non-germinal center B cells; CD: Cluster of differentiation; Ki: Kiel proliferation index; CI: Confidence interval; NK: Nature killer.

Table 3 Treatments and outcomes of patients in the No-gastrointestinal complications *vs* gastrointestinal complications groups of aggressive primary gastrointestinal non-Hodgkin lymphomas

Characteristics	No-GICs (*n* = 64)	GICs (*n* = 60)	*P* value
Chemotherapy, *n* (%)			0.000
No	3 (4.7)	17 (28.3)	
Yes	61 (95.3)	43 (71.7)	
Radiotherapy, *n* (%)			0.022
No	49 (76.6)	55 (91.7)	
Yes	15 (23.4)	5 (8.3)	
Surgery, *n* (%)			0.000
No	61 (95.3)	5 (11.7)	
Yes	3 (4.7)	53 (88.3)	
Treatment, *n* (%)			
Without CT	3 (4.7)	17 (28.3)	0.000
CT alone	43 (67.2)	4 (6.7)	
CT + Sur/RT	18 (28.1)	39 (65.0)	
EFS time, M (IQR)	21.0 (11.0, 36.0)	17.0 (5.5, 36.0)	0.580
OS time, M (IQR)	28.5 (14.5, 45.0)	24.5 (6.5, 47.5)	0.335
Death toll, *n* (%)	20 (31.3)	26 (43.3)	0.164

GICs: Gastrointestinal complications; CT: Chemotherapy; RT: Radiotherapy; Sur: Surgery; EFS: Event-free survival; OS: Overall survival; IQR: Interquartile range.

common anatomical site in the GICs group was the small intestine (43.3%). Some studies suggested that primary small intestinal DLBCL was indeed more likely to develop intestinal obstruction or perforation[28]. In our GICs group, bulky lymphoma accounted for a relatively high proportion, the maximum tumor diameter in this group was approximately 7.03 cm ± 3.7 cm, which was also a factor that leaded to more GIO in this group. In our study, GICs occurred in 11 cases (91.7%) of 12 patients with PGI-TNKL, while 49 patients (43.8%) of the 112 patients with PGI-B cell lymphoma developed GICs, which including 36 cases of GIO, 9 cases of GIP, 12 cases of GIB, and 3 cases of GIP and GIB. ITCL was more prone to perforation than intestinal B-cell lymphoma[14,29].

Multivariate Logistic regression analysis showed that the four independent risk factors for GICs were the primary site of small intestine, pathological type PGI-NKTL, large tumor diameter, invasion of the serosal layer and beyond. First, regarding the discussion of the most common primary site, a study suggested that the small intestine was the most common site, accounting for 59%[22]. In the single-center study on intestinal lymphoma complicated with perforation, 55% (51/92) of the cases occurred after chemotherapy[30]. Most patients had GIO before chemotherapy, accounting for 60% (36/60), and there were 20% (12/60) cases with intestinal perforation before or after chemotherapy. In our study, the highest occurrence site of GICs was also in the small intestine, accounting for 43.3%. The decrease in perforation rate in our study may be related to our early surgical intervention. Second, regarding the most common histopathological types, a study suggested that aggressive B-cell lymphoma had a higher risk of perforation than indolent B-cell lymphoma, particularly DLBCL[22]. In addition, although the incidence of enteric T-cell lymphoma was lower, GIP was higher in

Table 4 Univariate and multivariate analyses of factors associated with overall survival in aggressive primary gastrointestinal non-Hodgkin lymphoma patients with gastrointestinal complications

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Age				
≥ 60	1.29 (0.58-2.84)	0.535		
< 60	Ref.	-		
Gender				
Male	1.83 (0.77-4.36)	0.174		
Female	Ref.	-		
Primary site				
Esophagus or stomach	Ref.	-		
Small intestine	1.53 (0.55-4.31)	0.417		
Colon	1.25 (0.41-3.82)	0.697		
Histopathology				
ITCL + NKTCL	5.58 (2.40-13.00)	< 0.001	8.75 (2.87-26.71)	< 0.001
DLBCL + BL	Ref.	-	Ref.	-
Max tumor diameter	1.03 (0.93-1.14)	0.598		
Invasive depth				
Inside serous membrane	Ref.	-		
Serosal layer and outside	1.15 (0.53-2.49)	0.728		
LDH				
Elevated	3.33 (1.51-7.33)	0.003	3.90 (1.50-10.12)	0.005
Normal	Ref.	-	Ref.	-
Virus				
Yes	2.13 (0.73-6.23)	0.169		
No	Ref.	-		
Stage				
I/II	Ref.	-	Ref.	-
III/IV	6.29 (2.35-16.82)	< 0.001	5.49 (1.58-19.09)	0.007
ECOG				
0-1	Ref.	-	Ref.	-
≥ 2	13.40 (5.39-33.35)	< 0.001	24.14 (6.28-92.70)	< 0.001
N-GCB	0.28 (0.09-0.94)	0.040		
CD5 +	1.47 (0.51-4.28)	0.479		
CD30 +	1.58 (0.57-4.92)	0.347		
Double expression				
Yes	1.55 (0.58-4.12)	0.380		
No	Ref.	-		
KI 67 ≥ 70%				
Yes	0.95 (0.42-2.12)	0.891		
No	Ref.	-		
Treatment				

Without CT	Ref.	-	Ref.	-
CT alone	0.85 (0.24-2.97)	0.800	1.82 (0.44-7.50)	0.407
CT + Sur/RT	0.07 (0.03-0.19)	< 0.001	0.07 (0.02-0.24)	< 0.001

LDH: Lactate dehydrogenase, ECOG: Eastern cooperative oncology group; DLBCL: Diffuse large B-cell lymphoma; BL: Burkitt lymphoma; ITCL: Intestinal T-cell lymphoma; NKTCL: Natural killer/T-cell lymphoma; N-GCB: Non-germinal center B cells; CD: Cluster of differentiation; Ki: Kiel proliferation index; CI: Confidence interval; NK: Nature killer; CT: Chemotherapy; RT: Radiotherapy; Sur: Surgery; HR: Hazard ratio.

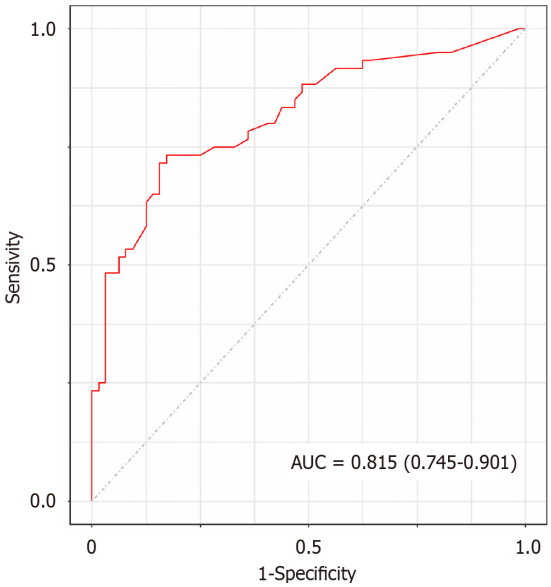

Figure 4 The predictive performance of the risk nomogram for predicting gastrointestinal complications in aggressive gastrointestinal non-Hodgkin lymphoma. Receiver operating characteristic curves displayed that this nomogram discriminated well. AUC: Area under the curve.

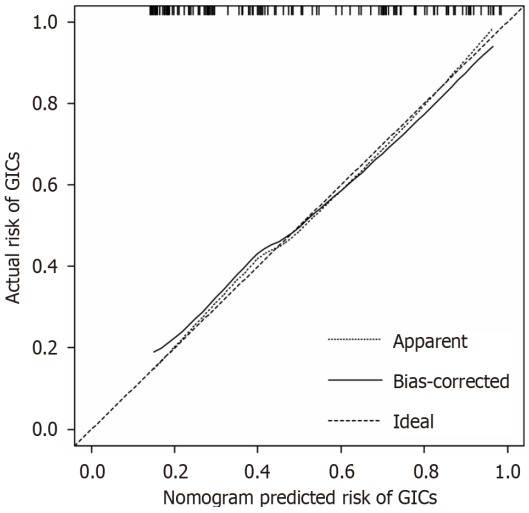

Figure 5 The calibration curve of risk nomogram of gastrointestinal complications in aggressive gastrointestinal non-Hodgkin lymphoma. GICs: Gastrointestinal complications.

aggressive T-cell lymphoma[31]. In our study, the most common histological type of GICs group was DLBCL (81.7%), followed by ITCL (16.7%), and NKTCL (1.6%). However, GICs occurred in 11 of the 12 patients with PGI-TNKL, especially intestinal perforation, required emergency surgery. At the end of our follow-up, only one patient in PGI-TNKL survived, and the others died within a year.

Third, the depth of tumor invasion of the tube wall was related to the occurrence of GICs. Studies have suggested that patients with gastrointestinal cancer were prone to perforation once the tumor encroached on the serosal layer[32]. Microscopic findings showed that alterations in gastrointestinal lymphoma began in the submucosal layer where they

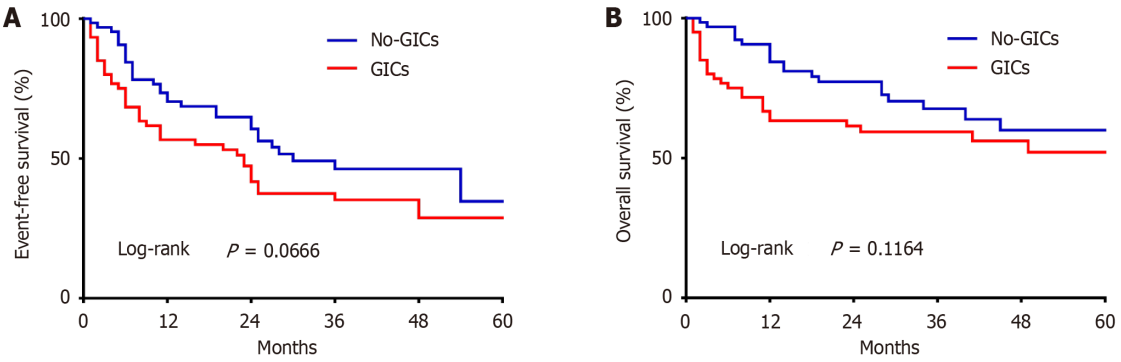

Figure 6 Survival analysis of the gastrointestinal complications and No-gastrointestinal complications groups in primary gastrointestinal non-Hodgkin lymphomas. A: Event-free survival curves for the comparison between gastrointestinal complications (GICs) and No-GICs group; B: Overall survival curves for the comparison between GICs and No-GICs group ($P > 0.05$). GICs: Gastrointestinal complications.

were most extensive, rarely spread to the muscular layer, and serosal layer[33]. Fourth, regarding the correlation between the maximum tumor diameter and the risk of GICs, the main reason was digestive tract obstruction caused by bulky mass. In a retrospective study of DLBCL, patients with tumor mass ≥ 10 cm, or intestinal involvement had significantly higher risk of severe GICs as initial manifestations[7].

Summarizing our study and previous clinical studies, these four factors did have GICs correlation, and we developed a nomogram to predict risk of GICs in aggressive PGINHL patients based on independent risk factors. The higher the total scores of the corresponding nomogram, the greater the risk of GICs. The high risk of GICs suggested that clinicians needed to take emergency measures such as surgical intervention and postponing chemotherapy. DCA mapping showed that our predictive model provided better clinical benefits. The ROC and calibration curves for the GICs probability exhibited an optimal agreement. Currently, there were no predictive GICs risk models for aggressive intestinal lymphoma. A small sample study proposed a risk scoring system that included indolent PGI lymphoma patients to predict severe GICs events, patients with high scores were candidates for elective surgery to prevent GIC events[34]. Our predictive GICs risk nomogram covered a more susceptible population than the few previously reported, with a relatively many predictors. Regarding prognostic markers for large B-cell lymphoma (Double expression, CD5, no-GCB), these markers did not hold up in multivariate analyses in this study population and it may be worthwhile to look specifically at their role in patients with large B-cell lymphoma. The limitation of this study was that there was a certain bias in the study data because it was retrospective. Also, it was the absence of external validation. In the future, multi-center and prospective studies are needed to verify and improve these results.

Unlike the patients with nodal or other primary extra nodal lymphomas, PGINHL patients with GICs required immediate intervention. because GICs were life-threatening acute abdomens such as GIO, GIB, and GIP. In particular, the perforation rate of small intestine was more than 50%, and the mortality rate was high[30]. How to adjust the treatments of those patients? Priority surgery, delayed surgery, or reduced-dose chemotherapy? In the GICs cohort we studied emergency surgery was performed, accounting for 88.3%. In the No-GICs group, three patients underwent surgery because the tumors were not detected by endoscopy but highly suspected by imaging. The majority of patients with GICs were given combination therapy. Due to identification of emergency and timely treatment of GICs, local surgery can prevent spontaneous perforation and/or bleeding during chemotherapy or radiotherapy and provide opportunities for subsequent systemic treatment. Patients with good recovery after total resection were treated with immunochemo-therapy. Adjuvant radiotherapy was given to bulky patients who could not be totally resected. For inoperable patients, attention should be paid to the initial chemotherapy starting from a low dose and gradually increasing to a standard dose. The tumor regression should be closely observed. Lymphoma was sensitive to immunochemotherapy and retreated rapidly; and normal tissue could not be repaired because of bulky or deep invasion lesions in the gastrointestinal tract. Thus, GICs caused by tumor regression after high-dose chemotherapy should be avoided as much as possible. Even if they undergo emergency surgery, faced severe myelosuppression after chemotherapy and had a poor prognosis. Therefore, we need to summarize the characteristics of patients with aggressive PGINHL and expand the sample size to avoid small sample bias.

Our survival analysis in GICs group, suggested that histopathology, LDH, stage, ECOG, chemotherapy combined with surgery or radiotherapy were independent factor. It had been reported that high LDH, advanced stage, and high ECOG in the international prognostic index scoring system were associated with poor OS of NHL[35,36]. In our study, ten cases of PGI-TNKL died of intestinal perforation and bleeding, the prognosis were poor. A multi-center prospective study in Asia suggested that PGI-TNKL showed aggressive behavior and poor OS[37]. Aggressive clinicopathological features of PGI-TNKL were different from indolent T-cell lymphoproliferative disorder[4]. Microscopic examination showed a diffuse transmural lymphoid infiltrate with accompanying mucosal ulceration[38]. Therefore, both clinicians and pathologists must be aware of the distinct characteristics of these lesions to ensure that appropriate care was provided. In our study, combination therapy did improve survival of the patients with GICs, EFS and OS were close to those in the No-GICs group. However, Surgery was controversial in GI lymphoma[39-41], we should grasp the surgical indications and avoid meaningless invasive surgery in low-risk populations.

CONCLUSION

The complication rate of GICs in patients with aggressive PGINHL was relatively high, especially in those with PGI-DLBCL. The independent risk factors for GICs were the small intestine, PGI-TNKL, bulky tumor, and depth of invasion. A preliminary risk model for predicting GICs was established. We analyzed therapeutic interventions for patients with a high incidence of GICs. Comprehensive treatment including surgery improved the OS of patients with GICs. We hope to further validate the nomogram with larger samples in the future.

FOOTNOTES

Author contributions: Liu XH and Liu H designed the study and interpreted the data; Chen G, Ke XK, Ke D, Tan W and Cao DD contributed to the acquisition of the clinical samples and data; Hu YG, Cao DD, Liu XH and Xu XM performed the analytic calculations and wrote the manuscript; All authors have read and approved the final manuscript.

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Informed consent statement: Patient consent was waived by the IRB because all patient data was de-identified.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: Pathology data and the statistical analyses for the current study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Retrospective Study

Endoscopic and pathological features of neoplastic transformation of gastric hyperplastic polyps: Retrospective study of 4010 cases

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Abstract

BACKGROUND

Hyperplastic polyps, which represent 30%-93% of all gastric epithelial polyps, are the second most common type of gastric polyps after fundic gland polyps. They were previously considered to have no risk of neoplastic transformation. Recently, an increasing number of cases of gastric hyperplastic polyps (GHPs) combined with neoplastic changes have been reported; however, the specific mechanism underlying their transformation has not been thoroughly explored.

AIM

To investigate the clinical, endoscopic, and pathological characteristics of the neoplastic transformation of GHPs and explore the risk factors.

METHODS

A retrospective analysis was performed on 4010 cases of GHPs diagnosed by gastroscopy and pathological examination at the hospital from 2005 to 2021. In total, 3874, 119, and 17 cases were in the group without intraepithelial neoplasia (IN), with low-grade IN, and with high-grade IN, respectively. The data analysis examined the association of endoscopic and pathological features with risk factors for neoplastic transformation. Factors with significant differences were entered into univariate logistic regression, followed by multivariate logistic regression analysis.

RESULTS

Univariate analysis revealed diameter, multiple polyp presence, redness, rough surface, lobulation, erosion, Yamada classification, location, and gastric mucosa were risk factors for neoplastic transformation. Multivariate analysis showed that age > 65 years [odds ratio (OR) = 1.789; 95% confidence interval (CI): 1.227-2.609; $P = 0.003$], male sex (OR = 1.680; 95%CI: 1.158-2.438; $P = 0.006$), multiple polyps (OR = 1.851; 95%CI: 1.230-2.784; $P = 0.003$), pedunculated or semi-pedunculated shape (OR = 2.722; 95%CI: 1.689-4.388; $P < 0.001$), and polyp diameter were significantly associated with GHPs that demonstrated neoplastic transformation. Compared with chronic superficial gastritis, autoimmune gastritis, atrophic gastritis, and gastritis with IN were independent risk factors for neoplastic transformation [(OR = 2.672; 95%CI: 1.559-4.579; $P < 0.001$), (OR = 1.876; 95%CI: 1.134-3.103; $P = 0.014$), and (OR = 5.299; 95%CI: 3.173–8.849; $P < 0.001$), respectively].

CONCLUSION

Male sex, age > 65 years, multiple polyps, pedunculated or semi-pedunculated shape, polyp size > 1 cm, and specific background gastric mucosa are key indicators for predicting neoplastic transformation of GHPs.

Key Words: Endoscopy; Gastric hyperplastic polyps; Neoplastic transformation; Pathology; Risk factors; Tumour

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Core Tip: Our results show that larger diameter, the presence of multiple polyps, pedunculated or semi-pedunculated shape, and specific background gastric mucosa were risk factors for neoplastic transformation. Furthermore, age > 65 years and male sex were important indicators for predicting the risk of malignant transformation of gastric hyperplastic polyps. Our findings suggest that for polyps with the abovementioned endoscopic and pathological features, clinicians should be alert to the possibility of neoplastic transformation to improve the diagnosis rate of the neoplastic transformation of gastric hyperplastic polyps. Additionally, our study showed that *Helicobacter pylori* infection was not associated with the risk.

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INTRODUCTION

Gastric hyperplastic polyps (GHPs) are the second most common type of gastric polyps after fundic gland polyps[1]. They typically do not cause obvious clinical symptoms and were previously considered to have no risk of neoplastic transformation. Recently, an increasing number of reports have emerged on GHPs combined with neoplastic change; however, the specific mechanism has not been thoroughly explored[2,3]. While our understanding of the neoplastic transformation mechanism of GHPs remains limited, knowledge regarding this condition is continuously advancing. Further research will contribute to a better understanding of the development of GHPs and provide more accurate diagnostic and treatment strategies for patients. Therefore, this study aimed to identify the endoscopic and pathological features of GHPs and discuss the risk of neoplastic transformation associated with these features to assist in clinical diagnosis and treatment.

MATERIALS AND METHODS

Study design and patients

This was a retrospective, single-centre study conducted at Peking University Third Hospital from 1 January 2005 to 31 December 2021. All patients were treated by endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD), or endoscopic forceps removal and were pathologically diagnosed as hyperplastic polyps. The inclusion criteria for patients were age ≥ 18 years, polyp diagnosis based on gastroscopy morphology, and hyperplastic polyp diagnosis based pathology. In contrast, the exclusion criteria were familial adenomatous polyposis, juvenile polyposis, Peutz-Jeghers syndrome, and Cronkhite–Canada syndrome. Ultimately, 4010 cases were enrolled in this study.

The Ethics Committee (No. M2023153) of the Peking University Third Hospital approved this clinical study and its protocol was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was not required from the patients due to the retrospective nature of the study.

Data collection

Patient information, such as age and sex, was retrospectively collected from medical records. Detailed characteristics, including the location, presence of single or multiple polyps, size, endoscopic appearance of polyp (Yamada's classification of polyps, mucosal erosion, lobulation, and surface roughness), and pathological features (presence or absence of dysplasia and adenocarcinoma) of polyps were examined. Background gastric mucosal characteristics (chronic superficial gastritis, chronic atrophic gastritis with or without intraepithelial neoplasia, and autoimmune gastritis) were also considered. A skilled pathologist assessed the gastric mucosal background. The location of GHPs in the stomach was classified as the lower third comprising the gastric antrum and angle; the middle third consisting of the lower and mid-body regions of the stomach; and the upper third comprising the fundus, cardia, and high-body of the stomach. Additionally, the presence or absence of *Helicobacter pylori* (*H. pylori*) was assessed using histological examination of biopsy specimens, *H. pylori* Warthin-Starry silver staining, or ^{13}C-urea breath tests. *H. pylori* status was considered positive if any of these test results were positive. GHPs with neoplasia were defined as those with histopathological confirmation of dysplasia or cancerous lesions in the endoscopically resected specimens. At least two pathologists confirmed each case with neoplasia. Further details are presented in Figure 1.

Research methods

Patients' basic information, in addition to gastroscopic and histopathological data, was retrospectively analyzed. According to histopathological results, hyperplastic polyps were categorised into hyperplastic polyps without intraepithelial neoplasia, with low-grade intraepithelial neoplasia, and with high-grade intraepithelial neoplasia, in which low-grade intraepithelial neoplasia included mild and moderate dysplasia whereas high-grade intraepithelial neoplasia comprised a high-grade dysplasia and carcinogenesis. Factors that influence neoplastic transformations were also analyzed. Neoplastic transformations were defined histologically by the presence of dysplasia or adenocarcinoma within GHPs. Further details are presented in Figure 1.

Statistical analysis

All statistical analyses were performed using Statistical Product and service solutions statistics for windows, version 26.0 (international business machines corporation, Armonk, NY, United States). Normally distributed measurement data are expressed as mean ± SD, and comparisons between groups were made using ordinary analysis of variance and independent sample *t*-test. Non-normally distributed measurement data are presented as median (range). Count data are expressed as percentages, and χ^2 or Fisher's exact test was used for comparisons between groups. Factors with significant differences were entered into univariate logistic regression, followed by multivariate logistic regression analysis. The results were determined using odds ratio (OR) and 95% confidence interval (CI). Statistical significance was set at $P < 0.05$.

RESULTS

General patient information

Between 2005 and 2021, 4010 cases of GHPs were confirmed based on gastroscopy and pathological examination at our hospital. Among these, 3874, 119, and 17 cases were in the groups without intraepithelial neoplasia, with low-grade intraepithelial neoplasia, and with high-grade intraepithelial neoplasia (5 cases with high-grade dysplasia and 12 with carcinogenesis), respectively. The mean ages of patients in the hyperplastic polyp, low-grade intraepithelial neoplasia, and high-grade intraepithelial neoplasia groups were 57.86 ± 0.22, 64.49 ± 1.13, and 67.93 ± 2.55 years, respectively. Continuous variables were transformed into grade variables, namely age (≤ 45, 45-65, and > 65 years), revealing significant differences. The age of patients in the neoplastic transformation group increased significantly. In terms of sex, GHPs without intraepithelial neoplasia were found more frequently in females (63%). In total, 49% of patients were male in the intraepithelial neoplasia group. The number of male patients significantly differed among the groups ($P < 0.05$). *H. pylori* infection was observed in 20%, 17%, and 12% of patients in the GHPs without intraepithelial neoplasia, with low-grade intraepithelial neoplasia, and with high-grade intraepithelial neoplasia groups, respectively, with no significant difference. Further details are presented in Table 1 and Table 2.

Endoscopic features

In terms of polyp size, the mean diameters of GHPs without intraepithelial neoplasia, with low-grade intraepithelial neoplasia, and with high-grade intraepithelial neoplasia were 0.66 ± 0.01, 1.25 ± 0.07, and 2.2 ± 0.32 cm, respectively, with significant difference ($P < 0.001$). Continuous variables were transformed into grade variables, namely polyp size (≤ 1 cm, 1 cm-2 cm, and > 2 cm), revealing significant differences. The diameter of GHPs with intraepithelial neoplasia was significantly larger than in the GHPs group ($\chi^2 = 203.055$, $P < 0.001$), with 82% of GHPs with high-grade intraepithelial neoplasia having a diameter of > 1 cm, which was significantly more frequent than in the low-grade intraepithelial neoplasia group ($P < 0.05$). Among GHPs without intraepithelial neoplasia, 70.6% were mainly single, while the proportion of multiple polyps increased with lesion progression. Multiple polyps were common (65%) in the high-grade intraepithelial neoplasia group.

According to Yamada's classification of polyps, simple GHPs were most commonly classified as Yamada type I (64%), while the proportion of polyps that exhibited intraepithelial neoplasia was significantly reduced, only 26% ($\chi^2 = 169.676$, $P < 0.001$). High-grade intraepithelial neoplasia polyps showed a difference with pedunculated or semi-pedunculated

Table 1 Features of gastric hyperplastic polyps in different groups and univariate logistic regression analysis of risk factors for neoplastic transformation

	GHPs without IN (*n* = 3874)	GHPs with IN (*n* = 136)	χ^2 test		Univariate analysis	
			χ^2 value	*P* value	*P* value	OR (95%CI)
Age (mean ± SD), years	57.86 ± 0.22	64.76 ± 1.04	37.178	< 0.001	< 0.001	1.761 (1.459-2.126)
≤ 45 years, *n* (%)	699 (18)	9 (6)				
45-65 years, *n* (%)	1988 (51)	54 (40)				
> 65 years, *n* (%)	1187 (31)	73 (54)				
Sex, *n* (%)			9.057	0.011	0.003	1.672 (1.188-2.356)
Male	1423 (37)	67 (49)				
Female	2451 (63)	69 (51)				
H. pylori infection, *n* (%)	782 (20)	22 (16)	1.865	0.172	0.174	0.721 (0.450-1.155)
Multiple polyps, *n* (%)	1122 (29.4)	62 (46)	23.162	< 0.001	< 0.001	2.275 (1.614-3.207)
Polyp size (mean ± SD), cm	0.66 ± 0.01		203.055	< 0.001	< 0.001	3.634 (2.948-4.478)
≤ 1 cm	3406 (88)	66 (48)				
1 cm-2 cm	374 (10)	53 (39)				
> 2 cm	94 (2)	17 (13)				
Endoscopic color, *n* (%)			18.626	< 0.001	0.03	0.671 (0.472-0.972)
Normal	2113 (55)	53 (40)				
Red	1488 (40)	82 (60)				
White	275 (5)	1 (0)				
Mucosal erosion, *n* (%)	289 (8)	30 (22)	38.701	< 0.001	< 0.001	3.538 (2.318-5.402)
Polyp lobulation, *n* (%)	185 (5)	25 (18)	49.109	< 0.001	< 0.001	4.491 (2.840-7.102)
Mucosal roughness, *n* (%)	864 (22)	69 (51)	59.494	< 0.001	< 0.001	3.588 (2.542-5.064)
Endoscopic classification, *n* (%)			169.676	< 0.001	< 0.001	2.604 (2.210-3.067)
I	2480 (64)	35 (26)				
II	1007 (26)	42 (31)				
III	233 (6)	27 (20)				
IV	155 (4)	32 (23)				
Location, *n* (%)			23.926	< 0.001	< 0.001	1.750 (1.384-2.212)
Upper third	1457 (38)	31 (23)				
Middle third	1715 (44)	60 (44)				
Lower third	702 (18)	45 (33)				
Background gastric mucosa, *n* (%)			81.877	< 0.001	< 0.001	1.699 (1.472-1.960)
Autoimmune gastritis	364 (9.4)	29 (21)				
Chronic superficial gastritis	2466 (64)	42 (31)				
Chronic atrophic gastritis	712 (18)	31 (23)				
Gastritis with IN	332 (9)	34 (25)				

OR: Odds ratio; CI: Confidence interval; GHPs: Gastric hyperplastic polyps; IN: Intraepithelial neoplasia; *H. pylori*: *Helicobacter pylori*.

Table 2 Multivariate logistic regression analysis of risk factors for neoplastic transformation of gastric hyperplastic polyps

Risk factors	*P* value	OR	95%CI
Age			
≤ 65 years		1	
> 65 year	0.003	1.789	1.227-2.609
Sex			
Female		1	
Male	0.006	1.680	1.158-2.438
Number			
Single	1		
Multiple ($n \geq 2$)	0.003	1.851	1.230-2.784
Polyp size			
≤ 1 cm		1	
1 cm-2 cm	< 0.001	3.565	2.177-5.838
> 2 cm	< 0.001	3.756	1.909-7.392
Endoscopic color-red	0.701	0.916	0.619-1.356
Mucosal erosion	0.271	0.75	0.454-1.255
Polyp lobulation	0.264	0.73	0.432-1.263
Mucosal roughness	0.128	1.38	0.912-2.093
Shape			
Non-pedunculated		1	
Pedunculated or semi-pedunculated	< 0.001	2.722	1.689-4.388
Location, *n* (%)			
Upper third		1	
Middle third	0.066	0.609	0.368-1.011
Lower third	0.055	1.624	0.968-2.724
Background gastric mucosa			
Chronic superficial gastritis		1	
Chronic atrophic gastritis	0.014	1.876	1.134-3.103
Gastritis with intraepithelial neoplasia	< 0.001	5.299	3.173-8.849
Autoimmune gastritis	< 0.001	2.672	1.559-4.579

OR: Odds ratio; CI: Confidence interval.

shapes, as Yamada types III and IV classifications accounted for 47% and 41% of these polyps, respectively.

GHPs without intraepithelial neoplasia were observed in the upper and middle third of the stomach (38% and 44%, respectively). Polyps with intraepithelial neoplasia were more likely to occur in the middle and lower third of the stomach (44% and 33%, respectively), with significant differences between the groups ($\chi^2 = 23.926$, $P < 0.001$). Additionally, Polyps with high-grade intraepithelial neoplasia were more likely to occur in the middle third of the stomach (65%).

Regarding polyp colour, GHPs without intraepithelial neoplasia were mainly the colour of the surrounding mucosa. The proportion of polyps with redness increased with lesion progression (58% and 76% of polyps in the low-grade and high-grade intraepithelial neoplasia groups, respectively), with significant differences ($\chi^2 = 18.626$, $P < 0.001$). Regarding endoscopic morphology, lesion progression was accompanied by mucosal erosion, increased lobulation, and greater surface roughness, with significant differences between the groups ($P < 0.001$). Further details are presented in Table 1.

Pathological features

We analyzed the pathological results of all polyps. The incidence of polyps with high-grade dysplasia and carcinogenesis was 3.1% (124/4010) and 0.3% (12/4010), respectively. In the analysis of the background gastric mucosa, significant

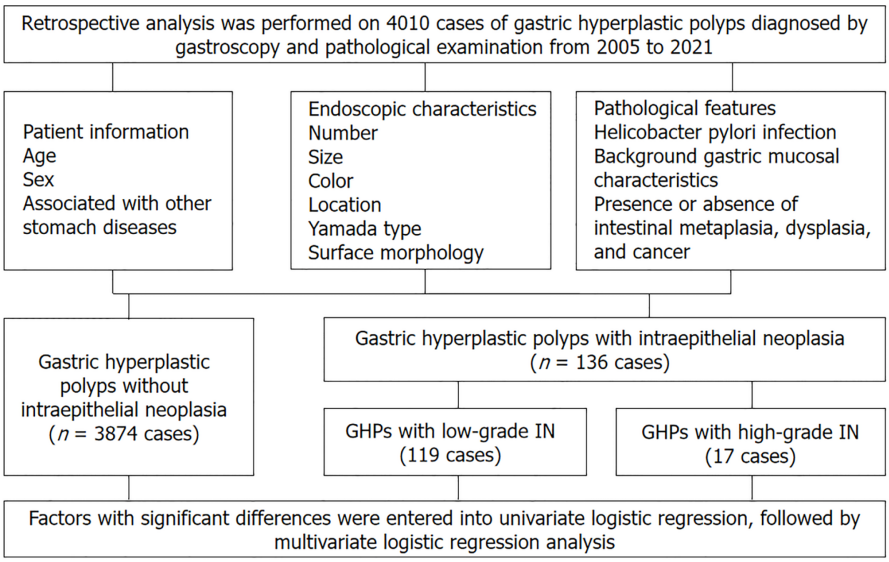

Figure 1 Flow diagram of date collection. GHPs: Gastric hyperplastic polyps; IN: Intraepithelial neoplasia.

differences were observed between the groups. The background gastric mucosa of GHPs mainly demonstrated chronic superficial gastritis, accounting for 64%. However, in the group with intraepithelial neoplasia, autoimmune gastritis, atrophic gastritis, and gastritis with intraepithelial neoplasia of the surrounding gastric mucosa were present in 21%, 23%, and 25%, respectively, with significant differences (χ^2 = 81.877, P < 0.001) (Table 1). Further details are presented in Figure 2.

Univariate and multivariate analyses of the potential risk factors for neoplastic transformation

Univariate and multivariate logistic regression analyses were performed to explore potential associations between risk factors and the presence of neoplastic transformation in GHPs. Low-grade and high-grade intraepithelial neoplasia polyps were combined into one group (GHPs with intraepithelial neoplasia group) and compared with the GHPs without intraepithelial neoplasia group. In the univariate analysis, significant differences were observed in the age and sex of patients, and diameter, endoscopic classification, location, surface morphology (mucosal erosion, lobulation, and surface roughness), and background gastric mucosa of polyps. The differences in these factors between groups were significant (P < 0.05). More specifically, male sex, larger diameter, the presence of multiple polyps, red polyps, rough surface, erosion, and lobulation in the middle third of the stomach, in addition to Yamada type III and IV classifications, with special background gastric mucosa were risk factors for neoplastic transformation. However, no significant difference was observed in terms of *H. pylori* infection (P > 0.05) (Table 1).

Statistically significant risk factors were included in multivariate logistic regression analysis. They were categorised into two groups according to age (< 65 and ≥ 65 years), while polyps with pedunculated or semi-pedunculated shape (corresponding to Yamada type III and IV classifications) were classified into one group and others polyps were categorised into another group (corresponding to Yamada types I and II). The results showed that age > 65 years (OR = 1.789; 95%CI: 1.227-2.609; P = 0.003], male sex (OR = 1.680; 95%CI: 1.158-2.438; P = 0.006), multiple polyps (OR = 1.851; 95%CI: 1.230-2.784; P = 0.003), and pedunculated or semi-pedunculated shape (OR = 2.722; 95%CI: 1.689-4.388; P < 0.001) were significantly associated with GHPs that demonstrated neoplastic transformation. Additionally, polyp diameter was an independent risk factor for harbouring a neoplasm in GHP. Compared with a size of ≤ 1 cm, diameters of 1 cm-2 cm and > 2 cm significantly differed [(OR = 3.565; 95%CI: 2.177-5.838; P < 0.001), (OR = 3.756; 95%CI: 1.909-7.392; P < 0.001), respectively]. Multivariate analysis also showed that specific background gastric mucosa was an independent risk factor for harbouring a neoplasm in GHPs. Compared with chronic superficial gastritis, autoimmune gastritis, atrophic gastritis, and gastritis with intraepithelial neoplasia were significantly different (OR = 2.672; 95%CI: 1.559-4.579; P < 0.001), (OR = 1.876; 95%CI: 1.134-3.103; P = 0.014), and (OR = 5.299; 95%CI: 3.173-8.849; P < 0.001), respectively] (Table 2). Further details are presented in Table 2. We generated a forest map based on independent risk factors, as shown in Figure 3.

GHPs with low-grade and high-grade intraepithelial neoplasia were selected for comparison and univariate regression analysis. The result showed that compared with a size of ≤ 1 cm, a diameter of 1 cm-2 cm significantly differed (OR = 6.956; 95%CI: 1.159-41.729; P < 0.05), and pedunculated or semi-pedunculated shape (OR = 7.375; 95%CI: 1.615-33.671; P < 0.05) were significantly associated with GHPs that demonstrated high-grade intraepithelial neoplasia. No significant difference was found in other univariate regression analyses between GHPs with low-grade and high-grade intraepithelial neoplasia, as shown in Table 3.

Treatment

Regarding treatment, the total and curative resection rates of 17 patients with high-grade intraepithelial neoplasia were 100% each. These rates are considered to be due to the fact that the cancer focus was mostly located in the polyp, the boundary was clear, and the operation was easy. The postoperative complication rate of ESD and EMR was 0, suggesting

Table 3 Analysis of gastric hyperplastic polyps with low-grade and high-grade intraepithelial neoplasia

	GHPs with low-grade IN (*n* = 119)	GHPs with high-grade IN (*n* = 17)	χ^2 test		Univariate analysis	
			χ^2 value	*P* value	*P* value	OR (95%CI)
Age (mean ± SD), years	64.49 ± 1.13	67.93 ± 2.55		0.662[1]	0.404	1.461 (0.600-3.555)
≤ 45 years, *n* (%)	8 (7)	1 (6)				
45-65 years, *n* (%)	49 (41)	5 (30)				
> 65 years, *n* (%)	62 (52)	11 (65)				
Sex (male), *n* (%)	57 (48)	10 (59)	0.710	0.399	0.402	1.544 (0.554-4.355)
H. pylori infection, *n* (%)	20 (17)	2 (12)		0.654[1]	0.655	0.702 (0.148-3.322)
Multiple polyps, *n* (%)	51 (43)	11 (65)	2.035	0.154	0.542	2.133 (0.741-6.145)
Polyp size (mean ± SD), cm	1.25 ± 0.07	2.2 ± 0.32	16.624	0.024	0.017	
≤ 1 cm	63 (52)	3 (18)			-	1
1 cm-2 cm	46 (39)	7 (41)			0.034	6.956 (1.159-41.729)
> 2 cm	10 (8)	7 (41)			0.490	1.766 (0.352-8.865)
Endoscopic color, *n* (%)			3.382	0.184	0.232	2.455 (0.826-7.294)
Normal	50 (42)	4 (24)				
Red	69 (58)	13 (76)				
Mucosal erosion, *n* (%)	23 (20)	7 (41)	4.403	0.051	0.224	2.891 (0.994-8.411)
Polyp lobulation, *n* (%)	19 (16)	6 (35)	3.704	0.054	0.086	2.781 (0.947-8.703)
Mucosal roughness, *n* (%)	58 (49)	11 (65)	1.517	0.218	0.237	1.928 (0.670-5.552)
Endoscopic classification, *n* (%)				0.020[1]	0.331	
I	35 (29)	0 (0)				
II	40 (34)	2 (12)				
III	19 (16)	8 (47)				
IV	25 (21)	7 (41)				
Shape, *n* (%)			8.600	0.003		
Non-pedunculated	75 (63)	2 (12)			-	1
Pedunculated or semi-pedunculated	44 (37)	15 (82)			0.010	7.375 (1.615-33.671)
Location, *n* (%)			4.451	0.103	0.302	0.632 (0.316-1.263)
Upper third	27 (23)	4 (24)				
Middle third	49 (41)	11 (65)				
Lower third	43 (36)	2 (12)				
Background gastric mucosa, *n* (%)				0.553[1]	0.644	1.788 (0.362-8.388)
Autoimmune gastritis	24 (20)	5 (29)				
Chronic superficial gastritis	39 (33)	3 (18)				

Chronic atrophic gastritis	26 (22)	5 (29)
Gastritis with IN	30 (25)	4 (24)

[1]*P* value means using Fisher's exact test.

OR: Odds ratio; CI: Confidence interval; GHPs: Gastric hyperplastic polyps; IN: Intraepithelial neoplasia; *H. pylori*: *Helicobacter pylori*.

that endoscopic treatment was effective.

DISCUSSION

Gastric polyps are a simple type of stomach polyp that usually cause mucosal damage, most commonly in cases of chronic and autoimmune gastritis caused by *H. pylori* infection. They are generally considered benign; however, in a few cases, they may progress to dysplasia (0.2%-10%) and adenocarcinoma (0.6%-3%)[2]. The neoplastic transformation of gastric polyps is diagnosed based on the current Nakamura criteria as follows: (1) Benign and neoplastic lesions coexist in the same polyp; (2) Sufficient evidence indicates that the benign part has the characteristics of benign polyps, and (3) The neoplastic part has obvious cellular and structural atypia[3]. In this study, the tissue carcinogenesis rate of GHPs was 0.3%, and the probability of concurrent dysplasia and intestinal metaplasia occurrence was 3.1% and 5%, respectively, which is broadly consistent with previous findings[4].

Regarding clinical features, the incidence of GHPs increased with age. The mean age of the patients in this study was 58 years, of which 45-65 years were the age groups with the highest incidence (51%). Furthermore, the incidence of GHPs was higher in females (63%). We observed significant differences in the age and sex of patients among the groups. The probability of neoplastic transformation of polyps increased with older age, whereas the proportion of neoplastic transformation was significantly higher in males with polyps.

Regarding the endoscopic features, an increasing number of reports have recently emerged on GHPs combined with neoplastic changes. A polyp size of > 1 cm is considered a risk factor for neoplastic transformation[3]. The erosive morphology differs significantly between hyperplastic polyps with neoplastic transformation and simple hyperplastic polyps ($P < 0.005$)[5]. In our study, large polyps, the presence of multiple polyps, rough surface, lobulation, mucosal erosion, and Yamada type III and IV classifications were considered risk factors for neoplastic transformation, suggesting that polyp morphology should be considered. In several international studies, multivariate analysis revealed a diameter of > 25 mm (OR = 84; 95%CI: 7.4-954), peripheral mucosal findings, accompanied by intestinal metaplasia (OR = 7.6; 95%CI: 1.0-55) and dysplasia (OR = 86; 95%CI: 10-741) to be significantly correlated with the neoplastic transformation of polyps[6].

Furthermore, the relationship between *H. pylori* infection and GHPs remains unclear. A large database study in the United States showed that the rate of *H. pylori* infection in the hyperplastic polyp group was lower than that in the control group[7]. However, considering the factors influencing the background gastric mucosa and the possibility of previous eradication of *H. pylori* infection is essential in the treatment of hyperplastic polyps[8]. The British gastroenterological of society strongly recommends the eradication of *H. pylori* in patients with hyperplastic polyps and endoscopic follow-up after 3-6 months of treatment[9]. *H. pylori* is considered a carcinogen of gastric cancer; however, in our study on the neoplastic transformation of GHPs, *H. pylori* infection was not found to be a significant risk factor ($P > 0.05$) after comparison between the groups. Even when compared with the group without intraepithelial neoplasia, it showed a gradually decreasing trend. Therefore, the carcinogenic mechanism may differ from that of *H. pylori* causing gastric cancer, which is an interesting finding.

GHPs are usually associated with inflammatory lesions of the local gastric mucosal tissue, particularly long-standing *H. pylori* infection-associated gastritis and autoimmune metaplastic atrophic gastritis[4], which are used as markers of an abnormal background gastric mucosa rather than an isolated pre-neoplastic lesion. According to Orlowska *et al*[10], the risk of developing neoplastic tumours in the gastric mucosa outside the polyps is slightly higher than that in the polyps. Markowski *et al*[4] reported a 7.1% chance of neoplastic transformation of the mucosa around the gastric polyp, whereas the polyp was neoplastic with a conversion rate of 2.1%.

In our study, multivariate analysis showed that specific background gastric mucosa was an independent risk factor for harbouring a neoplasm in GHPs. Compared with chronic superficial gastritis, autoimmune gastritis, atrophic gastritis, and gastritis with intraepithelial neoplasia were significantly different [(OR = 2.672; 95%CI: 1.559-4.579; $P < 0.001$), (OR = 1.876; 95%CI: 1.134-3.103; $P = 0.014$), and (OR = 5.299; 95%CI: 3.173-8.849; $P < 0.001$), respectively] (Table 2). In the high-grade intraepithelial neoplasia group, chronic atrophic gastritis with intraepithelial neoplasia accounted for 24% of cases (4/17), of which two were cases of gastric cancer. Autoimmune gastritis in the background gastric mucosa accounted for 29% of cases (5/17) in the high-grade intraepithelial neoplasia group, which is consistent with previous studies showing that patients with autoimmune gastritis are prone to polyp. Although the mechanism remains unclear, some studies suggest that it is related to mucosal atrophy or hypergastrinemia blood syndrome[11]. In Japan, a case of hyperplastic polyp carcinogenesis with submucosal and lymphatic invasion occurring on the basis of gastritis has been reported[12]. Therefore, the association between the background gastric mucosa and hyperplastic polyps should be emphasised in the clinical diagnosis of gastric polyps, and an adequate biopsy of the surrounding mucosa is recommended to evaluate any underlying gastric disease[13].

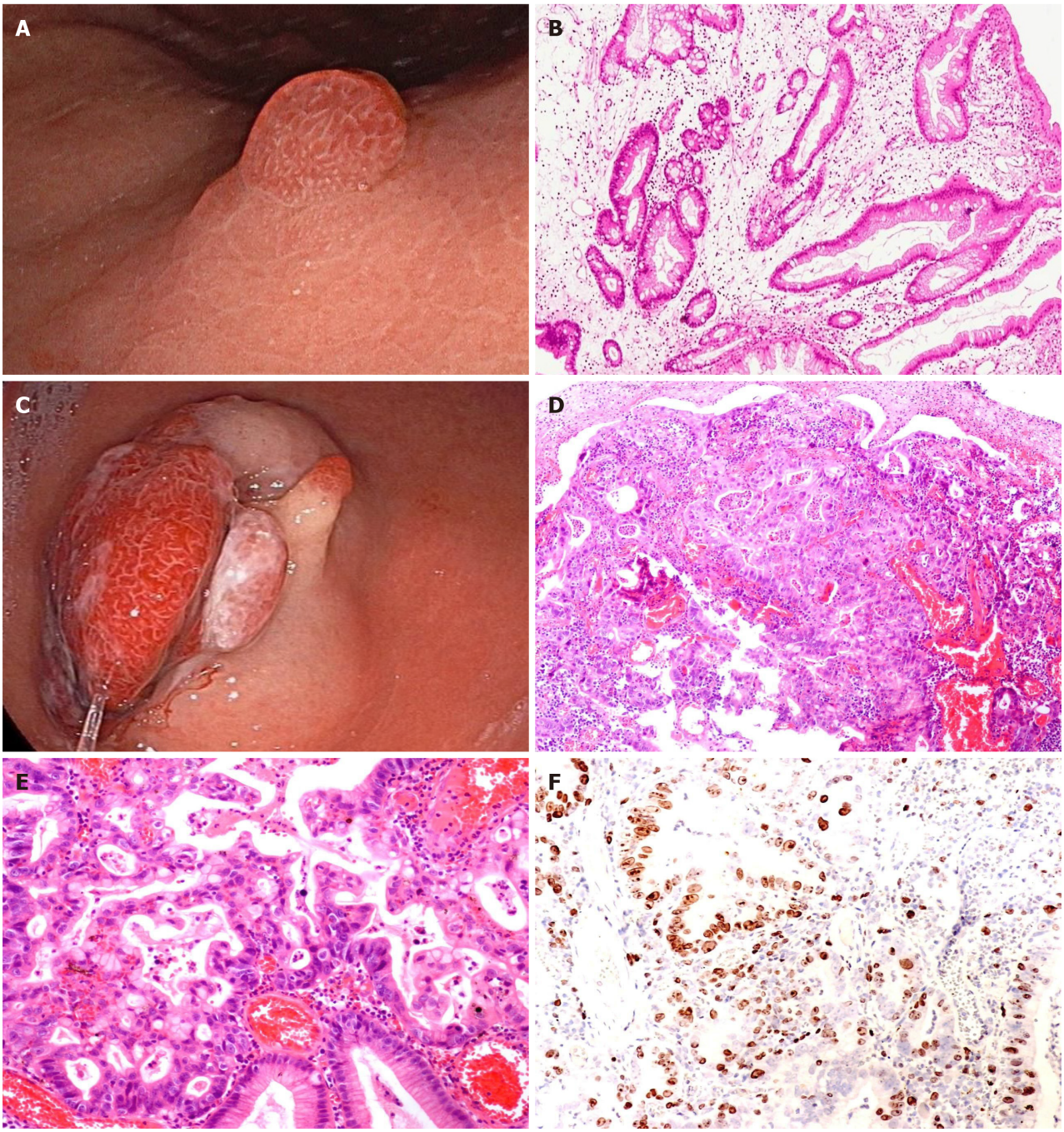

Figure 2 Endoscopic and pathological features of gastric hyperplastic polyps. A: Gastric hyperplastic polyp, smooth surface, same color as surrounding mucosa; B: Pathological manifestation of gastric hyperplastic polyps (Hematoxylin-eosin staining); C: Gastric hyperplastic polyp with carcinomatous transformation, with multiple pedunculated/sub-pedunculated shape, measuring > 3 cm in diameter, rough and red surface; D and E: The pathology of gastric hyperplastic polyp with carcinomatous transformation (Hematoxylin-eosin staining); F: Ki-67 positive of the lesion (immunohistochemical staining).

Studies have shown that cancers associated with GHPs are highly differentiated. Among the 17 patients in our study, except for 5 cases of high-grade intraepithelial neoplasia, the rest were differentiated cancers. Four cases were tubular adenocarcinoma, and one was papillary adenocarcinoma, all of which were differentiated adenocarcinomas. However, the remaining seven cases could not be conclusively diagnosed with a specific pathological type, and no poorly differentiated or undifferentiated cancers were found. These findings align with the results of previous literature and are also comparable to those reported in other studies. Of these cases, immune combination analysis revealed that two cases were caudal type homeobox (CDX)-2 (-) and one was CDX-2 (+). Currently, the exact mechanism underlying the carcinogenesis of hyperplastic polyps remains unclear. Previous studies have suggested that the most simple tissue type of hyperplastic polyps is differentiated adenocarcinoma[14]. A small number of poorly differentiated adenocarcinomas have been reported. Imura *et al*[15] studied six cases of cancerous polyps. Mucin (MUC) 5AC was detected in the normal, dysplastic, and cancerous parts of the polyp, and MUC2 was negative, supporting the diagnosis of adenocarcinoma as the gastric phenotype[15]. Terada[16] found that all cancerous lesions in GHPs were p53 positive with high expression of the Ki-67 marker and that 82% of 51 patients with GHP dysplasia were also p53 positive with dysplastic lesions, exhibiting a higher

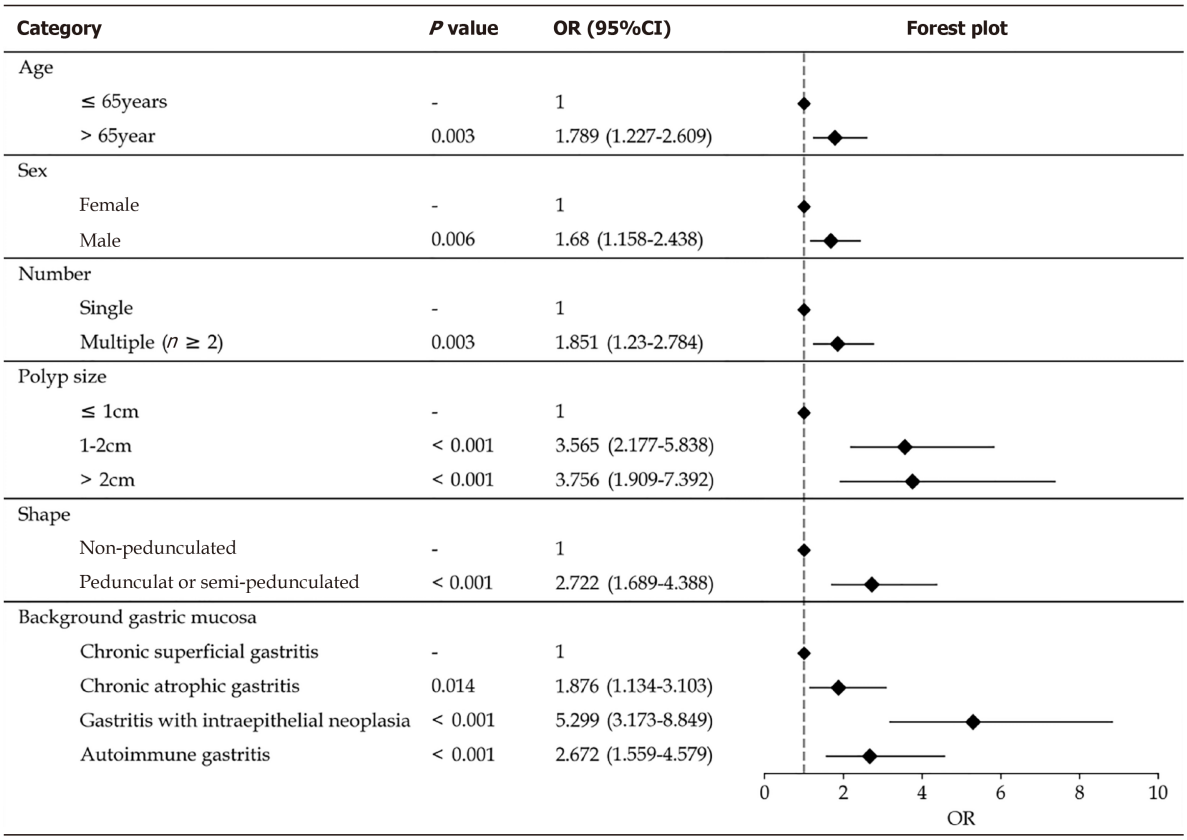

Figure 3 Forest plot of risks for neoplastic transformation of gastric hyperplastic polyps. OR: Odds ratio; CI: Confidence interval.

Ki-67 Labelling index. However, the intestinal metaplasia within GHPs that were p53-negative showed low Ki-67 staining. Their study suggests that intestinal metaplasia is unrelated to the neoplastic transformation of GHPs, contradicting the theory of the GHP enteric-dysplasia-carcinogenic sequence and strongly suggesting the presence of hyperplasia-dysplasia-adenocarcinoma sequences.

This study has some limitations. First, the overall number of carcinogenesis cases in this study was small. Therefore, further expanding the sample size is necessary for more in-depth research to explore the risk factors for neoplastic transformation. Other limitations of the study include its single-center and retrospective design. Additionally, basic experiments such as specific immunohistochemical experiments or analyses of gene expression are needed to further explore the specific mechanisms underlying the carcinogenesis of hyperplastic polyps. However, the overall sample size of this study was large, and we believe that the results will contribute to the clinical treatment of GHPs.

CONCLUSION

GHPs pose a risk of neoplastic transformation; however, the mechanism remains unclear and needs to be further explored. Polyps with large endoscopic diameter (> 1 cm), multiple polyps, pedunculated or semi-pedunculated shape, diameter of > 1 cm, specific background gastric mucosa, age > 65 years, and male sex were independent risk factors. Clinicians should be alert to the possibility of neoplastic transformation to improve the diagnosis rate of the neoplastic transformation of GHPs. The association between the background gastric mucosa and the neoplastic transformation of GHPs should be emphasised in the clinical diagnosis. During endoscopy, the background mucosa should also be carefully observed if necessary. Furthermore, *H. pylori* infection was not found to be a significant risk factor ($P > 0.05$) after comparison between the groups. Even when compared with the group without intraepithelial neoplasia, it showed a gradually decreasing trend, which is an interesting finding.

FOOTNOTES

Author contributions: Ding SG and Zhang J designed the study; Zhang DX and Wu YH collected data; Shi YY provided guidance and assistance in data analysis; Zhang HJ verified pathological data; Niu ZY, Wang Y, and Zu M assisted in reading and clarifying endoscopy reports; Zhang DX organized and analyzed data, wrote the initial draft of the manuscript; Zhang J assisted in manuscript revision; Ding SG critically reviewed the manuscript and approved its final version; All authors read, approved, and agreed to submit the final manuscript for publication.

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ORIGINAL ARTICLE

Basic Study

BIRC3 induces the phosphoinositide 3-kinase-Akt pathway activation to promote trastuzumab resistance in human epidermal growth factor receptor 2-positive gastric cancer

Shu-Liang Li, Pei-Yao Wang, Yang-Pu Jia, Zhao-Xiong Zhang, Hao-Yu He, Peng-Yu Chen, Xin Liu, Bang Liu, Li Lu, Wei-Hua Fu

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Abstract

BACKGROUND

Trastuzumab-targeted therapy is currently the standard of care for advanced human epidermal growth factor receptor 2 (HER2)-positive gastric cancer. However, the emergence of resistance to trastuzumab poses significant challenges.

AIM

To identify the key genes associated with trastuzumab resistance. These results provide a basis for the development of interventions to address drug resistance and improve patient outcomes.

METHODS

High-throughput sequencing and bioinformatics were used to identify the differentially expressed pivotal gene *BIRC3* and delineate its potential function and pathway regulation. Tumor samples were collected from patients with HER2-positive gastric cancer to evaluate the correlation between *BIRC3* expression and trastuzumab resistance. We established gastric cancer cell lines with both highly expressed and suppressed levels of *BIRC3*, followed by comprehensive *in vitro* and *in vivo* experiments to confirm the involvement of *BIRC3* in trastuzumab

resistance and to elucidate its underlying mechanisms.

RESULTS

In patients with HER2-positive gastric cancer, there is a significant correlation between elevated *BIRC3* expression in tumor tissues and higher T stage, tumor node metastasis stage, as well as poor overall survival and progression-free survival. *BIRC3* is highly expressed in trastuzumab-resistant gastric cancer cell lines, where it inhibits tumor cell apoptosis and enhances trastuzumab resistance by promoting the phosphorylation and activation of the phosphoinositide 3-kinase-Akt (PI3K-AKT) pathway in HER2-positive gastric cancer cells, both *in vivo* and *in vitro*.

CONCLUSION

This study revealed a robust association between high *BIRC3* expression and an unfavorable prognosis in patients with HER2-positive gastric cancer. Thus, the high expression of *BIRC3* stimulated PI3K-AKT phosphorylation and activation, stimulating the proliferation of HER2-positive tumor cells and suppressing apoptosis, ultimately leading to trastuzumab resistance.

Key Words: Gastric cancer; Human epidermal growth factor receptor 2; Trastuzumab; Drug-resistance; *BIRC3*

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Core Tip: Our study discovered that the overexpression of *BIRC3* leads to the stimulation of phosphoinositide 3-kinase-Akt phosphorylation and activation. Consequently, this enhances the proliferation of human epidermal growth factor receptor 2-positive tumor cells and inhibits apoptosis, resulting in resistance to trastuzumab.

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INTRODUCTION

Gastric cancer is a highly prevalent and lethal form of cancer worldwide[1,2]. As our understanding of the molecular landscape of gastric cancer continues to improve, new therapeutic targets and drugs have been discovered and developed, leading to improved treatment outcomes. In the context of cancer, human epidermal growth factor receptor 2 (HER2) expression is considered to be significantly associated with cancer development and the escalation of resistance to chemotherapy drugs[3]. Trastuzumab is considered the standard and optimal treatment for patients with HER2-positive metastatic gastric cancer[4].

Trastuzumab inhibits the growth of HER2-positive tumor cells through immune-related mechanisms, such as antibody-dependent or complement-dependent cytotoxicity[5,6]. However, with the continued use of HER2-targeted therapies such as trastuzumab, most patients experience reduced therapeutic sensitivity resulting in tumors more tolerant to HER2-targeted therapies, leading to disease relapse and progression, which significantly affects patient prognosis. Several potential mechanisms of trastuzumab resistance have been proposed. These factors include HER2 heterogeneity, formation of HER2 heterodimers, and alterations in intracellular signaling, among others. Notably, the abnormal activation of the phosphoinositide 3-kinase (PI3K) pathway is recognized as a significant mechanism underlying resistance to HER2-targeted therapy[7].

Multiple studies have identified a new PI3K signaling pathway-related protein: *BIRC3*, also known as baculoviral inhibitor of apoptosis (IAP) repeat containing 3. *BIRC3* encodes cIAP2 (cellular IAP 2) and belongs to the IAP family[8,9]. These apoptosis-inhibitory proteins are highly conserved and inhibit apoptosis by suppressing caspase activity and regulating immune-related signaling pathways. Studies have shown that IAPs are frequently overexpressed in cancer, and that their expression levels are associated with tumorigenesis, chemotherapy resistance, disease progression, and survival differences[10].

Previous studies have demonstrated that high *BIRC3* expression is linked to clinicopathological characteristics and poor prognosis in colorectal cancer, bladder cancer, and glioblastoma[11,12]. Furthermore, *BIRC3* overexpression is associated with chemotherapy resistance in breast cancer and oral squamous cell carcinoma. However, its role in gastric cancer, particularly in relation to resistance to trastuzumab-targeted therapy in HER2-positive gastric cancer, remains unexplored. Hence, our study aimed to investigate the involvement of *BIRC3* in the development of trastuzumab-targeted therapy resistance and to unravel the underlying mechanism with the goal of overcoming trastuzumab resistance in HER2-positive gastric cancer.

MATERIALS AND METHODS

Patients and tissue samples

We examined 28 HER2-positive tumor tissue specimens from patients with gastric cancer who underwent curative gastrectomy in the Department of General Surgery at Tianjin Medical University General Hospital (Tianjin, China) between March 2018 and May 2021. The expression intensity of HER2 was assessed using immunohistochemistry (IHC), employing a four-grade scoring system (ranging from 0 to 3 +) to determine the proportion of stained tumor cells. HER2 overexpression was characterized by an IHC score of 3 +. In instances where HER2 staining was ambiguous (IHC 2 +), Fluorescence in situ hybridization was employed to validate the amplification status of the HER2. None of the enrolled patients underwent neoadjuvant therapy before gastrectomy. Follow-up evaluations were conducted every three months during the initial two years post-radical surgery and every six months thereafter.

High-throughput sequencing and subsequent bioinformatics analysis

Two-terminal sequencing was performed using an Illumina NovaSeq 6000 (LC Bio Technology Co., Ltd. Hangzhou, Zhejiang Province, China) according to standard protocols in the PE150 sequencing mode. Subsequent bioinformatics analyses were performed on the sequencing data, and R language was used to conduct correlation analyses of gene expression within the samples.

Plasmids and RNA oligonucleotide transfection

To identify the "*BIRC3*" gene in the gene bank of the national center for biotechnology information website, we selected the transcript-id "NM_001165.5". This enabled us to obtain the coding sequence. The BamHI and NotI restriction sites were selected for plasmid generation. The expression plasmid pLVX-IRES-puro-*BIRC3* was synthesized by Hongxun Biotechnology Co., Ltd (Suzhou, Jiangsu Province, China). For the siRNA, featuring the sequence hs-*BIRC3*-si: Forward 5'-CAGUUCGUACAUUUCUUUCAUdTdT-3,' design and synthesis was carried out by Hongxun Biotechnology Co., Ltd (Suzhou, Jiangsu Province, China). Verification of the sequencing results ensured alignment with the original sequence sequenced by Hongxun Biotechnology Co., Ltd (Suzhou, Jiangsu Province, China).

Cell culture

In this study, the NCI-N87 cell line, originating from human HER2-positive gastric carcinoma, was used. The cell line was procured from the Institute of General Surgery (Tianjin, China). To induce resistance to trastuzumab, the concentration of trastuzumab was systematically increased from 10 μg/mL to 1000 μg/mL, and viability was examined using the cell counting kit-8 (CCK8) assay. The resultant trastuzumab-resistant cell line was denoted as NCI-N87R and was sustained for approximately half a year. The cells were grown at 37 °C under a 5% carbon dioxide atmosphere in a humid setting. Transfection trials were performed in a the trastuzumab-resistant NCI-N87R cell line using *BIRC3* siRNA and a non-coding siRNA (siRNA-NC). Additionally, the trastuzumab-sensitive NCI-N87 cell line was transfected with a *BIRC3* overexpression plasmid pLVX-IRES-puro-*BIRC3* or the empty plasmid pLVX-IRES-puro-NC. The efficacy of transfection was confirmed through quantitative reverse transcription polymerase chain reaction (RT-qPCR) and western blot analyses, and alterations in the cellular proliferation capability were examined using CCK8 and 5-ethynyl-2'-deoxyuridine (EdU) assays.

CCK8 assay

To evaluate cell viability, a manual lauryl sodium sulfate cell counting kit-8 (CCK8) assay was performed. We plated 2500 cells per well in 96-well plates. Various concentrations of trastuzumab were introduced into the culture medium, and after four days, a CCK8 assay was carried out to quantify cell viability at optical density 450 nm. A total of nine trastuzumab concentration gradients in five replicate wells were established in the medium, ranging from 0 μg/mL to 1000 μg/mL: 0 μg/mL, 1 μg/mL, 10 μg/mL, 20 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 500 μg/mL, and 1000 μg/mL.

RT-qPCR

BIRC3 levels were analyzed using RT-qPCR. The ChamQ universal synergetic binding reagent qPCR master mix was used for RT-qPCR assessments, with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) used as the reference gene. The primers utilized for *BIRC3* in the RT-qPCR analysis were as follows: Forward 5'-TTTCCGTGGCTCTTATTCAAACT-3' and reverse 5'-GCACAGTGGTAGGAACTTCTCAT-3c'; for *GAPDH*, forward 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse 5'-TGGTGAAGACGCCAGTGGA-3'. The RT-qPCR process was repeated three times, and the data were analyzed using GraphPad Prism 9.0 to determine the ^{2}Ct values. Statistical significance was set at $P \leq 0.05$.

IHC staining and analysis

IHC analyses were performed to assess *BIRC3* protein expression in paraffin-embedded sections of tissues obtained from patients with gastric cancer. Additionally, the expression levels of *BIRC3*, phosphorylated AKT (pAKT), and caspase 3 in paraffin sections of tumors formed from different groups of mice were analyzed. The IHC scoring formula was as follows: IHC score = staining intensity score (a) × percentage score of positive cells (b), where (a) represents staining intensity, with a negative score of 0, a weak positive score of 1, a positive score of 2, and a strong positive score of 3, and (b) represents the proportion of positive cells, with a score of 0 for 0% positive cells, a score of 1 for 1%-25% positive cells, a score of 2 for 26%-50% positive cells, a score of 3 for 51%-75% positive cells, and a score of 4 for 76%-100% positive cells.

Low expression was indicated by an IHC score of less than 4 points, whereas high expression was indicated by a score of 4 points or more.

In vivo tumor model

In the dynamic tumor model, the transgenic strain N87-LV-*BIRC3* with stable overexpression was developed by lentiviral transfection of the *BIRC3* gene. Subsequently, the transgenic strain N87R-LV-*BIRC3*-si with a stable knockdown of *BIRC3* was created by lentiviral transfection with *BIRC3* siRNA.

For the *in vivo* tumorigenesis experiment, specific pathogen free (SPF) BALB/c nude mice were divided into five groups. All appropriate measures were taken to minimize pain or discomfort, and comply with ARRIVE guidelines. The group that received NCI-N87 cell inoculation was designated with the N87-NC group. The group that received NCI-N87 cells transfected with the overexpressed LV-GFP-puro-*BIRC3* virus was labeled the N87-LV-*BIRC3* group. The group that received NCI-N87 cells transfected with the overexpressed LV-GFP-puro-*BIRC3* virus and treated with an allosteric AKT inhibitor MK-2206 was denoted as the N87-*BIRC3*-AKTi group. The NCI-N87R group, inoculated with drug-resistant cells, was designated as the N87R group. The group that received NCI-N87R cells transfected with the knockout LV-GFP-puro-*BIRC3*-si virus was named N87R-LV-*BIRC3*-si.

All experimental groups received a trastuzumab injection (200 μg/kg) into the abdominal cavity every two days. Furthermore, the N87-LV-*BIRC3*-AKTi group received 10 mg/kg AKT inhibitor (MK-2206) at the same intervals for seven administrations.

Statistical analysis

Each experiment was replicated at least three times, and the data presented reflect the average outcomes. Statistical analysis was performed using χ^2 test for IHC scores and patient pathological features. the statistical review of the study was performed by a biomedical statistician. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Construction of a trastuzumab-resistant tumor model

We created a trastuzumab-resistant variant HER2-positive gastric cancer cell line NCI-N87, designated NCI-N87R. The morphology of NCI-N87R cells closely mirrored that of its parental cell line, NCI-N87 (Figure 1A). As shown in Figure 1B, NCI-N87R cells exhibited remarkable resistance to trastuzumab when subjected to a concentration of 1000 μg/mL, as determined by a CCK-8 assay. The proliferative activity of NCI-N87R was (105.4 ± 8.240), whereas that of NCI-N87 cells was (64.34 ± 6.371, $P < 0.0001$). Consequently, we successfully generated a trastuzumab-resistant cell line, NCI-N87R, by using a low-concentration stepwise addition method.

High-throughput sequencing and bioinformatics analyses for hub gene screening

Following the successful generation of a trastuzumab-resistant cell line, we conducted high-throughput sequencing and bioinformatics analyses. By comparing the NCI-N87R cells with normal NCI-N87 cells and applying a filter with $|\log_2$ (fold change)$| \geq 2$ and $P \leq 0.05$, we identified a total of 506 up-regulated genes and 501 down-regulated genes. A volcano map depicting these differentially expressed genes is shown in Figure 2A. Subsequently, we performed gene ontology and protein interaction network analyses of these differentially expressed genes, as shown in Figure 2B and C, respectively. We then utilized the maximum neighborhood component centrality (DMNC) and MNC algorithms in Cytoscape 3.9.1 software to identify the top ten differentially expressed genes. The molecular complex detection algorithm was employed to screen genes, resulting in the identification of the two modules with the highest scores (score = 3.33), as illustrated in Figure 2D, Table 1 and Table 2. After consolidating the genes, we visualized the top ten genes selected using different algorithms from Cyto Hubba using a Venn plot (Figure 2E). We identified *BIRC3* as a pivotal gene that may contribute to trastuzumab resistance in patients with HER2-positive gastric cancer.

Expression of BIRC3 in human gastric cancer cell lines and trastuzumab-resistant cell lines

RT-qPCR analysis revealed a significant upregulation in the mRNA levels of *BIRC3* in NCI-N87R cells compared to that in control parental NCI-N87 cells ($P < 0.0001$) (Figure 3A). Western blotting confirmed that the protein levels of *BIRC3* were significantly higher in NCI-N87R cells than in NCI-N87 cells ($P < 0.001$) (Figure 3B). These findings suggest that trastuzumab-resistant gastric cancer cells exhibit increased expression of *BIRC3* compared to trastuzumab-sensitive cells.

BIRC3 facilitates trastuzumab resistance in HER2-positive gastric cancer by promoting tumor cell proliferation

To investigate the role of *BIRC3* in the response of human HER2-positive gastric cancer cells to trastuzumab, we used *BIRC3*-siRNA to downregulate the levels of *BIRC3* in NCI-N87R cells. Cell viability was evaluated using CCK8 assays, revealing significant reductions in cell survival (66.38 ± 6.93 *vs* 98.78 ± 14.54, $P = 0.0047$) ($P < 0.0001$). Notably, the degradation of *BIRC3* markedly decreased cell growth in NCI-N87R cells when exposed to 1000 μg/mL of trastuzumab compared to the control group (Figure 4A). Consequently, the decreased expression of *BIRC3* results in diminished trastuzumab resistance in trastuzumab-resistant gastric cancer cells.

Subsequently, we introduced the *BIRC3* overexpressing plasmid pLVX-IRES-puro-*BIRC3* or control empty plasmid pLVX-IRES-puro-NC into the HER2-positive gastric cancer cell line NCI-N87, designated N87-*BIRC3*-OE and N87-*BIRC3*-NC, respectively. The mRNA and protein levels of *BIRC3* were significantly higher in N87-*BIRC3*-OE cells than in N87-

Table 1 Screening TOP 10 gene by different algorithms of cytohubba

DMNC	MNC
HLA-DRB5	*HLA-DRB1*
PTPN22	*HLA-DRA*
CD74	*MUC6*
CD86	*MUC5B*
HLA-A	*HLA-DRB5*
HLA-DRB1	*PER1*
HLA-DRA	*CSNK1E*
PER1	*BIRC3*
CSNK1E	*NOD2*
BIRC3	*PTPN22*

DMNC: Density of maximum neighborhood component; MNC: Maximum neighborhood component.

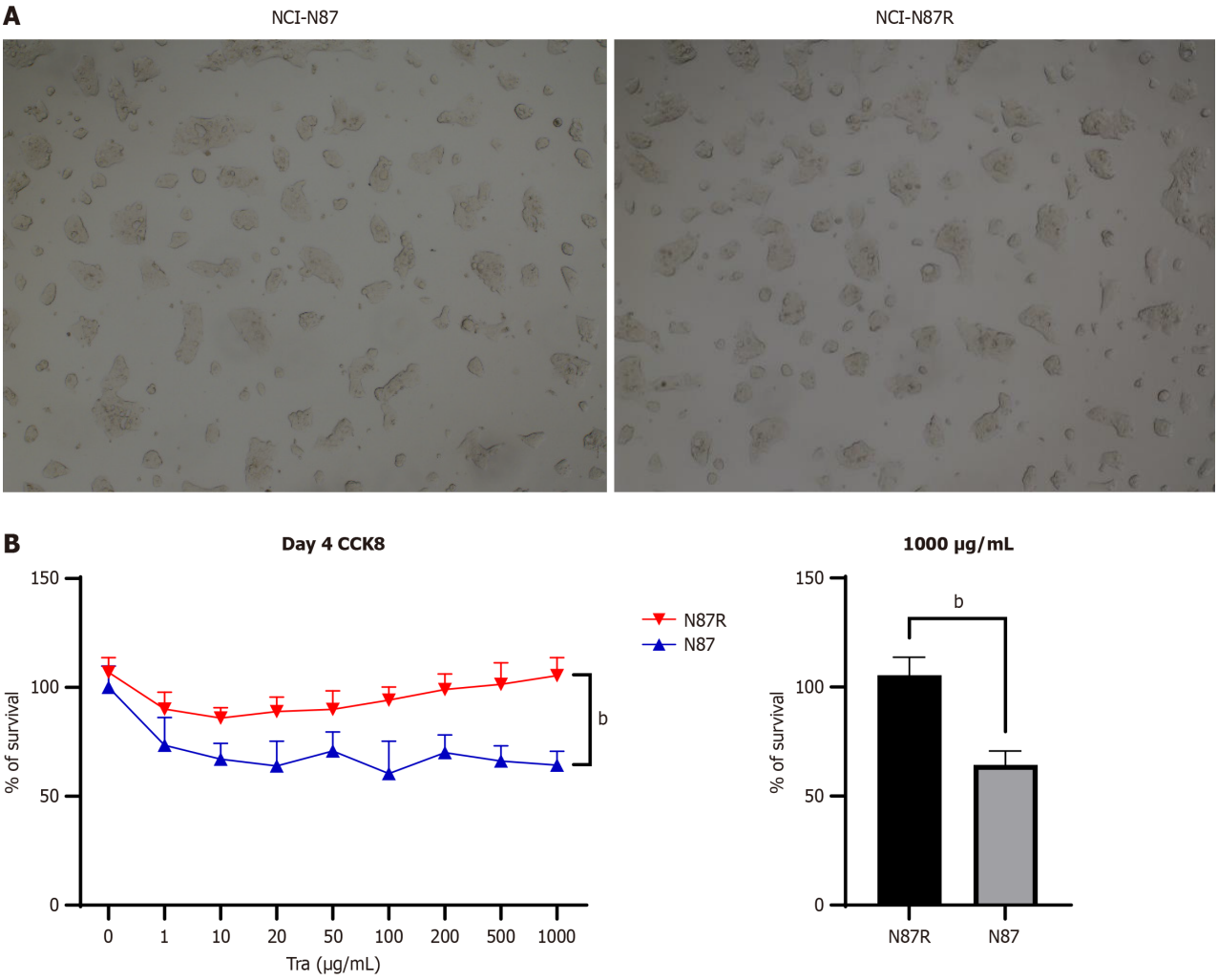
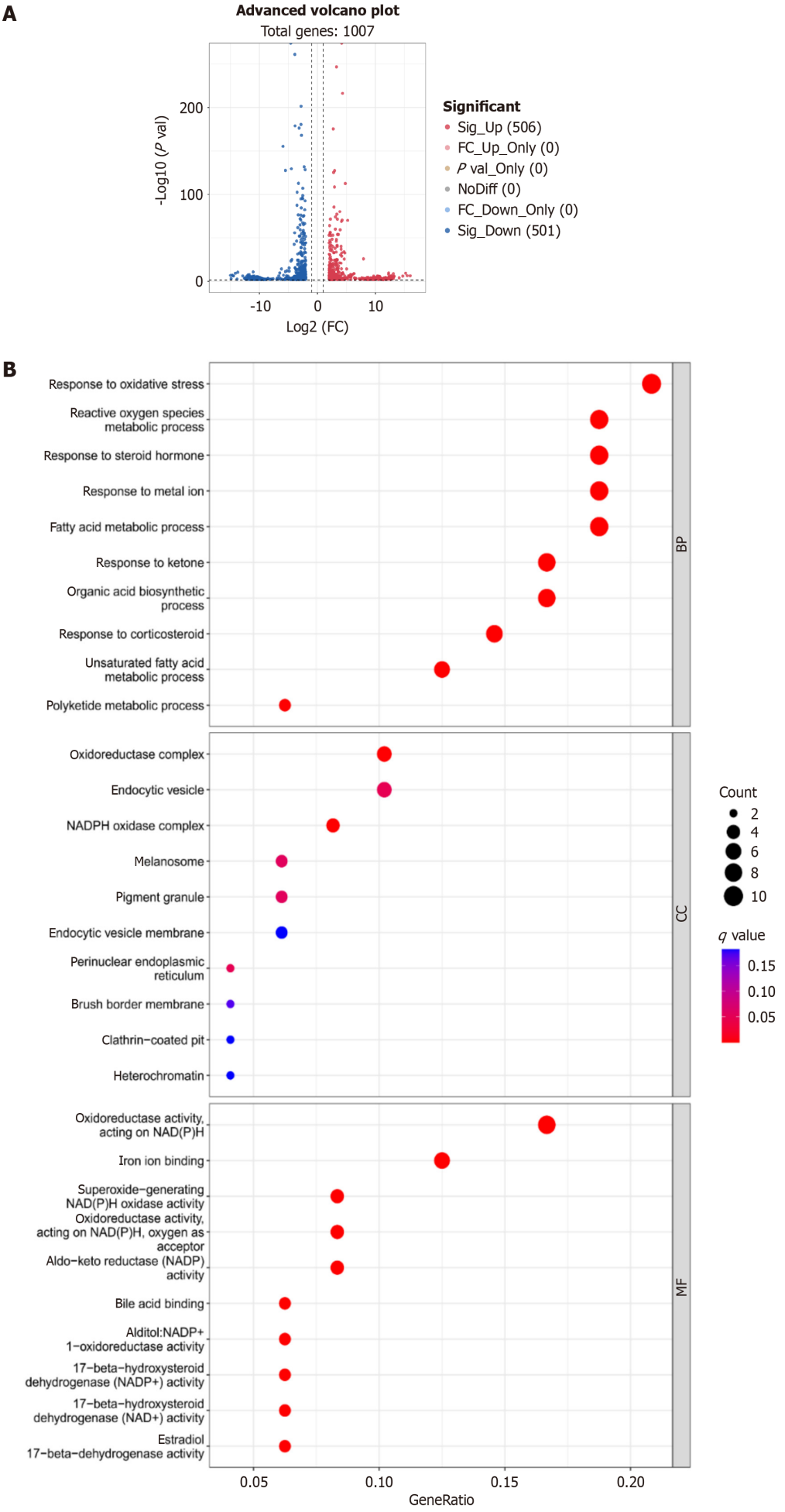


Figure 1 Construction of trastuzul tumor resistance model. A: The morphology of the resistant strain NCI-N87R is similar to that of the parent cell line NCI-N87; B: Cells were treated with 1000 μg/mL trastuzumab for 4 days, and cell viabilities were detected by cell counting kit-8 assay. $^{b}P < 0.0001$. CCK8: Cell counting kit-8; Tra: Trastuzumab.


A
Advanced volcano plot
Total genes: 1007
-Log10 (P val)
200
100
0
-10
0
10
Log2 (FC)
Significant
Sig_Up (506)
FC_Up_Only (0)
P val_Only (0)
NoDiff (0)
FC_Down_Only (0)
Sig_Down (501)
B
Response to oxidative stress
Reactive oxygen species metabolic process
Response to steroid hormone
Response to metal ion
Fatty acid metabolic process
Response to ketone
Organic acid biosynthetic process
Response to corticosteroid
Unsaturated fatty acid metabolic process
Polyketide metabolic process
BP
Oxidoreductase complex
Endocytic vesicle
NADPH oxidase complex
Melanosome
Pigment granule
Endocytic vesicle membrane
Perinuclear endoplasmic reticulum
Brush border membrane
Clathrin-coated pit
Heterochromatin
CC
Oxidoreductase activity, acting on NAD(P)H
Iron ion binding
Superoxide-generating NAD(P)H oxidase activity
Oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor
Aldo-keto reductase (NADP) activity
Bile acid binding
Alditol:NADP+ 1-oxidoreductase activity
17-beta-hydroxysteroid dehydrogenase (NADP+) activity
17-beta-hydroxysteroid dehydrogenase (NAD+) activity
Estradiol 17-beta-dehydrogenase activity
MF
0.05
0.10
0.15
0.20
GeneRatio
Count
2
4
6
8
10
q value
0.15
0.10
0.05

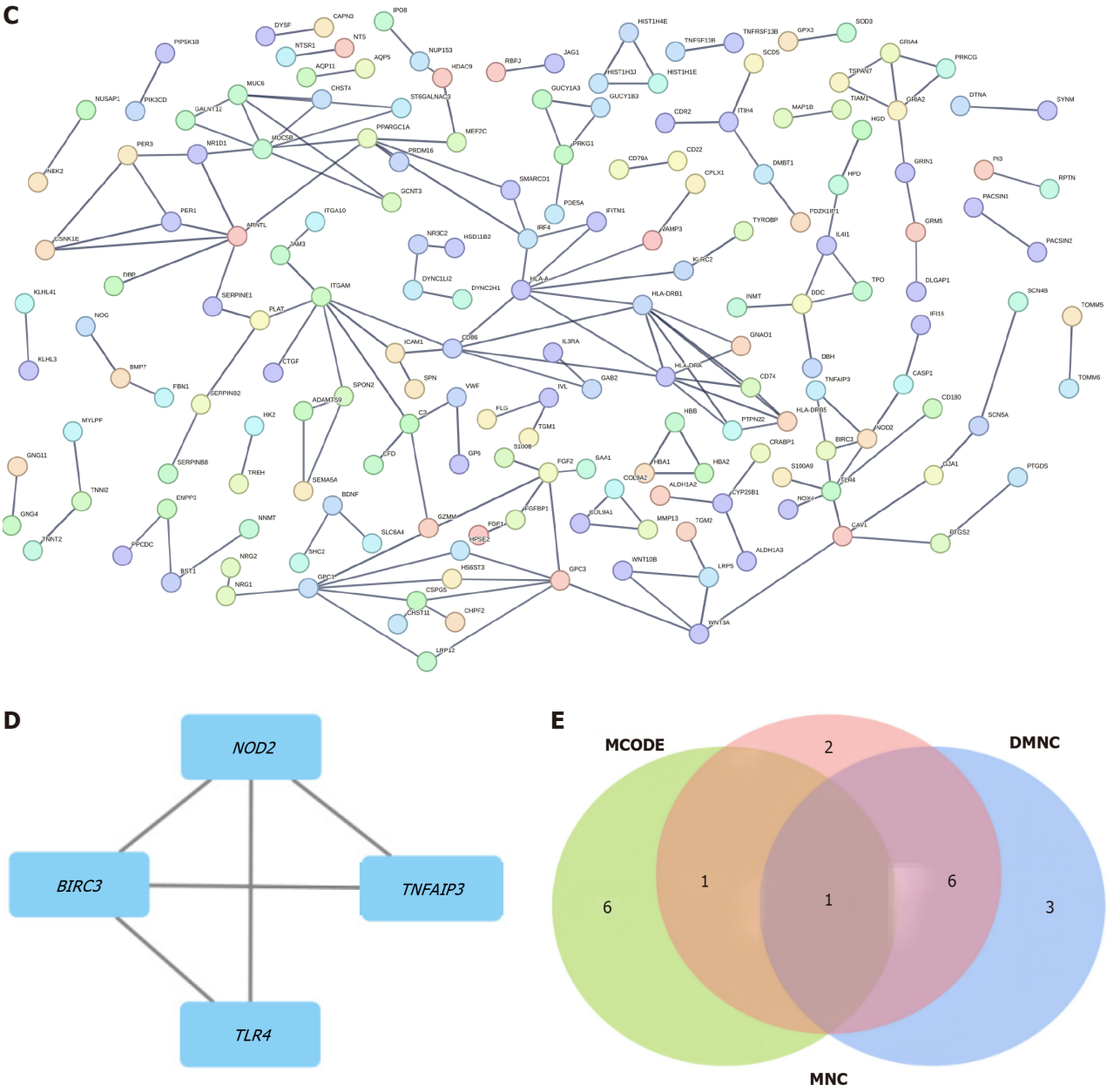


Figure 2 High throughput sequencing and bioinformatics analysis screening Hub genes. A: The volcano plot was generated from mRNA sequencing analysis of NCI-N87 and NCI-N87R; B: Gene pathway analysis was performed; C: Protein interaction network analysis for *BIRC3* was performed using the STRING online tools; D: the top differentially expressed genes were identified by the maximum neighborhood component centrality and neighborhood component centrality algorithms; E: Venn diagram was presented to show the top 10 genes selected using different algorithms, based on the CytoHubba online tools. FC: Fold change; DMNC: Density of maximum neighborhood component; MNC: Maximum neighborhood component.

BIRC3-NC cells, as shown in Figure 4B and C. Upon exposure to trastuzumab, both cell types exhibited decreased cell viability. However, the decline in cell viability was less pronounced in N87-*BIRC3*-OE cells compared to N87-*BIRC3*-NC cells, as demonstrated by the CCK8 assay (104.2 ± 14.59 *vs* 72.06 ± 6.859, $P = 0.0021$) and EdU assay ($P < 0.0001$) (Figure 4D and E). Therefore, the overexpression of *BIRC3* promoted trastuzumab resistance in the HER2-positive gastric cancer cell line NCI-N87.

BIRC3 is correlated with trastuzumab resistance and poor prognosis in patients with HER2-positive gastric cancer

To further investigate this correlation, we procured 28 paraffin-embedded tissue samples from individuals diagnosed with HER2-positive gastric cancer for IHC analysis of *BIRC3* protein levels. Notably, *BIRC3* was predominantly localized to the cytoplasm, characterized by discernible brown particles (Figure 5A). Subsequently, these gastric cancer specimens were stratified into two distinct cohorts based on their *BIRC3* expression profiles: The *BIRC3* high and *BIRC3* Low groups. To further elucidate the relationship between *BIRC3* expression and prognosis among patients with HER2-positive gastric cancer, sophisticated analytical tools, such as the gene expression omnibus and Kaplan-Meier plotter, were employed. The results are presented in Table 3 and underscore a significant association, revealing that elevated *BIRC3* levels were statistically associated with advanced T stage ($P = 0.030$), elevated tumor node metastasis stage ($P = 0.016$), increased risk

Table 2 Genes screened by molecular complex detection

Cluster	Score	Nodes	Edges	Node IDs
1	3.333	4	5	*GRIA2, GRIA4, TSPAN7, PRKCG*
2	3.333	4	5	*NOD2, TNFAIP3, BIRC3, TLR4*
3	3	3	3	*WNT10B, LRP5, WNT3A*
4	3	3	3	*HIST1H1E, HIST1H4E, HIST1H3J*
5	3	3	3	*HBB, HBA2, HBA1*
6	3	3	3	*GUCY1B3, GUCY1A3, PRKG1*
7	3	3	3	*HLA-DRA, GNAO1, HLA-DRB1*
8	3	3	3	*TPO, DDC, IL4I1*
9	3	3	3	*PER3, CSNK1E, PER1*
10	3	3	3	*MMP13, COL9A1, COL9A2*
11	3	3	3	*SPON2, ADAMTS9, SEMA5A*

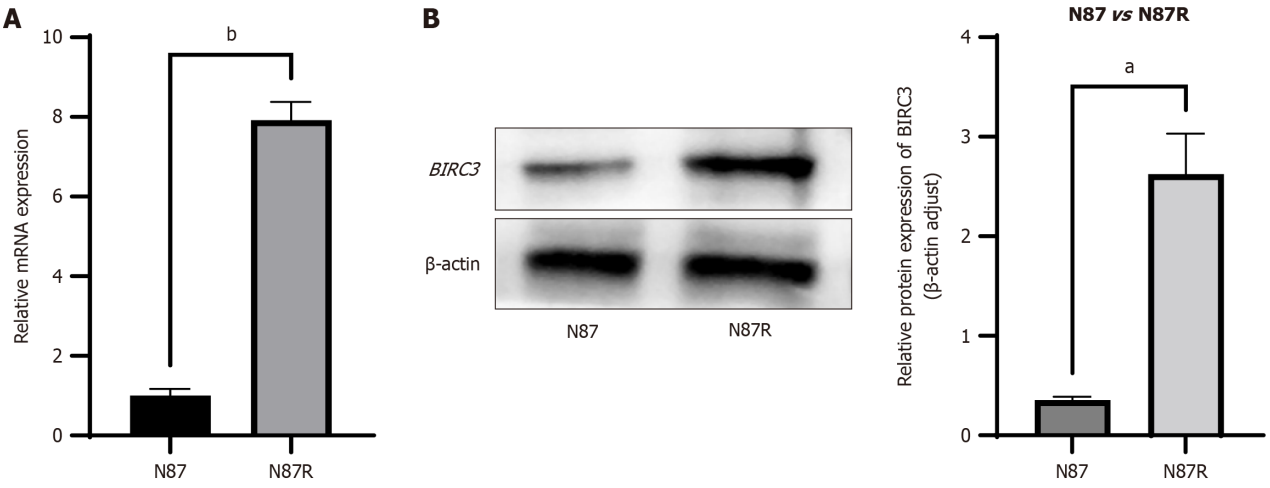


Figure 3 Expression of *BIRC3* in human gastric cancer cell lines and trastuzul resistant cell lines. A: mRNA levels of *BIRC3* in NCI-N87, and NCI-N87R cells were examined by reverse transcription polymerase chain reaction; *GAPDH* was used as a control; B: Protein levels of *BIRC3* in NCI-N87, and NCI-N87R cells were examined by western blotting, β-actin was a negative control. $^{a}P < 0.001$; $^{b}P < 0.0001$.

of overall survival ($P = 0.038$), and decreased disease-free survival rate ($P = 0.046$) within the cohort of 28 HER2-positive gastric cancer patients (Figure 5B). To verify the robustness of our findings, a parallel analysis was conducted on 32 HER2-negative patients, yielding results that were not statistically different (Table 4).

Within the scope of this study, 20 of 28 patients received a treatment regimen that combined trastuzumab with chemotherapy (Figure 5C). Among these, 10 of 20 patients received capecitabine and oxaliplatin chemotherapy, 5 of 20 received tegafur gimeracil oteracil potassium capsule and oxaliplatin chemotherapy, and 5 of 20 received treatment with Tegio or capecitabine chemotherapy. Following trastuzumab therapy, four patients displayed disease progression within a 3-month timeframe, while the remaining 13 patients exhibited sensitivity to trastuzumab during the same period. Additionally, at the time of analysis, three patients had not yet completed the full 3-month treatment protocol. IHC results indicated markedly elevated expression levels of *BIRC3* in the trastuzumab-resistant group compared to the trastuzumab-sensitive group.

These findings underscore the correlation between *BIRC3* expression in tumor cells, resistance to trastuzumab treatment, and adverse prognostic implications in patients diagnosed with gastric cancer.

Tumor cell-derived BIRC3 enhances the proliferation of HER2-positive gastric cancer cells treated with trastuzumab by activating AKT pathway

In this study, we explored the mechanism underlying *BIRC3* action in HER2-positive gastric cancer cells. Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis revealed that the differentially expressed genes were predominantly enriched in the PI3K-AKT and Ras pathways (Figure 6A). Existing literature has documented that aberrant activation of the PI3K-AKT pathway in HER2-positive tumor cells can trigger resistance to targeted trastuzumab therapy. Moreover, it has been observed that anomalous expression of *BIRC3* can stimulate the activation of the AKT

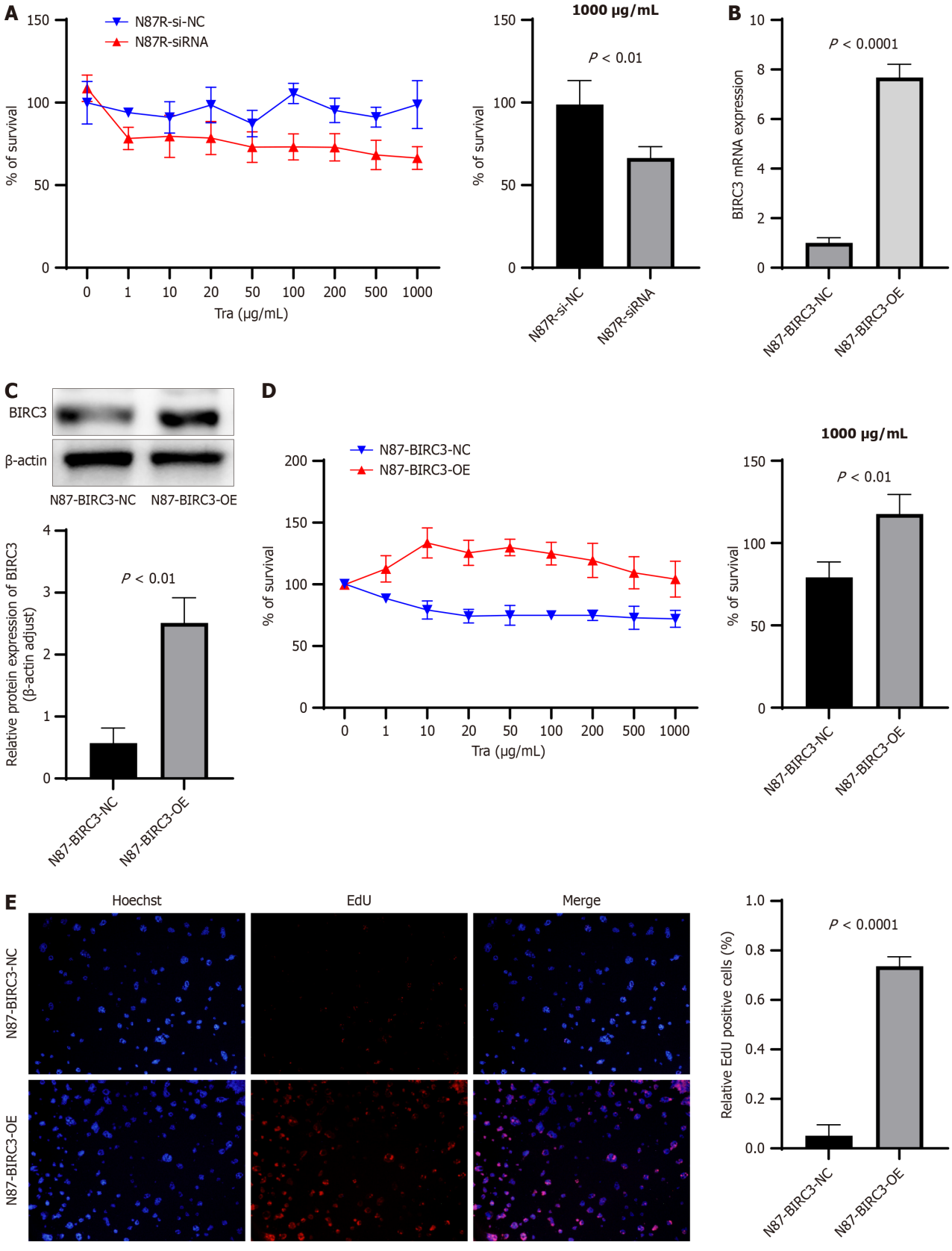


Figure 4 *BIRC3* promotes the resistance of human epidermal growth factor receptor 2-position gastric cancer to trastuzumab by promote the proliferation of tumor cell. A: *BIRC3* was depleted using *BIRC3*-siRNA in NCI-N87R cells. Cell viabilities of N87R-siRNA cells and N87R-siNC 1000 μg/mL trastuzumab were examined by cell counting kit-8 assay; B: mRNA levels of *BIRC3* in N87 cells after transfection of *BIRC3* overexpressing plasmids or control empty plasmids (designated as N87-*BIRC3*-OE and N87-*BIRC3*-NC) were examined by reverse transcription polymerase chain reaction; C: Protein levels of *BIRC3* in N87-*BIRC3*-OE and N87-*BIRC3*-NC were examined by western blotting. β-actin was a negative control; D: Cell viabilities of N87-*BIRC3*-OE and N87-*BIRC3*-NC cells 1000 μg/mL trastuzumab were examined by cell counting kit-8 assay; E: Cell viabilities of N87-*BIRC3*-OE and N87-*BIRC3*-NC cells 1000 μg/mL trastuzumab were examined by 5-ethynyl-2'-deoxyuridine assay. Tra: Trastuzumab; EdU: 5-ethynyl-2'-deoxyuridine.

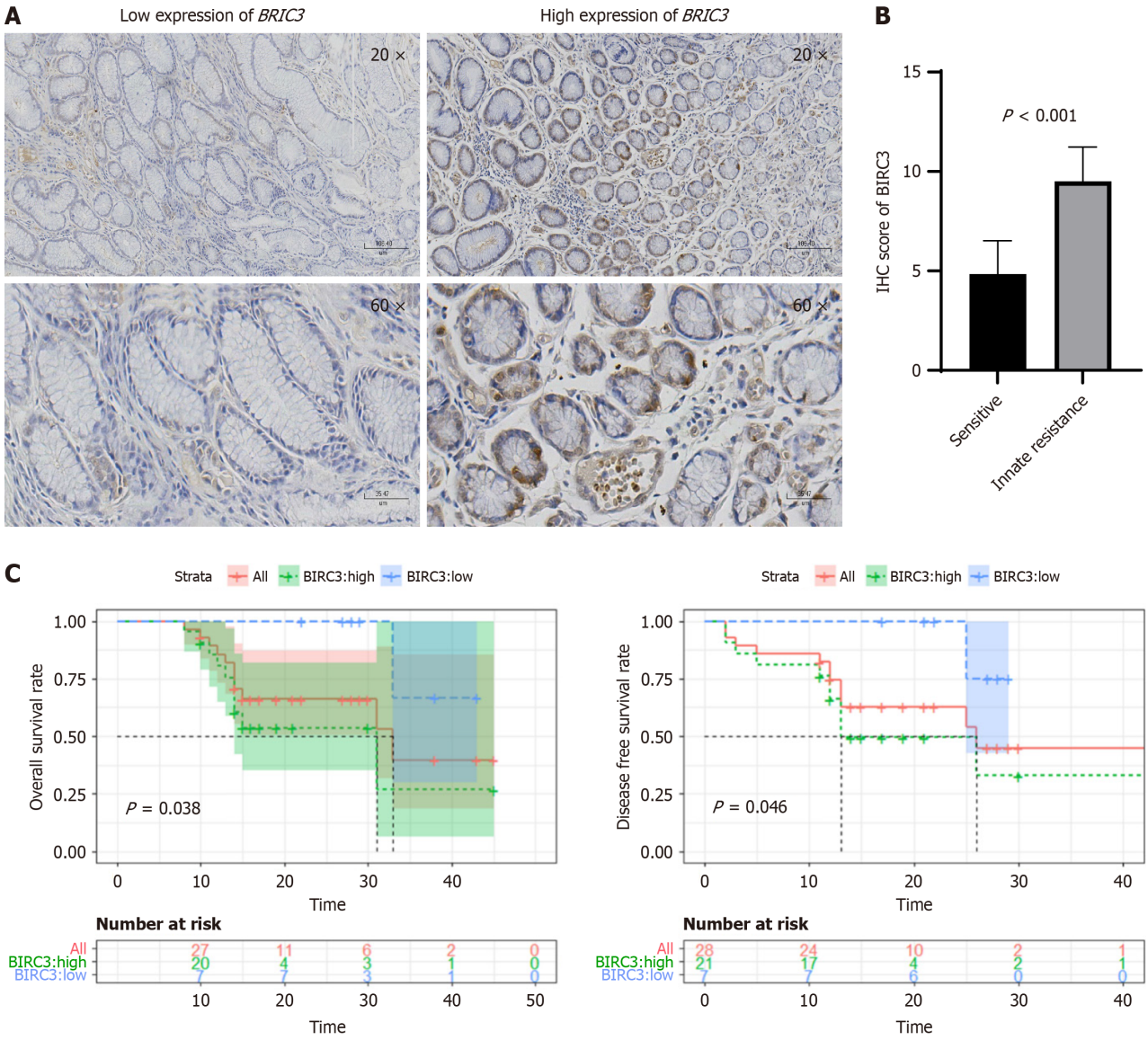


Figure 5 *BIRC3* is correlated with trastuzumab resistance and poor prognosis and the in human epidermal growth factor receptor 2-positive gastric cancer patients. A: Immunohistochemistry (IHC) staining for *BIRC3* in gastric cancer (GC) tissues were shown; B: 28 human epidermal growth factor receptor 2 (HER2)-positive patients were stratified into negative and positive groups according to IHC staining for *BIRC3*; C: The IHC results demonstrated significantly higher expression levels of *BIRC3* in the trastuzumab resistant group compared to the sensitive group. Kaplan-Meier analysis of overall survival in HER2-positive GC patients was performed based on *BIRC3* levels. IHC: Immunohistochemistry.

signaling pathway.

BIRC3 siRNA or siRNA-NC was transfected into NCI-N87R cells, resulting in two distinct cohorts: N87-siRNA and N87-si-NC. We conducted qPCR to assess the expression of *BIRC3*. As illustrated in Figure 6B, the N87-siRNA group displayed a notable decrease in *BIRC3* protein expression compared to the N87-si-NC group (0.6134 ± 0.06462 *vs* 0.9811 ± 0.03973, $P = 0.0011$). Furthermore, western blotting indicated that the downregulation of *BIRC3* significantly diminished the expression of AKT and pAKT in NCI-N87R cells (0.4776 ± 0.01184 *vs* 1.282 ± 0.1165, $P = 0.0003$). Additionally, Figure 6B shows that the expression levels of *BIRC3* (1.037 ± 0.1829 *vs* 0.4307 ± 0.09833, $P = 0.0072$), AKT, and pAKT proteins (0.9639 ± 0.04096 *vs* 0.3095 ± 0.01921, $P < 0.0001$) were markedly elevated in N87-*BIRC3*-OE cells compared to those in N87-*BIRC3*-NC cells.

To investigate the activation of the AKT pathway by *BIRC3* and its role in trastuzumab resistance, we conducted an AKT pathway blockade experiment. NCI-N87 cells were pretreated with MK-2206, an AKT inhibitor, and transfected with the overexpression plasmid pLVX-IRES-puro-*BIRC3*, to generate N87-AKTi-OE cells. As illustrated in Figure 6C and D, the CCK8 and EdU detection assays demonstrated a significantly higher cell proliferation activity in the N87-*BIRC3*-OE group compared to the N87-NC and N87-AKTi-OE groups, with statistically significant differences ($P < 0.001$). However, there was no discernible difference in cell proliferation between the N87-NC and N87-AKTi-OE groups. These results indicated that transfection with *BIRC3* significantly enhanced the proliferative capacity of N87-NC cells, which was attenuated by AKT inhibitors ($P < 0.001$). Furthermore, western blot analysis revealed a substantial increase in AKT phosphorylation in the sensitive cell line NCI-N87 upon *BIRC3* overexpression, whereas AKT inhibitors reduced AKT phosphorylation (Figure 6E). This suggested that *BIRC3* augmented the proliferative ability of NCI-N87 cells by

Table 3 Relationship between *BIRC3* and clinical pathology in human epidermal growth factor receptor 2 (+) group, *n* (%)

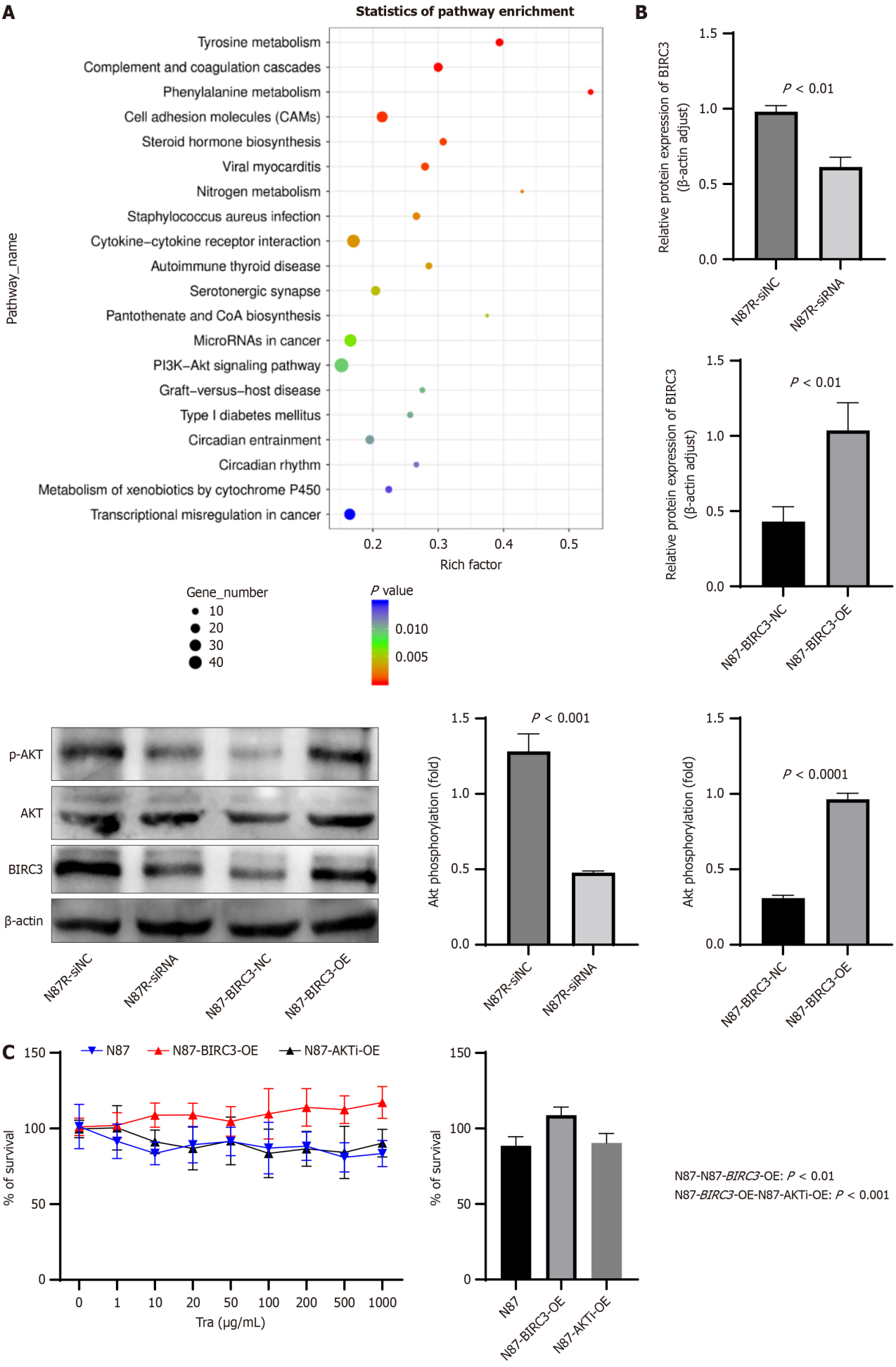
	BIRC^{low} group (*n* = 7)	*BIRC*^{high} group (*n* = 21)	*P* value
Sex (male)	6 (85.7)	19 (90.5)	1.000
Age (year)	63.71 ± 8.45	63.95 ± 8.52	0.949[2]
BMI (kg/m^2)	22.94 ± 2.81	24.56 ± 3.40	0.270[2]
Tumor size (cm)	3.64 ± 2.34	4.90 ± 1.78	0.146[2]
Differentiation			0.429[1]
Poor	3 (42.9)	14 (66.7)	
Moderate	4 (50.0)	6 (28.6)	
High	0 (0.0)	1 (4.8)	
T stage			0.030
1	1 (14.3)	0 (0)	
2	2 (28.6)	2 (9.5)	
3	4 (57.1)	2 (9.5)	
4	0 (0.0)	17 (81.0)	
N stage			0.245
0	3 (42.9)	5 (23.8)	
1	2 (28.6)	9 (42.9)	
2	2 (28.6)	3 (14.3)	
3	0 (0.0)	4 (19.0)	
M stage			-
0	7 (100.0)	19 (90.5)	
1	0 (0.0)	2 (9.5)	
TNM stage			0.016
I	1 (4.8)	0 (0.0)	
II	2 (9.5)	0 (0.0)	
III	4 (19)	19 (90.5)	
IV	0 (0.0)	2 (9.5)	
Survival			0.571[1]
Live	6 (85.7)	11 (52.4)	
Dead	1 (14.3)	10 (47.6)	
OS (month)			
mean ± SD	31.43 ± 7.138	17.0 ± 8.63	0.001[2]
Median (range)	29 (22-43)	14 (8-45)	-
Recurrence			0.571[1]
No	6 (85.7)	10 (47.6)	
Recurrence	1 (14.3)	11 (52.4)	
DFS (month)			
mean ± SD	24.14 ± 4.34	14.76 ± 9.89	0.002[2]
Median (range)	25 (17-29)	13 (2-45)	-

[1]*P* calculated by fisher test.
[2]*P* calculated by *t* text.
BMI: Body mass index; TNM: Tumor node metastasis; OS: Overall survival; DFS: Disease-free survival.

Table 4 Relationship between *BIRC3* and clinical pathology in human epidermal growth factor receptor 2 (-) group, *n* (%)

	BIRC^{low} group (*n* = 23)	BIRC^{high} group (*n* = 37)	*P* value
Sex (male)	19 (82.6)	24 (81.1)	0.270[1]
Age (year)	65.43 ± 10.77	64.97 ± 7.58	0.110[2]
BMI (kg/m^2)	23.46 ± 3.43	24.84 ± 3.77	0.892[2]
HER2			0.047
HER2 (-)	16 (69.6)	16 (43.2)	
HER2 (+)	7 (30.4)	21 (56.8)	
Tumor size (cm)	3.43 ± 2.02	4.59 ± 1.84	0.500[2]
Differentiation			0.489
Poor	9 (39.1)	24 (64.9)	
Moderate	14 (60.9)	10 (27.0)	
High	0 (0.0)	3 (8.1)	
T stage			0.001
1	5 (13.5)	1 (2.7)	
2	7 (18.9)	6 (16.2)	
3	10 (27.0)	11 (29.7)	
4	1 (2.7)	19 (51.4)	
N stage			0.208
0	11 (29.7)	8 (21.6)	
1	10 (27.0)	20 (54.1)	
2	2 (5.4)	5 (13.5)	
3	0 (0.0)	4 (10.8)	
M stage			0.69[1]
0	23 (100.0)	35 (94.6)	
1	0 (0.0)	2 (5.4)	
TNM stage			0.007
I	4 (10.8)	1 (2.7)	
II	5 (13.5)	1 (2.7)	
III	12 (32.4)	24 (64.9)	
IV	2 (5.4)	11 (29.7)	
OS (month)			
mean ± SD	32.2 ± 16.33	21.81 ± 15.17	0.015[2]
Median (range)	33 (3-53)	15 (5-53)	-
DFS (month)			
mean ± SD	26.74 ± 14.80	18.57 ± 14.21	0.040[2]
Median (range)	26 (3-52)	15 (2-53)	-

[1]*P* calculated by fisher test.
[2]*P* calculated by *t* text.
BMI: Body mass index; TNM: Tumor node metastasis; OS: Overall survival; DFS: Disease-free survival.


A
Statistics of pathway enrichment
Tyrosine metabolism
Complement and coagulation cascades
Phenylalanine metabolism
Cell adhesion molecules (CAMs)
Steroid hormone biosynthesis
Viral myocarditis
Nitrogen metabolism
Staphylococcus aureus infection
Cytokine–cytokine receptor interaction
Autoimmune thyroid disease
Serotonergic synapse
Pantothenate and CoA biosynthesis
MicroRNAs in cancer
PI3K–Akt signaling pathway
Graft–versus–host disease
Type I diabetes mellitus
Circadian entrainment
Circadian rhythm
Metabolism of xenobiotics by cytochrome P450
Transcriptional misregulation in cancer
Pathway_name
0.2
0.3
0.4
0.5
Rich factor
Gene_number
10
20
30
40
P value
0.010
0.005
B
Relative protein expression of BIRC3 (β-actin adjust)
P < 0.01
N87R-siNC
N87R-siRNA
Relative protein expression of BIRC3 (β-actin adjust)
P < 0.01
N87-BIRC3-NC
N87-BIRC3-OE
p-AKT
AKT
BIRC3
β-actin
N87R-siNC
N87R-siRNA
N87-BIRC3-NC
N87-BIRC3-OE
Akt phosphorylation (fold)
P < 0.001
N87R-siNC
N87R-siRNA
Akt phosphorylation (fold)
P < 0.0001
N87-BIRC3-NC
N87-BIRC3-OE
C
N87
N87-BIRC3-OE
N87-AKTi-OE
% of survival
Tra (μg/mL)
0
1
10
20
50
100
200
500
1000
% of survival
N87
N87-BIRC3-OE
N87-AKTi-OE
N87-N87-*BIRC3*-OE: P < 0.01
N87-*BIRC3*-OE-N87-AKTi-OE: P < 0.001

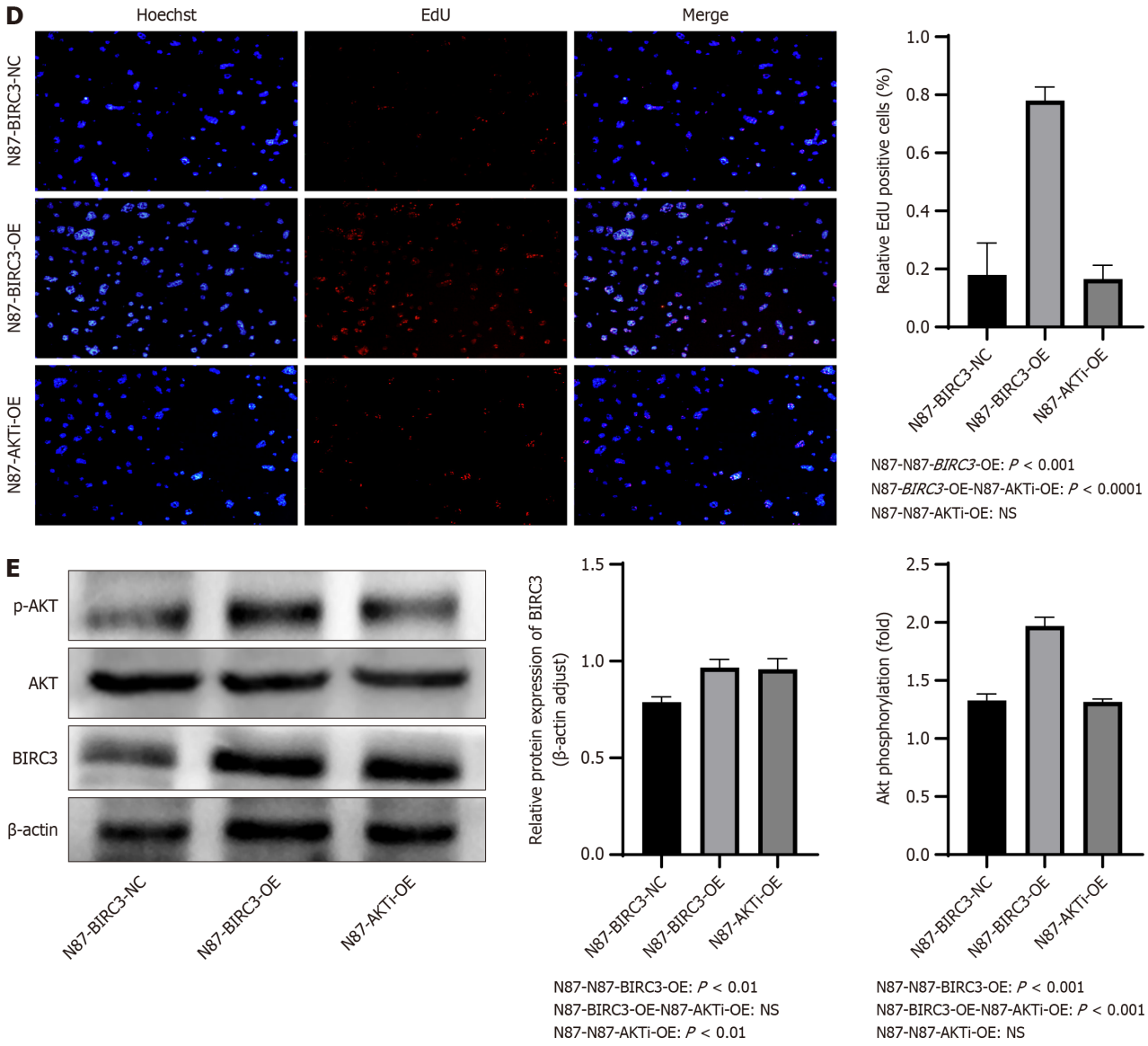


Figure 6 Tumor cell-derived *BIRC3* enhancing the proliferation of human epidermal growth factor receptor 2-positive gastric cancer cell treated with trastuzumab activating AKT pathway. *BIRC3* siRNA or siRNA-NC was transfected into NCI-N87R cells (designated as N87R-siRNA and N87R-siNC). NCI-N87 cells were pretreated with an Akt inhibitor (MK-2206) and transfected with the overexpression plasmid pLVX-IRES-puro-*BIRC3* (designated as N87-AKTi-OE). A: Revealed that the differential genes were mainly enriched in pathways by the Kyoto encyclopedia of genes and genomes enrichment analysis; B: Protein levels of *BIRC3* and AKT, p-AKT were examined by western blotting. β-actin was a negative control; C: Cell viabilities of N87, N87-*BIRC3*-OE and N87-*BIRC3* -NC cells 1000μg/mL trastuzumab were examined by cell counting kit-8 assay; D: Cell viabilities of N87, N87-*BIRC3*-OE and N87-*BIRC3*-NC cells 1000μg/mL trastuzumab were examined by 5-ethynyl-2'-deoxyuridine assay; E: Protein levels of *BIRC3* and AKT, p-AKT in N87,N87-*BIRC3*-OE, and N87-AKTi-OE cells were examined by western blotting. β-actin was a negative control. Tra: Trastuzumab; EdU: 5-ethynyl-2'-deoxyuridine; NS: No significance.

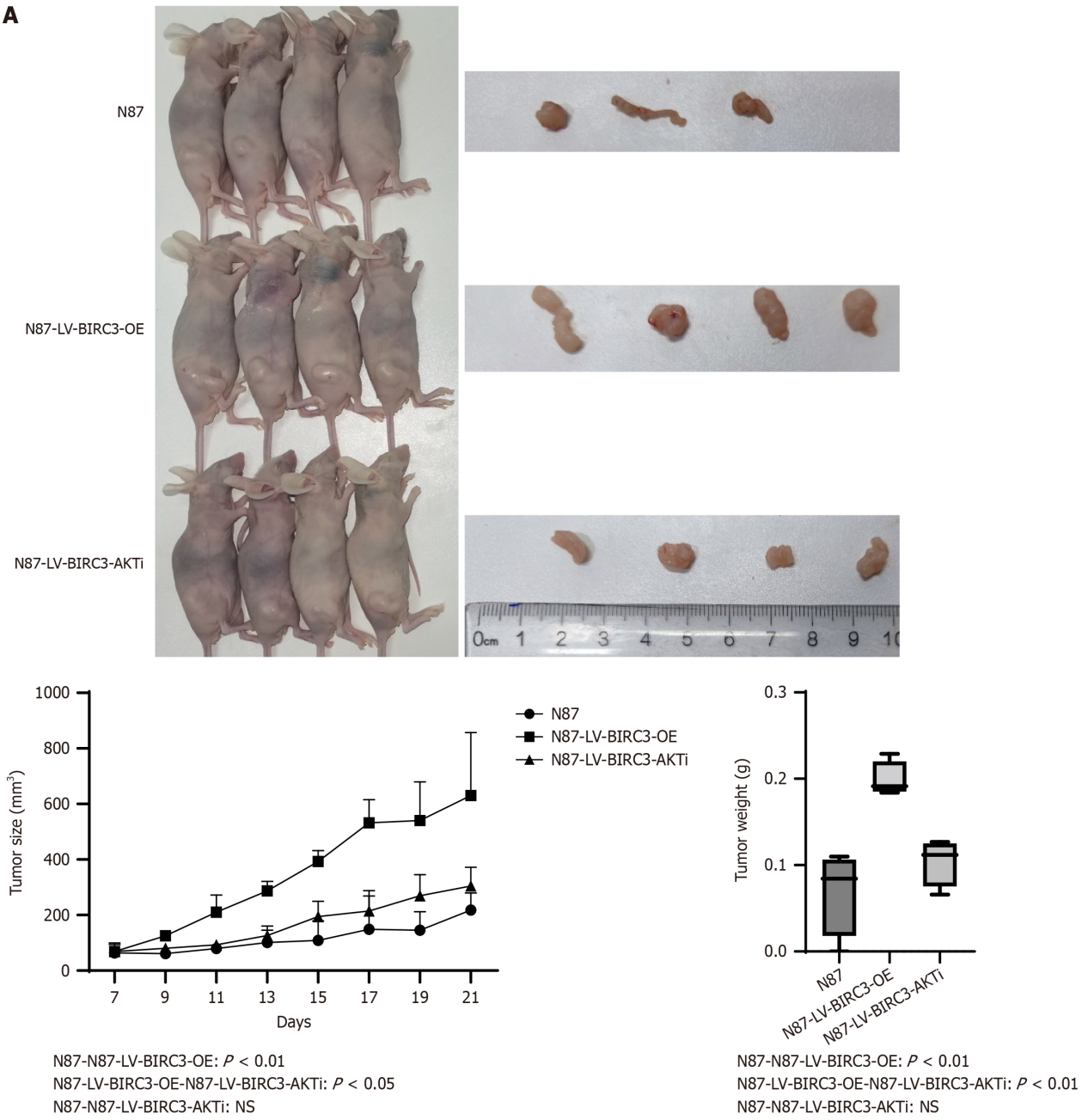
activating the AKT pathway.

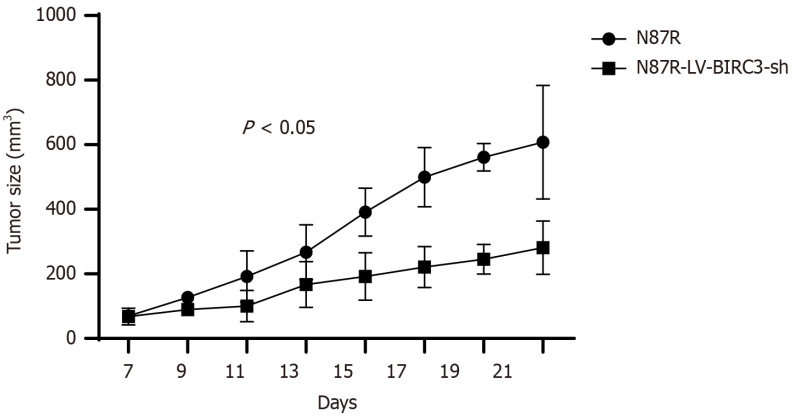
Tumor cell-derived BIRC3 inhibits apoptosis by activating the AKT pathway, leading to trastuzumab resistance

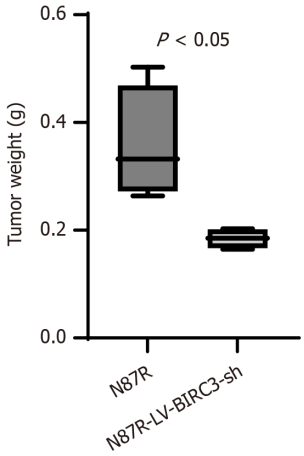
To examine tumorigenesis, SPF BALB/c nude mice were inoculated with the corresponding cells according to the described grouping plan. Tumor growth was monitored in nude mouse on the 7th day. Following the experimental design, the treatments were intraperitoneally administered every two days for two weeks.

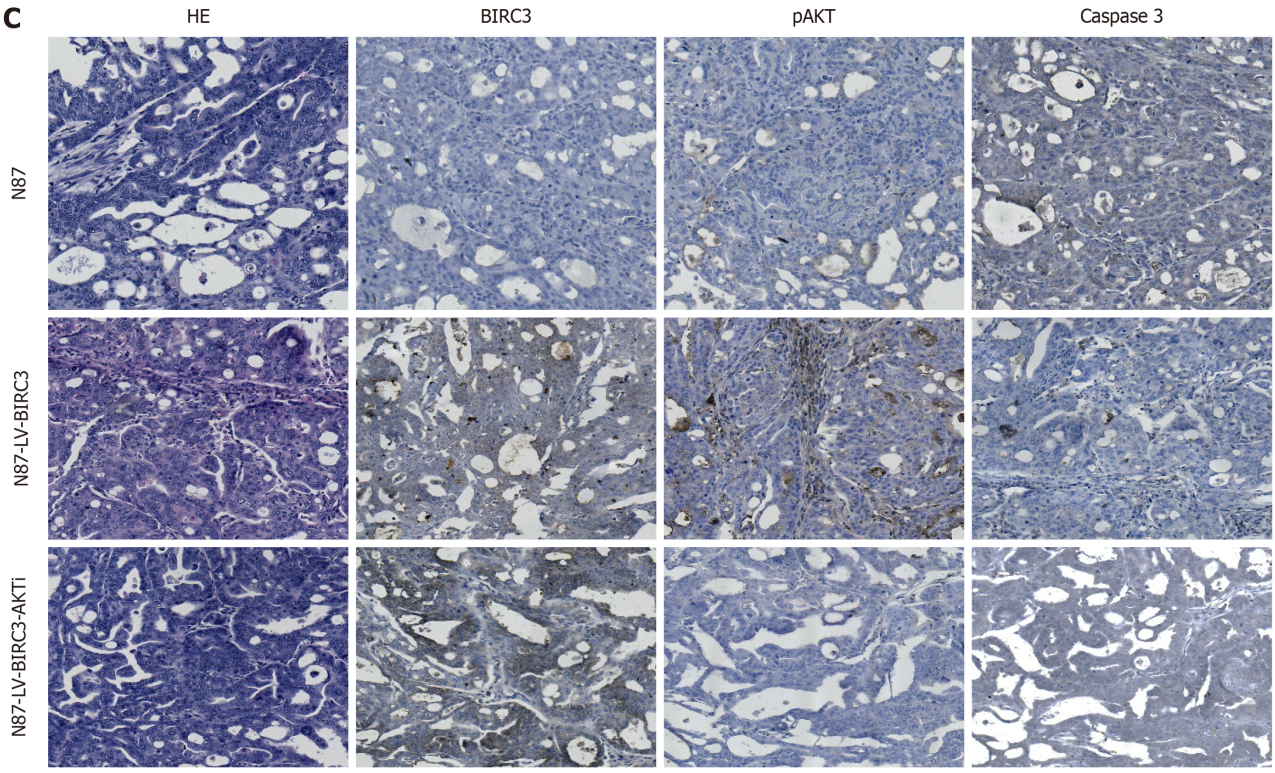
As shown in Figure 7A, one nude mouse in the N87-NC group was administered trastuzumab for two weeks after tumor formation, resulting in complete tumor regression. The N87-LV-*BIRC3* group displayed a significantly accelerated tumor growth rate and increased weight compared with both the N87-NC (P = 0.0084) and N87-LV-*BIRC3*-AKTi groups (P = 0.0044). However, no significant differences were observed between the N87-NC and N87-LV-*BIRC3*-AKTi groups. Besides, as depicted in Figure 7B, the NCI-N87R group exhibited accelerated tumor growth and greater weight relative to the N87R-LV-*BIRC3*-si group (0.3580 ± 0.1057 *vs* 0.1848 ± 0.01859, P = 0.0018).

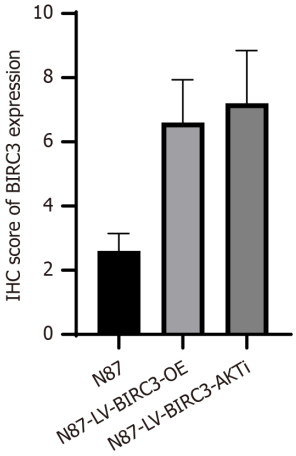
According to the IHC results, the expression of *BIRC3* was substantially enhanced in the N87R-LV-*BIRC3*-si (2.600 ± 0.5477 *vs* 6.7 ± 1.342, P = 0.0003) and N87-LV-*BIRC3*-AKTi (2.600 ± 0.5477 *vs* 7.20 ± 1.643, P = 0.0003) groups compared to the N87-NC group. Conversely, caspase 3 staining exhibited the opposite trend (P < 0.001). Additionally, the expression


A
N87
N87-LV-BIRC3-OE
N87-LV-BIRC3-AKTi
Tumor size (mm³)
Days
N87
N87-LV-BIRC3-OE
N87-LV-BIRC3-AKTi
N87-N87-LV-BIRC3-OE: P < 0.01
N87-LV-BIRC3-OE-N87-LV-BIRC3-AKTi: P < 0.05
N87-N87-LV-BIRC3-AKTi: NS
Tumor weight (g)
N87-N87-LV-BIRC3-OE: P < 0.01
N87-LV-BIRC3-OE-N87-LV-BIRC3-AKTi: P < 0.01
N87-N87-LV-BIRC3-AKTi: NS
B
N87R
N87R-LV-BIRC3-sh

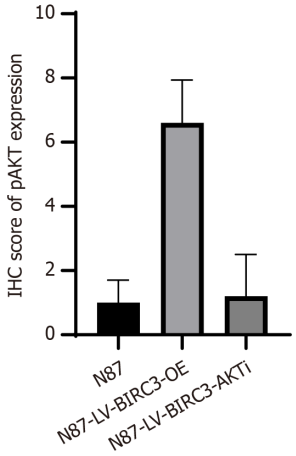




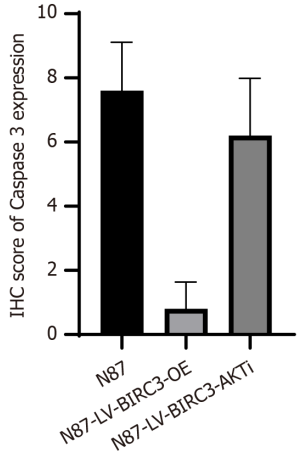




N87-N87-LV-BIRC3-OE: $P < 0.001$
N87-LV-BIRC3-OE-N87-LV-BIRC3-AKTi: NS
N87-N87-LV-BIRC3-AKTi: $P < 0.001$



N87-N87-LV-BIRC3-OE: $P < 0.0001$
N87-LV-BIRC3-OE-N87-LV-BIRC3-AKTi: $P < 0.001$
N87-N87-LV-BIRC3-AKTi: NS



N87-N87-LV-BIRC3-OE: $P < 0.0001$
N87-LV-BIRC3-OE-N87-LV-BIRC3-AKTi: $P < 0.001$
N87-N87-LV-BIRC3-AKTi: NS

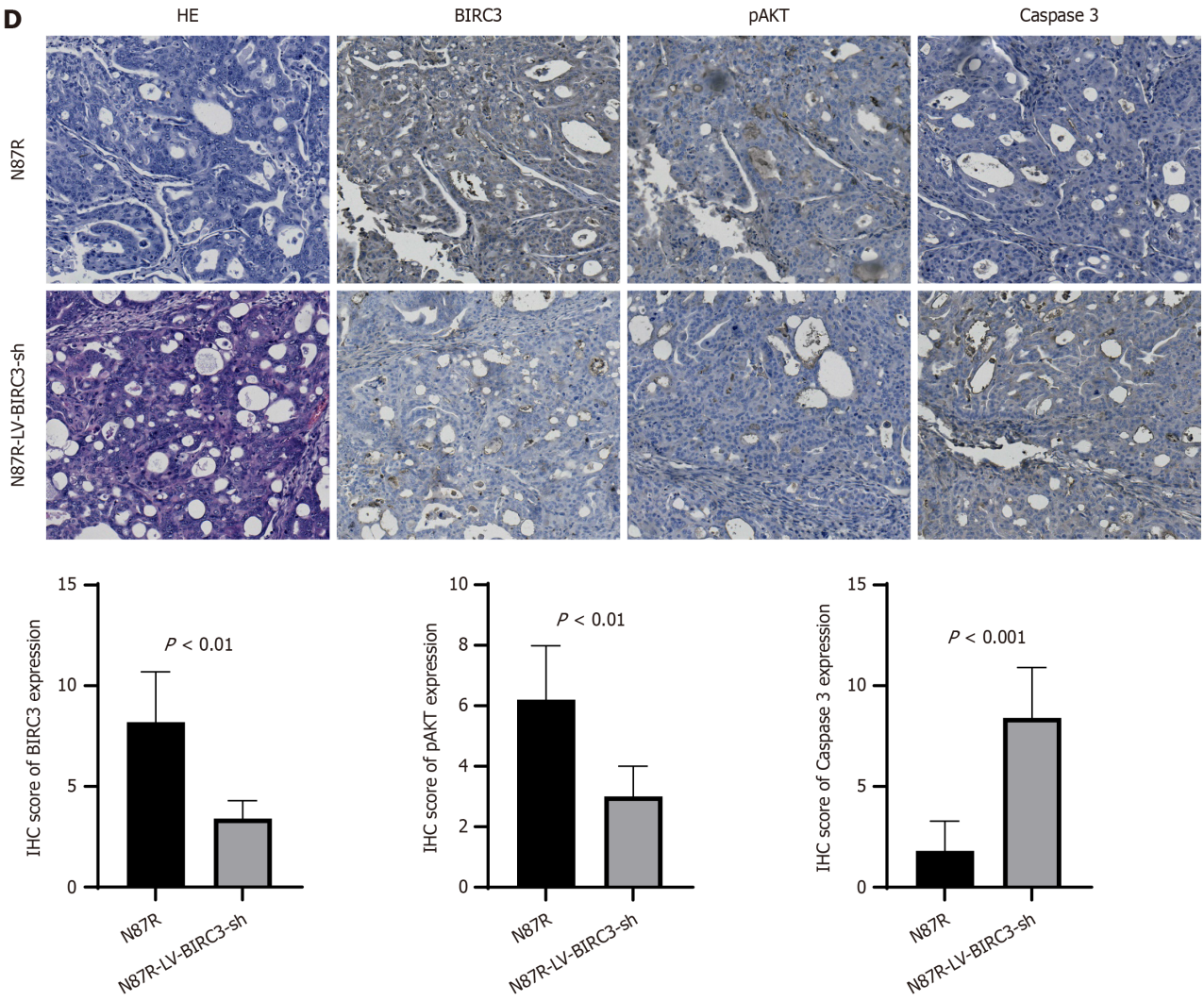


Figure 7 Tumor cell-derived *BIRC3* inhibits apoptosis by activating AKT pathway, lead to drug resistance to trastuzumab. A: Experimental in Balb/c nude mice. Tumor volume images, tumor weight and tumor growth curves of each group were shown. The different treatment groups included: (1) The stable control NCI-N87 cells (N87 group); (2) The stable *BIRC3* overexpressing NCI-N87 cells with the trastuzumab treatment (N87-LV-*BIRC3* group); and (3) The NCI-N87 transfected with the overexpressed virus LV-GFP-puro-*BIRC3* and treated with AKT inhibitor (MK-2206) (N87-LV-*BIRC3*-AKTi group); B: (1) The NCI-N87 was developed into a trastuzumab-resistant cell line(N87R group); (2) The group inoculated with NCI-N87R cells transfected with the knockout virus LV-GFP-puro-*BIRC3*-si (N87-LV-*BIRC3* group); C and D: Immunohistochemistry staining for *BIRC3*, pAKT and caspase 3 was presented, and the immunohistochemistry scores for *BIRC3*, pAKT and caspase 3 were statistically analyzed. IHC: Immunohistochemistry; HE: Hematoxylin-eosin; NS: No significance.

of pAKT in the N87-LV-*BIRC3* group was notably higher than that in the N87-NC (6.600 ± 1.342 *vs* 1.000 ± 0.7071, $P < 0.0001$) and N87-LV-*BIRC3*-AKTi groups (6.600 ± 1.342 *vs* 1.200 ± 1.304, $P = 0.0002$) (Figure 7C). Furthermore, when compared to the N87R group, the expression of *BIRC3* (8.200 ± 2.490 *vs* 3.400 ± 0.8944, $P = 0.0036$) and pAKT (6.200 ± 1.789 *vs* 3.000 ± 1.000, $P = 0.0082$) in the N87R-LV-*BIRC3*-si group significantly decreased, whereas the caspase 3 staining exhibited an opposite trend (1.800 ± 1.483 *vs* 8.400 ± 2.510, $P = 0.0010$) (Figure 7D).

These findings suggest that *BIRC3* potentiates the inhibition of apoptosis and stimulates tumor cell proliferation through activation of the pAKT pathway, ultimately leading to the resistance of HER2-positive gastric cancer cells to trastuzumab.

DISCUSSION

In this study, we established a trastuzumab-resistant cell line, NCI-N87R, using advanced sequencing and bioinformatics analyses. Our screening process identified the key gene, *BIRC3*, which is responsible for resistance to targeted therapy with trastuzumab.

We investigated the functional role of *BIRC3* in human gastric cancer, by focusing on its relationship with HER2. HER2 plays a crucial role in the development of gastric cancer, with approximately 5%-25% of gastric cancer cases exhibiting overexpression of the HER2. Studies have indicated that HER2 overexpression is an independent risk factor for advanced gastric cancer, leading to poor outcomes and shorter survival times[13,14]. The specific ligand of HER2 is unknown, but it is known to form hetero- or homodimers with itself or with other members of its family (*EGFR*, *HER3*, and *HER4*). This

dimerization leads to the phosphorylation of the tyrosine kinase domain, activating downstream signaling pathways such as the PI3K-AKT and *MAPK* pathways. These pathways play a crucial role in promoting cell proliferation, preventing cell apoptosis, and influencing cell growth, survival, and differentiation[15-17].

BIRC3 has been shown to have an anti-apoptotic role, as it directly binds and inhibits caspases 3, 7, and 9. Additionally, *BIRC3* can bind to polyubiquitin linked with lysine through its ubiquitin-related domain and activate the NF-κB signal pathway. This activation helps protect cells from tumor necrosis factor (TNF)-α induced cell apoptosis and maintain the survival of tumor cells[18]. Several studies have reported that *BIRC3* is overexpressed in more than 70% of human gastric cancers[19]. *BIRC3* knockout in gastric cancer cell lines has been shown to increase apoptosis, decrease proliferation, and delay cell migration[20]. Yoon *et al*[21] discovered that high *BIRC3* expression plays a crucial role in gastric cancer development after *Helicobacter pylori* infection. Furthermore, various studies have demonstrated that *BIRC3* exhibits similar effects in different tumor types. For example, high *BIRC3* expression is associated with clinicopathological characteristics and poor prognosis in colon cancer, pancreatic cancer, bladder cancer, and glioblastoma[9,22-25]. *BIRC3* overexpression plays a significant role in chemotherapy resistance in breast cancer, temozolomide resistance in glioblastoma multiforme, and cisplatin resistance induced by *COL11A1* in ovarian cancer[26-29]. These findings strongly suggest that *BIRC3* acts as a tumor promoter and has the potential to be a prognostic indicator in patients with tumors. Moreover, it may serve as a novel target for tumor treatment.

According to current literature, resistance to trastuzumab treatment in HER2-positive tumors can be attributed to various mechanisms. One important mechanism is abnormal activation of the downstream PI3K-AKT pathway[30,31]. Once AKT is phosphorylated and activated, it regulates several cellular processes including cell proliferation, differentiation, apoptosis, and angiogenesis through the involvement of Iκ-B kinase, procaspase 9, and mammalian target of rapamycin phosphorylation at the serine/threonine site[27-29]. Previous studies have demonstrated that AKT activation is associated with progressive disease and poor prognosis in certain tumor types[32]. In triple-negative breast cancer, overexpression of *BIRC3* activates the AKT signaling pathway, leading to tumor cell proliferation, metastasis, and poor prognosis[33]. Furthermore, research on resistance to HER2-positive tumor-targeted drugs has revealed that various molecular abnormalities, such as changes in receptor structure, co-expression with other transmembrane receptors, and abnormal activation of downstream pathways, can contribute to trastuzumab resistance[34-37].

The experimental results convincingly demonstrated that overexpression of *BIRC3* and AKT in N87 cells elicited a notable increase in cell proliferation and concurrent resistance to trastuzumab. Conversely, upon silencing *BIRC3* expression, the inverse effect was observed. Furthermore, inhibition of the AKT pathway resulted in a discernible decline in N87 cell proliferation, accompanied by increased susceptibility to trastuzumab. These findings suggest that *BIRC3* amplifies the proliferative potential of HER2-positive gastric cancer cells by activating the AKT pathway, thereby inducing resistance to targeted therapy with trastuzumab. Moreover, *in vitro* cytological experiments substantiated these observations, affirming that increased *BIRC3* expression activates the AKT pathway, resulting in enhanced proliferation of previously responsive cells after trastuzumab treatment, consequently fostering drug resistance. To confirm these conclusions, tumorigenesis experiments were conducted in nude mice. Additionally, a responsive experimental group was designed to provide further compelling evidence supporting the assertion that *BIRC3* induces trastuzumab-targeted drug resistance through the activation of the AKT pathway.

In the clinical data analysis section, several limitations are evident. This study is a single-center investigation and lacks data from larger multicenter samples. Consequently, a greater number of gastric cancer patients must be evaluated to enhance the robustness of the results. Although we conducted multiple repeated experiments to ensure the accuracy of our findings, additional results from diverse experimental methods are necessary for further improvement. Ultimately, more work is required to provide tangible benefits to clinical patients.

CONCLUSION

In conclusion, our findings illustrate that the knockdown of *BIRC3* diminishes the resistance of the trastuzumab-resistant cell line NCI-N87R. Conversely, high *BIRC3* expression induced resistance to trastuzumab. Moreover, upon transfection with the *BIRC3* overexpression vector, N87 cells exhibited resistance to trastuzumab. In addition, resistance to trastuzumab could be counteracted by AKT inhibitors, suggesting that *BIRC3* may trigger resistance to trastuzumab therapy by activating the AKT pathway.

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FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Impact and mechanism study of dioscin on biological characteristics of colorectal cancer cells

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Abstract

BACKGROUND

Colorectal cancer (CRC) is a considerable global health issue. Dioscin, a compound found in several medicinal plants, has shown potential anticancer effects.

AIM

To find the relationship between CRC cells (HCT116) and diosgenin and clarified their mechanisms of action.

METHODS

CRC cell line HCT116 was cultured by dividing cells into control and dioscin groups (dioscin + Jagged 1 group; Jagged 1 group, 5 μg/mL; and dioscin group, 2.5 μg/mL). The dioscin groups were given different concentrations of dioscin. Cell Counting Kit-8 was chosen for testing cell viability in different groups. Flow cytometry was established to undiscover the apoptosis rate of human liver cancer cell line 11. Real-time PCR as well as Western blot analyses were applied to reveal the expression levels of caspase-3, Notch, and other proteins. Transwell and scratch experiments were conducted to assess cell migration and invasion abilities.

RESULTS

This study indicated that dioscin restricted the growth of HCT116 cells, boosted cell apoptosis, and rose the *Bax*/*Bcl-2* ratio as well as the expression of *Caspase-3*. Dioscin also inhibited physiological activities, for instance cell migration, and significantly reduced the expression levels of proteins for instance Notch1 ($P < 0.05$). Dioscin partially reversed the effects of Jagged 1.

CONCLUSION

Dioscin exerts a certain inhibitory effect on HCT116, and its mechanism of action may be linked, with the inhibition of the Notch1 signaling pathway.

Key Words: Dioscin; Colorectal cancer; Notch1; Cell apoptosis; Cell proliferation

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Core Tip: This study investigates the anticancer effects of dioscin on colorectal cancer (CRC) cells (HCT116). Our findings reveal that dioscin inhibits cell viability, promotes apoptosis, and suppresses migration and invasion by modulating the Notch1 signaling pathway. These results highlight the potential of dioscin as a therapeutic agent against CRC.

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INTRODUCTION

Analysis of malignant tumors can confirm if they are cancer, and colorectal cancer (CRC) is responsible for the deaths of approximately 600000 individuals globally each year[1]. In developing countries, new cases of CRC will reach 2.5 million by 2035[2]. In China, the incidence and mortality rates of CRC increase every year, and the affected population is young individuals because of rapid economic development, aging population, and changes in residents' lifestyles and dietary patterns[3]. CRC originates from the epithelium and glands of the colorectal mucosa. Abnormal expression or structural changes in core regulatory pathway molecules within cells lead to disruptions in cell growth and metabolic functions. This process prompts the transformation of normal colonic mucosa into colorectal adenomas or polyps, which, with the gradual accumulation of abnormal molecules, can further develop into CRC[4]. Chemotherapy is the most widely used clinical treatment for CRC, but it can cause severe toxic side effects and complications in the body, for instance nausea, vomiting, bone marrow suppression, and liver and kidney toxicity[5]. Therefore, effective treatments should be developed so patients can achieve good treatment results.

The Notch1 signaling pathway plays a crucial role in the advancement as well as progression of CRC. Notch1 is a transmembrane receptor that interacts with ligands for instance Jagged1 and Delta-like proteins. When the Notch1 signaling pathway is activated, it triggers the cleavage of the Notch intracellular domain (NICD). The NICD subsequently moves into the nucleus, where it initiates the expression of downstream target genes such as Hes1 as well as Hey1. These target genes regulate cell proliferation, differentiation, as well as apoptosis. Dysregulation of the Notch1 pathway has been implicated in the promotion of CRC cell growth and survival.

Traditional Chinese medicine has unique advantages in natural resources, and its pharmacological effects are very extensive. Various Chinese medicines exert anti-tumor activity and have significant advantages in clinical treatment. Dioscin is widely present in medicinal plants of the *Dioscoreaceae*, *Liliaceae*, *Caryophyllaceae*, and *Rosaceae* families, especially in the rhizomes of *Dioscoreaceae* plants, for instance *Dioscorea nipponica*, *Dioscorea panthaica*, and *Dioscorea futschauensis*[6]. Modern pharmacology has shown that dioscin has preventive and therapeutic effects on various diseases, including anti-tumor, blood glucose reduction, lipid-lowering, anti-osteoporosis, liver protection, anti-inflammatory, and analgesic properties[7–9]. An increasing number of research suggests that dioscin has significant anti-tumor activity. Dioscin affects tumor cells, inhibit cell growth, and effectively promote cell apoptosis, thereby inhibiting tumor metastasis *in vivo* as well as *in vitro*. Dioscin can have an impact on the cell cycle, regulate the expression of corresponding proteins, and thus disrupt the proliferation of malignant tumor cells, thereby affecting cell division[10]. Dioscin suppresses the development of CRC cells by inhibiting Skp2 S72 phosphorylation, which in turn increases Skp2 ubiquitination and destruction in a manner that is reliant on Cdh1[11]. It inhibits MMP2 and MMP9, causes cell cycle arrest at the S phase, and reduce the invasive capacity of human laryngeal cancer cells[12]. I can induce apoptosis in lung cancer cells (A549, Caco-2) through multiple pathways, for instance the prostaglandin E2 pathway, according to *in vitro* tests[13]. Therefore, dioscin holds great promise as an anticancer agent.

A few studies investigated the ability of dioscin to suppress CRC. Limited data are available on the inhibitory effects of *Dioscoreaceae* medicinal plants on CRC cells *in vitro*. The systematic effects of dioscin on CRC and its underlying mechanisms still need further exploration. Therefore, this study aims to determine how dioscin affects CRC and would provide new insights for the treatment of CRC.

MATERIALS AND METHODS

Drugs and reagents

Dioscin (cat. no. JOT-10207) was purchased from Chengdu Pufei De Biotech Co., Ltd. Notch1 pathway activator Jagged 1 (cat. no. HY-P1846A) was acquired from MedChemExpress. Cell counting kit-8 (CCK-8) staining solution (cat. no. C0037) was obtained from Beyotime Biotechnology. qRT-PCR amplification (cat. no. RR820Q) and reverse transcription reagent

kit (cat. no. RR047A) as well as annexin V-FITC/PI cell apoptosis detection kit (cat. no. 630109) were provided by Takara. Primers for Bax, Bcl-2, as well as caspase-3 were synthesized by Shanghai Jima Technology Co., Ltd. Rabbit anti-Notch1 (cat. no. ab52627), Jagged1 (cat. no. ab109536), Hes1 (cat. no. ab108937), and GAPDH (cat. no. ab8245) antibodies and goat anti-rabbit secondary antibody coupled with horseradish peroxidase (HRP) (cat. no. ab181662) were supplied by Abcam (Shanghai, China).

Cell culture and grouping

CRC cells HCT116 were sourced from the Shanghai Cell Bank of the Chinese Academy of Sciences. These cells were cultured at 37 °C in an atmosphere containing 5% CO_2, using RPMI-1640 medium enriched with 10% fetal bovine serum. Upon reaching 80% cell confluence, the culture medium was disposed. The cells were washed carefully with phosphate buffered saline (PBS) and added with 1 mL of 0.25% trypsin for digestion. Digestion was stopped by adding culture medium when the cell gaps widened and some adherent cells detached. The adherent cells were detached by gentle pipetting to achieve a single-cell suspension and set aside for further use. In this study, cells were divided into control, dioscin groups (1.25, 2.5, 5 μg/mL), Jagged 1 group (5 μg/mL), and dioscin (2.5 μg/mL) + Jagged 1 (5 μg/mL) group.

Viability of HCT116 cells

HCT116 cells were seeded at a density of 1×10^5 cells per milliliter in a 96-well plate and allowed to grow for a duration of 24 hours. Each well was added with culture media containing different agents (1.25, 2.5, 5 μg/mL dioscin, 5 μg/mL Jagged 1, 2.5 μg/mL dioscin + 5 μg/mL Jagged 1). An equivalent volume of drug-free growth media was given to the control group. The cells were cultivated in a carbon dioxide incubator at 37 °C for 48 hours. The cells were then added with 10 μL of CCK-8 solution per well and cultured again for 60 minutes. Absorbance was detected at 450 nm. Cell viability was calculated by the formula: OD value of dioscin-treated group/OD value of control group × 100.

Measurement of apoptosis rate in HCT116 cells

The cells (2×10^4 cells/mL) were cultivated in a 24-well microplate for 24 hours Each well was added with culture media containing different concentrations of dioscin (1.25,2.5,5 μg/mL), 5 μg/mL Jagged 1, and a mixture of 2.5 μg/mL dioscin and 5 μg/mL Jagged 1. The control group received the same volume of culture medium without drugs. The cells were incubated at 37 °C in a 5% CO_2 cell culture incubator for 48 hours. After the cells were broken down by 0.25% trypsin, they were centrifuged at 1500 rpm for 3 minutes and then revived with 300 μL of binding buffer. The cell suspension (100 μL) was incubated at room temperature with 1 μL of PI and 5 μL of annexin V for 15 minutes.

Determination of the invasive capability of HCT116 cells

The lower chamber of the 24-well plate was added with 600 μL of standard culture media with serum. HCT116 cells were seeded at a density of 2×10^4/mL in serum-free medium at the top chamber of the Transwell. The upper chamber was added with different serum-free media containing dioscin (1.25, 2.5, 5 μg/mL), 5 μg/mL Jagged 1, and a mixture of 2.5 μg/mL dioscin and 5 μg/mL Jagged 1. An equivalent amount of serum-free medium was administered to the blank control group. After 24 hours in a cell culture incubator, the upper chamber was removed. The cells were washed with PBS, fixed with methanol pre-cooled in the bottom chamber for 30 minutes, stained with crystal violet for 10 minutes, and observed under an inverted microscope to check for cell invasion.

Detection of HCT116 cell migration

In a 12-well plate, cells (3×10^5 cells/mL) were cultivated until a monolayer formed. A straight incision was made at the center of the cell pore by using the tip of a sterile micropipette. After 48 hours, the cells were viewed under a microscope to determine the healing status of the wound. The rate of cell migration was determined using the formula: Migration rate = (Area at 0 hours - Area at 48 hours) / Area at 0 hours.

Assessment of apoptosis-related gene expression in HCT116 cells

Trizol method was used to extract total cellular RNA, and cDNA was synthesized according to the instructions of the reagent kit. The overall reaction volume was 20 μL, and the reaction conditions were set at 25 °C for 30 min, 42 °C for 30 minutes, and 85 °C for 5 minutes. About 2 μL of cDNA was taken as the amplification template, with an overall reaction volume of 20 μL. Amplification was conducted with the following steps: Pre-denaturation at 95 °C for 5 minutes, denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 60 seconds, for a total of 30 cycles, and a final extension at 72 °C for 1 minute. Relative gene expression changes were calculated using $2^{-\triangle\triangle CT}$ method. The primers used are shown in Table 1.

Western blot assay

After extracting all of the cellular proteins from each group, the BCA assay kit was used to quantify the proteins. The samples were concentrated with loading buffer as well as heated at 100 °C for 10 minutes to denature the proteins after their concentrations were adjusted to the same amount. SDS-PAGE was conducted at 100 V for 50 minutes, followed by wet transfer at 320 mA for 90 minutes. The membrane was placed in a room-temperature environment and sealed using 5% skim milk. The membrane was shaken for 60 minutes and washed using TBST. The membrane was placed in the antibody diluted by 1000 times and then placed in a humidification chamber for 12 hours. The membrane was removed, washed using TBST, placed in the second diluted antibody, and shaken for 120 minutes. Signal was detected using the ECL chemiluminescence detection kit. A gel imaging system was used to detect and analyze the image. The optical density ratio of the internal reference standard and the target strip was calculated and statistically analyzed.

Table 1 Primer sequences

Gene	Forward primer	Reverse primer
Bax	ATGGAGCTGCAGAGGATGATT	TGATGGTTCTGATCAGCTCGG
Bcl-2	GCGTCAACAGGGAGATGTCA	TTCCACAAAGGCATCCCAGC
Caspase-3	TCTACCGCACCCGGTTACTA	TCAAATTCCGTGGCCACCTT
GAPDH	GAAGGTGAAGGTCGGAGTCA	GAAGATGGTGATGGGATTTC

Statistical analysis

After completing data collection, statistical analysis was conducted using SPSS 18.0 software. Measurement data were described by mean ± SD. Normality of continuous variables was tested using the Shapiro-Wilk test. Different groups of data were compared using one-way ANOVA. Values with a P value of less than 0.05 were deemed to be statistically significant.

RESULTS

Suppressive effect of dioscin on CRC HCT116 cell viability

To explore the response of dioscin on the viability of HCT116 cells, we conducted a series of experiments. As shown in Figure 1, different concentrations of dioscin inhibited the viability of HCT116 cells. With increasing concentrations of dioscin, the cell viability of each group gradually reduced (0 μg/mL: 100.90% ± 1.18%, 1.25 μg/mL: 73.34% ± 2.09%, 2.5 μg/mL: 53.00% ± 1.49%, 5 μg/mL: 45.76% ± 1.89%), Cell viability was statistically significantly different between the control group and the dioscin groups ($P < 0.05$).

Dioscin promotes apoptosis in CRC HCT116 cells

To elucidate the impact of dioscin on apoptosis in HCT116 cells, we applied flow cytometry analysis. As shown in Figure 2, various dioscin doses increased the rate of cell apoptosis relative to the control group ($P < 0.05$). With increasing dioscin concentration, the degree of apoptosis in each group gradually increased (0 μg/mL: 100.90 ± 1.18, 1.25 μg/mL: 73.34 ± 2.09, 2.5 μg/mL: 53.00 ± 1.49, 5 μg/mL: 45.76 ± 1.89). This finding indicates that dioscin promotes apoptosis in HCT116 cells.

As shown in Figure 3A, with increasing dioscin concentration, the *Bax*/*Bcl-2* ratio (0 μg/mL: 1.00 ± 0.03, 1.25 μg/mL: 1.48 ± 0.29, 2.5 μg/mL: 2.34 ± 0.48, 5 μg/mL: 2.62 ± 0.44) and *Caspase-3* mRNA expression (0 μg/mL: 1.62 ± 0.26, 1.25 μg/mL: 1.37 ± 0.20, 2.5 μg/mL: 2.16 ± 0.26, 5 μg/mL: 2.21 ± 0.11) significantly increased ($P < 0.01$). This finding indicates that dioscin promotes the expression of apoptotic genes in HCT116 cells.

As shown in Figure 3B, compared with the control group, the expression of the Bax protein in the dioscin group (0 μg/mL: 1.00 ± 0.00, 1.25 μg/mL: 1.16 ± 0.01, 2.5 μg/mL: 1.87 ± 0.01, 5 μg/mL: 2.10 ± 0.00) significantly increased ($P < 0.05$), while the expression of the Bcl-2 protein (0 μg/mL: 1.00 ± 0.08, 1.25 μg/mL: 0.78 ± 0.05, 2.5 μg/mL: 0.47 ± 0.02, 5 μg/mL: 0.38 ± 0.03) significantly reduced ($P < 0.05$). The expression of the Caspase-3 protein (0 μg/mL: 1.00 ± 0.01, 1.25 μg/mL: 0.97 ± 0.02, 2.5 μg/mL: 1.21 ± 0.01, 5 μg/mL: 2.07 ± 0.01) significantly increased, except that at 1.25 μg/mL ($P < 0.05$). This finding indicates that dioscin promotes has the expression of apoptotic proteins.

As shown in Figure 3C, the protein expression levels of Bax (0 μg/mL: 1.00 ± 0.00, 1.25 μg/mL: 1.16 ± 0.01, 2.5 μg/mL: 1.87 ± 0.01, 5 μg/mL: 2.10 ± 0.00) and Caspase-3 (0 μg/mL: 1.00 ± 0.01, 1.25 μg/mL: 0.97 ± 0.02, 2.5 μg/mL: 1.21 ± 0.01, 5 μg/mL: 2.07 ± 0.01) significantly increased, whereas Bcl-2 (0 μg/mL: 1.00 ± 0.08, 1.25 μg/mL: 0.78 ± 0.05, 2.5 μg/mL: 0.47 ± 0.02, 5 μg/mL: 0.38 ± 0.03) significantly decreased with increasing dioscin concentration ($P < 0.05$). These results indicate that dioscin promotes the expression of pro-apoptotic proteins.

Dioscin inhibits the invasion and migration of CRC HCT116 cells

To investigate the effect of dioscin on the invasion and migration of HCT116 cells, we performed Transwell assays and wound healing assays. As shown in Figure 4, dioscin markedly reduced the rate of tumor cell invasion in comparison with the control group (0 μg/mL: 100.00% ± 5.43%, 1.25 μg/mL: 97.57% ± 28.72%, 2.5 μg/mL: 73.53% ± 25.89%, 5 μg/mL: 71.40% ± 25.81%; $P < 0.05$). This finding suggests that dioscin inhibits the invasive ability of HCT116 cells.

As shown in Figure 5, after 48 hours, diosgenin significantly inhibited the migration of HCT116 cells (0 μg/mL: 86.23% ± 0.26%, 1.25 μg/mL: 72.01% ± 0.46%, 2.5 μg/mL: 57.66% ± 0.28%, 5 μg/mL: 18.89% ± 0.55%; $P < 0.001$).

Dioscin inhibits the activity of the Notch 1 signaling pathway in CRC HCT116 cells

To extra understand the molecular mechanisms underlying the inhibitory effects of dioscin on HCT116 cells, we examined the expression of proteins involved in the Notch1 signaling pathway. The Notch1 signaling pathway is recognized as a vital factor in the governance of cell proliferation, differentiation, and apoptosis within CRC. As shown in Figure 6, the cells treated by dioscin had significantly lower expression of the Notch1 protein compared with the control group ($P < 0.05$). Dioscin can have a certain impact on cells and significantly inhibit the activity of related protein signaling pathways. Moreover, the diosin + Jagged 1 group had significantly lower protein expression levels than the Jagged 3

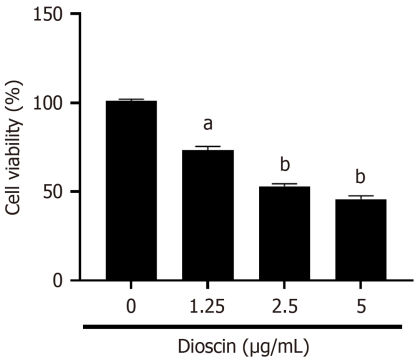


Figure 1 Effect on dioscin on HCT116 activity. Dioscin at different doses (0, 1.25, 2.5, and 5 μg/mL) was applied to HCT116 cells for 48 hours. The cell counting kit-8 kit was used to measure the dioscin inhibition rate. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.01$, and $^{b}P < 0.001$ compare to the control group.

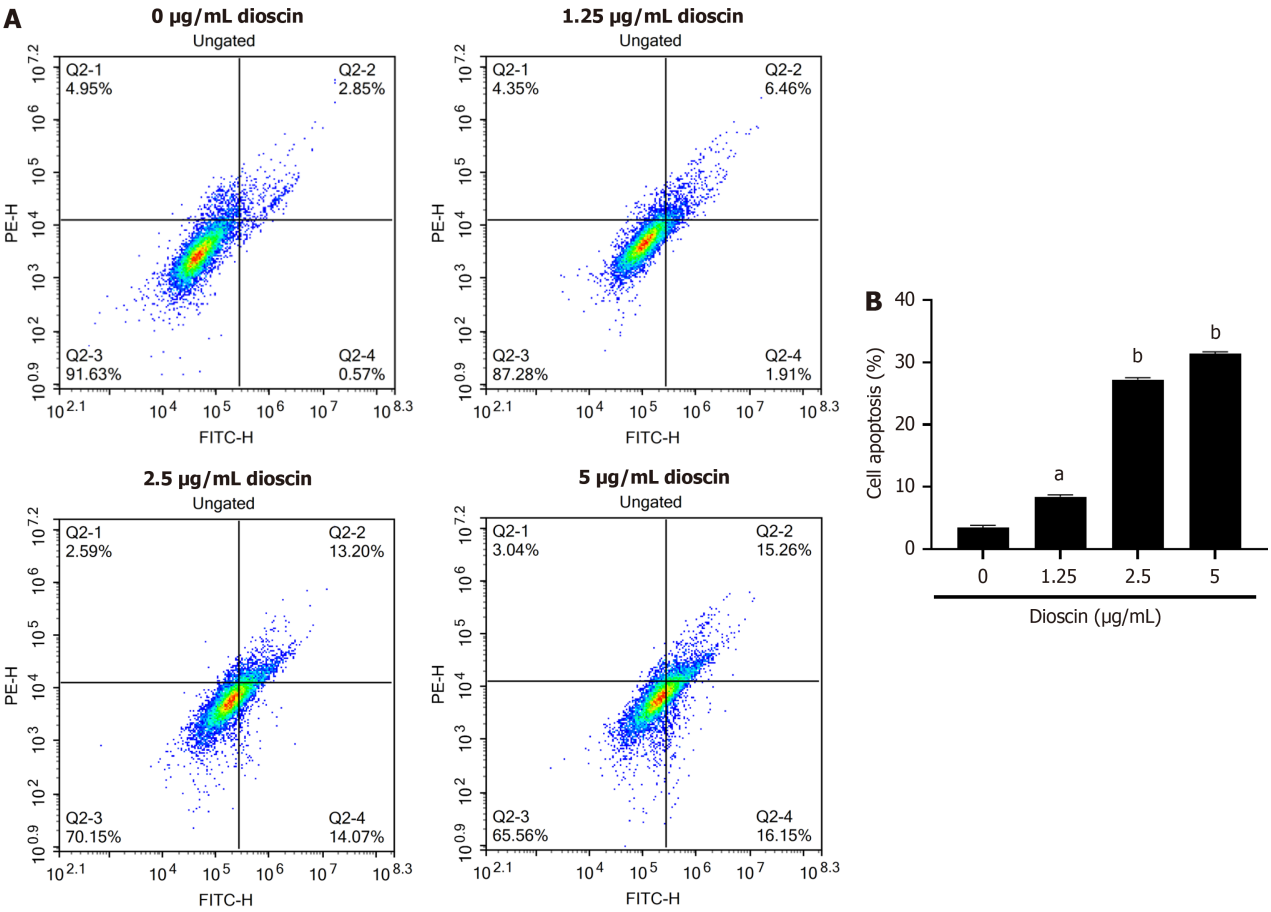


Figure 2 Effect of dioscin on HCT116 cell apoptosis. A: Annexin V-FITC and PI staining was utilized to assess apoptosis in various concentrations (0, 1.25, 2.5 and 5 μg/mL) of dioscin-treated HCT116 cells after 48 hours by using flow cytometry; B: Quantification of apoptosis in HCT116 cells. Three biological repeats were reported as mean ± SD. $^{a}P < 0.01$, $^{b}P < 0.001$ compare to the control group.

group, ($P < 0.05$). Hence, diosin can exert a blocking effect when the Notch 1 signaling pathway was activated.

Inhibitory effect of dioscin on HCT116 involves the participation of Notch

Basing on preliminary experiments, we believe that dioscin has a certain inhibitory effect on HCT116 cells and it could be related to the regulation of the Notch 1 signaling pathway. Therefore, we treated cells with the Notch 1 activator Jagged 1 to further explore the relationship between dioscin and Notch 1. In the preliminary experiments, the difference in cell viability and apoptosis level was relatively small when dioscin was increased from 2.5 μg/mL to 5 μg/mL. Therefore, we chose a concentration of 2.5 μg/mL dioscin for subsequent experiments.

As shown in Figure 7A-C, the apoptosis ratio of cells in the Jagged 1 group dramatically reduced ($P < 0.05$) compared with the control group (Jagged 1 group: 6.11% ± 0.44%, control group: 13.64% ± 0.72%). The Bax/Bcl-2 ratio (control

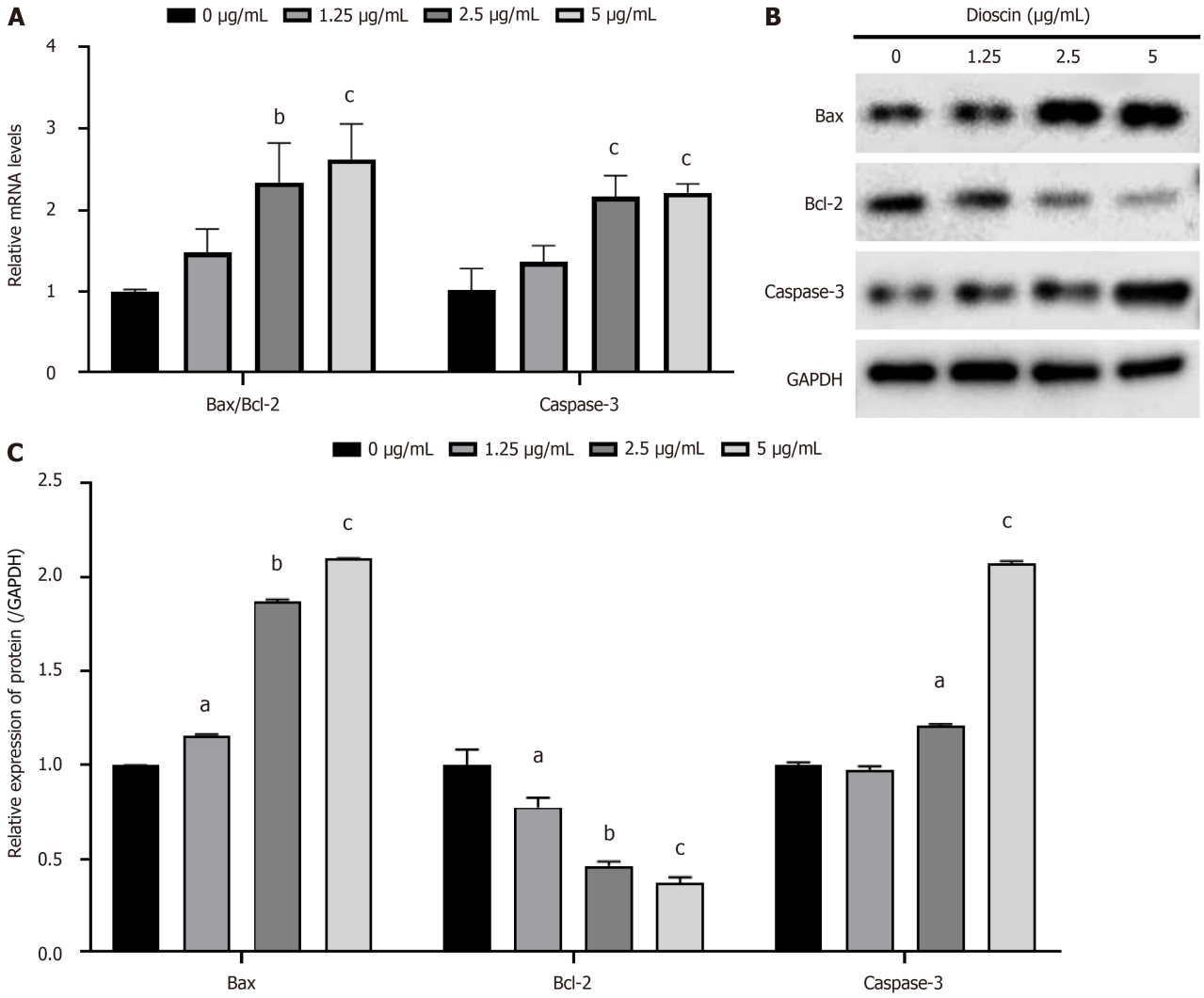


Figure 3 Effect of dioscin on apoptotic gene and protein expression in HCT116 cells. HCT116 cells were exposed to varying concentrations of dioscin (1.25, 2.5 and 5 μg/mL) for 48 hours. A: RT-qPCR study of Bax/Bcl-2 ratio as well as Caspase-3 mRNA expression; B: Western blot examination of the expression of the proteins Caspase-3, Bcl-2, and Bax; C: Quantification of Caspase-3, Bcl-2, and Bax protein expression levels from the Western blot results. Three biological repeats were reported in mean ± SD format. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compare to the control group.

group: 1.00 ± 0.04, Jagged 1 group: 0.69 ± 0.18) and caspase-3 gene expression (control group: 1.01 ± 0.17, Jagged 1 group: 0.62 ± 0.20) significantly reduced ($P < 0.05$). The protein expression levels of Bax (control group: 1.00 ± 0.01, Jagged 1 group: 0.37 ± 0.02) and caspase-3 (control group: 1.00 ± 0.04, Jagged 1 group: 0.78 ± 0.04) significantly reduced ($P < 0.05$), while that of Bcl-2 (control group: 1.00 ± 0.01, Jagged 1 group: 1.14 ± 0.011) significantly increased ($P < 0.01$). Compared with the Jagged 1 group, the dioscin + Jagged 1 group (Jagged 1 group: 6.11% ± 0.44%, dioscin + Jagged 1 group: 25.60% ± 0.68%) showed a significant increase in the apoptosis ratio ($P < 0.001$). The Bax/Bcl-2 ratio (Jagged 1 group: 0.69 ± 0.18, dioscin + Jagged 1 group: 1.60 ± 0.13) and caspase-3 gene expression (Jagged 1 group: 0.62 ± 0.20, dioscin + Jagged 1 group: 1.17 ± 0.11) significantly increased ($P < 0.05$). This finding indicates that dioscin reversed the inhibitory effect of Jagged 1 on cell apoptosis and suppressed the expression of pro-apoptotic genes and proteins.

As shown in Figure 7D and E, the protein expression levels of Bax (control group: 1.00 ± 0.00, Jagged 1 group: 0.37 ± 0.02, dioscin + Jagged 1 group: 1.87 ± 0.01) and Caspase-3 (control group: 1.00 ± 0.04, Jagged 1 group: 0.78 ± 0.04, dioscin + Jagged 1 group: 2.07 ± 0.01) significantly increased, whereas Bcl-2 (control group: 1.00 ± 0.01, Jagged 1 group: 1.14 ± 0.011, dioscin + Jagged 1 group: 0.47 ± 0.02) significantly decreased with increasing dioscin concentration ($P < 0.05$).

As shown in Figure 7F and G, the rates of cell invasion (control group: 100% ± 10.60%, Jagged 1 group: 131.32% ± 3.56%, dioscin + Jagged 1 group: 84.86% ± 25.36%) and cell migration (control group: 68.79% ± 0.83%, Jagged 1 group: 88.27% ± 0.02%, dioscin + Jagged 1 group: 53.52% ± 0.66%) significantly increased in the Jagged 1 group compared with those in the control group ($P < 0.05$). The rates of cell invasion and cell migration significantly reduced in the dioscin + Jagged 1 group compared with those in the Jagged 1 group ($P < 0.05$). Hence, dioscin can reverse the stimulating impact of Jagged 1 on tumor cell invasion and migration.

As shown in Figure 7H and I, the inhibition of HCT116 cell migration and invasion was quantified using the wound healing and Transwell assays, respectively. The dioscin + Jagged 1 group showed a significant reduction in migration and invasion compared to the Jagged 1 group ($P < 0.05$).

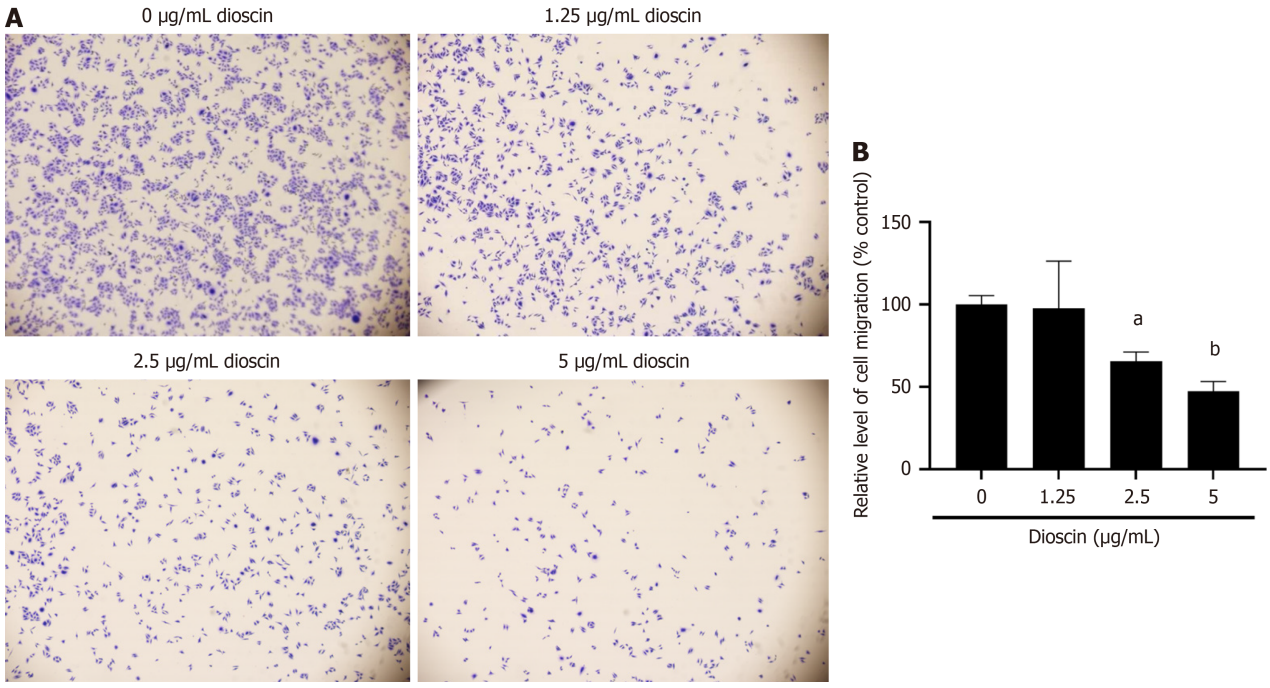


Figure 4 Effect of dioscin on HCT116 cells' invasion (40 ×). A: Transwell method was used to explore the relationship between diosgenin and HCT116 cell invasion and clarify the inhibitory effect of the former on the latter; B: Quantification of HCT116 cell invasion after diosgenin treatment using the Transwell assay. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$ compare to the control group.

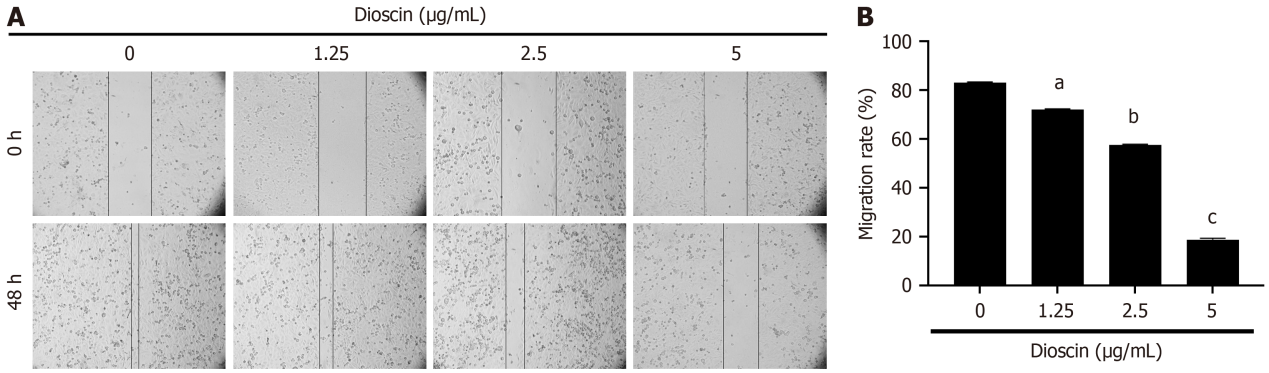


Figure 5 Effect of dioscin on HCT116 cells' migration (100 ×). A: Wound healing assay was conducted to explore the relationship between diosgenin and HCT116 cell migration and clarify the inhibitory effect of the former on the latter; B: Quantification of HCT116 cell migration following diosgenin treatment using the wound healing assay. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compare to the control group.

Notch 1 participates in the Notch 1/Hes 1 signaling pathway mediated by dioscin

To examine the involvement of Notch 1 in the Notch 1/Hes 1 signaling pathway, we analyzed the protein expression levels of Notch 1, Jagged 1, and Hes 1 using Western blot analysis. As demonstrated in Figure 8, the combination of dioscin and Jagged 1 group resulted in lower ($P < 0.05$) protein expression of Notch 1, Jagged 1, as well as Hes 1 than the Jagged 1 group. This finding suggests that dioscin can reverse the activation of the Notch 1/Hes 1 signaling pathway by Jagged 1.

DISCUSSION

CRC is as one of the prevalent malignant neoplasms that affect the digestive system and presents a notable risk to humans. A noteworthy increase in the incidence and mortality rates of CRC has been reported in China; such increase could have been propelled the ongoing enhancement of socio-economic conditions and shifts in lifestyle and dietary patterns. This surge affects individuals in younger age groups[14]. In China, a common approach to the treatment of CRC involves the integration of traditional Chinese medicine. Traditional Chinese medicine has been essential in oncology, significantly aiding in the management of tumors. It has proven effective in alleviating clinical symptoms, boosting

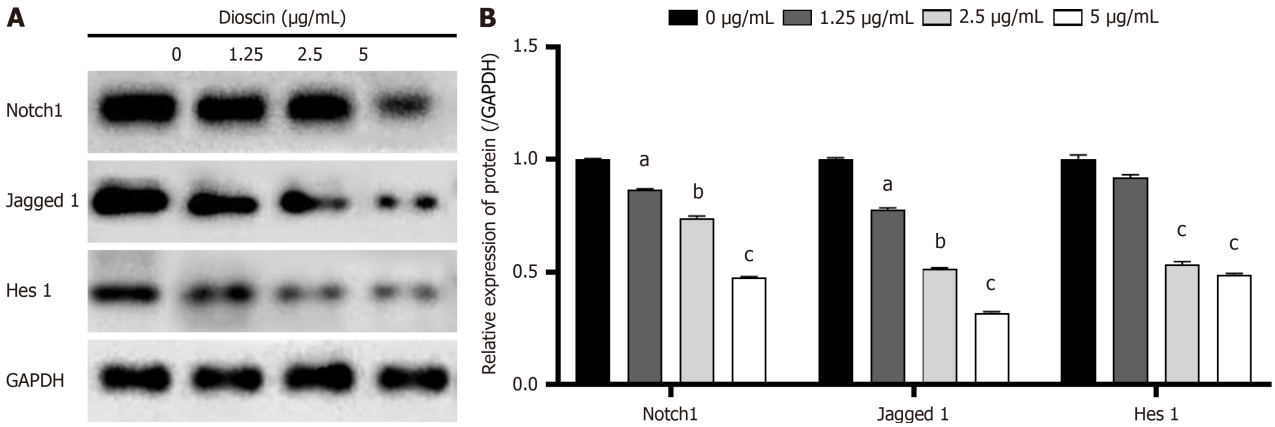


Figure 6 Effect of dioscin on HCT116 cells' expression of Notch 1 signaling pathway-related proteins. A: Cells were treated with dioscin for 48 hours. Western blot analysis was conducted to determine protein levels, with GAPDH as control; B: Quantification of protein expression levels from the Western blot results. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compare to the control group.

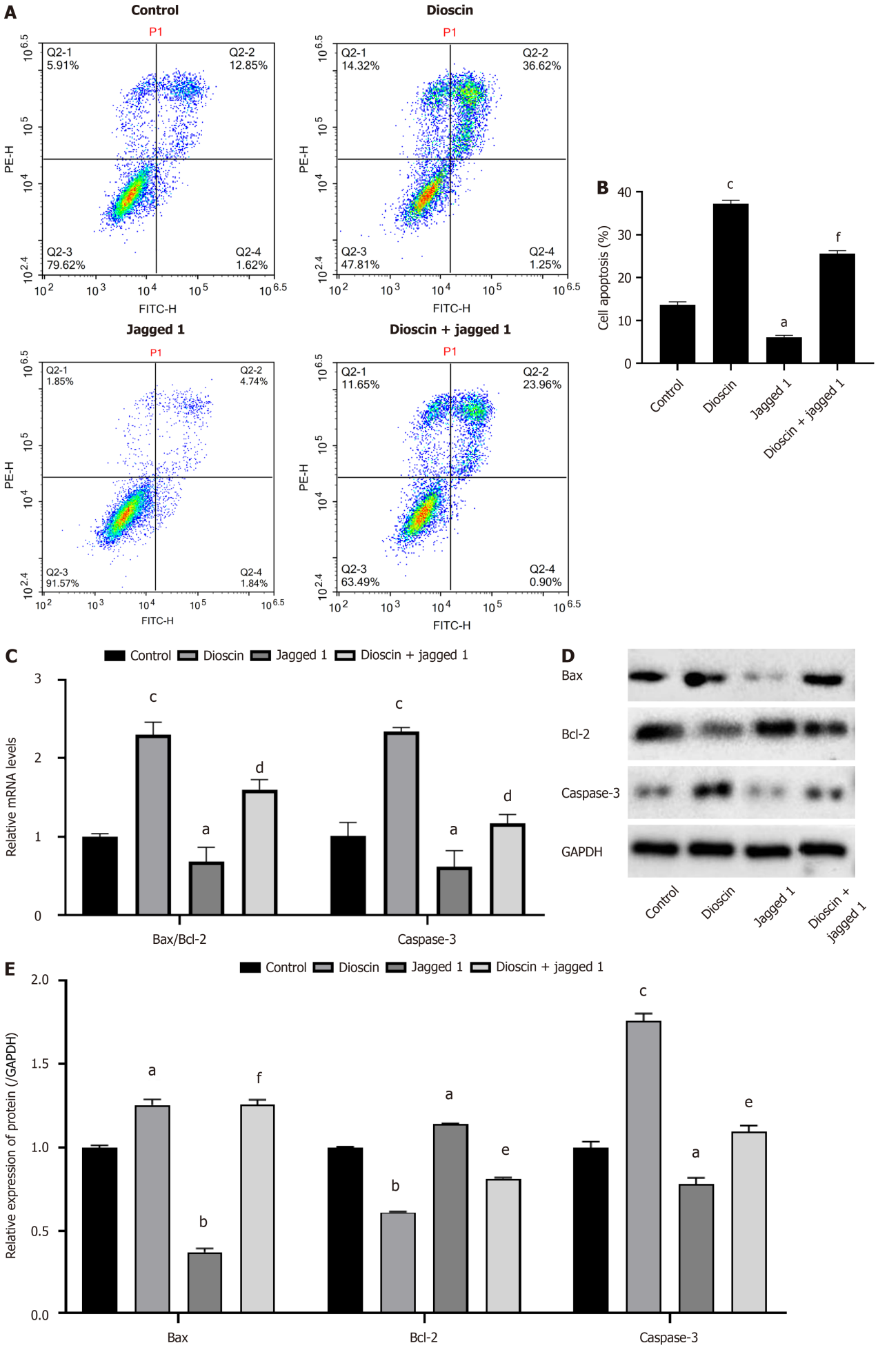
immune response, overcoming chemotherapy resistance, and reducing the likelihood of tumor recurrence and metastasis [15]. Dioscin, an active compound extracted from traditional Chinese herbs, exhibits anticancer activity against multitudinal cancer cell types, including SKOV3 ovarian cancer cells, A549 human lung adenocarcinoma cells, and tumor cells NCI-H446 and NCI-H460[16,17]. The present study initially explored the biological effects and mechanisms of dioscin in the CRC cell line HCT116.

The occurrence and development of tumors are primarily associated with abnormal cell proliferation, low apoptosis rates, and strong invasiveness. Dioscin significantly inhibits the viability of colorectal HCT116 cancer cells, and this inhibition is concentration-dependent, consistent with previous findings. Li *et al*[18] inquisited the sequels of dioscin on the proliferation of human CRC cells SW480, SW620, RKO, and Caco-2 and reported its dose-dependent inhibitory effect.

CRC has diverse etiological factors that involve numerous cellular pathways, for instance Notch signaling pathway. This pathway plays a great role in the initiation as well as progression of CRC[19,20]. The combination of different ligands and receptors can ultimately form the Notch signaling pathway. As a transmembrane protein, Notch receptors can release the binding domain of Notch receptors after binding to related ligands, which will then enter the nucleus, where it binds to RBP-Jk; the binding initiates the transcription of downstream genes and participates in the regulation of processes for instance tumor cell proliferation, apoptosis, invasion, and metastasis[21,22]. The Notch 1 signaling pathway activator, Jagged 1, can increase the number of main ligands in the Notch 1 signaling pathway, thereby directly activating the Notch 1 pathway. A number of research proved that diosgenin can inhibit signaling pathways and physiological activity of thyroid cancer cells, induce cell apoptosis, and promote cell apoptosis[23]. In the present study, dioscin intervention promotes apoptosis in CRC cells HCT116. However, adding the activator Jagged1 will affect the action of diosgenin by weakening its pro-apoptotic effect. Diosgenin can act on CRC cells possibly because it can affect the signaling pathway and inhibit its activity, thereby promoting cell apoptosis.

In the cascade of apoptosis, caspases assume a pivotal function, with caspase-3 situated downstream in this protein family. Once activated, it promotes cell apoptosis[24]. Bax is an important regulatory factor in the activation of caspase-3, and its pro-apoptotic effect may be achieved by enhancing the activity of caspase-3[25]. Bcl-2 can cause glutathione to accumulate in the cell nucleus, leading to an imbalance in nuclear redox status, thereby reducing caspase activity and protecting the cell. Bcl-2 is a substrate for the activated caspase-3[26]. Bcl-2 can bind to Bax, thereby diminishing the pro-apoptotic outcome of Bax and restraining the progression of apoptosis. The equilibrium in the Bcl-2 to Bax ratio functions as a regulatory factor in the initiation of apoptosis[27]. Dioscin can impede the proliferation of HepG2 Liver cancer cells and induce apoptosis by downregulating Bcl-2 expression and concurrently increasing Bax expression[28]. This study found that dioscin intervention significantly increased the *Bax*/*Bcl-2* ratio, promoted *caspase-3* gene expression at the gene level, and increased the expression of Bax and Caspase-3 proteins while downregulating Bcl-2 protein expression. Hence, dioscin affects the mitochondrial apoptosis pathway and regulates the expression of Bcl-2 as well as Bax, thereby inducing apoptosis in cells and the development of CRC. The Notch 1 pathway can be regulated by miRNAs, which participate in tumor progression. miRNA-449a has a negative regulatory effect on Notch1. If miRNA-449a is overexpressed, then it will have a significant effect on Bcl-2, causing a significant reduce in its expression level and a significant increase in Bax expression level[29]. The present work shows that Jagged1 can activate signaling pathways. After intervention with dioscin, it can have various effects on cell HCT116, significantly reducing the expression levels of genes for instance *caspase-3* and related proteins. Hence, diosgenin can inhibit the activeness of signaling pathways and induce the apoptosis of HCT116 cells.

Invasion and migration are two different but related processes by which cancer cells invade local tissues and spread to distant sites. Dioscin can hinder the movement and infiltration of A549 Lung cancer cells by suppressing TGF-β1-induced epithelial-mesenchymal transition (EMT)[30]. Dioscin can also inhibit the invasion and EMT process in lung adenocarcinoma by modulating the AKT/GSK3β/mTOR signaling pathway[31]. In the present experiment, dioscin inhibited the invasion as well as migration of CRC cells HCT116. However, the addition of Jagged1, an activator of the Notch signaling pathway, significantly attenuated the inhibitory effect of dioscin. Hence, dioscin can inhibit cell invasion and migration


A
Control
Dioscin
Jagged 1
Dioscin + jagged 1
P1
Q2-1
Q2-2
Q2-3
Q2-4
5.91%
12.85%
79.62%
1.62%
14.32%
36.62%
47.81%
1.25%
1.85%
4.74%
91.57%
1.84%
11.65%
23.96%
63.49%
0.90%
PE-H
FITC-H
B
Cell apoptosis (%)
C
Relative mRNA levels
Bax/Bcl-2
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D
Bax
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Caspase-3
GAPDH
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Relative expression of protein (/GAPDH)
Bax
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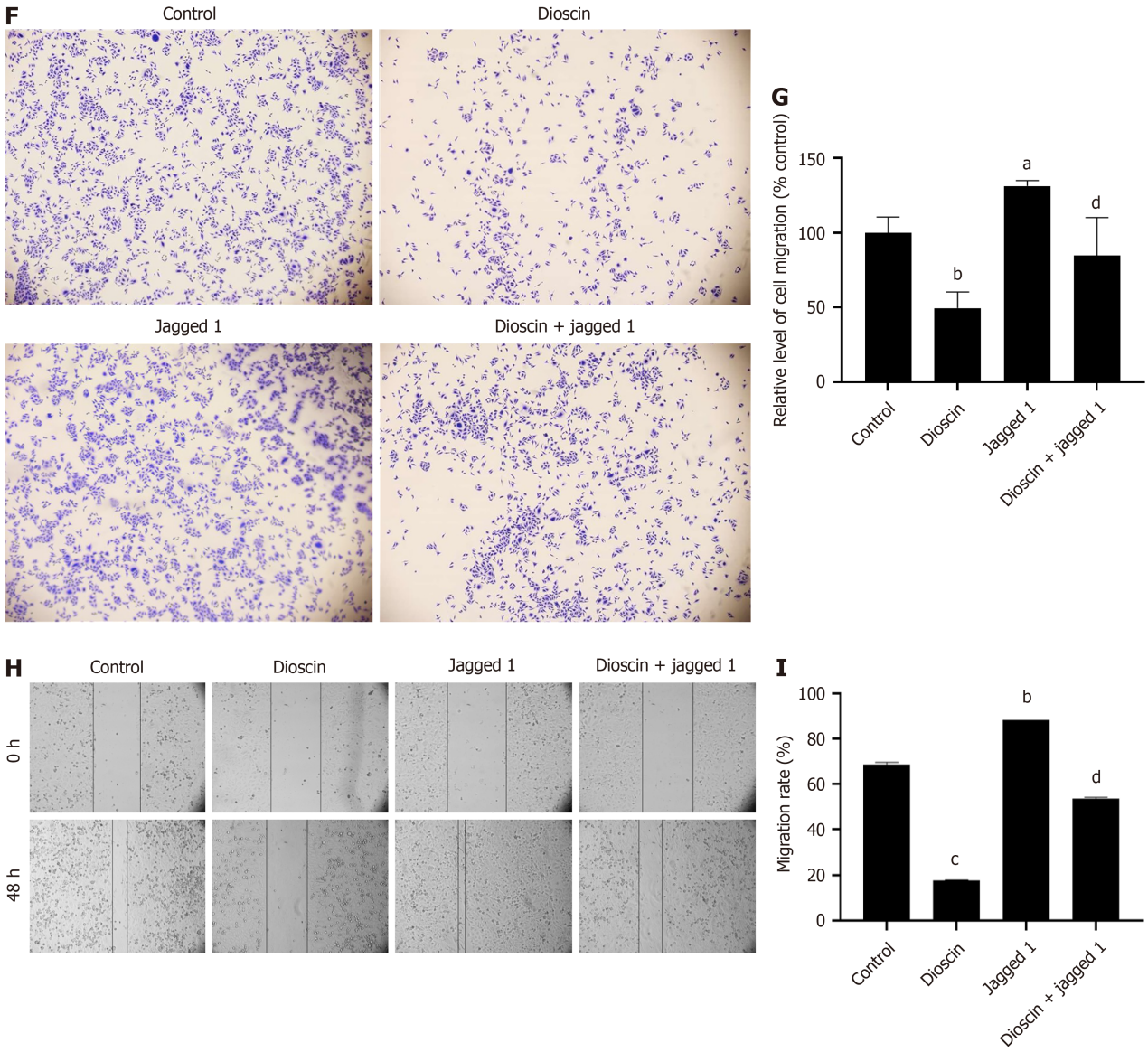

Figure 7 Notch 1 is involved in the inhibitory effect of dioscin on HCT116. HCT116 cells were treated with dioscin (5 μg/mL), Jagged 1 (5 μg/mL), dioscin (2.5 μg/mL) + Jagged 1 (5 μg/mL) for 48 hours. A: Apoptosis in HCT116 cells was revealed using flow cytometry as well as annexin V-FITC as well as PI staining methods; B: Quantification of apoptosis in HCT116 cells from flow cytometry results; C: RT qPCR analysis was performed using caspase-3 mRNA expression and Bax/Bcl-2 ratio; D: Expression levels of Bax, Bcl-2, as well as other proteins were detected using Western blot analysis; E: Quantification of Bax, Bcl-2, and other protein expression levels from the Western blot analysis; F: Transwell method was used to detect the level of inhibition of HCT116 cell invasion; G: Quantification of HCT116 cell invasion inhibition following dioscin treatment using the Transwell assay; H: Wound healing assay was used to detect the inhibition of HCT116 cell migration; I: Quantification of HCT116 cell migration inhibition following treatment using the wound healing assay. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compare to the control group, $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.01$ compare to the dioscin group.

by suppressing the activity of the Notch 1 signaling pathway.

The loss of Jagged-1 is an important factor that promotes the development of CRC by inhibiting tumor cell differentiation and inducing angiogenesis[32]. Hes1 is a downstream factor of Notch-1 and can inhibit Kruppel-like factor 4 (KLF4). High expression of KLF4 has an inhibitory effect on CRC[33]. Previous studies indicated that the Notch1 protein is overexpressed in CRC tissues[34]. In the present work, CRC cells HCT116 were treated with dioscin, and the protein expression levels of Notch1, Jagged-1, and downstream target gene HES-1 in the Notch1 signaling pathway lessened. The inhibitory effect of dioscin was significantly attenuated when the Notch1 signaling pathway was energized by the addition of Jagged1. Hence, dioscin can inhibit the occurrence and development of CRC by suppressing related signaling pathways.

CONCLUSION

In summary, dioscin can inhibit the growth, migration, and other activities of HCT116 cells, thereby effectively promoting cell apoptosis, by preventing the activation of relevant signaling pathways.

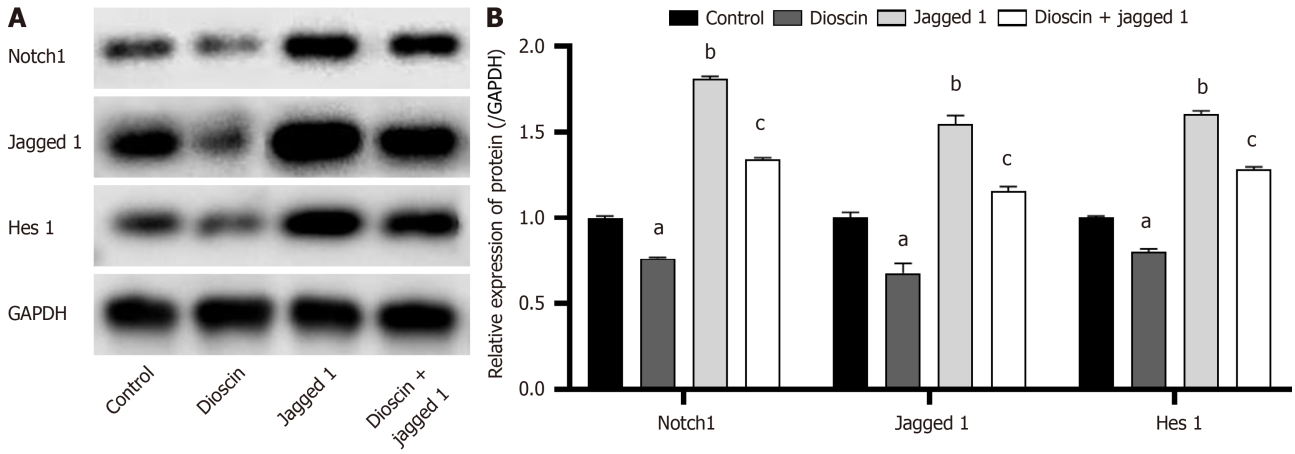

Figure 8 Notch 1 participates in the inhibitory effect of dioscin on the Notch 1/Hes 1 pathway. A: Western blot analysis was adopted to dig up protein expression levels, with GADPH as the control; B: Quantification of protein expression levels from Western blot analysis. Three biological repeats were used and reported as mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$ compare to the control group, $^{c}P < 0.05$ compare to the dioscin group.

FOOTNOTES

Author contributions: Cai XX and Yu J initiated research and conducted data collection; Huang ZF and Tu FY recorded, organized data, and conducted statistical analysis; Cai XX and Yu J wrote the original manuscript and revised the paper; all authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Basic Study

Effects of invigorating-spleen and anticancer prescription on extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathway in colon cancer mice model

Wei Wang, Jing Wang, Xiu-Xiu Ren, Hai-Long Yue, Zheng Li

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Abstract

BACKGROUND

Colon cancer (CC) is one of the most common malignant tumors in the gastrointestinal system. Overall, CC had the third highest incidence but the second highest mortality rate globally in 2020. Nowadays, CC is mainly treated with capecitabine chemotherapy regimen, supplemented by radiotherapy, immunotherapy and targeted therapy, but there are still limitations, so Chinese medicine plays an important role.

AIM

To investigate the effects of invigorating-spleen and anticancer prescription (ISAP) on body weight, tumor inhibition rate and expression levels of proteins in extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway in CC mice model.

METHODS

The CC mice model were established and the mice were randomly divided into 5 groups, including the control group, capecitabine group, the low-dose, medium-dose and high-dose groups of ISAP, with 8 mice in each group, respectively. After 2 weeks of intervention, the body weight and tumor inhibition rate of mice were observed, and the expression of *RAS*, *ERK*, phosphorylated *ERK* (*p-ERK*), *C-MYC* and matrix metalloproteinase 2 (MMP2) proteins in the tissues of tumors were

detected.

RESULTS

Compared with the control group, the differences of body weight before and after treatment was much smaller in the groups of ISAP, with the smallest difference in the high-dose group of ISAP, while the capecitabine group had the greatest difference, indicating ISAP had a significant inhibiting effect on the growth of transplanted tumor in mice. The expression of RAS protein was decreased in the low- and medium-dose groups of ISAP, and the change of *p-ERK* was significant in the medium- and high- dose groups of ISAP. MMP2 protein expression was significantly decreased in both the low-dose and medium-dose groups of ISAP. There were no significant changes in *ERK* in the ISAP group compared to the capecitabine group, while RAS, MMP2, and C-MYC protein expression were reduced in the ISAP group. The expression level of C-MYC protein decreased after treated with ISAP, and the decrease was the most significant in the medium-dose group of ISAP.

CONCLUSION

ISAP has a potential inhibiting effect on transplanted tumor in mice, and could maintain the general conditions, physical strength and body weight of mice. The expression levels of *RAS*, *p-ERK*, *MMP2* and *c-myc* were also decreased to a certain extent. By inhibiting the expression of upstream proteins, the expression levels of downstream proteins in ERK/MAPK signaling pathway were significantly decreased. Therefore, it can be concluded that ISAP may exert an anti-tumor effect by blocking the ERK/MAPK signaling pathway and inhibiting the expression of MMP2 and c-myc proteins.

Key Words: Colon cancer; Invigorating-spleen and anticancer formula; Extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathway; Mice model; C-MYC

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Core Tip: The incidence and mortality of intestinal cancer is increasing year by year. Due to the limited therapeutic means and low survival rate of advanced patients, and the easy development of drug resistance to chemotherapy, targeting and immunotherapy, we have found that spleen-healthy anticancer formula can play an anti-tumor role through the extracellular-signal-regulated kinase/mitogen-activated protein kinase signaling pathway and inhibit the expression of matrix metalloproteinase 2 and *c-myc*.

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INTRODUCTION

Colon cancer (CC) is among the most common malignant tumors of the digestive tract, ranking third and second worldwide in 2020 in terms of incidence and mortality, respectively[1]. CC typically arises from epithelial cells that line the lumen of the colon, which renew themselves every five days from a stem cell population located at the base of colonic epithelial cell crypts, and is the consequence of a multistep neoplastic process that extends over several years. Treatment of CC is largely based on the chemotherapeutic drug capecitabine, while there have also been remarkable advances in surgery, immunotherapy, stereotactic radiotherapy and new chemotherapy drugs; however, the incidence rate of and mortality from CC have continued increasing. The rising incidence of CC in younger people is related to dietary patterns, excess body weight, and lifestyle factors[2,3]. Further, drug resistance develops in nearly all patients with CC, leading to a decreased in the therapeutic efficacy of anticancer agents[4]. In addition, the presence of nodal involvement (stage III) predicts for a 60% likelihood of recurrence. Surgical resection is highly effective for early-stage CC, providing cure rates of over 90% and 75% in patients with stage I and II disease, respectively[5]. However, approximately 30% of the patients with CC have distant metastasis at diagnosis, indicating that they are unsuitable for surgical treatment[6,7]. The 5-Fu derivative, capecitabine, is an important agent for treatment of CC at all stages. In traditional Chinese medicine, CC is categorized as "abdominal mass" and "perianal abscess". The basic pathogenesis of CC superficially involves asthenia, while deficiency of vital Qi is the pathogenic characteristic of the whole disease process, with “cancer toxin”, “phlegm and blood stasis”, and “dampness and heat” as relevant pathogenic factors. Invigorating-spleen and anticancer prescription (ISAP) has shown good therapeutic effects against CC[8]. Previous cell experiments have demonstrated that ISAP can block the cell cycle and inhibit tumor cell growth and migration[9]. *In vivo* experiments showed that levels of vascular endothelial growth factor and microvascular density, which are closely related to angiogenesis, are significantly

inhibited in tumor-bearing mice; however, the anti-tumor mechanism underlying these effects of ISAP remains unclear. In this study, we evaluated the effects of different doses of ISAP on body weight and tumor inhibition rate in a CC mouse model, and detected the expression levels of proteins in the extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway, to explore the mechanism underlying the effects of ISAP in CC.

MATERIALS AND METHODS

Animals and cells

A total of 40 Kunming mice (4-6 weeks old, 18-20 g) were purchased from Liaoning Changsheng Biotechnology Co., LTD. CT26WT CC cells were purchased from Dalian Meilun Biotechnology Co., LTD.

Reagents and instruments

BCA Kit (Beyotime, China), RAS antibody (Abcam, United Kingdom), ERK monoclonal antibody, pERK monoclonal antibody (Cell Singnaling, United States), matrix metalloproteinase 2 (MMP2) monoclonal antibody (Boster, China), myc antibody (Abcam, United States), Goat anti-rabbit IgG (ZSGB bio, China), β-actin (AbCAM-AbBOT, United States), protein Marker (Fermetas, Germany); Automatic microplate reader (Omega, United States), Electrophoresis apparatus (BIO-RAD, United States), tissue dehydrator (Leica Microsystem, China), microelectronic balance (METTLER TOLEDO, Swiss), low speed centrifuge (Eppendorf, Germany).

Experimental drugs

ISAP is mainly composed of *Heterophylla falsestarwort* root 15 g, *Poria cocos* 15 g, *Atractylodes macrocephala* 15 g, *Prepared licorice* 10 g, dried *Tangerine* 10 g, *Rhizoma Pinellinae Praeparata* 10 g, *Bulbus iphigeniae indicae* 15 g, *Smilax glabra Roxb* 15 g, *Hedyotis diffusa* 15 g, *Fritillaria thunbergii* 15 g, and *Jobstears* seed 30 g and purchased from Outpatient Pharmacy of The First Affiliated Hospital of Liaoning University of Traditional Chinese Medicine. All the drugs were in accordance with the description of China pharmacopoeia 2015. After immersed in warm water for 1 hour, drugs were boiled 3 times for 1 hour each time. Subsequently, the combined decoction was centrifuged to remove the residue of drugs and stored at 4 °C for reserve. Tablets of capecitabine was purchased from Qilu Pharmaceutical Company, China. The specific dosage for mice were converted referring to the coefficient between human and mice.

CC mice model

Cell suspension (cell number: 5×10^6/mL) was prepared from the CT26WT CC cells. A 1 mL syringe was used to suck the cell suspension and subcutaneously injected into the right axilla until a bulge was observed. Finally, palpable ovoid masses under the skin indicated the CC mice model was successfully established. The CC mice were numbered, and randomly divided into 5 groups, including the control group, capecitabine group, low-dose, medium-dose and high-dose groups of ISAP using the table of random numbers. Different interventions were administered for 2 weeks. Grouping and doses were shown in Table 1.

At the end of the second week, mice were euthanatized by cervical dislocation. Subsequently, mice were placed in supine position and their limbs were fixed with big nails. The tumor tissues were collected and then cleaned with 0.9% sodium chloride, followed by weighing and photographing. Moreover, the general condition of mice in each group (including the food intake, skin color and activity) was observed. The changes of body weight in each group were observed before and after interventions. Tumor inhibition rate = (1 - tumor weight in the treatment group/tumor weight in the control group) × 100%.

Western blotting for the expression levels of related proteins in the MAPK/ERK signaling pathway

Appropriate amount of tumor tissues obtained from each group were cut into pieces and put into protein lysate for further homogenization. Then the mixture was sucked by pipette and transferred to centrifugal tube. After centrifuged, the total protein was collected from the supernatant and the protein concentration was determined by a BCA kit. A total of 100 μg proteins were sampled, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto polyvinylidene fluoride (PVDF) membrane and blocked with antibodies. The primary antibodies, including *RAS*, *ERK*, *pERK*, *C-MYC*, and *MMP2*, were added to the PVDF membrane at a dilution ratio of 1:1000 and incubated overnight. Then the secondary antibodies were diluted by tris-buffered saline at a ratio of 1:1000 and added to the membrane. After the incubation at room temperature for 2 hours, chemiluminescence was used for color development. The film was scanned and imaged by a scanner, and the molecular weight and gray values of the target bands in the image were analyzed by an Alpha gel image processing system.

Statistical analysis

SPSS 26.0 was used for statistical analysis of the experimental data. All experimental data were expressed as mean ± SD. One-way ANOVA was used for comparison among groups. Student's *t*-test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

Table 1 Grouping and interventions for mice

Group	Number	Dose, g/kg
Control group	8	/
Low-dose group of ISAP	8	9.5
Medium-dose group of ISAP	8	19
High-dose group of ISAP	8	38
Capecitabine group	8	19

ISAP: Invigorating-spleen and anticancer prescription.

RESULTS

Comparison of the general conditions in groups

Two weeks after intervention, the tumor-bearing mice in the capecitabine group had a poorer mental state, obvious emaciation and dull skin color, with less food intake. Compared with the control group, mice in the low-, medium- and high-dose groups of ISAP all showed better mental state, good activity and no obvious abnormality in skin color, especially in the high-dose group.

Comparison of body weight in groups

There was no significant difference in body weight before modeling among all the groups. After interventions, the body weight in all groups decreased compared with that on day D0 before treatment, especially in the control group and capecitabine group ($P < 0.05$; Table 2). The difference of body weight before and after treatment in the low-, medium- and high-dose groups of ISAP was significantly smaller than that in control group (Table 2).

Comparison of tumor inhibition rates in groups

As shown in Table 3 and Figure 1, different doses of ISAP all showed significant inhibitory effect on the growth of transplanted tumor in mice. The average tumor weight of the high- and medium-dose groups was significantly lower than that of the control group, especially in the high-dose group. There was no significant difference of the average tumor weight between the low-dose group and the control group.

Comparison of the expression levels of RAS, ERK and pERK protein in ERK/MAPK signal pathway in groups

As shown in Figure 2A and B, compared with the control group, the expression levels of RAS protein were significantly decreased in both the low- and medium- dose groups of ISAP, but not in the high-dose group of ISAP. There was no significant change in the protein expression of ERK before and after the low-, medium- and high-dose treatment of ISAP. However, the phosphorylated *ERK* (*p-ERK*) decreased significantly with the increase of the dose of ISAP, especially in the medium- and high-dose groups of ISAP.

Comparison of MMP2 and C-MYC protein expression in groups

As shown in Figure 2C and D, the expression of MMP2 protein was significantly decreased in both the low- and medium-dose groups of ISAP compared to the control group. The protein expressions of c-myc were all significantly decreased after treated with low-, medium- and high- dose ISAP ($P < 0.05$), with the most significant decrease in the medium-dose group of ISAP. There was no statistical difference between the medium- and high-dose groups.

DISCUSSION

Traditional Chinese medicine theory holds that the occurrence of CC is mainly related to the deficiency of vital Qi and the invasion of external pathogens. As written in Jing Yue's Complete Work - Miscellaneous internal diseases - Abdominal mass, most of the patients with splenogastric asthenia and related debilitating disorders have lumps in the abdomen, accompanied by pain or swelling. Further, as written in Lingshu - Nine Needles, pathogenic wind from four seasons and eight directions invading the meridians of the human body result in blood stagnation and stubborn diseases, highlighting the potential for exogenous pathogens in qi and blood pathways inside the human body to cause tumor-related diseases. CC is caused by the combination of internal and external factors, and specifically originates from the deficiency of vital Qi, with invasion of external pathogens another important pathogenic factor. ISAP was developed based on the above etiology and pathogenesis of CC described above, and is mainly composed of a decoction of six noble herbs, supplemented with other traditional Chinese medicines which can dissipate masses, such as *Herba Hedyotidis*, *Scutellariae Barbatae Herba*, *Cremastrae Pseudobulbus*, *Glabrous Greenbrier Rhizome*, *Thunberg Fritillary Bulb*, *Coicis Semen* among others; all these drugs have been demonstrated to have clear anti-tumor effect[10-13]. In addition, clinical studies have shown that a modified decoction of six noble herbs can significantly improve anorexia in patients with malignant tumors after

Table 2 The effect of invigorating-spleen and anticancer prescription on body weight of mice (mean ± SD, g)

Group	D0	D14	D14 - D0
Control group	20.22 ± 0.01	18.15 ± 0.03[a]	2.07 ± 0.03
Low-dose group of ISAP	20.47 ± 0.71	18.89 ± 0.34[a]	1.58 ± 0.83
Medium-dose group of ISAP	20.28 ± 0.02	19.08 ± 0.34[a]	1.20 ± 0.34
High-dose group of ISAP	20.39 ± 0.05	19.51 ± 0.59[a,b,c]	0.87 ± 0.07
Capecitabine group	20.46 ± 0.01	16.29 ± 0.08[a]	4.18 ± 0.06

[a]$P < 0.05$ compared to the control group.
[b]$P < 0.05$ compared to the low-dose group of invigorating-spleen and anticancer prescription (ISAP).
[c]$P < 0.05$ compared to the medium-dose group of ISAP.
ISAP: Invigorating-spleen and anticancer prescription; D0: Day 0; D14: Day 14.

Table 3 The effect of invigorating-spleen and anticancer prescription on tumor inhibition rate of mice

Group	Tumor weight (g; mean ± SD)	Tumor inhibition rate (%)
Control group	1.38 ± 0.12	/
Low-dose group of ISAP	1.22 ± 0.03	0.12 ± 0.02
Medium-dose group of ISAP	1.07 ± 0.34[a]	0.31 ± 0.08[b]
High-dose group of ISAP	0.9 ± 0.08[a]	0.34 ± 0.05[b]
Capecitabine group	0.53 ± 0.13[a]	0.59 ± 0.03[b]

[a]$P < 0.05$ compared to the control group.
[b]$P < 0.05$ compared to the low-dose group of invigorating-spleen and anticancer prescription.
ISAP: Invigorating-spleen and anticancer prescription.

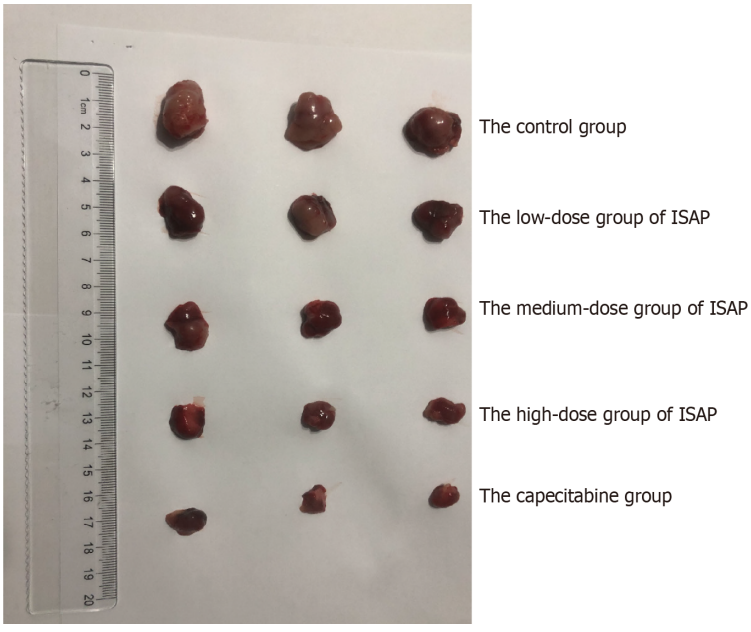

Figure 1 Tissues of transplanted tumors in mice. ISAP: Invigorating-spleen and anticancer prescription.

chemotherapy[14,15], and achieve the effects of invigorating qi, strengthening spleen, anti-cancer and detoxification through the purging-tonifying therapy based on the combinated application of all these drugs. After the spleen and stomach was strengthened, the healthy qi might be restored and the cancerous poison could be exorcised. In this study, results showed that the difference of body weight before and after treatment in different groups of ISAP were much smaller compared to the control group or the capecitabine group, indicating that the body weight of mice was effectively

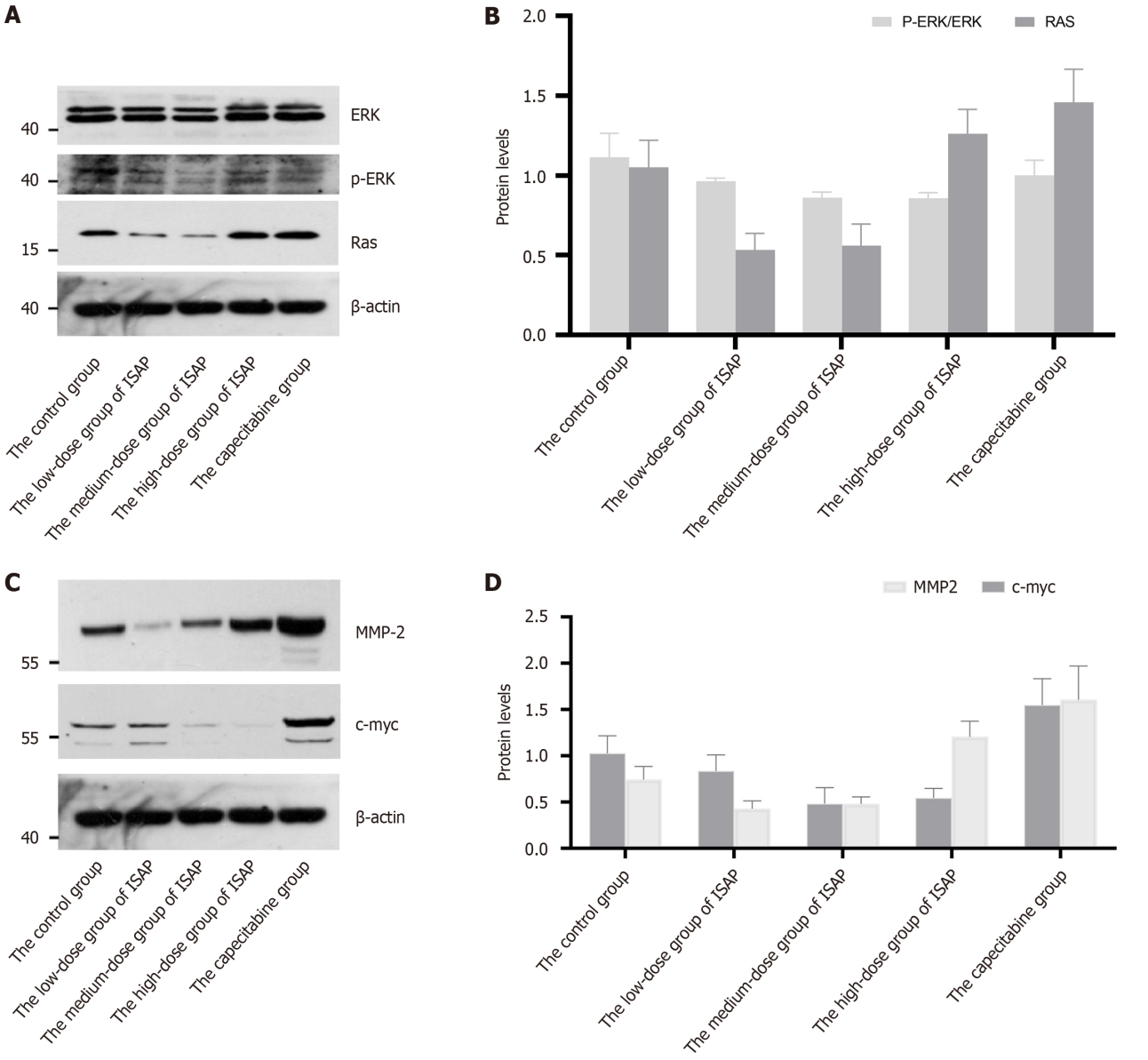

Figure 2 Protein expressions in groups. A: Protein expressions of RAS, extracellular-signal-regulated kinase (ERK) and Perk in groups; B: Comparison of the phosphorylated-ERK/ERK and RAS protein expressions in groups; C: Comparison of matrix metalloproteinase 2 (MMP2) and c-myc protein expressions in groups; D: Comparison of MMP2 and c-myc protein expressions in groups. ISAP: Invigorating-spleen and anticancer prescription; ERK: Extracellular-signal-regulated kinase; p-ERK: Phosphorylated extracellular-signal-regulated kinase; MMP2: Matrix metalloproteinase 2.

stabilized by ISAP, and the general conditions of mice was further maintained. Besides, ISAP had a significant inhibitory effect on the growth of transplanted tumor in mice, and the tumor inhibition rate in the high- and medium-dose groups of ISAP was significantly higher than that in the control group, with the highest in the high dose group, showing a dose-effect relationship to some extent.

MAPK signaling pathway is one of the most widely studied signal transduction pathways, which extensively exists in various human cells. MAPK family proteins in mammals are mainly consisted by *ERK1/2*, *JNK1/2* and *P38 MAPK*; *ERK* mainly has two forms, namely *ERK1* and *ERK2*, and it is activated by the upstream activators of *Ras/Raf* in the sigaling pathway. Together, they constitute the classical Ras-Raf-MAPK signaling pathway, which involves cell proliferation, survival, invasion, migration, apoptosis, glucose metabolism, and DNA repair, are up-regulated oncogenic cascade signals in a variety of tumors[16]. The abnormal activation of Ras-Raf-MAPK signaling pathway promotes cell proliferation, inhibits cell apoptosis, and promote the invasion of tumor tissues to the surrounding or distant areas[17,18]. Studies have demonstrated that MAPK signaling pathway is closely associated with the pathogenesis of colorectal cancer [19], and RAS/MAPK/ERK pathway plays an important regulatory role in cell proliferation and tumor metastasis. *Ras* is involved in transmembrane signal transduction. Activated *Ras* rapidly phosphorylates Raf, turns extracellular signals into specific intracellular signals and links them to nuclear response, thereby amplifying signaling cascade. After binding to *MEK*, *Raf* phosphorylates *MEK*, and phosphorylated *MEK* further phosphorylates *ERK* at two sites (Thr^{188} and Tyr^{190})[20, 21]. Ultimately, *p-ERK* can be transferred from the cytoplasm to the nucleus to activate related transcription factors. In this study, we found that ISAP could inhibit the expression of *RAS* as well as *p-ERK*, both of which were significantly reduced in ISAP group tumors compared with those from control and capecitabine group mice. Expression of the

upstream regulator *RAS*, was not reduced in the high-dose ISAP group, likely due to the greater toxicity effects on this indicator of the drug when used at the high dose, which in turn had an effect on this indicator. However, high and low doses of ISAP did not have a greater effect on the downstream *p-ERK* molecule. We speculate that *ERK* may function synergistically with other molecules. Overall, ISAP can affect CC through the ERK/MAPK signaling pathway.

MMPs are proteolytic enzymes secreted by both tumor cells and normal human cells, among which *MMP-2* and *MMP-9* are the most common subtypes, expressed in the tissues of various malignant tumor and could destroy the histological barriers to facilitate cell invasion. *MMPs* have been found to be closely related to tumor metastasis in liver cancer. As reported by Chen *et al*[22], the high expression of *MMP2* in the tissues of liver cancer could promote the formation of tumor thrombus, which is one of the most reliable indicators of invasion and metastasis of liver cancer. In this experiment, we selected *MMP2* for detection, and found that it was significantly reduced in low and medium dose ISAP groups compared with the control and capecitabine groups, while its levels were not reduced in the high dose ISAP group. We consider that the reasons for these findings may be similar to those discussed above regarding the effects of ISAP on *RAS* levels; that is, they may be due to drug toxicity; however, overall, our data indicate that ISAP can reduce *MMP2* levels.

C-myc is the most ubiquitous member of the myc oncogene family, and participate in the regulation a variety of processes, including the apoptosis, growth and invasion of tumor cells, as well as the angiogenesis and differentiation in tumors[23-26]. A wide variety of naturally occurring tumors exhibit both chromosomal translocations and amplification of the *c-myc* locus that result in constitutive overexpression of myc proteins[27], indicating the inhibition of *c-myc* overexpression could inhibit the occurrence and development of tumors. *C-myc* is also deeply involved in MAPK signaling pathway[28]. The activated ERK is able to activate its downstream substrates, resulting in the changes in gene expression and the levels of various transcription factors[29]. The uncontrolled and abnormal expression of *c-myc* has been found in more than half of the tumors in human. Excessive amplification and abnormal expression of *c-myc* have also been discovered in CC[30].

CONCLUSION

Our experimental data reveal that ISAP can reduce *C-MYC* expression and, taken together, indicated that ISAP may exert antitumor effects by inhibiting ERK/MAPK signaling pathway activation.

FOOTNOTES

Author contributions: Li Z conceived the work, supervised the writing, and provided intellectual input; Wang W and Wang J were responsible for most of the manuscript; All authors conducted the literature search.

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SYSTEMATIC REVIEWS

Prognostic value of neutrophil-to-lymphocyte ratio in gastric cancer patients undergoing neoadjuvant chemotherapy: A systematic review and meta-analysis

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Abstract

BACKGROUND

In recent studies, accumulating evidence has revealed a strong association between the inflammatory response and the prognosis of many tumors. There is a certain correlation of neutrophil-to-lymphocyte ratio (NLR) with the prognosis in gastric cancer (GC) patients undergoing neoadjuvant chemotherapy (NAC). However, the existing research results have remained controversial.

AIM

To explore the relationship between NLR ratio and prognosis of GC patients receiving NAC.

METHODS
A thorough systematic search was performed in databases such as PubMed, Embase, Web of Science, and Cochrane Library, the search is available until February 29, 2024, and studies exploring the interaction of NLR with clinical outcomes were collected. Relevant studies meeting pre-defined inclusion and exclusion criteria were carefully chosen. The outcomes included progression-free survival (PFS), relapse-free survival, disease-free survival (DFS), and overall survival (OS). The hazard ratio (HR) and its corresponding 95% confidence interval (CI) were utilized for estimation.

RESULTS
Our analysis encompassed 852 patients and incorporated data from 12 cohort studies. The comprehensive analysis revealed a significant association of high NLR with reduced OS (HR = 1.76; 95%CI: 1.22-2.54, P = 0.003), relapse-free survival (HR = 3.73; 95%CI: 1.74-7.96, P = 0.0007), and PFS (HR = 2.32; 95%CI: 1.42-3.81, P = 0.0008) in patients. However, this correlation in disease-free survival was not significant. NLR demonstrated its crucial role in effectively predicting the OS of GC patients undergoing NAC at different detection times, ages, regions, and NLR thresholds.

CONCLUSION
In GC patients receiving NAC, an elevated NLR is strongly associated with reduced OS and PFS. NLR has become an effective biomarker for patient prognosis evaluation, providing valuable insights for the treatment strategies of NAC in GC patients.

Key Words: Neutrophil-to-lymphocyte ratio; Gastric cancer; Neoadjuvant chemotherapy; Prognostic factors; Meta-analysis

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Core Tip: This study systematically evaluated the relationship between neutrophil to lymphocyte ratio (NLR) and prognosis in patients with gastric cancer (GC) receiving neoadjuvant chemotherapy (NAC). This is the first meta-analysis to evaluate the association between NLR and prognosis in patients with NAC for GC. In summary, NLR levels are highly correlated with the prognosis of GC patients receiving NAC, and NLR can be used as an effective biomarker for prognosis assessment of GC patients.

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INTRODUCTION

Gastric cancer (GC) is a malignancy affecting the digestive tract and poses a significant health risk to individuals. Based on the latest global cancer statistics from the International Agency for Research on Cancer of the World Health Organization (GLOBOCAN2022), GC ranks fifth among all malignancies in terms of both incidence and mortality, involving 980000 new cases and 650000 deaths[1]. The emergence and progression of GC can be intricately linked to factors such as our dietary habits, lifestyle, and genetic factors. Surgery is an important approach for the treatment of GC, and there is a comprehensive treatment plan including chemoradiotherapy, immunology, and targeted therapy. In recent years, diagnostic techniques, surgical techniques, and the concept of accelerated recovery during perioperative period have been continuously developed, and there has been a notable enhancement in the 5-year survival among patients with GC. However, a considerable proportion of GC patients at progressive or advanced stage are unable to undergo surgery after diagnosis or relapse after radical gastrectomy, and the treatment effect is not satisfactory. Therefore, the application of neoadjuvant chemotherapy (NAC) will be beneficial for patients with progressive or advanced GC. NAC is a recommended therapeutic strategy for managing locally advanced GC. It serves multiple purposes, including reducing tumor stage and volume, enhancing the R0 resection rate, addressing micrometastasis, and evaluating the sensitivity and tolerance of chemotherapy drugs. Importantly, NAC does not contribute to an increase in the incidence or mortality of the disease[2]. Although there are many NAC regimens for GC, the most frequently employed combinations include S-1 + oxaliplatin and oxaliplatin + capecitabine[3-5]. To identify individuals who can potentially derive benefits from NAC, it becomes imperative to discover precise predictors that can effectively determine NAC outcomes, which is of great significance for improving patients' survival outcomes and providing better treatment measures.

Recently, extensive investigations have been conducted to explore the correlation of systemic inflammatory response with the occurrence and development of tumors, revealing a significant correlation between systemic inflammatory

response and prognosis[6-8]. They exert a pivotal influence at every stage of tumor development. The proliferation, migration, and invasive potential of malignant cells, the failure and metastasis of an anti-tumor immune response, and other characteristics will change with changes in inflammatory cells[7]. Studies have demonstrated that certain peripheral blood parameters, including white blood cell, neutrophil, lymphocyte, monocyte, and platelet count, as well as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), can serve as potential indicators of the inflammatory status associated with tumors[9,10]. It has been reported that NLR has been proven to be a valuable prognostic indicator for predicting patient outcomes in the context of GC treatment[11,12], which indicates the correlation of neutrophil count with lymphocyte count. In addition, this indicator can effectively reflect the inflammation and immune status of the body when exposed to external pathogens or internal physiological changes. The study by Gong *et al*[13] on 91 patients with locally advanced GC undergoing NAC and D2 gastrectomy showed that NLR holds promise as a dependable prognostic marker for predicting survival outcomes in locally advanced GC patients prior to NAC. NLR and PLR were significantly decreased, patient overall survival (OS) was prolonged, and NLR levels were negatively correlated with the survival prognosis after NAC[13]. It can be concluded that exploring the changes of NLR in GC patients receiving NAC is expected to provide novel insight and methodology for improving patient survival.

Although most researchers have emphasized the prognostic value of NLR for GC patients undergoing radical gastrectomy, only a few researchers have focused on the importance of NLR for GC patients receiving NAC. To date, limited research has been conducted to investigate the prognostic relevance of the NLR specifically in patients undergoing NAC. Therefore, this meta-analysis aims to evaluate the impact of NLR on the prognosis of GC patients receiving NAC.

MATERIALS AND METHODS

Literature search

The study methodology was duly registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42024505051), adhering to the recommended guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Network Meta-Analyses (PRISMA2009)[14]. The formulation of search strategies was carried out collaboratively by three investigators, namely Wei ZH, Tuo M, and Ye C. Subject words and keywords were selected to search multiple databases such as PubMed, Embase, Cochrane Library, and Web of Science. The search encompassed the entire database inception period up until February 29, 2024. A wide range of phrases were used in the search, such as "gastric cancer", "neutrophil", "neutrophil-to-lymphocyte ratio (NLR)", "neoadjuvant chemoradiotherapy". Literature retrieval strategies are presented in Supplementary Table 1.

Study selection

The studies included in the analysis should comply with PICOS principles: (1) P: GC patients undergoing NAC (which refers to systemic chemotherapy performed before local treatment. The purpose of this therapy is to reduce the tumor size and kill invisible metastatic cells early, thus facilitating subsequent surgery, radiotherapy, and other treatments for GC patients); (2) I: Relatively high NLR value; (3) C: Relatively low NLR value; (4) O: Patient's prognosis [OS, relapse-free survival (RFS), disease-free survival (DFS), and progression-free survival (PFS)]; and (5) S: Observational study. The following criteria were used for study exclusion: (1) Reviews, comments, conference abstracts, case reports, and letters; (2) Studies that lacked adequate information to calculate the hazard ratio (HR) and its corresponding 95% confidence interval (CI); (3) Studies that did not provide survival data; (4) Studies using duplicate data or overlapping databases. Three researchers (Wei ZH, Tuo M, and Ye C) conducted a thorough review of the titles and abstracts of the studies obtained from the database, followed by the retrieval and evaluation of full-text articles to identify eligible studies for inclusion. During the process of study selection, any disagreements were resolved through consensus during the study selection process.

Data extraction

Data extraction was carried out independently by two investigators (Wei ZH and Tuo M). Any disagreements were resolved through consensus of co-authors. The extracted information included first author's name, study period, country (study site), study type, NLR detection time, study population, NAC regimen, total sample size, sample size for different genders, patient age, tumor-node-metastasis staging of GC, NLR threshold, as well as HRs (95%CI) for OS, RFS, DFS, and PFS. Importantly, the collected data were stratified into high NLR/low NLR group. In cases where the research data reported as low NLR/high NLR group[15,16], the reciprocals of the corresponding HR values and their CIs were collected, and the upper and lower confidence limits were interchanged accordingly. This ensured that the low NLR/high NLR group was appropriately converted to the high NLR/low NLR group for subsequent statistical analysis.

Quality assessment

The assessment of studies included in the meta-analysis was conducted using the Newcastle Ottawa Scale, which evaluated three aspects: Selection, comparability, and outcomes. Each study could obtain a maximum score of 9 on the scale[16]. Studies with scores ranging from 7 to 9 were categorized as high-quality[17].

Statistical analysis

To assess the prognostic significance of the NLR in GC patients undergoing NAC, pooled HRs and their corresponding 95%CIs were calculated. Heterogeneity was measured through Cochran's *Q* test and higgins I^2 statistic[18]. A random-effects model was adopted for data merging. In addition, subgroup analysis and sensitivity analysis were conducted to validate the robustness of results pertaining to OS and PFS and to explore potential factors contributing to the observed heterogeneity. To evaluate the potential presence of publication bias, funnel plots and the Egger's test were adopted. Statistical significance was considered when the *P* value was less than 0.05. All statistical analyses were conducted through STATA 15.0 and Review Manager (Version 5.4).

RESULTS

Study characteristics

After the initial search of the databases, a total of 203 articles were identified. Following a thorough examination, 34 articles were excluded due to duplicate publications. Upon assessing the titles and abstracts of the residual studies, 156 studies were ruled out. Subsequently, a comprehensive assessment was conducted on the full texts of 13 articles. 4 studies were ruled out primarily because they lacked the necessary data required for conducting survival analysis. In the final analysis, altogether 9 studies, comprising 852 patients, were enrolled in the meta-analysis[13,15-17,19-23] (Figure 1).

Among the 9 eligible studies, geographic diversity was observed, with 1 study performed in Japan, 1 in Egypt, 1 in Rome, 1 study in Poland, and the remaining 5 in China. It was worth noting that each article included 2 cohort studies in the 3 eligible articles[17,20,22], with a total of 12 cohort studies. One study was prospective[21], with the remaining 11 being retrospective[13,15-17,19,20,22,23]. Between 2020 and 2023, a total of 9 cohort studies were published in English. Additionally, 3 were published in English in 2013, 2014, and 2017, respectively. Of the 12 cohort studies, the oldest was published in 2013 by Jin *et al*[17]. All studies adopted NAC and were grouped into high NLR/low NLR group for analysis. Regarding the measurement of NLR, 2 studies measured NLR before treatment[16,19], and 10 studies examined NLR before treatment and after NAC[13,15,17,20-23]. In terms of NLR evaluation, 10 studies explored the prognostic significance of NLR for OS[13,15,16,19-23], 4 studies explored its prognostic significance for PFS[17,19,23], 2 studies explored its prognostic significance for RFS[20], and 2 studies explored its prognostic significance for DFS[15,16]. Characteristics of the included studies are listed in Table 1.

Study quality

The Newcastle Ottawa Scale scores of all 12 studies were between 7-8, indicating the high quality of the studies (Supplementary Table 2).

Meta-analysis results

NLR and OS: Our investigation focused on exploring the correlation of NLR with OS in a total of 959 patients across 10 cohort studies. In these studies, 2 only provided NLR values before the treatment, while 8 provided NLR values both before treatment and after NAC. Given the significant heterogeneity observed among the studies (I^2 = 58%, *P* = 0.01), a random-effects model was employed (Figure 2A). The analysis revealed a significant association of high NLR with shorter OS in GC patients undergoing NAC (HR = 1.76, 95%CI: 1.22-2.54; *P* = 0.003, Figure 2A).

To test potential heterogeneity, subgroup analysis was performed based on the detection time, age, region, and NLR threshold. The findings of these analyses are presented in Table 2. Firstly, significant associations were found between elevated NLR and shorter OS in both pre-treatment (HR = 1.76; 95%CI: 1.22-2.54; *P* = 0.003) and post-treatment (HR = 1.83; 95%CI: 1.22-2.75; *P* = 0.004) studies. Secondly, age-based subgroup analysis revealed that the predictive value of NLR for OS was limited to patients aged 60 years and above (HR = 2.13, 95%CI: 1.24-3.67; *P* = 0.006), while for patients under 60 years, the predictive significance of NLR for OS was not marked (HR = 1.40, 95%CI: 0.79-2.48; *P* = 0.250). Thirdly, region-based subgroup analysis revealed that NLR had no significant predictive significance for OS in the Asian population (HR = 1.36; 95%CI: 0.94-1.97; *P* = 0.100). However, NLR showed marked predictive significance for OS in populations in Africa (HR = 3.26; 95%CI: 1.14-9.28; *P* = 0.03) and Europe (HR = 2.36; 95%CI: 1.02-5.44; *P* = 0.04). In addition, subgroup analyses of both high NLR threshold (≥ 2.0) (HR = 1.99; 95%CI: 1.01-3.94; *P* = 0.049) and low NLR threshold (< 2.0) (HR = 1.65; 95%CI: 1.11-2.46; *P* = 0.01) indicated that NLR could predict OS in GC patients receiving NAC.

NLR and RFS: Two studies provided data on NLR and RFS, involving 94 patients, and both studies provided NLR values before and after treatment. Consistent with our OS analysis results, high NLR was significantly correlated with shorter RFS (HR = 3.73, 95%CI: 1.74-7.96; *P* = 0.0007, Figure 2B). There was no significant evidence of heterogeneity among the included studies (I^2 = 0%, *P* = 0.52).

NLR and DFS: The correlation of NLR with DFS was investigated, with data from two studies involving 202 patients. Among them, one study only provided NLR values before treatment, while the other study provided NLR values before and after treatment. The studies did not demonstrate a marked predictive value of NLR for DFS (HR = 1.08, 95%CI: 0.46-2.56; *P* = 0.86, Figure 2C), which required further exploration.

NLR and PFS: Finally, four studies involved data on the correlation between NLR and PFS, involving 227 patients. Among them, one study only provided NLR values before treatment, while the remaining three studies provided NLR

Table 1 Baseline characteristics of the included literature

Ref.	Study period	Country	Study design	Time of test	Population	Neoadjuvant chemotherapy	Patients (*n*)	Gender		Age	NLR threshold
								Male	Female		
Gong *et al* [13]	2007-2015	China	Retrospective	Before and after NAC	II-III	DOF, DF, FOLFOX	91	69	22	55	1.05
Jin *et al*[17]	2004-2009	China	Retrospective	Before and after NAC	III-IV	FOLFOX	46	36	10	60	2.50
el Aziz[19]	2010-2014	Egypt	Retrospective	Before NAC	III-IV	FOLFOX	70	47	23	53	3.00
Liu *et al* [15]	2016-2019	China	Retrospective	Before and after NAC	II-III	SOX, XELOX	111	83	28	58	1.75
Kasahara *et al*[20]	2011-2016	Japan	Retrospective	Before and after NAC	II	S-1	47	30	17	66	2.41
Chen *et al* [16]	2008-2015	China	Retrospective	Before NAC	II-III	SOX, XELOX	91	70	21	57	2.17
Zurlo *et al* [23]	2012-2017	Rome	Retrospective	Before and after NAC	III B-III C	XELOX, DOF	65	41	24	63	2.50
Pikuła *et al* [22]	2012-2020	Poland	Retrospective	Before and after NAC	II-III	EOX, FLOT-4	106	65	41	61	1.94
Li *et al*[21]	2014-2018	China	Prospective	Before and after NAC	II-IV A	DOF	225	172	53	60	2.57

NAC: Neoadjuvant chemotherapy; NLR: Neutrophil to lymphocyte ratio; DOF: Doxorubicin + oxaliplatin + fluorouracil; DF: Doxorubicin + fluorouracil; FOLFOX: Fluorouracil + oxaliplatin + calcium folinate; SOX: S-1 + oxaliplatin; XELOX: Oxaliplatin + capecitabine; S-1: Tegafur; EOX: Epirubicin + oxaliplatin + capecitabine; FLOT-4: Fluorouracil + oseltamivir + folinate + tegafur.

values before and after treatment. The research findings all indicated that NLR could predict the PFS of GC patients receiving NAC, and NLR was markedly correlated to PFS (HR = 2.32, 95%CI: 1.42-3.81; P = 0.0008, Figure 2D).

Sensitivity analysis

We conducted a sensitivity analysis to assess the robustness of our analysis findings and determine the clinical relevance of NLR. Given the limited availability of studies investigating RFS and DFS, we only conducted sensitivity analysis on OS and PFS. The results showed that after sequentially deleting each study, the effect size consistently fell within the original range. These findings suggested that no individual study had a disproportionate influence on the results of OS (Figure 3A) and PFS (Figure 3B), which validated the reliability of the analysis outcomes.

Publication bias

Publication bias was evaluated through the utilization of funnel plots and Egger's test. The symmetric funnel plot provided evidence of the absence of publication bias in the analysis of PFS (Egger: P = 0.224) (Figure 4A). Conversely, the results obtained from the Egger's test indicated the presence of publication bias in the analysis of OS (Egger: P = 0.026) (Figure 4B). However, the limited number of studies (lee than 3 studies) for the remaining analysis precluded us from conducting a comprehensive assessment of publication bias.

DISCUSSION

The majority of GC patients are diagnosed an advanced stage of the disease, and many patients have missed the optimal time for surgical treatment. Multimodal systemic comprehensive therapies, such as perioperative chemotherapy, radiation therapy, and targeted immunotherapy[24], for advanced GC have markedly contributed to improving the survival rates of patients[25]. We currently have a range of treatment options for GC, but predicting the prognosis of patients remains a huge challenge. In recent years, an increasing number of studies have highlighted the strong association of systemic inflammatory response with patient prognosis in numerous malignant tumors. GPS, PLR, NLR, and MLR are relatively reliable indicators for predicting the prognosis of GC patients[26,27]. A recent research by Lin *et al*

Table 2 Subgroup analysis of overall survival in patients with neoadjuvant chemotherapy for gastric cancer

Subgroup	OS			
	Study	HR (95%CI)	*P* value	I^2
Total	10	1.76 (1.22-2.54)	0.003	58%
Time of test				
Pre-NAC	10	1.76 (1.22-2.54)	0.003	58%
Post-NAC	8	1.83 (1.22-2.75)	0.004	59%
Age				
≥ 60 years	6	2.13 (1.24-3.67)	0.006	70%
< 60 years	4	1.40 (0.79-2.48)	0.25	37%
Region				
Asia	6	1.36 (0.94-1.97)	0.1	37%
Africa	1	3.26 (1.14-9.28)	0.03	NA
Europe	3	2.36 (1.02-5.44)	0.04	65%
NLR cut-off				
≥ 2	5	1.99 (1.01-3.94)	0.049	73%
< 2	5	1.65 (1.11-2.46)	0.01	12%

NAC: Neoadjuvant chemotherapy; NLR: Neutrophil to lymphocyte ratio; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval.

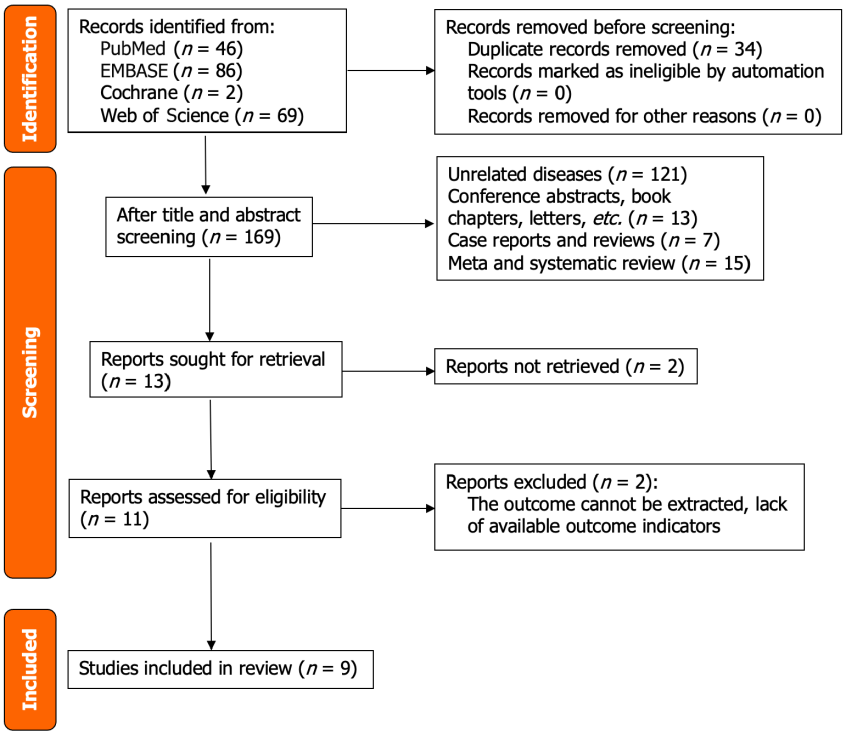

Figure 1 Flow chart of literature screening.

[28] suggested that patients with advanced GC who received a 2-week combined chemotherapy regimen of Docetaxel and S-1 achieved better chemotherapy outcomes in the low Glasgow Prognostic Score group, indicating a significant correlation between Glasgow Prognostic Score and patient prognosis. The study by Nguyen *et al*[29] showed that inflammatory markers such as NLR, MLR, and PLR could predict the prognosis of GC patients, and the combined detection by NLR, MLR, PLR, and tumor markers (carcinoembryonic antigen) improved the diagnosis rate of GC. It had high sensitivity and specificity in the diagnosis of GC, which was helpful for early diagnosis, detection, and treatment of GC

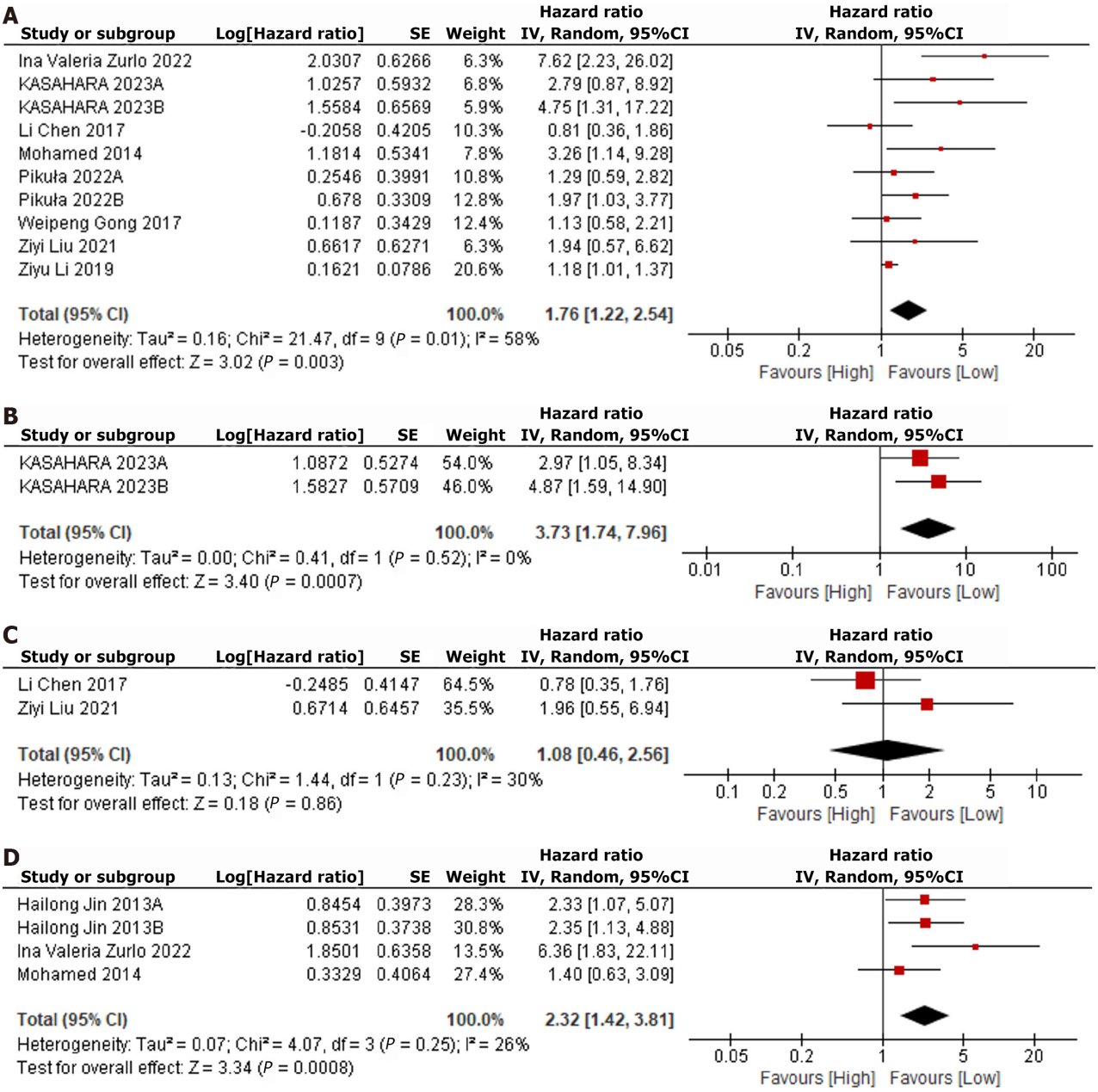

Figure 2 Forest plots for the association between neutrophil to lymphocyte ratio and overall survival, relapse-free survival, disease-free survival, progression-free survival. A: Forest plots for the association between neutrophil to lymphocyte ratio (NLR) and overall survival; B: Forest plots for the association between NLR and relapse-free survival; C: Forest plots for the association between NLR and disease-free survival; D: Forest plots for the association between NLR and progression-free survival. CI: Confidence interval.

patients. Compared with other prognostic indicators, NLR can comprehensively reflect the inflammatory status of the body[30] (involving not only the intensity of the inflammatory response, but also the overall function of the immune system). Therefore, NLR has high accuracy and reliability in predicting the prognosis of locally advanced or late stage GC patients. The study by Liu *et al*[15] has shown that an increase in NLR values is closely related to poor prognosis in GC patients. A high NLR value may indicate an exacerbation of inflammation in the patient's body, which may directly affect the growth and spread of tumors, or indirectly exacerbate the condition by affecting the body's immune response. A study by el Aziz *et al*[19] has revealed a correlation of elevated levels of NLR with poorer prognosis, shorter survival, and a relatively higher risk of recurrence. Therefore, as a reliable indicator for predicting the prognosis of GC, NLR holds marked potential for developing personalized treatment regimens, optimizing treatment strategies, and reducing recurrence and mortality rates. To date, limited research has been conducted to investigate the prognostic significance of NLR in GC patients undergoing NAC. Subsequently, an analysis was conducted to examine the association of NLR scores with the prognosis and survival in GC patients undergoing NAC.

In this meta-analysis, a comprehensive cohort of 852 patients was included to assess the prognostic significance of NLR in GC patients undergoing NAC. In the included original studies, patients were divided into a relatively high NLR group and a relatively low NLR group based on different thresholds, and the findings demonstrated a marked negative correlation of NLR with OS, RFS, and PFS, that is, the higher level of NLR indicted the worse prognosis. Kishi *et al*[31]

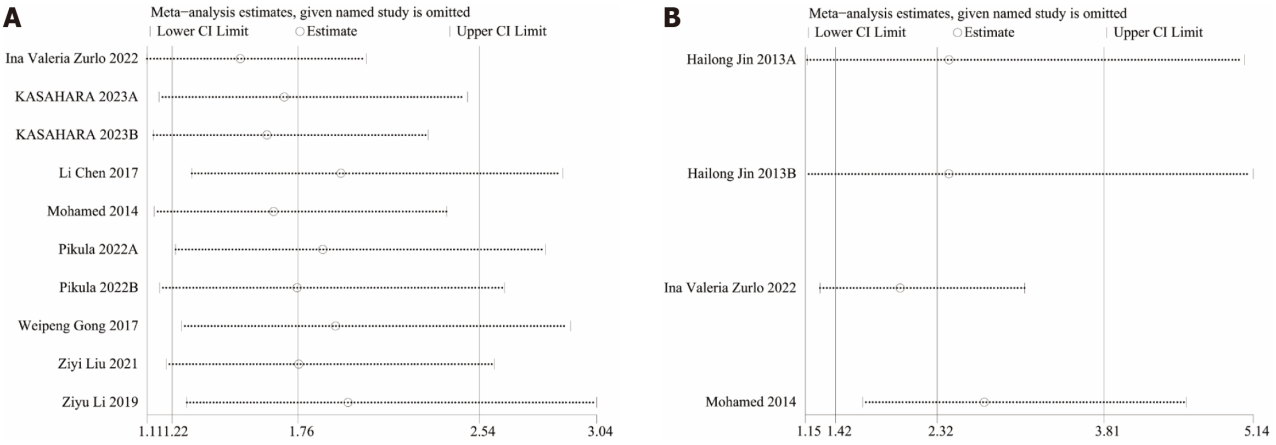

Figure 3 Sensitivity analysis of overall survival and progression-free survival. A: Sensitivity analysis of overall survival; B: Sensitivity analysis of progression-free survival. CI: Confidence interval.

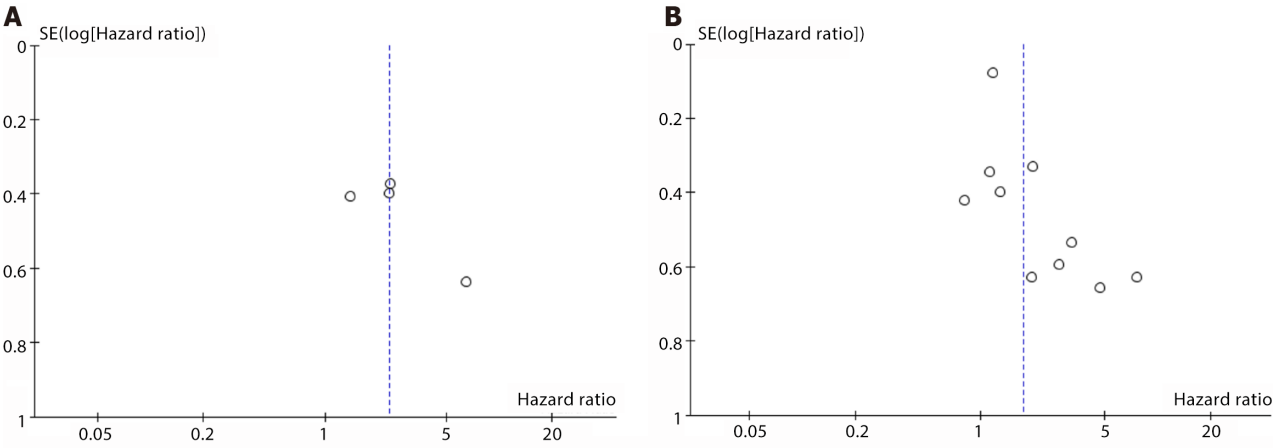

Figure 4 Funnel plot for the evaluation of publication bias for progression-free survival and overall survival. A: Funnel plot for the evaluation of publication bias for progression-free survival; B: Funnel plot for the evaluation of publication bias for overall survival.

reported on 38 patients with GC undergoing radical gastrectomy and postoperative chemotherapy showed that postoperative chemotherapy could normalize preoperative high levels of NLR, thereby improving patient survival. Therefore, NLR is a reliable factor in predicting the prognosis of GC patients undergoing chemotherapy. Another study showed that ovarian cancer patients undergoing NAC had better OS and PFS with lower NLR levels (HR = 1.72, 95%CI: 1.18-2.51)[32]. The results from above studies were consistent with our conclusion. It showed that after NAC, the lower level of NLR in GC patients indicated better OS and PFS. It could be concluded that the prognosis of patients could be evaluated by detecting the level of NLR. In contrast, our study focused on evaluating the prognosis of GC patients who lost the timing for surgery and only received NAC. After undergoing NAC in different malignant tumor, patient prognosis has also been significantly improved, indicating that NAC is particularly important in the treatment of malignant tumors. In this study, the relationship between NLR and DFS was not significant. This difference might be due to the fact that a very small number of GC patients could achieve DFS after receiving NAC, but there was a lack of studies on NLR data for these patients. It might also be related to differences in NLR detection time. We found that many studies conducted blood sampling within 2 to 6 weeks after the initial administration of chemotherapy drugs. Research evidence has suggested that activated white blood cells usually take at least 4 weeks to fully exert their effects after entering the bloodstream[33]. Thus, the reliability of the data obtained from blood sampling within 4 weeks after chemotherapy remains to be explored. Future research can verify whether changes in NLR detection time indeed affect clinical outcomes, and whether differences in detection time can affect patient prognosis.

To provide a more detailed analysis, subgroup analysis was conducted to further explore the relationship between NLR and OS. According to the study findings, NLR demonstrated predictive value for all indicators, except for those under 60 years old and Asian patients. For individuals under 60 years old, the predictive significance of NLR for OS was not marked (HR = 1.40, 95%CI: 0.79-2.48; P = 0.250), which might be correlated to the patient's immune system function and prognosis. Compared with older patients (≥ 60 years old), younger patients (< 60 years old) had a stronger immune system function, stronger anti-tumor ability, and better prognosis. Given the restricted number of included articles, a definitive conclusion regarding the predictive significance of NLR for OS in patients under 60 years old is currently lacking. Therefore, further studies are warranted to confirm whether NLR can predict the prognosis of patients under 60

years old. In addition, our research has shown that the predictive value of NLR for OS is significantly better in Europe and Africa than in Asia, which may be due to differences in the epidemiology, histopathological characteristics, and immune response of GC patients from different countries and races. First, there are significant differences in the incidence rate and OS rate of GC among Asia, Europe and other countries. The incidence rate and mortality rate of GC in Asia are generally high, while the incidence rate in Europe is relatively low[1]. This geographical difference may be related to specific genetic risk factors and different infectious agents (*Helicobacter pylori* infection is one of the leading pathogenic factors of GC in Asia) in the population. Secondly, there are differences in clinical characteristics among GC patients in different regions. According to the study by Janjigian *et al*[34], there are differences in the initial site, histological classification, and surgical approach of GC patients in Eastern and Western countries. These differences reflect the specificality of different populations in the occurrence and progression of GC, thereby affecting the application value of NLR in predicting GC in different regions.

The interplay between the systemic inflammatory response and tumor development is evident. The progression of tumors depends on the degree of systemic inflammatory response, which is an important component of the tumor microenvironment. Systemic inflammatory response can to some extent reflect the prognosis of patients. Some studies explored the mechanism of action of inflammatory factors in various malignant tumors, and found that a multifaceted interplay exists between tumor host immune and systemic inflammatory response[35-37]. When tumors damaged body tissues, it could trigger the local or systemic release of inflammatory factors from the immune system to fight against tumor invasion. In addition, various immune cells are polarized again in inhibiting the recruitment of cytotoxic immune cells, which disrupts the dynamic balance in tumor-host immune and promotes the occurrence and development of tumors. Various inflammatory indicators such as white blood cell, neutrophil, lymphocyte, monocyte, NLR, and PLR, are closely related to different types of tumors[38-42]. NLR has previously been identified as a reliable indicator for predicting the prognosis of GC patients. Saito *et al*[43] first proposed the correlation of NLR with survival in GC patients, and it was found that patients with high levels of NLR had poorer prognosis. Sahinli and Türker[44] have showed that the higher preoperative NLR level indicates the worse postoperative DFS and OS in GC patients. We need to explore why NLR can be an important indicator for predicting patient prognosis. When our tissues are infiltrated by tumor cells, our immune system will activate the systemic inflammatory response and release inflammatory cells to fight against the tumor microenvironment. As the main immune cells in the body, neutrophils and lymphocytes exert distinct effects during the anti-tumor immune response. Neutrophils inhibit our immune system and promote tumor progression, while lymphocytes inhibit the proliferation and migration of tumor cells through cytotoxic cell death. In particular, neutrophils exert promoting effects on tumor progression through various mechanisms. They contribute to the stimulation of tumor suppressor gene mutations, facilitate angiogenesis, secrete enzymes and cytokines that enhance tumor cell proliferation and metastasis, and actively reshape the extracellular matrix[45,46]. Lymphocytes play an important role in the anti-tumor process. When the systemic inflammatory effect of the body is triggered, a large amount of inflammatory factors are released, which further expose the suppression of innate cellular immune responses, resulting in a decrease in T8 lymphocytes and an increase in T4 helper lymphocytes, thus enhancing the anti-tumor effect[47]. Therefore, NLR shows the dynamic process of the body's inflammatory and immune response regulation. In addition, as an inflammatory indicator, NLR also reflects the transformation of this balance. When the body's inflammatory response and immune system are imbalanced, there is a chance to promote tumor growth. It can be concluded that high levels of NLR can promote tumor growth, weaken anti-tumor effects, and ultimately lead to poor prognosis in patients.

Although our meta-analysis provided reliable information, there were also some limitations. Firstly, the majority of the included studies in our analysis were performed in Asia, especially in China and Japan. One study was conducted in Africa, and three studies were conducted in Europe. Hence, it is essential to interpret our conclusions within the specific geographical contexts of the included studies. Caution is warranted when extrapolating our findings to patients residing in different regions, particularly the Americas and other geographical areas. Secondly, the majority of studies included employed a retrospective design rather than a prospective design. The retrospective design might introduce confounders that would affect the reliability of our results. Another limitation of the included studies was the variability in the cutoff values of NLR used. These thresholds ranged from 1.05 to 3. Due to inconsistency of data, inherent heterogeneity might be introduced in our meta-analysis. A subgroup analysis was conducted based on the NLR threshold, and it was found that the heterogeneity of the low NLR threshold (< 2.0) group (HR = 1.99; 95%CI: 1.01-3.94; P = 0.049; I^2 = 12%) was lower than that of the high NLR threshold (≥ 2.0) group (HR = 1.65; 95%CI: 1.11-2.46; P = 0.01; I^2 = 73%). Therefore, it can be concluded that the heterogeneity of OS may be related to different NLR thresholds. In the future clinical research process, we may consider the balance among multiple factors such as patient age, region, and NLR threshold, include patients of different age groups, determine multiple NLR thresholds, apply different analysis methods to determine the optimal NLR threshold, and conduct multi-center studies to avoid heterogeneity differences. In addition, there was publication bias in certain indicators in our study, indicating that the credibility of the evidence for these indicators needed further investigation. In order to improve reliability and comparability in future research, researchers must set up a standardized threshold for NLR.

CONCLUSION

To summarize, our meta-analysis findings indicate that in GC patients receiving NAC, high NLR is significantly correlated with shorter OS and PFS. However, the studies we included in the analysis still have issues such as a large number of retrospective studies, small sample sizes, high heterogeneity, and limited study areas. Consequently, there is a crucial need for a substantial number of prospective studies to confirm the efficacy and predictive value of NLR in GC

patients undergoing NAC.

FOOTNOTES

Author contributions: All authors contributed to the study conception and design; Wei ZH contributed to the writing - original draft preparation; Wei ZH, Tuo M, Ye C, Liu G, and Xiang T were involved in the writing - review and editing; Wei ZH and Xiang T took part in the conceptualization; Ren WZ participated in the methodology of this manuscript; Wei ZH, Ye C, and Wu XF contributed to the formal analysis and investigation of this study; Tuo M, Liu G, and Xiang T contributed to the funding acquisition; Wang HH was involved in the resources of this study; Liu G and Xiang T took part in the supervision; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Wei ZH and Tuo M contributed equally to this manuscript, they are the co-first authors of this study. Zhenhua Wei was responsible for data analysis and paper writing; Tuo M was responsible for data collection and compilation. Liu G and Xiang T contributed equally, they are the co-corresponding authors of this article. Liu G was responsible for writing guidance, review and project funding, and Xiang T was responsible for writing revision, review and project funding.

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SCIENTOMETRICS

Bibliometric analysis of olaparib and pancreatic cancer from 2009 to 2022: A global perspective

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Abstract

BACKGROUND

Genetic screening for breast cancer gene 1 (*BRCA*)*1/2* mutations can inform breast/ovarian/pancreatic cancer patients of suitable therapeutic interventions. Four to seven percent of pancreatic cancer patients have germline *BRCA* mutations. *BRCA* genes aid in DNA repair, especially homologous recombination, which impacts genomic stability and cancer cell growth. *BRCA1* regulates the cell cycle, ubiquitination, and chromatin remodeling, whereas *BRCA2* stimulates the immune response. They predict the efficacy of platinum chemotherapy or polymerase (PARP) inhibitors such as olaparib.

AIM

To determine the trends and future directions in the use of olaparib for pancreatic cancer treatment.

METHODS

To evaluate the trends in how olaparib works in pancreatic cancer, we performed a bibliometric analysis. One hundred and ninety-six related publications were accessed from the Web of Science Core Collection and were published between 2009 and 2022. The analytic parameters included publications, related citations, productive countries and institutes, influential authors, and keyword development.

RESULTS

This study visualizes and discusses the current research, including the present global trends and future directions in olaparib and pancreatic cancer. Overall, this study sheds light on optimizing the use of olaparib in pancreatic cancer treatment,

offering valuable guidance for researchers in this field.

CONCLUSION

Our findings identified trends in olaparib and pancreatic cancer, with China and the USA leading and with global cooperation tightening. O'Reilly EM's team and Memorial Sloan-Kettering had the highest output. The *Journal of Clinical Oncology* was the most cited journal. More PARP inhibitors are emerging, and combination therapy is suggested for future therapeutic trends.

Key Words: Olaparib; Pancreatic cancer; Bibliometric analysis; Breast cancer susceptibility gene; Poly (adenosine diphosphate–ribose) polymerase

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Core Tip: Breast cancer gene (*BRCA*)*1* and *BRCA2* mutations affect 4%-7% of pancreatic cancer patients and influence their response to therapies such as platinum chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors such as olaparib. A bibliometric analysis of 196 publications highlights growing global research interest, with China and the United States leading. The key contributors include O'Reilly EM's team and Memorial Sloan-Kettering, whereas the *Journal of Clinical Oncology* stands out for citations. Future trends point toward increased use of PARP inhibitors and combination therapies, offering valuable insights for advancing olaparib research in pancreatic cancer.

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INTRODUCTION

Pancreatic cancer is a relatively uncommon malignant tumor that mostly originates from exocrine pancreatic ductal cells [1]. The risk factors for pancreatic cancer include smoking, obesity, alcohol abuse, diabetes, and chronic pancreatitis[2]. Approximately 60430 new diagnoses are expected in 2021 in the United States, and it is the 4th leading cause of cancer-related deaths worldwide[3]. However, as the disease has nonspecific symptoms, most patients progress to the advanced stage with no chance of surgery when first diagnosed. The current standard first-line chemotherapy regimen is FOLFIRINOX (fluorouracil, irinotecan, leucovorin, oxaliplatin), which is associated with a median progression-free survival (mPFS) of 6.4 months[4]. As metastatic pancreatic cancer is resistant to many treatments, even after radical resection, the prognosis after initial diagnosis remains poor, with a 5-year survival rate of 11%[5].

Not all pancreatic cancer patients have a low chance of survival. Approximately 4%–7% of pancreatic cancer patients harbor mutations in the genes encoding loss of function of breast cancer susceptibility gene (*BRCA*)1, *BRCA2*, or both. *BRCA1* mutations are responsible for inherited cancers, including 40% of breast cancers and over 80% of ovarian cancers [6]. *BRCA* genes play a non-neglectable role in regulating cell replication, repairing DNA damage, and normal cell growth. *BRCA* genes also act as important tumor suppressors, the encoded protein of which interacts with other tumor suppressors[7]. Cells with *BRCA* mutations are relatively sensitive to poly(adenosine diphosphate-ribose) polymerase (PARP) inhibition. If the PARP gene is inhibited, *BRCA* gene mutations in patients make homologous recombination repair unable to proceed normally, eventually leading to cell apoptosis. Therefore, *BRCA* and PARP meet the definition of synthetic lethality. When the *BRCA* gene is mutated, the DNA repair pathway depends on the PARP-1 enzyme, and PARP inhibitors prevent DNA repair and eventually die[8]. The application of PARP inhibitors in patients is expected to kill tumor cells. However, the presence of *BRCA* in normal cells can still repair DNA and allow cells to survive, so PARP inhibitors can be used as targeted drugs to selectively kill *BRCA*-mutant cells[9]. Olaparib, a PARP inhibitor, has shown promising efficacy in terms of mPFS (7.0 months *vs* 4.2 months with standard treatment) in patients with metastatic breast cancer and a g *BRCAmt*[10]. Moreover, olaparib has certain antitumor effects on patients with *BRCA* mutation–metastatic pancreatic cancer with a heavy pretreatment history, extending the mPFS from 3.8 months to 7.4 months (placebo *vs* olaparib)[11]. Olaparib became the first pancreatic cancer-targeted drug approved, bringing new hope for the survival of patients with pancreatic cancer. However, several critical knowledge gaps exist regarding olaparib and its use in treating pancreatic cancer. This calls for a comprehensive overview of the evolving landscape of olaparib research, including publication and citation trends over time. Additionally, there is a need to understand the geographical distribution of research efforts and identify the most productive countries and institutions involved in this field, along with the most influential scholars and their contributions, particularly those focusing on novel aspects such as the role of ATM variants and homologous recombination deficiency (HRD) in pancreatic cancer. Furthermore, researchers are working to understand the transition from single-agent to combination therapies involving olaparib, including the integration of immunotherapies.

Bibliometric analysis helps researchers conveniently acquire the most complete information in a complex field, providing information such as the main publishing institutions, the most influential scholars and their collaborations, the most representative research, and the keywords in a certain period. Bibliometric analysis has the advantage of mapping approaches aimed at predicting the development of future directions in some areas and is disciplined in a newly structured, transcended systemic review, which mainly concerns the development of a segment over a while[12]. Therefore, it has strengths from a global perspective, providing a more comprehensive view of the development history of certain fields[13].

Many studies have been published from 2009 to 2022 in the field of olaparib and pancreatic cancer. In this article, we provide an overview and scientific analysis of published olaparib and pancreatic cancer research *via* data obtained from the Web of Science Core Collection (WoSCC). The indices included annual changes in publications and citations, high-output countries/regions and institutions, the most influential and highly cited authors, mainstream journals, and keyword changes over time, which were demonstrated and visualized *via* several bibliometric software programs. Through this analysis, we will understand the development of olaparib and pancreatic cancer, which will help us obtain a good reference when researching studies in this field.

MATERIALS AND METHODS

Data collection

First, data from 2009 to 2022 were retrieved from the WoSCC. We collected data within one day (September 8, 2022). The search queries were set as follows: "Topic Search (TS) = (olaparib)", AND "TS = (pancreatic cancer)", AND "Language: English AND Reference Type: Article OR Review". After deleting duplicates and irrelevant papers, a total of 196 references were extracted from the WoSCC and then used to perform the bibliometric analysis. The screening process is shown in Figure 1.

Data analysis

Visual bibliometric analysis was performed *via* HisCite (version 2.1), CiteSpace (version 6.1. R2), VOSviewer (version 1.6.18), SCImago Graphica (version 1.0.23), and the Bibliometrix 4.1.0 package in R. First, the total number of publications, total local citation score, and total global citation score for each publication year, top countries, journals, authors, and institutions were analyzed by HisCite. VOSviewer was employed to visualize the collaborative network, which included countries, institutions, and authors, and a density map of keywords. With the assistance of VOSviewer, SCImago Graphica could be utilized to visualize the co-occurrence network between institutions and international collaborations of olaparib in pancreatic cancer. Cluster analysis, keyword bursts, and timeline views were obtained *via* CiteSpace. More importantly, modularity $Q > 0.3$ and weighted mean silhouette > 0.5 of cluster analysis indicate that the clustering results are sufficiently comprehensive and convincing. The Bibliometrix package is a tool designed in the R language used for bibliometric analysis and was applied to analyze the production of top authors or countries over time, the authors' *H*-index, and three-field plots of institutions, authors, and keywords.

RESULTS

Annual publication and citations

A total of 196 documents, including 138 (70.4%) original studies and 58 (29.6%) reviews associated with olaparib in pancreatic cancer, were screened from the WoSCC database. These documents received 6399 citations before September 8, 2022, with an average number of citations of 32.65 per item. The annual counts of publications and citations are shown in Figure 2. Although there was one publication in 2009 (written by Vasiliou *et al*[14]), one in 2011 (written by Vance *et al*[15]) and fewer than 10 on olaparib in pancreatic cancer published annually from 2009 to 2016, there was an overall growth trend in olaparib use among pancreatic cancer patients from 2017 to 2022. Moreover, the growth of publications has accelerated distinctly since 2020, indicating that the concept has gradually been accepted by researchers since then. In particular, the number of publications in 2020 (n = 45) was slightly greater than that from 2016 to 2019 (n = 42). The substantial surge in publications in 2020 can be attributed to several potential factors. Notably, the emergence of critical clinical trials and data releases demonstrating the efficacy and safety of olaparib in treating pancreatic cancer could have significantly fueled this growth, sparking heightened interest and subsequent publications. For example, the positive outcomes from a trial showing the efficacy of olaparib maintenance therapy in patients with metastatic pancreatic cancer and germline *BRCA* mutations contributed to a burgeoning interest in olaparib[11]. Additionally, the regulatory approvals for the application of olaparib in pancreatic cancer have reportedly escalated research activities. The approval of olaparib by the United States Food and Drug Administration (FDA) for the treatment of *gBRCAm* metastatic pancreatic adenocarcinoma on December 27, 2019, provides further rationale for the increased number of publications in this domain.

Moreover, amplified funding for pancreatic cancer research, extensive media coverage, and public awareness campaigns regarding new treatments for pancreatic cancer likely played pivotal roles in driving the upsurge in publications. It is reasonable to posit that the combined influence of these factors substantively contributed to the marked increase in publications related to olaparib in the context of pancreatic cancer. As shown in Figure 2, the annual number of citations of publications, like the number of publications, presented an overall increasing trend. For 2022, the data were

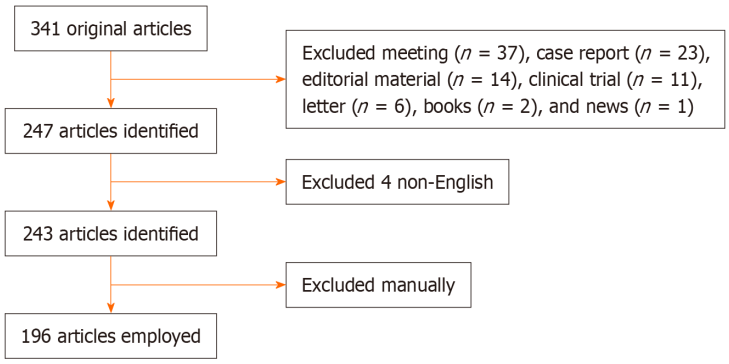


Figure 1 Flowchart of the screening process. A total of 196 references extracted from the Web of Science Core Collection were used to perform a bibliometric analysis.

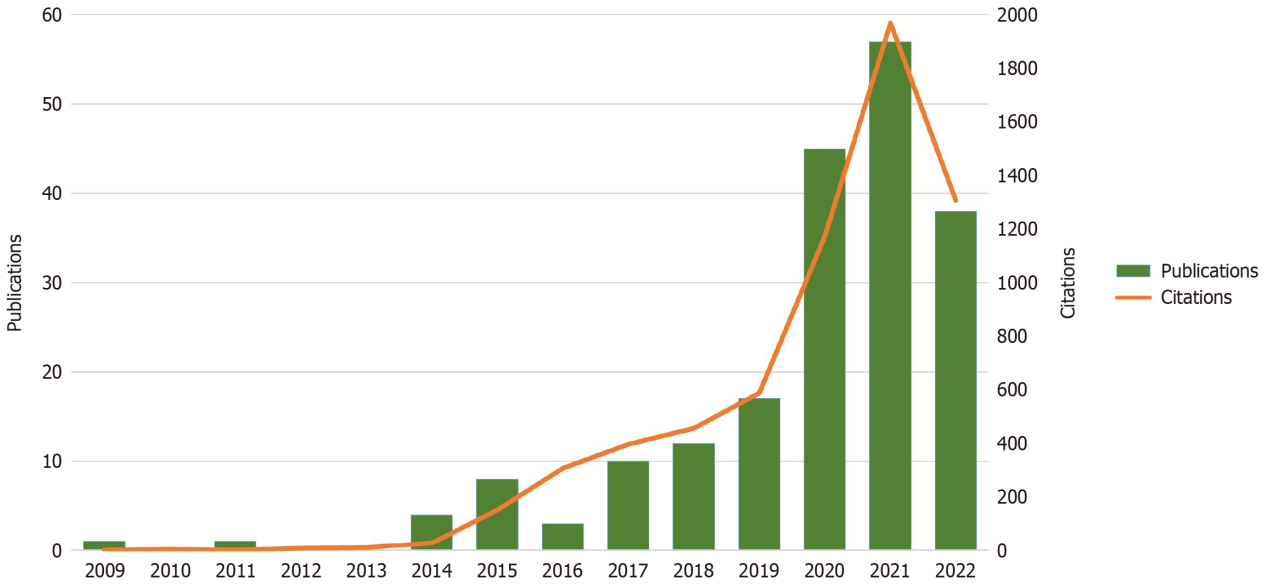


Figure 2 The number of papers published annually and the total number of citations of publications related to olaparib in pancreatic cancer. The annual number of citations of publications, similar to the number of publications, showed an overall increasing trend.

incomplete because the remaining three months were included. Therefore, the increased number of annual publications and citations highlights the potential of olaparib for the treatment of pancreatic cancer.

Countries/regions

A total of 27 countries conducted relevant studies on olaparib in pancreatic cancer patients from 2009 to 2022. First, we analyzed the production of Olaparib in five countries over time and found that the production trend in each country was rising, especially in the United States and China (Figure 3A). A adjusted geographical distribution map of global productivity is displayed in Figure 3B, where the area and color of each country are rescaled and adjusted according to the number of publications. The top 10 most prolific countries/regions associated with olaparib use in patients with pancreatic cancer are listed in Table 1. The authors from the United States published the most articles associated with olaparib in pancreatic cancer in recent years (n = 83). Next, China (n = 48) and the United Kingdom (n = 31) took second and third place, respectively. Additionally, the country with the most citations was the United States, with 4760 citations, followed by the United Kingdom, with 3748 citations, and Spain, with 2352 citations (Table 1).

Furthermore, a geographical collaboration map and cooperation network of countries/regions with a minimum of 3 publications were generated by SCImago Graphica and VOSviewer, respectively. The United States has the broadest collaboration network, mainly presenting scientific relationships with China and some European countries (Figure 3C). As shown in Figure 3D, the overvisualization of the cooperation network among countries/regions demonstrated that cooperation between countries has changed over time. The United States has collaborated most closely with some European countries, such as Germany, France, and Italy, in recent years. This may be due to shared scientific interests, funding opportunities, and historical ties. However, the United States has mainly cooperated closely with China in recent years. This increased collaboration with China underscores the growing recognition of the importance of investigating the efficacy of olaparib in Asian populations, particularly in light of genetic variations and disease prevalence. In the context of China, Chinese scientists have established extensive collaborative partnerships with Germany and France in recent

Table 1 The top 10 most popular countries/regions conducting research on olaparib and pancreatic cancer

Rank	Country	Counts	TLCS	TGCS
1	United States	83	320	4760
2	China	48	16	366
3	United Kingdom	31	241	3748
4	France	19	140	1537
5	Italy	19	124	1040
6	Germany	16	192	2254
7	Spain	12	186	2352
8	Australia	9	75	1746
9	Belgium	9	130	1391
10	Israel	9	179	2120

TLCS: Total local citation score; TGCS: Total global citation score.

years, demonstrating a keen interest in cutting-edge biotechnology and pharmaceutical development. Furthermore, earlier research endeavors and collaborations with Australia and Spain represent the diverse areas of study.

Authors

A total of 1639 authors have published 196 papers on olaparib in pancreatic cancer, and the top 20 prolific authors are presented in Table 2. All the authors came from foreign countries. Among them, O'Reilly EM, from the Memorial Sloan-Kettering Cancer Center, was the most productive author with 11 papers, followed by Golan T (n = 9), Hammel P (n = 8), Macarulla T (n = 7), Hochhauser D (n = 5), Seufferlein T (n = 5), and Van Cutsem E (n = 5). O'Reilly EM's research encompasses a wide range of topics related to olaparib in the context of pancreatic cancer. This includes investigations of olaparib's efficacy in clinical trials, its pharmacological effects, and its impact on patient outcomes. Moreover, the most cited author is Balmana J, with 1407 citations, followed by O'Reilly EM (1177 citations) and Golan T (979 citations).

We can see from the coauthorship map visualized by VOSviewer that the highly cited authors O'Reilly EM, Golan T, and Hammel P were at the center and collaborated closely (Figure 4A). The coauthorship map (Figure 4A) illustrates a close collaboration between O'Reilly EM, Golan T, and Hammel P. This suggests that their combined efforts positively influence the impact of their work. Collaborations allow for the sharing of resources, expertise, and patient populations, ultimately improving the quality and scope of research. However, some researchers were scattered independently from other researchers, especially Colle E and De Mestier L, who are in the lower left corner of Figure 4A. Therefore, the collaboration between researchers on olaparib in pancreatic cancer urgently needs to be promoted in the future. The timeline of authors who published studies on olaparib in pancreatic cancer is visualized in Figure 4B *via* the Bibliometrix package in the R language, revealing the time points at which each of the top 15 prolific authors published papers about olaparib in pancreatic cancer. As shown in Figure 4B, Lawrence TS, Morgan MA, and Parsels LA started research on olaparib in pancreatic cancer after publishing their papers in 2011 and continuing until 2021. The most prolific authors, O'Reilly EM and Golan T, began relevant research in 2015 and 2019, respectively, and continued into 2021. Most authors engaged in the study of olaparib in pancreatic cancer after 2019, and the number of publications of most authors also increased significantly in the same year. The H-index is another metric reflecting the number of publications and citations in a single number and is indicative of the authors' research achievements in this study. The highly cited authors O'Reilly EM, Golan T, and Hammel P have the highest H-indices, meaning the H-index is positively correlated with having more citations and the citation advantage of more popular authors (Figure 4C). To clarify the relationships among institutions, authors, and research fields (keywords) and promote potential cooperation between various experts from different institutions in this study, a three-field plot of institutions, authors, and keywords was generated *via* the R bibliometrix package (Figure 5), which also demonstrated that cooperation among more authors should be further enhanced.

Institutions

Four hundred and ninety-four institutions have contributed to research on olaparib in pancreatic cancer. The top 15 institutions ranked by number of publications are listed in Table 3. Most scientific research institutions were from the United States, the United Kingdom, or Israel. Among them, Memorial Sloan-Kettering Cancer Center (n = 14) in the United States was the leading institution in terms of publication outputs, followed by AstraZeneca (n = 13) in the United Kingdom. Strengthening collaboration between academic institutions and industry partners, such as AstraZeneca, is essential for translating research findings into clinical practice. These collaborations can accelerate the development of new therapeutic strategies and improve patient care. The visualization map for institutions' collaboration was generated with a minimum of 3 publications by VOSviewer, and 54 institutions formed 5 clusters with different colors, in which there were active collaborations between the institutions (Figure 6A).

Table 2 The top 20 most popular authors conducting research on olaparib and pancreatic cancer

Rank	Author	Counts	TLCS	TGCS
1	O'Reilly EM	11	135	1177
2	Golan T	9	132	979
3	Hammel P	8	130	956
4	Macarulla T	7	127	947
5	Hochhauser D	5	127	944
6	Seufferlein T	5	17	140
7	Van Cutsem E	5	127	944
8	Algul H	4	131	976
9	Brody JR	4	22	240
10	Cornelissen B	4	4	72
11	Kleger A	4	17	135
12	Lawrence TS	4	18	247
13	Morgan MA	4	18	247
14	Park JO	4	127	942
15	Parsels LA	4	18	247
16	Perkhofer L	4	17	135
17	Arnold D	3	118	919
18	Balmana J	3	68	1407
19	Biankin AV	3	5	49
20	Cavalli A	3	6	36

TLCS: Total local citation score; TGCS: Total global citation score.

The collaborative network between institutions was subsequently constructed by SCImago Graphica and VOSviewer (Figure 6B). The network revealed that the institutions in the upper left area cooperation more closely with one another than those in other areas, such as the University of Chicago, University of Paris VII, and Sungkyunkawan University. To enhance future collaborations, several areas and institutions stand out as potential targets for enhancement. First, institutions within closely integrated clusters should strive to broaden their collaboration by actively engaging with institutions outside of their immediate networks. This can involve the establishment of joint research programs, shared facilities, and collaborative grants aimed at facilitating the exchange of ideas and resources. Second, institutions located in less connected areas, such as those in the lower right region of the visualization map (Figure 6B), should be incentivized to establish new partnerships with leading institutions. This can be achieved by implementing mentorship programs, international symposia, and funding initiatives that foster cross-institutional and cross-border collaborations. In summary, collaboration among authors still needs to be strengthened in the future.

Journals

A total of 130 journals published papers focused on olaparib and pancreatic cancer. The top 10 journals ranked by publications are listed in Table 4. The top journal, *Cancers* [impact factor (IF) 2021, 6.575], published the most publications (n = 13) from 2009 to 2022, followed by Clinical Cancer Research (IF 2021, 13.801, n = 7), the *International Journal of Molecular Sciences* (IF 2021, 6.208, n = 5) and *Annals of Oncology* (IF 2021, 51.769, n = 4). Some academic journals receive a high volume of publications for several reasons. First, journals such as *Cancers*, which cover all aspects of cancer research, tend to attract many submissions because they provide a platform for a wide range of research topics. Second, well-reputed journals, as indicated by a high IF, are more likely to receive high-quality submissions, as authors want to publish in respected venues. Third, journals that align with current research trends, such as olaparib in pancreatic cancer, benefit from increased interest and activity in the field, resulting in a higher volume of submissions. Although the number of publications on olaparib in pancreatic cancer in journals was not significant, the majority of the top 10 journals had scientific influence, with high IFs 2021. The *Journal of Clinical Oncology* was the most-cited journal (1293 times) and had the highest IF (IF 2021, 50.717).

Most co-cited references

References reflect the solid foundation laid by predecessors, which may drive progression and achieve breakthroughs in

Table 3 The 15 most productive institutions regarding olaparib and pancreatic cancer research

Rank	Institution	Country	Counts	TLCS	TGCS
1	Memorial Sloan-Kettering Cancer Center	United States	14	147	1667
2	AstraZeneca	United Kingdom	13	208	2755
3	University of Michigan	United States	10	36	752
4	Harvard Medical School	United States	7	15	173
5	University of Bologna	Italy	7	6	92
6	University of Pennsylvania	United States	7	62	1254
7	Dana-Farber Cancer Institute	United States	6	21	633
8	Beaujon Hospital	France	6	121	931
9	Tel Aviv University	Israel	6	110	907
10	University of Oxford	United Kingdom	6	18	192
11	University of Texas, MD Anderson Cancer Center	United States	6	27	859
12	Vall d'Hebron University Hospital	Spain	6	130	1163
13	Sheba Medical Center	Israel	5	70	1220
14	University College London	United Kingdom	5	127	1150
15	University of Glasgow	United Kingdom	5	7	105

TLCS: Total local citation score; TGCS: Total global citation score.

Table 4 The top 10 journals publishing research on olaparib and pancreatic cancer

Rank	Journal	Counts	IF 2021	TLCS	TGCS
1	*Cancers*	13	6.575	0	94
2	*Clinical Cancer Research*	7	13.801	16	169
3	*International Journal of Molecular Sciences*	5	6.208	0	14
4	*Annals of Oncology*	4	51.769	43	566
5	*European Journal of Medicinal Chemistry*	4	7.088	8	139
6	*Journal of Clinical Oncology*	4	50.717	74	1293
7	*BMC Cancer*	3	4.638	0	13
8	*British Journal of Cancer*	3	9.075	6	99
9	*Cancer Biology & Therapy*	3	4.875	0	6
10	*Cancer Research*	3	13.312	11	75

TLCS: Total local citation score; TGCS: Total global citation score.

scientific research. In this study, we analyzed the top 10 most co-cited studies on olaparib in pancreatic cancer, the results of which are listed in Table 5. The article with the most co-citations, titled "Maintenance Olaparib for Germline *BRCA*-Mutated Metastatic Pancreatic Cancer", was written by Golan *et al*[11] and published in *The New England Journal of Medicine* in 2019 (103 citations). This article is the result of a clinical trial and revealed that PFS was longer with maintenance olaparib compared to the placebo in patients with *gBRCAmt* and metastatic pancreatic cancer[11]. This reference holds paramount significance within the field for several reasons. First, this study provides substantial evidence of the clinical efficacy of olaparib in a distinct cohort of patients afflicted by pancreatic cancer-particularly those harboring germline *BRCA* mutations. This discovery has profound implications for personalized medicine, emphasizing the pivotal role of genetic testing in tailoring treatments for individual patients. Furthermore, the study's rigorous design, exemplified by its randomized controlled trial framework, fortifies the credibility of the findings and the therapeutic advantages of olaparib. Consequently, this has had a discernible impact on regulatory determinations, as evidenced by the FDA endorsement of olaparib for this specific indication. The journals *Nature*, the *Journal of Clinical Oncology*, *The New England Journal of Medicine*, and *Cancer Research* have tremendous scientific influence on scholars and academics in this

Table 5 The top 10 cocited references related to olaparib and pancreatic cancer

Rank	Ref.	Title	Citations	Journal
1	Golan *et al*[11], 2019	Maintenance Olaparib for Germline *BRCA*-Mutated Metastatic Pancreatic cancer	103	*The New England Journal of Medicine*
2	Kaufman *et al*[35], 2015	Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation	56	*Journal of Clinical Oncology*
3	Farmer *et al*[36], 2005	Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy	52	*Nature*
4	Robson *et al*[10], 2017	Olaparib for Metastatic Breast Cancer in Patients with a Germline *BRCA* Mutation	46	*The New England Journal of Medicine*
5	Bryant *et al*[37], 2005	Specific killing of *BRCA2*-deficient tumours with inhibitors of poly(ADP-ribose) polymerase	44	*Nature*
6	Murai *et al*[38], 2012	Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors	40	*Cancer Research*
7	Waddell *et al*[39], 2015	Whole genomes redefine the mutational landscape of pancreatic cancer	39	*Nature*
8	Fong *et al*[40], 2009	Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers	37	*The New England Journal of Medicine*
9	Moore *et al*[21], 2018	Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer	37	*The New England Journal of Medicine*
10	Conroy *et al*[4], 2011	FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer	36	*The New England Journal of Medicine*

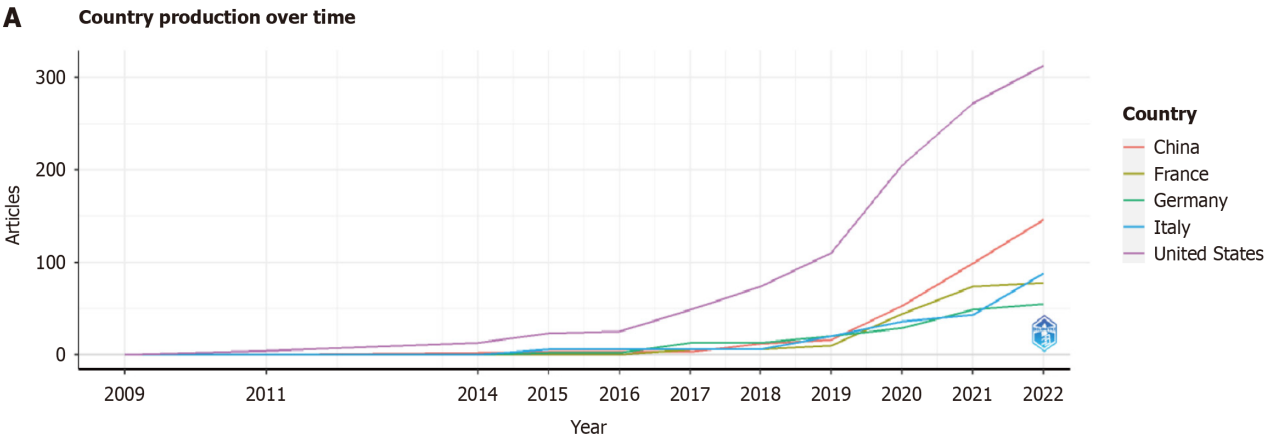
field, and all highly cocited papers are published in these high-quality journals. These references have significantly influenced subsequent research by establishing a standard for the design and implementation of clinical trials, emphasizing the importance of biomarker-driven therapies and providing directions for developing novel treatment approaches. They have also influenced clinical guidelines and recommendations for managing pancreatic cancer. Additionally, these findings have inspired further investigations into the synergistic effects of olaparib in combination with other therapies, the discovery of additional predictive biomarkers, and the examination of olaparib's potential application in earlier stages of the disease.

Keyword analysis

VOSviewer was used to present the map of the co-occurrence of keywords. Eight hundred and sixty-eight keywords were extracted, 89 of which appeared > 5 times and 44 appeared > 10 times. As shown in Figure 7A, the density map revealed high-frequency keywords, which occurred more than 10 times, indicating the hotspots and research trends in the field of olaparib in pancreatic cancer. The color represents the number of keyword occurrences. Among them, the top 5 keywords in terms of occurrence were "olaparib", "PARP inhibitors", "gemcitabinel", "survival", and "brcachemotherapy". These keywords highlight the central themes of the research, with "olaparib" and "PARP inhibitors" emphasizing the drug and its mechanism of action, "gemcitabine" representing standard chemotherapy, "survival" indicating the clinical outcomes of interest, and "*BRCA* chemotherapy" pointing toward the importance of genetic markers in treatment selection.

CiteSpace was used to perform cluster analysis and visualize the timeline map and keyword burst diagram. First, the modularity Q value was 0.7119, and the weighted mean silhouette S value was 0.8902, demonstrating the excellence of the cluster analysis. A total of 12 clusters with the highest K values were obtained (Figure 7B), which included "chemotherapy", "DNA repair", *etc.* We then performed cluster analysis graphically in the timeline view. As shown in Figure 7C, research on the DNA damage response, PARP inhibitors, *BRCA* mutations, precision medicine, and phase III trials has recently become a concern of researchers, who are predicting research frontiers and concerns. These topics collectively suggest the research frontiers and concerns in the field, with a particular emphasis on the integration of targeted therapies and personalized medicine in pancreatic cancer treatment.

Finally, keyword analysis with strong burst strength was performed to reflect recent emerging trends (Figure 7D). We found that "ADP ribose polymerase", "synthetic lethality", and "inhibitors" were related to drug mechanisms, whereas "*BRCA2* mutation", "combination", "chemotherapy", and "cisplatin" were related to clinical therapy. In particular, the citation burst time of the keyword "therapy" (2020-2022) has continued to 2021, which is still ongoing, suggesting that articles on olaparib in clinical research have attracted the attention of researchers. These findings suggest that future research will likely concentrate on optimizing the use of olaparib in combination with other agents, refining treatment strategies on the basis of genetic markers, and further elucidating the molecular mechanisms underlying the drug's efficacy.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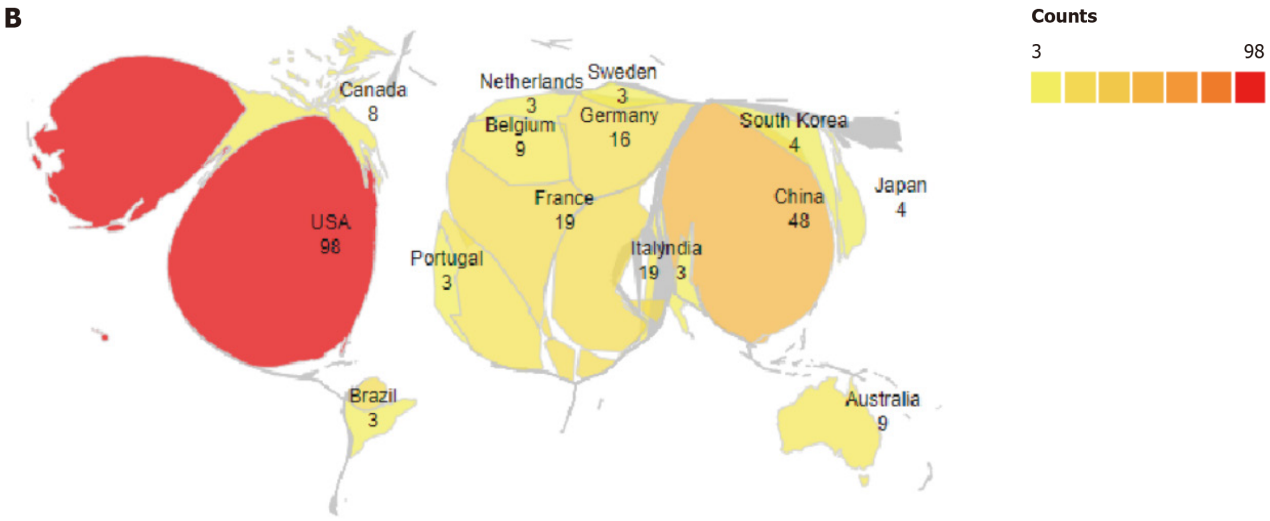
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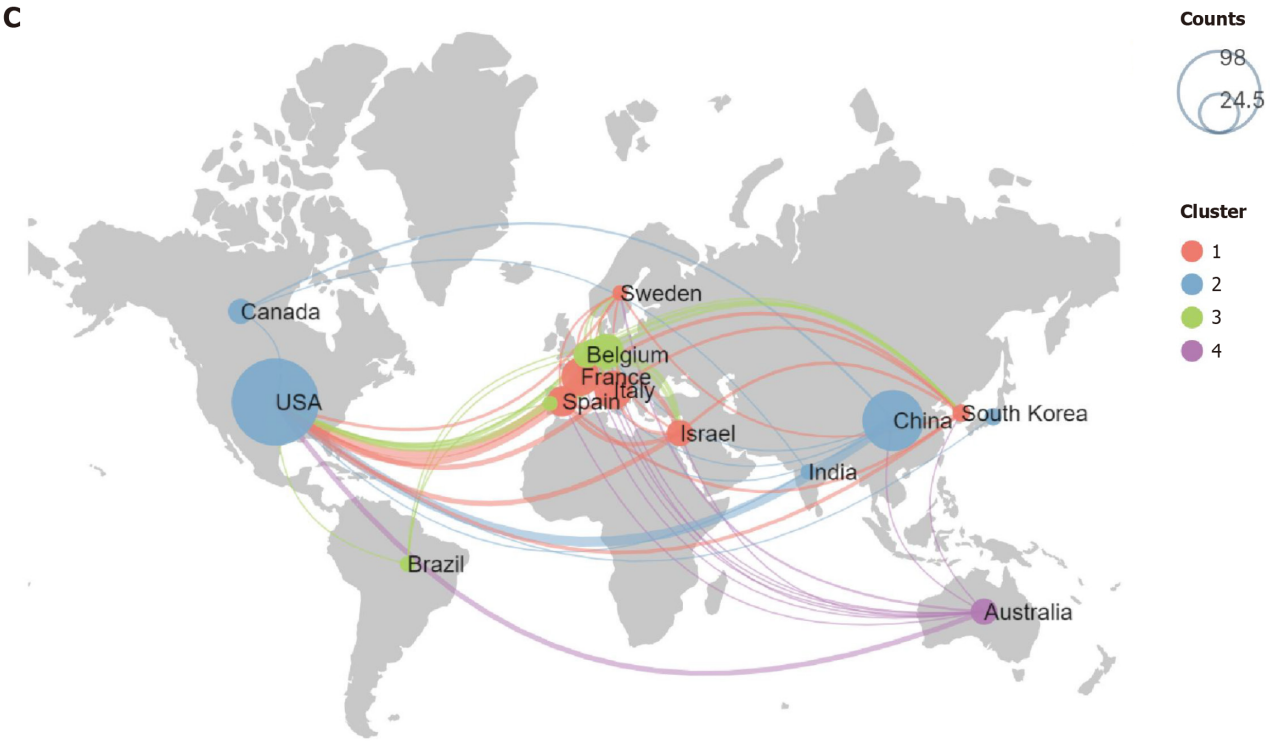
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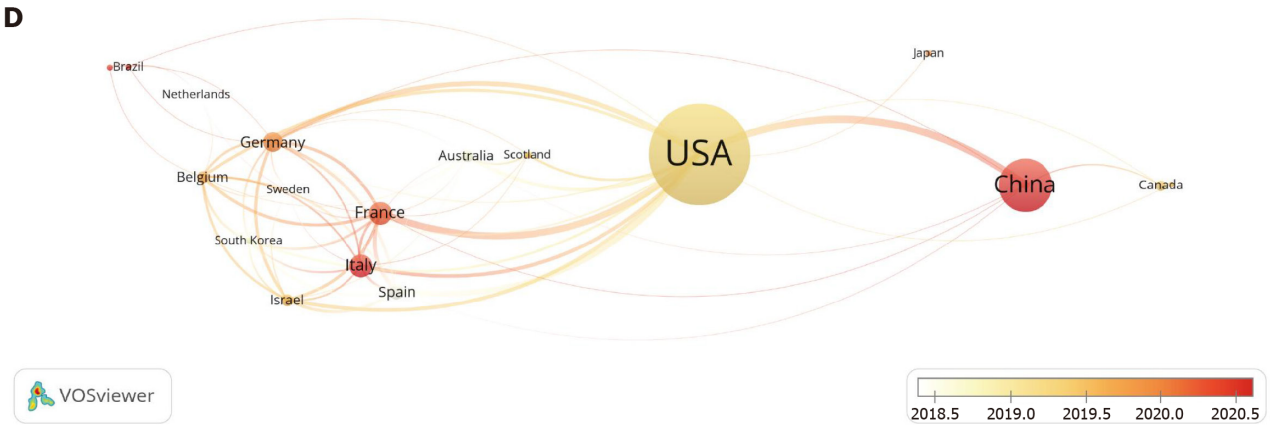

Figure 3 Analysis of countries/regions. A: Countries' production throughout the year; B: Deformed geographical distribution map of global productivity related to olaparib in pancreatic cancer. The area and color represent the number of publications; C: Geographical distribution map and collaboration map of publications related to olaparib in pancreatic cancer. Node size and color represent the number of publications; D: Global collaboration and time evolution of countries/regions in this field. The colors of the nodes represent the average number of publications annually.

DISCUSSION

In our study, we analyzed the main overview and development trends of olaparib and pancreatic cancer. Since the usage of olaparib in cancer patients with *BRCA* mutations, annual production and citations have increased. The United States is the most productive country, and the top 12 of the 30 most productive institutions are located in the United States. The most influential scholar is O'Reilly EM. She is the lead specialist in pancreatic cancer, specializing in the tumor microenvironment of pancreatic cancer and integrating immunotherapy into the treatment of pancreatic cancer[16-18]. Her latest study involved categorizing gATMmt, sATMmt, and zygosity and their roles in HRD. She discovered that ATM variants in pancreatic cancer represent a distinct biological feature with better overall survival (OS) but are not relevant to the HRD signature[19]. The most productive institution is the Memorial Sloan-Kettering Cancer Center (14 published articles), which is among the top 6 most productive institutions. The latest publication concerning OS results from a phase III study of active maintenance therapy with olaparib *vs* placebo in *gBRCAmt* metastatic pancreatic cancer patients was named the Pancreatic Cancer Olaparib Ongoing (POLO) trial[20].

Our analysis shows that the keywords from 2009 to 2022 were "olaparib", "parp inhibitor", "gemcitabine", "breast cancer", "ovarian cancer", "adenocarcinoma", "platinum", "brachemotherapy", and "maintenance therapy" (Figure 7A). Maintenance therapy means reaching the goal of extending PFS and OS without compromising a patient's quality of life. The idea of maintenance therapy was new to pancreatic cancer, but it has already been applied in ovarian cancers and many other cancers with significant benefits[21,22]. The POLO trial was conducted to evaluate the efficacy of olaparib as a maintenance treatment for metastatic pancreatic cancer patients with germline *BRCA* mutations that had not progressed during previous platinum-based first-line chemotherapy[11]. The results certainly support that olaparib as maintenance therapy benefits pancreatic cancer patients with no refractory previous platinum-based chemotherapy, as it prolongs the disease progression rate in 2 years from 9.6% to 22.1% compared with that in the placebo group. Notably, olaparib as a maintenance therapy has few adverse effects, suggesting that pancreatic cancer patients have maintained their quality of life. From 2014 to 2016, the keywords started with the biological function of *BRCA* genes in clinical trials, from "DNA damage", "apoptosis" and "DNA repair" to "veliparib", "gemcitabine", "multicenter" and "AZD2281". From 2017 to 2022, the keywords changed to "aphidicolin glycinate", "bard1 expression", "atm protein", "secondary mutation rad51", "frfg1", and "checkpoint" (Figure 7B). This finding indicates that research in this field has changed from single-regimen treatment to combination therapy. In preclinical studies, an antiangiogenic phenomenon has been reported in *PARPi*- and *PARP-1*-knockout mice[23]. Downregulation of *RAD51*, another homologous recombination gene, was observed to be downregulated in the setting of hypoxia and was associated with increased PARPi sensitivity[24]. A randomized phase II study of a combination of cediranib (FGFR1 inhibitor) and olaparib *vs* olaparib monotherapy for women with recurrent platinum-sensitive ovarian cancer revealed improvement in mPFS[25]. The mPFS increased from 9.0 months to 17.7 months with the addition of cediranib. Surprisingly, patients with *gBRCAwt*/u status also benefitted from the addition of cediranib with prolonged mPFS compared with olaparib monotherapy. Lai *et al*[26] reported that FGFR1 inhibitor-resistant pancreatic cancer cells are sensitive to the *FGFR1* inhibitor-- PD173074 after olaparib treatment, indicating that *FGFR1*/PARP can mediate synthetic lethality *in vitro*. However, in a clinical study including 19 patients with metastatic *gBRCAmt* pancreatic cancer, no objective response (OR) was observed after oral treatment with cediranib or olaparib 2 times daily[27]. The median OS was 3.4 months, which suggested that the combination of cediranib and olaparib does not result in clinically effective outcomes in patients with metastatic *gBRCAmt* pancreatic cancer.

For immunotherapy, one potential synergy between PARP inhibitors and immune checkpoint inhibitors, namely PARP inhibitors, is mediated through interferon-independent mechanisms such as PD-L1 upregulation[28,29]. With this underlying mechanism, the use of CTLA-4 and PARP inhibitors in *BRCA1*-deficient tumors produces significant preclinical responses. Long-term inhibition of PARP enzymes results in persistent DNA damage, altering the epigenetics of tumor cells and making them more readily recognized and eliminated by T cells and NK cells, ultimately resulting in

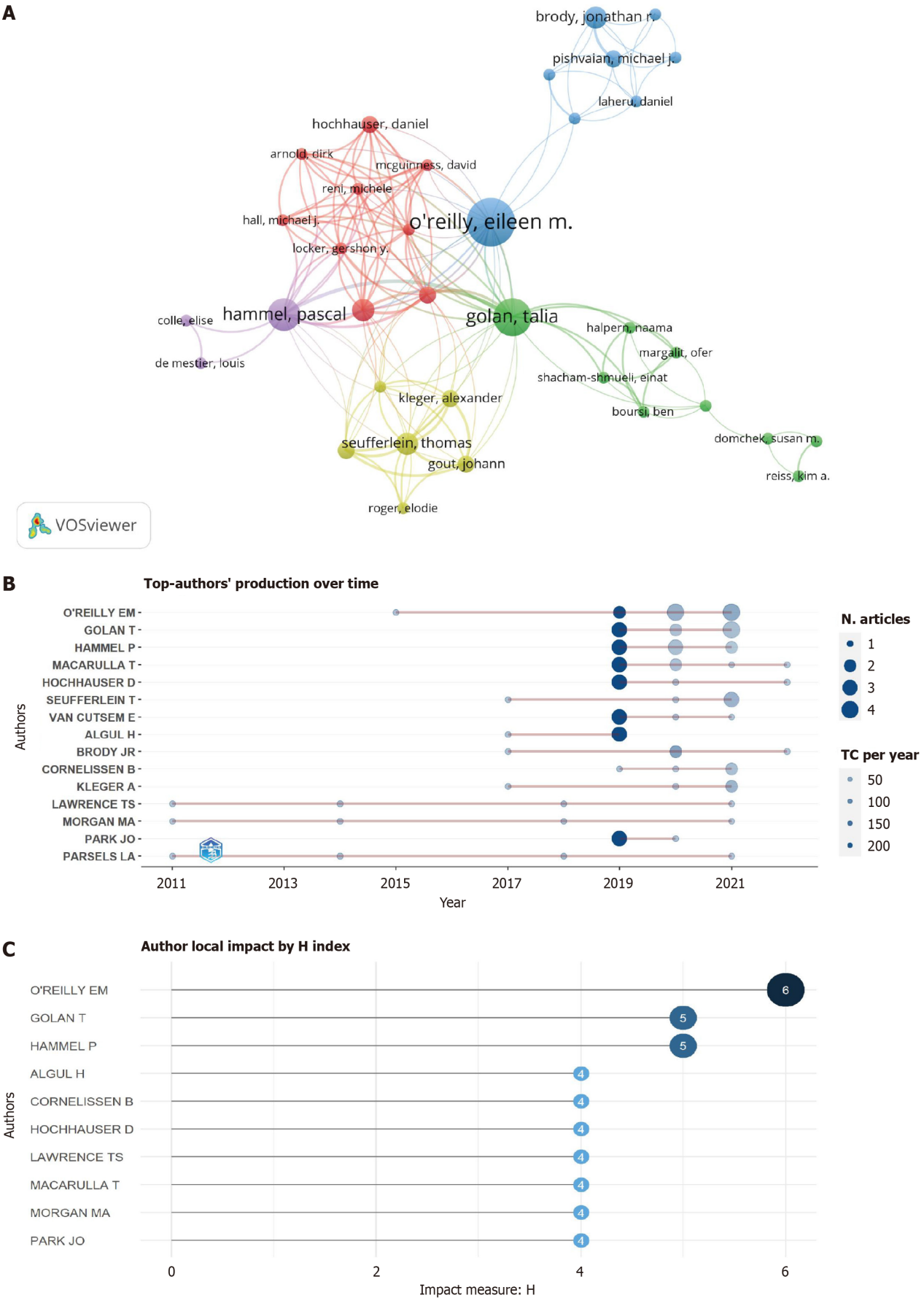

Figure 4 Illustrations of the collaborative network in olaparib and pancreatic cancer. A: Cooperative network and cluster analysis of authors. The thickness of the line indicates the strength of cooperation; B: The top 15 authors' production over time; C: Author local impact according to the H index.

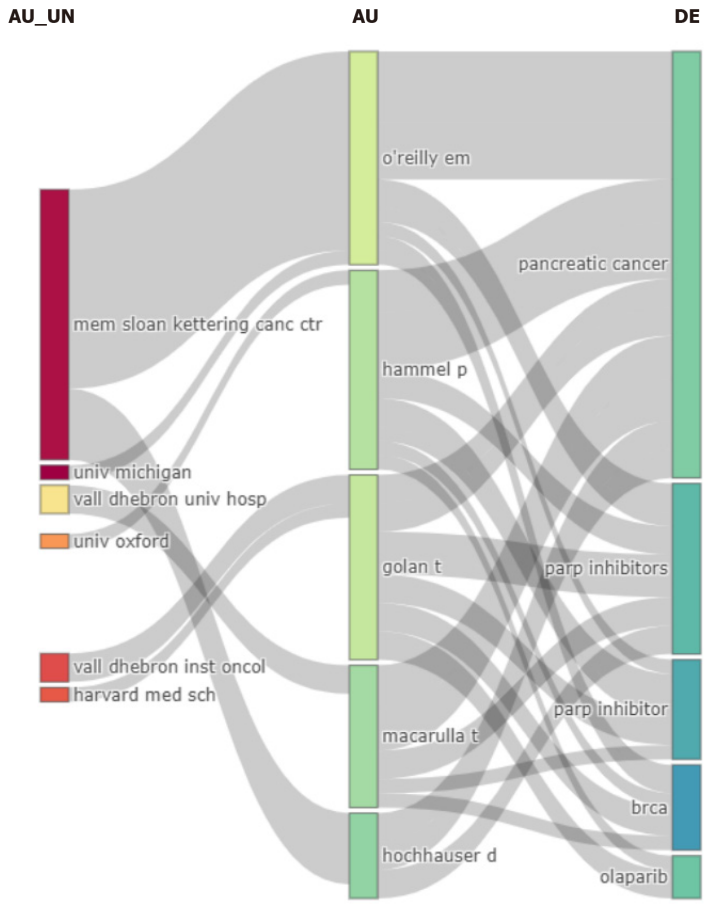

Figure 5 Relationships among institutions, authors, and keywords. Three-field plot of institutions, authors, and keywords related to olaparib in pancreatic cancer.

increased intrinsic immunogenicity of tumor cells[30-32]. Combination therapy with anti-PD-1 (pembrolizumab) and a PARP inhibitor (niraparib) is more effective than therapy with wild-type *BRCA1/2* in *BRCA*-mutated ovarian and triple-negative breast cancer (TNBC) tumors in the TOPACIO trial[33]. Among all 60 patients, the OR rate (ORR) was 25%, the disease control rate (DCR) was 68%, and nearly one-third of patients with platinum-resistant ovarian cancer achieved a response. The ORR and DCR were elevated to 45% and 73%, respectively, in *BRCAmt* tumors. However, as pancreatic cancer has a low response rate to checkpoint inhibitors, investigations have been launched to identify opportunities to increase immunotherapy efficacy *via* combination approaches. The currently ongoing phase Ib/II study named PARPVAX is designed for patients with locally advanced/metastatic pancreatic cancer who do not progress after platinum-based first-line chemotherapy. Eligible patients were included in the niraparib with nivolumab (anti-PD-1) group or the niraparib with ipilimumab group. The primary outcome measure was the 6-month PFS in 2 arms (NCT03404960). Our analysis revealed that combination therapy is a trend in the field of olaparib and pancreatic cancer, and the results of the combination of immunotherapies and PARP inhibitors are promising.

As can be seen from Figure 7D, clinical medication dominates the top 11 most-cited keywords, such as "combination", "chemotherapy", "clinical trial", and "cisplatin", "synthetic lethality". In recent years, more PARP inhibitors have sprung up gradually. The inhibition and trapping of PARP1 alone would be enough to achieve antitumor ability, while selectively inhibiting PARP2 could improve targeted killing of tumor cells with DNA repair deficiency and protect normal cells more effectively. Unlike first-generation PARPi, the newly invented drug AZD5305 minimizes hematological side effects and kills tumor cells with DNA repair deficiency more precisely[34]. This trend means that an increasing number of PARP inhibitors will appear, and an increasing number of combination therapies will be united.

CONCLUSION

By analyzing publication and citation numbers, productive countries, influential authors and institutions, mainstream journals, representative works, co-occurrence keywords, and frontier hotspots over the past 23 years, we can identify both historical and future research trends in olaparib use for pancreatic cancer. The results section highlights the leading roles played by institutions from China and the USA, with other countries and institutions showing steady participation. Global cooperation is becoming increasingly high and productive over time. The team led by O'Reilly EM has contributed the most to this field, with the Memorial Sloan-Kettering Cancer Center having the highest output of articles. The Journal

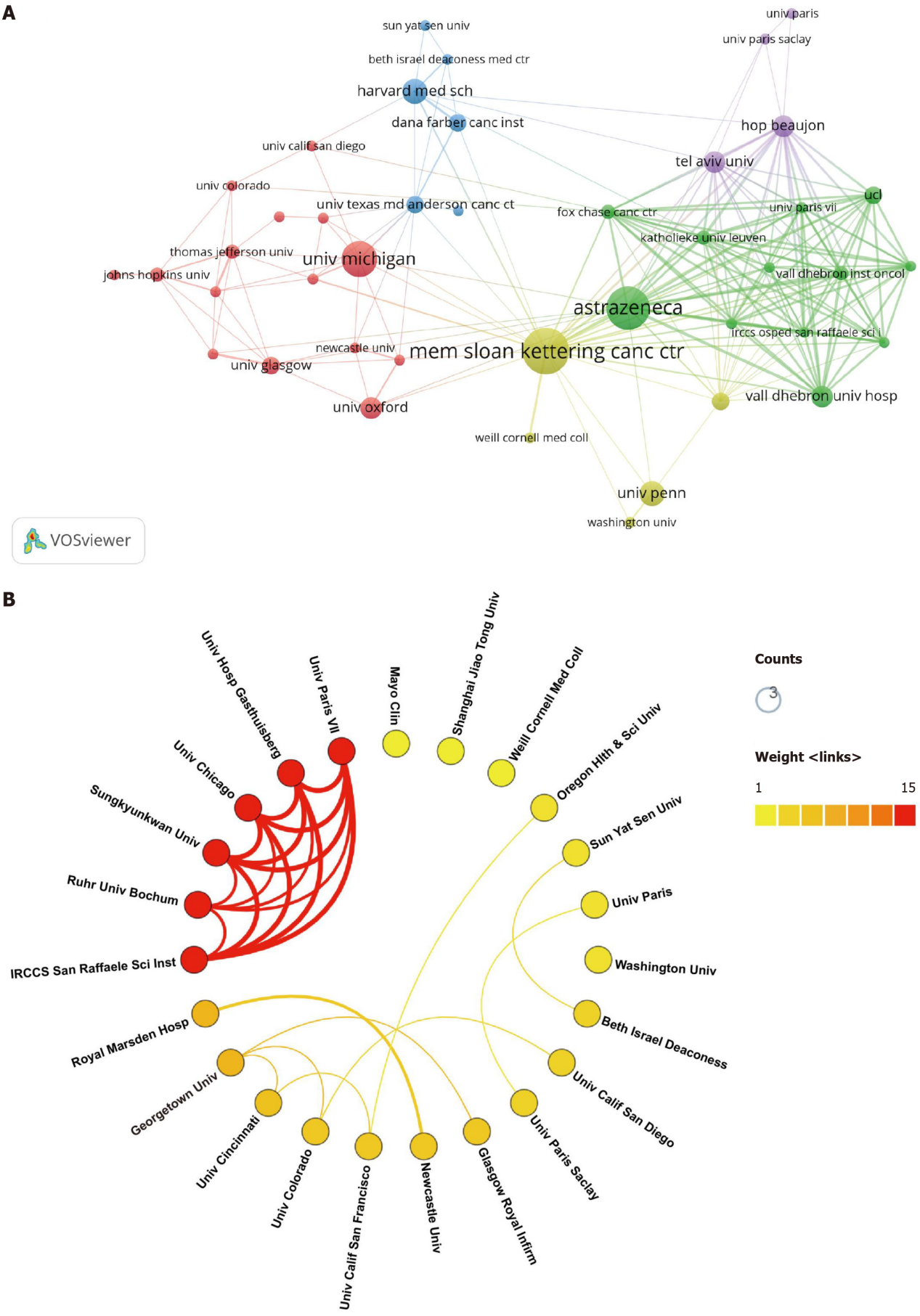

Figure 6 Illustrations of cluster analysis of cooperation among institutions and collaboration networks. A: Visual cluster analysis of cooperation among institutions *via* VOSviewer; B: Cross-institution collaboration network. The color indicates the strength of cooperation between institutions.

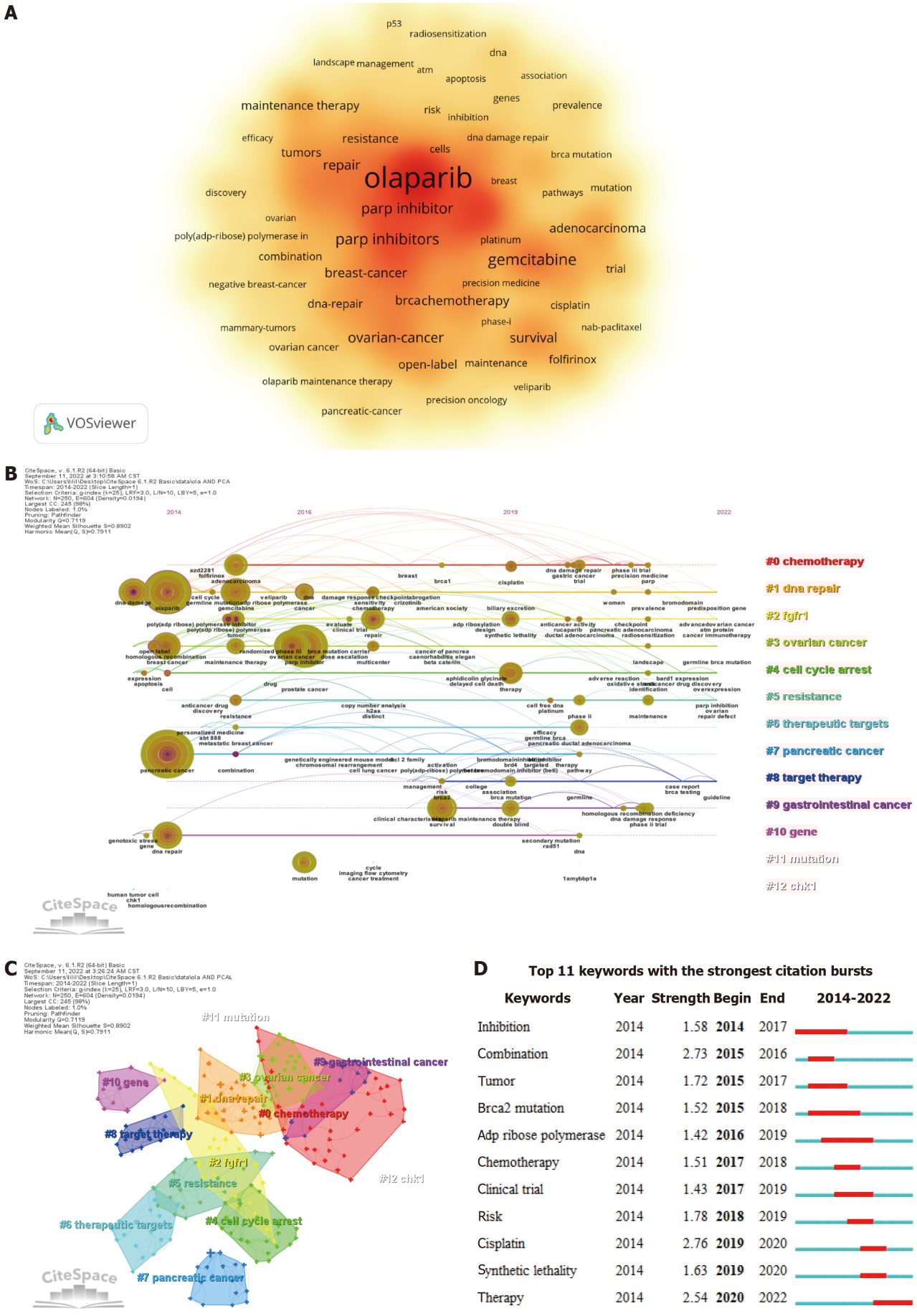

Figure 7 The top 12 clustering terms of olaparib and pancreatic cancer and the keyword trends. A: Density map of the co-occurrence analysis of

keywords. The word size, circle size, and opacity of color are positively related to frequency; B: Cluster analysis of keywords; C: Keyword timeline map of 12 clusters; D: The top 11 keywords with the strongest bursts.

of Clinical Oncology was the most highly cited journal. Keyword trends suggest the emergence of more articles on PARP inhibitors, and combination therapy is becoming a therapeutic trend. In light of practical implications, the emerging trend of a combination therapy underscores the necessity for clinicians to investigate the viability of incorporating olaparib in conjunction with other pharmaceutical agents, such as gemcitabine, to improve the prognosis of individuals afflicted with pancreatic cancer. Furthermore, the discernment of these patterns underscores the importance of personalized medicine, wherein genetic screening for *BRCA* mutations and other pertinent biomarkers can inform the identification of suitable therapeutic interventions.

FOOTNOTES

Author contributions: Feng X and Jiang KX were responsible for conceptualization; Chai YH and Pan Y were responsible for methodology; Jiang KX and Feng X were responsible for investigation; Pan Y, Feng X and Chai YH were responsible for visualization; Chen WC, Jiang WB and Feng X were responsible for supervision; Feng X and Chen WC were responsible for writing – original draft; Pan Y, Feng X and Jiang WB were responsible for writing – review & editing. Feng X and Chai YH are listed as co-first authors for their contributions to both the research and the manuscript. They share responsibility as well as accountability for the work delivered and the research that has been conducted, including substantial contributions to the conception or design of the work, drafting the work or revising it critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Chen WC and Pan Y are listed as co-corresponding authors for their contributions to this manuscript. They have read and agreed to the published version of the manuscript, ensuring that all aspects of the work are accurately represented and that all conditions for authorship are met.

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CASE REPORT

Pathologic complete response to conversion therapy in hepatocellular carcinoma using patient-derived organoids: A case report

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Abstract

BACKGROUND

For primary liver cancer, the key to conversion therapy depends on the effectiveness of drug treatment. Patient-derived tumor organoids have been demonstrated to improve the efficacy of conversion therapy by identifying individual-targeted effective drugs, but their clinical effects in liver cancer remain unknown.

CASE SUMMARY

We described a patient with hepatocellular carcinoma (HCC) who achieved pathologic complete response (pCR) to conversion therapy guided by the patient-derived organoid (PDO) drug sensitivity testing. Despite insufficiency of the remaining liver volume after hepatectomy, the patient obtained tumor reduction after treatment with the PDO-sensitive drugs and successfully underwent radical surgical resection. Postoperatively, pCR was observed.

CONCLUSION

PDOs contributes to screening sensitive drugs for HCC patients to realize the personalized treatment and improve the conversion therapy efficacy.

Key Words: Tumor organoids; Hepatocellular carcinoma; Drug sensitivity testing; Conversion therapy; Pathological response; Case report

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Core Tip: Patient-derived tumor organoids have been demonstrated to improve the efficacy of conversion therapy by identifying individual-targeted effective drugs. Here we described a patient with hepatocellular carcinoma (HCC) who achieved pathologic complete response to conversion therapy guided by the patient-derived organoid (PDO) drug sensitivity testing. This typical case suggests that PDO-based drug sensitivity testing contributes to screening sensitive drugs for HCC patients to realize the personalized treatment and to improve the efficacy of conversion therapy, which may change the previous experiential therapy and serve as a novel treatment mode in liver cancer.

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INTRODUCTION

Primary liver cancer (PLC) is a common malignant tumor in the digestive system, and the treatment modalities for PLC include hepatectomy, liver transplantation, ablation therapy, transarterial chemoembolisation (TACE), radiation therapy, systemic anti-tumor therapy, and more. At present, radical surgical resection remains the only completely cured method for PLC[1,2]. Selection of appropriate treatment methods for liver cancer patients at different stages can maximize the therapeutic effect[3]. Conversion therapy is an effective technique for patients with advanced liver cancer to achieve radical resection and long-term survival, which can transform the liver cancer with poor oncological characteristics into that with good oncological characteristics, thereby reducing postoperative recurrence and prolonging the survival[4,5]. Currently, the most common conversion therapies for liver cancer include systemic anti-tumor therapy, local therapy, and radiotherapy[4]. However, one of the key issues that urgently need to be solved is the selection of conversion therapy regimens. Current treatment mainly depends on traditional experience or clinical trial results. On the one hand, an effective treatment regimen for most patients may be ineffective for a certain case at the individual level. On the other hand, how to choose the most effective regimen among the various options is a core concern of conversion therapy.

Tumor organoids, a kind of three-dimensional (3D) microstructures formed by *in vitro* culture of tumor tissues from patients under a highly similar condition to the human microenvironment, can maintain the biological behaviors and functions of original tumors while retaining pathohistological and genetic features, which provide an effective platform for tumor research and drug discovery[6,7]. Unlike patient-derived xenografts, patient-derived organoids (PDOs) require less time and tissue for establishment and can faithfully recapitulate the key characteristics of original tumors even after long-term passaging[8]. By comparing responses to antitumor agents from PDOs and PDO-based xenograft models with those of patients in clinical trials, Vlachogiannis *et al*[9] demonstrated that PDOs effectively retained the patients' clinical response and could be used in precision medicine protocols. Importantly, the PDOs can be co-cultured with immune cells, cancer-associated fibroblasts and vasculatures to model the tumor microenvironment (TME), thereby allowing for more effective screening of personalized drugs[10].

Currently, organoid-based drug sensitivity testing is gradually being applied in various scenarios, including neoadjuvant and/or palliative chemotherapy, ineffective first-line treatment, advanced and rare cancers, *etc*[11]. Here, we reported a patient with hepatocellular carcinoma (HCC) who achieved pathologic complete response (pCR) to conversion therapy under the guidance of the PDO-based drug sensitivity testing.

CASE PRESENTATION

Chief complaints

A 55-year-old woman was admitted to hospital because of abdominal pain and fever for 10 days.

History of present illness

The patient had abdominal pain and fever for 10 days.

History of past illness

The patient previously received multiple surgeries, including choledochocystectomy, biliary-enteric anastomosis and cholecystectomy for congenital choledochal cysts, as well as left lateral hepatic lobectomy and bile duct exploration for left intrahepatic bile duct stones.

Personal and family history

The patient denied any family history of malignant tumors.

Laboratory examinations

Laboratory examinations included: Carbohydrate antigen (CA) 19-9 of 49.09 U/mL, CA125 of 83.8 U/mL, alpha fetoprotein of 1.64 ng/mL, carcinoembryonic antigen of 1.3 ng/mL, and CA15-3 of 8.5 U/mL (Table 1).

Imaging examinations

The computed tomography (CT) scan showed a slightly low-density mass shadow in the right lobe of the liver, suggesting the possibility of tumors or metastatic lesions (Figure 1A-C). Positron emission tomography/CT further indicated a liver tumor without distant metastasis.

FINAL DIAGNOSIS

In combination with relevant examinations and previous history of surgery, it was speculated that the tumor was located in the right posterior lobe of the liver, with a diameter of 9.0 cm × 6.4 cm. After multi-disciplinary team (MDT) discussion, a needle biopsy was performed, and HCC was confirmed (Figure 2).

TREATMENT

After the patient and her family members were informed consent, tumor tissues from needle biopsies were collected for organoid culture. First, the tumor tissues were rinsed using precooled phosphate-buffered saline, and then minced. Second, cell pellets were collected through centrifugation following 30-minute digestion. When Matrigel was added, cells and Matrigel suspension were both seeded onto 6-well plates (2 mL per well) using pipettes, and the plates were placed in a 37 °C incubator for 15 minutes. Third, the culture medium [Kingbio Medical (Chongqing) Co., Ltd., China] was supplemented after the droplets were fully solidified, and the plates were again placed into an incubator (37 °C, 5% CO_2) for culture. Notably, the culture medium was replaced every 2-3 days. Subsequently, the organoids conforming to requirements were seeded in 96-well plates, with corresponding drugs added. There were at least 3 compound pores. Meanwhile, a negative control was set up[12]. Finally, the organoid activity values were read using a multimode reader after treatment with drugs, and the drug sensitivity was calculated.

During organoid culture, TACE with lobaplatin (50 mg) was used as the initial treatment option in combination with Lenvatinib (40 mg/d) and tislelizumab (200 mg per time, once every three weeks). However, the increased levels of tumor markers CA19-9 and CA125 were observed after treatment (Table 1). Based on the organoid drug sensitivity testing, we found that the PDO was more sensitive to doxorubicin compared with other agents (Figure 3), thus lobaplatin was replaced by doxorubicin in TACE, but the usage and dosage of Lenvatinib and tislelizumab were unchanged. After two cycles of treatment, the levels of tumor markers CA19-9 and CA125 were decreased significantly (Table 1). CT and magnetic resonance imaging examinitions both showed a signifcantly reduced tumor diameter in the liver (Figure 1D-I), which was assessed resectable according to Response Evaluation Criteria in Solid Tumors (version 1.1). In addition, the liver functional reserve indicators of the patient were also improved. Prior to neoadjuvant therapy, the patient had Child-Pugh B, indocyanine green retention rate at 15 minutes (ICG-R15) of 15% and China liver cancer staging of Ib. After neoadjuvant therapy, the patient was assessed as Child-Pugh A and ICG-R15 of 8.3%.

Through MDT discussion, the sufficient remaining liver volume was calculated after lesion resection. Subsequently, right posterior hepatic lobectomy and partial phrenectomy were performed following communication with the patient. The time interval between the last preoperative TACE and surgery was 4 weeks. Postoperative pathology suggested partial liver cell edema, chronic inflammation in the portal area, fibrous hyperplasia, and focal deposition of hemosiderin, without definite residual cancer tissues and cancer cells in the cutting edge, suggesting pCR.

OUTCOME AND FOLLOW-UP

The patient attended our hospital for further consultation 7 months after surgery, and no recurrence or metastasis was observed based on CT examinations (Figure 1J-L).

DISCUSSION

The PLC originating from the epithelial or mesenchymal tissues of the liver is one of the most common malignancies, of which HCC accounts for 75%-85%[13]. Currently, radical surgery remains the main treatment modality for liver cancer patients to achieve long-term survival[3]. However, most liver cancer patients are initially diagnosed at the middle and advanced stage, inappropriate for surgical resection. Although some patients have undergone surgery, the risk of postoperative recurrence and metastasis is high.

Conversion therapy aims to converting unresectable liver cancer into resectable liver cancer to remove the tumor, which is one of the most promising ways to perform radical surgery and improve the long-term survival of patients with liver cancer[4]. At present, use multimodal and high-intensity anti-tumor therapies is recommended to promote the

Table 1 Changes of tumor markers at different time points

Indicators	AFP (ng/mL)	CA19-9 (U/mL)	CEA (ng/mL)	CA125 (U/mL)	CA15-3 (U/mL)
On admission	1.64	49.09	1.30	83.80	8.50
After the initial treatment	2.07	55.00	1.66	91.00	9.00
After organoid-based treatment	2.45	25.67	1.21	34.20	10.20
7 months after surgery	2.37	27.41	1.36	23.95	10.60

AFP: Alpha fetal protein; CA: Carbohydrate antigen; CEA: Carcinoembryonic antigen.

conversion of the PLC, with consideration of the treatment safety[5]. The commonly used anti-tumor treatment modalities for liver cancer include TACE, targeted therapy, immunotherapy, radiotherapy, or a combination of multiple therapies, among which chemotherapy mainly focusing on liver tumor perfusion/embolization and targeted therapy are one of the most important methods for liver tumor conversion. However, there is still a lack of precise methods regarding the selection of specific drugs for liver tumors. The selection strategy of each center is to choose the most effective plan based on guidelines or clinical experience, or to choose a new plan based on the latest clinical research. Notably, there exist several problems. First, the most effective plan, as proven by experience, may be effective for most cases, but may be inefficient or even ineffective in some patients. Second, for individual cases, there may be several effective chemotherapy or targeted regimens available simultaneously. At present, choosing the most effective regimen to achieve the optimal conversion efficiency is still difficult.

In recent years, the emergence of 3D culture technology represented by PDOs has opened up a new method for studying tumor evolution and evaluating treatment response. For liver cancer, several major subtypes of organoids have been successfully established, including HCC, cholangiocarcinoma, biliary tract cancer, etc. However, the success rate of generating liver cancer organoids is only about 30%[14], significantly lower than 75%-85% for pancreatic cancer organoids[15] and 90% for colorectal cancer organoids[16], which may partially be explained by epithelial-mesenchymal transition and limited HCC subpopulations. In our study, HCC organoids from needle biopsies were successfully generated, supported by the data from the study by Nuciforo *et al*[14].

Currently, liver cancer organoids have been demonstrated not only to retain the histological and molecular features of original tumors, but also help identify the drug sensitivity of individual patients[17]. Saltsman *et al*[18] presented the potential of hepatoblastoma organoids in improving treatment options for a subset of hepatoblastoma patients irresponsive to existing treatments. Additionally, based on pharmaco-proteogenomic profiling of the liver cancer organoids, Ji *et al*[19] identified potential drug combination therapies, thus offering guidance for clinical patient selection and drug combination therapies. Although a strong association between use of PDOs and clinical outcomes in predicting chemotherapy and/or radiotherapy efficacy has been confirmed in multiple cancer types[9,20,21], there is lack of evidence in HCC, especially in use of PDOs to guide conversion therapy. In our study, the initial treatment of TACE with lobaplatin in combination with tislelizumab and Lenvatinib did not yield satisfactory therapeutic effects. According to organoid drug sensitivity testing results, one of the chemotherapy drugs during TACE was adjusted to doxorubicin, with unchanged usage and dosage of Lenvatinib and tislelizumab. After treatment, tumor markers returned to normal, and the tumor significantly reduced. The patient achieved pCR after radical resection, and no recurrence or metastasis was observed 7 months after surgery. Collectively, the organoid drug sensitivity testing helps to screen suitable chemotherapy drugs for liver cancer patients, thereby promoting conversion therapy.

Notably, surgical safety should be considered for HCC patients successfully achieving conversion therapy. However, for different preoperative treatments the timing of surgical resection is different. It is reported that continuous use of small molecule drugs, such as tyrosine kinase inhibitors, cannot increase the incidence of postoperative complications [22]. If conversion therapy based on immune checkpoint inhibitors is applied for HCC preoperatively, surgery should be performed within 4 weeks after the last dose[23]. If adverse reactions occur during neoadjuvant therapy, surgery will be performed after drug withdrawal until grade I or no adverse reactions[24]. Additionally, TACE-based neoadjuvant therapy can cause liver inflammation, increase the amount of intraoperative bleeding and the difficulty of surgical procedures. It is recommended in Chinese expert consensus on conversion and perioperative therapy of PLC that the interval between the last preoperative TACE and surgery should be greater than 4 weeks[24]. In our case, the duration between the preoperative last TACE and surgery was just 4 weeks.

To the best of our knowledge, this is the first case to select the most effective regimen to promote conversion therapy using the PDO model and the first case to transform empirical modes into precision modes. During organoid culture, traditional empirical models including TACE plus targeted therapy were first used. Three weeks later, precise treatment was employed based on the organoid drug sensitivity results, and pCR was obtained. Notably, there are still some challenges in the use of PDOs in clinical practice, including sample availability and processing, standardization of culture protocols, turnaround time and scalability, recapitulation of the TME, optimization of culture medium, *etc*[11]. With technical development and continuous research, the combination of the PDOs with other advanced technologies, such as organ-on-a-chip, 3D bioprinting and CRISPR-HOT, allows for modeling more complicated and realistic state, which may help to overcome the above challenges and create more appropriate model systems for cancer treatment.

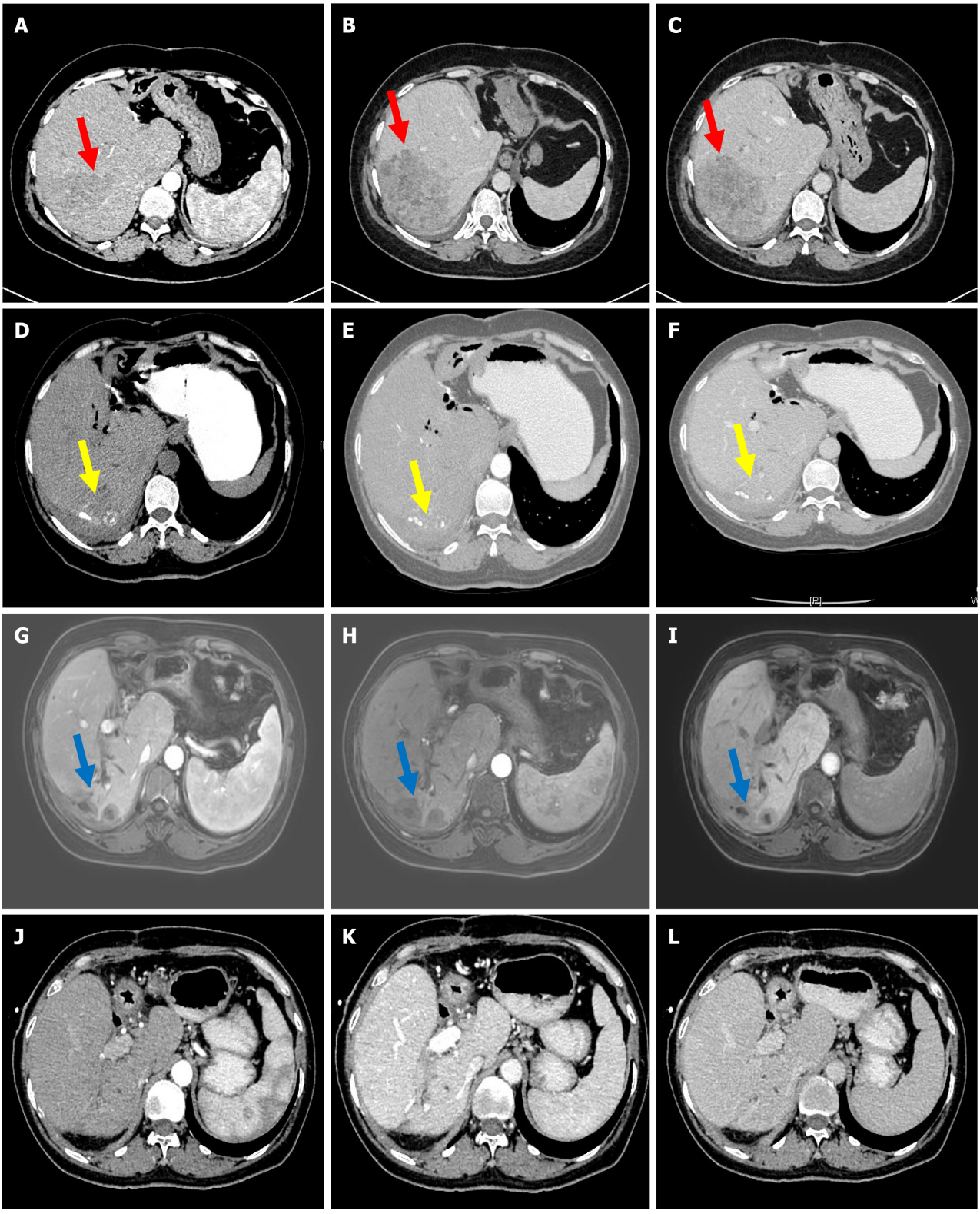

Figure 1 Radiological images of the patient before and after conversion therapy. A-C: computed tomography (CT) examination shows that the tumor is in the right lobe of the liver before conversion therapy; D-F: CT and magnetic resonance imaging; G-I: Examinations both indicate a decreased tumor in the right lobe of the liver before surgery; J-L: Seven months after surgery, no recurrence or metastasis was observed under the CT. The red, yellow, and blue arrows all head towards the tumor.

CONCLUSION

PDO-based drug sensitivity testing contributes to screening sensitive drugs for HCC patients to realize personalized treatment and to improve the effectiveness of conversion therapy, which may change the previous experiential therapy and become a novel treatment mode in liver cancer.

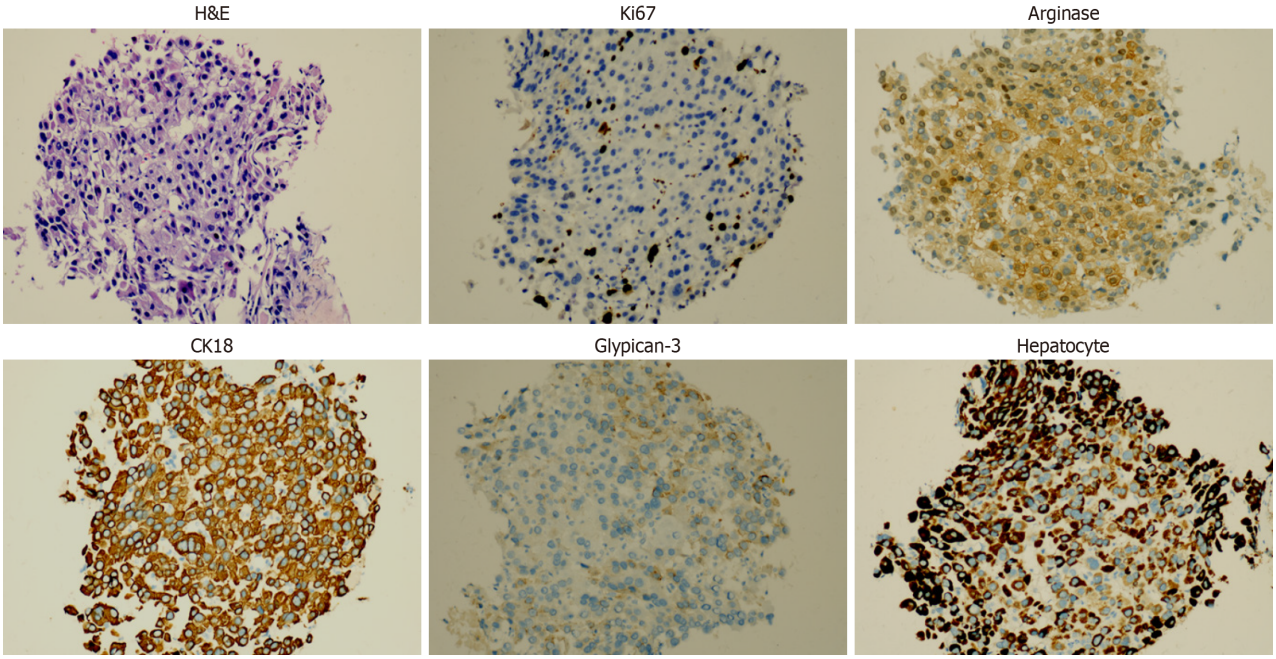

Figure 2 Images of HE and immunohistochemical staining of the biopsy specimen (× 200).

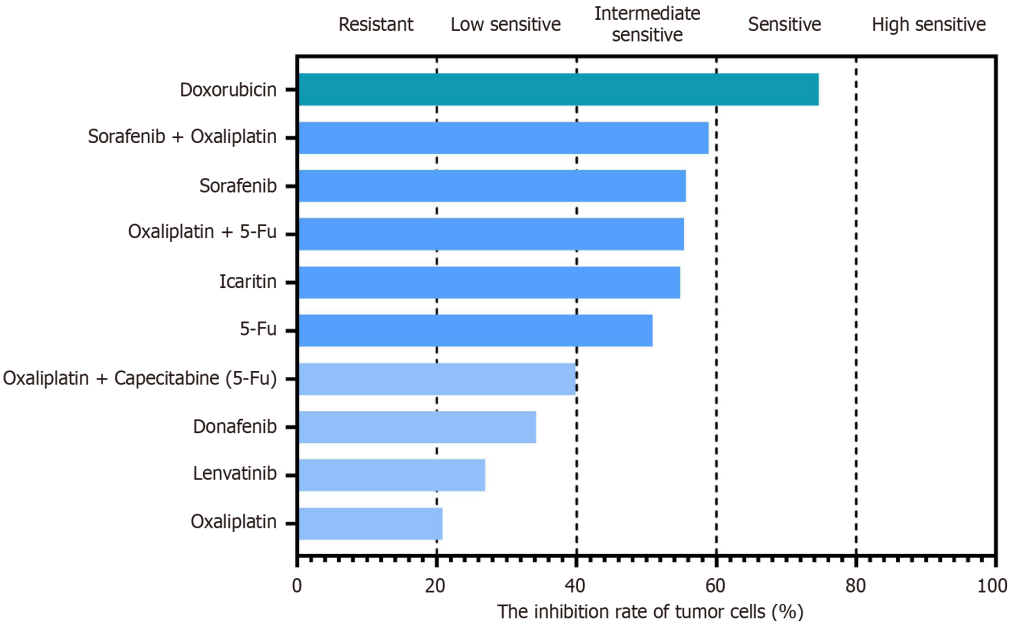

Figure 3 The drug sensitivity results of the patient-derived organoid from hepatocellular carcinoma.

FOOTNOTES

Author contributions: He YG, Li J and Wang Z were responsible for writing the first draft; Xi W and Zhao CY participated in data acquisition and investigation. Supervision was performed by Huang XB and Zheng L. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All the authors contributed to the study conception and design. He YG and Wang Z contributed equally to this work as co-first authors.

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LETTER TO THE EDITOR

Vascular endothelial growth factor pathway's influence on bevacizumab efficacy in metastatic colorectal cancer treatment

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Abstract

In this article, an article published in the *World Journal of Gastrointestinal Oncology*, which focuses on whether the expression of programmed death-ligand 1 (PD-L1) affects the effectiveness of chemotherapy regimens, including bevacizumab, in treating patients with colorectal cancer (CRC). Through neutralization of vascular endothelial growth factor (VEGF), bevacizumab inhibits tumor angiogenesis, impairing neovascularization and thereby depriving the tumor of essential nutrients and oxygen. Conversely, PD-L1 binding to VEGF receptor 2 promotes angiogenesis, supporting tumor vasculature. The interplay between these pathways complicates the assessment of bevacizumab's efficacy in cancer therapy, notably in CRC, where VEGF and PD-L1 significantly affect treatment response. This review examines metastatic CRC treatment strategies, focusing on bevacizumab's mechanism of action and the role of PD-L1 in this therapeutic context.

Key Words: Bevacizumab; Chemotherapy; Metastatic colorectal cancer; PD-1/PD-L1 axis; Therapeutic approach; Vascular endothelial growth factor

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Core Tip: In the management of colorectal carcinoma, bevacizumab wields its therapeutic impact *via* the neutralization of vascular endothelial growth factor (VEGF), a paramount mediator of intratumoral angiogenesis. This inhibitory action on VEGF obstructs neovascularization, consequently sequestering the essential sustenance of nutrients and oxygen requisite for tumoral proliferation and viability. Contrarily, the interaction between programmed death-ligand 1 and VEGF receptor 2 catalyzes the genesis of novel vasculature that sustains and nurtures the neoplasm, thereby potentiating angiogenic processes.

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TO THE EDITOR

Colorectal cancer (CRC), a malignancy that predominantly affects the colon and rectal regions, is the third most common cancer worldwide. As per the authoritative global cancer statistics documented in 2024, the disease trajectory of CRC has undergone a remarkable shift since the late 1990s. Initially, it was identified as the fourth foremost contributor to cancer-related fatalities among men and women under the age of 50 years. However, in contemporary times, CRC has ascended to the apex as the primary cause of cancer-related mortality in males, it also ranks second in females, underscoring its escalating impact on public health across demographics[1]. Among patients diagnosed with CRC, nearly one-quarter present with metastatic disease (metastatic CRC, mCRC) at the onset of symptoms, and within this group, approximately 20% were initially diagnosed with localized disease that subsequently progressed to stage IV. Stage IV diseases portend a poor prognosis, with an estimated 5-year survival rate of merely 14%[2]. Presently, the therapeutic landscape for CRC is multifaceted, featuring a spectrum of treatment modalities designed to address the complex nature of this malignancy. These modalities include definitive surgical resection, cytotoxic chemotherapy, precision-targeted therapies, and immunotherapeutic interventions. Contemporary oncological practice often employs a multimodal approach, integrating multiple treatment strategies in a coordinated fashion to optimize patient outcomes and survival rates. This comprehensive care model acknowledges the heterogeneity of CRC and tailors the therapeutic plan to the unique characteristics of each patient's disease, aiming to maximize therapeutic efficacy while minimizing adverse effects[3]. Therapeutic strategies for CRC are customized to match the disease's stage. In stage IV mCRC, where malignancy spreads systemically, surgery alone is inadequate. Initial treatment thus favors a combined therapy approach, integrating chemotherapy, targeted treatments, and immunotherapies to optimize control and improve the quality of life of patients with mCRC.

Combination therapy for MCRC

In efforts to enhance frontline therapies for resectable mCRC, core research aims at immediate tumor containment, symptom alleviation, disease stabilization, and minimalization of metastatic spread before surgery. Therapeutic approaches now synthesize traditional chemotherapy with innovative molecularly targeted drugs, remakably improving response rates, extending progression-free survival (PFS) periods, and crucially enhancing overall survival (OS) outcomes for patients with mCRC, signaling a pivotal evolution in the treatment of advanced colorectal malignancies[4]. Regarding chemotherapy protocol choices in mCRC, FOLFOX and FOLFIRI exhibit comparable efficacy but distinct toxicity profiles. Their combination with targeted therapies is essential in mCRC management[5-7]. Selecting between them demands a thorough assessment of patient demographics, health status, comorbidities, personal inclinations, and considerations of drug-related toxicities and accessibility. On the basis of the distinct stages of mCRC, a comprehensive evaluation approach is adopted, integrating molecular biological characteristics, drug tolerance, tumor burden, and the patient's overall health condition to formulate the most appropriate treatment regimen. Currently, a plethora of combination therapy options are available, encompassing various permutations, such as quadruple therapy with FOLFIRINOX plus bevacizumab or cetuximab; triple therapy involving fluoropyrimidines in conjunction with oxaliplatin or irinotecan and either bevacizumab, cetuximab, or panitumumab; and dual therapy consisting of capecitabine paired with bevacizumab [3]. Comparable in prolonging PFS, these regimens exhibit unique variations in adverse effects and response rates. The most suitable treatment is matched through meticulous patient-specific assessments. The targeted drugs within these combinations inhibit critical pathways, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and B-Raf proto-oncogene, serine/threonine kinase (BRAF), which synergistically attack tumors for enhanced and expanded therapeutic effects, delivering anticipated clinical successes.

Targeted therapeutic pathways for MCRC

Angiogenesis in tumors signifies cancer progression[8,9]. Physiologically, angiogenesis is regulated by a balance between pro- and anti-angiogenic factors[10], with VEGF signaling being a cardinal pathway. The VEGF signaling network encompasses secreted proteins, such as VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor, along with receptor tyrosine kinases, including VEGFR-1, VEGFR-2, and VEGFR-3[10,11]. These components serve as pivotal regulators of the angiogenic process and play a significant role in the therapy of mCRC, marking them as potential therapeutic targets[12,13]. Bevacizumab, as an angiogenesis-targeting agent, exerts its therapeutic effects primarily by precisely obstructing the neovascularization process in tumors through specifically binding to VEGF-A, effectively neutralizing VEGF's biological activity and thereby preventing its interaction with surface receptors, ultimately inhibiting the formation of new blood vessels[14]. Given that tumor proliferation and metastasis are heavily reliant on nutrients and oxygen delivered *via* neo-angiogenesis, this mechanism significantly retards tumor progression. As a result, when combined with chemotherapy, bevacizumab, which is an anti-VEGF monoclonal antibody, becomes a cornerstone treatment strategy for mCRC because it has been proven to substantially improve OS in patients. Despite the proven extension of survival in many patients through anti-angiogenic therapy, particularly the blockade of VEGF-A, angiogenesis eventually resumes in most individuals, a phenomenon attributed to various known resistance mechanisms impacting VEGF-targeted treatments[15]. Currently, research on the biomarkers for mCRC is actively underway[16].

However, no biomarker has yet been identified that can accurately predict which patients may respond to anti-angiogenic therapy and develop resistance. Therefore, although significant strides have been achieved in the field, further exploration is still required regarding how to more precisely select patients suitable for such therapies and how to overcome resistance. The article approaches immunotherapy as a pivotal point, focusing on the modulation of tumor immune responses through targeting programmed death 1 (PD-1) and its ligand PD-ligand 1 (PD-L1). The expression of PD-L1 is regulated by multiple pathways, and lower expression typically correlates with reduced T-cell infiltration, often leading to superior therapeutic outcomes compared with that in tumors with higher expression levels[17,18]. Anti-VEGF monoclonal antibodies not only suppress angiogenesis and reduce tumor activity but also induce hypoxia, which facilitates enhanced sensitivity of effector T cells to PD-1/PD-L1 inhibition[5,19,20]. PD-L1 may engage in interactions with VEGF, thereby facilitating angiogenesis and metastatic processes in neoplastic cells[21]. Hence, the interplay between PD-L1 and VEGF signaling pathways indicates that anti-PD-L1/PD-1 therapy may have a potential combined effect with anti-angiogenic therapy in various types of tumors. The study concludes that the efficacy of bevacizumab is not correlated with PD-L1 expression, thus, irrespective of the expression levels of PD-L1, the combination of chemotherapy with bevacizumab can be deemed a first-line therapeutic option for patients with metastatic CRC. Although the article does not fully achieve its research goals, it provides novel perspectives for subsequent clinical treatments by highlighting the connection between PD-L1 immune checkpoint regulation and VEGF-mediated angiogenesis pathways, thereby broadening the therapeutic landscape in clinical practice.

CONCLUSION

Within the intricate landscape of the tumor microenvironment, hypoxic conditions spur cancer cells and vascular endothelial cells to secrete VEGF. This potent cytokine catalyzes angiogenesis, fueling tumor expansion, tumor invasion, and the dissemination of malignant cells to distant sites. The advent of VEGF inhibitors has revolutionized cancer therapy by reprogramming the immunosuppressive milieu of tumors, fostering an environment amenable to immune activation. Emerging evidence suggests that combining VEGF inhibition with the blockade of the interaction between PD-L1 and its receptor PD-1 may produce additive or even cooperative benefits, thereby enhancing the efficacy of cancer treatments. This study specifically investigated the implications of PD-L1 expression in the context of mCRC therapy, utilizing bevacizumab—a targeted VEGF inhibitor—concomitantly with chemotherapy. Special emphasis was placed on elucidating the interconnections among VEGF signaling, PD-L1, and VEGFR2. Although PD-L1 expression did not markedly influence the clinical outcomes of bevacizumab in combination with chemotherapy, this finding illuminated promising avenues for future research. It encourages the scientific community to delve deeper into the complex interplay between diverse biomarkers and pivotal oncologic pathways, encompassing VEGF, EGFR, and BRAF mutations, with the ultimate goal of unearthing novel therapeutic strategies. Advancements in therapeutic optimization and precision medicine are anticipated through meticulous analysis of these biomarkers as conduits and evaluation of the synergistic potential of inhibitors paired with chemotherapy in mCRC. Such endeavors not only shed light on the convoluted molecular underpinnings of cancer but also herald a new era of personalized medicine, wherein tailored treatment regimens are crafted in accordance with the unique biomarker signatures of individual patients. This comprehensive strategy holds the promise of significantly boosting survival rates and improving the quality of life of those battling mCRC while offering critical insights that could inform the development of treatment paradigms for a broadened spectrum of malignancies.

FOOTNOTES

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LETTER TO THE EDITOR

From biomarker discovery to combined therapies: Advancing hepatocellular carcinoma treatment strategies

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Abstract

This editorial reviews advances in hepatocellular carcinoma (HCC) treatment, focusing on a triple therapy approach and biomarker discovery. Zhang *et al* discuss the synergistic potential of transarterial chemoembolization combined with tyrosine kinase inhibitors and PD-1 inhibitors. Meanwhile, Li *et al* identify protein tyrosine phosphatase non-receptor II (PTPN2) as a biomarker for poor prognosis and immune evasion in HCC. The studies highlight the importance of combined therapies and biomarkers in improving HCC treatment efficacy and patient outcomes, with PTPN2 emerging as a potential therapeutic target. This article supplements the aforementioned studies with more recent research advancements, focusing on the molecular mechanisms and clinical applications of biomarkers.

Key Words: Hepatocellular carcinoma; Triple therapy; Transarterial chemoembolization; Protein tyrosine phosphatase non-receptor II

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Core Tip: This article reviews the integration of novel combined therapies and biomarker identification in hepatocellular carcinoma (HCC) management. The studies by Zhang *et al* and Li *et al* explore the efficaciousness of a triple therapy involving transarterial chemoembolization, tyrosine kinase inhibitors, and PD-1 inhibitors, and the prognostic value of protein tyrosine phosphatase non-receptor II (PTPN2), respectively. These investigations underscore the significance of PTPN2 as a potential therapeutic target and highlight the promise of synergistic treatment strategies in enhancing HCC patient outcomes.

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TO THE EDITOR

Hepatocellular carcinoma (HCC) is a significant contributor to cancer-related mortality worldwide. As our understanding of its molecular underpinnings grows, so too does the development of targeted and personalized treatment strategies. This editorial delves into recent breakthroughs in HCC therapy, highlighting the role of biomarkers and the emerging concept of triplet therapy.

The study by Zhang *et al*[1] explored the synergistic potential of transarterial chemoembolization (TACE) combined with tyrosine kinase inhibitors and PD-1 inhibitors. This integrated therapy not only targets tumor angiogenesis but also enhances the body's antitumor immune response through checkpoint inhibitors, offering new hope for HCC treatment. Simultaneously, Li *et al*[2] identified non-receptor type II protein tyrosine phosphatase (PTPN2) as a biomarker of poor prognosis and immune escape in HCC. The discovery of PTPN2 not only provides a new prognostic indicator for HCC but also suggests potential therapeutic targets. These studies underscore the significance of integrated therapies and biomarkers in enhancing treatment efficacy and prognosis in HCC patients.

Unveiling the molecular basis

The genomic landscape of HCC: HCC is a complex disease characterized by a diverse array of genetic and epigenetic alterations[3]. The genomic profile of HCC features a variety of mutations, copy number variations, and changes in gene expression, driving tumor initiation, progression, and metastasis. In HCC, cell cycle regulators and apoptosis-related gene families, such as CCND1 and CDK4, are key molecular players[4]. Their overexpression, as shown by Lee *et al*[5], is typically associated with poor prognosis and aggressive phenotypes in HCC. Moreover, the dysregulation of growth factors and their receptors, such as the VEGF family, crucial for angiogenesis, is a necessary condition for tumor growth and invasion[6]. The intricate interplay among these molecular components forms the foundation of HCC heterogeneity, requiring deeper understanding for the development of targeted therapies.

The role of lymph node metastasis-related genes: The propensity of HCC for lymph node metastasis is a major contributor to its poor prognosis[7]. As revealed by the genome-wide analysis by Lee *et al*[5] published in the *World Journal of Gastroenterology*, the upregulation and downregulation of genes related to HCC lymph node metastasis provide new insights into the molecular mechanisms of tumor metastasis. These genes, including *MET*, *EPHA2*, and *MMP2*, are involved in processes such as cell adhesion, migration, and extracellular matrix degradation[3,8]. The identification of these genes not only sheds light on the molecular mechanisms of tumor cell metastasis but also presents potential therapeutic targets. For instance, inhibiting matrix metalloproteinases (MMPs) like MMP2 and MMP13 may help limit the invasive capabilities of HCC cells, restricting metastasis and improving patient outcomes[9].

Integration of biomarkers and triplet therapy: The discovery of biomarkers in HCC has paved the way for personalized medicine, with therapies tailored to individual molecular characteristics. The integration of biomarkers with triplet therapy is an emerging frontier in HCC treatment. For instance, the discovery of immunotherapy biomarkers has transformed the systemic treatment of advanced HCC, exemplified by the success of the atezolizumab and bevacizumab combination[10]. Moreover, the combination of regional therapy, such as TACE, with immunotherapy is under active investigation. TACE, by increasing the release of tumor antigens, may enhance the efficacy of immunotherapy[11]. Ongoing phase 3 clinical trials, such as IMbrave150 and EMERALD-1, will provide critical evidence for this combined treatment approach[12].

Clinical translation of biomarker research

The clinical translation of biomarkers is a critical component of precision medicine, especially for HCC, a highly heterogeneous malignancy. Biomarkers in HCC not only reveal the molecular features of tumors but also predict patient responses to specific treatments, providing essential insights for clinical decision-making. In the latest IMbrave150 clinical trial, ISS and ISS10 were demonstrated to be promising predictive biomarkers that can enhance treatment outcomes for HCC patients receiving combination immunotherapy. These markers are crucial for optimizing patient stratification and personalized treatment approaches to improve the efficacy of standard care regimens[13]. The identification of

biomarkers is typically based on comprehensive analysis of the tumor genome, transcriptome, proteome, and metabolome[14]. For example, through high-throughput microarray technology, previous studies conducted gene expression analysis on tumor and non-tumor tissues from 32 HCC patients, uncovering gene expression patterns associated with lymph node metastasis[15]. These genes include cell membrane receptors, intracellular signaling, and cell adhesion-related genes, such as *MET*, *EPHA2*, *CCND1*, *MMP2*, and *MMP13*. Abnormal expression of these genes may promote tumor cell invasion and metastasis, offering new molecular targets for HCC treatment. Recent study have identified minichromosome maintenance 4, as a member of the minichromosome maintenance protein family, as a potential biomarker in pan-cancer analyses[16].

Despite the promise of biomarker discovery, their clinical application faces significant challenges. Firstly, biomarker validation requires testing in larger patient populations to ensure their generalizability and reproducibility. Additionally, biomarker detection methods need to be highly sensitive, specific, and user-friendly to meet clinical testing demands. For instance, previous studies confirmed microarray analysis results using real-time quantitative reverse transcription-polymerase chain reaction, a method that is accurate but may be complex and costly for clinical application[17]. Therefore, the development of simpler, more cost-effective detection methods is crucial for the clinical application of biomarkers.

Moreover, the clinical application of biomarkers must consider individual patient variations, such as genetic background, tumor subtype, and environmental factors[18]. These factors may influence biomarker expression and function, affecting their predictive value in clinical settings. Future research should further explore the relationship between these factors and biomarkers to improve the clinical effectiveness of biomarker applications. With the advancement of precision medicine, the study of personalized biomarkers will become a focal point. Personalized biomarkers can provide more accurate treatment predictions and guidance based on specific patient conditions, such as genetic background, tumor characteristics, and environmental factors. Individualized treatment strategies based on specific gene mutations or expression profiles may significantly enhance treatment outcomes while reducing adverse reactions.

CONCLUSION

This editorial discusses the pivotal role of biomarkers in HCC treatment and their transformative impact on precision medicine. Despite the potential of biomarkers identified in preliminary studies, their clinical application requires rigorous validation through large-scale, multicenter clinical trials to ensure their reliability and generalizability. The transition of biomarkers from research to clinical practice presents challenges, including the optimization of detection methods, consideration of individual differences, assessment of therapeutic efficacy and safety, and compliance with regulatory standards. The future of personalized treatment is promising, with biomarkers guiding precise therapeutic directions based on patient-specific conditions. Technological innovations, like artificial intelligence, big data analysis, and novel detection technologies, expedite the discovery, validation, and clinical application of biomarkers, enhancing detection accuracy and efficiency. However, it is important to emphasize that the reproducibility and variability of biomarker efficacy across different patient populations still pose challenges, necessitating further research for support.

FOOTNOTES

Author contributions: Kong MW and Yu Y wrote the manuscript; Designation of Kong MW and Yu Y as joint first authors are based on three main reasons; Kong MW and Yu Y contributed efforts of equal substance throughout the research process. Selecting these researchers as joint first authors acknowledge and respect this equal contribution, while recognizing the spirit of teamwork and collaboration of this study; Zhang CX provided crucial suggestions and guidance for the writing; Wan Y and Gao Y reviewed and revised the manuscript; all authors read and approved the final manuscript.

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LETTER TO THE EDITOR

Are preoperative inflammatory and nutritional markers important for the prognosis of patients with peritoneal metastasis of colorectal cancer?

Isabella Sforzin, Mitesh Borad, Pedro Luiz Serrano Uson Junior

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Abstract

Colorectal cancer (CRC) is a type of cancer that grows from polypoid lesions developing over the years. It has a high incidence of about 1.8 million new cases annually. While screening and lifestyle modifications have stabilized the rate of CRC in high-income countries, the incidence of early-onset CRC is increasing globally. The worst prognosis for this cancer is linked to recurrence and metastasis, with peritoneal metastasis occurring in 8% to 20% of cases. In these cases, treatment with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy is indicated. However, this approach is risky and requires careful selection of patients who will truly benefit from it. This article will discuss the correlation between nutrition and inflammation in patients with peritoneal metastasis and advanced CRC, emphasizing the importance of nutritional and inflammatory markers for assessing disease status. Finally, we will highlight the main biomarkers in the field.

Key Words: Colorectal cancer; Peritoneal metastasis; Inflammation; Nutrition; Biomarkers; Prognosis

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Core Tip: In this study, the clinical data of patients with colorectal cancer (CRC) from a single center were retrospectively analyzed. A high neutrophil-to-lymphocyte ratio and low hemoglobin levels were independent predictive risk factors for poor prognosis in patients with peritoneal metastasis (PM) of CRC. The established nomogram including CA 19-9 levels and patient age accurately predicted the overall survival of patients having PM, indicating its usefulness as a valuable prognostic tool for this patient cohort.

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TO THE EDITOR

In this letter, we comment on the interesting article published by Wu *et al*[1] addressing the association between preoperative inflammatory biomarkers and prognosis in patients with colorectal cancer (CRC) with peritoneal metastasis (PM). CRC accounts for approximately 10% of diagnosed cancers[2], with about 1.8 million new cases annually[3]. Its risk factors include increasing age, a positive family history of the disease, hereditary diseases such as Lynch Syndrome, and long-standing inflammatory bowel disease among others[2]. Adherence to screening and disease tracking as well as lifestyle changes have helped stabilize the incidence of CRC in high-income countries[4]. However, over the last several decades, the incidence of early-onset CRC (*i.e.* in patients under the age of 50 years) has increased worldwide[3]. CRC develops from polypoid lesions with malignant potential over 10 years to 15 years. These lesions can occur *via* distinct pathways: the adenoma-carcinoma sequence (70%-90% of cases) and the serrated neoplasia pathway (10%-20% of cases)[3]. CRC can be classified into four consensus molecular subtypes (CMS), which offer a feasible prognostic framework: immune (CMS1), canonical (CMS2), metabolic (CMS3), and mesenchymal (CMS4), although these have not yet been validated as predictive biomarkers. Right-sided CRC is often associated with poorer survival outcomes[2]. Approximately half of the patients with CRC are diagnosed at an advanced stage, with distant metastases already present. The liver and lungs are the most common metastatic sites[3,5]. PM is a common site of recurrence and is associated with worse survival outcomes[6]. For a long time, CRC with PM was considered a terminal stage of the disease. However, the introduction of Sugarbaker's treatment, combining peritonectomy and hyperthermal intraperitoneal chemotherapy (HIPEC), has offered a treatment option for PM, considering systemic chemotherapy is often ineffective for these cases[3,6]. Although PM can be asymptomatic, severe symptoms such as ascites and intestinal obstruction can occur[6]. Therefore, timely disease staging at diagnosis is critical to assess tumor burden and disease severity, enabling early implementation of therapies such as peritonectomy and HIPEC that can improve the patient's prognosis. At this stage, preoperative assessments, including scores and markers of inflammatory and nutritional status through blood tests, can help identify patients who are most likely to benefit from these treatment strategies[3].

Case series

The article by Wu *et al*[1] describes a retrospective study evaluating 133 patients (66.9% male) from the Sixth Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) diagnosed with CRC with PM. Each patient underwent assessment for combined treatment with cytoreductive surgery (CRS) followed by HIPEC. This treatment has been reported to offer improved survival outcomes. However, its complexity is associated with high morbidity. Therefore, careful patient selection is crucial for optimizing the success of this procedure (Table 1). Although there are prognostic scoring systems for patients with PM, they rely on intraoperative or postoperative data (Table 2). Therefore, preoperative clinical assessment may also help identify subgroups of more vulnerable patients[1]. In this context, Wu *et al*[1] aimed to evaluate inflammatory and nutritional markers as prognostic factors in preoperative assessments. Among the markers they examined were the neutrophil-to-lymphocyte ratio (NLR) and hemoglobin (Hb). Neutrophils were evaluated because they are a crucial part of white blood cells that contribute to remodeling the cellular matrix to promote tumor growth. Previous data have shown that elevated NLR is associated with poorer overall survival in various cancer types[7]. In this study, about 40% of patients exhibited a high NLR and had an inferior median overall survival (mOS) of 7.9 months. In contrast, those with a low NLR had an average mOS of 17.5 months. Additionally, patients with normal Hb levels had an mOS that was 12.2 months longer compared to those with low Hb levels[1]. These markers were also significantly associated with the success of CRS and albumin levels. Therefore, this study concluded that high NLR and low Hb are independent prognostic risk factors. Furthermore, a nomogram with these biomarkers, along with patient age, CA 19-9 levels, and the peritoneal cancer index was validated to predict 1-year and 2-year survival probabilities[1].

For the discussion, in July 2024, we searched PubMed for pertinent articles, using various combinations of the search terms "peritoneal carcinomatosis," "colorectal cancer," "inflammatory markers," "nutritional markers," "cytoreductive surgery," "anemia," and "albumin." The articles most relevant to the purpose of this study have been reviewed and discussed below.

Discussion

Despite advances in various approaches, including surgery, chemotherapy, and radiotherapy, mortality rates for CRC

Table 1 Summary of the results found in the original article

Parameter	*n* among 133 patients	Most common age in years	Most common sex	Normal albumin, ≥ 35 g/L	Tumor most prevalent location	Most common histology	Number of patients with CC 0/1[1]	Number of patients treated with HIPEC[2]
High NLR	54 (40.6)	≥ 60 (54.1)	Men	46 (85.1)	Left side of the colon	Adenocarcinoma	30 (55.6)	16 (29.6)
Low NLR	79 (59.4)			53 (67.1)			27 (34.1)	19 (24.1)
Normal Hb	94 (70.7)	< 60 (58.5)		78 (82.9)			49 (52.1)	29 (30.8)
Low Hb	39 (29.3)	≥ 60 (64.1)		21 (53.9)			8 (20.5)	6 (15.3)

Data are *n* (%).

[1]Patients with cytoreduction scores of 0 or 1 were considered eligible for hyperthermal intraperitoneal chemotherapy.

[2]Among the patients pre-selected for hyperthermal intraperitoneal chemotherapy, only those deemed capable of tolerating the procedure received the treatment.

CC: Cytoreduction; Hb: Hemoglobin; HIPEC: Hyperthermal intraperitoneal chemotherapy; NLR: Neutrophil-to-lymphocyte ratio.

Table 2 Clinical relevance of the association between neutrophil-to-lymphocyte ratio, hemoglobin, and colorectal cancer prognosis

Parameter	High NLR	Low Hb
Clinical associated factors	Higher secretion of chemokines and inflammatory cytokines	Anemia and iron losses (bleeding, ingestion and absorption deficiency)
Clinical outcomes	Suppression of apoptosis, increased proliferation, migration, and invasion of tumor cells, and organ dysfunction	Reduced erythropoiesis, low iron release, cachexia
Number of patients admitted with CRC-PM (poor prognosis)	40.6% (54/133)	70.6% (94/133)

CRC: Colorectal cancer; Hb: Hemoglobin; NLR: Neutrophil-to-lymphocyte ratio.

with PM remain high[1]. Therefore, carefully selecting patients who may benefit from intensive treatments is necessary [5]. In this group, screening patients eligible for CRS and HIPEC is important to weigh the risks of the treatment against potential survival benefits. Therefore, the decision to proceed should be based on biomarkers capable of predicting unfavorable prognoses[8,9]. The evaluation of patients who may be candidates for CRS and HIPEC includes scores like the PCI and Peritoneal Surface Disease Severity Score, the latter of which also incorporates clinical factors. Considering that cancer-related systemic inflammatory and nutritional responses are often linked to tumor progression, measuring these biological markers could be important for predicting negative outcomes that influence patient survival after treatment[3,5]. Patients with metastatic disease have a significantly higher likelihood of dying compared to those with non-metastatic cancer. Nonetheless, the factors determining cancer mortality are complex and involve the dysfunction of various interconnected systems in the body[1]. Metastatic cancer is associated with the dysfunction of multiple organ systems. This occurs due to the intense activation of local and systemic inflammatory pathways, as well as tissue repair and immunosuppressive mechanisms[10]. Chronic inflammation leads to a persistent leukocyte infiltrate that causes pathological inflammation, resulting in tissue damage and an increase in mutagenic agents. This creates an environment conducive to dysplasia and tumor proliferation[11]. In this context, inflammation markers detectable through blood tests can assess the prognosis of patients[5,7]. Neutrophils secrete chemokines and inflammatory cytokines that contribute to cancer development and organ dysfunction[5]. As a result, there is suppression of apoptosis and increased proliferation, migration, and invasion of tumor cells[11]. In contrast, lymphocytes are involved in the cytotoxic immune response to cancer through cell-mediated immunity[5,8,12]. In summary, serum inflammation markers can be divided into upregulators (neutrophils, platelets, and C-reactive protein [CRP]) and downregulators (lymphocytes). The comparative relationship between their values enables the assessment of the patient's inflammatory prognosis. Commonly used ratios include the NLR, lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR), and lymphocyte-CRP ratio. Other scores that are evaluated include the Glasgow prognostic score (GPS), which evaluates serum levels of CRP and albumin, the Systemic Inflammation Score (SIS), which correlates serum albumin levels with LMR, and the Prognostic Nutritional Index (PNI)[5,7,8]. These biomarkers provide a general assessment of the patient and are potential predictive tools for inflammation indices and deterioration in the performance of patients with cancer. NLR and PLR are markers of systemic inflammation related to toxicity and thrombocytosis, respectively, and their prognostic value for various types of cancer, along with their ease of calculation, make them useful measures for assessing inflammatory status in clinical practice. Similarly, markers related to the measurement of monocyte quantities (such as the LMR) also reflect inflammatory activity. Additionally, GPS, SIS, and PNI scores propose a combination of these defense cell indices with other established laboratory markers of inflammation and nutrition (such as CRP and albumin). Therefore, they allow for clinical comparison of predictive performance for patient prognosis. Since these immune status coefficients are known to be correlated with prognostic outcomes, they can be explored as possibly associated with patient risk stratification.

Owing to the differing roles of neutrophils and lymphocytes in tumor development, the NLR has become a robust prognostic marker. A high NLR is associated with poorer overall survival and recurrence-free survival. Monocytes differentiate into tumor-associated macrophages, creating a favorable tumor microenvironment. Therefore, a low LMR, due to increased monocyte count relative to lymphocytes, is linked to poorer prognosis[5,7,8]. Since CRP is an inflammation marker, a low lymphocyte-CRP ratio can also be a sensitive biomarker for adverse outcomes. Finally, platelet release factors (such as vascular endothelial growth factor, transforming growth factor beta, and platelet-derived growth factor) contribute to inflammation and tumorigenesis[7]. A high platelet count (high PLR) also suggests a poorer prognosis[5,7,12].

Two other markers have been proposed for the concurrent evaluation of different immune cells: the systemic immune-inflammation index (SII) and, more recently, the systemic inflammation response index (SIRI). Both scores assess neutrophils and lymphocytes, but the SII also considers platelet count, while the SIRI includes monocyte count. A high SII value indicates neutrophilia and thrombocytosis (pro-tumor cells) along with lymphopenia (anti-tumor cells), indicating a pro-tumor inflammatory state and a weakened immune surveillance. Therefore, high systemic inflammation measured by the SII is a marker of poor outcome[9]. This index has been proposed as having better prognostic performance compared to NLR and PLR[5]. Conversely, a high SIRI value driven by monocytosis allows for the assessment of a favorable tumor microenvironment. However, it remains a less extensively studied marker in CRC. In summary, these markers indicate a decreased immune response against the tumor or an increased likelihood of tumor dissemination or recurrence[9].

A meta-analysis published in 2024 gathered data from studies aiming to validate the prognostic significance of both scores in CRC. Elevated levels of both SII and SIRI were associated with worse overall survival. However, the validation of these scores as prognostic predictors may still be inconsistent and vary for metastatic CRC, primarily depending on the location of the metastasis (especially considering the inflammatory characteristics of peritoneal carcinomatosis), patient heterogeneity, and the different treatments used, including decisions related to CRS and HIPEC[9].

The presence of metastatic disease is related to increased metabolic demands on the body. The combination of increased catabolism and energy expenditure, along with decreased caloric intake in patients with metastatic cancer, culminates in cachexia and sarcopenia, which are complex nutrient loss syndromes[10]. Caloric deficits induced by oncological treatments may also contribute to nutritional and immunological impairment. Therefore, this nutritional deficiency is multifactorial. In addition to being associated with the propensity of the metastatic tumor environment, elevated levels of cytokines, such as tumor necrosis factor and interleukins 1 and 6, also influence this nutritional loss. Conversely, altered metabolism also contributes to the immune dysfunction observed in some cancer patients[10]. The association between immune and inflammatory markers is widely studied. The Hb, albumin, lymphocyte, and platelet score has emerged as a biomarker that integrates routinely collected indicators for various types of cancer, including CRC [12]. Although it is still largely theoretical and research-focused, it is worth noting that it proposes the combined use of markers known to be related to cancer prognosis. This suggests that these markers should indeed be incorporated into the prognosis of overall survival, progression-free survival, and disease recurrence[12]. Albumin, a protein that decreases in both malnutrition and cases of exacerbated inflammation secondary to cancer, is particularly significant. Therefore, hypoalbuminemia is associated with a poor prognosis in patients with cancer[5].

As previously mentioned, the GPS, SIS, and PNI scores associate inflammatory markers with albumin to predict outcomes in patients with CRC with PM. A high GPS reflects both systemic inflammation (elevated CRP) and poor nutritional status (hypoalbuminemia)[5,12]. A high SIS indicates a worse prognosis as it corresponds to low albumin levels. Finally, in advanced tumors, a decreased PNI may predict poor patient status and the occurrence of postoperative complications[5]. Chronic disease-associated anemia is a well-recognized condition in patients with cancer. The release of inflammatory cytokines affects erythropoiesis, increases hepcidin secretion, and reduces iron release. In gastrointestinal cancers (including CRC), deficiencies in intake, absorption, and blood loss can also lead to anemia. Therefore, anemia is often present in both acute and advanced stages of cancer and serves as an important marker in patients with advanced CRC[12,13]. Additionally, anemia and hypoalbuminemia, commonly seen in cancer-related malnutrition, often accompany cachexia. Therefore, Hb becomes an important marker of the patient's nutritional status, crucial for clinical assessment. Several studies have evaluated the Hb, albumin, lymphocyte, and platelet score in patients with CRC. These assessments were both prospective and retrospective, aiming to predict patient survival after curative surgery. The results indicated that a higher Hb, albumin, lymphocyte, and platelet score was a statistically significant predictor of better survival outcomes[12]. Although this marker requires further investigation before being incorporated into clinical practice, these findings suggest the importance of integrating nutritional and immunological biomarkers into the preoperative assessment of patients with CRC, especially those with metastatic disease. However, the exact association between these markers and their cutoff values still needs to be better established[3]. Considering the high incidence of CRC, the selection of patients who will benefit from surgical intervention must be meticulous. Therefore, incorporating biological biomarkers (such as NLR and Hb) into disease status assessment scores is of great importance and aligns with the current understanding of their prognostic value.

Conclusion

The study presented by Wu *et al*[1] is relevant as it reiterates the importance of using nutritional and inflammatory markers as predictors of prognosis in patients with CRC and PM who undergo CRS and HIPEC.

Future directions

Despite the various biomarkers mentioned, solid clinical evidence to establish their use as robust prognostic markers for CRC remains limited. Randomized clinical trials are still needed to more conclusively test and validate these markers in the literature. At present, most studies rely on retrospective assessments of patients with CRC and PM and are therefore

subject to biases related to the progression of the disease and the impact of external factors, such as chronic inflammation and organ failure. Recent studies have focused on elucidating ctDNA, which are fragments of DNA released by tumor cells, as a promising prognostic biomarker for CRC, particularly in cases with PM. Peritoneal fluid biopsy shows a strong resemblance to tissue biopsies and generally has a high mutation detection rate. Moreover, there is a well-known significant association between the detection of ctDNA, and disease recurrence and poorer prognosis. Therefore, detecting ctDNA in primary or metastatic sites of peritoneal disease could serve as a complementary prognostic surveillance tool to clinical markers. It could also assist in predicting responses to immunotherapy, detecting recurrence, or uncovering associated systemic disease[14]. However, detecting ctDNA in primary or metastatic sites of peritoneal disease remains complex and has variable sensitivity. It is also crucial to determine whether this biomarker, along with other biomarkers, has robust evidence to predict poor outcomes and guide early interventions. Considering the ongoing efforts to improve treatments for disease progression, address residual disease, and enhance oncological outcomes, there is a strong rationale to focus on establishing reliable prognostic markers[14].

FOOTNOTES

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LETTER TO THE EDITOR

Elevated *ETV4* expression in cholangiocarcinoma is linked to poor prognosis and may guide targeted therapies

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Abstract

Cholangiocarcinoma (CCA), a highly aggressive bile duct cancer, is associated with late-stage diagnosis and limited treatment options, leading to poor patient outcomes. Early detection and personalized treatment strategies are crucial. The study by Wang *et al* highlights the prognostic potential of the PEA3 subfamily genes (*ETV1*, *ETV4*, and *ETV5*) in CCA, identifying ETV4 as a particularly promising biomarker. Their bioinformatic analysis revealed that elevated *ETV4* expression correlates with poorer survival, positioning it as a strong indicator of disease progression. These findings suggest that ETV4 could enhance prognostic precision and guide personalized therapies, although further validation through large-scale clinical trials is essential. Challenges in clinical application include the need for comprehensive experimental validation and addressing the tumor heterogeneity in CCA. Future research should focus on validating these biomarkers in diverse cohorts and developing targeted therapies, especially in regions where CCA is endemic.

Key Words: Prognostic biomarkers; Cholangiocarcinoma; Survival rates; *ETV4* expression; PEA3 subfamily; Precision medicine; Targeted therapy

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Core Tip: Cholangiocarcinoma is a highly aggressive cancer with limited treatment options. The recent study by Wang *et al* highlights the prognostic value of the PEA3 subfamily genes (*ETV1*, *ETV4*, and *ETV5*), especially *ETV4*, as key indicators of poor survival. Elevated *ETV4* expression is linked to aggressive tumor behavior and worse outcomes. These findings offer potential for personalized treatment strategies, but further large-scale validation is required to integrate *ETV4* as a prognostic biomarker and therapeutic target in clinical practice, particularly in high-incidence regions.

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TO THE EDITOR

Cholangiocarcinoma (CCA) is one of the most aggressive and lethal gastrointestinal cancers, largely due to its late detection and limited treatment options. The global burden of CCA varies significantly, with the highest incidence rates found in parts of Asia[1]. Northeast Thailand reports age-standardized rates of 85 cases *per* 100000, followed by North and Central Thailand (14.5 *per* 100000), and Gwangju, South Korea (8.8 *per* 100000). In contrast, Western countries exhibit lower incidence rates, ranging from 0.5 to 3.4 *per* 100000[2].

Mortality trends show a similar pattern, with intrahepatic CCA (iCCA) mortality rates rising over the past decade in Europe, North America, and Oceania. Countries like Ireland, the United Kingdom, and Portugal have some of the highest rates, while Baltic nations, including Latvia and Lithuania, have seen sharp increases, with annual mortality changes exceeding 18%. Given the rising global burden and median survival rates often less than 24 months in advanced stages, early detection and precision oncology approaches are pivotal. In this context, the identification of novel prognostic markers is critical to advancing personalized medicine in CCA. The recent study by Wang *et al*[3] provides valuable insights into the potential role of the PEA3 subfamily genes (*ETV1*, *ETV4*, and *ETV5*) as biomarkers for CCA prognosis, offering a promising avenue for personalized medicine.

The PEA3 subfamily, part of the E26 transformation-specific family of transcription factors, plays a significant role in cancer progression, influencing proliferation, invasion, and metastasis. While these genes have been implicated in other cancers like liver, colorectal, and lung cancer, Wang *et al*'s study uniquely highlights the prognostic significance of *ETV1*, *ETV4*, and *ETV5* in CCA[3]. These findings are especially valuable for improving diagnosis and treatment, particularly in high-incidence regions like Asia.

KEY FINDINGS AND CLINICAL IMPLICATIONS

Through bioinformatic analysis of data from the Cancer Genome Atlas and Genotype-Tissue Expression project, Wang *et al*[3] discovered that *ETV1*, *ETV4*, and *ETV5* were significantly overexpressed in CCA tissues compared to healthy controls. Notably, high expression levels of *ETV1* and *ETV4* were associated with shorter overall survival, positioning these genes as potential indicators of disease progression. Among these, *ETV4* emerged as a particularly strong predictor of poor prognosis, with higher expression levels correlating with significantly worse survival outcomes. The high *ETV4* expression group had a *P*-value of 0.04, indicating a statistically significant association.

Mechanistically, *ETV4* has been implicated in driving tumorigenesis through its involvement in key oncogenic pathways, particularly in prostate and breast cancers[4]. It promotes cancer cell proliferation, migration, and invasion by influencing signaling pathways such as MAPK/ERK and PI3K/Akt. These pathways are crucial for regulating cell survival, growth, and differentiation, making *ETV4* a potent driver of aggressive tumor behavior in CCA. *ETV4* can also influence epithelial-mesenchymal transition (EMT), a process that enhances metastatic potential and resistance to apoptosis, further contributing to its role in poor prognosis.

In terms of clinical relevance, the identification of these genetic markers holds promise for more personalized therapeutic approaches in CCA. Stratifying patients based on *ETV4* expression could allow for tailored treatment plans, as those with high *ETV4* expression may be identified as a high-risk group likely to experience more aggressive disease progression and thus benefit from intensified therapeutic regimens. Integrating ETV4 expression analysis into routine diagnostic and prognostic workflows could provide valuable tools for early risk stratification, particularly through its incorporation into initial biopsy assessments and companion diagnostic tests. This can be achieved *via* immunohistochemistry or quantitative polymerase chain reaction to assess *ETV4* expression levels in CCA tissue samples.

For prognostic purposes, *ETV4* levels could serve as biomarkers to estimate overall survival and guide decision-making regarding aggressive *vs* conservative treatment approaches. Moreover, longitudinal tracking of *ETV4* expression through non-invasive techniques, such as liquid biopsy [*e.g.*, circulating tumor DNA (ctDNA) from blood samples], could allow clinicians to monitor changes over time without the need for repeated tissue biopsies. This would aid in assessing treatment response and detecting early signs of recurrence, enabling more dynamic and responsive clinical management.

Additionally, ETV4 has been shown to potentially induce treatment resistance in CCA, particularly chemoresistance, through its activation of pathways such as PI3K/Akt and MAPK/ERK, which promote survival and growth in tumor cells. Hence, therapeutic strategies targeting ETV4 could prove beneficial in overcoming this resistance, and future studies could explore the role of PEA3 gene silencing to enhance chemosensitivity and improve the efficacy of CCA therapies.

IMPLICATIONS FOR PROGNOSTIC PRECISION

The identification of *ETV4* as a prognostic biomarker is particularly significant in light of CCA's treatment challenges. Current therapeutic options for CCA have shown limited efficacy, and no established prognostic markers are routinely used in clinical practice. Early attempts to target the vascular endothelial growth factor and epidermal growth factor receptor pathways with agents such as bevacizumab, cediranib, erlotinib, sunitinib, and vandetanib failed to improve survival outcomes[5]. Therefore, the discovery of *ETV4* as a strong predictor of poor survival in CCA patients marks a critical advancement. Wang *et al*'s functional assays in animal models demonstrated that silencing PEA3 subfamily genes, particularly *ETV4*, effectively suppressed invasion and metastasis in CCA cells, leading to reduced tumor proliferation and growth[3]. This suggests a promising therapeutic target for future treatment.

Several strategies for targeting *ETV4* in CCA treatment approach are promising. One potential approach includes gene silencing techniques such as RNA interference or *CRISPR-Cas9* gene editing, which could directly inhibit the expression of *ETV4*, thereby reducing its pro-tumorigenic effects on invasion and metastasis. Additionally, small molecule inhibitors that disrupt the *ETV4* transcriptional activity or block its interactions with key regulatory proteins in cancer pathways may offer another therapeutic avenue. Such inhibitors could reduce *ETV4*-driven signaling cascades involved in tumor proliferation, migration, and EMT, processes that are critical for metastasis[6].

The Cox regression analysis conducted in this study further validated the prognostic relevance of *ETV4*, showing that high *ETV4* expression is associated with a significantly higher risk of early mortality in CCA patients. Specifically, the analysis revealed that patients with elevated *ETV4* expression had a hazard ratio of 3.00 (1.05-8.58, $P = 0.004$), indicating more than double the risk of poor survival compared to those with lower *ETV4* expression. While these findings are promising, they remain preliminary and require validation in larger clinical trials before broad clinical application.

CHALLENGES IN CLINICAL APPLICATION AND FUTURE DIRECTIONS

The integration of *PEA3* genes, particularly *ETV4*, into clinical practice faces several challenges. CCA is a highly heterogeneous disease, both anatomically and genetically, which could impact the reliability of *ETV4* as a prognostic biomarker. CCA tumors are classified into intrahepatic (iCCA) and extrahepatic subtypes (eCCA), with eCCA further subdivided into perihilar and distal. Each subtype presents distinct molecular and histological characteristics. For instance, tumors may harbor varying mutations, such as K-ras, TP53, and others, with mutations differing based on underlying etiologies like parasitic infections or chronic inflammation. The heterogeneity of CCA means that *ETV4* expression may not be uniform across all subtypes, potentially affecting its reliability as a universal biomarker.

To address this challenge, several strategies should be considered. Subgroup analysis based on CCA subtype and genetic mutations (*e.g.*, TP53, K-ras) could provide a more precise understanding of how *ETV4* expression correlates with outcomes across different tumor types. Combining *ETV4* with other biomarkers, such as those related to genetic mutations or tumor morphology, could create a more robust prognostic tool. Validating *ETV4* expression across diverse populations and genetic backgrounds is also critical, particularly given the variation in CCA incidence and etiology across regions, such as Southeast Asia *vs* Western countries. Additionally, longitudinal tracking of *ETV4* expression through non-invasive methods like ctDNA could offer a dynamic approach to monitoring disease progression and treatment response, enhancing its clinical applicability despite tumor heterogeneity. Demographic and genetic diversity may significantly influence the applicability of *ETV4* profiling across different populations. In East Asia, where CCA is often linked to liver fluke infection and hepatitis[7,8], *ETV4* expression patterns could vary compared to Western populations, where CCA arises from different risk factors like primary sclerosing cholangitis[9]. In high-incidence regions such as Southeast Asia, the use of *ETV4* as a biomarker could be particularly impactful by enabling earlier diagnosis and risk stratification in populations facing endemic CCA. These areas have the highest global rates of CCA due to environmental and infectious risk factors, and the aggressive nature of the disease makes early detection vital. Implementing *ETV4* testing in these regions could significantly improve clinical outcomes by identifying high-risk patients sooner and enabling more personalized treatment plans.

Future studies should validate *ETV4* as a biomarker across diverse populations and CCA subtypes, ensuring its global relevance. Additionally, developing *ETV4*-targeted therapies and conducting clinical trials tailored to genetic diversity will be crucial for personalized CCA treatment. Ethical considerations such as cost, accessibility, and patient consent for genetic testing must also be addressed as the field advances toward clinical implementation. This is particularly urgent in CCA-endemic regions, where early diagnosis could have the greatest impact on patient survival. Moreover, leveraging advanced technologies like CRISPR for gene editing, coupled with machine-learning algorithms for predictive modeling, could enhance the precision of genetic screening and targeted treatment in CCA.

CONCLUSION

The expression of *PEA3* subfamily genes, particularly *ETV4*, represents a significant advancement in the understanding and treatment of CCA. This study highlights the potential of *ETV4* as a key prognostic marker and emphasizes the therapeutic value of targeting *PEA3* genes in clinical practice. However, further comprehensive clinical studies are needed due to limited experimental validation. As the molecular mechanisms of this aggressive cancer are further unraveled, integrating such biomarkers into routine care could lead to improved prognosis, personalized treatments, and better survival outcomes for CCA patients.

In this regard, the next steps for researchers looking to build upon Wang *et al*'s findings would involve several key areas of focus[3]. First, conducting large-scale, multi-center clinical trials to validate the prognostic utility of *ETV4* and other *PEA3* subfamily genes across diverse CCA populations is essential. Additionally, studies should aim to explore the therapeutic potential of targeted therapies against these genes, particularly investigating their role in modulating tumor progression and resistance mechanisms. In parallel, researchers should delve deeper into combinatorial treatment strategies, where *PEA3*-targeted therapies are integrated with current standards of care such as chemotherapy or immunotherapy, to determine synergistic effects. Furthermore, leveraging advanced molecular tools like CRISPR-based gene editing and RNA interference could offer new insights into how *PEA3* genes contribute to CCA pathogenesis and reveal novel drug targets.

By building on these findings, this research lays a strong foundation for future therapeutic developments, ushering in a new era of precision oncology for CCA, where biomarker-driven approaches could greatly enhance personalized treatment plans and improve survival outcomes.

FOOTNOTES

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