ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2023 September 15; 15(9): 1505-1674

Published by Baishideng Publishing Group Inc

WJGO World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 15 Number 9 September 15, 2023

World Journal of Gastrointestinal Oncology

Monthly Volume 15 Number 9 September 15, 2023

Contents

World Journal of Gastrointestinal Oncology

Monthly Volume 15 Number 9 September 15, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Qiang Lin, MD, PhD, Professor, Department of Oncology, North China Petroleum Bureau General Hospital, Hebei Medical University, Renqiu 062552, Hebei Province, China. billhappy001@163.com

AIMS AND SCOPE

The primary aim of *World Journal of Gastrointestinal Oncology* (*WJGO*, *World J Gastrointest Oncol*) is to provide scholars and readers from various fields of gastrointestinal oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

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*.

INDEXING/ABSTRACTING

The *WJGO* is now abstracted and indexed in PubMed, PubMed Central, Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJGO* as 3.0; IF without journal self cites: 2.9; 5-year IF: 3.0; Journal Citation Indicator: 0.49; Ranking: 157 among 241 journals in oncology; Quartile category: Q3; Ranking: 58 among 93 journals in gastroenterology and hepatology; and Quartile category: Q3. The *WJGO*'s CiteScore for 2022 is 4.1 and Scopus CiteScore rank 2022: Gastroenterology is 71/149; Oncology is 197/366.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Jia-Ru Fan*.

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Florin Burada

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

PUBLICATION DATE
September 15, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1505-1519

DOI: 10.4251/wjgo.v15.i9.1505

ISSN 1948-5204 (online)

REVIEW

Deoxyribonucleic acid methylation driven aberrations in pancreatic cancer-related pathways

Akash Bararia, Amlan Das, Sangeeta Mitra, Sudeep Banerjee, Aniruddha Chatterjee, Nilabja Sikdar

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Niu ZS, China; Jin S, China

Received: April 18, 2023
Peer-review started: April 18, 2023
First decision: May 12, 2023
Revised: May 29, 2023
Accepted: August 1, 2023
Article in press: August 1, 2023
Published online: September 15, 2023

Akash Bararia, Nilabja Sikdar, Human Genetics Unit, Indian Statistical Institute, Kolkata 700108, India

Amlan Das, Department of Biochemistry, Royal Global University, Assam 781035, India

Sangeeta Mitra, Department of Biochemistry and Biophysics, University of Kalyani, West Bengal 741235, India

Sudeep Banerjee, Department of Gastrointestinal Surgery, Tata Medical Center, Kolkata 700160, India

Aniruddha Chatterjee, Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin 9054, New Zealand

Aniruddha Chatterjee, School of Health Sciences and Technology, University of Petroleum and Energy Studies, Dehradun 248007, India

Corresponding author: Nilabja Sikdar, PhD, Research Scientist, Doctor, Human Genetics Unit, Indian Statistical Institute, 203, B.T. Road, Kolkata 700108, India. snilabja@isical.ac.in

Abstract

Pancreatic cancer (PanCa) presents a catastrophic disease with poor overall survival at advanced stages, with immediate requirement of new and effective treatment options. Besides genetic mutations, epigenetic dysregulation of signaling pathway-associated enriched genes are considered as novel therapeutic target. Mechanisms beneath the deoxyribonucleic acid methylation and its utility in developing of epi-drugs in PanCa are under trails. Combinations of epigenetic medicines with conventional cytotoxic treatments or targeted therapy are promising options to improving the dismal response and survival rate of PanCa patients. Recent studies have identified potentially valid pathways that support the prediction that future PanCa clinical trials will include vigorous testing of epigenomic therapies. Epigenetics thus promises to generate a significant amount of new knowledge of biological and medical importance. Our review could identify various components of epigenetic mechanisms known to be involved in the initiation and development of pancreatic ductal adenocarcinoma and related precancerous lesions, and novel pharmacological strategies that target these components could potentially lead to breakthroughs. We aim to highlight the possibilities that exist and the potential therapeutic interventions.

Key Words: Methylation driven pathways; Pancreatic cancer methylation markers; Signaling pathway targeted therapy; PanCa enriched methylated pathway; Pre-cancer methylated pathways

Core Tip: Given the limited commercial availability of targeted epi-drugs and pathway-based biomarkers, it is important to generalize them for appropriate treatment of pancreatic cancer and related precancerous lesions. We also highlighted the clinical use of these therapeutic targets based on methylation driven pathways. This review will successfully help readers address current issues and support cutting-edge development of targeted therapies using epigenetically regulated pathways.

Citation: Bararia A, Das A, Mitra S, Banerjee S, Chatterjee A, Sikdar N. Deoxyribonucleic acid methylation driven aberrations in pancreatic cancer-related pathways. *World J Gastrointest Oncol* 2023; 15(9): 1505-1519
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1505.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1505

INTRODUCTION

Pancreatic cancer (PanCa) is one of the fatal malicious carcinomas globally. Currently, PanCa is one of the foremost causes of death by cancer especially in the United States[1]. According to GLOBACON 2020, PanCa is the 12th most common cause of cancer with 495000 new cases worldwide, of which approximately 47% of new cases occurred in Asia and another significant proportion, 28%, in Europe[2]. By the end of 2022, the incidence could increase by 70%, equivalent to about 844000 new cases per year[3]. Recent studies elucidate deoxyribonucleic acid (DNA) methylation depiction from inflammatory diseases thus opening a new profile for the biomarker development in early prognosis. Cell-free DNA methylation, in particular, could be used to identify pre-neoplastic features in individuals with suspected pancreatic disorders. This is a clear non-invasive approach of PanCa pre-diagnosis[4]. It is observed through the years that PanCa consists of extremely fatal malignancies, having less than 5 year of survival rate. Early detection and treatment of this disease is hampered due to a lack of reliable diagnostic and prognostic markers[3]. It has been noted that there is epigenetic variance between populations which can be accounted for by a variety of racial, demographic, and vocational characteristics. Only a few research have examined the Pancreatic ductal Adenocarcinoma (PDAC) progression stage globally and the shifting epigenetic landscape in various ethnic groups[5]. Recent research has demonstrated the dynamic changes in the global DNA methylation and gene expression patterns play important roles in cancer development, including PanCa development. These findings offer important new information for understanding the onset and progression of this malignancy[5].

PanCa is clinically allied with an elevated rate of mortality. In terms of geographic features, Northern America and Europe show the maximum prevalence of PanCa where more males tend to get affected. There are an estimated 62210 (male) and 32970 (female) new cases in the United States alone in 2022, with an estimated 25970 male and 23860 PanCa-related deaths[2,3]. In South Eastern countries like India, the rate of incidence of PanCa are comparatively lower compared to the Western world. According to per year statistics in Eastern countries like in India, the rate of prevalence of PanCa seems to be 0.5 to 2.4 out of 100000 women and 0.2 to 1.8 out of 100000 men. Although, regardless of the prevalence of this deadly disease, patient survivability with PanCa is comparatively downcast with 1 to 5-year of relative survival rates for all stages[6]. The main reason for such miserable and prolonged consequences is perhaps because of the fact that this fatal disease is predominantly lacks any symptoms in the early stages. Meanwhile, the symptoms commence to expand largely and eventually tend to get metastatic in nature[7]. As a result, enucleating a metastatic tumor is frequently impossible. PanCa has a 1-year survival rate of 26%, and the 5-year survival rate is roughly 6% for advanced cancer and 22% for early stages when surgical removal of the tumor is still possible[8]. For this reason, few new therapeutic strategies like radiotherapy and chemotherapy are effective to mitigate the tumor size in selected PanCa patients[9]. Hypermethylation in DNA methylation can promote tumorigenesis. However, with histone and RNA methylation, both writers and erasers can be PanCa oncogenes such as *SMYD3, KDM1, MELLT3* and *FTO*[10].

The study of malignant genomic modifications has been ongoing over the past few decades, and it has become quite evident that epigenetics are crucial to carcinogenesis. DNA methylation, a key component of epigenetics, affects a variety of biological functions, including gene imprinting, genome stability, and cell differentiation. Hypermethylation and hypomethylation are the two categories of abnormal DNA methylation. DNA hypomethylation refers to less DNA methylation, which frequently causes disturbance of chromosome stability or increased aneuploidy. On the other hand, DNA hypermethylation refers to the buildup of methylation, which mostly results in transcriptional repression and reduced gene expression. Typically, abnormal DNA methylation can be seen in the promoter regions of transcription factors, which promotes the growth and metastasis of cancers[11,12]. DNA methylation plays a crucial role in the onset and progression of cancer. Early on in the tumorigenic process, DNA methylation alterations frequently take place. This phenomenon has been confirmed for the bladder, lung, breast, colorectal, and pancreatic pre-neoplastic lesions[13,14].

According to Thompson *et al*[15], 2015, out of around 250000 assessed CpG sites, 20000 hotspots were correlated with patient survival. The two categories that were survival (-) and survival (+) which represented the connection between higher methylation and survival. The survival (+) sites were more evenly dispersed intragenically, whereas the survival (-) sites tended to group close to the TSS (transcription start site), indicating hypermethylation of promoter regions. An increased methylation pattern, associated with shorter survival was observed in survival (-) groups, while reduced methylation led to longer survival times in survival (+) groups. Some of the important genes [within the top 10 in survival (-) groups] which were found to be hypermethylated were *FAM150A, ONECUT1, RASSF10, RNF207,* PanCa DH9. The tumor-suppressor role of these genes are well-established in other aggressive cancers. While the genes such as *PTPRN2, MAD1L1, CBFA2T3, COL5A1,* and *SHANK2* etc. made their way into the top ten differentially methylated genes in the survival (+) group[16]. Thus, this segmentation, together with the fact that promoter regions of genes are typically better defined and documented, led to the surviving sites producing a clearer recovery of functional annotation and genes overall[17]. There is growing evidence that DNA methylation can affect how genes are expressed, despite the fact that the majority of research on DNA methylation has focused on the methylation state of promoters and CpG islands. Importantly, a mutation of *KRAS* in acinar or ductal cells causes the development of pancreatic lesions, which is the causative genetic event in over 90% of PDAC cases (PanIN). Along with the *KRAS* mutation, subsequent deletion mutations or mutations of other types in tumor suppressor genes promote tumor growth and accelerate the course of the disease[10,18].

The Advantageous incidence of this systematic review is to summarize all the differential methylation pathways in several precancerous lesions of PanCa. The alterations in epigenetics occurring in PanCa also discussed in this review. This review also gives insight into landscapes of the early epigenetics in precursor lesions. In this review also highlighted the differentially methylated enriched signaling pathways and methylated modulators, and their therapeutic targets for precancerous as well as PanCa. In brief, we describe an overview of differentially methylated genes, highlighting their diagnostic or prognostic potential in PanCa related enriched pathways (Figure 1).

PRECANCEROUS LESIONS OF THE PANCREAS

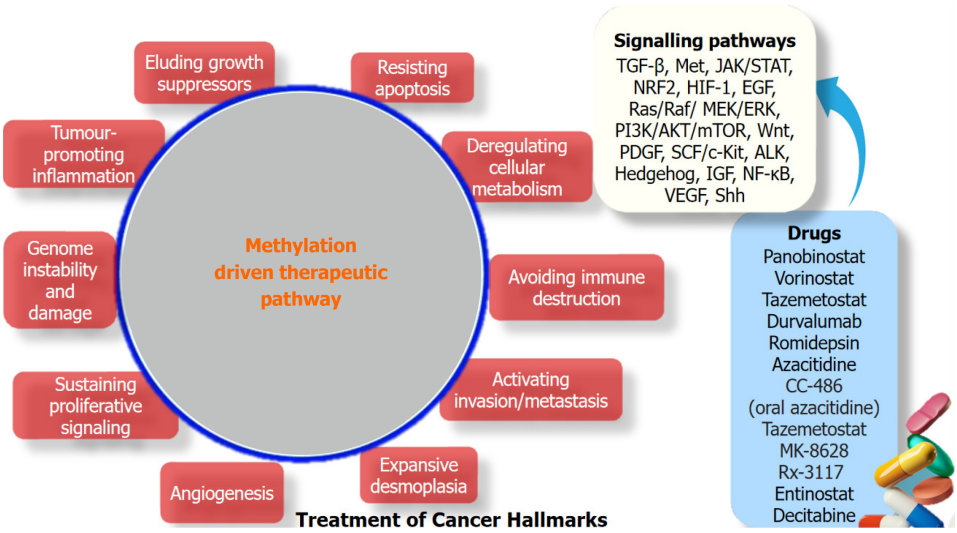
PanCa shows a proclivity for almost about 5 to 7 years of retention rate. Over the years it has been observed that a significant number of patients execute an immensely impoverished prophecy. Recent clinical studies clearly depict that throughout a long period of time; over 10 to 11 years, cellular level observation shows a clear tendency to originate various invasive proficiency. These series of phenomena conciliate the detection factors as well as root out the precursor lesions in PanCa[19]. From recent clinical studies, it is observed that the prior detection of these precancerous lesions put forward the possibility to reduce the death rate. The studies also delineate the fact that several non-intrusive prototype lesions exhibit malignant PanCa. From the surgical history of PanCa, it has been observed that patients having a previous history of PDAC may have microscopic pancreatic intraepithelial neoplasms (PanINs). Furthermore, these multifocal PanINs is clinically allied with diagnosable lobulocentric atrophy. Moreover, Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCN) are another couple of prominent pre-cancerous lesions which give rise to PanCa. These lesions are often considered to form cysts as well[20]. The surgical treatment and revelation of these MCN, IPMNs and PanINs can often create disturbance in the advancement to incursive PanCa. These eventually shows a high efficacy to save the lives of cancer patients[3,20].

PANCREATIC CANCER AND ITS EPIGENETICS LANDSCAPE

Due to epigenetic alterations, oncogenic signaling pathways specifically derived from transcriptional deregulation create a trademark to PanCa. 5-Hydroxymethylcytosine (5-hmC) is a chemical (epigenetic) modification of DNA at regulatory regions that result in the generation of 5-methylcytosine (5-mC) residue and has been thoroughly studied in PanCa. Genome analysis of 5-hmC occupied loci was done in the cell lines of short-passaged PanCa. As a result, surprising patterns of alteration were seen in neoplastic tissues in primary cancer patients[19,21]. It was observed that near the open chromatin regions, the 5-hmC was very much enhanced and thus tends to show upregulation of the allied transliteration [22]. The transcripts involve a few important oncogenic signaling pathways enmeshed in pancreatic neoplasia, such as *KRAS,* master regulator of cell cycle (*MYC), BRD4* and *VEGFA* where *BRD4* tends to be highly overexpressed in nature. In terms of functional approach, accession of 5-hmC at promoter *BRD4* was implicated along with the transcript expression elevation specifically in primary patient samples. It was also noticed that the in *in-vivo* experiments the growth of PanCa is highly inhibited by the *BRD4* blockage. Concisely, it can be said that in human PanCa and oncogenic enhancers, partisan enhancement and 5-hmC reallocation tend to be an important regulatory mechanism[22,23].

ROLE OF THE KEY PATHWAY MODULATORS IN PANCA AND ITS ASSOCIATED PRECANCEROUS LESSIONS

In PanCa, some important pathways are Raf/Ras/ERK. MEK interposes specific cellular responses to a few growth factor actions. In the past years, inhibitors emergence is highly noticed that directly target *KRAS*. This circumvents the long-harboured speculation that drugs cannot be produced by *KRAS*. In PDAC, several attempts have been made to target this



DOI: 10.4251/wjgo.v15.i9.1505

Figure 1 Comprehensive visualization showcasing interaction between epigenetic pathways and probable drug treatments concerning pancreatic cancer. EGF: Epidermal growth factor; Ras/Raf/MEK/ERK: Rat sarcoma virus/Rapidly Accelerated Fibrosarcoma/Mitogen-activated protein kinase/extracellular-signal-regulated kinase; PI3K/AKT/mTOR: Phosphoinositide 3-kinases/Ak strain transforming/Mammalian target of rapamycin; Wnt: Wingless-related integration site; PDGF: Platelet-derived growth factor; SCF/c-Kit: Stem cell factor/receptor tyrosine kinase; ALK: Anaplastic lymphoma kinase; TGF-β: Transforming growth factor beta; HGF: Hepatocyte growth factor; JAK/STAT: Janus kinase/signal transducers and activators of transcription; BTK: Bruton tyrosine kinase; Src: Tyrosine-protein kinase (sarcoma); COX-2: Cyclooxygenase 2; NRF2: Nuclear factor erythroid 2–related factor 2; HIF-1: Hypoxia-inducible factor-1; PKCδ-PKD1: Protein Kinase Cδ-Polycystin 1, Transient Receptor Potential Channel Interacting; IGF: Insulin like growth factor; VEGF: Vascular endothelial growth factor.

important oncogenic pathway in various approaches. The downstream regulation of frequently mutated *KRAS* is eventually considered to be an esoteric drug target[24,25]. On the other hand, owing to the offsetting mechanism that involves the enzyme, geranylgeranyl transferase, the upstream regulation of *KRAS,* using inhibitors like farnesyltransferase has been completely nugatory[26]. It is also observed that the inhibitors like *MRTX8, AMG510* and others specifically target only the *KRAS* mutant variant such as G12C[27,28]. Over 1%–5% of PDACs portray this kind of mutation and the progress is really promising. In PDAC, to regulate the antitumor pursuit in *KRAS*, the genetic inhibition of some autophagy regulators reciprocally enhances the propensity of *ERK* inhibitors (Table 1)[29].

EPIDERMAL GROWTH FACTOR PATHWAY MODULATORS

Epidermal growth factor receptor (EGFR) functions in a significant way in PanCa specifically in terms of tumorigenesis. Epidermal growth factor (EGF) is one of the classic pathways that works in a dysregulated manner in PDAC and is thus often considered as a potent therapeutic target. EGF signaling pathway inhibitors are considered as one of the efficient and significant regulators for cellular viability. These pathway regulators often mediate a wide range of signaling activities, precisely Jak-STAT, Akt/PI3K, Ras/Raf/ and MEK/ERK[29,30]. In PanCa patients, it is often noticed that affirmative activation and regulation of *EGFR* works effectively for the activation of *KRAS* and *ERK* and this persuades the formation of tumor more profoundly[31]. It is found that in Phase III clinical trial, the add-on of erlotinib elevates more positive improvements in cell survival in PanCa patients. The presence of *KRAS* gene (wild type) in PDAC tumors with a tiny proportion also leads to a significant improvement in PDAC patient survival[27,31].

WNT PATHWAY MODULATORS

In case of tissue development and maintenance in both embryos and adults, The Wnt signaling pathway plays a critical role. Digressive activation of this Wnt pathway has been closely associated with cancers like PanCa, specifically to the severely affected digestive tract. It is observed that Cancer stem cells are strongly associated with the activation of this pathway[32]. Furthermore, a precise monoclonal antibody named Wnt inhibitor vantictumab, which eventually targets the decrepitate receptor. This depicts a huge responsive activity of tumors and is often found to be combined with gemcitabine[33].

Table 1 Targeting various characteristics of pancreatic carcinomas and their associated therapeutic strategies

Potent therapeutics	Cancer Hallmarks
TGF-β pathway inhibitors HGF; Met pathway inhibitors	Activating invasion/metastasis
JAK/STAT pathway inhibitors; BTK inhibitors; Src inhibitors; COX-2 inhibitors	Tumour-promoting inflammation
NRF2 pathway inhibitors; HIF-1 pathway inhibitors; PKCδ-PKD1 inhibitors; Amino acid transporter inhibitors; α-Glucosidase inhibitors	Deregulating cellular metabolism
EGF pathway inhibitors; Ras/Raf/ MEK/ERK pathway inhibitors; PI3K/AKT/mTOR pathway inhibitors; Wnt pathway inhibitors; PDGF pathway inhibitors; SCF/c-Kit pathway inhibitors; ALK pathway inhibitors; Hedgehog pathway inhibitors	Sustaining proliferative signalling
IGF pathway inhibitors; NF-κB pathway inhibitors	Resisting apoptosis
VEGF pathway inhibitors	Inducing angiogenesis
Shh pathway inhibitors; FAK inhibitors; Src inhibitors; EGFR inhibitors	Expansive desmoplasia
Aurora kinase inhibitors; Cyclin-dependent kinase inhibitors	Eluding growth suppressors
PD-L1 inhibitors; CTLA-4 inhibitors	Avoiding immune destruction
PARP inhibitors Photodynamic agents; Bromodomain inhibitors; HDAC inhibitors	Genome instability and damage

EGF: Epidermal growth factor; Ras/Raf/MEK/ERK: Rat sarcoma virus/Rapidly Accelerated Fibrosarcoma/Mitogen-activated protein kinase/extracellular-signal-regulated kinase; PI3K/AKT/mTOR: Phosphoinositide 3-kinases/Ak strain transforming/Mammalian target of rapamycin; Wnt: Wingless-related integration site; PDGF: Platelet-derived growth factor; SCF/c-Kit: Stem cell factor/receptor tyrosine kinase; ALK: Anaplastic lymphoma kinase; TGF-β: Transforming growth factor beta; HGF: Hepatocyte growth factor; JAK/STAT: Janus kinase/signal transducers and activators of transcription; BTK: Bruton tyrosine kinase; Src: Tyrosine-protein kinase (sarcoma); COX-2: Cyclooxygenase 2; NRF2: Nuclear factor erythroid 2–related factor 2; HIF-1: Hypoxia-inducible factor-1; PKCδ-PKD1: Protein Kinase Cδ-Polycystin 1, Transient Receptor Potential Channel Interacting; IGF: Insulin like growth factor; VEGF: Vascular endothelial growth factor.

STEM CELL FACTOR/C-KIT PATHWAY MODULATORS

In several cell lines of PanCa, the occupancy of c-Kit has been clearly mentioned. The stem cell factor tends to reinforce the differentiation as well as the proliferation of cells and also seems to be expressing towards its ligands. Masitinib tends to strongly inhibit both the platelet derived growth factor and stem cell factor signaling pathways, thus delivering such extremely promising outcomes. This affirmative feedback is often found to be combined with gemcitabine[34]. Moreover, this c-Kit pathway inhibitors effluxes the overexpressed *ACOX1* marker which elucidates the efficiency in cancer patients [35].

PI3K/AKT/MTOR PATHWAY MODULATORS

The inhibitors of some specific signaling pathways like PI3K/mTOR and Akt bring into play some indispensable control over multifarious processes that are closely related to the growth and survival of cells in case of disease as well as health [36]. The mTOR/Akt and PI3 pathways also play distinctive key roles in several important cellular mechanisms like cell invasion, adhesion, and migration[37].

ROLE OF EPIGENETIC MODULATED PATHWAYS IN PANCA

Whole genome and exome sequencing has shown that a considerable portion of PDAC patients also carries non-germline mutations in chromatin remodelling complexes and epigenetic regulators, such as *ARID1A/B, MLL2/3/4, PBRM1, SMARCA2/4,* and *KDM6A* in addition to germline mutations. Moreover, the inactivation of *KDM6A, MLL3,* and *MLL5* (histone modification enzymes) and non-germline mutations in *ARID1A* occurred simultaneously with oncogenic *KRAS* in insertional mutagenesis screening of sleeping beauty transposon[38]. Vincent *et al*[39] discovered that the histone-modifying enzyme-coding genes were mutated in all of the malignancies in our screen. These mutations helped oncogenic *KRAS* accelerate the progression of PDAC, indicating that changes to the epigenome are crucial for accelerating PDAC. These results demonstrate the importance of epigenetic regulation in the progression of PanCa[40].

Transcriptional silencing is linked to abberant CpG island methylation of multiple tumor suppressor genes, including p16, in pancreatic and other carcinomas. In 15% of PanCa, the p16 gene is reported to be inactive due to hypermethylation of the CpG island. With higher PanIN grades, there is a greater tendency for the loss of p16 protein production, The *ppENK* gene exhibits anomalous methylations in pancreatic carcinomas, as was recently established using representational difference analysis and methylation CpG island amplification (MCA)[41].

PENK DNA methylation has been widely observed in precancerous lesions of varying severity, including extra- and intraluminal PTs and CPs, PanINs, IPMNs, and mucinous cystic neoplasms. Changes in PENK methylation increased with increasing coverage of tumor tissue, but were absent in autoimmune pancreatitis (AIP) and adjacent normal pancreatic tissue[3].

The m6A demethylase *ALKBH5* was found to be downregulated in a gemcitabine-treated patient-derived xenograft model, and its overexpression made PDAC cells more sensitive to treatment. Reduced *ALKBH5* levels predict poor clinical outcome in PDAC and other malignancies. Furthermore, both *in vitro* and *in vivo*, downregulation of *ALKBH5* greatly promotes PDAC cell proliferation, migration, and invasion, whereas overexpression has the opposite impact. The m6A global profile indicated changes in the expression of certain *ALKBH5* target genes, such as Wnt inhibitor 1 (WIF-1), which associated with Wnt signaling pathway mediation and WIF-1 transactivation[42].

Met-enkephalin, a tonically active inhibitory factor that interacts with the opioid growth factor receptor, is encoded by the *ppENK* gene. Met-enkephalin was found to slow the growth of various human cancers, including PanCa, according to Zagon and colleagues. Comb and associates claim that the CpG island methylation of *ppENK* directly prevented a positively active transcription factor from binding, which in turn suppressed the production of the gene. Given this, it is a possible outcome that cell growth and carcinogenesis of the pancreas are promoted because of the methylation of the ppEK gene[41]. Moreover, α-catenin, angiogenesis inhibitor *BAI3, CTNNA2, DPP6* (dipeptidyl-peptidase), *GUCY1A2* (guanylate cyclase), heterotrimeric G-protein-coupled receptor, protein kinases like *PRKCG,* and *Q9H5F0*- these genes were often altered at significantly lower frequencies[43]. According to the reports of Li *et al*[44] a total of 16420 genes having methylation information were found to be differently methylated, including 40 and 831 significantly hypomethylated and hypermethylated genes, respectively. *SARM1, IRX4, IRF4, FOXC2, EN2, ZSCAN23, PTPN5, HOXB4, CACNA1,* and *IGF2BP1* were the 10 genes with the most significantly different methylation patterns. The 10 genes with the most different methylation patterns were *REG4, C11orf34, BRD9, S100A16, HIST1H2BK, STATH, LRRC31, UBD, MIR548A1,* and *PSMG3*[45].

Processes like the differentiation of neurons in the CNS, neuropeptide signaling pathway and organ development at the embryonic stage, were the most observed enrichment functions. According to the study, these genes were primarily engaged in signaling pathways for neuroactive ligand-receptor interaction, cAMP, salivary secretions, glutamatergic synapses, calcium, morphine addiction, circadian rhythm, nicotine addiction, and pancreatic secretions. Genes that significantly affect survival were included as taxonomic features in order to define molecular subtypes of PDAC in relation to prognosis[46]. An important finding from the univariate Cox proportional hazards regression model developed for clinical factors indicated that age should be considered as a significant parameter related to patient survival. These 135 significantly differentially methylation genes were included in the above-mentioned multivariate regression model together with age as a covariate to find variables that independently influence prognosis. Using multivariate Cox regression models, 78 differentially methylation genes substantially linked with prognosis were discovered[46,47].

Chatterjee *et al*[5], identified "regulation of ion transport", "alpha/beta interferon signaling", "morphogenesis and development" and "transcriptional dysregulation" as the four most statistically significant extended terms. Voltage-gated ion channels are membrane proteins that selectively transport ions and are activated by changes in membrane potential. The activation of channels permits potassium ions to move along the electrochemical gradient. Hypermethylation of the *KCNA3* gene promoter may explain the poor expression of Kv1.3 in PDAC. The modulation of ion channels has been demonstrated to play a significant role in the regulation of cell death, evasion, and survival in the context of PDAC invasion and development.

In a study by Nones *et al*[48] 25 pathways were reported to be significantly affected by DNA methylation in PDACs. Axon guidance was one of the most significant (adjusted *P* value 5 1.91E-05) and was supported by MetaCore pathway analysis. This pathway was recently implicated in PDAC. Other pathways identified here as enriched for genes aberrantly methylated including cell adhesion, hedgehog signaling, TGF-b, integrin signaling and WNT/NOTCH signaling are well-known key cancer signaling previously described to be genetically altered in PDAC. WNT signaling has been reported to be aberrant methylated in PDAC cell lines. Our results confirm that this pathway is aberrantly methylated in this large cohort of PDAC. Stellate cell activation (adjusted *P* value3.26E-05) another interesting pathway identified here as significantly affected by DNA methylation deserves further investigation due its importance in PDAC. Pancreatic stellate cells are the main fibroblastic cells in PDAC and are known to interact with PanCa cells creating the fibrotic microenvironment of PDAC. It is hypothesized that the fibrous microenvironment of PDAC creates a barrier that impairs the delivery of chemotherapeutic drugs and promotes aggressive behaviour of tumor cells. Known genes are involved in astrocyte activation [cyclooxygenase-2, transforming growth factor-beta receptor 1, EGFR, tumor necrosis factor-alpha and MET were hypomethylated in PDAC and confirmed by bisulfite amplicon deep sequencing[48].

miRNAs are frequently suppressed in cancer cells and have the potential to act as tumor suppressors. Several miRNAs have been implicated in the development and spread of cancer in the pancreas, and it may one day be possible to stop the disease's progression by increasing the activity of a particular miRNA within a cell. The Food and Drug Administration (FDA) -approved drug Miravirsen, which employs miRNA to treat hepatitis C, has sparked interest in miRNA-based medicines for the treatment of PanCa. Regrettably, no treatments employing miRNAs or siRNAs that are comparable to them have been tried in clinical trials to treat PanCa, therefore miRNA will not be explored in great detail in this review. Nevertheless, we recommend individual study into the state of the art in miRNA studies[49].

In addition to confirming the mutations in the tumor suppressor and classical PDAC-associated oncogenes listed above, sequencing efforts have also revealed mutations in a variety of chromatin-modifying enzymes and complexes. The chromatin remodelers like, SWI/SNF family which alters nucleosome structure using ATP and accessibility of DNA in order to control gene transcription, includes the *ARID1A* component as one of their most often altered genes. 6% of the *ARID1A* mutations in human PDAC were found using multiplatform sequencing analysis. Although the role of *ARID1A*

as a PDAC-associated tumor suppressor gene is well documented, lymph node or distant metastases do not coincide with its expression levels. Instead, they are related to tumor stage and differentiation. When *ARID1A* is knocked out, acinar to ductal metaplasia and PanIN lesions develop as a result[41]. It's interesting to note that a recent study using genetically altered PanCa mice models demonstrated the importance of the survival gene Arid1a, whose absence inhibits cell development and causes cell death. In Ras-driven animal models, the deletion of *ARID1A* also prevents cell growth, leading to the emergence of inactive and low-quality cystic precursor lesions known as IPMNs[50]. The progression of Arid1a-deficient progenitor cells to adenocarcinomas, however, occurs during carcinogenesis *via* routes involving *Tp53* loss or Myc overexpression. PDAC has also been linked to mutations in the SWI/SNF subunits *SMARCB1, ARID1B, BRG1, PBRM1, SMARCA2,* and *SMARCA4.* In PanCa cell lines, *BRG1* inactivating mutations and deletions have been discovered. *BRG1,* a crucial entity of the SWI/SNF chromatin remodelling complex, is an ATP-dependent helicase. Neoplastic lesions that mirror human intraductal papillary mucinous neoplasms are produced as a result of *BRG1* deletion and *KRAS* mutation, which aids in the progression of PDAC[41,51].

Methylation at particular genomic locations may put patients at risk for tumor recurrence following total surgical removal and may be a sign of local and/or systemic metastasis. The surgical resection margin methylation profile may be used as a biologic marker in the absence of histologic disease to detect remaining pancreatic tissue that is susceptible to tumor recurrence or that harbours multi-focal disease throughout the gland. The link between methylation abnormalities and Auto immuno pancreatitis (AIP), a representative Immunoglobulin G4 (IgG4)-related illness, has yet to be determined. Through methylation array research using the Methylation 450K BeadChip array, the scientists discovered that sphingosine kinase inhibitor (SKI) may have a major methylation anomaly in AIP and explored the connection of SKI with AIP clinicopathological characteristics. AIP had a considerably lower SKI methylation ratio than PDAC and nurse practitioner (NP). Furthermore, the immunohistochemical staining-index (SI) score for SKI in AIP was substantially greater than in NP, despite no significant difference between AIP and PDAC[52]. Both the serum IgG4 concentration and the SKI methylation ratio showed a strong negative connection between the SI score and the methylation ratio. SI and the serum IgG4 concentration were shown to be somewhat positively correlated. Givien that SKI is regarded as an oncogene, hypomethylation of SKI and carcinogenesis may be connected to AIP[53]. Additionally, the association between serum IgG4 levels and SKI methylation raises the possibility that SKI plays the role in the aetiology of AIP. *NPTX2*, along with Cyclin D2, *FOXE1, TFPI2, ppENK,* and p16 all had hypermethylation events (10%) according to a research by Kinugawa *et al*[54]. However, compared to NCA and NP, AIP had a considerably greater*TFPI2* methylation ratio.

THERAPEUTIC ASPECTS OF EPIGENETIC MODULATED PATHWAYS IN PDAC AND ITS ASSOCIATED PRECANCEROUS LESIONS

PDAC is a deadly illness with few therapy options. According to new research, PDAC includes numerous layers of epigenetic alterations. Because the change is possibly reversible, it is a possible therapeutic target. Epigenetic changes can potentially affect the tumor microenvironment, modulating and enhancing treatment. Because epigenetic changes occur early in the disease, epigenetic markers can also be employed as diagnostic screening tools. Immunotherapy is being used more frequently to treat solid organ tumors, however there is no benefit for PDAC because most patients do not respond to these new treatments[55,56]. Because epigenetic processes regulate underlying immune cell activities, resulting in an anti-tumor response, combining immunotherapy and epigenetic therapy may improve patient outcomes even more. PDACs are currently classified as three to five subtypes according on the system used[57,58]. Using transcriptomic profiling, two primary molecular subtypes of PDAC were discovered: classical and basal[59]. The traditional kind has a better prognosis and clinical significance. Basal subtypes have altered the methylation of effectors and inhibitors of the Wnt signaling pathway by analyzing the epigenomic landscape. Classical tumors are hypomethylated, resulting in upregulation of the cholesterol transporter *NCP1L1*[60]. Furthermore, basal tumors were discovered to contain dysregulation of multiple genes related with established oncogenic signaling networks, including the *MYC*, erythroblastic oncogene B/*EGFR*, and transforming growth factor *(TGF)* signaling pathways. Chronic pancreatitis is a well-known risk factor for PDAC, which is consistent with the previously documented general link between tumor and inflammation[61,62]. Early stage PanCa caused by inflammation is linked to epigenetic alterations. Damage to the pancreatic epithelium during a pancreatitis episode results in long-lasting transcriptional and epigenetic remodelling that creates epithelial memory that protects against strokes in the future[63].

Reader proteins, have lately been identified as prospective therapeutic targets, in particular the chromatin adaptors of the bromo and extra C-terminal (BET) family, after directly engaging with the histone tails with acetylated lysine residues, these proteins with bromodomains can bind transcription factors to the DNA, boosting the acetylation-induced transcriptional activation. BET proteins use the epigenetic landscape in this way to support the growth of PDAC cells. Given the wide variety of abnormal epigenetic marks that are possible targets for the advancement of anticancer therapy, the study of and application of epigenetic enzyme inhibitors for the anti-cancer therapy show promise[49,64].

Cell interactions and released substances can cause epigenetic alternations. It has been demonstrated that PDAC cells induced DNA methylation of the *SOCS1* gene, acytokine supressor and cancer promoting growth factor, to boost tumor cell proliferation *in vitro*[63,65]. Clinical evidences demonstrating a higher 3-mo overall survival in patients missing *SOCS1* methylationl end credence to this. In PDAC, lysine demethylase 3A (*KDM3A*) is an effective epigenetic regulator of immunotherapy responses. This enzyme controls the *EGFR* expressions[66]. Tumors produced by cancer cells deficient in *KDM3A* have infiltrating immune cells that are responsive to immunotherapy. To distinguish between PDAC and cancer precursor phase, methylation -specific electrophoresis was used to determine the methylation status of the *MUC1, MUC2,* and *MUC4* genes in pancreatic fluids[67]. Additionally, the methylation status of the mucin genes was examined

using machine learning, and it was discovered that *MUC1* and *MUC4* hypomethylation levels were significantly correlated with poor prognosis[68].

Through the suppression of Hedgehog (Hh) signaling, improved gemcitabine delivery was shown in preclinical investigations. Clinical studies were conducted for a number of cancers, but they were unsuccessful and did not progress to phase III trials[69]. However, preclinical research using epigenetic targeting of the proteins known as BET bromodomains, which controls the transcriptional output of Hh signaling, demonstrated positive results *in vitro,* suggesting possible synergistic therapeutic approaches[70]. BET bromodomain proteins are thought to be crucial contribution to PDAC development and are a topic of active investigations[71]. Based on the transcription factor GATA-binding factor 6 (*GATA6*)'s function as a regulator of the traditional PDAC subtype identity, the method to induce subtype switching in PDAC has been further investigated. A basal state is provided in PDAC by *GATA6* depletion[72]. As a regulator of *GATA6* transcription in PDAC, the histone methyltransferase zeste homologue 2 (*EZH2*) enhancer prevents the decreased *EZH2-GATA6* and induced gene signatures present in traditional PDAC subtypes. Therefore, a potential target for PDAC treatment in the future is the *EZH2-GATA6* axis[73]. Tazemetostat, an *EZH2* inhibitor, has been FDA-approved for use in the treatment of advanced epithelioid sarcoma and is currently being investigated in a phase II research in conjunction with ICI in the treatment of other solid tumors, including PDAC (NCT04705818)[74]. A hostile squamous cell subtype is promoted to differentiate in PDAC by epigenetic silencing of *GATA6*. Using genome-wide epigenetic mapping of the alterations 5-methylcytosine and 5-hydroxymethylcytosine (5hmC), this epigenetic dysregulation was demonstrated[75]. Due to decreased production of the enzyme 5-methylcytosine hydroxylase *TET2*, these transcriptional subtypes exhibit a higher loss of 5hmC. In addition, reduction of *SMAD4* expression revealed decreased 5hmC and *GATA6,* resulting in a more squamous-like tumor. Blocking DNA methylation by utilizing the DNA methyltransferase (DNMT) inhibitor 5-azacytidine slows the growth of typical PDAC tumor. In contrast, utilising the same medication or DNMT knockdown *via* small interfering RNA boosted hyaluronic acid synthesis, ultimately increasing the advancement of PDACI[76]. Epithelial cells from normal pancreata and PDAC underwent transcriptomic and DNA methylomic analysis, which identified a subpopulation characterised by hypomethylation of repetitive regions, which in turn triggers an interferon-linked transcriptional programme[77]. The relationship between cell-of-origin and epigenetics and tumor heterogeneity can be seen in the fact that tumors with low methylation were more aggressive than tumors with high methylation, which kept more of their cell-of-origin characteristics[78].

A clinical trial examining the medication in solid tumor types, including PDAC, and the recent FDA approval of the *EZH2* inhibitor tazemetostat for the treatment of advanced epithelioid sarcoma show a potential clinical relevance of the found *EZH2-GATA6* axis in PDAC tumor[79]. Numerous researches have examined how DNA methylation mechanisms control the expression of genes in various TME components[80]. For instance, 5-azacytidine, a DNA methyltransferase (DNMT) inhibitor, inhibited global DNA methylation in epithelial PDAC cells and cancer-associated fibroblasts (CAFs), which slowed the evolution of PDAC[75]. In immunocompetent PDAC models, DNMT inhibition increased CD4 and CD8 T-cell infiltration and significantly reduced tumor size. Espinet *et al*[77] have discovered a link between low DNA methylation levels and subpar PDAC patient outcomes. They show that tumors with low levels of overall DNA methylation in the epithelial cells exhibit increased expression of endogenous retroviral transcripts, robust double-stranded RNA sensing machinery engagement, activation of an interferon signature, and stromal cell reprogramming that is pro-tumourigenic in the PDAC TME. Clinical trials for a sequential strategy based on HDAC/DNMT inhibition, chemotherapy, and then PD-L1 blocking are now being conducted in PDAC, and the findings are highly anticipated[81].

Specifically, nucleoside-like inhibitors induce cytotoxicity through DNA damage brought on by the creation of DNMT-DNA abducts, disrupt DNA methylation, and encourage the re-expression of dormant genes. Both outcomes support anticancer action[82]. Additionally, RNA modification of N6-methyladenosine (m6A) is a unique strategy for dynamic and reversible epigenetic control that has been discovered by researchers. By triggering the Wnt signaling cascade and changing Wnt I[82].

Inhibitory factor 1 (*WIF-1*), m6A accelerates the course of PanCa. Demethylase, m6A rubber, and the alkylation repair protein 5 (*ALKBH5*) homolog are increased in gemcitabine-treated sensitized PDAC cells. By demethylating m6A and consequently reducing *WIF-1* and deactivating Wnt signaling, it slows the growth of tumors. *In vitro* and *in vivo* development and invasiveness are accelerated when PanCa cells lack *ALKBH5*[42,83]. As a result, *ALKBH5* might be a brand-new target for PanCa treatment. Numerous studies have shown how DNMT inhibitors affect PanCa cell lines *in vitro* by inhibiting them, radiosensitizing them, and immunological sensitizing them. PanCa DNA repair regulation is mediated by H3K36 methylation. H3K36 is a SETD2-dependent protein that is essential for HR repair. Demethylating H3K36 by demethylase *KDM4A* alters heart rate. A transcription factor for *MHCII, RFXAP* has been linked to the inhibition of tumor growth. PDAC survival was favourably linked with *RFXAP* deficiency[84]. Ding *et al*[85] found that the natural flavonoid fisetin regulates H3K36 methylation to promote *RFXAP* and *KDM4A* expression and interferes with HR, leading to DNA damage and PDAC S-phase arrest[85]. Therefore, this tactic may constitute a cutting-edge therapeutic method for treating PanCa. DNMT inhibitors (DNMTis) are undertaking Phase I/II clinical trials in patients with PanCa and have been shown to sensitize PDAC cells to immune checkpoint blockade treatment and chemotherapy [86]. Decitabine, alongside 5-aza, and guadecitabine are DNMTs used for PDAC. Haematological malignancies are also accepted to be treated with HDAC inhibitors (HDACis)[87]. Another therapeutic epigenetic approach for PanCa patients is HDAC inhibition. In PanCa cells, HDAC is, which includes *SAHA* and *CUDC-101*, can downregulate apoptotic inhibitory proteins including survivin and XIAP. Additionally, these HDACs can make PanCa more radiosensitive and make it cytotoxic[88]. AR-42, which is another potent HDACi against PanCa cells, can inhibit cell proliferation *via* inducing cell cycle arrest at G2 phase. Additionally, it can induce DNA damage, apoptosis, and p53 expression, suggesting that it may have therapeutic promise for the treatment of PanCa[89]. In addition to that, reader proteins with different bromodomains that attract proteins implicated in tumor initiation and elongation are blocked by the BET inhibitor JQ1 from binding to the BET domain. In the framework of personalized medicine, Bian *et al*[90] defined a novel

technique for PDAC classification and management based on sensitivity to JQ1 treatment. In order to select PanCa patients with unregulated c-MYC signaling pathways and demonstrate that these selected tumors exhibited greater susceptibility to BET inhibitor JQ1 treatment, the technique involved molecularly characterizing patient xenografts. According to the study, administering BET inhibitors in conjunction with conventional anticancer regimens may constitute an efficient therapy option for individuals who have been carefully chosen and categorized (Table 2)[83,91].

Mechanisms of faulty negative control of cell proliferation, in particular immune evasion, can also produce abnormal proliferation in the development of gastrointestinal malignancies, in addition to unchecked proliferation brought on by cell cycle dysregulation[92]. For instance, it was discovered by researchers that during PanCa, H3K4me3 of the *BCL2L1, CFLAR,* and *MCL-1* gene promoters upregulates the production of the anti-apoptotic proteins *Bcl-x, FLIP,* and *MCL-1,* as well as the *BAK1, BAX,* and *BCL2L11* gene promoters of Bak and Bax. Proapoptotic proteins like the Bim protein, for example, have their expression downregulated[93]. These six apoptosis-controlling genes are all essential for PanCa growth and development[94].

Initial investigations with human pancreatic cell lines showed that silencing *KMT2D* lowered the number and proportion of cells in G0/G1, which was accompanied by a drop in H3K4me1/2/3, indicating that histone methylation is actually involved in cells cycle management[95]. Further research has primarily focused on CKI control. *P15* and *P21* genes, which encode two often reported CKIs, show higher levels of H3K27me3 and H3K9me3 and lower levels of H3K4me2/3 in gastrointestinal malignancies such as GC, CRC, HCC, and PanCa[96]. Upstream lncRNAs such as *BLACAT1, SNHG17,* and *CASC15* can decrease P15 and P21 expression and cause G0/G1 checkpoint deficit. DZNep (3-deazaneplanocin A), a powerful pharmacologic inhibitor of S-adenosylhomocysteine hydrolase, modifies chromatin accessibility *via* inhibiting histone methyltransferases such as *EZH2*[97,98]. It results in a large decrease in H3K27me3 (a primary repressive histone mark) levels, as well as a significant decrease in cell proliferation and migration in CRC. Similar effects can be seen in PanCa, with decreased global H3K27me3 levels leading to re-expression of miR-218, limiting cell growth, encouraging apoptosis, and finally triggering cell cycle arrest in PanCa cells[99]. Another study found that DZNep significantly modulates miR-663a and miR-4787-5p expression and consecutively suppresses TGFb1-induced EMT signaling in PanCa[98,100]. UNC1999, an EZH2-specific inhibitor, not only lowers the abnormal H3K27 methylation that characterizes PanCa cells, but it also slows cancer cell proliferation in three model systems[101]. Furthermore, chaetospirolactone has been shown to suppress the activity of the epigenetic regulator *EZH2* and consistently decrease H3K27me3 to allow for the transcription of *DR4*, which then binds to TRAIL and culminates in the activation of initiator caspase-8 and the formation of the death-inducing signaling complex[102]. As a result, diosgenin, garcinol, *FBW7* and curcumin analogue CDF have also been identified as potential agents targeting *EZH2* to prevent the development of PanCa[97,103]. Amalgamation treatment with the HMT inhibitor panH3K9me chaetocin and an aurorakinase A (*AURKA*) inhibitor reduces H3K9 methylation at the centrosome, generating mitotic abnormalities that eventually drive aberrant mitotic checkpoint responses and eventually mitotic catastrophe in PanCa[104].

CONCLUSION

Since PanCa patients have a dismal prognosis, understanding the molecular events that drive this terrible tumor disease is critical for developing alternative and more effective treatment regimens and determining trustworthy diagnostic indicators. The role of epigenetics in the initiation, development, and evolution of PDAC has been demonstrated by advances in high throughput sequencing and genome-wide association studies. This review covers the major epigenetic signaling pathways as well as how the epigenetic machinery is altered or 'hijacked' in PanCa. Recent epigenetic research has considerably expanded our understanding of the regulatory characteristics involved in PanCa initiation, and progression, along with metastasis tumor. As discussed in this article, DNA-based epigenetic processes have been shown to play a role in PanCa and may serve as potential therapeutic targets aimed at rectifying epigenetic dysregulation of cellular machinery. Initial clinical trials with DNMT inhibitors at stages I-III are presently underway, paving the path for the creation of innovative, and hopefully more successful, 'epidrugs' for patients with PanCa. As a result, we believe that targeting epigenetic regulators and modulators with successful pharmaceutical or even immunotherapeutic techniques would be a game changer in the fight against this aggressive cancer. One significant restriction of using such epigenetic reprogramming of PDAC tumors is the danger of pleiotropic effects, which occur when certain components of the epigenetic machinery have opposite effects in different cellular compartments. Recent improvements in single-cell sequencing technologies that provide multi-omics information from the genome and transcriptome may be useful in determining the specific involvement of the several players in the epigenetic regulation of PDAC tumors. Overall, manipulating the epigenetic machinery, either alone or as part of a combination treatment plan, has the potential to reprogram the aggressive PDAC tumor profile to a less aggressive or easily identifiable and curable state, thereby benefiting patients in the future. In conclusion, we conclude that when cancer-associated signaling pathways are evaluated as a combined shift in "genomic-epigenomic-and-nuclear" structure, an even more realistic picture of PanCa will be obtained. Early preneoplastic lesions in this organ appear to require only a few mutations to initiate a process of aberrant organogenesis *via* self-reinforcing pathological loops. During metastatic progression, epigenomic landscapes defined by the differential acquisition of enhancers and super-enhancers appear to be required to maintain inheritable, cancer-associated gene expression patterns that support the heterogeneous differentiation of human PanCa tumors. This has given unique insights into an arsenal of novel, potentially actionable signaling pathways that were not previously achieved through genomic analyses, supporting the notion that effective future PDAC therapeutic regimens will require precision medicine approaches that include epigenomic targets.

Table 2 Current ongoing trails targeting epigenetic therapy (combination drugs) in Pancreatic Cancer

Drug names	Combination agents	Trail phase	NCT number
Panobinostat vorinostat	Various antineoplastic drugs	Phase 1	NCT03878524
Vorinostat	Capecitabine + radiation	Phase 1/2	NCT00948688
Tazemetostat	Durvalumab/gemcitabine	Phase 2 recruiting	NCT04705818
Durvalumab	Tazemetostat	Phase 2	NCT04705818
Romidepsin, azacitidine	Durvalumab, lenalidomide, nab-paclitaxel	Phase 1/2 recruiting	NCT04257448
Azacitidine	Chemotherapy after progression	Phase 2 active	NCT01845805
Vorinostat	Gemcitabine, sorafenib +/−, radiation	Phase 1 active	NCT02349867
CC-486 (oral azacitidine)	–	Phase 2 active	NCT01845805
Azacitidine, not recruiting	Pembrolizumab	Phase 2 active	NCT03264404
Tazemetostat	Durvalumab	Phase 2	NCT04705818
MK-8628	-	Phase 1 completed	NCT02259114
Rx-3117	Nab-paclitaxel	1,2	NCT03189914
Entinostat	Nivolumab	Phase 2 completed	NCT03250273
Decitabine	Tetrahydrouridine	Phase 1 completed	NCT02847000
Vorinostat	Capecitabine	Phase 1 completed	NCT00983268
Azacitidine	nab-Paclitaxel, carboplatin	Phase 1 completed	NCT01478685
Vorinostat	NPI-0052 (marizomib)	Phase 1 completed	NCT00667082
Azacitidine	Pembrolizumab	Phase 2 recruiting	NCT03264404
Azacitidine	Abraxane, gemcitabine	Phase 2 active	NCT01845805
Entinostat	Nivolumab	Phase 2 active	NCT03250273

ACKNOWLEDGEMENTS

First, we would like to thank all the authors and clinicians whose valuable work has supported us in writing this review. Second, we also acknowledge clinicians from Tata Medical Center namely Dr. Paromita Roy along with clinicians from Apollo Multispecialty Hospital namely Dr. Sumit Gulati and Dr. Supriyo Ghatak. We would also like to thank Prof. Bitan Kumar Chattopadhay from Department of General Surgery, IPGMER & SSKM Hospital, Kolkata and Prof. Shibajyoti Ghosh from Department of General Surgery, Medical College and Hospital, Kolkata, India. Dr. Aniruddha Chatterjee would like to thank Rutherford Discovery Fellowship from the Royal Society of New Zealand, Department of Pathology, Dunedin School of Medicine, University of Otago for their support. The authors specifically thank and acknowledge Mr. Saraswan Sikdar.

FOOTNOTES

Author contributions: Bararia A, Das A, and Mitra S wrote the first draft of the paper and constructed the flow chart and tables; Chatterjee A, Banerjee S, and Sikdar N provided valuable input, suggestions, comments and guidance while writing the review manuscript and contributed to proofreading and editing; Sikdar N constructed, conceptualized, and edited the review manuscript.

Supported by the Department of Biotechnology, Government of India Grant Sanction, Ramalingaswami Re-entry Fellowship, No. RLS/BT/Re-entry/05/2012.

Conflict-of-interest statement: All the authors declare no conflicts of interest.

Country/Territory of origin: India

ORCID number: Akash Bararia 0000-0002-9069-3116; Amlan Das 0000-0002-9295-3743; Sangeeta Mitra 0000-0002-0651-2280; Sudeep Banerjee

0000-0002-3157-4184; Aniruddha Chatterjee 0000-0001-7276-2248; Nilabja Sikdar 0000-0003-4465-472X.

S-Editor: Liu JH
L-Editor: A
P-Editor: Xu ZH

REFERENCES

1 **Ying L**, Sharma A, Chhoda A, Ruzgar N, Hasan N, Kwak R, Wolfgang CL, Wang TH, Kunstman JW, Salem RR, Wood LD, Iacobuzio-Donahue C, Schneider EB, Farrell JJ, Ahuja N. Methylation-based Cell-free DNA Signature for Early Detection of Pancreatic Cancer. *Pancreas* 2021; **50**: 1267-1273 [PMID: 34860810 DOI: 10.1097/MPA.0000000000001919]

2 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

3 **Bararia A**, Chakraborty P, Roy P, Chattopadhay BK, Das A, Chatterjee A, Sikdar N. Emerging role of non-invasive and liquid biopsy biomarkers in pancreatic cancer. *World J Gastroenterol* 2023; **29**: 2241-2260 [PMID: 37124888 DOI: 10.3748/wjg.v29.i15.2241]

4 **Thompson ED**, Roberts NJ, Wood LD, Eshleman JR, Goggins MG, Kern SE, Klein AP, Hruban RH. The genetics of ductal adenocarcinoma of the pancreas in the year 2020: dramatic progress, but far to go. *Mod Pathol* 2020; **33**: 2544-2563 [PMID: 32704031 DOI: 10.1038/s41379-020-0629-6]

5 **Chatterjee A**, Bararia A, Ganguly D, Mondal PK, Roy P, Banerjee S, Ghosh S, Gulati S, Ghatak S, Chattopadhay BK, Basu P, Chatterjee A, Sikdar N. DNA methylome in pancreatic cancer identified novel promoter hyper-methylation in NPY and FAIM2 genes associated with poor prognosis in Indian patient cohort. *Cancer Cell Int* 2022; **22**: 334 [PMID: 36329447 DOI: 10.1186/s12935-022-02737-1]

6 **Hu JX**, Zhao CF, Chen WB, Liu QC, Li QW, Lin YY, Gao F. Pancreatic cancer: A review of epidemiology, trend, and risk factors. *World J Gastroenterol* 2021; **27**: 4298-4321 [PMID: 34366606 DOI: 10.3748/wjg.v27.i27.4298]

7 **O'Neill RS**, Stoita A. Biomarkers in the diagnosis of pancreatic cancer: Are we closer to finding the golden ticket? *World J Gastroenterol* 2021; **27**: 4045-4087 [PMID: 34326612 DOI: 10.3748/wjg.v27.i26.4045]

8 **Khalaf N**, El-Serag HB, Abrams HR, Thrift AP. Burden of Pancreatic Cancer: From Epidemiology to Practice. *Clin Gastroenterol Hepatol* 2021; **19**: 876-884 [PMID: 32147593 DOI: 10.1016/j.cgh.2020.02.054]

9 **Schnelldorfer T**, Ware AL, Sarr MG, Smyrk TC, Zhang L, Qin R, Gullerud RE, Donohue JH, Nagorney DM, Farnell MB. Long-term survival after pancreatoduodenectomy for pancreatic adenocarcinoma: is cure possible? *Ann Surg* 2008; **247**: 456-462 [PMID: 18376190 DOI: 10.1097/SLA.0b013e3181613142]

10 **Zhao Y**, Yang M, Wang S, Abbas SJ, Zhang J, Li Y, Shao R, Liu Y. An Overview of Epigenetic Methylation in Pancreatic Cancer Progression. *Front Oncol* 2022; **12**: 854773 [PMID: 35296007 DOI: 10.3389/fonc.2022.854773]

11 **Pandey S**, Gupta VK, Lavania SP. Role of epigenetics in pancreatic ductal adenocarcinoma. *Epigenomics* 2023; **15**: 89-110 [PMID: 36647796 DOI: 10.2217/epi-2022-0177]

12 **Zhang Z**, Zhu R, Sun W, Wang J, Liu J. Analysis of Methylation-driven Genes in Pancreatic Ductal Adenocarcinoma for Predicting Prognosis. *J Cancer* 2021; **12**: 6507-6518 [PMID: 34659542 DOI: 10.7150/jca.53208]

13 **Visani M**, Acquaviva G, De Leo A, Sanza V, Merlo L, Maloberti T, Brandes AA, Franceschi E, Di Battista M, Masetti M, Jovine E, Fiorino S, Pession A, Tallini G, de Biase D. Molecular alterations in pancreatic tumors. *World J Gastroenterol* 2021; **27**: 2710-2726 [PMID: 34135550 DOI: 10.3748/wjg.v27.i21.2710]

14 **Brancaccio M**, Natale F, Falco G, Angrisano T. Cell-Free DNA Methylation: The New Frontiers of Pancreatic Cancer Biomarkers' Discovery. *Genes (Basel)* 2019; **11** [PMID: 31877923 DOI: 10.3390/genes11010014]

15 **Thompson MJ**, Rubbi L, Dawson DW, Donahue TR, Pellegrini M. Pancreatic cancer patient survival correlates with DNA methylation of pancreas development genes. *PLoS One* 2015; **10**: e0128814 [PMID: 26039411 DOI: 10.1371/journal.pone.0128814]

16 **Thompson JK**, Bednar F. Clinical Utility of Epigenetic Changes in Pancreatic Adenocarcinoma. *Epigenomes* 2021; **5** [PMID: 34968245 DOI: 10.3390/epigenomes5040020]

17 **Bararia A**, Dey S, Gulati S, Ghatak S, Ghosh S, Banerjee S, Sikdar N. Differential methylation landscape of pancreatic ductal adenocarcinoma and its precancerous lesions. *Hepatobiliary Pancreat Dis Int* 2020; **19**: 205-217 [PMID: 32312637 DOI: 10.1016/j.hbpd.2020.03.010]

18 **Wang SS**, Xu J, Ji KY, Hwang CI. Epigenetic Alterations in Pancreatic Cancer Metastasis. *Biomolecules* 2021; **11** [PMID: 34439749 DOI: 10.3390/biom11081082]

19 **Neureiter D**, Jäger T, Ocker M, Kiesslich T. Epigenetics and pancreatic cancer: pathophysiology and novel treatment aspects. *World J Gastroenterol* 2014; **20**: 7830-7848 [PMID: 24976721 DOI: 10.3748/wjg.v20.i24.7830]

20 **Zamboni G**, Hirabayashi K, Castelli P, Lennon AM. Precancerous lesions of the pancreas. *Best Pract Res Clin Gastroenterol* 2013; **27**: 299-322 [PMID: 23809247 DOI: 10.1016/j.bpg.2013.04.001]

21 **Guler GD**, Ning Y, Ku CJ, Phillips T, McCarthy E, Ellison CK, Bergamaschi A, Collin F, Lloyd P, Scott A, Antoine M, Wang W, Chau K, Ashworth A, Quake SR, Levy S. Detection of early stage pancreatic cancer using 5-hydroxymethylcytosine signatures in circulating cell free DNA. *Nat Commun* 2020; **11**: 5270 [PMID: 33077732 DOI: 10.1038/s41467-020-18965-w]

22 **Fujikura K**, Alruwaii ZI, Haffner MC, Trujillo MA, Roberts NJ, Hong SM, Macgregor-Das A, Goggins MG, Roy S, Meeker AK, Ding D, Wright M, He J, Hruban RH, Wood LD. Downregulation of 5-hydroxymethylcytosine is an early event in pancreatic tumorigenesis. *J Pathol* 2021; **254**: 279-288 [PMID: 33870509 DOI: 10.1002/path.5682]

23 **Lomberk G**, Dusetti N, Iovanna J, Urrutia R. Emerging epigenomic landscapes of pancreatic cancer in the era of precision medicine. *Nat Commun* 2019; **10**: 3875 [PMID: 31462645 DOI: 10.1038/s41467-019-11812-7]

24 **Michael JV**, Goldfinger LE. Concepts and advances in cancer therapeutic vulnerabilities in RAS membrane targeting. *Semin Cancer Biol* 2019; **54**: 121-130 [PMID: 29203271 DOI: 10.1016/j.semcancer.2017.11.021]

25 **Saha G**, Singh R, Mandal A, Das S, Chattopadhyay E, Panja P, Roy P, DeSarkar N, Gulati S, Ghatak S, Ghosh S, Banerjee S, Roy B, Chaudhuri D, Arora N, Biswas NK, Sikdar N. A novel hotspot and rare somatic mutation p.A138V, at TP53 is associated with poor survival of

pancreatic ductal and periampullary adenocarcinoma patients. *Mol Med* 2020; **26**: 59 [PMID: 32552660 DOI: 10.1186/s10020-020-00183-1]

26 **Choi M**, Bien H, Mofunanya A, Powers S. Challenges in Ras therapeutics in pancreatic cancer. *Semin Cancer Biol* 2019; **54**: 101-108 [PMID: 29170065 DOI: 10.1016/j.semcancer.2017.11.015]

27 **Canon J**, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, Lanman BA, Werner J, Rapaport AS, San Miguel T, Ortiz R, Osgood T, Sun JR, Zhu X, McCarter JD, Volak LP, Houk BE, Fakih MG, O'Neil BH, Price TJ, Falchook GS, Desai J, Kuo J, Govindan R, Hong DS, Ouyang W, Henary H, Arvedson T, Cee VJ, Lipford JR. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 2019; **575**: 217-223 [PMID: 31666701 DOI: 10.1038/s41586-019-1694-1]

28 **Seton-Rogers S**. KRAS-G12C in the crosshairs. *Nat Rev Cancer* 2020; **20**: 3 [PMID: 31728026 DOI: 10.1038/s41568-019-0228-3]

29 **Bryant KL**, Stalnecker CA, Zeitouni D, Klomp JE, Peng S, Tikunov AP, Gunda V, Pierobon M, Waters AM, George SD, Tomar G, Papke B, Hobbs GA, Yan L, Hayes TK, Diehl JN, Goode GD, Chaika NV, Wang Y, Zhang GF, Witkiewicz AK, Knudsen ES, Petricoin EF 3rd, Singh PK, Macdonald JM, Tran NL, Lyssiotis CA, Ying H, Kimmelman AC, Cox AD, Der CJ. Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nat Med* 2019; **25**: 628-640 [PMID: 30833752 DOI: 10.1038/s41591-019-0368-8]

30 **Fitzgerald TL**, Lertpiriyapong K, Cocco L, Martelli AM, Libra M, Candido S, Montalto G, Cervello M, Steelman L, Abrams SL, McCubrey JA. Roles of EGFR and KRAS and their downstream signaling pathways in pancreatic cancer and pancreatic cancer stem cells. *Adv Biol Regul* 2015; **59**: 65-81 [PMID: 26257206 DOI: 10.1016/j.jbior.2015.06.003]

31 **Ardito CM**, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, Pal D, Briel T, Herner A, Trajkovic-Arsic M, Sipos B, Liou GY, Storz P, Murray NR, Threadgill DW, Sibilia M, Washington MK, Wilson CL, Schmid RM, Raines EW, Crawford HC, Siveke JT. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 2012; **22**: 304-317 [PMID: 22975374 DOI: 10.1016/j.ccr.2012.07.024]

32 **Zhan T**, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene* 2017; **36**: 1461-1473 [PMID: 27617575 DOI: 10.1038/onc.2016.304]

33 **Davis SL**, Cardin DB, Shahda S, Lenz HJ, Dotan E, O'Neil BH, Kapoun AM, Stagg RJ, Berlin J, Messersmith WA, Cohen SJ. A phase 1b dose escalation study of Wnt pathway inhibitor vantictumab in combination with nab-paclitaxel and gemcitabine in patients with previously untreated metastatic pancreatic cancer. *Invest New Drugs* 2020; **38**: 821-830 [PMID: 31338636 DOI: 10.1007/s10637-019-00824-1]

34 **Deplanque G**, Demarchi M, Hebbar M, Flynn P, Melichar B, Atkins J, Nowara E, Moyé L, Piquemal D, Ritter D, Dubreuil P, Mansfield CD, Acin Y, Moussy A, Hermine O, Hammel P. A randomized, placebo-controlled phase III trial of masitinib plus gemcitabine in the treatment of advanced pancreatic cancer. *Ann Oncol* 2015; **26**: 1194-1200 [PMID: 25858497 DOI: 10.1093/annonc/mdv133]

35 **Fang YT**, Yang WW, Niu YR, Sun YK. Recent advances in targeted therapy for pancreatic adenocarcinoma. *World J Gastrointest Oncol* 2023; **15**: 571-595 [PMID: 37123059 DOI: 10.4251/wjgo.v15.i4.571]

36 **Li Q**, Li Z, Luo T, Shi H. Targeting the PI3K/AKT/mTOR and RAF/MEK/ERK pathways for cancer therapy. *Mol Biomed* 2022; **3**: 47 [PMID: 36539659 DOI: 10.1186/s43556-022-00110-2]

37 **Karar J**, Maity A. PI3K/AKT/mTOR Pathway in Angiogenesis. *Front Mol Neurosci* 2011; **4**: 51 [PMID: 22144946 DOI: 10.3389/fnmol.2011.00051]

38 **Takeda H**, Rust AG, Ward JM, Yew CC, Jenkins NA, Copeland NG. Sleeping Beauty transposon mutagenesis identifies genes that cooperate with mutant Smad4 in gastric cancer development. *Proc Natl Acad Sci U S A* 2016; **113**: E2057-E2065 [PMID: 27006499 DOI: 10.1073/pnas.1603223113]

39 **Vincent A**, Omura N, Hong SM, Jaffe A, Eshleman J, Goggins M. Genome-wide analysis of promoter methylation associated with gene expression profile in pancreatic adenocarcinoma. *Clin Cancer Res* 2011; **17**: 4341-4354 [PMID: 21610144 DOI: 10.1158/1078-0432.CCR-10-3431]

40 **di Magliano MP**, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 2013; **144**: 1220-1229 [PMID: 23622131 DOI: 10.1053/j.gastro.2013.01.071]

41 **Fukushima N**, Sato N, Ueki T, Rosty C, Walter KM, Wilentz RE, Yeo CJ, Hruban RH, Goggins M. Aberrant methylation of preproenkephalin and p16 genes in pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma. *Am J Pathol* 2002; **160**: 1573-1581 [PMID: 12000709 DOI: 10.1016/S0002-9440(10)61104-2]

42 **Tang B**, Yang Y, Kang M, Wang Y, Bi Y, He S, Shimamoto F. m(6)A demethylase ALKBH5 inhibits pancreatic cancer tumorigenesis by decreasing WIF-1 RNA methylation and mediating Wnt signaling. *Mol Cancer* 2020; **19**: 3 [PMID: 31906946 DOI: 10.1186/s12943-019-1128-6]

43 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

44 **Li X**, Zhang X, Lin X, Cai L, Wang Y, Chang Z. Classification and Prognosis Analysis of Pancreatic Cancer Based on DNA Methylation Profile and Clinical Information. *Genes (Basel)* 2022; **13** [PMID: 36292798 DOI: 10.3390/genes13101913]

45 **Xiao M**, Liang X, Yan Z, Chen J, Zhu Y, Xie Y, Li Y, Li X, Gao Q, Feng F, Fu G, Gao Y. A DNA-Methylation-Driven Genes Based Prognostic Signature Reveals Immune Microenvironment in Pancreatic Cancer. *Front Immunol* 2022; **13**: 803962 [PMID: 35222383 DOI: 10.3389/fimmu.2022.803962]

46 **Zou T**, Shi D, Wang W, Chen G, Zhang X, Tian Y, Gong P. Identification of a New m6A Regulator-Related Methylation Signature for Predicting the Prognosis and Immune Microenvironment of Patients with Pancreatic Cancer. *Mediators Inflamm* 2023; **2023**: 5565054 [PMID: 37181810 DOI: 10.1155/2023/5565054]

47 **Zheng X**, Du Y, Liu M, Wang C. ITGA3 acts as a purity-independent biomarker of both immunotherapy and chemotherapy resistance in pancreatic cancer: bioinformatics and experimental analysis. *Funct Integr Genomics* 2023; **23**: 196 [PMID: 37270717 DOI: 10.1007/s10142-023-01122-z]

48 **Nones K**, Waddell N, Song S, Patch AM, Miller D, Johns A, Wu J, Kassahn KS, Wood D, Bailey P, Fink L, Manning S, Christ AN, Nourse C, Kazakoff S, Taylor D, Leonard C, Chang DK, Jones MD, Thomas M, Watson C, Pinese M, Cowley M, Rooman I, Pajic M; APGI, Butturini G, Malpaga A, Corbo V, Crippa S, Falconi M, Zamboni G, Castelli P, Lawlor RT, Gill AJ, Scarpa A, Pearson JV, Biankin AV, Grimmond SM. Genome-wide DNA methylation patterns in pancreatic ductal adenocarcinoma reveal epigenetic deregulation of SLIT-ROBO, ITGA2 and MET signaling. *Int J Cancer* 2014; **135**: 1110-1118 [PMID: 24500968 DOI: 10.1002/ijc.28765]

49 **Paradise BD**, Barham W, Fernandez-Zapico ME. Targeting Epigenetic Aberrations in Pancreatic Cancer, a New Path to Improve Patient Outcomes? *Cancers (Basel)* 2018; **10** [PMID: 29710783 DOI: 10.3390/cancers10050128]

50 **House MG**, Guo M, Iacobuzio-Donahue C, Herman JG. Molecular progression of promoter methylation in intraductal papillary mucinous neoplasms (IPMN) of the pancreas. *Carcinogenesis* 2003; **24**: 193-198 [PMID: 12584167 DOI: 10.1093/carcin/24.2.193]

51 **Grzenda A**, Ordog T, Urrutia R. Polycomb and the emerging epigenetics of pancreatic cancer. *J Gastrointest Cancer* 2011; **42**: 100-111 [PMID: 21336826 DOI: 10.1007/s12029-011-9262-4]

52 **Hamano H**, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaido T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**: 732-738 [PMID: 11236777 DOI: 10.1056/NEJM200103083441005]

53 **Umehara H**, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Sumida T, Mimori T, Tanaka Y, Tsubota K, Yoshino T, Kawa S, Suzuki R, Takegami T, Tomosugi N, Kurose N, Ishigaki Y, Azumi A, Kojima M, Nakamura S, Inoue D; Research Program for Intractable Disease by Ministry of Health, Labor and Welfare (MHLW) Japan G4 team. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. *Mod Rheumatol* 2012; **22**: 1-14 [PMID: 21881964 DOI: 10.1007/s10165-011-0508-6]

54 **Kinugawa Y**, Uehara T, Matsuda K, Kobayashi Y, Nakajima T, Hamano H, Kawa S, Higuchi K, Hosaka N, Shiozawa S, Ishigame H, Nakamura T, Maruyama Y, Nakazawa K, Nakaguro M, Sano K, Ota H. Promoter hypomethylation of SKI in autoimmune pancreatitis. *Pathol Res Pract* 2018; **214**: 492-497 [PMID: 29534839 DOI: 10.1016/j.prp.2018.03.005]

55 **Zhang CY**, Liu S, Yang M. Clinical diagnosis and management of pancreatic cancer: Markers, molecular mechanisms, and treatment options. *World J Gastroenterol* 2022; **28**: 6827-6845 [PMID: 36632312 DOI: 10.3748/wjg.v28.i48.6827]

56 **Jamieson NB**, Steele CW. Immuno-Oncology in Pancreatic Cancer. In: Textbook of Pancreatic Cancer. Cham: Springer International Publishing, 2021: 287–304 [DOI: 10.1007/978-3-030-53786-9_20]

57 **Bailey P**, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM; Australian Pancreatic Cancer Genome Initiative, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; **531**: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

58 **Moffitt RA**, Marayati R, Flate EL, Volmar KE, Loeza SG, Hoadley KA, Rashid NU, Williams LA, Eaton SC, Chung AH, Smyla JK, Anderson JM, Kim HJ, Bentrem DJ, Talamonti MS, Iacobuzio-Donahue CA, Hollingsworth MA, Yeh JJ. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet* 2015; **47**: 1168-1178 [PMID: 26343385 DOI: 10.1038/ng.3398]

59 **Malinova A**, Veghini L, Real FX, Corbo V. Cell Lineage Infidelity in PDAC Progression and Therapy Resistance. *Front Cell Dev Biol* 2021; **9**: 795251 [PMID: 34926472 DOI: 10.3389/fcell.2021.795251]

60 **Koni M**, Pinnarò V, Brizzi MF. The Wnt Signalling Pathway: A Tailored Target in Cancer. *Int J Mol Sci* 2020; **21** [PMID: 33080952 DOI: 10.3390/ijms21207697]

61 **Kirkegård J**, Mortensen FV, Cronin-Fenton D. Chronic Pancreatitis and Pancreatic Cancer Risk: A Systematic Review and Meta-analysis. *Am J Gastroenterol* 2017; **112**: 1366-1372 [PMID: 28762376 DOI: 10.1038/ajg.2017.218]

62 **Del Poggetto E**, Ho IL, Balestrieri C, Yen EY, Zhang S, Citron F, Shah R, Corti D, Diaferia GR, Li CY, Loponte S, Carbone F, Hayakawa Y, Valenti G, Jiang S, Sapio L, Jiang H, Dey P, Gao S, Deem AK, Rose-John S, Yao W, Ying H, Rhim AD, Genovese G, Heffernan TP, Maitra A, Wang TC, Wang L, Draetta GF, Carugo A, Natoli G, Viale A. Epithelial memory of inflammation limits tissue damage while promoting pancreatic tumorigenesis. *Science* 2021; **373**: eabj0486 [PMID: 34529467 DOI: 10.1126/science.abj0486]

63 **Garcia-Gomez A**, Rodríguez-Ubreva J, Ballestar E. Epigenetic interplay between immune, stromal and cancer cells in the tumor microenvironment. *Clin Immunol* 2018; **196**: 64-71 [PMID: 29501540 DOI: 10.1016/j.clim.2018.02.013]

64 **Natale F**, Vivo M, Falco G, Angrisano T. Deciphering DNA methylation signatures of pancreatic cancer and pancreatitis. *Clin Epigenetics* 2019; **11**: 132 [PMID: 31492175 DOI: 10.1186/s13148-019-0728-8]

65 **Xiao Q**, Zhou D, Rucki AA, Williams J, Zhou J, Mo G, Murphy A, Fujiwara K, Kleponis J, Salman B, Wolfgang CL, Anders RA, Zheng S, Jaffee EM, Zheng L. Cancer-Associated Fibroblasts in Pancreatic Cancer Are Reprogrammed by Tumor-Induced Alterations in Genomic DNA Methylation. *Cancer Res* 2016; **76**: 5395-5404 [PMID: 27496707 DOI: 10.1158/0008-5472.CAN-15-3264]

66 **Li J**, Yuan S, Norgard RJ, Yan F, Sun YH, Kim IK, Merrell AJ, Sela Y, Jiang Y, Bhanu NV, Garcia BA, Vonderheide RH, Blanco A, Stanger BZ. Epigenetic and Transcriptional Control of the Epidermal Growth Factor Receptor Regulates the Tumor Immune Microenvironment in Pancreatic Cancer. *Cancer Discov* 2021; **11**: 736-753 [PMID: 33158848 DOI: 10.1158/2159-8290.CD-20-0519]

67 **Yokoyama S**, Kitamoto S, Higashi M, Goto Y, Hara T, Ikebe D, Yamaguchi T, Arisaka Y, Niihara T, Nishimata H, Tanaka S, Takaori K, Batra SK, Yonezawa S. Diagnosis of pancreatic neoplasms using a novel method of DNA methylation analysis of mucin expression in pancreatic juice. *PLoS One* 2014; **9**: e93760 [PMID: 24714692 DOI: 10.1371/journal.pone.0093760]

68 **Yokoyama S**, Hamada T, Higashi M, Matsuo K, Maemura K, Kurahara H, Horinouchi M, Hiraki T, Sugimoto T, Akahane T, Yonezawa S, Kornmann M, Batra SK, Hollingsworth MA, Tanimoto A. Predicted Prognosis of Patients with Pancreatic Cancer by Machine Learning. *Clin Cancer Res* 2020; **26**: 2411-2421 [PMID: 31992588 DOI: 10.1158/1078-0432.CCR-19-1247]

69 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]

70 **Huang Y**, Nahar S, Nakagawa A, Fernandez-Barrena MG, Mertz JA, Bryant BM, Adams CE, Mino-Kenudson M, Von Alt KN, Chang K, Conery AR, Hatton C, Sims RJ 3rd, Fernandez-Zapico ME, Wang X, Lillemoe KD, Fernández-Del Castillo C, Warshaw AL, Thayer SP, Liss AS. Regulation of GLI Underlies a Role for BET Bromodomains in Pancreatic Cancer Growth and the Tumor Microenvironment. *Clin Cancer Res* 2016; **22**: 4259-4270 [PMID: 27169995 DOI: 10.1158/1078-0432.CCR-15-2068]

71 **Smith SG**, Zhou MM. The Bromodomain: A New Target in Emerging Epigenetic Medicine. *ACS Chem Biol* 2016; **11**: 598-608 [PMID:

26596782 DOI: 10.1021/acschembio.5b00831]

72 **Lomberk G**, Blum Y, Nicolle R, Nair A, Gaonkar KS, Marisa L, Mathison A, Sun Z, Yan H, Elarouci N, Armenoult L, Ayadi M, Ordog T, Lee JH, Oliver G, Klee E, Moutardier V, Gayet O, Bian B, Duconseil P, Gilabert M, Bigonnet M, Garcia S, Turrini O, Delpero JR, Giovannini M, Grandval P, Gasmi M, Secq V, De Reyniès A, Dusetti N, Iovanna J, Urrutia R. Distinct epigenetic landscapes underlie the pathobiology of pancreatic cancer subtypes. *Nat Commun* 2018; **9**: 1978 [PMID: 29773832 DOI: 10.1038/s41467-018-04383-6]

73 **Kloesch B**, Ionasz V, Paliwal S, Hruschka N, Martinez de Villarreal J, Öllinger R, Mueller S, Dienes HP, Schindl M, Gruber ES, Stift J, Herndler-Brandstetter D, Lomberk GA, Seidler B, Saur D, Rad R, Urrutia RA, Real FX, Martinelli P. A GATA6-centred gene regulatory network involving HNFs and ΔNp63 controls plasticity and immune escape in pancreatic cancer. *Gut* 2022; **71**: 766-777 [PMID: 33846140 DOI: 10.1136/gutjnl-2020-321397]

74 **Eyres M**, Lanfredini S, Xu H, Burns A, Blake A, Willenbrock F, Goldin R, Hughes D, Hughes S, Thapa A, Vavoulis D, Hubert A, D'Costa Z, Sabbagh A, Abraham AG, Blancher C, Jones S, Verrill C, Silva M, Soonawalla Z, Maughan T, Schuh A, Mukherjee S, O'Neill E. TET2 Drives 5hmc Marking of GATA6 and Epigenetically Defines Pancreatic Ductal Adenocarcinoma Transcriptional Subtypes. *Gastroenterology* 2021; **161**: 653-668.e16 [PMID: 33915173 DOI: 10.1053/j.gastro.2021.04.044]

75 **Shakya R**, Gonda T, Quante M, Salas M, Kim S, Brooks J, Hirsch S, Davies J, Cullo A, Olive K, Wang TC, Szabolcs M, Tycko B, Ludwig T. Hypomethylating therapy in an aggressive stroma-rich model of pancreatic carcinoma. *Cancer Res* 2013; **73**: 885-896 [PMID: 23204224 DOI: 10.1158/0008-5472.CAN-12-1880]

76 **Kohi S**, Sato N, Cheng XB, Koga A, Higure A, Hirata K. A novel epigenetic mechanism regulating hyaluronan production in pancreatic cancer cells. *Clin Exp Metastasis* 2016; **33**: 225-230 [PMID: 26589701 DOI: 10.1007/s10585-015-9771-9]

77 **Espinet E**, Gu Z, Imbusch CD, Giese NA, Büscher M, Safavi M, Weisenburger S, Klein C, Vogel V, Falcone M, Insua-Rodríguez J, Reitberger M, Thiel V, Kossi SO, Muckenhuber A, Sarai K, Lee AYL, Backx E, Zarei S, Gaida MM, Rodríguez-Paredes M, Donato E, Yen HY, Eils R, Schlesner M, Pfarr N, Hackert T, Plass C, Brors B, Steiger K, Weichenhan D, Arda HE, Rooman I, Kopp JL, Strobel O, Weichert W, Sprick MR, Trumpp A. Aggressive PDACs Show Hypomethylation of Repetitive Elements and the Execution of an Intrinsic IFN Program Linked to a Ductal Cell of Origin. *Cancer Discov* 2021; **11**: 638-659 [PMID: 33060108 DOI: 10.1158/2159-8290.CD-20-1202]

78 **Roalsø MTT**, Hald ØH, Alexeeva M, Søreide K. Emerging Role of Epigenetic Alterations as Biomarkers and Novel Targets for Treatments in Pancreatic Ductal Adenocarcinoma. *Cancers (Basel)* 2022; **14** [PMID: 35158814 DOI: 10.3390/cancers14030546]

79 **Patil S**, Steuber B, Kopp W, Kari V, Urbach L, Wang X, Küffer S, Bohnenberger H, Spyropoulou D, Zhang Z, Versemann L, Bösherz MS, Brunner M, Gaedcke J, Ströbel P, Zhang JS, Neesse A, Ellenrieder V, Singh SK, Johnsen SA, Hessmann E. EZH2 Regulates Pancreatic Cancer Subtype Identity and Tumor Progression *via* Transcriptional Repression of GATA6. *Cancer Res* 2020; **80**: 4620-4632 [PMID: 32907838 DOI: 10.1158/0008-5472.CAN-20-0672]

80 **Neesse A**, Bauer CA, Öhlund D, Lauth M, Buchholz M, Michl P, Tuveson DA, Gress TM. Stromal biology and therapy in pancreatic cancer: ready for clinical translation? *Gut* 2019; **68**: 159-171 [PMID: 30177543 DOI: 10.1136/gutjnl-2018-316451]

81 **Versemann L**, Hessmann E, Ulisse M. Epigenetic Therapeutic Strategies to Target Molecular and Cellular Heterogeneity in Pancreatic Cancer. *Visc Med* 2022; **38**: 11-19 [PMID: 35291698 DOI: 10.1159/000519859]

82 **Pechalrieu D**, Etievant C, Arimondo PB. DNA methyltransferase inhibitors in cancer: From pharmacology to translational studies. *Biochem Pharmacol* 2017; **129**: 1-13 [PMID: 27956110 DOI: 10.1016/j.bcp.2016.12.004]

83 **Ganji C**, Farran B. Current clinical trials for epigenetic targets and therapeutic inhibitors for pancreatic cancer therapy. *Drug Discov Today* 2022; **27**: 1404-1410 [PMID: 34952224 DOI: 10.1016/j.drudis.2021.12.013]

84 **Ettel M**, Zhao L, Schechter S, Shi J. Expression and prognostic value of NSD1 and SETD2 in pancreatic ductal adenocarcinoma and its precursor lesions. *Pathology* 2019; **51**: 392-398 [PMID: 31060750 DOI: 10.1016/j.pathol.2019.02.005]

85 **Ding G**, Xu X, Li D, Chen Y, Wang W, Ping D, Jia S, Cao L. Fisetin inhibits proliferation of pancreatic adenocarcinoma by inducing DNA damage *via* RFXAP/KDM4A-dependent histone H3K36 demethylation. *Cell Death Dis* 2020; **11**: 893 [PMID: 33093461 DOI: 10.1038/s41419-020-03019-2]

86 **Berdasco M**, Esteller M. Clinical epigenetics: seizing opportunities for translation. *Nat Rev Genet* 2019; **20**: 109-127 [PMID: 30479381 DOI: 10.1038/s41576-018-0074-2]

87 **Bennett RL**, Licht JD. Targeting Epigenetics in Cancer. *Annu Rev Pharmacol Toxicol* 2018; **58**: 187-207 [PMID: 28992434 DOI: 10.1146/annurev-pharmtox-010716-105106]

88 **Moertl S**, Payer S, Kell R, Winkler K, Anastasov N, Atkinson MJ. Comparison of Radiosensitization by HDAC Inhibitors CUDC-101 and SAHA in Pancreatic Cancer Cells. *Int J Mol Sci* 2019; **20** [PMID: 31269745 DOI: 10.3390/ijms20133259]

89 **De Souza C**, Chatterji BP. HDAC Inhibitors as Novel Anti-Cancer Therapeutics. *Recent Pat Anticancer Drug Discov* 2015; **10**: 145-162 [PMID: 25782916 DOI: 10.2174/1574892810666150317144511]

90 **Bian B**, Bigonnet M, Gayet O, Loncle C, Maignan A, Gilabert M, Moutardier V, Garcia S, Turrini O, Delpero JR, Giovannini M, Grandval P, Gasmi M, Ouaissi M, Secq V, Poizat F, Nicolle R, Blum Y, Marisa L, Rubis M, Raoul JL, Bradner JE, Qi J, Lomberk G, Urrutia R, Saul A, Dusetti N, Iovanna J. Gene expression profiling of patient-derived pancreatic cancer xenografts predicts sensitivity to the BET bromodomain inhibitor JQ1: implications for individualized medicine efforts. *EMBO Mol Med* 2017; **9**: 482-497 [PMID: 28275007 DOI: 10.15252/emmm.201606975]

91 **Bian B**, Juiz NA, Gayet O, Bigonnet M, Brandone N, Roques J, Cros J, Wang N, Dusetti N, Iovanna J. Pancreatic Cancer Organoids for Determining Sensitivity to Bromodomain and Extra-Terminal Inhibitors (BETi). *Front Oncol* 2019; **9**: 475 [PMID: 31231611 DOI: 10.3389/fonc.2019.00475]

92 **Lu C**, Yang D, Sabbatini ME, Colby AH, Grinstaff MW, Oberlies NH, Pearce C, Liu K. Contrasting roles of H3K4me3 and H3K9me3 in regulation of apoptosis and gemcitabine resistance in human pancreatic cancer cells. *BMC Cancer* 2018; **18**: 149 [PMID: 29409480 DOI: 10.1186/s12885-018-4061-y]

93 **Tan N**, Wong M, Nannini MA, Hong R, Lee LB, Price S, Williams K, Savy PP, Yue P, Sampath D, Settleman J, Fairbrother WJ, Belmont LD. Bcl-2/Bcl-xL inhibition increases the efficacy of MEK inhibition alone and in combination with PI3 kinase inhibition in lung and pancreatic tumor models. *Mol Cancer Ther* 2013; **12**: 853-864 [PMID: 23475955 DOI: 10.1158/1535-7163.MCT-12-0949]

94 **Abulwerdi F**, Liao C, Liu M, Azmi AS, Aboukameel A, Mady AS, Gulappa T, Cierpicki T, Owens S, Zhang T, Sun D, Stuckey JA, Mohammad RM, Nikolovska-Coleska Z. A novel small-molecule inhibitor of mcl-1 blocks pancreatic cancer growth in vitro and in vivo. *Mol Cancer Ther* 2014; **13**: 565-575 [PMID: 24019208 DOI: 10.1158/1535-7163.MCT-12-0767]

95 **Dawkins JB**, Wang J, Maniati E, Heward JA, Koniali L, Kocher HM, Martin SA, Chelala C, Balkwill FR, Fitzgibbon J, Grose RP. Reduced Expression of Histone Methyltransferases KMT2C and KMT2D Correlates with Improved Outcome in Pancreatic Ductal Adenocarcinoma.

Cancer Res 2016; **76**: 4861-4871 [PMID: 27280393 DOI: 10.1158/0008-5472.CAN-16-0481]

96 **Matsuura I**, Denissova NG, Wang G, He D, Long J, Liu F. Cyclin-dependent kinases regulate the antiproliferative function of Smads. *Nature* 2004; **430**: 226-231 [PMID: 15241418 DOI: 10.1038/nature02650]

97 **Batchu RB**, Qazi AM, Gruzdyn OV, Semaan A, Seward SM, Chamala S, Dhulipala VB, Bouwman DL, Weaver DW, Gruber SA. EZH2-shRNA-mediated upregulation of p21waf1/cip1 and its transcriptional enhancers with concomitant downmodulation of mutant p53 in pancreatic ductal adenocarcinoma. *Surgery* 2013; **154**: 739-46; discussion 746 [PMID: 24074410 DOI: 10.1016/j.surg.2013.06.041]

98 **Cheng Y**, He C, Wang M, Ma X, Mo F, Yang S, Han J, Wei X. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther* 2019; **4**: 62 [PMID: 31871779 DOI: 10.1038/s41392-019-0095-0]

99 **Kato S**, Schwaederle M, Daniels GA, Piccioni D, Kesari S, Bazhenova L, Shimabukuro K, Parker BA, Fanta P, Kurzrock R. Cyclin-dependent kinase pathway aberrations in diverse malignancies: clinical and molecular characteristics. *Cell Cycle* 2015; **14**: 1252-1259 [PMID: 25695927 DOI: 10.1080/15384101.2015.1014149]

100 **Mody HR**, Hung SW, AlSaggar M, Griffin J, Govindarajan R. Inhibition of S-Adenosylmethionine-Dependent Methyltransferase Attenuates TGFβ1-Induced EMT and Metastasis in Pancreatic Cancer: Putative Roles of miR-663a and miR-4787-5p. *Mol Cancer Res* 2016; **14**: 1124-1135 [PMID: 27624777 DOI: 10.1158/1541-7786.MCR-16-0083]

101 **Huang L**, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, Nostro C, Wang R, Muthuswamy LB, Crawford HC, Arrowsmith C, Kalloger SE, Renouf DJ, Connor AA, Cleary S, Schaeffer DF, Roehrl M, Tsao MS, Gallinger S, Keller G, Muthuswamy SK. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med* 2015; **21**: 1364-1371 [PMID: 26501191 DOI: 10.1038/nm.3973]

102 **Hu W**, Jia X, Gao Y, Zhang Q. Chaetospirolactone reverses the apoptotic resistance towards TRAIL in pancreatic cancer. *Biochem Biophys Res Commun* 2018; **495**: 621-628 [PMID: 29107694 DOI: 10.1016/j.bbrc.2017.10.144]

103 **Ashkenazi A**. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. *Nat Rev Drug Discov* 2008; **7**: 1001-1012 [PMID: 18989337 DOI: 10.1038/nrd2637]

104 **Chen Y**, Ren B, Yang J, Wang H, Yang G, Xu R, You L, Zhao Y. The role of histone methylation in the development of digestive cancers: a potential direction for cancer management. *Signal Transduct Target Ther* 2020; **5**: 143 [PMID: 32747629 DOI: 10.1038/s41392-020-00252-1]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1520-1530

DOI: 10.4251/wjgo.v15.i9.1520

ISSN 1948-5204 (online)

MINIREVIEWS

Metastasis-associated lung adenocarcinoma transcript 1 molecular mechanisms in gastric cancer progression

Daniel Mateus de Oliveira Batista, Jéssica Manoelli Costa da Silva, Carolina de Oliveira Gigek, Marília de Arruda Cardoso Smith, Paulo Pimentel de Assumpção, Danielle Queiroz Calcagno

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Agostino SD, Italy; Ma X, China

Received: May 2, 2023
Peer-review started: May 2, 2023
First decision: May 9, 2023
Revised: May 30, 2023
Accepted: July 27, 2023
Article in press: July 27, 2023
Published online: September 15, 2023

Daniel Mateus de Oliveira Batista, Jéssica Manoelli Costa da Silva, Paulo Pimentel de Assumpção, Danielle Queiroz Calcagno, Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém 66073-000, Pará, Brazil

Carolina de Oliveira Gigek, Departamento de Patologia, Universidade Federal de São Paulo, São Paulo 04023-062, São Paulo, Brazil

Marília de Arruda Cardoso Smith, Disciplina de Genética,Departamento de Morfologia e Genética, Universidade Federal de São Paulo, São Paulo 04023-900, São Paulo, Brazil

Corresponding author: Danielle Queiroz Calcagno, PhD, Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, R. dos Mundurucus, 4487-Guamá, Belém-PA, Belém 66073-000, Pará, Brazil. danicalcagno@gmail.com

Abstract

Gastric cancer (GC) remains among the most common cancers worldwide with a high mortality-to-incidence ratio. Accumulated evidence suggests that long non-coding RNAs (lncRNAs) are involved in gastric carcinogenesis. These transcripts are longer than 200 nucleotides and modulate gene expression at multiple molecular levels, inducing or inhibiting biological processes and diseases. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is one of the best-studied lncRNAs with comprehensive actions contributing to cancer progression. This lncRNA regulates gene expression at the transcriptional and posttranscriptional levels through interactions with microRNAs and proteins. In the present review, we discussed the molecular mechanism of MALAT1 and summarized the current knowledge of its expression in GC. Moreover, we highlighted the potential use of MALAT1 as a biomarker, including liquid biopsy.

Key Words: Long noncoding RNA; Gastric carcinogenesis; Transcriptional levels; Posttranscriptional levels; Prognostic biomarker; Liquid biopsy

Core Tip: Gastric cancer (GC) is one of the leading causes of cancer-related deaths globally, highlighting the need for novel biomarker for improved evaluation. The long noncoding RNAs metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) plays a crucial role in many cellular processes associated with GC progression, including proliferation, invasion, metastasis, and drug response. The current review summarizes the present knowledge of MALAT1 in GC, elucidating its molecular mechanisms of action and potential as a biomarker for the clinical management of GC.

Citation: Batista DMO, da Silva JMC, Gigek CO, Smith MAC, de Assumpção PP, Calcagno DQ. Metastasis-associated lung adenocarcinoma transcript 1 molecular mechanisms in gastric cancer progression. *World J Gastrointest Oncol* 2023; 15(9): 1520-1530
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1520.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1520

INTRODUCTION

Gastric cancer (GC) is the fifth most prevalent neoplasm and the fourth leading cause of cancer-related deaths worldwide. Despite advancements in treatment modalities, the prognosis for advanced GC remains poor. Therefore, one of the main factors related to the high incidence and mortality of GC is complex tumor heterogeneity at the molecular level, which poses a major challenge to comprehensively understanding the mechanisms underlying gastric tumorigenesis[1]. As such, identifying molecular biomarkers is critical for improving the clinical outcomes of GC patients.

Advanced RNA-sequencing techniques have allowed the discovery of novel contributors to tumor development, as noncoding RNAs (ncRNAs)[2]. NcRNAs are essential regulators of gene expression that play a vital role in the progression of GC, including mainly microRNAs (miRNAs) and long ncRNAs (lncRNAs)[3-5].

MiRNAs are a class of small RNAs with an average 22 nucleotides in length that modulate negatively the expression of target mRNAs by base-pairing complementarity. This interaction between the two nuclei acids is dynamic and dependent on many factors, such as subcellular location of miRNAs, the abundance of miRNAs and target mRNAs, and the affinity of miRNA-mRNA interactions. Interestingly, these ncRNAs may play an essential role in intercellular signaling. Mature miRNAs transported to the cytoplasm may cross gap junctions (intercellular channels present in the plasma membrane of solid tissues, allowing communication between adjacent cell) and target mRNAs in neighboring cells[6-8].

In contrast, lncRNAs are transcripts highly heterogeneous with more than 200 nucleotides[9] that play a crucial role as master regulators by interacting with DNA, RNA, or proteins to regulate gene expression[10,11].

Due to their complex characteristics, lncRNAs can be classificafied based on their genomic location relative to the nearest protein-coding genes. These classifications include: (1) Long intergenic ncRNAs, which do not overlap or are close to protein-coding genes; (2) Sense lncRNAs, which are on the same strand and transcribed in the same direction; (3) Antisense lncRNAs, which are situated on the opposite strand and overlap protein-coding genes; (4) Intronic lncRNAs, whose sequence is within the boundaries of introns; and (5) Bidirectional lncRNAs, positioned on the antisense strand and having their transcription start site (TSS) near the TSS of protein-coding genes, with transcription occurring in the opposite direction[12-14].

In addition, lncRNAs exhibit archetypes that distinguish them based on molecular functions: (1) Signals are stimuli expressed lncRNAs that interact with transcription factors or chromatin modifiers; (2) Decoy lncRNAs bind to regulatory factors, turning off their activity; (3) Guide lncRNAs recruit and direct chromatin modifiers or transcription factors to specific target genomic locations, either in cis (neighboring-genes) or in trans (distantly-located genes); and (4) Scaffold lncRNAs function as structural elements in the assembly and organization of ribonucleoprotein complexes[15].

Over recent years, evidence has suggested that lncRNAs are key players in the initiation, progression, and response to therapy in GC[16,17]. Regarding their role in cancer, lncRNAs participate in different biological processes, including cell proliferation, angiogenesis, autophagy, apoptosis, differentiation, and immune responses. Consequently, they may be potential targets for clinical applications[18].

Among the lncRNAs involved in GC, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has gained attention as a promoter of cancer progression and an inhibitor of cell sensitivity to therapies[19]. Here, we summarized the current knowledge regarding MALAT1 function and its putative role in biological processes, including GC. Furthermore, we explored the association of MALAT1 overexpression with the clinicopathological features of GC patients and highlighted its potential as a biomarker for diagnosis, prognosis, and prediction of response to therapy.

MALAT1

MALAT1, also known as nuclear enriched abundant transcript 2, is a transcript > 8.7 kbp encoded on human chromosome 11q13.1 widely expressed in normal tissues, especially in the lung and pancreas. Compared to other lncRNAs, MALAT1 exhibits a distinctive triple helix structure at its 3' end. This unique structural feature has been demonstrated to provide protection against exonucleases, contributing to the enhanced stability of MALAT1[20,21]. The subcellular localization determines the molecular functions of MALAT1. Generally, this lncRNA resides in nuclear speckles and specific nuclear bodies enriched with epigenetic regulators, splicing, and transcription factors. Within these nuclear bodies, MALAT1 can interact with various proteins, enabling it to exert regulatory control over alternative

splicing (AS) and transcription processes[22] (Figure 1).

MALAT1 has been shown modulate recruitment of pre-mRNA splicing factors, such as serine/arginine-rich (SR) proteins, acting as a sponge of these components. As illustrated in Figure 1, MALAT1 can influence endogenous pre-mRNA AS through the regulation of SR protein phosphorylation and dephosphorylation. This process leads to modifications in mRNA expression and subsequent alterations in cellular function[22,23].

Furthermore, MALAT1 plays a significant role in modulating gene expression through its interactions with transcription factors, such as members of the polycomb2 protein family and transcriptional enhanced factors with TEA/ATTS domain (TEAD). The crosstalk between MALAT1 and TEAD blocks their association with the coactivator Yes-associated protein, resulting in a negative modulation of gene transcription[24].

In addition to influencing splicing and transcription, MALAT1 also can act as a competitive endogenous RNA (ceRNA) or miRNA sponge to sequester miRNAs under various conditions. CeRNAs are genetic components that control gene expression at a posttranscriptional level. They share miRNA response elements and compete with mRNAs for miRNA binding[25]. Consequently, binding of ceRNAs to miRNAs releases the target mRNA, allowing their translation[21,26,27]. Accumulating evidence supports the regulatory role of MALAT1 in endothelial cell function and vascular growth. A study conducted by Michalik *et al*[28] reported that inhibiting MALAT1 has an antiproliferative and promigratory effect on endothelial cells. Moreover, this transcript differentiates bone marrow-derived mesenchymal stem cells from endothelial cells, contributing to endothelial repair[29]. However, further research is required to understand the role of MALAT1 in physiological processes.

Several studies have shown the involvement of MALAT1 in the molecular mechanisms of various complex diseases, including cardiovascular and neurodegenerative disorders, as well as solid tumors such as lung cancer, pancreatic cancer, breast cancer, and GC[30,31].

MALAT1 IN GC

MALAT1 overexpression has been linked with the clinical characteristics of GC patients, including histological subtype, tumor node metastasis stage, overall survival (OS), and drug resistance (Table 1).

Notably, drug resistance a major challenge in the clinical management of GC[32-36]. For instance, Zhang *et al*[36] showed that MALAT1 expression was noticeably higher in tissue samples from 24 GC patients with oxaliplatin (OXA) resistance than in GC patients without chemoresistance.

Recently, new avenues have opened in the complex field of GC-related lncRNAs. Circulating lncRNAs have attracted considerable attention as potential minimally invasive diagnostic, prognostic, and predictive biomarkers. Even in unfavorable circumstances such as severe potential of hydrogen and numerous freeze-thaw cycles, ncRNAs in body fluids are resistant to exonucleases and highly stable[16,36,37].

Notably, circulating MALAT1 levels in body fluids and clinicopathological traits of GC patients were related to in three studies. For example, Xia *et al*[38] identified that circulating MALAT1 expression in plasma from GC patients was significantly higher at later stages of tumor development and in tumors that had undergone extensive metastasis. In contrast, circulating MALAT1 levels in GC patients without metastasis showed no significant difference compared to healthy controls. Taken together, these results suggest that circulating MALAT1 expression is linked to widespread metastasis and tumor stage, indicating its potential as a prognostic biomarker for GC.

Moreover, in their study, Lu *et al*[39] observed higher circulating MALAT1 expression in sera from GC patients without metastasis than healthy controls. They also found that GC patients with advanced stage had higher levels of MALAT1 expression than GC patients within early stages, indicating the potential of MALAT1 as both a prognostic and diagnostic tool.

Similarly, Zhu *et al*[33] conducted research with plasma samples from 64 GC patients. Circulating MALAT1 was overexpressed in plasma samples from GC patients compared to healthy controls. An estimated area under the curve value of 0.898 from receiver operating characteristic analyses indicates that MALAT1 may effectively discriminate against GC patients from healthy controls. These findings support the utilization of lncRNAs as valuable tools for improving the clinical management of GC.

Overall, the collective results of these studies consistently indicate that MALAT1 overexpression in plasma and serum is correlated with patients clinicopathological characteristics, highlighting its importance as a valuable prognostic and diagnostic biomarker in GC.

MOLECULAR MECHANISMS OF MALAT1 IN GC

Several studies have also explored the molecular mechanism of MALAT1 using GC cell lines, highlighting that MALAT1 plays putative role in chemoresistance, metastasis, and angiogenesis (Table 2).

CHEMORESISTANCE

Cisplatin and OXA are platinum compounds and alkylating agents widely used in cancer treatment, and the latter is more commonly used in gastrointestinal malignancies. These molecules form metal adducts through their interaction

Table 1 MALAT1 overexpression and clinical characteristics in GC patients

Samples	Sample	Clinical implications	Ref.
61 GC/DM, 50 GC/NDM, 36 C	Plasma, tissue	Staging, Metastasis	Xia *et al*[38]
150 GC, 15 peritumoral paraffin-embedded	Tissue	OS, PFS	Li *et al*[49]
78 GC, 78 NTAT	Tissue	Staging, LNM	Li *et al*[52]
60 GC, 60 NTAT	Tissue	Staging, LNM, Tumor size	Zhang *et al*[53]
20 GC, 20 NTAT	Tissue	Metastasis	Chen *et al*[54]
70 GC, 70 C	Serum	Staging	Lu *et al*[39]
89 GC, 89 NTAT	Tissue	LNM, Tumor size	Yan *et al*[32]
64 GC, 64 NTAT, 64 C	Tissue, plasma	Metastasis	Zhu *et al*[33]
30 GC, 30 NTAT	Tissue	Vascular invasion, Lymphatic invasion	Esfandi *et al*[30]
37 GC, 37 NTAT	Tissue	Staging	Li *et al*[34]
30 GC, 30 NTAT	Tissue	OS	Dai *et al*[35]
24 GC, 24 NTAT, 24 GC/OXA	Tissue	Chemoresistance	Zhang *et al*[36]

C: control samples without cancer; GC: gastric cancer patients; GC/OXA: gastric cancer patients treated with oxaliplatin; GC/DM: gastric cancer patients with distant metastasis; CG/NDM: gastric cancer patients withoutmetastasis; LNM: lymph node metastasis; OS: overall survival; NTAT: nontumoral adjacent tissues of GC patients; PFS: progression-free survival.

with DNA, forming interstrand or intrastrand DNA crosslinks that disrupt DNA replication and transcriptional processes [40,41].

Among the observed miRNAs, miR-22-3p was the sole miRNA observed in more than one study. In GC, miR-22-3p acts as a tumor suppressor, effectively inhibiting cell proliferation and cell sensitivity to therapy[34,36]. In the context of OXA resistance, MALAT1 functions as a ceRNA for miR-22-3p, exerting control over ZPF91 expression and increasing GC cell resistance to OXA. Notably, overexpression of MALAT1 enhances cell proliferation, confers resistance to OXA, and inhibits cell death mechanisms[36]. Consistent with these findings, Zhang *et al*[36] also reported the relationship between MALAT1 and cellular sensitivity to OXA in GC cell lines. Knockdown of MALAT1 using small interfering RNA MALAT1 (siMALAT1) reduced the level of ZPF91 protein and increased miR-22-3p expression. Furthermore, transfection of miR-22-3p in OXA-resistant cell lines yielded similar results.

Additionally, MALAT1 regulates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/ serine/threonine-protein kinase (AKT) pathway promoting GC cell resistance to cisplatin. Knockdown of MALAT1 using siMALAT1 decreased PI3K and AKT activity, reducing GC cell proliferation, migration, and invasion. In contrast, GC cell lines treated with plasmid cloning DNA-MALAT1 (pcDNA-MALAT1) did not impact on the expression of PI3K, AKT, and signal transducer and activator of transcription 3[35].

These findings highlight the multifaceted involvement of MALAT1 in modulating drug resistance in GC, provide insights into the underlying mechanisms through which it influences cellular responses to therapy, and show the untapped potential of MALAT1 as a therapeutic target for GC treatment.

METASTASIS

From the data of several studies described in this review, epithelial-mesenchymal transition (EMT) markers were the most frequently reported proteins associated with MALAT1 overexpression in GC cell lines. Specifically, Vimentin and E-cadherin emerged as the most reported proteins linked to MALAT1 dysregulation in GC. EMT is a crucial stage in the metastatic process, characterized by losing epithelial properties and acquiring of mesenchymal characteristics[42].

MALAT1 upregulation led to a reduction in E-cadherin expression and an increase in vimentin. In GC, E-cadherin acts as a tumor suppressor by preserving cell adhesion and inhibiting cell migration and invasion, while vimentin enhances GC cell migration and invasion[43,44].

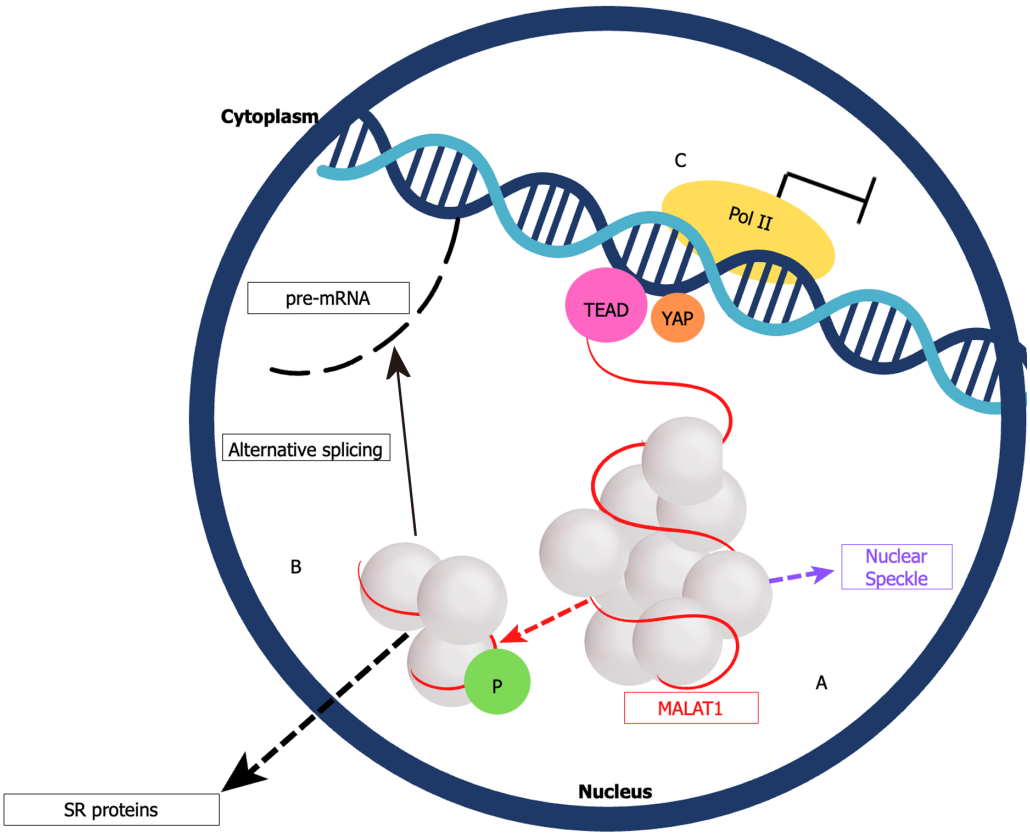
Moreover, chemokine ligand 21 may upregulate MALAT1, promoting the expression of serine and arginine-rich splicing factor 1 (SRSF1) and the activation of the mammalian target of rapamycin (mTOR) pathway, consequently facilitating EMT[45]. Transfection assays using overexpression vectors and siMALAT1 demonstrated that the upregulation of MALAT1 increased the expression of SRSF1 protein and the phosphorylation of the mTOR pathway, leading to the downregulation of E-cadherin and overexpression of vimentin, slug, snail, and twist. Furthermore, the role of MALAT1 as a ceRNA for miR-202-3p contributes to the positive regulation of SRSF1, enhancing EMT processes (Figure 2).

Table 2 MALAT1 molecular mechanism in GC

Cell line	Molecular interactions	Main discoveries	Ref.
MKN28, SGC7901, BCG823, GES1	EGFL7	MALAT1/EGFL7 axis promotes metastasis and cell invasion	Deng *et al*[46]
MKN45, AGS, GES1	EZH2/PCDH10	MALAT1 recruits EZH2 to inhibit the synthesis of cadherin PCDH10, promoting metastasis	Qi *et al*[55]
SGC7901, MKN 45, BGC823, CTC141, CTC105, GES1	miR-122/IGF1R	miR-122/IGF1R axis causes dysregulation of MALAT1, increasing cell invasion and migration of cells	Xia *et al*[38]
SGC7901, MKN45, BGC823, AGS, SGC7901NM, SGC7901M, GES1	E-cadherin, vimentin, SLUG, SNAIL, TWIST	MALAT1 contributes to cell migration, invasion, and proliferation by upregulating EMT markers and downregulating E-cadherin	Chen *et al*[54]
MKN28, MKN74, AGS	RASSF6, β-catenin	Dysregulation of MALAT1 improves the expression of β-catenin and other EMT markers, promoting metastasis	Lee *et al*[56]
BGC823, SGC7901, HEK293T, GES1	UPF1	Overexpression of MALAT1 causes hypermethylation of the UPF1 promoter, increasing cell migration, invasion, and proliferation	Li *et al*[57]
BGC823, SGC7901, MKN45, AGS, BGC803, MGC803, GES1	VE-cadherin/β-catenin, ERK/MMP, FAK/paxillin	MALAT1 promotes angiogenesis by through vasculogenic mimicry	Li *et al*[49]
BGC823, SGC7901, MKN45, MKN28, GES1	miR-1297/HMGB2	MALAT1/miR-1297 increases HMGB2 protein, promoting cell invasion and proliferation of cells	Li *et al*[52]
SGC7901, SGC7901/VCR, BGC823	miR-23b-3p/ATG12	MALAT1/miR-23b-3p, promotes drug resistance *via* the ATG12 protein	YiRen *et al*[37]
SGC7901, MKN45, MKN28, GES1	miR-202/GLI2	MALAT1/miR-202, increases GLI2 expression, inducing tumor progression and cell proliferation	Zhang *et al*[53]
BGC823, SGC7901, GES1	Vimentin, E-cadherin	MALAT1 decreases E-cadherin and increases vimentin expression, promoting EMT	Yang *et al*[58]
BGC823, HGC27, SGC7901, GES1	miR-183/SIRT1, PI3KCA/AKT/mTOR	MALAT1/miR-183 increases SIRT1 protein expression, increasing cell viability, and inhibiting cell apoptosis	Li *et al*[59]
MGC803, GES1	miR-181a-5p/AKT3	MALAT1/miR-181a-5p increases AKT3 protein expression, promoting cell proliferation and inhibiting cell apoptosis	Lu *et al*[39]
MKN45, SGC7901, GES1	Vimentin, E-cadherin, SOX2	MALAT1 increases cell stemness *via* the SOX2 protein, and promotes metastasis	Xiao *et al*[60]
BGC823, HGC27, MKN45, AGS, GES1	IL-21R/miR-125a	MALAT1/miR-125a increases IL-21R expression, increasing cell invasion	Yan *et al*[32]
AGS, SNU1	PI3KCA/AKT	MALAT1 contributes to cell proliferation, invasion, and migration through the PI3KCA/AKT pathway	Zhu *et al*[33]
MKN45, MKN28, MGC803, MGC803/CDDP, HGC27, NCIN87, AGS, GES1	PI3KCA/AKT	MALAT1 increases PI3KCA, AKT and STAT3 activity, promoting resistance to cisplatin	Dai *et al*[35]
SGC7901, BGC823, GES1	miR-22-3p/ErbB3	MALAT1/miR22-3p inhibits cell apoptosis	Li *et al*[34]
CTC141, CTC105, MKN45, GES1	miR-204/MAP1LC3B/TRPM3	MALAT1/miR-204 increases the expression of LC3B and TRPM3, promoting autophagy	Shao *et al*[18]
SGC7901, BGC823, SGC7901/OXA, BGC823/OXA	miR22-3p/ZFP91	MALAT1 increases resistance to OXA	Zhang *et al*[36]
SGC7901, SGC7901/CDDP	miR-30e/ATG5	MALAT1 acts as a ceRNA to miR-30e, raising cisplatin resistance and autophagy *via* the miR-30e/AGT5 axis	Zhang *et al*[61]

SGC7901, MGC803, HEK293T	CCL21, miR-202-3p/SRSF1, SRSF1/mTOR	MALAT1 promotes EMT through miR-202-3p/SRSF1/ mTOR	Fu *et al*[45]

AKT: erine/threonine-protein kinase; ATG5: Autophagy related 5; ATG12: autophagy related 12; CCL21: C-C Motif Chemokine Ligand 21; ceRNA: Competitive endogenous RNA; EGFL7: Epidermal growth factor-like domain-containing protein 7; ERBB3: Erb-b2 receptor tyrosine kinase 3; ERK: Extracellular signal-regulated kinase; EMT: Epithelial-mesenchymal transition; EZH2: Enhancer of zeste 2 polycomb repressive complex 2 subunit; FAK: Focal adhesion kinase; GLI2: GLI family zinc finger 2; HMGB2: High mobility group box 2; IGF1R: insulin like growth factor 1 receptor; IL-21R: Interleukin 21 receptor; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; MAP1LC3B: Microtubule Associated Protein 1 Light Chain 3 Beta; mTOR: Mammalian target of rapamycin; MMP: Matrix metalloproteinases; OXA: Oxaliplatin; PCDH10: Protocadherin 10; PI3KCA: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; RASSF6: Ras association domain family member 6; SIRT1: Sirtuin 1; SOX2: SRY-box transcription factor 2; SRSF1: Serine and arginine-rich splicing factor 1; STAT3: Signal transducer and activator of transcription 3; TRPM3: Transient receptor potential cation channel subfamily M member 3; UPF1: UPF1 RNA helicase and ATPase; ZFP91: Zinc finger protein 91.

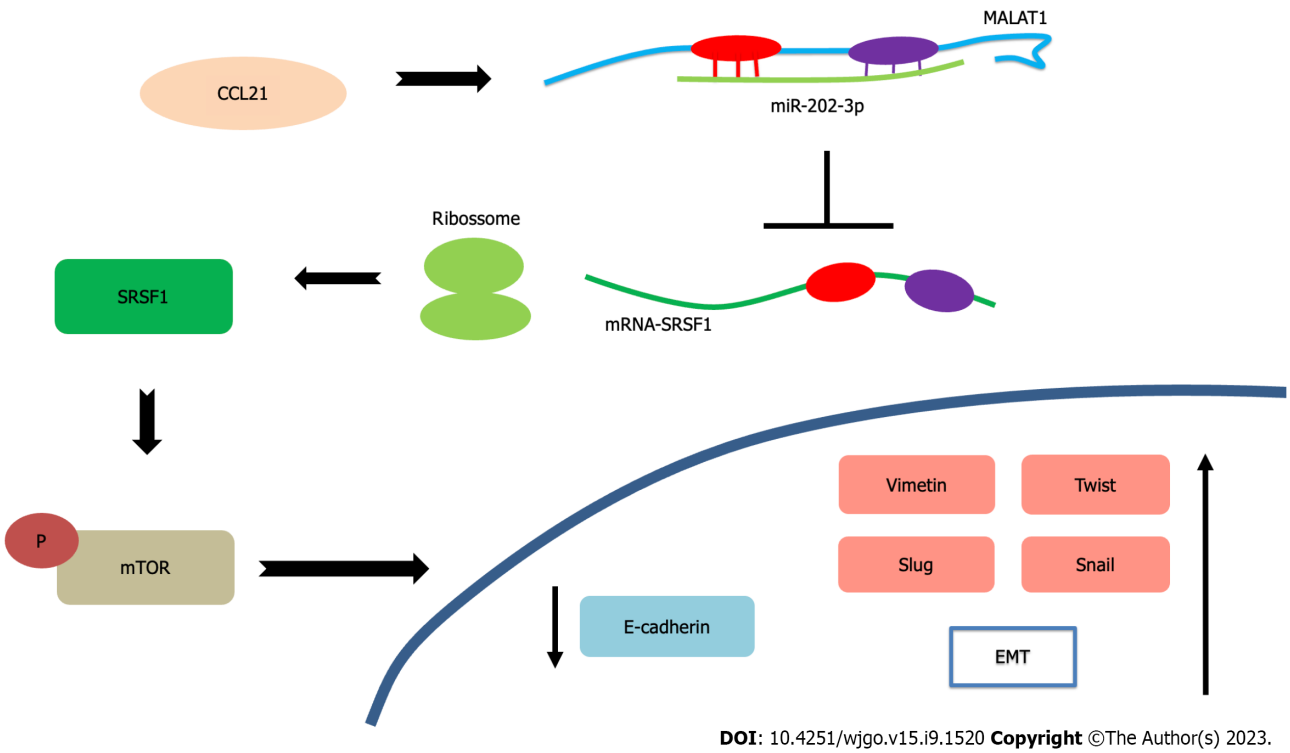


DOI: 10.4251/wjgo.v15.i9.1520 **Copyright** ©The Author(s) 2023.

Figure 1 Metastasis-associated lung adenocarcinoma transcript 1 subcellular location. A: Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is the red strand around the nuclear spots (white spheres), MALAT1 can interact with proteins present in nuclear speckles; B: MALAT1 can interact with serine/arginine proteins to modulate alternative splicing of pre-mRNAs; C: MALAT1 binds with transcriptional enhancer factor transcriptional enhanced factors with TEA/ATTS domain, blocking Yes-associated protein, inhibiting gene transcription. MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; SR: Serine/arginine-rich; TEAD: Transcriptional enhanced factors with TEA/ATTS domain; YAP: Yes-associated protein.

Additionally, MALAT1 overexpression significantly impacts themetastasis, invasion, and migration of GC cells through epidermal growth factor-like domain-containing protein 7 (EGFL7). Transfection assays with siMALAT1 in BGC823 cells demonstrated a reduction in acetylation of the promoter region EGFL7 located in histone H3, decreasing the EGFL7 protein level. Conversely, the injection of pcDNA-MALAT1 into MKN28 cells increased EGFL7 acetylation and EGFL7 protein concentration[46] (Figure 3).

Therefore, MALAT1 plays a pivotal role in promoting EMT, invasion, and migration of GC cells, suggesting its potential as a therapeutic target for metastasis and EMT. These compelling findings underscore the need for further research in this area, warranting exploration to understand its potential as a therapeutic target and assess its clinical significance.



DOI: 10.4251/wjgo.v15.i9.1520 **Copyright** ©The Author(s) 2023.

Figure 2 Metastasis-associated lung adenocarcinoma transcript 1 expression is influenced by protein CCL21. Metastasis-associated lung adenocarcinoma transcript 1 sponges miR-202-3p, then SRSF1 mRNA (serine and arginine-rich splicing factor 1) is translated in protein and activates mammalian target of rapamycin pathway improving epithelial-mesenchymal transition (EMT) factors and decreasing E-cadherin expression. EMT: epithelial-mesenchymal transition; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; SRSF1: serine and arginine-rich splicing factor 1; mTOR: Mammalian target of rapamycin.

ANGIOGENESIS

Angiogenesis comprises the growth of new blood vessels from preexisting vasculature, providing tissues with oxygen and nutrients essential to tumor progression[47,48]. Vasculogenic mimicry (VM) is a phenomenon observed in highly aggressive tumors, where malignant cells imitate endothelial cells, contributing to the formation of microvascular channels that supply blood to cancer cells[49]. A key player in this process is CDH5 or vascular endothelial-cadherin, a transmembrane protein commonly expressed in the endothelium that acts to form and maintain adherent junctions between endothelial cells [50,51].

Furthermore, Li *et al*[49] revealed that MALAT1 overexpression regulates the expression of CDH5 and β-catenin. Interestingly, the knockdown of MALAT1 *in vitro* showed a significant decrease in the expression of the CDH5/β-catenin complex. When upregulated, MALAT1 influenced the CDH5/β-catenin complex to initiate VM and increase vascular permeability.

MALAT1 expression was also associated with the extracellular signal-regulated kinase (ERK)/matrix metalloproteinase (MMP) and focal adhesion kinase (FAK)/paxillin complexes. Upregulation of MALAT1 increased the activity of ERK, FAK, and paxillin; and increased the expression of MMPs, enhancing VM.

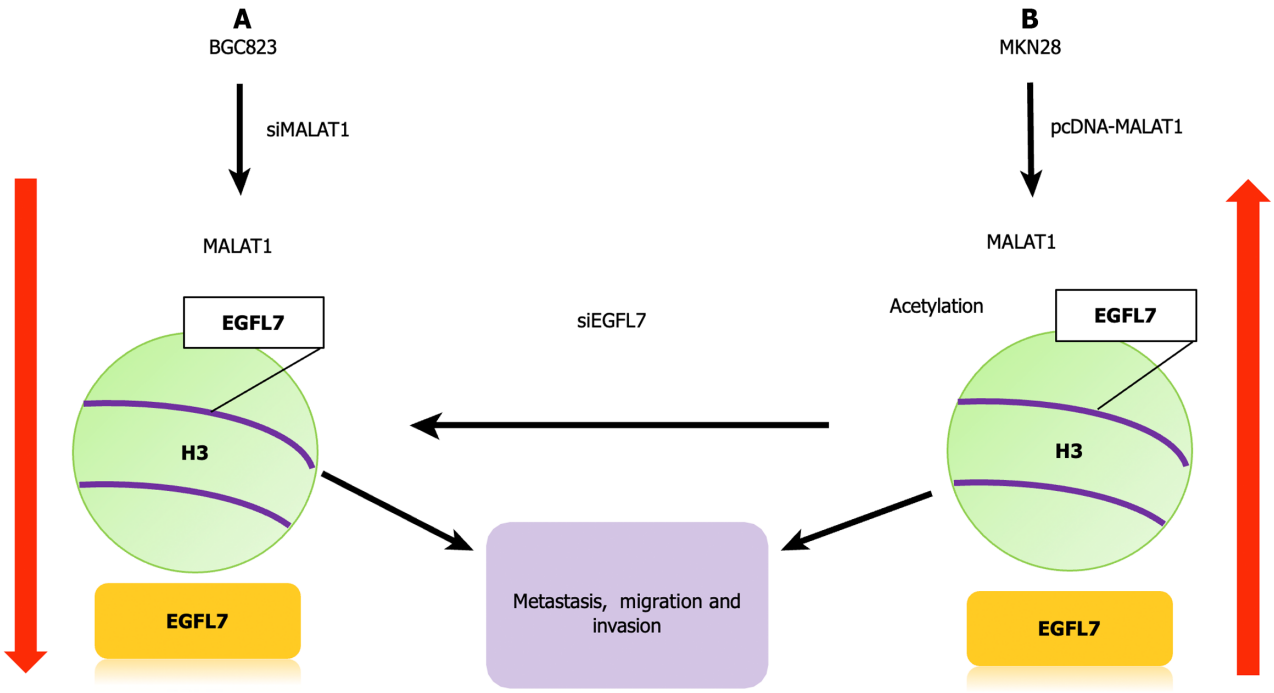
These insights provide valuable evidence for the involvement of MALAT1 in the modulation of these processes. However, further studies are warranted to clarify the intricate mechanism on how MALAT1 exerts influence over CDH5, which may offer potential avenues for targeted therapeutic interventions against VM and angiogenesis in GC.

CONCLUSION

In summary, MALAT1 is an antisense lncRNA that acts as a fundamental regulator of gene expression through interactions with proteins or miRNAs. In GC, MALAT1 has the potential to be a pivotal contributor to various molecular mechanisms, including EMT, apoptosis, proliferation, cell migration, and invasion.

Accumulating evidence has demonstrated a significant tumor suppressor role of miR-22-3p and its interaction with MALAT1 in GC, inhibiting cell apoptosis and increasing GC cell resistance to OXA.

Moreover, studies have correlated MALAT1 overexpression in the tissues and liquid biopsy samples of GC patients with metastasis, staging, worse OS, tumor size, and chemoresistance. The presence of MALAT1 in plasma and serum samples allows the use of minimally invasive collection methods. Although additional validations are needed, these findings show the potential of MALAT1 as a prognostic biomarker and therapeutic target. Further research to elucidate MALAT1 mechanisms of action may identify a new target of interest for translation into clinical applications, thereby



DOI: 10.4251/wjgo.v15.i9.1520

Figure 3 MALAT1 modulates acetylation in promoter region epidermal growth factor-like domain-containing protein 7 located in histone H3. A: Transfection of small interfering MALAT1 reduces acetylation on promoter region of *EGFL7* gene (Epidermal growth factor-like domain-containing protein 7), decreasing metastasis, cell invasion and migration; B: Plasmid cloning DNA-MALAT1 transfection increases *EGFL7* acetylation and protein expression, promoting migration, invasion, and metastasis of GC cells. EGFL7: Epidermal growth factor-like domain-containing protein 7; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; siMALAT1: Small interfering RNA MALAT1; pcDNA-MALAT1: Plasmid cloning DNA-MALAT1; siEGFL7: Small interfering RNA EGFL7.

improving the personalized clinical management of GC.

ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Amazônia de Amparo a Estudos e Pesquisas and the Programa de Pós-Graduação em Oncologia e Ciências Médicas for their help and support.

FOOTNOTES

Author contributions: Batista DMO, da Silva JMC, Calcagno DQ wrote the paper; Batista DMO designed the tables and the figures; Smith MAC, Gigek CO, and de Assumpção PP critically revised this paper; Calcagno DQ designed the article.

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

Country/Territory of origin: Brazil

ORCID number: Daniel Mateus de Oliveira Batista 0000-0001-9884-2641; Jéssica Manoelli Costa da Silva 0000-0002-4896-5034; Carolina de Oliveira Gigek 0000-0003-4857-687X; Marília de Arruda Cardoso Smith 0000-0002-1441-1033; Paulo Pimentel de Assumpção 0000-0003-3846-8445; Danielle Queiroz Calcagno 0000-0002-4429-2573.

S-Editor: Lin C
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **He X**, Liu X, Zuo F, Shi H, Jing J. Artificial intelligence-based multi-omics analysis fuels cancer precision medicine. *Semin Cancer Biol* 2023; **88**: 187-200 [PMID: 36596352 DOI: 10.1016/j.semcancer.2022.12.009]

3 **Chen D**, Ping S, Xu Y, Wang M, Jiang X, Xiong L, Zhang L, Yu H, Xiong Z. Non-Coding RNAs in Gastric Cancer: From Malignant Hallmarks to Clinical Applications. *Front Cell Dev Biol* 2021; **9**: 732036 [PMID: 34805143 DOI: 10.3389/fcell.2021.732036]

4 **Wu SR**, Wu Q, Shi YQ. Recent advances of miRNAs in the development and clinical application of gastric cancer. *Chin Med J (Engl)* 2020; **133**: 1856-1867 [PMID: 32649523 DOI: 10.1097/CM9.0000000000000921]

5 **Gao Y**, Wang JW, Ren JY, Guo M, Guo CW, Ning SW, Yu S. Long noncoding RNAs in gastric cancer: From molecular dissection to clinical application. *World J Gastroenterol* 2020; **26**: 3401-3412 [PMID: 32655264 DOI: 10.3748/wjg.v26.i24.3401]

6 **Lee S**, Jiang X. Modeling miRNA-mRNA interactions that cause phenotypic abnormality in breast cancer patients. *PLoS One* 2017; **12**: e0182666 [PMID: 28793339 DOI: 10.1371/journal.pone.0182666]

7 **Saliminejad K**, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *J Cell Physiol* 2019; **234**: 5451-5465 [PMID: 30471116 DOI: 10.1002/jcp.27486]

8 **Condrat CE**, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, Suciu N, Cretoiu SM, Voinea SC. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells* 2020; **9** [PMID: 31979244 DOI: 10.3390/cells9020276]

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

10 **Quinn JJ**, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 2016; **17**: 47-62 [PMID: 26666209 DOI: 10.1038/nrg.2015.10]

11 **Nandwani A**, Rathore S, Datta M. LncRNAs in cancer: Regulatory and therapeutic implications. *Cancer Lett* 2021; **501**: 162-171 [PMID: 33359709 DOI: 10.1016/j.canlet.2020.11.048]

12 **Hrdlickova B**, de Almeida RC, Borek Z, Withoff S. Genetic variation in the non-coding genome: Involvement of micro-RNAs and long non-coding RNAs in disease. *Biochim Biophys Acta* 2014; **1842**: 1910-1922 [PMID: 24667321 DOI: 10.1016/j.bbadis.2014.03.011]

13 **Spurlock CF 3rd**, Crooke PS 3rd, Aune TM. Biogenesis and Transcriptional Regulation of Long Noncoding RNAs in the Human Immune System. *J Immunol* 2016; **197**: 4509-4517 [PMID: 27913643 DOI: 10.4049/jimmunol.1600970]

14 **Amodio N**, Raimondi L, Juli G, Stamato MA, Caracciolo D, Tagliaferri P, Tassone P. MALAT1: a druggable long non-coding RNA for targeted anti-cancer approaches. *J Hematol Oncol* 2018; **11**: 63 [PMID: 29739426 DOI: 10.1186/s13045-018-0606-4]

15 **Wang KC**, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell* 2011; **43**: 904-914 [PMID: 21925379 DOI: 10.1016/j.molcel.2011.08.018]

16 **Uchôa Guimarães CT**, Ferreira Martins NN, Cristina da Silva Oliveira K, Almeida CM, Pinheiro TM, Gigek CO, Roberto de Araújo Cavallero S, Assumpção PP, Cardoso Smith MA, Burbano RR, Calcagno DQ. Liquid biopsy provides new insights into gastric cancer. *Oncotarget* 2018; **9**: 15144-15156 [PMID: 29599934 DOI: 10.18632/oncotarget.24540]

17 **Syllaios A**, Moris D, Karachaliou GS, Sakellariou S, Karavokyros I, Gazouli M, Schizas D. Pathways and role of MALAT1 in esophageal and gastric cancer. *Oncol Lett* 2021; **21**: 343 [PMID: 33747200 DOI: 10.3892/ol.2021.12604]

18 **Shao G**, Zhao Z, Zhao W, Hu G, Zhang L, Li W, Xing C, Zhang X. Long non-coding RNA MALAT1 activates autophagy and promotes cell proliferation by downregulating microRNA-204 expression in gastric cancer. *Oncol Lett* 2020; **19**: 805-812 [PMID: 31897197 DOI: 10.3892/ol.2019.11184]

19 **Li ZX**, Zhu QN, Zhang HB, Hu Y, Wang G, Zhu YS. MALAT1: a potential biomarker in cancer. *Cancer Manag Res* 2018; **10**: 6757-6768 [PMID: 30584369 DOI: 10.2147/CMAR.S169406]

20 **Zhang X**, Hamblin MH, Yin KJ. The long noncoding RNA Malat1: Its physiological and pathophysiological functions. *RNA Biol* 2017; **14**: 1705-1714 [PMID: 28837398 DOI: 10.1080/15476286.2017.1358347]

21 **McCown PJ**, Wang MC, Jaeger L, Brown JA. Secondary Structural Model of Human MALAT1 Reveals Multiple Structure-Function Relationships. *Int J Mol Sci* 2019; **20** [PMID: 31717552 DOI: 10.3390/ijms20225610]

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

23 **Arun G**, Aggarwal D, Spector DL. MALAT1 Long Non-Coding RNA: Functional Implications. *Noncoding RNA* 2020; **6** [PMID: 32503170 DOI: 10.3390/ncrna6020022]

24 **Chen Q**, Zhu C, Jin Y. The Oncogenic and Tumor Suppressive Functions of the Long Noncoding RNA MALAT1: An Emerging Controversy. *Front Genet* 2020; **11**: 93 [PMID: 32174966 DOI: 10.3389/fgene.2020.00093]

25 **Su K**, Wang N, Shao Q, Liu H, Zhao B, Ma S. The role of a ceRNA regulatory network based on lncRNA MALAT1 site in cancer progression. *Biomed Pharmacother* 2021; **137**: 111389 [PMID: 33601150 DOI: 10.1016/j.biopha.2021.111389]

26 **Zampetaki A**, Albrecht A, Steinhofel K. Long Non-coding RNA Structure and Function: Is There a Link? *Front Physiol* 2018; **9**: 1201 [PMID: 30197605 DOI: 10.3389/fphys.2018.01201]

27 **Graf J**, Kretz M. From structure to function: Route to understanding lncRNA mechanism. *Bioessays* 2020; **42**: e2000027 [PMID: 33164244 DOI: 10.1002/bies.202000027]

28 **Michalik KM**, You X, Manavski Y, Doddaballapur A, Zörnig M, Braun T, John D, Ponomareva Y, Chen W, Uchida S, Boon RA, Dimmeler S. Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circ Res* 2014; **114**: 1389-1397 [PMID: 24602777

DOI: 10.1161/CIRCRESAHA.114.303265]
29 **Sun X**, Luo L, Li J. LncRNA MALAT1 facilitates BM-MSCs differentiation into endothelial cells *via* targeting miR-206/VEGFA axis. *Cell Cycle* 2020; **19**: 3018-3028 [PMID: 33121336 DOI: 10.1080/15384101.2020.1829799]
30 **Esfandi F**, Salehnezhad T, Taheri M, Afsharpad M, Hafez AA, Oskooei VK, Ghafouri-Fard S. Expression assessment of a panel of long non-coding RNAs in gastric malignancy. *Exp Mol Pathol* 2020; **113**: 104383 [PMID: 31982396 DOI: 10.1016/j.yexmp.2020.104383]
31 **Ghafouri-Fard S**, Taheri M. Long non-coding RNA signature in gastric cancer. *Exp Mol Pathol* 2020; **113**: 104365 [PMID: 31899194 DOI: 10.1016/j.yexmp.2019.104365]
32 **Yan L**, Zhang J, Guo D, Ma J, Shui SF, Han XW. IL-21R functions as an oncogenic factor and is regulated by the lncRNA MALAT1/miR-125a-3p axis in gastric cancer. *Int J Oncol* 2019; **54**: 7-16 [PMID: 30387833 DOI: 10.3892/ijo.2018.4612]
33 **Zhu K**, Ren Q, Zhao Y. lncRNA MALAT1 overexpression promotes proliferation, migration and invasion of gastric cancer by activating the PI3K/AKT pathway. *Oncol Lett* 2019; **17**: 5335-5342 [PMID: 31186750 DOI: 10.3892/ol.2019.10253]
34 **Li X**, Zhao J, Zhang H, Cai J. Silencing of LncRNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 Inhibits the Proliferation and Promotes the Apoptosis of Gastric Cancer Cells Through Regulating microRNA-22-3p-Mediated ErbB3. *Onco Targets Ther* 2020; **13**: 559-571 [PMID: 32021298 DOI: 10.2147/OTT.S222375]
35 **Dai Q**, Zhang T, Li C. LncRNA MALAT1 Regulates the Cell Proliferation and Cisplatin Resistance in Gastric Cancer *via* PI3K/AKT Pathway. *Cancer Manag Res* 2020; **12**: 1929-1939 [PMID: 32214850 DOI: 10.2147/CMAR.S243796]
36 **Zhang Z**, Li M, Zhang Z. lncRNA MALAT1 modulates oxaliplatin resistance of gastric cancer *via* sponging miR-22-3p. *Onco Targets Ther* 2020; **13**: 1343-1354 [PMID: 32104001 DOI: 10.2147/OTT.S196619]
37 **YiRen H**, YingCong Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, Ende C, XiZhou L, Yanfan C. Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance *via* miR-23b-3p sequestration in gastric cancer. *Mol Cancer* 2017; **16**: 174 [PMID: 29162158 DOI: 10.1186/s12943-017-0743-3]
38 **Xia H**, Chen Q, Chen Y, Ge X, Leng W, Tang Q, Ren M, Chen L, Yuan D, Zhang Y, Liu M, Gong Q, Bi F. The lncRNA MALAT1 is a novel biomarker for gastric cancer metastasis. *Oncotarget* 2016; **7**: 56209-56218 [PMID: 27486823 DOI: 10.18632/oncotarget.10941]
39 **Lu Z**, Luo T, Pang T, Du Z, Yin X, Cui H, Fang G, Xue X. MALAT1 promotes gastric adenocarcinoma through the MALAT1/miR-181a-5p/AKT3 axis. *Open Biol* 2019; **9**: 190095 [PMID: 31480991 DOI: 10.1098/rsob.190095]
40 **Riddell IA**. Cisplatin and Oxaliplatin: Our Current Understanding of Their Actions. *Met Ions Life Sci* 2018; **18** [PMID: 29394020 DOI: 10.1515/9783110470734-007]
41 **Rogers BB**, Cuddahy T, Briscella C, Ross N, Olszanski AJ, Denlinger CS. Oxaliplatin: Detection and Management of Hypersensitivity Reactions. *Clin J Oncol Nurs* 2019; **23**: 68-75 [PMID: 30682002 DOI: 10.1188/19.CJON.68-75]
42 **Ribatti D**, Tamma R, Annese T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. *Transl Oncol* 2020; **13**: 100773 [PMID: 32334405 DOI: 10.1016/j.tranon.2020.100773]
43 **Yin S**, Chen FF, Yang GF. Vimentin immunohistochemical expression as a prognostic factor in gastric cancer: A meta-analysis. *Pathol Res Pract* 2018; **214**: 1376-1380 [PMID: 30078472 DOI: 10.1016/j.prp.2018.07.014]
44 **Bure IV**, Nemtsova MV, Zaletaev DV. Roles of E-cadherin and Noncoding RNAs in the Epithelial-mesenchymal Transition and Progression in Gastric Cancer. *Int J Mol Sci* 2019; **20** [PMID: 31212809 DOI: 10.3390/ijms20122870]
45 **Fu Q**, Tan X, Tang H, Liu J. CCL21 activation of the MALAT1/SRSF1/mTOR axis underpins the development of gastric carcinoma. *J Transl Med* 2021; **19**: 210 [PMID: 34001131 DOI: 10.1186/s12967-021-02806-5]
46 **Deng QJ**, Xie LQ, Li H. Overexpressed MALAT1 promotes invasion and metastasis of gastric cancer cells *via* increasing EGFL7 expression. *Life Sci* 2016; **157**: 38-44 [PMID: 27259812 DOI: 10.1016/j.lfs.2016.05.041]
47 **Li T**, Kang G, Wang T, Huang H. Tumor angiogenesis and anti-angiogenic gene therapy for cancer. *Oncol Lett* 2018; **16**: 687-702 [PMID: 29963134 DOI: 10.3892/ol.2018.8733]
48 **Kretschmer M**, Rüdiger D, Zahler S. Mechanical Aspects of Angiogenesis. *Cancers (Basel)* 2021; **13** [PMID: 34638470 DOI: 10.3390/cancers13194987]
49 **Li Y**, Wu Z, Yuan J, Sun L, Lin L, Huang N, Bin J, Liao Y, Liao W. Long non-coding RNA MALAT1 promotes gastric cancer tumorigenicity and metastasis by regulating vasculogenic mimicry and angiogenesis. *Cancer Lett* 2017; **395**: 31-44 [PMID: 28268166 DOI: 10.1016/j.canlet.2017.02.035]
50 **Vestweber D**. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arterioscler Thromb Vasc Biol* 2008; **28**: 223-232 [PMID: 18162609 DOI: 10.1161/ATVBAHA.107.158014]
51 **Delgado-Bellido D**, Serrano-Saenz S, Fernández-Cortés M, Oliver FJ. Vasculogenic mimicry signaling revisited: focus on non-vascular VE-cadherin. *Mol Cancer* 2017; **16**: 65 [PMID: 28320399 DOI: 10.1186/s12943-017-0631-x]
52 **Li J**, Gao J, Tian W, Li Y, Zhang J. Long non-coding RNA MALAT1 drives gastric cancer progression by regulating HMGB2 modulating the miR-1297. *Cancer Cell Int* 2017; **17**: 44 [PMID: 28396617 DOI: 10.1186/s12935-017-0408-8]
53 **Zhang Y**, Chen Z, Li MJ, Guo HY, Jing NC. Long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 regulates the expression of Gli2 by miR-202 to strengthen gastric cancer progression. *Biomed Pharmacother* 2017; **85**: 264-271 [PMID: 27887846 DOI: 10.1016/j.biopha.2016.11.014]
54 **Chen D**, Liu L, Wang K, Yu H, Wang Y, Liu J, Guo Y, Zhang H. The role of MALAT-1 in the invasion and metastasis of gastric cancer. *Scand J Gastroenterol* 2017; **52**: 790-796 [PMID: 28276823 DOI: 10.1080/00365521.2017.1280531]
55 **Qi Y**, Ooi HS, Wu J, Chen J, Zhang X, Tan S, Yu Q, Li YY, Kang Y, Li H, Xiong Z, Zhu T, Liu B, Shao Z, Zhao X. MALAT1 long ncRNA promotes gastric cancer metastasis by suppressing PCDH10. *Oncotarget* 2016; **7**: 12693-12703 [PMID: 26871474 DOI: 10.18632/oncotarget.7281]
56 **Lee NK**, Lee JH, Ivan C, Ling H, Zhang X, Park CH, Calin GA, Lee SK. MALAT1 promoted invasiveness of gastric adenocarcinoma. *BMC Cancer* 2017; **17**: 46 [PMID: 28077118 DOI: 10.1186/s12885-016-2988-4]
57 **Li L**, Geng Y, Feng R, Zhu Q, Miao B, Cao J, Fei S. The Human RNA Surveillance Factor UPF1 Modulates Gastric Cancer Progression by Targeting Long Non-Coding RNA MALAT1. *Cell Physiol Biochem* 2017; **42**: 2194-2206 [PMID: 28942451 DOI: 10.1159/000479994]
58 **Yang Z**, Xie Q, Chen Z, Ni H, Xia L, Zhao Q, Chen P. Resveratrol suppresses the invasion and migration of human gastric cancer cells *via* inhibition of MALAT1-mediated epithelial-to-mesenchymal transition. *Exp Ther Med* 2019; **17**: 1569-1578 [PMID: 30783423 DOI: 10.3892/etm.2018.7142]
59 **Li H**, He C, Wang X, Wang H, Nan G, Fang L. MicroRNA-183 affects the development of gastric cancer by regulating autophagy *via* MALAT1-miR-183-SIRT1 axis and PI3K/AKT/mTOR signals. *Artif Cells Nanomed Biotechnol* 2019; **47**: 3163-3171 [PMID: 31352788 DOI:

10.1080/21691401.2019.1642903]

60 **Xiao Y**, Pan J, Geng Q, Wang G. LncRNA MALAT1 increases the stemness of gastric cancer cells *via* enhancing SOX2 mRNA stability. *FEBS Open Bio* 2019; **9**: 1212-1222 [PMID: 31037832 DOI: 10.1002/2211-5463.12649]

61 **Zhang YF**, Li CS, Zhou Y, Lu XH. Propofol facilitates cisplatin sensitivity *via* lncRNA MALAT1/miR-30e/ATG5 axis through suppressing autophagy in gastric cancer. *Life Sci* 2020; **244**: 117280 [PMID: 31926239 DOI: 10.1016/j.lfs.2020.117280]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1531-1543

DOI: 10.4251/wjgo.v15.i9.1531

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

RNA-binding protein CPSF6 regulates IBSP to affect pyroptosis in gastric cancer

Xue-Jun Wang, Yong Liu, Bin Ke, Li Zhang, Han Liang

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Baryshnikova NV, Russia; Cabezuelo AS, Spain; Osera S, Japan; Yakar M, Turkey

Received: April 7, 2023
Peer-review started: April 7, 2023
First decision: July 19, 2023
Revised: July 31, 2023
Accepted: August 18, 2023
Article in press: August 18, 2023
Published online: September 15, 2023

Xue-Jun Wang, Yong Liu, Bin Ke, Li Zhang, Han Liang, Department of Gastric Oncology, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Tianjin's Clinical Research Center for Cancer, Tianjin Key Laboratory of Digestive Cancer, Tianjin 300060, China

Corresponding author: Xue-Jun Wang, MD, Professor, Department of Gastric Oncology, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Tianjin's Clinical Research Center for Cancer, Tianjin Key Laboratory of Digestive Cancer, Huanhu Xi Road, Tiyuan Bei, Hexi District, Tianjin 300060, China.
wangxuejun20202020@163.com

Abstract

BACKGROUND

Extensive evidence has illustrated the promotive role of integrin binding sialoprotein (IBSP) in the progression of multiple cancers. However, little is known about the functions of IBSP in gastric cancer (GC) progression.

AIM

To investigate the mechanism underlying the regulatory effects of IBSP in GC progression, and the relationship between IBSP and cleavage and polyadenylation factor 6 (CPSF6) in this process.

METHODS

The mRNA and protein expression of relevant genes were assessed through real-time quantitative polymerase chain reaction and Western blot, respectively. Cell viability was evaluated by Cell Counting Kit-8 assay. Cell invasion and migration were evaluated by Transwell assay. Pyroptosis was measured by flow cytometry. The binding between CPSF6 and IBSP was confirmed by luciferase reporter and RNA immunoprecipitation (RIP) assays.

RESULTS

IBSP exhibited higher expression in GC tissues and cell lines than in normal tissues and cell lines. IBSP knockdown suppressed cell proliferation, migration, and invasion but facilitated pyroptosis. In the exploration of the regulatory mechanism of IBSP, potential RNA binding proteins for IBSP were screened with catRAPID omics v2.0. The RNA-binding protein CPSF6 was selected due to its higher expression in stomach adenocarcinoma. Luciferase reporter and RIP assays revealed that CPSF6 binds to the 3'-untranslated region of IBSP and regulates its

expression. Knockdown of CPSF6 inhibited cell proliferation, migration, and invasion but boosted pyroptosis. Through rescue assays, it was uncovered that the retarded GC progression mediated by CPSF6 knockdown was reversed by IBSP overexpression.

CONCLUSION

Our study highlighted the vital role of the CPSF6/IBSP axis in GC, suggesting that IBSP might be an effective bio-target for GC treatment.

Key Words: Integrin binding sialoprotein; Cleavage and polyadenylation factor 6; Pyroptosis; Gastric cancer

Core Tip: This study, for the first time, revealed the crucial role of the cleavage and polyadenylation factor 6 (CPSF6)/integrin binding sialoprotein (IBSP) axis in gastric cancer (GC). This discovery might shed light on GC treatment. However, although this study explored this regulatory axis on cell proliferation, metastasis, and pyroptosis in GC, its data regarding the regulatory effects of CPSF6/IBSP on GC progression are limited. In the future, the regulatory effects of the CPSF6/IBSP axis on stemness, autophagy, and inflammation should be investigated through more experiments.

Citation: Wang XJ, Liu Y, Ke B, Zhang L, Liang H. RNA-binding protein CPSF6 regulates IBSP to affect pyroptosis in gastric cancer. *World J Gastrointest Oncol* 2023; 15(9): 1531-1543

URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1531.htm

DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1531

INTRODUCTION

Similar to other malignant tumors, gastric cancer (GC) is featured by immoderate cell proliferation and delayed cell apoptosis[1,2]. The activation of oncogenes and the inactivation of tumor suppressor genes are the main inducements for tumors[3]. At present, treatments for GC are mainly surgery, chemotherapy, and radiotherapy[4,5]. However, there are no ideal treatment strategies. GC cells are often resistant to chemotherapy or radiotherapy, which is the main reason for tumor recurrence after treatment[6,7]. In view of the serious threat of GC to patient lives, there is an urgent need to look for effective bio-targets for GC treatment.

Different factors [proteins, long non-coding/circular RNAs (lnc/circRNAs), microRNAs (miRNAs), *etc.*] play critical roles in the progression of cancers, including GC[8-11]. For example, SRY-box transcription factor 4 accelerates transforming growth factor β-stimulated epithelial-mesenchymal transition and stemness in GC[12]. Tripartite motif containing 58 inactivates β-catenin signaling through ubiquitination to suppress tumor growth in GC[13]. Besides, the lncRNA bladder cancer associated transcript 1/microRNA 361 (miR-361)/ATP binding cassette subfamily B member 1 (ABCB1) competitive exogenous RNA axis contributes to oxaliplatin resistance in GC[14]. Centromere protein U promotes GC cell proliferation and glycolysis by modulating high mobility group box 2[15]. Integrin binding sialoprotein (IBSP) serves as a member of the small integrin-binding ligand, N-linked glycoprotein family, and the gene encoding this protein is located on 4q21.1[16,17]. IBSP has higher expression and important function in various types of cancers. For instance, IBSP modulates the Fyn/β-catenin signaling pathway to aggravate colorectal cancer progression[18]. Exosomal miR-19a interacts with IBSP in estrogen receptor-positive breast cancer to stimulate osteolytic bone metastasis[19]. Besides, overexpression of IBSP results in a poor prognosis in esophageal squamous cell carcinoma patients[20]. However, the functions and related regulatory mechanism of IBSP are still unclear in GC. Some studies have confirmed the oncological function of cleavage and polyadenylation factor 6 (CPSF6) in various kinds of cancers[21-24]. However, the relationship between IBSP and CPSF6 in GC progression remains to be investigated.

This study aimed to investigate the mechanism underlying the regulatory effects of IBSP in GC progression, and the relationship between IBSP and CPSF6 in this process. Our study revealed that CPSF6-mediated IBSP facilitated cell proliferation, invasion, and migration and reduced cell pyroptosis in GC. This discovery is of great clinical significance for identifying promising bio-targets for GC treatment.

MATERIALS AND METHODS

Tissue samples

Thirty paired GC tissues and adjacent non-cancer tissues were obtained from January 2020 to March 2023 from patients who had undergone surgery at Tianjin Medical University Cancer Institute and Hospital, Tianjin, China. All patients were histologically or pathologically verified as having GC by two independent pathologists and did no receive prior anti-cancer treatments. The study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute

and Hospital (E2020216) and written informed consent was obtained from all patients. Resected tissues were immediately frozen in liquid nitrogen and then stored at -80 °C.

Cell lines and cell culture

GC cell lines (HGC-27, MKN45, SGC7901, and BGC823) and the human normal gastric mucosal cell line (GES-1) were obtained from American Type Culture Collection (ATCC, Manassas, VA, United States). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO, United States) with 10% fetal bovine serum (FBS; Gibco, Waltham, MA, United States) and 1% penicillin-streptomycin at 37 °C in a humidified incubator with 5% CO_2.

Transfection

Small interfering RNAs (siRNAs) against IBSP (si-IBSP#1 and si-IBSP#2) and siRNAs against CPSF6 (si-CPSF6#1 and si-CPSF6#2) were designed for silencing IBSP and CPSF6, respectively. A negative control siRNA (si-NC) was also used. To overexpress IBSP, pcDNA3.1/IBSP (OV-IBSP) plasmid was constructed, and the empty vector pcDNA3.1 was used as the negative control. These vectors were obtained from Genepharma (Shanghai, China) and transfected into GC cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States).

Real-time quantitative polymerase chain reaction

TRIzol reagent (Invitrogen, Carlsbad, CA, United States) was utilized to isolate total RNA from GC tissues or cells. The ReverTra Ace quantitative polymerase chain reaction (RT-qPCR) RT Kit (Takara, Beijing, China) was employed to synthesize cDNA from RNA. The SYBR Green Real-time PCR Master Mix (Takara, Beijing, China) was used for qPCR amplification on the ABI 7500 real-time PCR system (Applied Biosystems, Bedford, MA, United States). β-actin was used as the internal reference. The $2^{-\Delta\Delta Ct}$ method was used for calculating gene expression.

Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8) assay was performed to examine the viability of GC cells as described previously[25,26]. In brief, GC cells (1×10^4 cells/well) were plated into 96-well plates and then incubated for 0 h, 24 h, 48 h, and 72 h. The CCK8 solution (10 μL, Dojindo, Japan) was added into each well and incubated for 2 h, and the absorbance (450 nm) was then measured with a microplate reader.

Transwell assay

GC cells (1×10^4 cells/well) were seeded into the upper chamber (8 μm pore size; Millipore, Billerica, MA, United States) coated with (for invasion assay) or without Matrigel (for migration assay). The culture medium with 10% FBS was added into the lower chamber. The invading and migrating cells were fixed with methanol and dyed with crystal violet. Subsequently, a microscope (Olympus, Tokyo, Japan) was employed to count these cells.

Flow cytometry analysis

Cell apoptosis was assessed by flow cytometry with the propidium iodide (PI) and FITC Annexin V Apoptosis Detection Kit (BD Biosciences, San Jose, CA, United States) as described previously[27]. GC cells were cultured for 72 h, followed by washing with cold phosphate-buffered saline and resuspending in 1 × binding buffer. Annexin V-FITC (5 μL) was utilized for dyeing the cells, followed by mixing with PI (5 μL) in the darkness. FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, United States) was employed for evaluating cell apoptosis.

Luciferase reporter assay

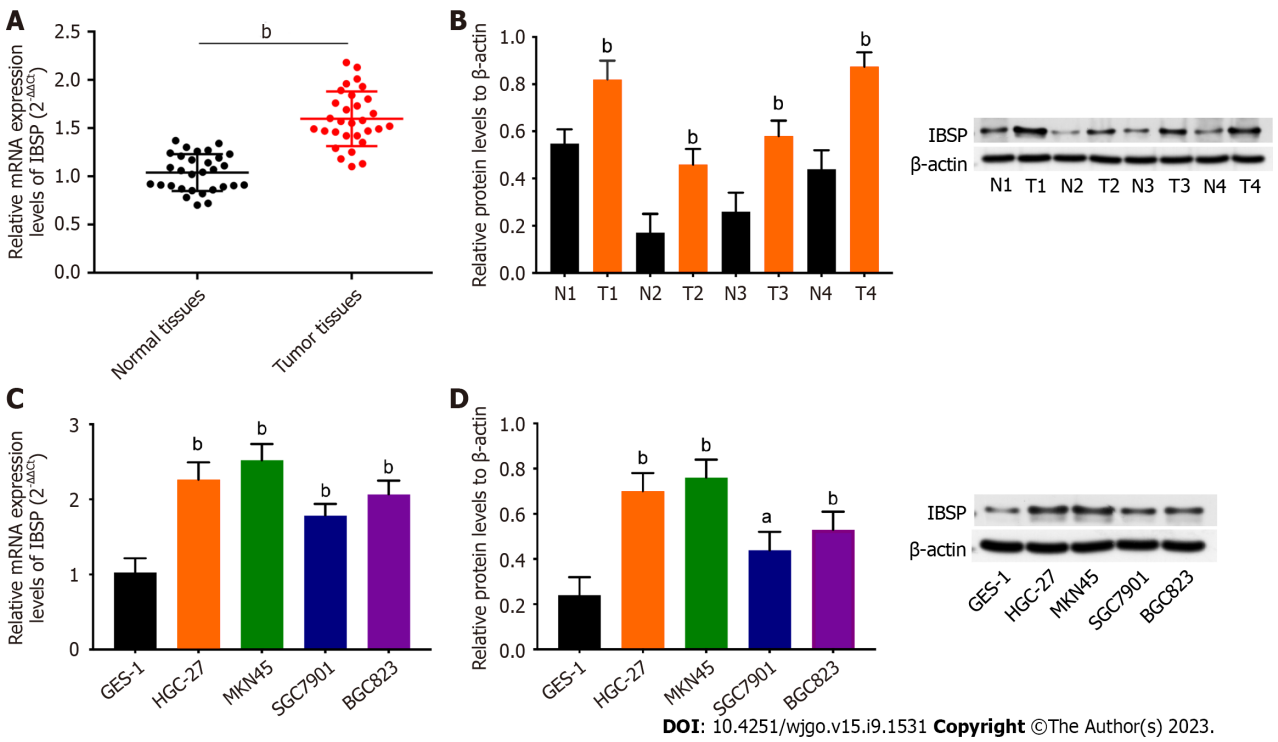
Luciferase reporter assay was performed as described in previous studies[28,29]. The wild-type (wt) and mutant-type (mut) 3′-untranslated region (3′-UTR) sequences of IBSP (IBSP 3′-UTR-wt/mut) were inserted into psiCHECK2 dual-luciferase vector (Promega, Madison, United States) to generate reporter vectors. Then, IBSP 3′-UTR-wt or mut reporters were separately transfected with pcDNA3.1 or pcDNA3.1-CPSF6 into GC cells. After 48 h, the luciferase reporter assay system (Promega, Madison, Wisconsin, United States) was applied to measure the luciferase activity.

RNA immunoprecipitation assay

RNA immunoprecipitation (RIP) assay was performed following previous studies[30,31]. GC cells were lysed with the lysis buffer. Cell lysate was mixed with anti-CPSF6 or anti-immunoglobulin G (IgG) antibodies, and then magnetic beads were added to immunoprecipitate the RNA-protein immunocomplexes. After washing, IBSP expression was assessed by RT-qPCR.

Western blot analysis

GC cells were lysed with RIPA lysis buffer. Then, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride or polyvinylidene difluoride membranes (Amersham, United States). After blocking with non-fat milk, the membranes were incubated with primary antibodies, including those against IBSP, CPSF6, NLR family pyrin domain containing 3 (NLRP3), cleaved caspase-1, interleukin (IL)-18, IL-1β, and β-actin, at 4 °C overnight. Subsequently, the secondary antibody was added and incubated for 2 h. All antibodies were bought from Abcam (Shanghai, China). After washing, the ECL detection (ThermoScientific, Waltham, MA, United States) was utilized to visualize protein bands.



DOI: 10.4251/wjgo.v15.i9.1531

Figure 1 Integrin binding sialoprotein shows higher expression in gastric cancer tissues and cell lines. A: The mRNA expression of integrin binding sialoprotein (IBSP) in gastric cancer (GC) tissues and normal adjacent tissues was examined by real-time quantitative polymerase chain reaction (RT-qPCR); B: The protein expression of IBSP in four pairs of GC tissues and normal adjacent tissues was detected by Western blot; C and D: The mRNA and protein expression levels of IBSP in gastric epithelial cell line (GES-1) and GC cell lines (HGC-27, MKN45, SGC-7901, and BGC823) were detected by RT-qPCR and Western blot, respectively. $^{a}P < 0.05$, $^{b}P < 0.01$. IBSP: Integrin binding sialoprotein.

In vivo assay

Male BALB/c nude mice (4-wk-old, n = 15) were purchased from Vital River (Beijing, China). Mice were randomly divided into three groups (n = 5 for each group; si-NC, si-CPSF6, and si-CPSF6 + OV-IBSP groups). The transfected GC cells were injected into the right flanks of mice. After 4 wk, tumor size, volume, and weight were assessed. This work was approved by the Animal Care and Use Committee of Beijing Viewsolid Biotechnology Co. LTD (VS212601449).

Statistical analysis

Data are shown as the mean ± SD. Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, United States). The correlation between IBSP and CPSF6 expression was assessed by Pearson correlation analysis. The comparison between two groups or among multiple groups was done by Student's t test and one-way analysis of variance, respectively. $P < 0.05$ was considered statistically significant.

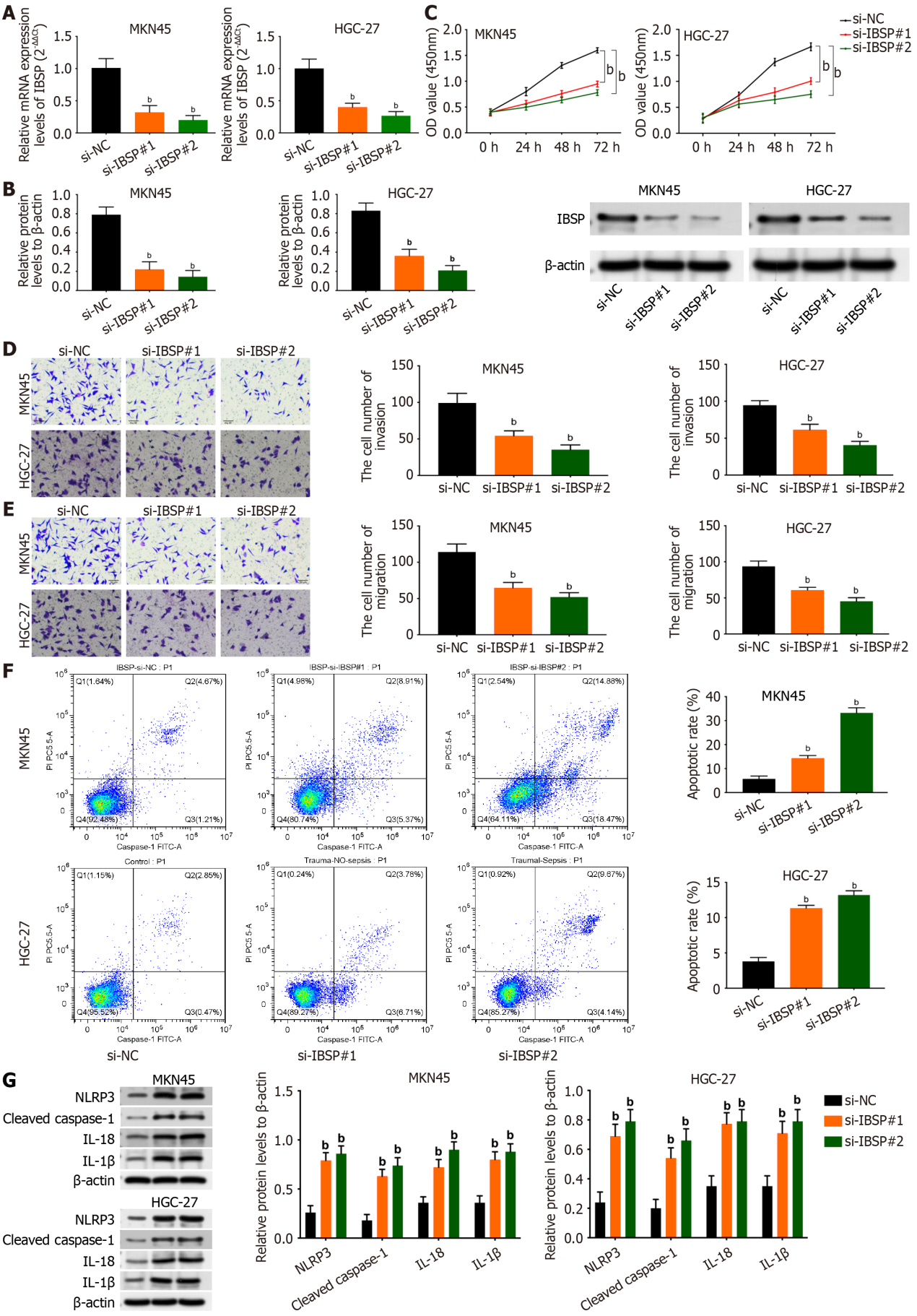
RESULTS

IBSP shows higher expression in GC tissues and cell lines

As shown in Figure 1A and B, the mRNA and protein expression levels of IBSP were higher in the GC tissues than in the normal tissues. The correlation between GC patients' clinicopathological features and IBSP expression is shown in Table 1. IBSP expression was not significantly correlated with age, gender, or distant metastasis but was significantly related with tumor diameter and TNM stage ($P < 0.05$). The mRNA and protein expression levels of IBSP were up-regulated in GC cell lines (HGC-27, MKN45, SGC-7901, and BGC823) compared with the human normal gastric mucosal cell line GES-1 (Figure 1C and D). Additionally, the prognosis of GC patients with high IBSP expression was poor (Supplementary Figure 1A). Taken together, IBSP shows higher expression in GC tissues and cell lines.

IBSP downregulation suppresses cell proliferation, migration, and invasion and facilitates pyroptosis

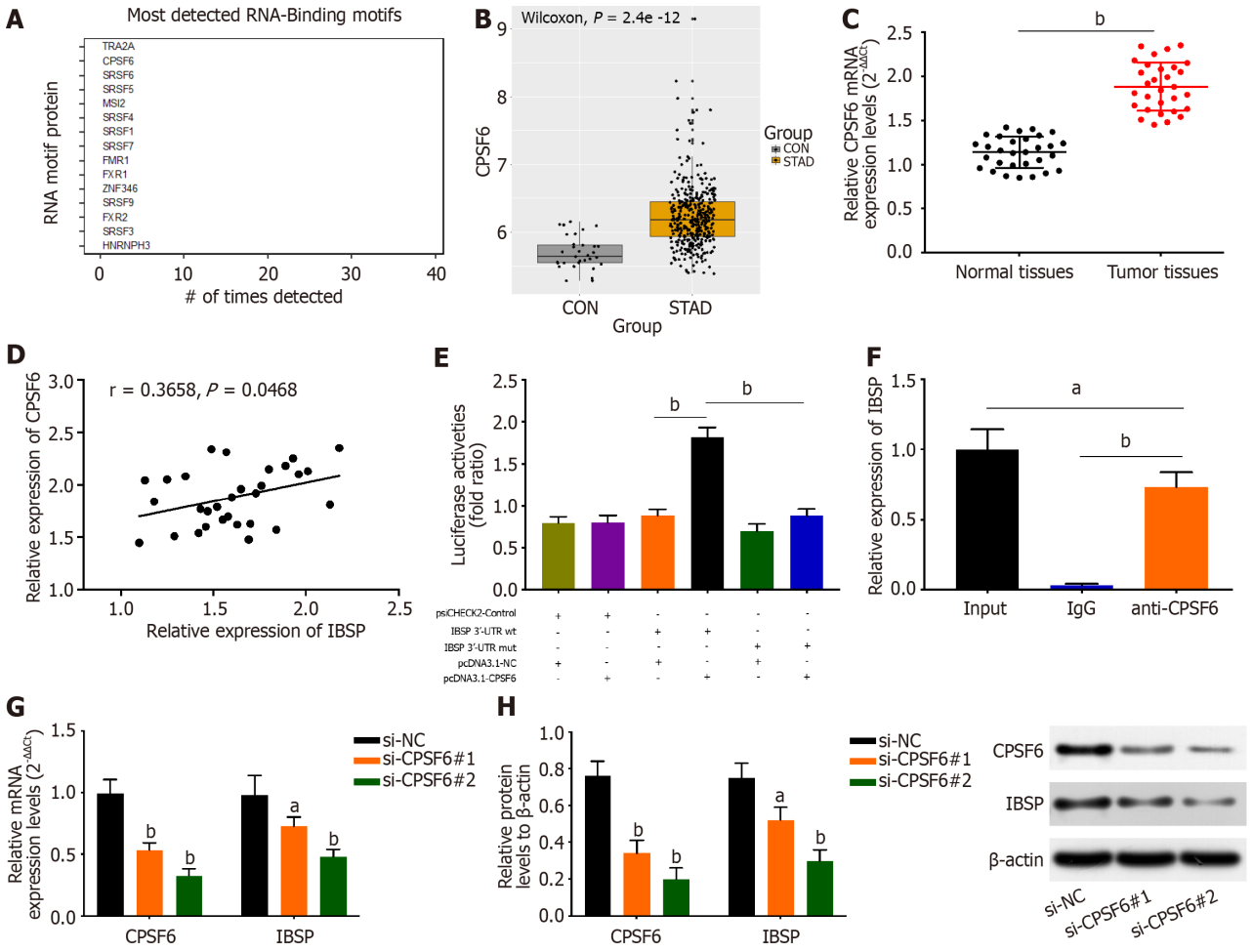
The efficiency of IBSP knockdown was verified by the decreased mRNA and protein expression levels of IBSP after IBSP silencing (Figure 2A and B). Cell viability was attenuated after suppressing IBSP in MKN45 and HGC-27 cells (Figure 2C). Furthermore, the invasion and migration of MKN45 and HGC-27 cells were weakened after IBSP inhibition (Figure 2D and E). The cell apoptosis rate was increased after IBSP knockdown in MKN45 and HGC-27 cells (Figure 2F). In addition, the protein levels of NLRP3, cleaved caspase-1, IL-18, and IL-1β were all upregulated after inhibiting IBSP in MKN45 and HGC-27 cells (Figure 2G). Thus, MKN45 cells were used for further experiments. These findings demonstrate that IBSP



DOI: 10.4251/wjgo.v15.i9.1531

Figure 2 Integrin binding sialoprotein downregulation suppresses cell proliferation, migration, and invasion and facilitates pyroptosis. A

and B: The mRNA and protein expression levels of integrin binding sialoprotein (IBSP) were assessed in the si-NC, si-IBSP#1, and si-IBSP#2 groups by real-time quantitative polymerase chain reaction and Western blot in MKN45 and HGC-27 cells, respectively; C: Cell viability was detected after silencing IBSP by Cell-Counting Kit-8 assay in MKN45 and HGC-27 cells; D and E: Cell invasion and migration were evaluated by Transwell assay in MKN45 and HGC-27 cells; F: Pyroptosis was measured after IBSP knockdown by flow cytometry in MKN45 and HGC-27 cells; G: The protein expression levels of NLR family pyrin domain containing 3, cleaved caspase-1, interleukin-18 (IL-18), and IL-1β were examined by Western blot after suppressing IBSP in MKN45 and HGC-27 cells. $^{b}P < 0.01$. IBSP: Integrin binding sialoprotein; IL: Interleukin; NLRP3: NLR family pyrin domain containing 3.



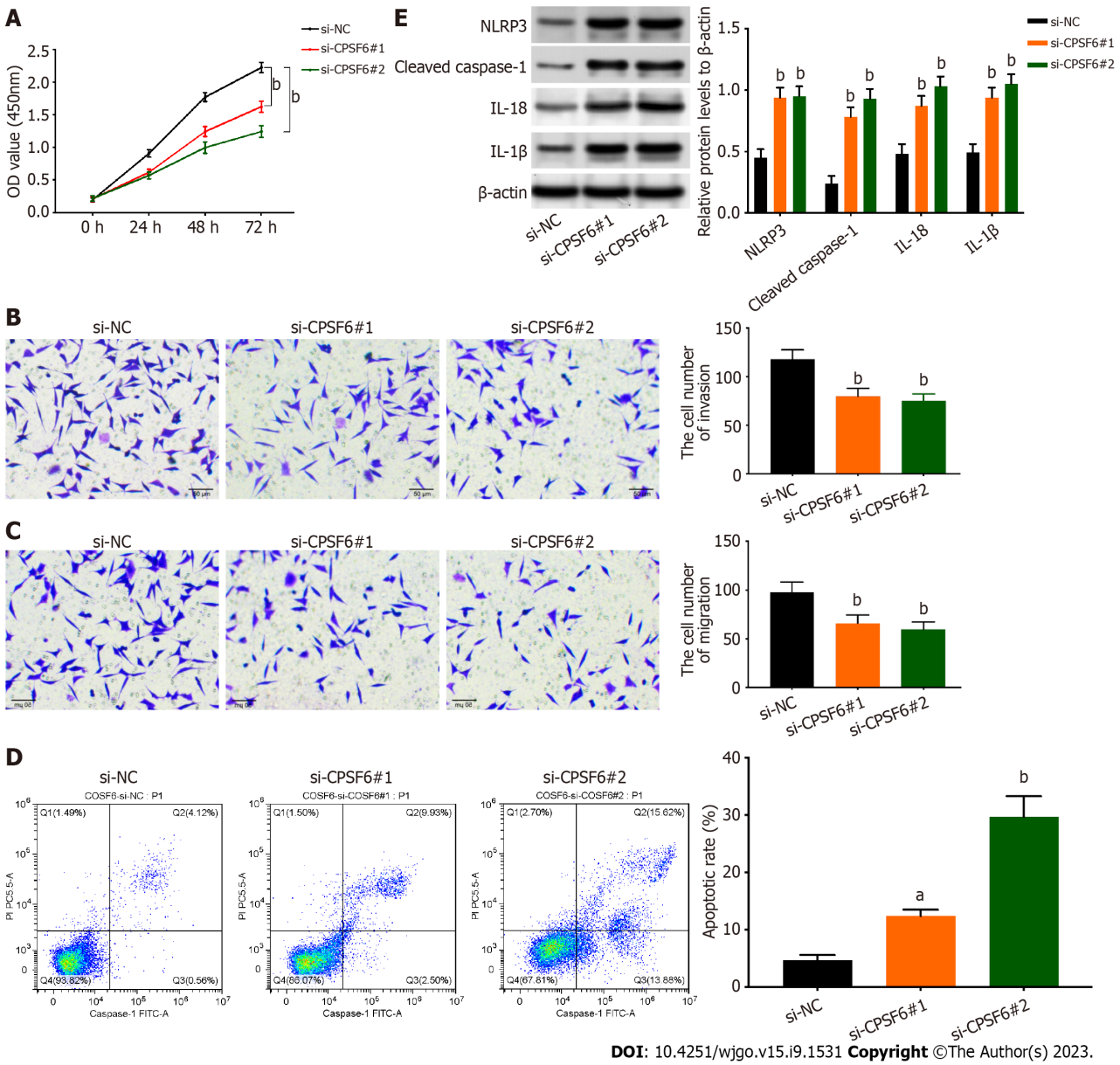
DOI: 10.4251/wjgo.v15.i9.1531

Figure 3 The RNA-binding protein cleavage and polyadenylation factor 6 binds to the 3'-untranslated region of integrin binding sialoprotein and regulates its expression. A: Potential RNA-binding proteins that can bind to integrin binding sialoprotein (IBSP) were analyzed through bioinformatic analysis; B: The levels of cleavage and polyadenylation factor 6 (CPSF6) in stomach adenocarcinoma; C: The mRNA expression of CPSF6 was detected in GC tissues and normal adjacent tissues by real-time quantitative polymerase chain reaction (RT-qPCR); D: The correlation between CPSF6 and IBSP was verified; E and F: The binding ability between CPSF6 and IBSP was confirmed by luciferase reporter and RNA immunoprecipitation chip assays; G and H: The mRNA and protein expression of CPSF6 and IBSP was measured in the si-NC, si-CPSF6#1, and si-CPSF6#2 groups by RT-qPCR and Western blot, respectively. $^{a}P < 0.05$, $^{b}P < 0.01$.

downregulation suppresses cell proliferation, migration, and invasion and facilitates pyroptosis.

CPSF6 binds to 3'-UTR of IBSP to regulate its expression

catRAPID omics v2.0 was used to predict and screen potential RNA binding proteins for IBSP (Figure 3A). CPSF6 ranked second in the binding ability to IBSP and was differentially expressed in GC. The transformer 2 alpha homolog, which ranked first in binding ability, was not differentially expressed in GC. Thus, CPSF6 was selected for the subsequent study. The expression of CPSF6 was upregulated in stomach adenocarcinoma tissues (Figure 3B). Similarly, CPSF6 expression was higher in GC tissues and was positively correlated with IBSP expression (Figure 3C and D). Moreover, the prognosis of GC patients with high CPSF6 expression was poor (Supplementary Figure 1B). The luciferase activity of IBSP-wt reporters was increased after overexpressing CPSF6, but that of IBSP-mut reporters had no noticeable change (Figure 3E). RIP assay revealed that CPSF6 binds to IBSP (Figure 3F). The mRNA and protein levels of CPSF6 and IBSP were reduced after silencing CPSF6 (Figure 3G and H). Thus, CPSF6 binds to the 3'-UTR of IBSP and regulates its expression.



DOI: 10.4251/wjgo.v15.i9.1531

Figure 4 Knockdown of cleavage and polyadenylation factor 6 inhibits cell proliferation, migration, and invasion but boosts pyroptosis. A: Cell viability was verified after suppressing cleavage and polyadenylation factor 6 (CPSF6) by Cell Counting Kit-8 assay; B and C: Cell migration and invasion were detected after inhibiting CPSF6 by Transwell assay; D: Cell apoptosis was examined after silencing CPSF6 by flow cytometry; E: The protein expression levels of NLR family pyrin domain containing 3, cleaved caspase-1, interleukin-18 (IL-18), and IL-1β were examined after suppressing CPSF6 by Western blot. $^{a}P < 0.05$, $^{b}P < 0.01$. CPSF6: Cleavage and polyadenylation factor 6; NLRP3: NLR family pyrin domain containing 3; IL: Interleukin.

Knockdown of CPSF6 inhibits cell proliferation, migration, and invasion but boosts pyroptosis

The proliferation of GC cells was weakened after repressing CPSF6 (Figure 4A). In addition, cell invasion and migration were reduced after silencing CPSF6 (Figure 4B and C). In contrast, cell apoptosis was strengthened after CPSF6 suppression (Figure 4D). The protein levels of NLRP3, cleaved caspase-1, IL-18, and IL-1β were increased after CPSF6 knockdown (Figure 4E). Collectively, knockdown of CPSF6 represses cell proliferation, migration, and invasion but boosts pyroptosis.

CPSF6 regulates IBSP to affect GC progression

Rescue assays were conducted to verify the interaction between CPSF6 and IBSP. IBSP expression was decreased after CPSF6 knockdown, but this effect could be reversed by IBSP overexpression (Figure 5A and B). The reduced cell viability mediated by CPSF6 inhibition was rescued by IBSP overexpression (Figure 5C). Additionally, the weakened cell invasion and migration induced by CPSF6 suppression were counteracted by IBSP upregulation (Figure 5D and E). Cell apoptosis was reduced after repressing CPSF6, but this effect was offset by overexpressing IBSP (Figure 5F). Besides, the protein levels of NLRP3, cleaved caspase-1, IL-18, and IL-1β were upregulated after CPSF6 knockdown, but these changes were neutralized by IBSP overexpression (Figure 5G). Tumor size, volume, and weight were decreased after CPSF6 inhibition, but these effects were rescued by IBSP upregulation (Figure 5H-J).

Table 1 Correlation between integrin binding sialoprotein expression and clinicopathologic characteristics in gastric cancer patients

Parameter	*n*	Low IBSP expression (*n* = 8)	High IBSP expression (*n* = 22)	*P* value
Age (yr)				
≥ 60	11	4	7	0.361
< 60	19	4	15	
Tumor diameter (cm)				
≥ 3	10	6	4	0.004[a]
< 3	20	2	18	
Gender				
Male	14	3	11	0.544
Female	16	5	11	
TNM stage				
I + II	7	5	2	0.002[a]
III + IV	23	3	20	
Distant metastasis				
M0	15	5	10	0.409
M1	15	3	12	

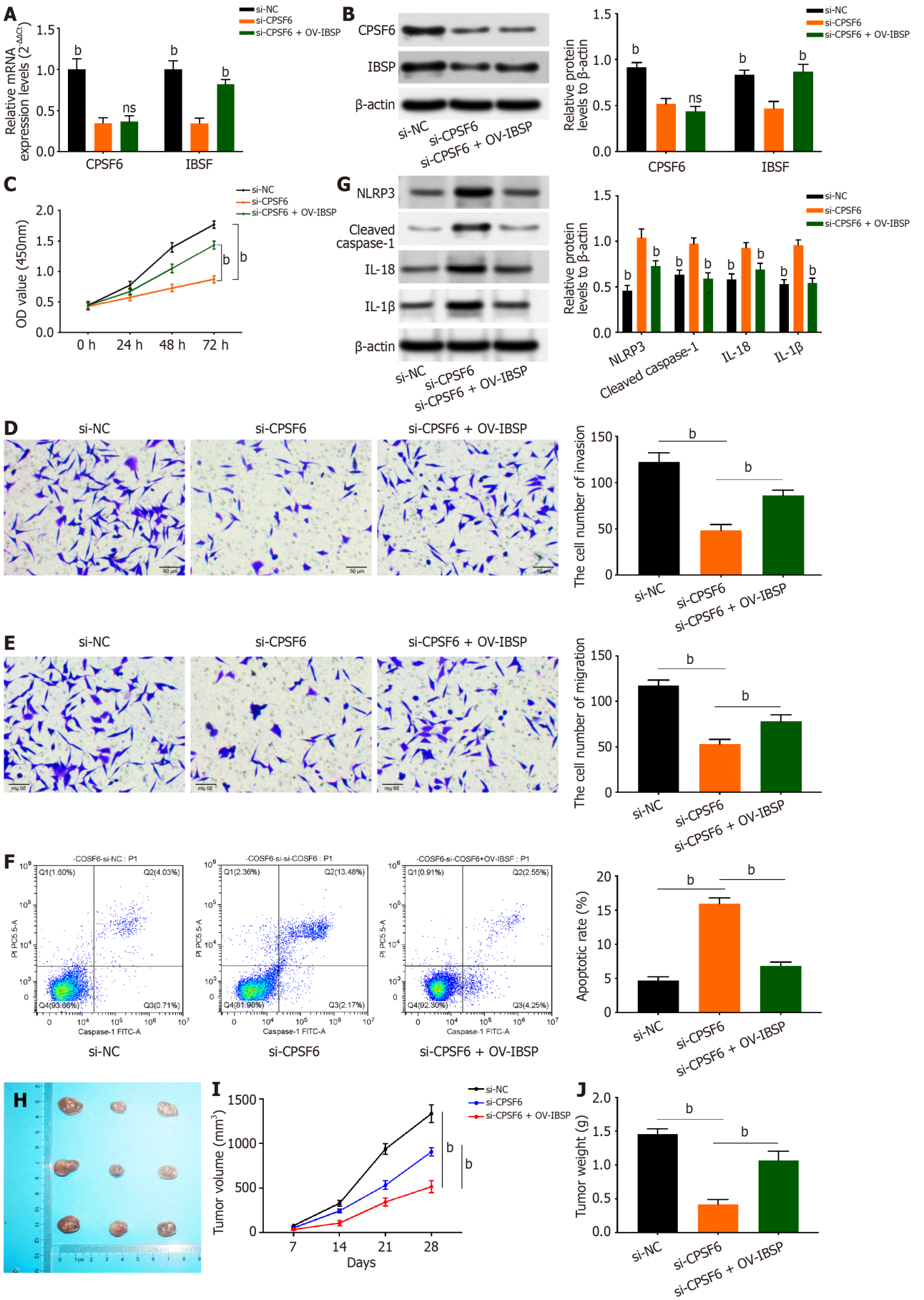
[a]$P < 0.05$ was considered to have a significant difference.
Categorical variables were compared by the chi-square test. IBSP: Integrin binding sialoprotein.

DISCUSSION

GC is one of the most common cancers[32]. Most GC patients are diagnosed at the advanced stage, and the 5-year survival rate of advanced GC patients is less than 15%[33,34]. The emergence of novel bio-targets can improve the early diagnosis and treatment of GC. IBSP has been discovered to exhibit higher expression and important regulatory function in various types of cancers[18-20]. However, the regulatory functions of IBSP in GC progression remain unclear. Similar to the above studies, our study demonstrated that IBSP showed higher expression in GC tissues and cell lines. In addition, IBSP knockdown suppressed cell proliferation, migration, and invasion and facilitated cell apoptosis. In addition, IBSP knockdown strengthened pyroptosis.

RNA-binding protein could bind to the 3′-UTR of mRNAs to regulate their expression levels in various cancers. For instance, the RNA-binding protein NONO post-transcriptionally regulates S-phase kinase associated protein 2 and E2F transcription factor 8 to boost breast cancer tumorigenesis[35]. Additionally, the RNA-binding protein sorbin and SH3 domain containing 2 (SORBS2) stabilizes RAR related orphan receptor A (RORA) mRNA to repress tumor growth and metastasis in hepatocellular carcinoma[36]. The RNA-binding protein DAZ-associated protein 1 interacts with solute carrier family 7 member 11 (SLC7A11) mRNA to aggravate hepatocellular carcinoma progression and modulate ferroptosis[37]. The RNA-binding protein SORBS2 strengthens microtubule associated scaffold protein 1 (MTUS1) mRNA stability to inhibit metastasis in clear cell renal cell carcinoma[38]. Previous studies also verified the oncological function of CPSF6 in acute myeloid leukemia and breast cancer[21,24]. Inhibition of CPSF6 enhances apoptosis by shortening human von Hippel-Lindau (VHL) 3′-UTR in GC[22]. Also, nudix hydrolase 21 (NUDT21) regulates CPSF6 to inhibit tumorigenesis in breast cancer[23]. Similar to these previous reports, this study also revealed that CPSF6 expression was upregulated in GC tissues.

Similar regulatory mechanism (RNA-binding protein-mRNA) also exists in GC. For example, the RNA-binding protein RNPC1 stabilizes aurora kinase B (AURKB) mRNA to enhance GC progression[39]. The RNA binding protein Lin28B interacts with neuropilin-1 to affect stemness in GC[40]. LINC00668 interacts with human antigen R (HuR) to upregulate protein kinase N2 (PKN2) and facilitate GC metastasis[41]. The lncRNA small nucleolar RNA host gene 12 (SNHG12) aggravates cisplatin resistance by regulating the HuR/X-linked inhibitor of apoptosis protein axis in non-small cell lung cancer[42]. In this work, potential RNA binding proteins that can bind to IBSP were predicted and screened using catRAPID omics v2.0. The RNA-binding protein CPSF6 was selected due to its higher expression in GC. However, the relationship between IBSP and CPSF6 has not been studied in GC progression. CPSF6 expression was positively correlated with IBSP expression. Furthermore, through luciferase reporter and RIP assays, it was showed that CPSF6 binds to the 3′-UTR of IBSP and positively regulates IBSP expression. Knockdown of CPSF6 inhibited cell proliferation, migration, and invasion but boosted pyroptosis. Rescue assays revealed that the retarded GC progression mediated by CPSF6 knockdown was reversed by IBSP overexpression.



DOI: 10.4251/wjgo.v15.i9.1531 **Copyright** ©The Author(s) 2023.

Figure 5 Cleavage and polyadenylation factor 6 regulates integrin binding sialoprotein to affect gastric cancer progression. Cells were

divided into the si-NC, si-cleavage and polyadenylation factor 6 (si-CPSF6), and si-CPSF6 + OV-integrin binding sialoprotein (IBSP) groups. A and B: The mRNA and protein expression of CPSF6 and IBSP was detected by real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot, respectively; C: Cell viability was examined by Cell Counting Kit-8 assay; D and E: Cell invasion and migration were tested by transwell assay; F: Cell apoptosis was measured by flow cytometry; G: The protein expression levels of NLR family pyrin domain containing 3, cleaved caspase-1, interleukin-18 (IL-18), and IL-1β were detected by Western blot; H-J: Tumor size, volume, and weight were measured. $^{b}P < 0.01$. IBSP: Integrin binding sialoprotein; CPSF6: Cleavage and polyadenylation factor 6; NLRP3: NLR family pyrin domain containing 3; IL: Interleukin.

CONCLUSION

This study, for the first time, revealed the crucial role of the CPSF6/IBSP axis in GC progression, shedding light on GC treatment. The main findings in previous studies and our findings in this study are shown in Table 2. However, some limitations exist in this study: The luciferase reporter assay is unable to determine whether the protein directly interacts with DNA itself; the RIP assay used native immunoprecipitation without any form of cross-linking; the number of human samples and animal samples was not large; and other phenotypes (such as stemness, exosome, autophagy, and glycolysis) were not assessed. In the future, the regulatory effects of the CPSF6/IBSP axis on these phenotypes will be investigated through more experiments.

Table 2 Cleavage and polyadenylation factor 6 regulates integrin binding sialoprotein to aggravate gastric cancer progression

No.	Findings in previous studies	Findings in this work
1	IBSP has been discovered to exhibit higher expression and important regulatory function in colorectal cancer, breast cancer, and esophageal squamous cell carcinoma. However, the regulatory functions of IBSP in GC progression remain unclear	IBSP exhibits higher expression in GC tissues and cell lines. IBSP facilitates GC cell proliferation, migration, and invasion but suppresses pyroptosis
2	Previous studies have verified the oncological function of the RNA-binding protein CPSF6 in acute myeloid leukemia, breast cancer, and GC. However, no reports have focused on the regulatory effects of CPSF6 on metastasis and pyroptosis	CPSF6 promotes cell proliferation, migration, and invasion but boosts pyroptosis
3	The RNA-binding protein CPSF6 binds to the 3′-UTR of genes to participate in the progression of hepatocellular carcinoma, lung adenocarcinoma, and GC. But, the relationship between IBSP and CPSF6 has not been studied in GC progression	CPSF6 binds to the 3′-UTR of IBSP and positively regulates IBSP expression
4	The regulatory mechanism (RNA binding protein-mRNA 3′-UTR) exists in GC progression. However, the regulatory effects of CPSF6/IBSP remain unclear	The retarded GC progression mediated by CPSF6 knockdown is reversed by IBSP overexpression

IBSP: Integrin binding sialoprotein; CPSF6: Cleavage and polyadenylation factor 6; 3′-UTR: 3′-untranslated region; GC: Gastric cancer.

ARTICLE HIGHLIGHTS

Research background

Previous studies have illustrated that integrin binding sialoprotein (IBSP) exhibits a promotive role in the progression of cancers. However, the regulatory functions of IBSP in gastric cancer (GC) progression remain unclear.

Research motivation

To find effective bio-targets for GC prognosis and treatment.

Research objectives

To probe the regulatory effects and underlying molecular mechanism of IBSP in GC progression.

Research methods

Real-time quantitative polymerase chain reaction and Western blot were used to detect the mRNA and protein expression of IBSP, respectively. The prognosis of GC patients with high or low IBSP expression was evaluated. The regulatory effects of IBSP in GC progression was assessed *via in vitro* and *in vivo* experiments. The molecular mechanism of the IBSP/cleavage and polyadenylation factor 6 (CPSF6) axis was validated.

Research results

IBSP exhibited higher expression in GC, and IBSP knockdown suppressed cell proliferation, migration, and invasion but facilitated pyroptosis. Moreover, the results revealed that CPSF6 binds to the 3′-untranslated region of IBSP and positively regulates IBSP expression in GC.

Research conclusions

Other regulatory functions and related mechanisms of ISBP in GC may be investigated in the future, and its application in

GC treatment will be explored.

Research perspectives

IBSP expression is upregulated in GC tissues and cells, which results in a poor prognosis in GC. CPSF6 positively regulates IBSP to affect pyroptosis and aggravate tumor growth in GC.

FOOTNOTES

Author contributions: Wang XJ designed and wrote the manuscript; Liu Y, Ke B, Zhang L, and Liang H collected and analyzed the data; and all authors read and approved the final manuscript.

Supported by the National Key R&D Program of China, No. 2016YFC1303202.

Institutional review board statement: The study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (Approval No. E2020216).

Institutional animal care and use committee statement: This work was reviewed and approved by the Animal Care and Use Committee of Beijing Viewsolid Biotechnology Co. LTD (Approval No. VS212601449).

Informed consent statement: Written informed consent was obtained from all patients.

Conflict-of-interest statement: The authors report no conflict of interest for this article.

Data sharing statement: The data that support the findings of this study are available from the corresponding author.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Country/Territory of origin: China

ORCID number: Xue-Jun Wang 0000-0001-7215-5149; Han Liang 0000-0002-5674-0994.

S-Editor: Chen YL
L-Editor: Wang TQ
P-Editor: Zhao S

REFERENCES

1 **Guggenheim DE**, Shah MA. Gastric cancer epidemiology and risk factors. *J Surg Oncol* 2013; **107**: 230-236 [PMID: 23129495 DOI: 10.1002/jso.23262]
2 **Smyth EC**, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet* 2020; **396**: 635-648 [PMID: 32861308 DOI: 10.1016/S0140-6736(20)31288-5]
3 **Baylin SB**, Jones PA. Epigenetic Determinants of Cancer. *Cold Spring Harb Perspect Biol* 2016; **8** [PMID: 27194046 DOI: 10.1101/cshperspect.a019505]
4 **Machlowska J**, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric Cancer: Epidemiology, Risk Factors, Classification, Genomic Characteristics and Treatment Strategies. *Int J Mol Sci* 2020; **21** [PMID: 32512697 DOI: 10.3390/ijms21114012]
5 **Ilson DH**. Advances in the treatment of gastric cancer: 2019. *Curr Opin Gastroenterol* 2019; **35**: 551-554 [PMID: 31436556 DOI: 10.1097/MOG.0000000000000577]
6 **Chen Z**, Li Y, Tan B, Zhao Q, Fan L, Li F, Zhao X. Progress and current status of molecule-targeted therapy and drug resistance in gastric cancer. *Drugs Today (Barc)* 2020; **56**: 469-482 [PMID: 32648857 DOI: 10.1358/dot.2020.56.7.3112071]
7 **Huang T**, Song C, Zheng L, Xia L, Li Y, Zhou Y. The roles of extracellular vesicles in gastric cancer development, microenvironment, anti-cancer drug resistance, and therapy. *Mol Cancer* 2019; **18**: 62 [PMID: 30925929 DOI: 10.1186/s12943-019-0967-5]
8 **Li D**, Wang J, Zhang M, Hu X, She J, Qiu X, Zhang X, Xu L, Liu Y, Qin S. LncRNA MAGI2-AS3 Is Regulated by BRD4 and Promotes Gastric Cancer Progression *via* Maintaining ZEB1 Overexpression by Sponging miR-141/200a. *Mol Ther Nucleic Acids* 2020; **19**: 109-123 [PMID: 31837602 DOI: 10.1016/j.omtn.2019.11.003]
9 **Li D**, She J, Hu X, Zhang M, Sun R, Qin S. The ELF3-regulated lncRNA UBE2CP3 is over-stabilized by RNA-RNA interactions and drives gastric cancer metastasis *via* miR-138-5p/ITGA2 axis. *Oncogene* 2021; **40**: 5403-5415 [PMID: 34274947 DOI: 10.1038/s41388-021-01948-6]
10 **Wang J**, Zhang M, Hu X, She J, Sun R, Qin S, Li D. miRNA-194 predicts favorable prognosis in gastric cancer and inhibits gastric cancer cell growth by targeting CCND1. *FEBS Open Bio* 2021; **11**: 1814-1826 [PMID: 33605558 DOI: 10.1002/2211-5463.13125]
11 **Li D**, Xu M, Wang Z, Huang P, Huang C, Chen Z, Tang G, Zhu X, Cai M, Qin S. The EMT-induced lncRNA NR2F1-AS1 positively

modulates NR2F1 expression and drives gastric cancer *via* miR-29a-3p/VAMP7 axis. *Cell Death Dis* 2022; **13**: 84 [PMID: 35082283 DOI: 10.1038/s41419-022-04540-2]

12 **Peng X**, Liu G, Peng H, Chen A, Zha L, Wang Z. SOX4 contributes to TGF-β-induced epithelial-mesenchymal transition and stem cell characteristics of gastric cancer cells. *Genes Dis* 2018; **5**: 49-61 [PMID: 30258935 DOI: 10.1016/j.gendis.2017.12.005]

13 **Liu X**, Long Z, Cai H, Yu S, Wu J. TRIM58 suppresses the tumor growth in gastric cancer by inactivation of β-catenin signaling *via* ubiquitination. *Cancer Biol Ther* 2020; **21**: 203-212 [PMID: 31747856 DOI: 10.1080/15384047.2019.1679554]

14 **Wu X**, Zheng Y, Han B, Dong X. Long noncoding RNA BLACAT1 modulates ABCB1 to promote oxaliplatin resistance of gastric cancer *via* sponging miR-361. *Biomed Pharmacother* 2018; **99**: 832-838 [PMID: 29710482 DOI: 10.1016/j.biopha.2018.01.130]

15 **Deng T**, Jiang X, He Z, Cai M, Chen C, Xu Z. Centromere protein U (CENPU) promotes gastric cancer cell proliferation and glycolysis by regulating high mobility group box 2 (HMGB2). *Bioengineered* 2021; **12**: 10194-10202 [PMID: 34872447 DOI: 10.1080/21655979.2021.2002018]

16 **Malaval L**, Aubin JE, Vico L. Role of the small integrin-binding ligand N-linked glycoprotein (SIBLING), bone sialoprotein (BSP) in bone development and remodeling. *Osteoporos Int* 2009; **20**: 1077-1080 [PMID: 19340496 DOI: 10.1007/s00198-009-0869-2]

17 **Fisher LW**, Jain A, Tayback M, Fedarko NS. Small integrin binding ligand N-linked glycoprotein gene family expression in different cancers. *Clin Cancer Res* 2004; **10**: 8501-8511 [PMID: 15623631 DOI: 10.1158/1078-0432.CCR-04-1072]

18 **Chen Y**, Qin Y, Dai M, Liu L, Ni Y, Sun Q, Li L, Zhou Y, Qiu C, Jiang Y. IBSP, a potential recurrence biomarker, promotes the progression of colorectal cancer *via* Fyn/β-catenin signaling pathway. *Cancer Med* 2021; **10**: 4030-4045 [PMID: 33987980 DOI: 10.1002/cam4.3959]

19 **Wu K**, Feng J, Lyu F, Xing F, Sharma S, Liu Y, Wu SY, Zhao D, Tyagi A, Deshpande RP, Pei X, Ruiz MG, Takahashi H, Tsuzuki S, Kimura T, Mo YY, Shiozawa Y, Singh R, Watabe K. Exosomal miR-19a and IBSP cooperate to induce osteolytic bone metastasis of estrogen receptor-positive breast cancer. *Nat Commun* 2021; **12**: 5196 [PMID: 34465793 DOI: 10.1038/s41467-021-25473-y]

20 **Wang M**, Liu B, Li D, Wu Y, Wu X, Jiao S, Xu C, Yu S, Wang S, Yang J, Li Y, Wang Q, Luo S, Tang H. Upregulation of IBSP Expression Predicts Poor Prognosis in Patients With Esophageal Squamous Cell Carcinoma. *Front Oncol* 2019; **9**: 1117 [PMID: 31709184 DOI: 10.3389/fonc.2019.01117]

21 **Binothman N**, Hachim IY, Lebrun JJ, Ali S. CPSF6 is a Clinically Relevant Breast Cancer Vulnerability Target: Role of CPSF6 in Breast Cancer. *EBioMedicine* 2017; **21**: 65-78 [PMID: 28673861 DOI: 10.1016/j.ebiom.2017.06.023]

22 **Shi X**, Ding K, Zhao Q, Li P, Kang Y, Tan S, Sun J. Suppression of CPSF6 Enhances Apoptosis Through Alternative Polyadenylation-Mediated Shortening of the VHL 3'UTR in Gastric Cancer Cells. *Front Genet* 2021; **12**: 707644 [PMID: 34594359 DOI: 10.3389/fgene.2021.707644]

23 **Wang BJ**, Liu DC, Guo QY, Han XW, Bi XM, Wang H, Wu ZS, Wu WY. NUDT21 Suppresses Breast Cancer Tumorigenesis Through Regulating CPSF6 Expression. *Cancer Manag Res* 2020; **12**: 3069-3078 [PMID: 32431549 DOI: 10.2147/CMAR.S239664]

24 **Zhang Z**, Jiang M, Borthakur G, Luan S, Huang X, Tang G, Xu Q, Ji D, Boyer AD, Li F, Huang R, You MJ. Acute myeloid leukemia with a novel CPSF6-RARG variant is sensitive to homoharringtonine and cytarabine chemotherapy. *Am J Hematol* 2020; **95**: E48-E51 [PMID: 31769058 DOI: 10.1002/ajh.25689]

25 **Chen MS**, Lo YH, Chen X, Williams CS, Donnelly JM, Criss ZK 2nd, Patel S, Butkus JM, Dubrulle J, Finegold MJ, Shroyer NF. Growth Factor-Independent 1 Is a Tumor Suppressor Gene in Colorectal Cancer. *Mol Cancer Res* 2019; **17**: 697-708 [PMID: 30606770 DOI: 10.1158/1541-7786.MCR-18-0666]

26 **Wang H**, Wu M, Lu Y, He K, Cai X, Yu X, Lu J, Teng L. LncRNA MIR4435-2HG targets desmoplakin and promotes growth and metastasis of gastric cancer by activating Wnt/β-catenin signaling. *Aging (Albany NY)* 2019; **11**: 6657-6673 [PMID: 31484163 DOI: 10.18632/aging.102164]

27 **Zhao Z**, Xiao S, Yuan X, Yuan J, Zhang C, Li H, Su J, Wang X, Liu Q. AHNAK as a Prognosis Factor Suppresses the Tumor Progression in Glioma. *J Cancer* 2017; **8**: 2924-2932 [PMID: 28928883 DOI: 10.7150/jca.20277]

28 **Shan Z**, An N, Qin J, Yang J, Sun H, Yang W. Long non-coding RNA Linc00675 suppresses cell proliferation and metastasis in colorectal cancer *via* acting on miR-942 and Wnt/β-catenin signaling. *Biomed Pharmacother* 2018; **101**: 769-776 [PMID: 29524886 DOI: 10.1016/j.biopha.2018.02.123]

29 **Shu D**, Xu Y, Chen W. Knockdown of lncRNA BLACAT1 reverses the resistance of afatinib to non-small cell lung cancer *via* modulating STAT3 signalling. *J Drug Target* 2020; **28**: 300-306 [PMID: 31359792 DOI: 10.1080/1061186X.2019.1650368]

30 **Guo M**, Zhang X. LncRNA MSTO2P promotes colorectal cancer progression through epigenetically silencing CDKN1A mediated by EZH2. *World J Surg Oncol* 2022; **20**: 95 [PMID: 35346226 DOI: 10.1186/s12957-022-02567-5]

31 **Hu S**, Song Y, Zhou Y, Jiao Y, Li G. METTL3 Accelerates Breast Cancer Progression *via* Regulating EZH2 m(6)A Modification. *J Healthc Eng* 2022; **2022**: 5794422 [PMID: 35392146 DOI: 10.1155/2022/5794422]

32 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362 [PMID: 16489633 DOI: 10.3748/wjg.v12.i3.354]

33 **Song Z**, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. *Tumour Biol* 2017; **39**: 1010428317714626 [PMID: 28671042 DOI: 10.1177/1010428317714626]

34 **Tan Z**. Recent Advances in the Surgical Treatment of Advanced Gastric Cancer: A Review. *Med Sci Monit* 2019; **25**: 3537-3541 [PMID: 31080234 DOI: 10.12659/MSM.916475]

35 **Iino K**, Mitobe Y, Ikeda K, Takayama KI, Suzuki T, Kawabata H, Suzuki Y, Horie-Inoue K, Inoue S. RNA-binding protein NONO promotes breast cancer proliferation by post-transcriptional regulation of SKP2 and E2F8. *Cancer Sci* 2020; **111**: 148-159 [PMID: 31733123 DOI: 10.1111/cas.14240]

36 **Han L**, Huang C, Zhang S. The RNA-binding protein SORBS2 suppresses hepatocellular carcinoma tumourigenesis and metastasis by stabilizing RORA mRNA. *Liver Int* 2019; **39**: 2190-2203 [PMID: 31365778 DOI: 10.1111/liv.14202]

37 **Wang Q**, Guo Y, Wang W, Liu B, Yang G, Xu Z, Li J, Liu Z. RNA binding protein DAZAP1 promotes HCC progression and regulates ferroptosis by interacting with SLC7A11 mRNA. *Exp Cell Res* 2021; **399**: 112453 [PMID: 33358859 DOI: 10.1016/j.yexcr.2020.112453]

38 **Lv Q**, Dong F, Zhou Y, Cai Z, Wang G. RNA-binding protein SORBS2 suppresses clear cell renal cell carcinoma metastasis by enhancing MTUS1 mRNA stability. *Cell Death Dis* 2020; **11**: 1056 [PMID: 33311452 DOI: 10.1038/s41419-020-03268-1]

39 **Ji CM**, Zhang X, Fang W, Meng L, Wei X, Lu C. RNA-binding protein RNPC1 acts as an oncogene in gastric cancer by stabilizing aurora kinase B mRNA. *Exp Cell Res* 2021; **406**: 112741 [PMID: 34302858 DOI: 10.1016/j.yexcr.2021.112741]

40 **Wang X**, Hu H, Liu H. RNA binding protein Lin28B confers gastric cancer cells stemness *via* directly binding to NRP-1. *Biomed Pharmacother* 2018; **104**: 383-389 [PMID: 29787985 DOI: 10.1016/j.biopha.2018.05.064]

41 **Li J**, Dong W, Jiang Q, Zhang F, Dong H. LINC00668 cooperated with HuR dependent upregulation of PKN2 to facilitate gastric cancer metastasis. *Cancer Biol Ther* 2021; **22**: 311-323 [PMID: 33879018 DOI: 10.1080/15384047.2021.1905138]

42 **Tan D**, Li G, Zhang P, Peng C, He B. LncRNA SNHG12 in extracellular vesicles derived from carcinoma-associated fibroblasts promotes cisplatin resistance in non-small cell lung cancer cells. *Bioengineered* 2022; **13**: 1838-1857 [PMID: 35014944 DOI: 10.1080/21655979.2021.2018099]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1544-1555

DOI: 10.4251/wjgo.v15.i9.1544

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Osteopontin promotes gastric cancer progression *via* phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway

Yue-Chao Qin, Xin Yan, Xiao-Lin Yuan, Wei-Wei Yu, Fan-Jie Qu

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Baryshnikova NV, Russia; Kukongviriyapan V, Thailand

Received: April 11, 2023
Peer-review started: April 11, 2023
First decision: July 9, 2023
Revised: July 14, 2023
Accepted: July 28, 2023
Article in press: July 28, 2023
Published online: September 15, 2023

Yue-Chao Qin, Xin Yan, Wei-Wei Yu, Fan-Jie Qu, Department of Oncology, Affiliated Dalian Third People's Hospital of Dalian Medical University, Dalian 116033, Liaoning Province, China

Yue-Chao Qin, Xiao-Lin Yuan, Research Center, Affiliated Zhongshan Hospital of Dalian University, Dalian 116001, Liaoning Province, China

Corresponding author: Fan-Jie Qu, MD, Professor, Department of Oncology, Affiliated Dalian Third People's Hospital of Dalian Medical University, No. 40 Qianshan Road, Dalian 116033, Liaoning Province, China. wyb960419@163.com

Abstract

BACKGROUND

Gastric cancer (GC) is one of the most common malignant tumors. Osteopontin (OPN) is thought to be closely related to the occurrence, metastasis and prognosis of many types of tumors.

AIM

To investigate the effects of OPN on the proliferation, invasion and migration of GC cells and its possible mechanism.

METHODS

The mRNA and protein expression of OPN in the GC cells were analyzed by real-time quantitative-reverse transcription polymerase chain reaction and western blotting, and observe the effect of varying degree expression OPN on the proliferation and other behaviors of GC. Next, the effects of OPN knockdown on GC cells migration and invasion were examined. The short hairpin RNA (shRNA) and negative control shRNA targeting OPN-shRNA were transfected into the cells according to the manufacturer's instructions. Non transfected cells were classified as control in the identical transfecting process. 24 h after RNA transfection cell proliferation activity was detected by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide assay, and cell invasiveness and migration were detected by Trans well assay. Meanwhile, the expression of protein kinase B (AKT), matrix metalloproteinase 2 (MMP-2) and vascular endothelial growth factor (VEGF) in the human GC cell lines was detected by reverse transcription polymerase chain reaction and western blotting.

RESULTS
The results of this study revealed that OPN mRNA and protein expression levels were highly expressed in SGC-7901 cells. OPN knockdown by specific shRNA noticeably reduced the capabilities of proliferation, invasion and migration of SGC-7901 cells. Moreover, in the experiments of investigating the underlying mechanism, results showed that OPN knockdown could down-regulated the expression of MMP-2 and VEGF, it also decreased the phosphorylation of AKT. Meanwhile, the protein expression levels of MMP-2, VEGF and phosphorylated AKT was noticeable lower than that in control group in the GC cells after they were added to phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002).

CONCLUSION
These results suggested that OPN though PI3K/AKT/mammalian target of rapamycin signal pathway to up-regulate MMP-2 and VEGF expression, which contribute SGC-7901 cells to proliferation, invasion and migration. Thus, our results demonstrate that OPN may serve as a novel prognostic biomarkers as well as a potential therapeutic targets for GC.

Key Words: Osteopontin; Proliferation; Invasion; Migration; Gastric cancer; Phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway

Core Tip: We investigated the effects of osteopontin (OPN) on the proliferation, invasion and migration of gastric cancer (GC) cells and its possible mechanism. The results of this study revealed that OPN mRNA and protein expression levels were highly expressed in SGC-7901 cells. OPN knockdown by specific short hairpin RNA noticeably reduced the capabilities of proliferation, invasion and migration of SGC-7901 cells. Moreover, our results showed that OPN though phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway signal pathway to up-regulate matrix metalloproteinase 2 and vascular endothelial growth factor expression, which contribute SGC-7901 cells to proliferation, invasion and migration. These results demonstrate that OPN may serve as a novel prognostic biomarkers as well as a potential therapeutic targets for GC.

Citation: Qin YC, Yan X, Yuan XL, Yu WW, Qu FJ. Osteopontin promotes gastric cancer progression *via* phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway. *World J Gastrointest Oncol* 2023; 15(9): 1544-1555
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1544.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1544

INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide[1]. At present, the development of comprehensive treatment strategies has greatly improved the therapeutic effect of GC patients. Because the accurate diagnosis of early GC is difficult[2], the prognosis of most GC patients is still poor, and the 5-year survival rate of patients with advanced GC is approximately 25%[3]. Therefore, more accurate identification of prognostic biomarkers and molecular basis of GC invasion and metastasis has important clinical value for understanding GC and developing new effective treatment strategies.

Osteopontin (OPN), an extracellular matrix (ECM) phosphoglycoprotein, is expressed at elevated levels in a variety of malignant tumors (such as breast cancer, lung cancer, urogenital tumors, head and neck cancer, osteosarcoma, *etc.*) and is involved in many pathophysiological processes including tumorigenesis, leading to poor prognosis. Since it is involved in promoting aggressive and metastatic progression of many cancers, it is considered as a potential important biomarker for monitoring cancer progression[4-13]. In addition, the up-regulation of OPN expression is also closely related to the occurrence, metastasis and prognosis of tumors in the digestive system, and even the size and grade of tumors[14-18].

With the ongoing study of OPN, OPN is also being explored as a potential therapeutic target. For example, reducing OPN expression could provide novel strategies for the treatment of patients with various types of metastatic cancer[15,19-22].

A number of studies have reported that the expression of OPN in GC tissues is significantly higher than that in non-tumor tissues, and is closely related to the invasion, metastasis and prognosis of GC[15,23-28]. But there are conflicting stories. Tang *et al*[29] concluded that the expression of OPN in GC tissues was not related to prognosis.

Previous studies of our research group have found[30] that OPN is significantly up-regulated in GC tissues, and its expression level is closely related to clinicopathological parameters, overall survival (OS) and disease-free survival of patients, suggesting that OPN is closely related to poor prognosis of GC. The results are consistent with those of Sun *et al*[31]. Final results in a meta-analysis showed that high OPN expression was associated with poor OS, suggesting that OPN is a promising prognostic biomarker for GC[32].

These data indicated that OPN may play a crucial role in the carcinogenesis of GC. Despite increasing insights into the function of OPN-promoted progression of GC, the exact mechanism of OPN-promoted invasion and progression in GC remains unclear.

Phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway is one of the most widely studied signaling pathways. PI3K/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway is involved in cell proliferation, invasion and metastasis after abnormal activation of malignant tumors[33]. PI3K/AKT/mTOR signaling pathway is considered to be one of the most common regulatory pathways in GC molecular mechanism studies [33-35].

However, whether OPN can regulate the PI3K-AKT-mTOR signaling pathway in GC cells has not been reported in the literature. Therefore, our study comprehensively analyzed whether OPN in GC cells regulates the expression of PI3K and phosphorylation of its downstream signal transduction pathway protein by exerting its kinase activity, thus promoting the proliferation, migration and invasion of GC cells. This study aims to explore the mechanism of the regulation of PI3K-AKT-mTOR signal transduction pathway by OPN in GC cells, so as to provide a new theoretical basis for elucidate the metastasis and invasion mechanism of GC and further develop the targeted treatment of GC with protein kinase inhibitors.

MATERIALS AND METHODS

Reagents

The GC cell lines (SGC-7901, HGC-27, and AGS) and normal gastric mucosa epithelial cell line (GES-1) were provided by China Center for Type Culture Collection (China); roswell park memorial institute (RPMI) 1640 cell culture medium, Ham's F 12 nutrient medium (F12), and 0.25% trypsin-ethylenediaminetetraacetic acid were provided by HYclone (United States); fetal bovine serum (FBS) was offered by Haoyang (China); OPN-short hairpin RNA (shRNA) interference vector and negative control shRNA (NC-shRNA) interference vector were designed and developed by Sangon (China); RNAiso Plus TB Green™ Premix Ex Taq™ II PrimeScript™ RT reagent kit with gDNA Eraser were provided by Takara (Japan); The primer sequence of OPN, matrix metalloproteinase 2 (MMP-2), vascular endothelial growth factor (VEGF) and β-actin were designed and fabricated by Sangon (China); primary monoclonal antibodies for mTOR (Abp54398), OPN (Abp52084), AKT (Abp50636), phosphorylated AKT (p-AKT) (phosphorySer473), β-actin (A01010) and horseradish peroxidase (HRP) conjugated secondary antibodies (A21010, A21020) were offered by Abbkine (United States); primary monoclonal antibodies for MMP-2 (BS-0412R) and VEGF (BS-0279R) were provided by Bioss (China); LY294002 inhibitor was purchased from Meilun (China).

Cells Preparation

Human GC cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS and antibiotics (100 U/mL streptomycin and 100 μg/mL penicillin); the AGS cells underwent culture process in F-12 medium supplemented with 10% FBS and antibiotics (100 U/mL streptomycin and 100 μg/mL penicillin). All of the cells were grown in a humid incubator with 5% CO_2 at 37 °C, besides, the adherent cells were cleaned twice consecutively with phosphate-buffered saline. Cells were harvested with 0.25% trypsin and passaged at a ratio of 1: 3 every three days.

Gene silencing of OPN with shRNA interference vectors construction

The shRNA targeting OPN-shRNA and the NC-shRNA were transiently transfected cells with Ultra Fectin according to the instruction of the manufacturer. Non transfected cells were classified as control in the identical transfecting process. *TTCAAGAA* was taken as the loop structure of shRNA template to avoid the formation of termination signal, and T6 structure acted as the transcription termination sequence of shRNA. ShRNA expression vector covered the expression framework of green fluorescent protein, which can be expressed after being transferred into cells. The transfection efficiency can be easily determined under a fluorescence microscopy or by flow cytometry. Cells were cultured for 24 h or 48 h and subsequently harvested for further experiments.

The sequences of the shRNAs include: OPN-shRNA1: Sense: *5'-CACCGAGGAGTTGAATGGTGCATACTTCAAGAGAGTATGCACCATTCAACTCCTCTTTTTTG-3'*, Anti-sense: *5'-AGCTCAAAAAAGAGGAGTTGAATGGTGCATACTCTCTTGAAGTATGCACCATTCAACTCCTC-3'*; OPN-shRNA2: Sense: *5'-CACCGTAAGGAAGAAGATAAACACCTTCAAGAGAGGTGTTTATCTTCTTCCTTACTTTTTTG-3'*, Anti-sense: *5'-AGCTCAAAAAAGTAAGGAAGAAGATAAACACCTCTCTTGAAGGTGTTTATCTTCTTCCTTAC-3'*; OPN-shRNA3: Sense: *5'-CACCGTGCATCTTCTGAGGTCAATTTTCAAGAGAAGACCTCAGAAGATGCACTTTTTTG-3'*, Anti-sense: *5'-AGCTCAAAAAAGTGCATCTTCTGAGGTCAATTTCTCTTGAAAATTGACCTCAGAAGATGCAC-3'*; NC-shRNA: Sense: *5'-CACCGTTCTCCGAACGTGTCACGTCAAGAGATTACGTGACACGTTCGGAGAATTTTTTG-3'*, Anti-sense: *5'-AGCTCAAAAAATTCTCCGAACGTGTCACGTAATCTCTTGACGTGACACGTTCGGAGAAC-3'*.

Cell proliferation detection by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide (MTT) assay

24 h after RNA transfection, human GC cell line (SGC-7901) was seeded in 96-plates (4×10^3 cells/well). For this experiment, 1 h post-cell seeding was defined as the 0 h time point. After 0, 24, 48, 72, 96 h, the cells were incubated with MTT solution (5 mg/mL) in an incubator for 4 h, respectively. The formed formazan crystals were dissolved with 200 μL of dimethyl sulfoxide and then mixed well. The optical density of each sample was determined at 490 nm with Epoch™ Microplate Spectrophotometer (United States).

Cell invasion and migration determination by Transwell assays

The transwell chamber was placed into a 24-well plates. In the upper chamber coated with matrigel (for invasion assay), SGC-7901 (4×10^4) cells in 200 μL serum-free XGI-1640 medium were added. In the upper chamber without being coated with matrigel (for migration assay), SGC-7901 (2×10^4) cells in 200 μL serum-free XGI-1640 were added. The lower chamber was filled with 600 μL conditioned media. After incubated for 48 h (for invasion) or 24 h (for migration), the cells were incubated with formaldehyde 4% for 20 min at ambient temperature. Subsequently, cells were stained with 1% crystal violet for 30 min at ambient temperature. Next, the images of various fields (n = 3) at 100 × magnification for each insert were counted.

RNA extraction and Real-time polymerase chain reaction (PCR)

Total RNA was extracted from human gastric cell lines using RNAiso Plus and then quantified spectrophotometrically by its absorbance at 260 nm. Forward primer *5'-AGCGAGGAGTTGAATGGTGCATAC-3'* reverse primer *5'-AATCTGGACT-GCTTGTGGCTGTG-3'*; MMP-2, forward primer *5'-GGCGGTCACAGCTACTTCTTCAAG-3'*, reverseprimer *5'-ATCGAAG-GCAGTGGAGAGGAAGG-3'*; VEGF, forwardprimer *5'-CCTTCGCTTACTCTCACCTGCTTC-3'*, reverse primer *5'-GGCTGCTTCTTCCAACAATGTGTC-3'*; β-actin, human beta-actin Endogenous Reference Genes Primers, 10μM (B661102-0001, Sangon Biotech). Reverse transcription was performed with PrimeScript™ RT reagent Kit with gDNA Eraser (perfect real time) following the directives of the manufacturer. RT-PCR was performed with the following protocol: An initial pre-denaturation step at 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 5 s, and then annealing process at 60 °C for 30 s. Meantime, the β-actin RNA was amplified and acted as an internal control. The cycle threshold values for β-actin RNA the samples were calculated by computer software.

Detection of protein by Western blot assay

Human GC cell lines and treated SGC-7901 cells were harvested and lysed with ice-cold radio immunoprecipitation assay solution, and then added to phenylmethanesulfonyl fluoride (99: 1) as well as phosphatase inhibitors (99: 1), Then, the resulting homogenate was centrifuged for 5 min at 12000 rmp and 4 °C. Subsequently, the total protein in the supernatant was quantified with a protein quantification kit protein assay kit, resolved by 10% SDS-PAGE and then transferred to a polyvinylidene difluoride membrane. Next, the protein was blocked by 5% BSA blocking buffer or evaporated milk at ambient temperature for 1-2 h, incubated overnight with polyclonal antibody at 4 °C under gentle agitation, subsequently, it was incubated with HRP-conjugated goat anti-rabbit immunoglobin (H + L) at ambient temperature for 1 h. After being cleaned with tris buffered saline-0.1% 3 times, the membrane was developed by enhanced chemiluminescence (Sigma) and then exposed to Xray films scanned and determined with imagej software to quantify protein expression.

Statistical analysis

Each experiment was performed at least three times. Data (mean ± SE) were studied by one-way ANOVA or independent t-test. All of the calculations were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, United States). The level of significance was set at $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$.

RESULTS

Expression levels of OPN and capacity of proliferation in human GC cell lines

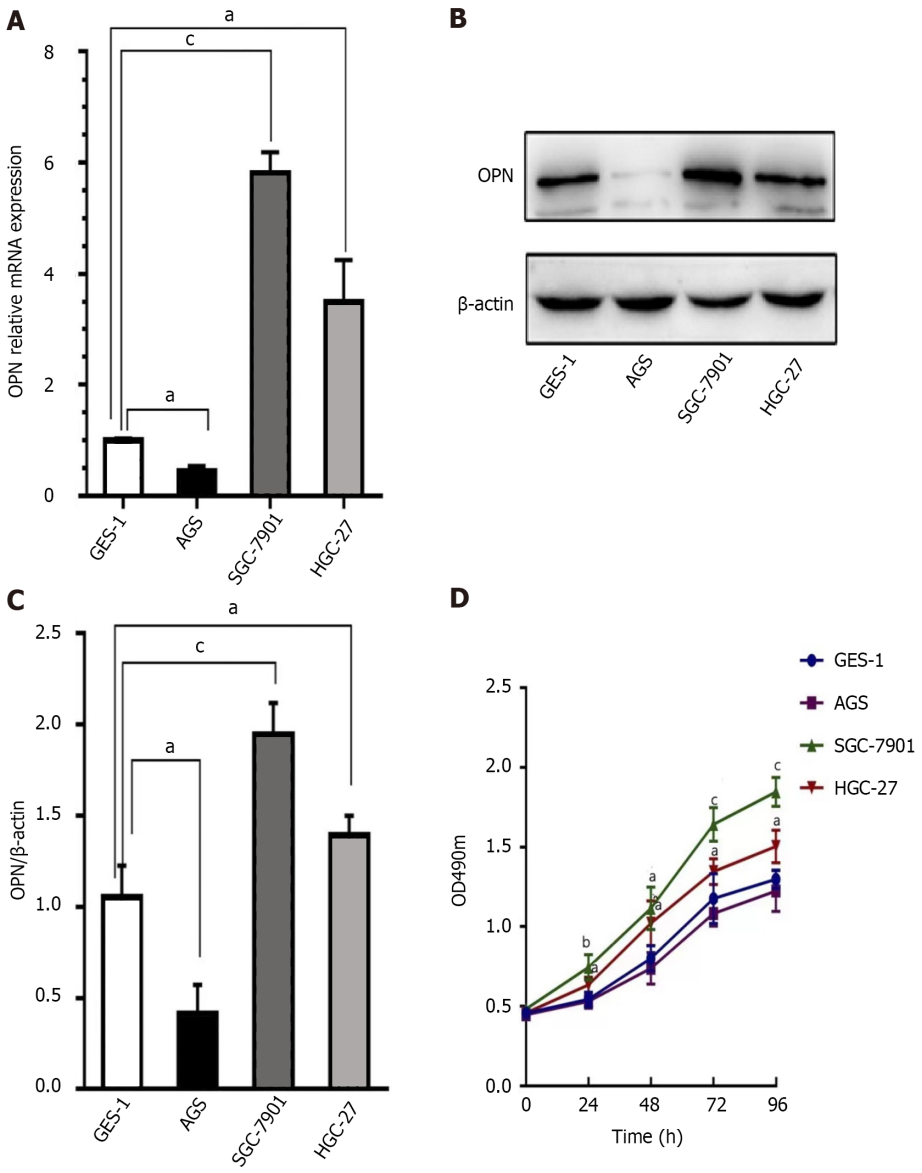
The mRNA and protein expression of OPN in the GC cells and GES-1 cells were analyzed by real-time quantitative-reverse transcription and western blotting, respectively. Results revealed that GC cell lines (AGS, SGC-7901 and HGC-27) and normal cell line GES-1 expressed OPN mRNA and protein to varying degree. The mRNA and protein expression levels of OPN in the GC cell lines (SGC-7901, HGC-27) were markedly higher than those in GES-1 cells (Figure 1A-C). Moreover, we examined the effects of OPN expression on GC cell proliferation by MTT assay; results revealed the capacity of proliferation related with the OPN expression levels; SGC-7901 cells with high level expressed OPN possess strong capacity of proliferation (Figure 1D).

OPN knockdown inhibits proliferation, invasion and metastasis of GC cells

To explore the effects of OPN on GC cells, the proliferation, invasion and metastasis capacities of high level OPN expression SGC-7901 cells and low level OPN expression SGC-7901 cells were examined. In order to create low level OPN expression SGC-7901 cells, three OPN-shRNA transfect vector were built. The transfection efficiency can be easily determined under a fluorescence microscopy (Figure 2A). Results reveal that the expression of OPN of all three sequences of OPN-shRNA-transfected SGC-7901 cells were significantly lower than that of control (blank control) cells(Figure 2B-D). And the OPN-shRNA3 exhibited the optimal interference efficiency of OPN, revealing that it acts as a right model for ascertaining the effects of OPN knockdown (Figure 2B-D). Meanwhile, there were no noticeable differences in the expression between control and NC-shRNA-transfected SGC-7901 cells (Figure 2B-D).

The MTT assay and transwell assay revealed that capacities of proliferation, invasion and metastasis of OPN-shRNA3-transfected group were significantly lower than that of control group.

Meanwhile, there were no differences between in the control group and NC-shRNA-transfected group (Figure 2E-I). These results demonstrated that OPN play a key role in promoting SGC-7901 cells proliferation, invasion and migration.



DOI: 10.4251/wjgo.v15.i9.1544 **Copyright** ©The Author(s) 2023.

Figure 1 Expression levels of osteopontin and capacity of proliferation in human gastric cancer cell lines. A: real-time quantitative-reverse transcription analysis of osteopontin (OPN) mRNA levels in the gastric cancer (GC) cell lines (AGS, SGC-7901, HGC-27) and normal human gastric mucosal epithelial cell line (GES-1); B: Western blot assay of OPN protein expression levels in various human GC cell lines; C: Densitometry analysis of the protein bands of OPN proteins; D: The relative proliferation rate at 0, 24, 48 and 96 h in various GC cells by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide assay. Data are shown as the means ± SE (n = 3). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. OPN: Osteopontin.

OPN upregulates MMP-2 and VEGF expression

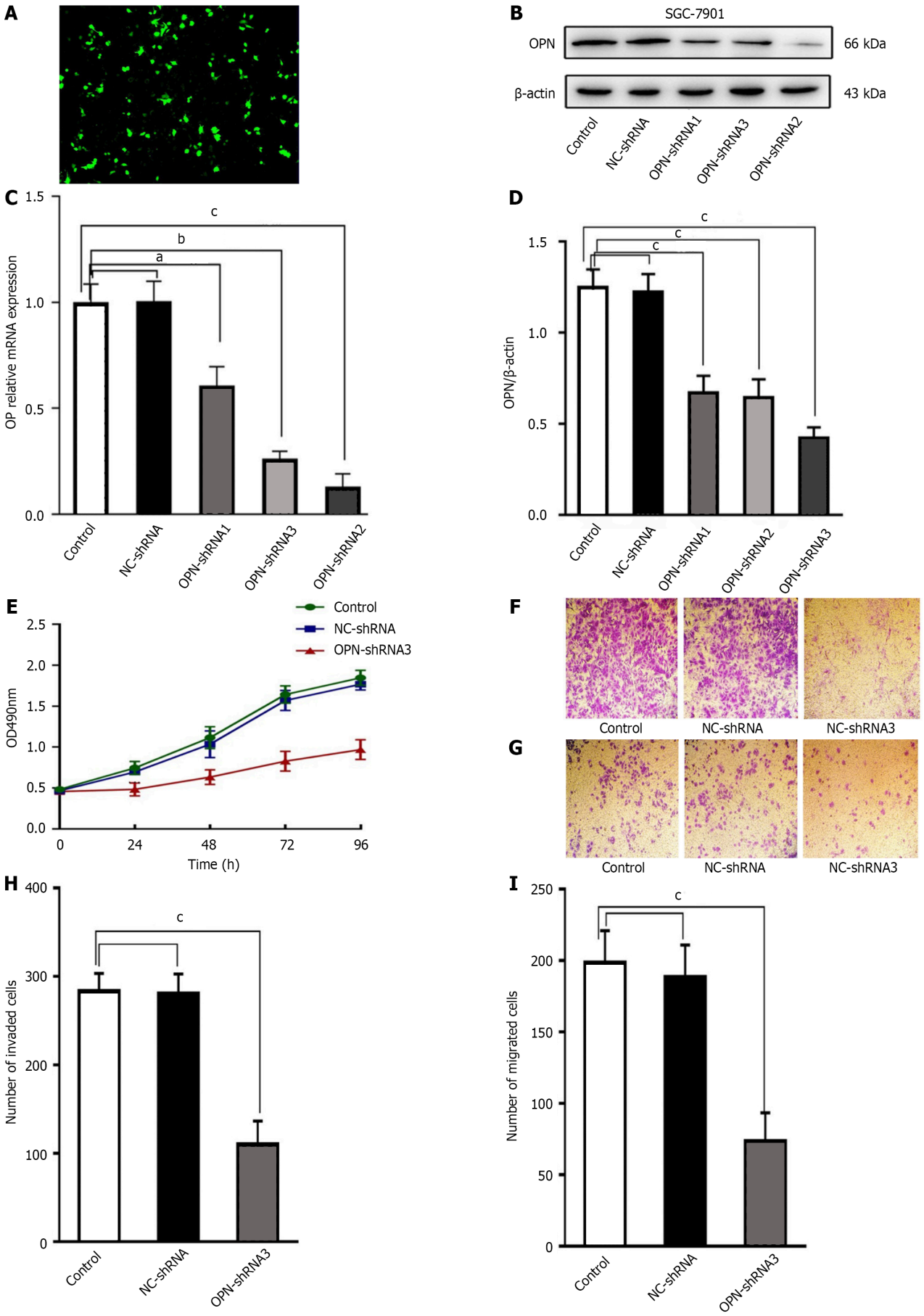
In order to observe the relevance between OPN and the expression MMP-2 and VEGF, the effect of OPN on regulating MMP-2 and VEGF expression was studied. Results show that the MMP-2 and VEGF mRNA in OPN-shRNA3 group were significantly down-regulated by 52.6% and 49.0% compared with control group, respectively (Figure 3), the result revealed that OPN knockdown could down-regulate the expressions of MMP-2 and VEGF.

OPN regulates the MMP-2 and VEGF expression via PI3K/AKT/mTOR pathway

To further investigate the underlying mechanism of OPN in proliferation, invasion and migration of GC cells. We analyzed the protein expression levels of mTOR, AKT, p-AKT, MMP-2, VEGF in the SGC-7901 after they were OPN knockdown or added to PI3K inhibitor (LY294002).

The western blotting analysis revealed that protein expression levels of total mTOR and AKT among control, NC-shRNA and OPN-shRNA3 group remained constant, while that of p-AKT in OPN-shRNA3 group was noticeably lower than that in control group (Figure 4A and B). Meanwhile, MMP-2 and VEGF expression in OPN-shRNA3 were lower than the groups of control (Figure 4A and B).

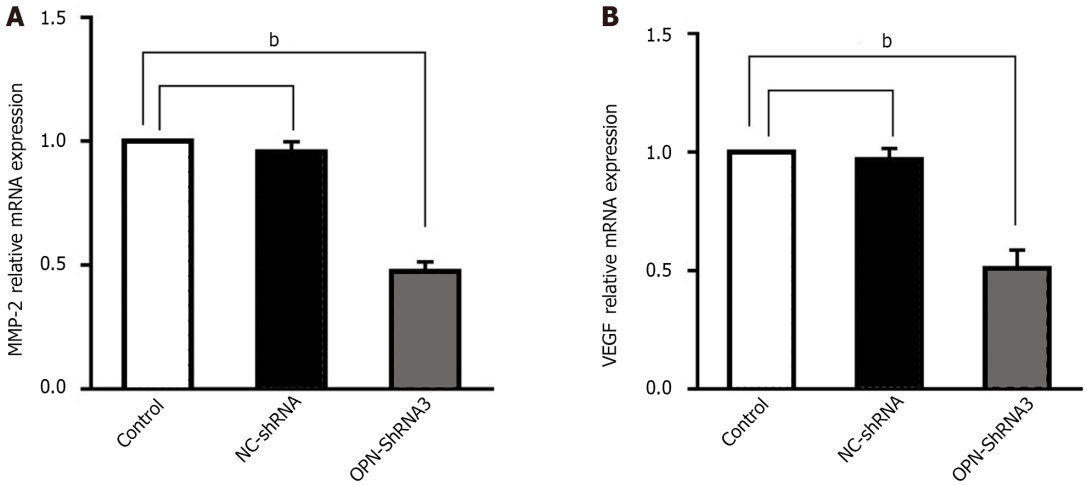
In order to further study the relationship between OPN and PI3K/AKT/mTOR pathway, we administrated SGC-7901 cells with PI3K inhibitor (LY294002). As shown in Figure 4C and D, the protein expression levels of total mTOR and AKT in control and LY294002 group remained constant, while the protein expression levels of p-AKT, MMP-2 and VEGF in



DOI: 10.4251/wjgo.v15.i9.1544

Figure 2 Osteopontin knockdown inhibits proliferation, invasion and metastasis of gastric cancer cells. A: Fluorescence microscope

observation transfection efficiency of SGC-7901 cells after being transfected with osteopontin-short hairpin RNA (shRNA) (× 100); B: 3distinct, sequence-specific OPN shRNAs (OPN-shRNA1; OPN-shRNA2; OPN-shRNA3) and negative control shRNA were designed, and the OPN-shRNA3 has the best interference efficiency of OPN; C: Western blot assay of OPN protein levels in SGC-7901 cells 48 h after transfection; D: Densitometry analysis of the protein bands of OPN proteins in SGC-7901 cells 48 h after they were transfected; E: 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide assay observation the relative proliferation rate at 0, 24, 48 and 96 h in SGC-7901 cells following transfection, compared with control group; F: Microscope observation effect of the invasive ability of OPN knockdown in SGC-7901 cells was assessed by the Transwell matrigel-coated assay (× 100); G: Microscope observation of the migrative ability of OPN knockdown on SGC-7901 cells was assessed by the Transwell assay (× 100); H and I: Cells invading and migrating through the membrane were counted in 3 random fields for respective group. Data are shown as the means ± SE (n = 3). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. OPN: Osteopontin; shRNA: Short hairpin RNA; NC-shRNA: Negative control shRNA.



DOI: 10.4251/wjgo.v15.i9.1544

Figure 3 Osteopontin upregulates matrix metalloproteinase 2 and vascular endothelial growth factor expression. A: Matrix metalloproteinase 2 mRNA level was tested by real-time quantitative-reverse transcription polymerase chain reaction (PCR) and normalized to β-Actin expression; B: Vascular endothelial growth factor mRNA level was detected by quantitative PCR and normalized to β-Actin expression. Data are shown as the means ± SE (n = 3). $^{b}P < 0.01$. MMP-2: Matrix metalloproteinase 2; VEGF: Vascular endothelial growth factor; NC-shRNA: Negative control shRNA.

LY294002 group significantly decreased as compared with control group. These results suggested that OPN up-regulate the expressions of MMP-2 and VEGF *via* PI3K/AKT/mTOR signaling pathway, thus promote the proliferation, invasion and migration of GC SGC-7901 cells.

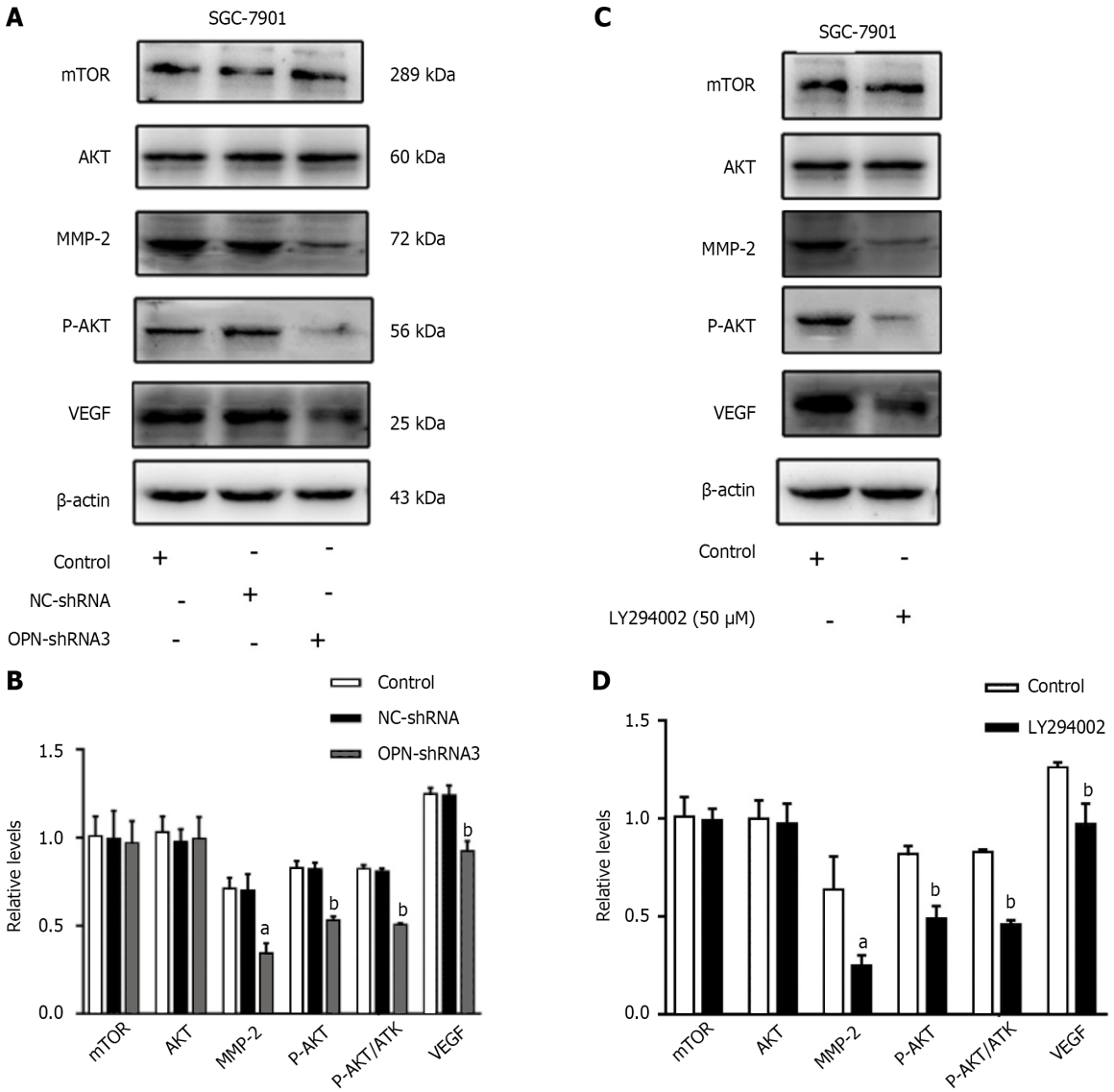
DISCUSSION

It is well known that patients with metastatic GC have a poor prognosis[3], so it is of great clinical value to search for more accurate prognostic markers to better understand the molecular mechanism of the occurrence and development of GC and develop new therapeutic strategies. In this study, the effects of OPN on proliferation, invasion and migration of GC SGC-7901 cells were investigated through a number of in vitro experiments, and the mechanism of the regulation of PI3K/AKT/mTOR signaling pathway by OPN in GC cells was also explored, providing a new theoretical basis for clarifying the metastasis and invasion mechanism of GC and further developing the targeted treatment of GC with protein kinase inhibitors.

In recent years, numerous studies demonstrated that OPN overexpressed and promoted the cancer progression in various cancers *via* various signaling pathways[4-18]. With the ongoing study of OPN, OPN is also being explored as a potential therapeutic target. For example, reducing OPN expression could provide novel strategies for the treatment of patients with various types of metastatic cancer[19-22]. Wang *et al*[36] reported that silencing the expression of OPN in GC cell line SGC7901 inhibited the growth and metastasis of GC. Park *et al*[37] also reported that the migration ability of GC cells with OPN knockdown was reduced.

We found in our study that the mRNA and protein expression levels of OPN were highly expressed in GC SGC-7901 cells (Figure 1), and OPN knockdown inhibits cell proliferation, invasion and migration in SGC-7901 cells (Figure 2). Our results indicated that OPN as an inducer of cell proliferation, invasion and migration.

Another important finding of our study was that overactivation of the PI3K/AKT signaling pathway was associated with OPN-induced progression of GC cells. Several studies have demonstrated that OPN promotes tumor invasion and metastasis by inducing activation of signaling pathways that regulate cell migration and tumor progression, such as mitogen-activated protein kinase and PI3K/AKT[38].



DOI: 10.4251/wjgo.v15.i9.1544

Figure 4 Osteopontin regulates the matrix metalloproteinase 2 and vascular endothelial growth factor expression *via* phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin pathway. A: Western blotting analysis of mammalian target of rapamycin (mTOR), protein kinase B (AKT), phosphorylated AKT (p-AKT) (Ser473), matrix metalloproteinase 2 (MMP-2) and vascular endothelial growth factor (VEGF) protein expression levels in SGC-7901 cells after transfected into negative control short hairpin RNA (shRNA) (negative control-shRNA) or osteopontin-shRNA3. β-actin expression was used as a loading control and for normalization; B: Densitometry analysis of the protein bands of mTOR, AKT, MMP-2, p-AKT, p-AKT and AKT, VEGF; C: Western blotting assay of SGC-7901 cells administrated LY294002 inhibitor on mTOR, AKT, p-AKT (Ser473), MMP-2 and VEGF protein expression levels; D: Relative protein expression of mTOR, AKT, MMP-2, p-AKT, p-AKT/AKT and VEGF. Data are shown as the means ± SE (n = 3). $^{a}P < 0.05$; $^{b}P < 0.01$. AKT: Protein kinase B; mTOR: Mammalian target of rapamycin; MMP-2: Matrix metalloproteinase 2; VEGF: Vascular endothelial growth factor; NC-shRNA: Negative control short hairpin RNA; OPN: Osteopontin.

PI3K/AKT signaling pathway is one of the most widely studied signaling pathways. Some studies have shown that the PI3K/AKT/mTOR signaling pathway is involved in the proliferation, invasion and metastasis of GC cells[34,35]. In an in vitro study of breast cancer, OPN expression was found to increase with the aggressiveness of the breast cancer phenotype. Knockdown of OPN may reduce breast cancer metastasis by regulating αv and β3 integrin expression and inhibiting PI3K/AKT/mTOR signaling pathway[39].

PKB/AKT, a serine/threonine (Ser/Thr) protein kinase, is the main effector downstream of PI3K and its activity is regulated by phosphorylation. P-AKT, the active form of AKT, affects a variety of cellular functions. Abnormal activation of AKT has been detected in a variety of malignant tumor cells[40].

In our study, western blot results showed that phosphorylation of AKT in SGC-7901 cells decreased after OPN silencing (Figure 4). This further supports the idea. Similarly, GC cell lines with stable overexpression of OPN were incubated with the PI3K inhibitor LY294002, and phosphorylation of AKT was significantly reduced (Figure 4). In conclusion, OPN may promote GC invasion and migration by activating PI3K/AKT/mTOR signaling pathway. To our knowledge, this study is the first to demonstrate a relevance between OPN and the PI3K/AKT signaling pathway in human GC cell lines.

ECM degradation is a key step in tumor invasion and migration and ECM degradation mainly depends on MMPs (such as MMP-2 and MMP-9), which bind to adhesion molecules and digest ECM-related components during cell migration, thus facilitating the movement of cancer cells[41]. MMP-2 is a proteolytic enzyme that mainly degrades type IV collagen, leading to destruction of basement membrane, infiltration of tumor cells into connective tissue matrix, infiltration of small blood vessels and lymphatic vessels, and thus metastasis[42]. MMPs are highly regulated by growth factors, cytokines and ECM proteins. OPN, as an ECM protein, can induce the production and activation of MMP-2 in cells, and the increased expression of MMP-2 further enhances the ability of tumor cells to digest ECM-related components, and ultimately leads to the promotion of tumor cell invasion and metastasis[43,25].

Tumor growth and metastasis depend on angiogenesis, and VEGF can induce angiogenesis[44,45]. It has been reported that increased VEGF expression can promote angiogenesis, thereby enhancing the ability of tumor cells to enter circulation and ultimately promote tumor metastasis to other organs[45]. Tang *et al*[29] found that OPN and VEGF were co-expressed in GC tissues, and their expression levels were significantly correlated with tumor node metastasis staging, lymph node metastasis and distant metastasis ($P < 0.05$). Xu *et al*[46] also studied the effect of OPN on VEGF expression in articular cartilage and found that OPN may directly up-regulate VEGF expression through PI3K/AKT and mitogen-activated protein kinase 1 pathways.

In this study, we found that mRNA and protein expression levels of MMP-2 and VEGF were inhibited in SGC-7901 cells treated with OPN knockdown and LY294002 inhibitor (Figures 3 and 4). Consequently, we speculate that OPN promoting the invasion and migration of GC SGC-7901 cells, which might be related to the increasing expression of MMP-2 and VEGF. The results of our study are similar to those of previous studies[47].

In our study, we detected a strange phenomenon that OPN was also expressed in normal gastric epithelial GES-1 cells (Figure 1A-C). This phenomenon may be related to the existence of OPN splicing variants (a, b, c). Studies have found that normal gastric GES-1 cells mainly express OPN-a subtype, and GC cell lines mainly express OPN-c subtype[48]. Another study also showed that OPN-c subtypes were overexpressed in GC and correlated with the prognosis of GC, while the other two subtypes were not associated with the progression of GC[31]. These studies may explain why OPN is expressed in GES-1 cells.

In this study, we preliminarily analyzed the effects of OPN on the proliferation, invasion and migration of GC cells, which is similar to previous literature. Our study further found that OPN knockdown and LY294002 inhibitor inhibited the activation of PI3K/AKT/mTOR pathway and down-regulated the mRNA and protein expression of MMP-2 and VEGF in GC-7901 cells, ultimately inhibiting the proliferation, invasion and migration of GC cells.

CONCLUSION

In conclusion, OPN may promote the progression of GC by activating PI3K/AKT/mTOR signaling pathway and up-regulating the expression of MMP-2 and VEGF. Our findings suggest that OPN is a new prognostic marker and potential therapeutic target for GC.

There are some limitations to our study. Due to the limitation of time and funds, the experimental design was somewhat simple, and only in vitro experiments were designed. In the future, with the support of further research funding, we hope to conduct some in vivo studies to verify this, such as animal trials. Although more remains to be learned about the mechanism, it is clear that OPN is a promising biomarker. OPN targeting therapy may be an effective way to overcome treatment failure and significantly enhance anti-tumor activity. Currently, several OPN inhibitors are under preclinical study for the treatment of solid tumors such as bowel cancer and lung cancer[49,50]. Although initial results from different OPN inhibitors are encouraging, clinical benefits remain to be demonstrated.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer (GC) is one of the most common malignant tumors. Osteopontin (OPN) is thought to be closely related to the occurrence, metastasis and prognosis of many types of tumors.

Research motivation

To search for a potential prognostic biomarker for GC as well as a potential therapeutic target.

Research objectives

The purpose of this study was to investigate the effects of OPN on the proliferation, invasion and migration of GC cells and its possible mechanism.

Research methods

The mRNA and protein expression of OPN in the GC cells were analyzed by real-time quantitative-reverse transcription and western blotting, and observe the effect of varying degree expression OPN on the proliferation and other behaviors of GC. Next, the effects of OPN knockdown on GC cells migration and invasion were examined. The short hairpin RNA (shRNA) and negative control shRNA targeting OPN-shRNA were transfected into the cells according to the manu-

facturer's instructions. Non transfected cells were classified as control in the identical transfecting process. 24 h after RNA transfection cell proliferation activity was detected by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide assay, and cell invasiveness and migration were detected by Trans well assay. Meanwhile, the expression of protein kinase B (AKT), matrix metalloproteinase 2 (MMP-2) and vascular endothelial growth factor (VEGF) in the human GC cell lines was detected by reverse transcription polymerase chain reaction and western blotting.

Research results

The results of this study revealed that OPN mRNA and protein expression levels were highly expressed in SGC-7901 cells. OPN knockdown by specific shRNA noticeably reduced the capabilities of proliferation, invasion and migration of SGC-7901 cells. Moreover, in the experiments of investigating the underlying mechanism, results showed that OPN knockdown could down- regulated the expression of MMP-2 and VEGF, it also decreased the phosphorylation of AKT. Meanwhile, the protein expression levels of MMP-2, VEGF and phosphorylated AKT was noticeable lower than that in control group in the GC cells after they were added to phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002).

Research conclusions

These results suggested that OPN though PI3K/AKT/mammalian target of rapamycin signal pathway to up-regulate MMP-2 and VEGF expression, which contribute SGC-7901 cells to proliferation, invasion and migration. Thus, our results demonstrate that OPN may serve as a novel prognostic biomarkers as well as a potential therapeutic targets for GC.

Research perspectives

The effects of OPN on proliferation, invasion and migration of GC cells were confirmed by preliminary evidence, which may be used as a prognostic biomarker and potential therapeutic target in the future.

FOOTNOTES

Author contributions: Qu FJ and Yuan XL designed this study; Qin YC and Yan X directed the experiment technology; Qin YC and Yu WW performed the experiments; Qin YC prepared the figures and drafted the manuscript; Qu FJ helped to revising of manuscript; All authors read and approved the final manuscript.

Supported by Liaoning Provincial Natural Science Foundation, No. 201602209.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of the Affiliated Dalian Third People's Hospital of Dalian Medical University.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Fan-Jie Qu 0000-0003-3977-5068.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **Wu CW**, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AF, Lui WY, Whang-Peng J. Nodal dissection for patients with gastric cancer: a randomised controlled trial. *Lancet Oncol* 2006; **7**: 309-315 [PMID: 16574546 DOI: 10.1016/S1470-2045(06)70623-4]

3 **Dassen AE**, Lemmens VE, van de Poll-Franse LV, Creemers GJ, Brenninkmeijer SJ, Lips DJ, Vd Wurff AA, Bosscha K, Coebergh JW. Trends in incidence, treatment and survival of gastric adenocarcinoma between 1990 and 2007: a population-based study in the Netherlands. *Eur J Cancer* 2010; **46**: 1101-1110 [PMID: 20219351 DOI: 10.1016/j.ejca.2010.02.013]

4 **Psyrri A**, Kalogeras KT, Wirtz RM, Kouvatseas G, Karayannopoulou G, Goussia A, Zagouri F, Veltrup E, Timotheadou E, Gogas H, Koutras A, Lazaridis G, Christodoulou C, Pentheroudakis G, Economopoulou P, Laskarakis A, Arapantoni-Dadioti P, Batistatou A, Sotiropoulou M, Aravantinos G, Papakostas P, Kosmidis P, Pectasides D, Fountzilas G. Association of osteopontin with specific prognostic factors and survival

in adjuvant breast cancer trials of the Hellenic Cooperative Oncology Group. *J Transl Med* 2017; **15**: 30 [PMID: 28193231 DOI: 10.1186/s12967-017-1134-7]

5 **Rizwan A**, Paidi SK, Zheng C, Cheng M, Barman I, Glunde K. Mapping the genetic basis of breast microcalcifications and their role in metastasis. *Sci Rep* 2018; **8**: 11067 [PMID: 30038419 DOI: 10.1038/s41598-018-29330-9]

6 **Yan CH**, Lv M, Li H, Song X, Yan F, Cao S, Ren X. Osteopontin is a novel prognostic biomarker in early-stage non-small cell lung cancer after surgical resection. *J Cancer Res Clin Oncol* 2015; **141**: 1371-1378 [PMID: 25555465 DOI: 10.1007/s00432-014-1902-1]

7 **Hao C**, Cui Y, Chang S, Huang J, Birkin E, Hu M, Zhi X, Li W, Zhang L, Cheng S, Jiang WG. OPN promotes the aggressiveness of non-small-cell lung cancer cells through the activation of the RON tyrosine kinase. *Sci Rep* 2019; **9**: 18101 [PMID: 31792339 DOI: 10.1038/s41598-019-54843-2]

8 **Xu ST**, Guo C, Ding X, Fan WJ, Zhang FH, Xu WL, Ma YC. Role of osteopontin in the regulation of human bladder cancer proliferation and migration in T24 cells. *Mol Med Rep* 2015; **11**: 3701-3707 [PMID: 25591934 DOI: 10.3892/mmr.2015.3202]

9 **Tilli TM**, Bellahcène A, Castronovo V, Gimba ER. Changes in the transcriptional profile in response to overexpression of the osteopontin-c splice isoform in ovarian (OvCar-3) and prostate (PC-3) cancer cell lines. *BMC Cancer* 2014; **14**: 433 [PMID: 24928374 DOI: 10.1186/1471-2407-14-433]

10 **Song JY**, Lee JK, Lee NW, Yeom BW, Kim SH, Lee KW. Osteopontin expression correlates with invasiveness in cervical cancer. *Aust N Z J Obstet Gynaecol* 2009; **49**: 434-438 [PMID: 19694703 DOI: 10.1111/j.1479-828X.2009.01027.x]

11 **Xu C**, Li H, Yin M, Yang T, An L, Yang G. Osteopontin is involved in TLR4 pathway contributing to ovarian cancer cell proliferation and metastasis. *Oncotarget* 2017; **8**: 98394-98404 [PMID: 29228698 DOI: 10.18632/oncotarget.21844]

12 **Qin X**, Yan M, Wang X, Xu Q, Zhu X, Shi J, Li Z, Zhang J, Chen W. Cancer-associated Fibroblast-derived IL-6 Promotes Head and Neck Cancer Progression *via* the Osteopontin-NF-kappa B Signaling Pathway. *Theranostics* 2018; **8**: 921-940 [PMID: 29463991 DOI: 10.7150/thno.22182]

13 **Liang S**, Li Y, Wang B. The cancer-related transcription factor Runx2 combined with osteopontin: a novel prognostic biomarker in resected osteosarcoma. *Int J Clin Oncol* 2021; **26**: 2347-2354 [PMID: 34546483 DOI: 10.1007/s10147-021-02025-4]

14 **Kolb A**, Kleeff J, Guweidhi A, Esposito I, Giese NA, Adwan H, Giese T, Büchler MW, Berger MR, Friess H. Osteopontin influences the invasiveness of pancreatic cancer cells and is increased in neoplastic and inflammatory conditions. *Cancer Biol Ther* 2005; **4**: 740-746 [PMID: 15970685 DOI: 10.4161/cbt.4.7.1821]

15 **Cao DX**, Li ZJ, Jiang XO, Lum YL, Khin E, Lee NP, Wu GH, Luk JM. Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. *World J Gastroenterol* 2012; **18**: 3923-3930 [PMID: 22912540 DOI: 10.3748/wjg.v18.i30.3923]

16 **Ng L**, Wan T, Chow A, Iyer D, Man J, Chen G, Yau TC, Lo O, Foo CC, Poon JT, Poon RT, Pang R, Law WL. Osteopontin Overexpression Induced Tumor Progression and Chemoresistance to Oxaliplatin through Induction of Stem-Like Properties in Human Colorectal Cancer. *Stem Cells Int* 2015; **2015**: 247892 [PMID: 26106421 DOI: 10.1155/2015/247892]

17 **Lee SH**, Park JW, Woo SH, Go DM, Kwon HJ, Jang JJ, Kim DY. Suppression of osteopontin inhibits chemically induced hepatic carcinogenesis by induction of apoptosis in mice. *Oncotarget* 2016; **7**: 87219-87231 [PMID: 27888617 DOI: 10.18632/oncotarget.13529]

18 **Loosen SH**, Roderburg C, Kauertz KL, Pombeiro I, Leyh C, Benz F, Vucur M, Longerich T, Koch A, Braunschweig T, Ulmer TF, Heidenhain C, Tacke F, Binnebösel M, Schmeding M, Trautwein C, Neumann UP, Luedde T. Elevated levels of circulating osteopontin are associated with a poor survival after resection of cholangiocarcinoma. *J Hepatol* 2017; **67**: 749-757 [PMID: 28668580 DOI: 10.1016/j.jhep.2017.06.020]

19 **Bandopadhyay M**, Bulbule A, Butti R, Chakraborty G, Ghorpade P, Ghosh P, Gorain M, Kale S, Kumar D, Kumar S, Totakura KV, Roy G, Sharma P, Shetti D, Soundararajan G, Thorat D, Tomar D, Nalukurthi R, Raja R, Mishra R, Yadav AS, Kundu GC. Osteopontin as a therapeutic target for cancer. *Expert Opin Ther Targets* 2014; **18**: 883-895 [PMID: 24899149 DOI: 10.1517/14728222.2014.925447]

20 **Shevde LA**, Samant RS. Role of osteopontin in the pathophysiology of cancer. *Matrix Biol* 2014; **37**: 131-141 [PMID: 24657887 DOI: 10.1016/j.matbio.2014.03.001]

21 **Shi L**, Wang X. Role of osteopontin in lung cancer evolution and heterogeneity. *Semin Cell Dev Biol* 2017; **64**: 40-47 [PMID: 27578008 DOI: 10.1016/j.semcdb.2016.08.032]

22 **Zhao H**, Chen Q, Alam A, Cui J, Suen KC, Soo AP, Eguchi S, Gu J, Ma D. The role of osteopontin in the progression of solid organ tumour. *Cell Death Dis* 2018; **9**: 356 [PMID: 29500465 DOI: 10.1038/s41419-018-0391-6]

23 **Wu CY**, Wu MS, Chiang EP, Wu CC, Chen YJ, Chen CJ, Chi NH, Chen GH, Lin JT. Elevated plasma osteopontin associated with gastric cancer development, invasion and survival. *Gut* 2007; **56**: 782-789 [PMID: 17148500 DOI: 10.1136/gut.2006.109868]

24 **Dai N**, Bao Q, Lu A, Li J. Protein expression of osteopontin in tumor tissues is an independent prognostic indicator in gastric cancer. *Oncology* 2007; **72**: 89-96 [PMID: 18004082 DOI: 10.1159/000111108]

25 **Song G**, Ouyang G, Mao Y, Ming Y, Bao S, Hu T. Osteopontin promotes gastric cancer metastasis by augmenting cell survival and invasion through Akt-mediated HIF-1alpha up-regulation and MMP9 activation. *J Cell Mol Med* 2009; **13**: 1706-1718 [PMID: 19602039 DOI: 10.1111/j.1582-4934.2008.00540.x]

26 **Imano M**, Satou T, Itoh T, Sakai K, Ishimaru E, Yasuda A, Peng YF, Shinkai M, Akai F, Yasuda T, Imamoto H, Okuno K, Ito H, Shiozaki H, Ohyanagi H. Immunohistochemical expression of osteopontin in gastric cancer. *J Gastrointest Surg* 2009; **13**: 1577-1582 [PMID: 19582521 DOI: 10.1007/s11605-009-0955-y]

27 **Di Bartolomeo M**, Pietrantonio F, Pellegrinelli A, Martinetti A, Mariani L, Daidone MG, Bajetta E, Pelosi G, de Braud F, Floriani I, Miceli R. Osteopontin, E-cadherin, and β-catenin expression as prognostic biomarkers in patients with radically resected gastric cancer. *Gastric Cancer* 2016; **19**: 412-420 [PMID: 25862567 DOI: 10.1007/s10120-015-0495-y]

28 **Yazici O**, Dogan M, Ozal G, Aktas SH, Demirkazik A, Utkan G, Senler FC, Icli F, Akbulut H. Osteopontin is a Prognostic Factor in Patients with Advanced Gastric Cancer. *Comb Chem High Throughput Screen* 2021; **24**: 1229-1235 [PMID: 32881660 DOI: 10.2174/1386207323666200902135349]

29 **Tang H**, Wang J, Bai F, Zhai H, Gao J, Hong L, Xie H, Zhang F, Lan M, Yao W, Liu J, Wu K, Fan D. Positive correlation of osteopontin, cyclooxygenase-2 and vascular endothelial growth factor in gastric cancer. *Cancer Invest* 2008; **26**: 60-67 [PMID: 18181047 DOI: 10.1080/07357900701519279]

30 **Yu WW**, Wang H, Zhang J, Miao JF, Kong Y, Qu FJ. Expression of osteoblastin, matrix metalloproteinase 2 and vascular endothelial growth factor in gastric cancer tissues and its prognostic value. *Zhongliu YanjiuYu Linchuang* 2019; **031**: 390-394 [DOI: 10.3760/cma.j.issn.1006-9801.2019.06.007]

31 **Sun X**, Wang L, Hou W, Li Y, Liu L, Zuo W, Yu J. [Expression of osteopontin splice variant and its clinical significance in gastric cancer]. *Zhonghua Zhong Liu Za Zhi* 2015; **37**: 427-430 [PMID: 26463145]

32 **Gu X**, Gao XS, Ma M, Qin S, Qi X, Li X, Sun S, Yu H, Wang W, Zhou D. Prognostic significance of osteopontin expression in gastric cancer: a meta-analysis. *Oncotarget* 2016; **7**: 69666-69673 [PMID: 27626167 DOI: 10.18632/oncotarget.11936]

33 **Aoki M**, Fujishita T. Oncogenic Roles of the PI3K/AKT/mTOR Axis. *Curr Top Microbiol Immunol* 2017; **407**: 153-189 [PMID: 28550454 DOI: 10.1007/82_2017_6]

34 **Singh SS**, Yap WN, Arfuso F, Kar S, Wang C, Cai W, Dharmarajan AM, Sethi G, Kumar AP. Targeting the PI3K/Akt signaling pathway in gastric carcinoma: A reality for personalized medicine? *World J Gastroenterol* 2015; **21**: 12261-12273 [PMID: 26604635 DOI: 10.3748/wjg.v21.i43.12261]

35 **Riquelme I**, Tapia O, Espinoza JA, Leal P, Buchegger K, Sandoval A, Bizama C, Araya JC, Peek RM, Roa JC. The Gene Expression Status of the PI3K/AKT/mTOR Pathway in Gastric Cancer Tissues and Cell Lines. *Pathol Oncol Res* 2016; **22**: 797-805 [PMID: 27156070 DOI: 10.1007/s12253-016-0066-5]

36 **Wang ZM**, Cui YH, Li W, Chen SY, Liu TS. Lentiviral-mediated siRNA targeted against osteopontin suppresses the growth and metastasis of gastric cancer cells. *Oncol Rep* 2011; **25**: 997-1003 [PMID: 21286666 DOI: 10.3892/or.2011.1168]

37 **Park JW**, Lee SH, Go du M, Kim HK, Kwon HJ, Kim DY. Osteopontin depletion decreases inflammation and gastric epithelial proliferation during Helicobacter pylori infection in mice. *Lab Invest* 2015; **95**: 660-671 [PMID: 25867766 DOI: 10.1038/labinvest.2015.47]

38 **Rangaswami H**, Bulbule A, Kundu GC. Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol* 2006; **16**: 79-87 [PMID: 16406521 DOI: 10.1016/j.tcb.2005.12.005]

39 **Zhang H**, Guo M, Chen JH, Wang Z, Du XF, Liu PX, Li WH. Osteopontin knockdown inhibits αv,β3 integrin-induced cell migration and invasion and promotes apoptosis of breast cancer cells by inducing autophagy and inactivating the PI3K/Akt/mTOR pathway. *Cell Physiol Biochem* 2014; **33**: 991-1002 [PMID: 24714122 DOI: 10.1159/000358670]

40 **Vivanco I**, Sawyers C L. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nature Reviews Cancer* 2002; **2**: 489-501 [DOI: 10.1038/nrc839]

41 **Jabłońska-Trypuć A**, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *J Enzyme Inhib Med Chem* 2016; **31**: 177-183 [PMID: 27028474 DOI: 10.3109/14756366.2016.1161620]

42 **Liotta LA**, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991; **64**: 327-336 [PMID: 1703045 DOI: 10.1016/0092-8674(91)90642-c]

43 **Philip S**, Bulbule A, Kundu GC. Osteopontin stimulates tumor growth and activation of promatrix metalloproteinase-2 through nuclear factor-kappa B-mediated induction of membrane type 1 matrix metalloproteinase in murine melanoma cells. *J Biol Chem* 2001; **276**: 44926-44935 [PMID: 11564733 DOI: 10.1074/jbc.M103334200]

44 **Holash J**, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* 1999; **284**: 1994-1998 [PMID: 10373119 DOI: 10.1126/science.284.5422.1994]

45 **Niu G**, Chen X. Vascular endothelial growth factor as an anti-angiogenic target for cancer therapy. *Curr Drug Targets* 2010; **11**: 1000-1017 [PMID: 20426765 DOI: 10.2174/138945010791591395]

46 **Xu J**, Yi Y, Li L, Zhang W, Wang J. Osteopontin induces vascular endothelial growth factor expression in articular cartilage through PI3K/AKT and ERK1/2 signaling. *Mol Med Rep* 2015; **12**: 4708-4712 [PMID: 26099282 DOI: 10.3892/mmr.2015.3975]

47 **Liu J**, Liu Q, Wan Y, Zhao Z, Yu H, Luo H, Tang Z. Osteopontin promotes the progression of gastric cancer through the NF-κB pathway regulated by the MAPK and PI3K. *Int J Oncol* 2014; **45**: 282-290 [PMID: 24756245 DOI: 10.3892/ijo.2014.2393]

48 **Tang X**, Li J, Yu B, Su L, Yu Y, Yan M, Liu B, Zhu Z. Osteopontin splice variants differentially exert clinicopathological features and biological functions in gastric cancer. *Int J Biol Sci* 2013; **9**: 55-66 [PMID: 23289017 DOI: 10.7150/ijbs.5280]

49 **Zagani R**, Hamzaoui N, Cacheux W, de Reyniès A, Terris B, Chaussade S, Romagnolo B, Perret C, Lamarque D. Cyclooxygenase-2 inhibitors down-regulate osteopontin and Nr4A2-new therapeutic targets for colorectal cancers. *Gastroenterology* 2009; **137**: 1358-66.e1 [PMID: 19549529 DOI: 10.1053/j.gastro.2009.06.039]

50 **Zhang J**, Yamada O, Kida S, Matsushita Y, Murase S, Hattori T, Kubohara Y, Kikuchi H, Oshima Y. Identification of brefelamide as a novel inhibitor of osteopontin that suppresses invasion of A549 lung cancer cells. *Oncol Rep* 2016; **36**: 2357-2364 [PMID: 27498705 DOI: 10.3892/or.2016.5006]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1556-1566

DOI: 10.4251/wjgo.v15.i9.1556

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

MicroRNA-363-3p inhibits colorectal cancer progression by targeting interferon-induced transmembrane protein 1

Yun Wang, Shao-Kai Bai, Tao Zhang, Cheng-Gong Liao

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Chuang WL, Taiwan; Grassi G, Italy; Janvilisri T, Thailand; Sato H, Japan; Stojanovic B, Serbia

Received: May 5, 2023
Peer-review started: May 5, 2023
First decision: July 9, 2023
Revised: July 21, 2023
Accepted: August 18, 2023
Article in press: August 18, 2023
Published online: September 15, 2023

Yun Wang, Shao-Kai Bai, Tao Zhang, Cheng-Gong Liao, Department of Oncology, Tangdu Hospital, Air Force Medical University, Xi'an 710038, Shaanxi Province, China

Corresponding author: Cheng-Gong Liao, PhD, Chief Physician, Department of Oncology, Tangdu Hospital, Air Force Medical University, No. 569 Xinsi Road, Xi'an 710038, Shaanxi Province, China. liaochenggong@163.com

Abstract

BACKGROUND

The molecular mechanisms of colorectal cancer development and progression are far from being elucidated.

AIM

To investigate the role of microRNA-363-3p (miR-363-3p) in the progression of colorectal cancer.

METHODS

Real-time polymerase chain reaction was performed to detect miRNA expression in human colorectal cancer tissues and paired normal colorectal tissues. PITA 6 was utilized to predict the targets of miR-363-3p. Dual-luciferase reporter system was used to validate the target of miR-363-3p. Plate colony formation assay and wound-healing assay were performed to evaluate cancer cells' clonogenic survival ability and migration ability, respectively. Cell proliferation was examined by cell counting kit-8 assay. Immunohistochemical staining was used to determine the expression level of interferon-induced transmembrane protein 1 (IFITM1) in colorectal cancer tissues and adjacent tissues. The TCGA and GTEx databases were used to compare the expression levels of *IFITM1* mRNA in colorectal cancer tissues and normal colorectal tissues and analyze the correlation between the expression levels of IFITM1 mRNA and overall survival and disease-free survival of patients. A colorectal cancer cell line with a deficiency of IFITM1 was constructed, and the regulation effect of IFITM1 on the clonogenic growth of colorectal cancer cells was clarified.

RESULTS

MiR-363-3p was decreased in colorectal cancer tissues compared to normal colorectal tissues. IFITM1 was characterized as a direct target of miR-363-3p. Overexpression of miR-363-3p led to decreased clonogenic survival, proliferation, and migration of colorectal cancer cells, which could be reversed by forced

IFITM1 expression.

CONCLUSION
MiR-363-3p can constrain clonogenic survival, proliferation, and migration of colorectal cancer cells *via* targeting IFITM1.

Key Words: MicroRNA-363-3p; Proliferation; Clonogenic survival; Colorectal cancer; Interferon-induced transmembrane protein 1

Core Tip: MicroRNAs (miRNAs) have been implicated in almost all known cancer processes. Although many algorithms can predict target genes for miRNA, the exact regulatory relationships still need to be experimentally verified. In this study, we investigated the role of miR-363-3p in clonogenic survival, proliferation, and migration of colorectal cancer cells and interferon-induced transmembrane protein 1 (IFITM1) was identified as a direct target of miR-363-3p. These findings widen and deepen the understanding of the molecular function of miR-363-3p and IFITM1.

Citation: Wang Y, Bai SK, Zhang T, Liao CG. MicroRNA-363-3p inhibits colorectal cancer progression by targeting interferon-induced transmembrane protein 1. *World J Gastrointest Oncol* 2023; 15(9): 1556-1566
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1556.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1556

INTRODUCTION

Colorectal cancer is the third most common cancer type worldwide; in 2020, almost 2 million cases were diagnosed. Colorectal cancer is the second most common cause of cancer death, leading to almost 1 million deaths per year, accounting for approximately 10% of new tumor cases and deaths[1]. In 2019, China (607900), the United States (227242), and Japan (160211) had the highest number of new cases of colorectal cancer, and China (261777), India (79098), and the United States (84026) had the highest number of colorectal cancer deaths[2]. Low-dairy diets (15.6%), smoking (13.3%), low-calcium diets (12.9%), and alcohol consumption (9.9%) are important risk factors for colorectal cancer[2], but the molecular mechanisms of colorectal cancer development and progression are far from being elucidated.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs (ncRNAs) of about 22 nucleotides in size. miRNAs play important roles in gene regulation *via* a posttranscriptional manner, and their dysregulation is implicated in various human diseases including cancer. It is estimated that miRNAs can target more than 60% of human protein-coding genes [3]. Mechanically, miRNAs prevent the translation of target mRNAs that are then sequestered into mRNA-processing bodies (P-bodies) and degraded. MiRNAs also contribute to the degradation of the target mRNAs without sequestration to P-bodies[4]. The specificity of miRNA - mRNA interaction is bestowed mainly by a miRNA's first eight nucleotides (known as seed sequence)[5]. Over the past period, miRNAs have been implicated in almost all known cancer processes. Depending on the target gene and tumor type, some miRNAs typically negatively affect oncogenes encoding proteins, while some other miRNAs can inhibit known tumor suppressors, so miRNAs can act as onco-miRNAs or tumor suppressor miRNAs[6]. For example, miR-100 and miR-125b coordinately repressed five Wnt/β-catenin negative regulators, resulting in increased Wnt signaling in colorectal cancer[7]. MiR-146a targets PTGES2 and suppresses colorectal cancer[8]. Recently, miR-363-3p was reported to participate in the regulation of a variety of diseases. In addition, the downregulation of miR-363-3p is closely correlated with the degree of differentiation, tumor-node-metastasis stage, and lymph node metastasis in gastric cancer[9]. Overexpression of miR-363-3p is a strong predictor of favorable prognosis in adenocarcinoma of the uterine cervix[10]. MiR-363-3p suppresses tumor growth and metastasis of colorectal cancer *via* targeting sphingosine kinase 2[11] and SRY-related high-mobility-group box 4 (SOX4)[12]. In contrast, the expression of miR-363-3p was increased in glioma[13] and pediatric T-cell acute lymphoblastic leukemia [14]. MiR-363-3p functions as onco-miRNA promotes cell proliferation, protects against apoptosis, and enhances invasion by directly targeting PDHB in glioma[13] and PTEN and BIM in leukemic cells[14]. Based on previous studies, we speculated that miR-363-3p might exert an essential effect on colorectal cancer progression.

Interferon-induced transmembrane protein 1 (IFITM1), also known as DSPA2a and CD225, is a member of the interferon-induced transmembrane protein family. Friedman *et al*[15] first identified the IFITM1 gene in neuroblastoma cells. The IFITM1-coding gene is located at 11p15.5, and the IFITM1 protein consists of 125 amino acid residues with a molecular weight of about 13.96 kDa, including the C-terminal extracellular domain, two transmembrane domains, and N-terminal intracellular domains. Li *et al*[16] found higher levels of IFITM1 expression in gallbladder adenocarcinoma and adenosquamous cell carcinoma tissues with high tumor-node-metastasis (TNM) stage and with lymph node metastasis and invasion. In estrogen receptor (ER)-positive breast cancer, IFITM1 expression levels are associated with TNM staging and poor prognosis[17]. Therefore, IFITM1 is closely related to the occurrence and development of tumors, but the regulation and clinical significance of IFITM1 in colorectal cancer tissues still need to be studied in depth. In this

study, we investigated the role and the underlying mechanisms of miR-363-3p in the clonogenic survival, proliferation, and migration of colorectal cancer cells. To our knowledge, this is the first study to identify IFITM1 as a direct target of miR-363-3p.

MATERIALS AND METHODS

Cell culture

Human colorectal cancer cell lines SW480, SW1116, Colo320, and Caco-2 were obtained from American Type Culture Collection and cultured in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, United States) containing 10% fetal bovine serum (Thermo Fisher Scientific) and Penicillin-Streptomycin (Thermo Fisher Scientific). IFITM1 knockout SW480 cell line (SW480KO) was generated using the CRISPR/Cas9 system. gRNA targeting sequence was 5′-CCGCTGT-GGTGTCCGGATGC-3′. SW480 cells were transfected with PX459 V2.0 containing the gRNA sequence using Lipofectamine 2000 (Invitrogen, Waltham, MA). After 48 h of transfection, the positive cells were selected with puromycin at 2 μg/mL for 5 d. The puromycin-resistant cells were seeded into a 96-well plate at one cell per well using CytoFLEX SRT (Beckman, Brea, CA). The knockout cells were confirmed by western blotting. All cultures were maintained at 5% CO_2 and 37 °C.

Tissue samples

Colorectal cancer tissues and adjacent normal tissues were obtained from patients at Tangdu Hospital, Air Force Medical University. All human individuals provided written informed consent. The study was approved by the Hospital Ethics Committee (202203-116). All participants (aged 42-76 years, 60% males, stages ranging from I to IVA) did not receive chemotherapy or radiation therapy before resection. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Agomir/antagomir transfection

Agomir-363-3p, agomir-NC, antagomir-363-3p and antagomir-NC were obtained from RiboBio (Guangzhou, China). Cells in the logarithmic growth phase were trypsinized, resuspended, and seeded into 6-well plates. After being cultured overnight, cells were transfected with 75 pmol agomir or antagomir using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 48 h, the cells were collected for subsequent analysis.

RNA isolation, cDNA transcription, and quantitative polymerase chain reaction

Total RNA was extracted using a Trizol reagent (Life Technologies, Carlsbad, CA) based on the supplier's instruction. MiRNA was reversely transcribed using the miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). mRNA was reversely transcribed using the SuperScript™ IV First-Strand Synthesis System with ezDNase™ Enzyme (Thermo Fisher Scientific). Quantitative polymerase chain reaction (qPCR) with specific primers was performed with the SYBR kit (TaKaRa, Shiga, Japan). Samples were normalized to housekeeping expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or snRNA U6 using the $2^{-\Delta\Delta Ct}$ method. The sequences of primers were as follows: IFITM1 sense, 5′-CCAAGGTCCACCGTGATTAAC-3′; antisense, 5′-ACCAGTTCAAGAAGAGGGTGTT-3′; GAPDH sense, 5′-GCACCGT-CAAGGCTGAGAAC-3′; antisense, 5′-TGGTGAAGACGCCAGTGGA-3′; miRNA363-3p sense, 5′-AATTGCACG-GTATCCA-3′; antisense, 5′-AGTGCAGGGTCCGAGGTATT-3′; snRNA U6 sense, 5′-CTCGCTTCGGCAGCACA-3′; antisense, 5′-AACGCTTCACGAATTTGCGT-3′.

Western blotting

Cells were lysed with RIPA lysis buffer containing protease inhibitors. Protein concentration was determined using a BCA kit (Thermo Fisher Scientific). The proteins were separated using standard gel electrophoresis and blotted onto polyvinylidene fluoride membranes. Membranes were blocked with 5% nonfat milk in poly(butylene succinate-butylene terephthalate) (PBST) and incubated in primary antibody solution at 4 °C overnight. After being washed with PBST three times, the membranes were incubated with a secondary antibody at 24 °C for 45 min. Immunoblots were developed using an ECL-chemiluminescence Kit (Merck Millipore, Watford, United Kingdom), according to the manufacturer's instructions. The primary antibody against IFITM1 (5B5E2) was obtained from Proteintech (Wuhan, China). α-tubulin was used as loading control and its antibody was purchased from Cell Signaling Technology (Danvers, MA). HRP-linked secondary antibodies were obtained from Thermo Fisher Scientific. The raw blots have been included in Supplementary Figure 1.

Dual-luciferase reporter assay

The predicted binding region of miR-363-3p in IFITM1 3′ untranslated region (UTR) (pGL3-wt) or mutated targeting sequence (pGL3-mt) was ligated into the pGL3-Basic vector. SW480 cells were transfected with pGL3 construct and pRL-TK (ratio of 50 to 1) using Lipofectamine 2000 (Invitrogen). Luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega, Madison, WI) according to the manufacturer's instructions. The relative luciferase activity was calculated by dividing the results from the Firefly luciferase assay over the Renilla luciferase assay.

Plate colony formation assay

Cells were digested to prepare single-cell suspensions and seeded at 300 cells/2 mL in 6-well plates. Cells were cultured

for about 2 wk until visible clones formed. The clones were fixed with 4% paraformaldehyde at 24 °C for 15 min. After being rinsed with phosphate buffered saline (PBS) three times, the clones were stained with Coomassie brilliant blue R250 at 24 °C for 20 min. The plates were washed with PBS several times to remove the residual dye. The clones in each well were counted.

Wound-healing assay

Cells were seeded at 3×10^5 cells per well in 24-well plates. After the cells became confluent, a 10 μL pipette tip was used to wound the monolayer by scratching and the cells in suspension were removed by changing the medium. With a cell-free gap prepared, a series of time-lapse images were acquired as cells migrated into the cell-free gap. The change in the wound width (the average distance between the two margins of the scratch) was measured using ImageJ.

Cell proliferation assay

Cell proliferation was determined using cell counting kit-8 (CCK-8, Solarbio, Beijing, China). Cells were digested to prepare single-cell suspensions and seeded at a density of 2×10^3 cells/100 μL in 96-well plates. 10 μL of the CCK-8 solution was added to each well of the plate. After incubating the plate for 2 h in the incubator, the absorbance at 450 nm was measured using a microplate reader (Fluoroskan FL, Thermo Fisher Scientific). Subtraction of the blank well absorbance (absorbance of wells containing medium and CCK-8) was performed before analysis.

Immunohistochemical staining

Sections 5 μm in thickness were prepared from formalin-fixed, paraffin-embedded tissues. Paraffin sections were deparaffinized, hydrated followed by a Tris-EDTA-based antigen retrieval step, and blocked against non-specific binding using normal goat serum (Cell Signaling Technology) followed by incubation using an anti-IFITM1 antibody (5B5E2, Proteintech) at 4 °C overnight. After being washed for 20 min, the sections were treated with universal biotinylated anti-mouse/rabbit/goat IgG derived from the horse (Vector Laboratories, Burlingame, CA) at 24 °C for 30 min. Signal development was performed using the NovaRED kit (Vector Laboratories) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (San Diego, CA, United States). Student's *t*-test was used to compare the two groups. The One-Way ANOVA was used to compare the means across three or more groups. Survival analysis was performed using the Kaplan-Meier method and compared using the log-rank test. Immunohistochemical (IHC) score was analyzed using a χ^2 test. Statistical significance was considered at $P < 0.05$.

RESULTS

MiR-363-3p is downregulated in human colorectal cancer tissues

We first determined the expression pattern of miR-363-3p in 50 colorectal cancer tissues and paired normal colorectal tissues using real-time PCR. As shown in Figure 1A, we found that the expression of miR-363-3p was decreased in colorectal cancer tissues compared to the paired normal colorectal tissues.

MiR-363-3p inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells

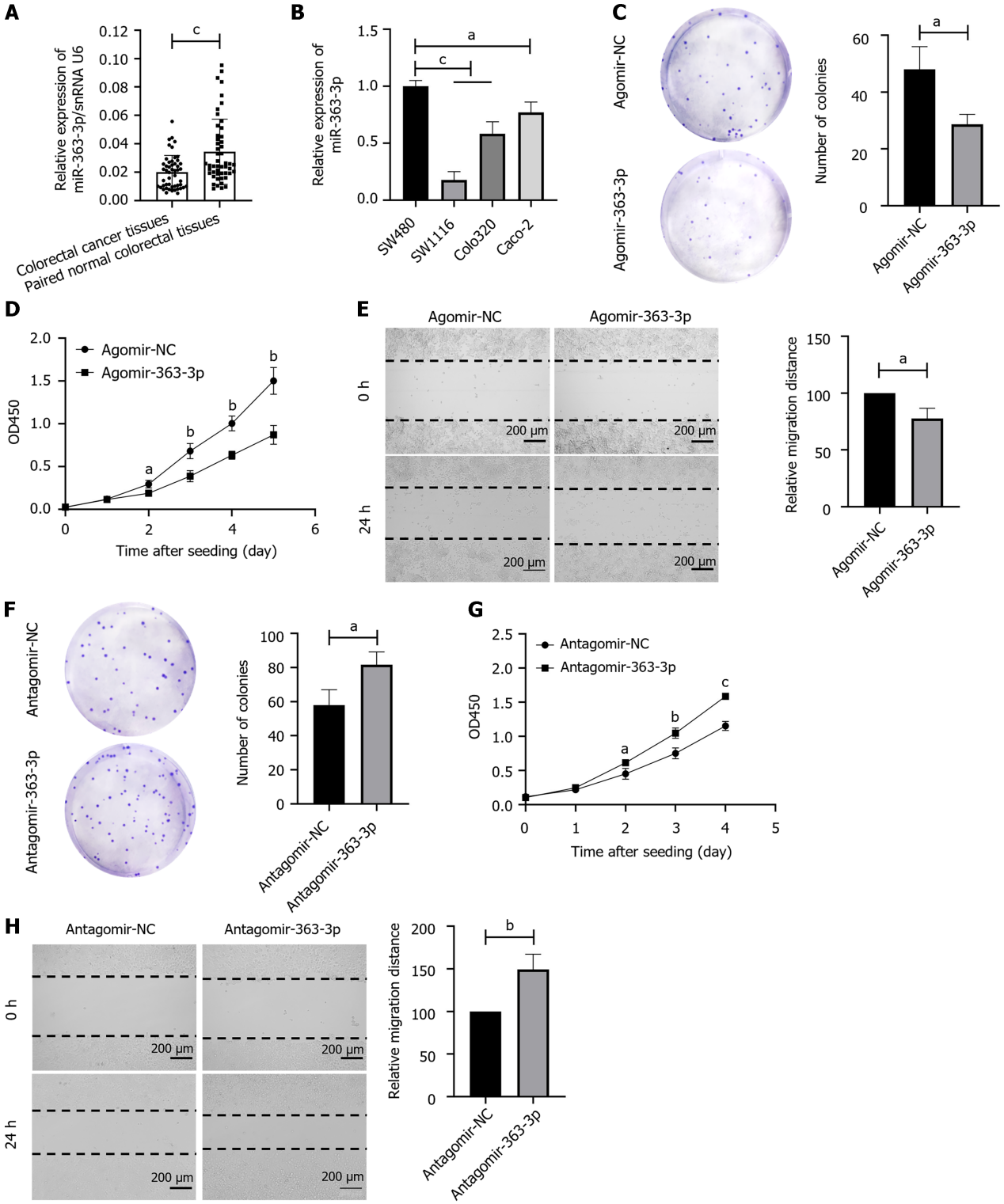
To determine the effect of miR-363-3p on colorectal cancer progression, we first determined the expression level of miR-363-3p in human colorectal cancer cell lines (Figure 1B). As SW480 and SW1116 had the highest and lowest expression of miR-363-3p, respectively, the two cell lines were used in the subsequent experiments. We transfected SW1116 cells with agomir-363-3p and found that overexpression of miR-363-3p led to decreased clonogenic survival (Figure 1C), proliferation (Figure 1D), and migration (Figure 1E). In contrast, transfection of SW480 cells with antagomir-363-3p resulted in enhanced clonogenic survival (Figure 1F), proliferation (Figure 1G), and migration (Figure 1H). All these data suggest that miR-363-3p is a suppressive player involved in colorectal cancer progression.

MiR-363-3p directly targets IFITM1

MiRNAs are supposed to regulate various cellular behaviors by targeting specific sites in mammalian mRNAs. Using PITA 6[18], IFITM1 was predicted as a promising target for miR-363-3p (Figure 2A). We found that overexpression of agomir-363-3p in SW1116 cells significantly decreased IFITM1 expression at both mRNA and protein levels (Figures 2B and C). As expected, inhibiting miR-363-3p *via* transfecting SW480 cells with antagomir-363-3p increased IFITM1 expression (Figures 2D and E). To examine the interaction between miR-363-3p and its targeting site in IFITM1 mRNA, luciferase reporter gene assays using constructs containing the predicted targeting sequence (pGL3-wt) and mutated targeting sequence (pGL3-mt) were performed. We found that co-transfection of agomir-363-3p and pGL3-wt in SW1116 cells led to decreased luciferase activity compared with the scramble control (Figure 2F), while co-transfection of agomir-363-3p and pGL3-mt in SW1116 cells showed luciferase activity comparable to that of the scramble control (Figure 2G). All these results demonstrate that IFITM1 is a direct target of miR-363-3p.

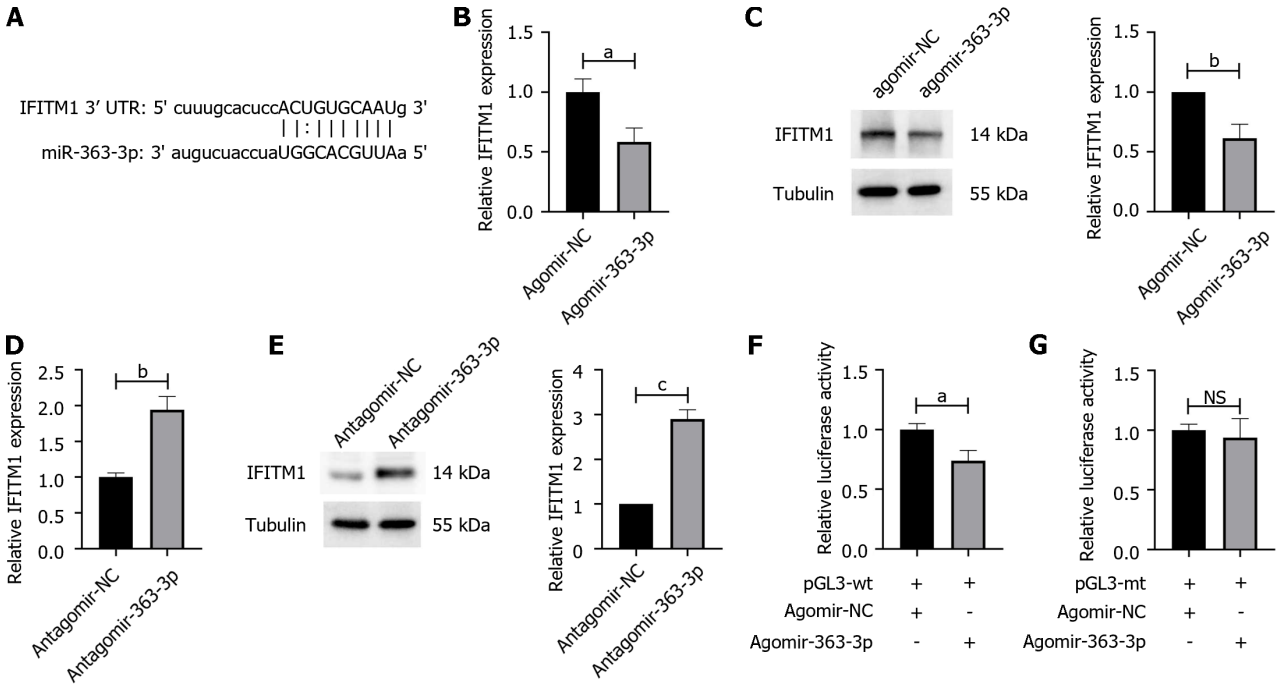
IFITM1 mediates the regulatory effects of miR-363-3p on colorectal cancer progression

As miR-363-3p modulates IFITM1 expression, next we determined whether IFITM1 contributed to the regulatory effects of miR-363-3p on colorectal cancer progression. We generated IFITM1 knockout SW480 cell line (SW480KO) using the



DOI: 10.4251/wjgo.v15.i9.1556 **Copyright** ©The Author(s) 2023.

Figure 1 MicroRNA-363-3p is downregulated in colorectal cancer tissues and inhibits clonogenic survival, proliferation and migration of colorectal cancer cells. A: Quantitative polymerase chain reaction (qPCR) analysis of microRNA-363-3p (miR-363-3p) expression in colorectal cancer tissues and paired normal colorectal tissues; B: qPCR analysis of miR-363-3p expression in human colorectal cancer cell lines; C: Representative images of clonogenic survival of SW1116 cells transfected with agomir-363-3p or agomir-NC. The graph shows the number of colonies; D: Proliferation curve of SW1116 cells transfected with agomir-363-3p or agomir-NC; E: Representative images of the gaps at 0 and 24 h after scratching. SW1116 cells were transfected with agomir-363-3p or agomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm; F: Representative images of clonogenic survival of SW480 cells transfected with antagomir-363-3p or antagomir-NC. The graph shows the number of colonies; G: Proliferation curve of SW480 cells transfected with antagomir-363-3p or antagomir-NC; H: Representative images of the gaps at 0 and 24 h after scratching. SW480 cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm. The results were shown as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. miR-363-3p: MicroRNA-363-3p; NC: Negative control.



DOI: 10.4251/wjgo.v15.i9.1556 **Copyright** ©The Author(s) 2023.

Figure 2 Interferon-induced transmembrane protein 1 is a direct target of microRNA-363-3p. A: The complementary sequences of microRNA-363-3p were identified in 3' untranslated region of interferon-induced transmembrane protein 1 (*IFITM1*) mRNA; B: Quantitative polymerase chain reaction (qPCR) analysis of *IFITM1* mRNA expression in SW1116 cells transfected with agomir-363-3p or agomir-NC; C: Western blot analysis of IFITM1 expression in SW1116 cells transfected with agomir-363-3p or agomir-NC. Graph shows quantification of relative levels of IFITM1 expression; D: qPCR analysis of *IFITM1* mRNA expression in SW480 cells transfected with antagomir-363-3p or antagomir-NC; E: Western blot analysis of IFITM1 expression in SW480 cells transfected with antagomir-363-3p or antagomir-NC. Graph shows quantification of relative levels of IFITM1 expression; F and G: Luciferase reporter gene assays using constructs containing the predicted targeting sequence (pGL3-wt) and mutated targeting sequence (pGL3-mt). SW1116 cells were transfected with the indicated constructs. The results were shown as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. UTR: Untranslated region; IFITM1: Interferon-induced transmembrane protein 1; miR-363-3p: MicroRNA-363-3p; NC: Negative control; NS: Not significant.

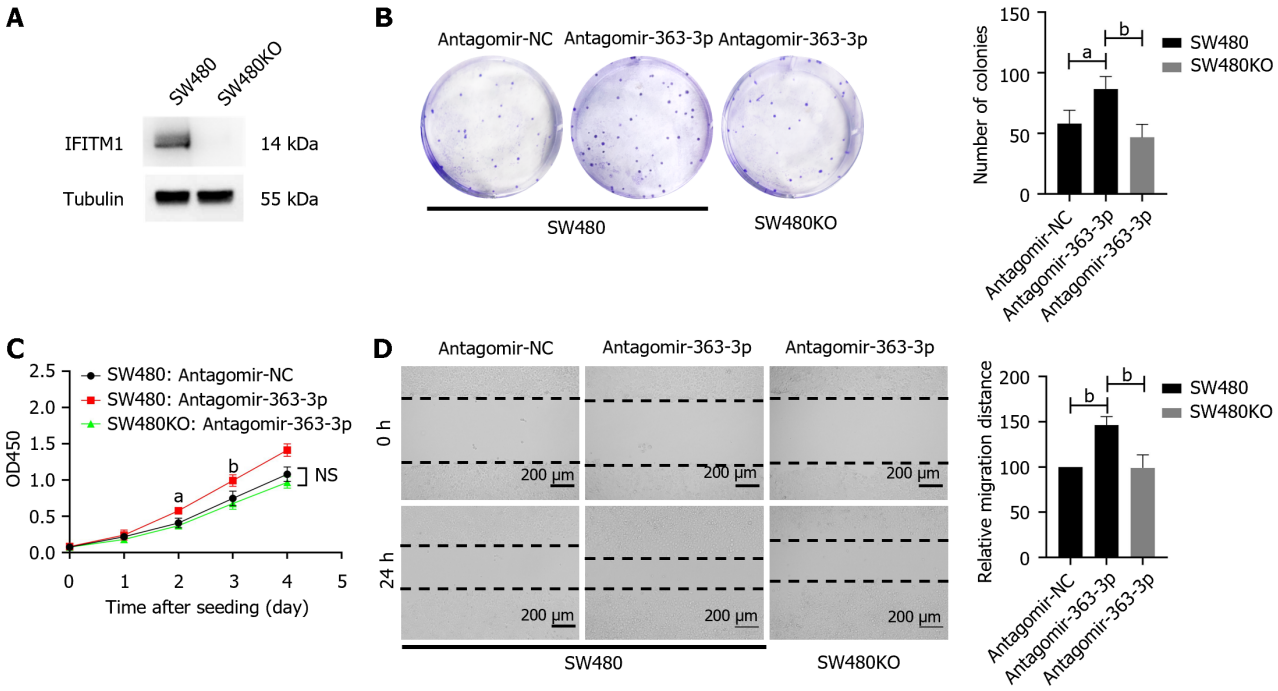
CRISPR-Cas9 system (Figure 3A) and inhibited miR-363-3p *via* transfecting antagomir-363-3p. As mentioned above, inhibiting miR-363-3p resulted in elevated clonogenic survival, proliferation, and migration in SW480 cells, in contrast, these effects became marginal in SW480KO cells (Figures 3B-D). These results suggest that IFITM1 contributes, at least partially, to the regulatory effects of miR-363-3p on clonogenic survival, proliferation, and migration of colorectal cancer cells.

The abundance of miR-363-3p displays the negative correlation with IFITM1 expression in human colorectal cancer tissues

We identified IFITM1 as a direct target of miR-363-3p using colorectal cancer cell lines. To make this conclusion more solid, we evaluated the expression of miR-363-3p and IFITM1 in human colorectal cancer tissues. We found that the mRNA and protein expression levels of IFITM1 in colorectal cancer tissues were significantly higher than those in normal colorectal tissues (Figures 4A and B, Supplementary Table 1); TCGA data also showed that *IFITM1* mRNA expression was increased in colorectal cancer tissues (Figure 4C) and was not associated with overall survival (OS) in patients with colorectal cancer (Figure 4D), but was positively correlated with disease-free survival (DFS) in patients with rectal cancer (Figure 4E). We also evaluated miR-363-3p and *IFITM1* mRNA expression in 24 colorectal cancer tissues using qPCR. We found that *IFITM1* mRNA expression was inversely correlated to miR-363-3p expression ($R^2 = 0.2216$, Figure 4F). These results indicate that miR-363-3p negatively modulates IFITM1 expression in colorectal cancer tissues.

DISCUSSION

MiR-363-3p has been reported to be dysregulated and exert a promoting or inhibiting effect on tumor development and progression in many types of cancers. MiR-363-3p was significantly decreased in hepatocellular carcinoma (HCC)[19], papillary thyroid carcinoma[20], lung cancer[21], osteosarcoma[22], gastric cancer[9], $CD133^+$ larynx cancer stem-like cells [23] and colorectal cancer[11]. However, the underlying mechanisms behind this dysregulation are far from clear. Li *et al* [24] reported that miR-363-3p is activated by its upstream transcription activator MYB in osteoporosis pathogenesis. MiR-363-3p could be sponged by circCTNNA1[25], circ_0002111[26], lncRNA NR2F1-AS1[27], lncRNA SNHG5[28], and lncRNA MALAT1[29] in colorectal cancer, papillary thyroid carcinoma, non-small cell lung cancer, and clear cell renal cell carcinoma.



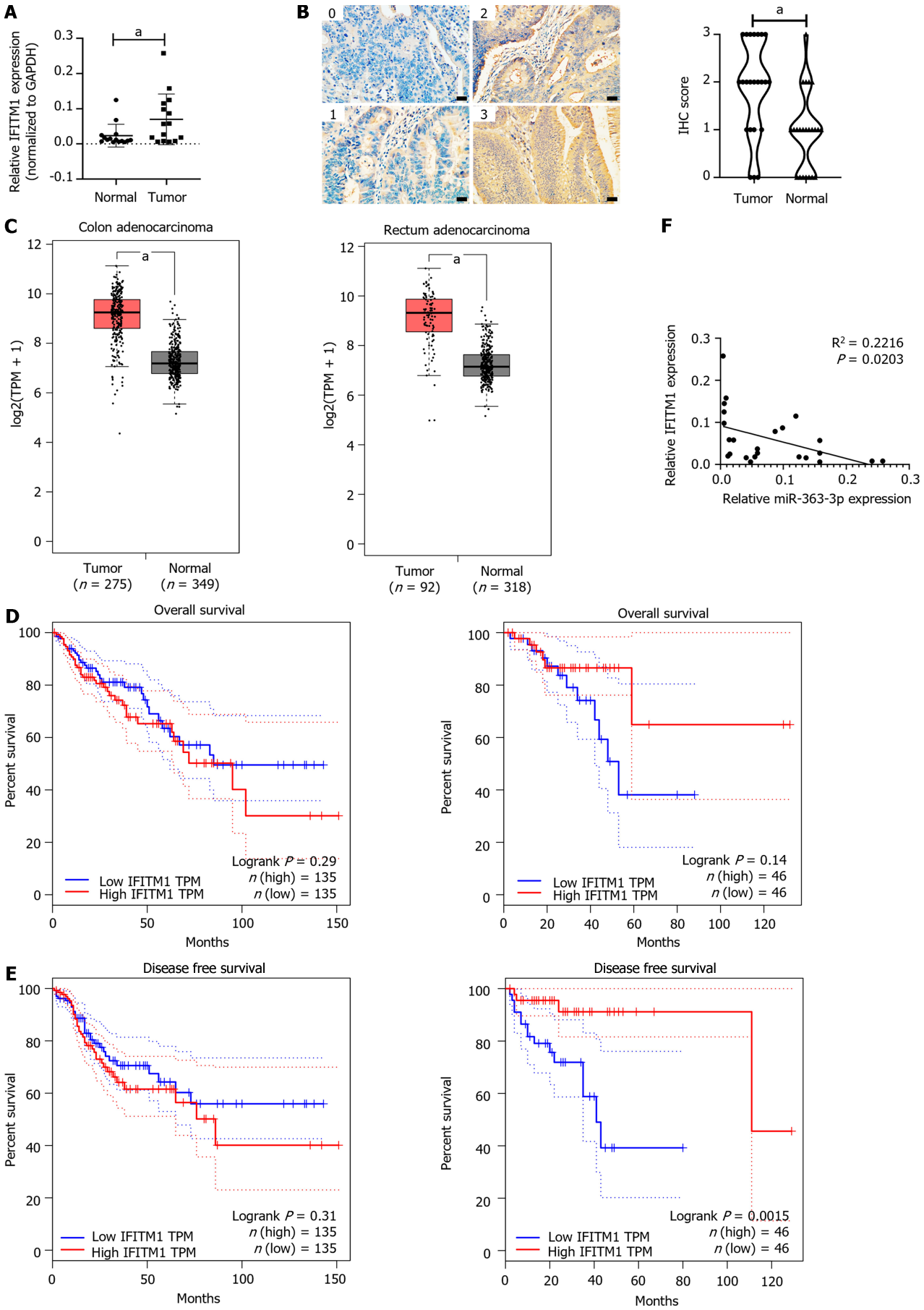
DOI: 10.4251/wjgo.v15.i9.1556

Figure 3 Interferon-induced transmembrane protein 1 mediates the regulatory effects of microRNA-363-3p on clonogenic survival, proliferation and migration of colorectal cancer cells. A: Western blot analysis of interferon-induced transmembrane protein 1 expression in SW480 and SW480KO cells; B: Representative images of clonogenic survival of SW480 or SW480KO cells. Cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the number of colonies; C: Proliferation curve of SW480 or SW480KO cells. Cells were transfected with antagomir-363-3p or antagomir-NC; D: Representative images of the gaps at 0 and 24 h after scratching. SW480 or SW480KO cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm. The results were shown as the mean ± SD. $^aP < 0.05$, $^bP < 0.01$. NS: No significance; IFITM1: Interferon-induced transmembrane protein 1; NC: Negative control.

MiR-363-3p inhibits tumorigenesis by directly targeting SOX4[19], high mobility group protein 2 (HMGA2)[30], USP28 [31], and specificity protein 1[32] in HCC. MiR-363-3p inhibits tumor growth by targeting mouse double minute 2[33], proliferating cell nuclear antigen[34], HMGA2[35], neural precursor cell-expressed developmentally down-regulated 9 and SOX4[36] in lung cancer. miR-363-3p suppresses anoikis resistance *via* targeting integrin alpha 6 in papillary thyroid carcinoma[20]. MiR-363-3p is induced by hypoxia-inducible factor 2alpha to promote the stemness of melanoma cells *via* inhibiting p21[37]. MiR-363-3p markedly inhibits the proliferation, migration, and invasion of osteosarcoma cells *via* targeting SOX4[22]. This study provides another piece of evidence supporting miR-363-3p as a tumor suppressor. We confirmed that miR-363-3p is downregulated in human colorectal cancer tissues and inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells.

IFITM1 belongs to a family of small homologous proteins, localized in the plasma and endolysosomal membranes, which regulate T cell differentiation and function and confer cellular resistance to many viruses[38]. There is mounting evidence that IFITM1 is an oncogene. IFITM1 expression is upregulated in gastric cancer[7], aromatase inhibitor-resistant breast cancer[8], triple-negative breast cancer[9], oral squamous cell carcinoma[10], and non-small cell lung cancer[11]. Furthermore, IFITM1 expression levels are closely related to patient outcomes[7,12]. IFITM1 regulates diverse aspects of tumorigenesis and progression, such as tumor cell proliferation, invasion, angiogenesis, metastasis, and therapeutic resistance, indicating that IFITM1 is a promising therapeutic target, and inhibiting IFITM1 (*e.g.*, blocking IFITIM1 by antibody, suppressing IFITM1 expression by oligonucleotides, targeted IFITIM1 degradation using bifunctional small molecules) may be a promising strategy for cancer treatment. In colorectal cancer, the elevated IFITM1 expression significantly correlates with colorectal cancer lymph node and distance metastasis, a more advanced clinical stage as well as a shorter OS[39]. However, TCGA data showed that increased *IFITM1* mRNA expression was not associated with OS in patients with colorectal cancer (Figure 4D), but was positively correlated with DFS in patients with rectal cancer (Figure 4E). He *et al*[40] reported that high expression of IFITM1 is associated with poor prognosis of rectal cancer, and no association was found between IFITM1 expression and the prognostic significance with patients with colon cancer. This discrepancy may be due to several factors. First, analysis using TCGA data focuses on *IFITM1* mRNA expression, however, the level of mRNA expression is not exactly the same as the level of protein expression. Secondly, the antibodies used for IFITM1 detection and the scoring criteria for IHC staining are not exactly the same. Finally, patient survival is associated with many factors, and tumors are highly heterogeneous.

In vitro assays revealed that IFITIM1 promotes migration[41] and invasion[39] of human colorectal cancer *via* caveolin-1. Apc mutation induces the expression of IFITM1 and high expression of IFITM1 reduces the uptake of fibroblast extracellular vesicles[42]. In this study, we identified miR-363-3p as a new epigenetic modulator of IFITM1. We revealed the binding site of miR-363-3p in IFITM1 3′ UTR region and proved that the expression of IFITM1 can be efficiently



DOI: 10.4251/wjgo.v15.i9.1556

Figure 4 Expression levels of significance and interferon-induced transmembrane protein 1 are negatively correlated in human colorectal

cancer tissues. A: Quantitative polymerase chain reaction analysis of interferon-induced transmembrane protein 1 (*IFITM1*) mRNA expression in colorectal cancer tissues and paired normal colorectal tissues; B: Representative images of IFITM1 expression detected by immunohistochemical staining. Immunohistochemical (IHC) scores are shown in the upper left corner, and the scale bar is 50 μm. Violin plots show statistical analysis of IHC scores; C: *IFITM1* mRNA expression levels in colon cancer and normal colon tissues (left panel), rectal cancer, and normal rectal tissues (right panel) were analyzed using TCGA and GTEx databases; D: Correlation analysis of *IFITM1* mRNA expression level and overall survival in patients with colon cancer (left panel) or rectal cancer (right panel); E: Correlation analysis of *IFITM1* mRNA expression level and disease-free survival in patients with colon cancer (left panel) or rectal cancer (right panel); F: Correlation analysis of *IFITM1* mRNA expression level and microRNA-363-3p expression level. The results were shown as the mean ± SD. $^{a}P < 0.05$. IHC: Immunohistochemical; IFITM1: Interferon-induced transmembrane protein 1; miR-363-3p: MicroRNA-363-3p; NC: Negative control.

inhibited by miR-363-3p and that the negative regulatory relationship exists in human colorectal cancer tissues. Moreover, a deficiency of IFITM1 can abolish the regulatory effects of miR-363-3p on clonogenic survival, proliferation, and migration of colorectal cancer cells. It would be interesting to further investigate whether the regulatory relationship between miR-363-3p and IFITM1 is common to other types of cancer, and the related lncRNA or circRNA.

CONCLUSION

Taken together, we identified that the expression of miR-363-3p and IFITM1 was downregulated and upregulated in colorectal cancer, respectively. Furthermore, IFITM1 is a direct target of miR-363-3p and the inhibitory effect of miR-363-3p on colorectal cancer progression is, at least partially, attributed to IFITM1 downregulation. We acknowledge several limitations in the present study. First, we didn't determine the contribution of the miR-363-3p/IFITM1 axis to colorectal cancer progression using *in vivo* models. Second, whether the negative regulatory relationship between miR-363-3p and IFITM1 is prevalent in different kinds of tumors has to be further studied. Last, miR-363-3p is dysregulated in numerous tumors including colorectal cancer, however, the underlying mechanism was not further explored in the current study.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer is the second most common cause of cancer death, however, the molecular mechanisms of tumorigenesis and development of colorectal cancer are far from being elucidated.

Research motivation

MicroRNAs play important roles in gene regulation and modulate numerous physical and pathological processes. The motivation of this study is to reveal the role of microRNA-363-3p (miR-363-3p) in the development of colorectal cancer and the underlying mechanisms.

Research objectives

Compare the expression of miR-363-3p between colorectal cancer tissues and adjacent normal tissues; clarify the role of miR-363-3p in clonogenic survival, migration, and proliferation of colorectal cancer cells; identify the direct target of miR-363-3p in colorectal cancer cells.

Research methods

Real-time polymerase chain reaction was performed to detect miRNA expression. PITA 6 was utilized to predict the targets of miR-363-3p. Dual-luciferase reporter system was used to validate the target of miR-363-3p. Plate colony formation and wound-healing assays were performed to evaluate cancer cells' clonogenic survival and migration ability, respectively. Cell proliferation was examined by cell counting kit-8 assay. Immunohistochemical staining was used to determine the expression level of interferon-induced transmembrane protein 1 (IFITM1).

Research results

MiR-363-3p was decreased in colorectal cancer tissues. IFITM1 was characterized as a direct target of miR-363-3p.

Research conclusions

MiR-363-3p inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells *via* targeting IFITM1.

Research perspectives

MiR-363-3p/IFITM1 axis may represent a therapeutic target in colorectal cancer.

FOOTNOTES

Author contributions: Wang Y and Bai SK designed and performed the assay; Zhang T analyzed the data; Liao CG designed the study and prepared the manuscript.

Supported by the Social Talent Fund Supporting Scheme of Tangdu Hospital, No. 2021SHRC001.

Institutional review board statement: The study was approved by the Hospital Ethics Committee (202203-116).

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

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Yun Wang 0000-0002-3892-8627; Shao-Kai Bai 0009-0002-1503-9525; Tao Zhang 0009-0004-3199-8034; Cheng-Gong Liao 0000-0002-7624-0196.

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

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **GBD 2019 Colorectal Cancer Collaborators**. Global, regional, and national burden of colorectal cancer and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol* 2022; **7**: 627-647 [PMID: 35397795 DOI: 10.1016/S2468-1253(22)00044-9]

3 **Friedman RC**, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; **19**: 92-105 [PMID: 18955434 DOI: 10.1101/gr.082701.108]

4 **Budakoti M**, Panwar AS, Molpa D, Singh RK, Büsselberg D, Mishra AP, Coutinho HDM, Nigam M. Micro-RNA: The darkhorse of cancer. *Cell Signal* 2021; **83**: 109995 [PMID: 33785398 DOI: 10.1016/j.cellsig.2021.109995]

5 **Grimson A**, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell* 2007; **27**: 91-105 [PMID: 17612493 DOI: 10.1016/j.molcel.2007.06.017]

6 **He B**, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S, Xie H, Peng X, Yin W, Tao Y, Wang X. miRNA-based biomarkers, therapies, and resistance in Cancer. *Int J Biol Sci* 2020; **16**: 2628-2647 [PMID: 32792861 DOI: 10.7150/ijbs.47203]

7 **Lu Y**, Zhao X, Liu Q, Li C, Graves-Deal R, Cao Z, Singh B, Franklin JL, Wang J, Hu H, Wei T, Yang M, Yeatman TJ, Lee E, Saito-Diaz K, Hinger S, Patton JG, Chung CH, Emmrich S, Klusmann JH, Fan D, Coffey RJ. lncRNA MIR100HG-derived miR-100 and miR-125b mediate cetuximab resistance *via* Wnt/β-catenin signaling. *Nat Med* 2017; **23**: 1331-1341 [PMID: 29035371 DOI: 10.1038/nm.4424]

8 **Garo LP**, Ajay AK, Fujiwara M, Gabriely G, Raheja R, Kuhn C, Kenyon B, Skillin N, Kadowaki-Saga R, Saxena S, Murugaiyan G. MicroRNA-146a limits tumorigenic inflammation in colorectal cancer. *Nat Commun* 2021; **12**: 2419 [PMID: 33893298 DOI: 10.1038/s41467-021-22641-y]

9 **Chen Z**, Liu X, Hu Z, Wang Y, Liu M, Li H, Ji R, Guo Q, Zhou Y. Identification and characterization of tumor suppressor and oncogenic miRNAs in gastric cancer. *Oncol Lett* 2015; **10**: 329-336 [PMID: 26171025 DOI: 10.3892/ol.2015.3179]

10 **Park H**, Lee MJ, Jeong JY, Choi MC, Jung SG, Joo WD, Lee C, An HJ. Dysregulated microRNA expression in adenocarcinoma of the uterine cervix: clinical impact of miR-363-3p. *Gynecol Oncol* 2014; **135**: 565-572 [PMID: 25230213 DOI: 10.1016/j.ygyno.2014.09.010]

11 **Dong J**, Geng J, Tan W. MiR-363-3p suppresses tumor growth and metastasis of colorectal cancer *via* targeting SphK2. *Biomed Pharmacother* 2018; **105**: 922-931 [PMID: 30021386 DOI: 10.1016/j.biopha.2018.06.052]

12 **Hu F**, Min J, Cao X, Liu L, Ge Z, Hu J, Li X. MiR-363-3p inhibits the epithelial-to-mesenchymal transition and suppresses metastasis in colorectal cancer by targeting Sox4. *Biochem Biophys Res Commun* 2016; **474**: 35-42 [PMID: 27084453 DOI: 10.1016/j.bbrc.2016.04.055]

13 **Xu DX**, Guo JJ, Zhu GY, Wu HJ, Zhang QS, Cui T. MiR-363-3p modulates cell growth and invasion in glioma by directly targeting pyruvate dehydrogenase B. *Eur Rev Med Pharmacol Sci* 2018; **22**: 5230-5239 [PMID: 30178846 DOI: 10.26355/eurrev_201808_15721]

14 **Drobna M**, Szarzyńska B, Jaksik R, Sędek Ł, Kuchmiy A, Taghon T, Van Vlierberghe P, Szczepański T, Witt M, Dawidowska M. hsa-miR-20b-5p and hsa-miR-363-3p Affect Expression of PTEN and BIM Tumor Suppressor Genes and Modulate Survival of T-ALL Cells In Vitro. *Cells* 2020; **9** [PMID: 32380791 DOI: 10.3390/cells9051137]

15 **Friedman RL**, Manly SP, McMahon M, Kerr IM, Stark GR. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. *Cell* 1984; **38**: 745-755 [PMID: 6548414 DOI: 10.1016/0092-8674(84)90270-8]

16 **Li D**, Yang Z, Liu Z, Zou Q, Yuan Y. DDR2 and IFITM1 Are Prognostic Markers in Gallbladder Squamous Cell/Adenosquamous Carcinomas and Adenocarcinomas. *Pathol Oncol Res* 2019; **25**: 157-167 [PMID: 29043607 DOI: 10.1007/s12253-017-0314-3]

17 **Lui AJ**, Geanes ES, Ogony J, Behbod F, Marquess J, Valdez K, Jewell W, Tawfik O, Lewis-Wambi J. IFITM1 suppression blocks

proliferation and invasion of aromatase inhibitor-resistant breast cancer in vivo by JAK/STAT-mediated induction of p21. *Cancer Lett* 2017; **399**: 29-43 [PMID: 28411130 DOI: 10.1016/j.canlet.2017.04.005]

18 **Kertesz M**, Iovino N, Unnerstall U, Gaul U, Segal E. The role of site accessibility in microRNA target recognition. *Nat Genet* 2007; **39**: 1278-1284 [PMID: 17893677 DOI: 10.1038/ng2135]

19 **Wang J**, Tang Q, Lu L, Luo Z, Li W, Lu Y, Pu J. LncRNA OIP5-AS1 interacts with miR-363-3p to contribute to hepatocellular carcinoma progression through up-regulation of SOX4. *Gene Ther* 2019; **27**: 495-504 [PMID: 32042127 DOI: 10.1038/s41434-020-0123-2]

20 **Pan Y**, Zhu X, Wang K, Chen Y. MicroRNA-363-3p suppresses anoikis resistance in human papillary thyroid carcinoma *via* targeting integrin alpha 6. *Acta Biochim Biophys Sin (Shanghai)* 2019; **51**: 807-813 [PMID: 31257410 DOI: 10.1093/abbs/gmz066]

21 **Wang Y**, Chen J, Lin Z, Cao J, Huang H, Jiang Y, He H, Yang L, Ren N, Liu G. Role of deregulated microRNAs in non-small cell lung cancer progression using fresh-frozen and formalin-fixed, paraffin-embedded samples. *Oncol Lett* 2016; **11**: 801-808 [PMID: 26870288 DOI: 10.3892/ol.2015.3976]

22 **Wang K**, Yan L, Lu F. miR-363-3p Inhibits Osteosarcoma Cell Proliferation and Invasion *via* Targeting SOX4. *Oncol Res* 2019; **27**: 157-163 [PMID: 29471893 DOI: 10.3727/096504018X15190861873459]

23 **Karatas OF**, Suer I, Yuceturk B, Yilmaz M, Oz B, Guven G, Cansiz H, Creighton CJ, Ittmann M, Ozen M. Identification of microRNA profile specific to cancer stem-like cells directly isolated from human larynx cancer specimens. *BMC Cancer* 2016; **16**: 853 [PMID: 27816053 DOI: 10.1186/s12885-016-2863-3]

24 **Li M**, Luo R, Yang W, Zhou Z, Li C. miR-363-3p is activated by MYB and regulates osteoporosis pathogenesis *via* PTEN/PI3K/AKT signaling pathway. *In Vitro Cell Dev Biol Anim* 2019; **55**: 376-386 [PMID: 31025251 DOI: 10.1007/s11626-019-00344-5]

25 **Zhang Y**, Zheng S, Liao N, Huang H, Chen W, Wu Z, Wu D. CircCTNNA1 acts as a ceRNA for miR-363-3p to facilitate the progression of colorectal cancer by promoting CXCL5 expression. *J Biol Res (Thessalon)* 2021; **28**: 7 [PMID: 33640021 DOI: 10.1186/s40709-021-00135-8]

26 **Zhang Z**, Mo Y, Wu L, Wang X, Liao G, Tan W, Li D. Circ_0002111 modulates the growth process of papillary thyroid carcinoma cells by targeting the miR-363-3p/HMGB1 axis. *Anticancer Drugs* 2022; **33**: 923-934 [PMID: 36136992 DOI: 10.1097/CAD.0000000000001382]

27 **Jin L**, Chen C, Huang L, Sun Q, Bu L. Long noncoding RNA NR2F1-AS1 stimulates the tumorigenic behavior of non-small cell lung cancer cells by sponging miR-363-3p to increase SOX4. *Open Med (Wars)* 2022; **17**: 87-95 [PMID: 34993348 DOI: 10.1515/med-2021-0403]

28 **Li WZ**, Zou Y, Song ZY, Wei ZW, Chen G, Cai QL, Wang Z. Long non-coding RNA SNHG5 affects the invasion and apoptosis of renal cell carcinoma by regulating the miR-363-3p-Twist1 interaction. *Am J Transl Res* 2020; **12**: 697-707 [PMID: 32194916]

29 **Xie JJ**, Li WH, Li X, Ye W, Shao CF. LncRNA MALAT1 promotes colorectal cancer development by sponging miR-363-3p to regulate EZH2 expression. *J Biol Regul Homeost Agents* 2019; **33**: 331-343 [PMID: 30972996]

30 **Wang J**, Liang H, Ge H, Guo X, Gu D, Yuan Y. MicroRNA3633p inhibits hepatocarcinogenesis by targeting HMGA2 and is associated with liver cancer stage. *Mol Med Rep* 2019; **19**: 935-942 [PMID: 30535489 DOI: 10.3892/mmr.2018.9711]

31 **Han H**, Sun D, Li W, Shen H, Zhu Y, Li C, Chen Y, Lu L, Zhang J, Tian Y, Li Y. A c-Myc-MicroRNA functional feedback loop affects hepatocarcinogenesis. *Hepatology* 2013; **57**: 2378-2389 [PMID: 23389829 DOI: 10.1002/hep.26302]

32 **Ying J**, Yu X, Ma C, Zhang Y, Dong J. MicroRNA-363-3p is downregulated in hepatocellular carcinoma and inhibits tumorigenesis by directly targeting specificity protein 1. *Mol Med Rep* 2017; **16**: 1603-1611 [PMID: 28627662 DOI: 10.3892/mmr.2017.6759]

33 **Rong H**, Chen B, Wei X, Peng J, Ma K, Duan S, He J. Long non-coding RNA XIST expedites lung adenocarcinoma progression through upregulating MDM2 expression *via* binding to miR-363-3p. *Thorac Cancer* 2020; **11**: 659-671 [PMID: 31968395 DOI: 10.1111/1759-7714.13310]

34 **Wang Y**, Chen T, Huang H, Jiang Y, Yang L, Lin Z, He H, Liu T, Wu B, Chen J, Kamp DW, Liu G. miR-363-3p inhibits tumor growth by targeting PCNA in lung adenocarcinoma. *Oncotarget* 2017; **8**: 20133-20144 [PMID: 28423618 DOI: 10.18632/oncotarget.15448]

35 **Jiang C**, Cao Y, Lei T, Wang Y, Fu J, Wang Z, Lv Z. microRNA-363-3p inhibits cell growth and invasion of nonsmall cell lung cancer by targeting HMGA2. *Mol Med Rep* 2018; **17**: 2712-2718 [PMID: 29207105 DOI: 10.3892/mmr.2017.8131]

36 **Chang J**, Gao F, Chu H, Lou L, Wang H, Chen Y. miR-363-3p inhibits migration, invasion, and epithelial-mesenchymal transition by targeting NEDD9 and SOX4 in non-small-cell lung cancer. *J Cell Physiol* 2020; **235**: 1808-1820 [PMID: 31332786 DOI: 10.1002/jcp.29099]

37 **Hao T**, Li CX, Ding XY, Xing XJ. MicroRNA-363-3p/p21(Cip1/Waf1) axis is regulated by HIF-2α in mediating stemness of melanoma cells. *Neoplasma* 2019; **66**: 427-436 [PMID: 30784290 DOI: 10.4149/neo_2018_180828N655]

38 **Yánez DC**, Ross S, Crompton T. The IFITM protein family in adaptive immunity. *Immunology* 2020; **159**: 365-372 [PMID: 31792954 DOI: 10.1111/imm.13163]

39 **Yu F**, Xie D, Ng SS, Lum CT, Cai MY, Cheung WK, Kung HF, Lin G, Wang X, Lin MC. IFITM1 promotes the metastasis of human colorectal cancer *via* CAV-1. *Cancer Lett* 2015; **368**: 135-143 [PMID: 26259513 DOI: 10.1016/j.canlet.2015.07.034]

40 **He J**, Li J, Feng W, Chen L, Yang K. Prognostic significance of INF-induced transmembrane protein 1 in colorectal cancer. *Int J Clin Exp Pathol* 2015; **8**: 16007-16013 [PMID: 26884876]

41 **Sari IN**, Yang YG, Phi LT, Kim H, Baek MJ, Jeong D, Kwon HY. Interferon-induced transmembrane protein 1 (IFITM1) is required for the progression of colorectal cancer. *Oncotarget* 2016; **7**: 86039-86050 [PMID: 27852071 DOI: 10.18632/oncotarget.13325]

42 **Kelemen A**, Carmi I, Oszvald Á, Lőrincz P, Petővári G, Tölgyes T, Dede K, Bursics A, Buzás EI, Wiener Z. IFITM1 expression determines extracellular vesicle uptake in colorectal cancer. *Cell Mol Life Sci* 2021; **78**: 7009-7024 [PMID: 34609520 DOI: 10.1007/s00018-021-03949-w]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1567-1594

DOI: 10.4251/wjgo.v15.i9.1567

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Clinical and Translational Research

Cellular senescence throws new insights into patient classification and pharmacological interventions for clinical management of hepatocellular carcinoma

Hou-Hong Wang, Wen-Li Chen, Ya-Yun Cui, Hui-Hui Gong, Heng Li

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Nagaya M, Japan; Ozturk M, Turkey

Received: February 13, 2023
Peer-review started: February 13, 2023
First decision: May 23, 2023
Revised: July 10, 2023
Accepted: August 6, 2023
Article in press: August 6, 2023
Published online: September 15, 2023

Hou-Hong Wang, Wen-Li Chen, Department of General Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, Bozhou 236800, Anhui Province, China

Ya-Yun Cui, Department of Cancer Radiotherapy, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China (Anhui Provincial Cancer Hospital), Hefei 230000, Anhui Province, China

Hui-Hui Gong, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford OX3 0BP, United Kingdom

Heng Li, Department of Comprehensive Surgery, Anhui Provincial Cancer Hospital, West District of The First Affiliated Hospital of USTC, Hefei 230000, Anhui Province, China

Corresponding author: Heng Li, Doctor, Professor, Department of Comprehensive Surgery, Anhui Provincial Cancer Hospital, West District of The First Affiliated Hospital of USTC, No. 17 Lujiang Road, Hefei 230000, Anhui Province, China. jxna36@163.com

Abstract

BACKGROUND

Cellular senescence, a state of stable growth arrest, is intertwined with human cancers. However, characterization of cellular senescence-associated phenotypes in hepatocellular carcinoma (HCC) remains unexplored.

AIM

To address this issue, we delineated cellular senescence landscape across HCC.

METHODS

We enrolled two HCC datasets, TCGA-LIHC and International Cancer Genome Consortium (ICGC). Unsupervised clustering was executed to probe tumor heterogeneity based upon cellular senescence genes. Least absolute shrinkage and selection operator algorithm were utilized to define a cellular senescence-relevant scoring system. *TRNP1* expression was measured in HCCs and normal tissues through immunohistochemistry, immunoblotting and quantitative real-time polymerase chain reaction. The influence of TMF-regulated nuclear protein (TRNP)1 on HCC senescence and growth was proven *via* a series of experiments.

RESULTS
TCGA-LIHC patients were classified as three cellular senescence subtypes, named C1–3. The robustness and reproducibility of these subtypes were proven in the ICGC cohort. C2 had the worst overall survival, C1 the next, and C3 the best. C2 presented the highest levels of immune checkpoints, abundance of immune cells, and immunogenetic indicators. Thus, C2 might possibly respond to immunotherapy. C2 had the lowest somatic mutation rate, while C1 presented the highest copy number variations. A cellular senescence-relevant gene signature was generated, which can predict patient survival, and chemo- or immunotherapeutic response. Experimentally, it was proven that TRNP1 presented the remarkable upregulation in HCCs. TRNP1 knockdown induced apoptosis and senescence of HCC cells and attenuated tumor growth.

CONCLUSION
These findings provide a systematic framework for assessing cellular senescence in HCC, which decode the tumor heterogeneity and tailor the pharmacological interventions to improve clinical management.

Key Words: Cellular senescence; Hepatocellular carcinoma; Prognosis; Subtypes; Tumor microenvironment; Gene signature; Pharmacological interventions

Core Tip: Cellular senescence, a state of stable growth arrest, is implicated in human cancers. Nevertheless, characterization of cellular senescence-associated phenotypes in hepatocellular carcinoma (HCC) is still indistinct. Here, we proposed a novel cellular senescence-based classification for HCC and identified TRNP1 as a novel therapeutic target.

Citation: Wang HH, Chen WL, Cui YY, Gong HH, Li H. Cellular senescence throws new insights into patient classification and pharmacological interventions for clinical management of hepatocellular carcinoma. *World J Gastrointest Oncol* 2023; 15(9): 1567-1594
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1567.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1567

INTRODUCTION

Cellular senescence is defined as an irreversible cessation of cellular division of cells with normal proliferation[1]. Human cells age due to progressive shortening of telomeres following cellular division, stress, oncogenes, *etc*[2]. Numerous genes have been implicated in cellular senescence as biomarkers and causal drivers[3,4]. Cellular senescence is a double-edged sword for cancer and its treatment[5,6]. The growth arrest and immunomodulatory features linked with senescence possess powerful antimalignant roles[7]. In addition, senescence bypass and secretory phenotype correlate to tumor progression and recurrence[8]. Research has unveiled the great potential for antiaging interventions as a novel antitumor strategy[9]. Nonetheless, the heterogeneity of senescence-related features makes the definition and targeting of treatment-induced senescent cells challenging[10].

Hepatocellular carcinoma (HCC) is a poorly managed malignancy with high mortality due to the lack of response to classical chemotherapy agents (doxorubicin, cisplatin, *etc.*) and targeted agents in the early stage[11]. For late-stage HCCs, single sorafenib or combination therapy remains the mainstay in first-line therapy, which improves overall survival by 3 mo[12]. The modest therapeutic success is largely attributable to sorafenib resistance[13]. Immunotherapy with checkpoint inhibitors (anti-PD-1/PD-L1) has displayed potent anti-HCC activity in a subset of patients[14]. The main unmet challenge in HCC immunotherapy is to discover and verify predictive biomarkers[15]. Accumulated evidence demonstrates that inducing tumor cells into senescence represents a potential anti-HCC therapy[16]. In HCCs, cellular senescence is primarily controlled by p53-dependent or -independent mechanisms[17]. Paradis *et al*[18] investigated replicative senescence in normal liver, chronic hepatitis C, and HCC, and demonstrated that chronic hepatitis C is a relevant model of accelerated replicative senescence and that accumulation of replicative senescent cells predispose to HCC progression[18]. Hepatic stellate cell activation and senescence also trigger the development of liver cirrhosis towards HCC[19]. Yildiz *et al*[20] found that cirrhosis and HCC exhibit expression patterns compatible with senescent and immortal phenotypes, respectively, while dysplasia is a transitional state. Senescence bypass exerts an essential role in hepatocellular carcinogenesis engendering systematic alteration in the transcription of genes modulating DNA repair, proliferation, differentiation, metabolism, *etc*[20]. Eggert *et al*[21] reported that while chemokines secreted by senescent hepatocytes inhibit liver cancer initiation, they enable to facilitate the growth of fully established HCC[21]. Due to the highly heterogeneous malignancy at the molecular and histological levels, characterization of cellular senescence-based classification might facilitate the personalized treatment of HCCs. Recently, cell senescence molecular subtypes have been conducted for predicting prognostic outcomes and immunotherapeutic responses of hepatitis B virus-related HCC patients[22]. A cellular-senescence-related classifier has been developed for inferring predicting prognosis, immunothera-

peutic responses, and candidate agents in HCCs[23]. However, these findings are based upon retrospective analysis, and lack of experimental validation. To address these problems, our integrative analysis classified HCCs as three cellular senescence subtypes and defined a cellular senescence-relevant scoring system, which decoded the tumor heterogeneity as well as tailored the pharmacological interventions to boost clinical management of HCC.

MATERIALS AND METHODS

Acquisition of cellular senescence genes

Totally, 279 human cellular senescence genes were acquired from the CellAge database (https://genomics.senescence.info/cells/)[3,4]. Genes that induce cellular senescence present the overexpression with age in human tissue samples and are notably overrepresented in antilongevity and tumor-suppressor genes; meanwhile, genes that inhibit cellular senescence overlap with prolongevity genes and oncogenes. The detailed information is listed in Supplementary Table 1.

Public HCC datasets

HCC patients were acquired from three public datasets, covering the Cancer Genome Atlas (TCGA-LIHC) database (https://portal.gdc.cancer.gov/projects/TCGA-LIHC) (n = 368), the International Cancer Genome Consortium (ICGC) portal (https://dcc.icgc.org/projects/LIRI-JP/) (n = 232), and the GSE14520 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14520). Clinicopathological traits of above datasets are summarized in Supplementary Table 2. All expression data were transformed o transcripts per kilobase million, followed by log-2 conversion.

Differential expression analysis

Utilizing limma package, differentially expressed cellular senescence genes were screened in HCC relative to normal liver tissues[24]. To prevent high false-positive rate, P values were adjusted *via* Benjamini–Hochberg approach. Adjusted P < 0.01 and $|\log_2$ fold change (FC)$|$ > 0.58 were regarded as the criteria of differentially expressed genes.

Functional annotation analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were annotated by use of clusterProfiler package[25]. GO terms and KEGG pathways with adjusted P < 0.05 were significantly enriched. The activity of fifty hallmark pathways was computed through GSVA package[26] based upon the gene sets of Molecular Signatures Database[27].

Unsupervised clustering

Based upon the prognostic differentially expressed cellular senescence genes derived from univariate Cox regression (P < 0.05), unsupervised clustering was implemented for TCGA-LIHC patients utilizing ConsensusClusterPlus package[28]. This process was conducted with 1000 iterations through sampling 80% of all the data for each iteration, thus ensuring clustering stability. The optimal number of clusters was identified utilizing consensus heatmap together with cumulative distribution function (CDF) curves. Principal component analysis (PCA) was utilized for recognizing and visualizing distinct subtypes.

Nearest template prediction for subtype verification

Nearest template prediction (NTP) method is flexible for evaluating class prediction confidence for patients. Up-regulated genes were regarded as markers of each subtype with adjusted P < 0.05, which were adopted in the NTP method derived from CMScaller package[29], thus assessing the reliability and stability of subtypes.

Tumor microenvironment estimation

Single-cell gene set enrichment analysis (ssGSEA), a deconvolution algorithm from GSVA package, was executed for quantifying the compositions within the tumor microenvironment (TME), comprising 22 immune cells and two stromal components (fibroblasts and endothelial cells). The ssGSEA score denoted the abundance of these TME components. The abundance of the TME components was also inferred through TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL, together with EPIC methods.

Genetic alteration analysis

The available mutation annotation format files from the TCGA were adopted for the analysis of somatic mutation utilizing maftools package[30]. Copy number variations (CNVs) of TCGA HCCs were stratified into three cellular senescence subtypes. Significant amplifications or deletions across the whole genome were assessed utilizing GISTIC2.0 [31].

Cellular senescence subtype-relevant gene selection

Genes with differential expression between subtypes were selected with the thresholds of adjusted P < 0.05 together with $|\log_2 FC|$ > 0.58. Cellular senescence subtype-relevant genes were determined following the intersection.

Gene signature establishment

Univariate Cox regression analysis was utilized for picking out cellular-senescence-relevant genes with prognostic implication based upon $P < 0.05$. Through least absolute shrinkage and selection operator (LASSO) algorithm, the best gene subset was found out *via* glmnet package[32]. The cellular senescence-relevant scoring system was defined following the formula: RiskScore = Σ(coefficient (β)*Expression β), where β denoted each selected prognostic cellular senescence-relevant gene. HCCs were stratified into low- and high-RiskScore groups with the median RiskScore.

Nomogram construction

Uni- and multivariate Cox regression analyses on the cellular-senescence-relevant gene signature and conventional clinicopathological variables were executed to select independent prognostic factors in the TCGA-LIHC cohort. A nomogram based upon independent factors was generated to predict the probability of overall survival through rms package. Decision curve analysis was conducted for validating the nomogram[33].

Therapeutic response prediction

The half-maximal inhibitory concentration (IC_{50}) value of commonly used chemotherapy or targeted therapy drugs was inferred utilizing pRRophetic package[34]. Immunotherapy response was inferred by use of Tumor Immune Dysfunction and Exclusion (TIDE)[35].

Patients and tissues

Thirty fresh HCC tumors together with adjacent normal tissues were harvested from The Affiliated Bozhou Hospital of Anhui Medical University. No patients experienced any preoperative adjuvant treatment. HCC diagnosis was confirmed pathologically. Written informed consent was provided by each patient. This project gained the approval of the Ethics Committee of The Affiliated Bozhou Hospital of Anhui Medical University (2022-17).

Immunohistochemistry

TRNP1 expression in HCC or normal tissues was tested through fixing tissue sections with 4% paraformaldehyde. The sections were sealed utilizing goat serum, followed by incubation with TRNP1 antibody (1:500; ab174303; Abcam, Cambridge, MA, USA) along with secondary antibody (1/1000; ab7090). After administration with diaminobenzidine tetrahydrochloride, images were acquired under a microscope (Zeiss, Germany).

Immunoblotting

Total protein extraction was analyzed utilizing immunoblotting. Protein content was measured utilizing BCA kit (Beyotime, Shanghai, China). Proteins were separated *via* 12% SDS-PAGE, and transferred onto PVDF membranes that were then probed with primary antibody against TRNP1 (1/5000; ab174303; Abcam), p16 (1/500; ab151303), p21 (1/1000; ab109199) or GAPDH (1/2500; ab9485) at 4°C overnight, and secondary antibody (1/1000; ab7090) at room temperature for 2 h. Proteins were developed using ECL reagent (Beyotime).

Quantitative real-time polymerase chain reaction

RNA extraction was achieved utilizing RNA easy mini kit (Invitrogen, Carlsbad, CA, USA), with cDNA preparation *via* PrimeScript RT Master Mix (Takara, Dalian, China). Quantitative real-time polymerase chain reaction (RT-qPCR) was conducted *via* ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China). The relative expression value was estimated with $2^{-\Delta\Delta Ct}$ approach as well as normalized to endogenous GAPDH.

Cell culture and transfection

RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (Gibco), and 1% penicillin–streptomycin (Gibco) was adopted for culturing SMMC-7721 and HepG2 HCC cells. All cells were maintained in an incubator at 37°C with 5% CO_2. For transfection, siRNAs of TRNP1 (si-TRNP1) and negative control (si-NC) were acquired from GenePharma. Cell transfection was conducted utilizing Lipofectamine 2000 (Thermo Fisher Scientific).

Flow cytometry

Apoptotic rate was tested through flow cytometry utilizing Annexin V–fluorescein isothiocyanate (apoptosis detection kit (BD Biosciences). Cells were harvested and the assay was performed. Next, samples were assessed instantly utilizing flow cytometry (Beckman Coulter).

SA-β-galactosidase staining

To investigate senescence, 10^4 cells were seeded onto a six-well plate. After being fixed, they were stained with senescence-associated -galactosidase activity (SA-β-gal) (Gibco).

Tumor xenograft

Female BALB/c nude mice (5-wk-old, 16–18 g; Beijing Vital River Laboratory Animal Technology Co. Ltd., China) were fed under a 12-h light/dark cycle. They were divided into three groups (n = 5 per group). SMMC-7721 cells ($n = 10^5$) with si-NC, si-TRNP1#1 or si-TRNP1#2 were inoculated into the armpit. After 36 d, they were killed, with subsequent tumor excision. Tumor volume was finally calculated. This experiment gained the approval the Animal Ethics Committee of The Affiliated Bozhou Hospital of Anhui Medical University (LLSC20232071).

Statistical analysis

For between-group comparisons, unpaired Student's *t*-test was adopted; Mann–Whitney *U* test was utilized for variables with non-normal distribution. Kaplan–Meier curves were utilized to estimate the overall survival of groups, with log-rank for testing the difference significance between groups. Survival analysis was executed utilizing survival and survminer packages. Receiver operator characteristic curves were plotted to evaluate the prediction efficacy in overall survival. Statistical analyses were implemented utilizing R packages and GraphPad Prism software. A two-sided $P < 0.05$ indicated statistical significance.

RESULTS

Differentially expressed cellular senescence genes in HCC and biological significance

We identified 146 differentially expressed cellular senescence genes in TCGA HCCs relative to normal tissues with adjusted $P < 0.01$ and $|\log_2 FC| > 0.58$ (Figure 1A and B; Supplementary Table 3), which might participate in HCC initiation or progression. They were linked with metabolic process, cellular senescence, cell cycle, and immunity pathways (Figure 1C–F). Their prognostic value was then assessed. Ninety-seven differentially expressed cellular senescence genes were significantly linked with HCC prognosis (Table 1).

Classification of HCC patients as three cellular senescence subtypes

Prognostic differentially expressed cellular senescence genes were utilized for probing HCC heterogeneity. Utilizing unsupervised clustering, TCGA-LIHC cases were initially assigned to 2–9 clusters. Combining consensus CDF and consensus matrix, the optimal number of clusters was generated when k = 3 (Figure 2A–C). Thus, HCCs were classified as three cellular senescence subtypes, named C1–3. Prognostic differentially expressed cellular senescence genes presented the highest transcript levels in C2, followed by C1 and C3 (Figure 2D). PCA proved the extensive discrepancy in transcript levels among three subtypes (Figure 2E). Additionally, we focused on the survival difference, with C2 having the worst overall survival, C1 the next, and C3 the best (Figure 2F). Based upon up-regulated markers of each subtype (Supplementary Table 4), the robustness and reproducibility of cellular senescence subtypes were verified utilizing NTP in the ICGC cohort (Figure 2G). The discrepancy in transcript levels and overall survival among subtypes was further proven in this cohort (Figure 2H and I).

Responses to immunotherapy and chemotherapy of three cellular senescence subtypes

To elucidate the underlying mechanisms among the three cellular senescence subtypes, the activity of 50 hallmark pathways was inferred. Tumorigenic pathways (DNA repair, MYC, PI3K–AKT–mTOR, mTORC1, *etc.*) exhibited the highest activity in C2, with the lowest activity of metabolism pathways (Figure 3A). C3 presented the lowest activity of tumorigenic pathways, as well as the highest activity of metabolism pathways. Additionally, it was found that immune checkpoints displayed the highest transcript levels in C2, with the highest abundance of immune cells (Figure 3B). Immunogenetic indicators were then observed. Aneuploidy score, cancer-testicular antigen score, homologous recombination defects, and intratumor heterogeneity displayed the highest levels in C2, followed by C1 and C3 (Figure 3C–F). TIDE score was utilized to estimate the response to immune checkpoint inhibitors. Among three subtypes, C3 presented the lowest TIDE score, indicating that this subtype was most likely to respond to immune checkpoint inhibitors (Figure 3G). It was also found that cisplatin, doxorubicin and gemcitabine showed the lowest IC_{50} values in C2 subtype (Figure 3H–J). Thus, C2 patients were most likely to benefit from above chemotherapeutic drugs.

Genetic alterations of three cellular senescence subtypes

Overall, somatic mutation rate was the lowest in C2 among three cellular senescence subtypes (Figure 4A–C). Additionally, C1 presented the highest copy number amplified and deleted alterations (Figure 4D–I). Altogether, there was remarkable heterogeneity in genetic alterations among three cellular senescence subtypes.

Identification of cellular senescence subtype-relevant genes and functional implications

To select cellular senescence subtype-relevant genes, we assessed the genes with differential expression between cellular senescence subtypes based upon adjusted $P < 0.05$ together with $|\log_2 FC| > 0.58$. After the intersection, 666 cellular senescence subtype-relevant genes were eventually acquired (Supplementary Table 5 and Figure 5A). We elucidated the underlying functional implications. Consequently, these cellular senescence subtype-relevant genes were remarkably linked with cell cycle, DNA replication, oocyte meiosis, homologous recombination, cellular senescence, Fanconi anemia pathway, p53 pathway, progesterone-mediated oocyte maturation, mismatch repair, *etc* (Figure 5B–E).

Definition and external verification of a cellular-senescence-relevant gene signature

To illustrate the relationships of the cellular-senescence-relevant genes and patient survival, univariate Cox regression method was adopted. A total of 511 cellular -enescence-relevant genes presented significant correlations to TCGA-LIHC prognosis (Supplementary Table 6). These prognostic genes were entered into LASSO analysis (Figure 6A and B). A 19-gene signature was generated in accordance with the optimal λ value. The cellular senescence-relevant scoring system was computed as follows: RiskScore = 0.0610156 ∗ transcript level of SLC1A5 + 0.049731458 ∗ transcript level of G6PD + 0.038762092 ∗ transcript level of PSRC1 + 0.104396819 ∗ transcript level of UCK2 + 0.004054037 ∗ transcript level of TCOF1 + 0.03040522 ∗ transcript level of CCT5 + 0.002669582 ∗ transcript level of DTYMK + 0.053080689 ∗ transcript level of

Table 1 Univariate-cox regression results of prognostic differentially expressed cellular senescence genes with *P* < 0.05 in TCGA-LIHC dataset

Gene	HR	95%lower	95%upper	*P* value	Gene	HR	95%lower	95%upper	*P* value
MCRS1	1.6954	1.2504	2.2988	0.0007	*TPR*	1.2593	1.0274	1.5436	0.0264
FASTK	1.3714	1.0147	1.8536	0.0399	*HDAC1*	1.8639	1.4442	2.4055	< 0.0001
AURKA	1.2794	1.1083	1.4770	0.0008	*SPOP*	1.4186	1.0061	2.0004	0.0461
PTTG1	1.3361	1.1782	1.5151	< 0.0001	*BTG3*	1.3304	1.0766	1.6440	0.0082
PSMB5	1.8929	1.3286	2.6969	0.0004	*IRF5*	1.4232	1.1118	1.8217	0.0051
MAP2K2	1.5424	1.1846	2.0083	0.0013	*IGFBP3*	1.1741	1.0435	1.3210	0.0076
E2F1	1.2175	1.0804	1.3721	0.0012	*CDKN2B*	1.3951	1.1619	1.6751	0.0004
CDK1	1.3097	1.1553	1.4847	< 0.0001	*AAK1*	1.3018	1.0160	1.6682	0.0371
GRK6	1.7940	1.3455	2.3920	< 0.0001	*ARPC1B*	1.3007	1.0918	1.5495	0.0032
EZH2	1.5722	1.3117	1.8845	< 0.0001	*LIMK1*	1.3450	1.1517	1.5706	0.0002
DPY30	1.9266	1.3785	2.6928	0.0001	*SFN*	1.1541	1.0714	1.2432	0.0002
CBX8	1.4643	1.1477	1.8684	0.0022	*CSNK2A1*	1.3825	1.0896	1.7541	0.0077
SMARCA4	1.4396	1.1405	1.8170	0.0022	*GLB1*	1.4265	1.1097	1.8336	0.0056
CDKN2A	1.1748	1.0571	1.3055	0.0028	*MOB3A*	1.4527	1.1459	1.8416	0.0020
IRF3	1.4048	1.0834	1.8216	0.0103	*BRD7*	1.3897	1.0401	1.8567	0.0260
HRAS	1.4678	1.1958	1.8016	0.0002	*PRKCD*	1.4989	1.2458	1.8035	< 0.0001
ADCK5	1.3095	1.0375	1.6527	0.0232	*CDK4*	1.4798	1.2301	1.7800	< 0.0001
RUVBL2	1.7508	1.3029	2.3528	0.0002	*BLVRA*	1.1790	1.0296	1.3500	0.0172
ACLY	1.3734	1.1160	1.6901	0.0027	*SENP1*	1.5189	1.1628	1.9840	0.0022
TACC3	1.3618	1.1752	1.5780	< 0.0001	*BMI1*	1.6112	1.2516	2.0742	0.0002
SIRT6	1.6515	1.2586	2.1671	0.0003	*DHX9*	1.3908	1.1069	1.7477	0.0046
SUPT5H	1.5227	1.1449	2.0252	0.0039	*RSL1D1*	1.7134	1.2421	2.3634	0.0010
FOXM1	1.2772	1.1287	1.4454	0.0001	*PAK4*	1.2908	1.0419	1.5990	0.0195
PSMD14	1.9715	1.5043	2.5838	< 0.0001	*PDCD10*	1.6080	1.2369	2.0903	0.0004
HJURP	1.4529	1.2514	1.6869	< 0.0001	*ASF1A*	1.7774	1.3749	2.2977	< 0.0001
TRIM28	1.6005	1.2816	1.9988	< 0.0001	*PNPT1*	1.6790	1.2487	2.2576	0.0006
P3H1	1.9307	1.5257	2.4431	< 0.0001	*MAPK12*	1.2163	1.0478	1.4119	0.0101
MAGOHB	1.4601	1.0477	2.0346	0.0254	*UBTD1*	1.3794	1.0584	1.7977	0.0173
RBX1	1.6692	1.2410	2.2451	0.0007	*KDM5B*	1.3241	1.1029	1.5895	0.0026
MAGOH	1.6449	1.1977	2.2592	0.0021	*USP1*	1.4478	1.1790	1.7778	0.0004
CENPA	1.4947	1.2958	1.7241	< 0.0001	*MAP3K7*	1.4740	1.1327	1.9180	0.0039
EWSR1	1.9373	1.3937	2.693	< 0.0001	*PKM*	1.2079	1.0979	1.3290	0.0001
HSPA5	1.3234	1.0428	1.6796	0.0212	*NINJ1*	1.4743	1.0929	1.9888	0.0110
CHEK1	1.5484	1.2766	1.8780	< 0.0001	*SERPINE1*	1.1201	1.0226	1.2269	0.0147
PIAS4	1.4493	1.0683	1.9663	0.0171	*BRCA1*	1.4378	1.1653	1.7741	0.0007
PRPF19	2.5587	1.7707	3.6974	< 0.0001	*DEK*	1.2103	1.0125	1.4468	0.0360
MAPKAPK5	2.0718	1.4388	2.9834	< 0.0001	*NDRG1*	1.2946	1.1412	1.4686	< 0.0001
MAD2L1	1.4390	1.2065	1.7164	< 0.0001	*SRC*	1.2011	1.0507	1.3730	0.0073
TFAP4	1.9651	1.4205	2.7184	< 0.0001	*ASPH*	1.2007	1.0488	1.3746	0.0081
G6PD	1.3897	1.2500	1.5450	< 0.0001	*STK32C*	1.5149	1.2235	1.8759	0.0001

GAPDH	1.5984	1.2892	1.9818	< 0.0001	*CDK2AP1*	1.2327	1.0010	1.5181	0.0490
SMARCB1	1.4393	1.1297	1.8336	0.0032	*KDM4A*	1.3363	1.0476	1.7045	0.0196
LEO1	1.4005	1.0359	1.8934	0.0286	*MMP9*	1.1360	1.0384	1.2428	0.0054
TXN	1.3040	1.0667	1.5941	0.0096	*HK3*	1.2356	1.0293	1.4832	0.0232
HDAC4	1.5029	1.1470	1.9692	0.0031	*VEGFA*	1.2906	1.0657	1.5630	0.0090
FXR1	1.6935	1.2509	2.2927	0.0007	*LGALS3*	1.2041	1.0826	1.3392	0.0006
RAD21	1.3480	1.0928	1.6629	0.0053	*AR*	0.8662	0.7749	0.9682	0.0115
SRSF1	1.7610	1.2427	2.4955	0.0015	*BAG3*	1.2740	1.0326	1.5718	0.0238

NEIL3 + 0.031271078 * transcript level of TRNP1 + (-0.037524429) * transcript level of ADH4 + 0.018409162 * transcript level of HMMR + 0.001793118 * transcript level of SMG5 + (-0.016621762) * transcript level of CLEC3B + 0.083327624 * transcript level of PLOD2 + 0.035535231 * transcript level of SPP1 + (-0.023169364) * transcript level of CFHR3 + 0.020042596 * transcript level of TMEM106C + (-0.019959706) * transcript level of ANXA10 + (-0.039577478) * transcript level of LCAT. Based upon the median RiskScore, TCGA-LIHC cases were classified as low- and high-RiskScore groups (Figure 6C). Expression levels of these selected genes exhibited the notable differences between groups. Next, K-M curves illustrated that high-RiskScore patients' overall survival was worse (Figure 6D). AUCs at 1-, 3- and 5-year survival all exceeded 0.75, demonstrating the excellent discrimination power of the gene signature (Figure 6E).

The ICGC and GSE14520 datasets were utilized to externally verify this signature. The current study stratified HCCs into low- and high-RiskScore groups based upon the median RiskScore in the ICGC dataset (Figure 6F). Overall survival rate of high-RiskScore group was prominently lower (Figure 6G). AUCs at 1- and 3-year survival were > 0.75 (Figure 6H). Above data proved the high reproducibility of the signature. The similar findings were also confirmed in the GSE14520 dataset (Figure 6I–K).

Generation of a prognostic nomogram for clinical practice

Univariate and multivariate Cox regression analyses were executed to select the independent prognostic parameters for HCCs. It was found that stage and the cellular-senescence-relevant RiskScore acted as independent risk factors of HCC prognosis (Figure 7A and B). As a visual representation of the prognostic model, a nomogram containing stage, and RiskScore was built to illustrate HCC patients' survival more intuitively. The nomogram showed that RiskScore had the highest influence on 1-, 3- and 5-year survival of HCC patients, followed by stage (Figure 7C). Decision curve analysis demonstrated that the nomogram can accurately predict 1-, 3- and 5-year clinical outcomes (Figure 7D–F).

Assessment of the cellular-senescence-relevant gene signature in predicting efficacy of pharmacological interventions

The IC_{50} of some chemotherapy or targeted therapy agents was estimated in TCGA-LIHC dataset. High-RiskScore HCCs presented the notably lower IC_{50} of cisplatin, doxorubicin, and gemcitabine relative to those with low RiskScore (Figure 8A–C). However, no significant difference in the IC_{50} of sorafenib was found between the two groups (Figure 8D). Accordingly, high-RiskScore HCCs more possibly responded to cisplatin, doxorubicin or gemcitabine chemotherapy.

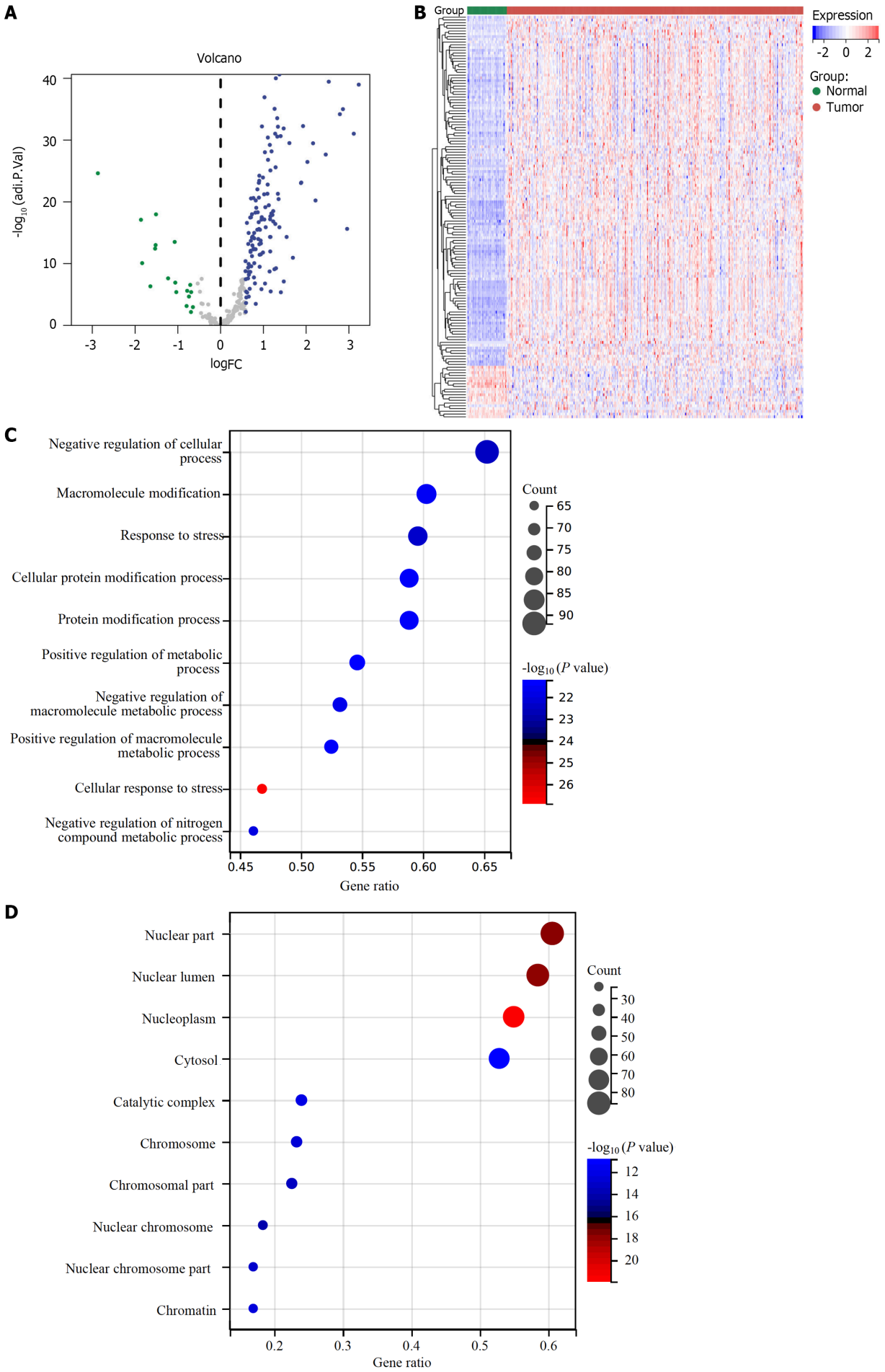
Some reliable computational approaches were adopted to infer the abundance of the TME elements across TCGA-LIHC samples. Overall, most immune cells exhibited the higher infiltration in high-risk HCCs (Figure 8E). The TIDE method was used to predict immunotherapy response. We did not observe any difference in carcinoma-associated fibroblasts between the groups (Figure 8F). Lower myeloid-derived suppressor cells, interferon gamma, exclusion score and TIDE score as well as higher dysfunction score were found in low-RiskScore HCCs (Figure 8G–K). This indicated that low-RiskScore HCCs more possibly benefited from immunotherapy.

Experimental verification of expression of TRNP1 in HCC

Among the genes in the cellular-senescence-relevant gene signature, the role of TRNP1 in HCC remains unclear. Therefore, we focused on TRNP1. It was proven that TRNP1 presented remarkable upregulation in HCCs relative to normal tissues in accordance with immunohistochemistry (Figure 9A and B), immunoblotting (Figure 9C and D) and RT-qPCR (Figure E). Specific siRNAs of TRNP1 were transfected into SMMC-7721 and HepG2 cells. Immunoblotting demonstrated the notable decrease in TRNP1 expression induced by siRNAs (Figure 9F–9H).

Suppression of TRNP1 induces apoptosis and senescence of HCC cells and attenuates tumor growth

Based upon flow cytometry, apoptotic rate of SMMC-7721 and HepG2 cells was prominently elevated by TRNP1 knockdown (Figure 10A–C). SA-β-Gal staining showed that TRNP1 knockdown notably induced cellular senescence of two HCC cells (Figure 10D–F). Additionally, two cellular senescence markers: p16 and p21 were overexpressed in HCC cells with TRNP1 knockdown (Figure 10G–K), further proving the role of TRNP1 in HCC senescence. *In vivo* tumorigenicity models were also developed for evaluating whether TRNP1 influenced tumor growth. As a result, TRNP1 knockdown was found to decrease *in vivo* tumor volume (Figure 10L and M).


A
Volcano
-log10 (adj.P.Val)
logFC
B
Group
Expression
Group:
Normal
Tumor
C
Negative regulation of cellular process
Macromolecule modification
Response to stress
Cellular protein modification process
Protein modification process
Positive regulation of metabolic process
Negative regulation of macromolecule metabolic process
Positive regulation of macromolecule metabolic process
Cellular response to stress
Negative regulation of nitrogen compound metabolic process
Gene ratio
Count
-log10 (P value)
D
Nuclear part
Nuclear lumen
Nucleoplasm
Cytosol
Catalytic complex
Chromosome
Chromosomal part
Nuclear chromosome
Nuclear chromosome part
Chromatin
Gene ratio
Count
-log10 (P value)

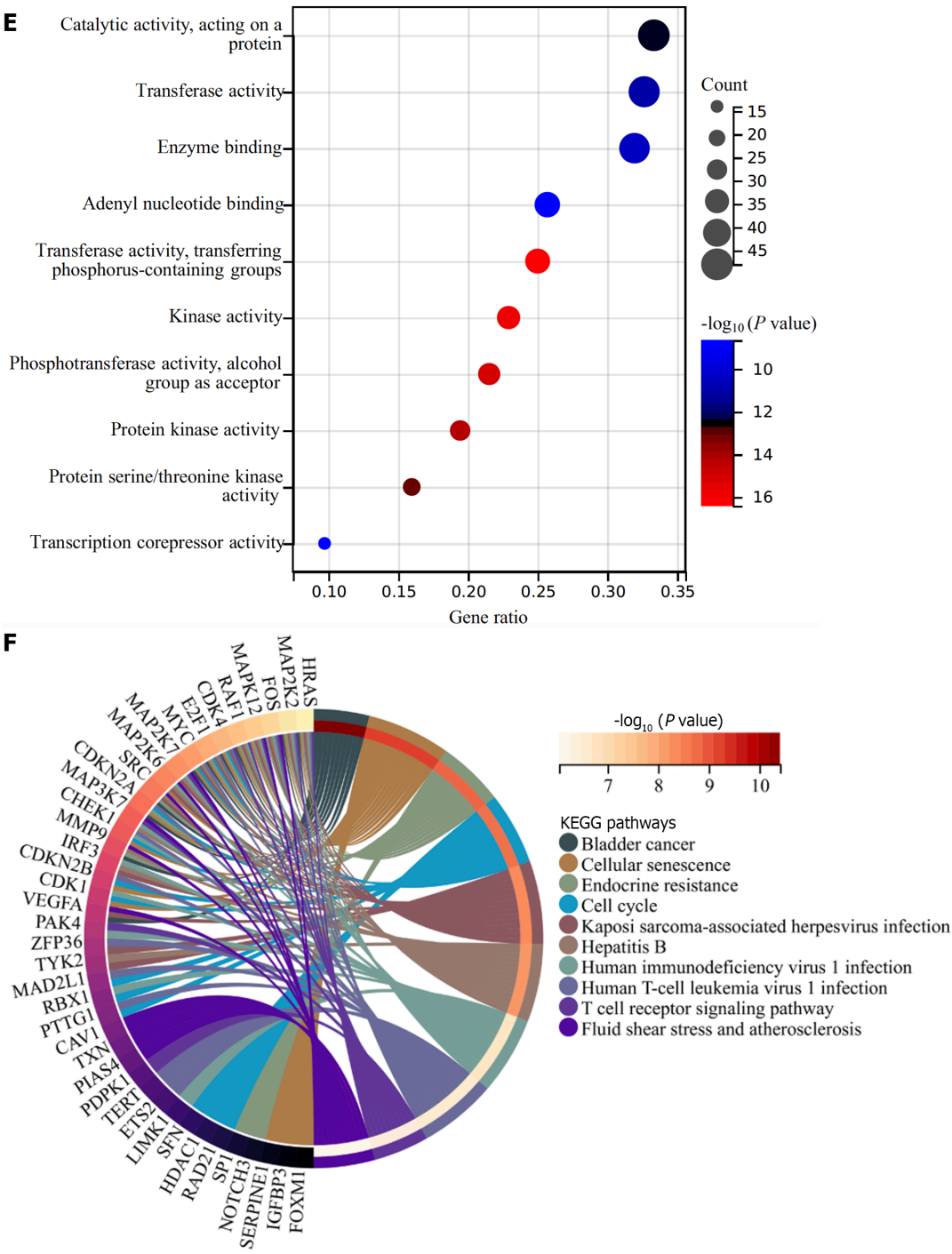


Figure 1 Differentially expressed cellular senescence genes in hepatocellular carcinoma and biological significance. A: Volcano diagram of the abnormal expression of cellular senescence genes in hepatocellular carcinoma (HCC) relative to normal liver tissues in the TCGA-LIHC dataset. Blue dots denote differentially expressed cellular senescence genes, with grey dots for the not differentially expressed genes; B: Heatmap of the transcript levels of differentially expressed cellular senescence genes in HCC and normal liver tissues; C–E: Bubble diagrams of the top 10 biological process, cellular component, molecular function terms enriched by differentially expressed genes. The bubble size indicates the count of genes enriched. The closer the color is to red, the smaller the adjusted p; F: Circle graph of the Kyoto Encyclopedia of Genes and Genomes pathways enriched by differentially expressed genes.

DISCUSSION

Cellular senescence is a permanent state of cell cycle arrest occurring in proliferating cells when face distinct stresses[36]. In cancers, senescence is usually an effective barrier against tumorigenesis because it prevents the division potential of cells[37]. Nonetheless, numerous research has demonstrated that senescent cells also have tumorigenic properties[38]. Thus, it is of significance to characterize key features of cellular senescence in HCC.

HCC is a typically fatal malignant tumor displaying genetic heterogeneity and limited therapy responses[39]. Based upon prognostic differentially expressed cellular senescence genes, we classified HCCs as three cellular senescence subtypes: C1–C3. The robustness and reproducibility of this classification were externally proven. C2 had the worst

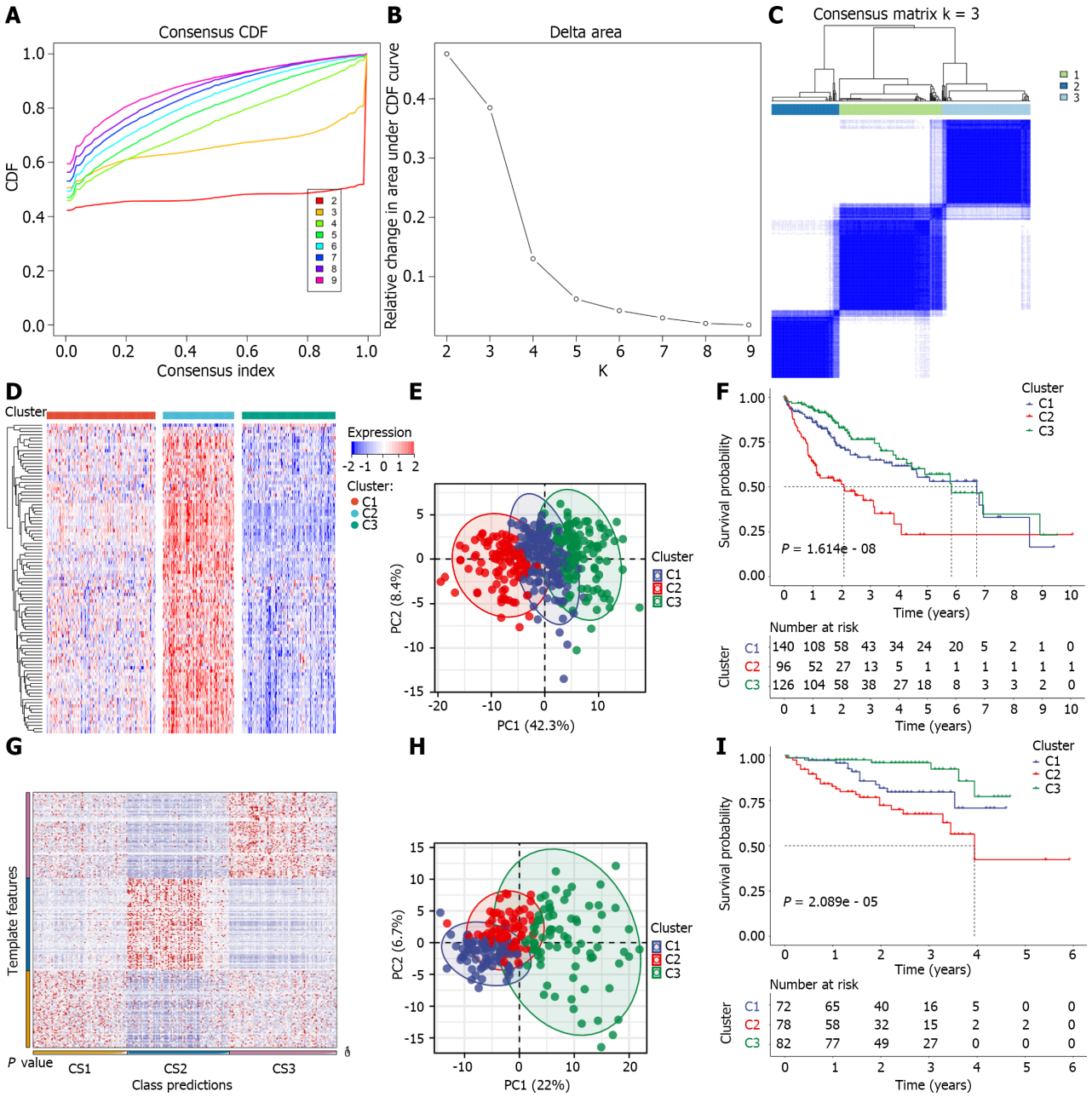
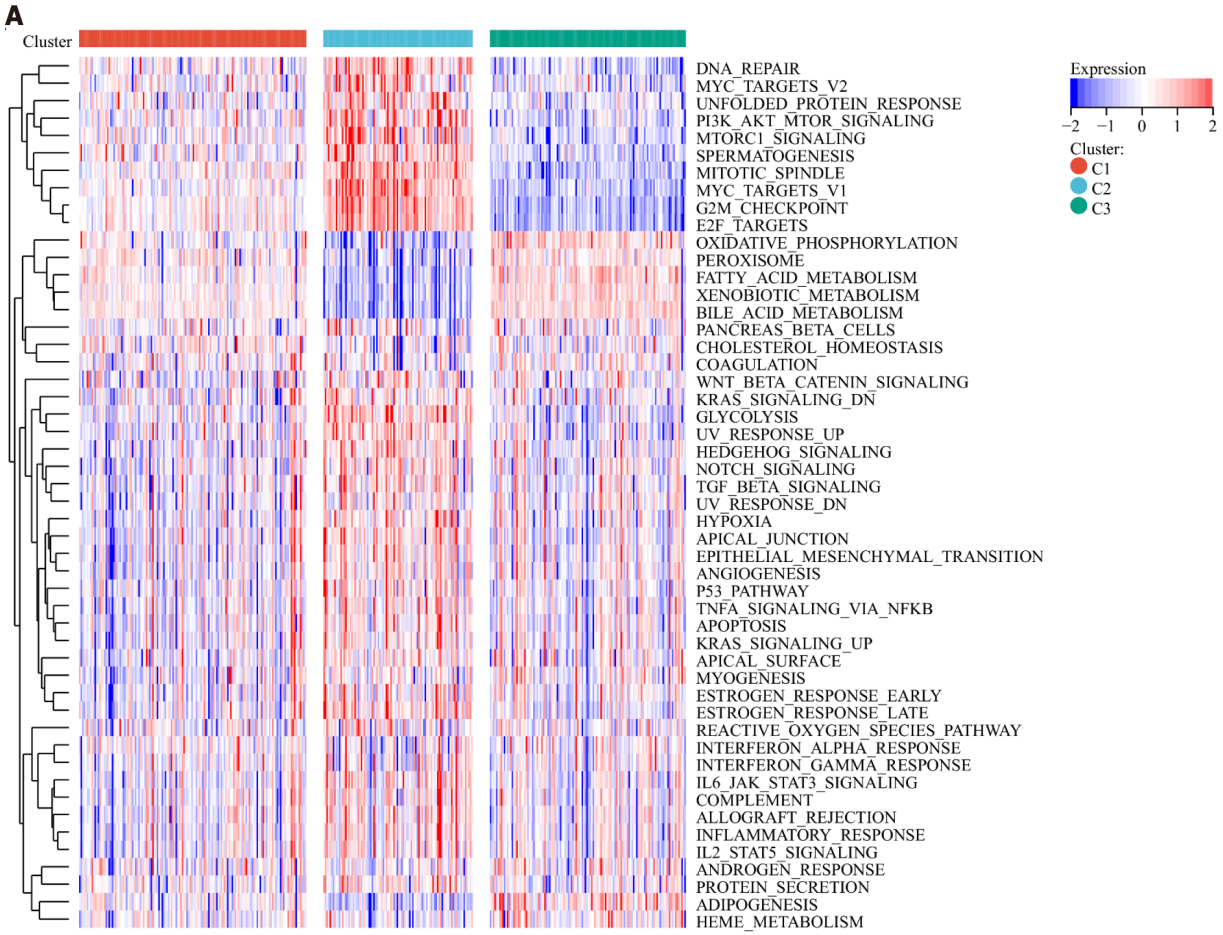
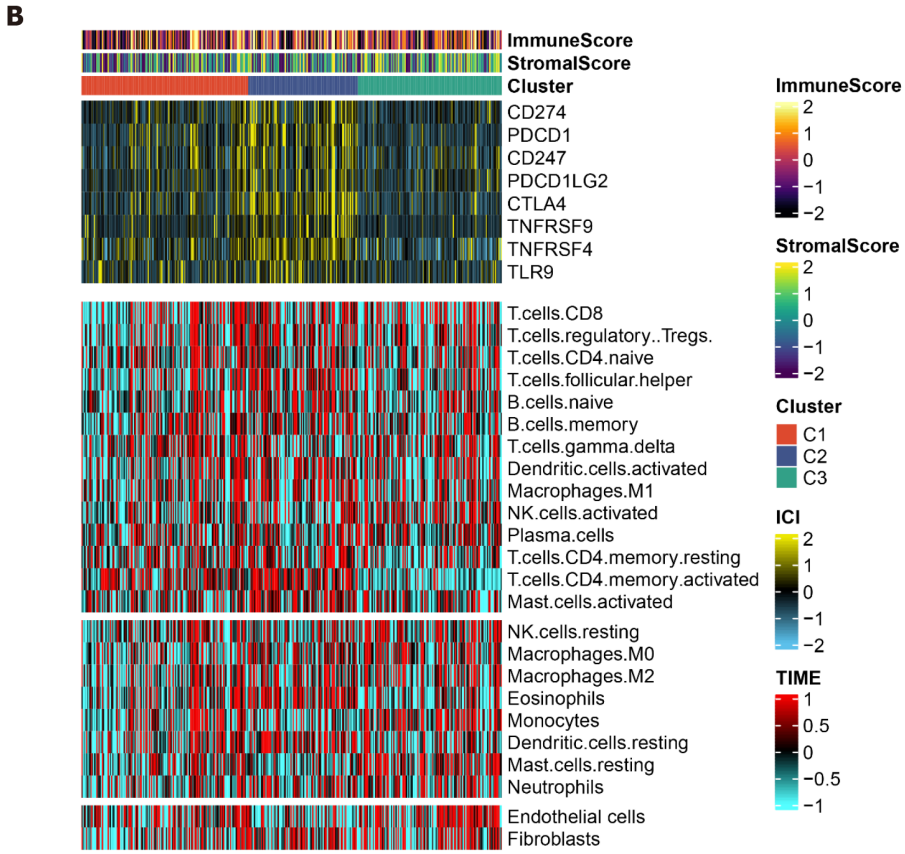


Figure 2 Classification of TCGA-LIHC patients as three cellular senescence subtypes and external dataset validation. A–C: Consensus cumulative distribution function (CDF), relative alteration in area under CDF curve, and consensus matrix k = 3 based upon the transcriptome of prognostic differentially expressed cellular senescence genes across TCGA-LIHC patients; D: The transcript levels of differentially expressed cellular senescence genes with prognostic implications across three subtypes; E: Principal component analysis (PCA) plots of the discrepancy in transcript levels among subtypes; F: Kaplan–Meier (K-M) curves of overall survival in TCGA-LIHC; G: Nearest template prediction for verifying the subtypes in the International Cancer Genome Consortium (ICGC) cohort; H and I: PCA plots of the transcriptome difference and K-M curves of overall survival among subtypes in the ICGC cohort.

overall survival, C1 the next, and C3 the best, revealing the heterogeneity in prognostic outcomes among subtypes. Single-agent anti-PD-1 immune checkpoint blockade showed ponent efficacy in early-phase trials, but the findings were not confirmed in phase III studies[40]. In accordance with the lowest TIDE score, and immunogenetic indicators, C3 HCCs might possibly respond to immunotherapy. Additionally, C2 HCCs were most likely to benefit from chemotherapy. Thus, this classification might assist clinical decision-making. Genetic mutations associate with HCC initiation and progression[41-43]. For instance, mutant TP53 is the most frequent in HCC, affecting patient survival, and immune response[44]. CTNNB1 mutation occupies a large proportion of human HCCs, which correlates to high TMB and AFP in HCCs[45]. Among three cellular senescence subtypes, C2 presented the lowest somatic mutation rate, while C1 had the highest frequent CNVs. Accordingly, cellular senescence subtypes appear to associate with genetic mutations.

We defined a novel cellular-senescence-relevant gene signature comprising SLC1A5, G6PD, PSRC1, UCK2, TCOF1, CCT5, DTYMK, NEIL3, TRNP1, ADH4, HMMR, SMG5, CLEC3B, PLOD2, SPP1, CFHR3, TMEM106C, ANXA10, and LCAT, with the excellent power in survival prediction in HCCs. Previous research has proven the biological implications of the cellular-senescence-relevant genes in HCC. For example, SLC1A5 regulated by DDR1 contributes to HCC


A
Cluster
DNA_REPAIR
MYC_TARGETS_V2
UNFOLDED_PROTEIN_RESPONSE
PI3K_AKT_MTOR_SIGNALING
MTORC1_SIGNALING
SPERMATOGENESIS
MITOTIC_SPINDLE
MYC_TARGETS_V1
G2M_CHECKPOINT
E2F_TARGETS
OXIDATIVE_PHOSPHORYLATION
PEROXISOME
FATTY_ACID_METABOLISM
XENOBIOTIC_METABOLISM
BILE_ACID_METABOLISM
PANCREAS_BETA_CELLS
CHOLESTEROL_HOMEOSTASIS
COAGULATION
WNT_BETA_CATENIN_SIGNALING
KRAS_SIGNALING_DN
GLYCOLYSIS
UV_RESPONSE_UP
HEDGEHOG_SIGNALING
NOTCH_SIGNALING
TGF_BETA_SIGNALING
UV_RESPONSE_DN
HYPOXIA
APICAL_JUNCTION
EPITHELIAL_MESENCHYMAL_TRANSITION
ANGIOGENESIS
P53_PATHWAY
TNFA_SIGNALING_VIA_NFKB
APOPTOSIS
KRAS_SIGNALING_UP
APICAL_SURFACE
MYOGENESIS
ESTROGEN_RESPONSE_EARLY
ESTROGEN_RESPONSE_LATE
REACTIVE_OXYGEN_SPECIES_PATHWAY
INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
IL6_JAK_STAT3_SIGNALING
COMPLEMENT
ALLOGRAFT_REJECTION
INFLAMMATORY_RESPONSE
IL2_STAT5_SIGNALING
ANDROGEN_RESPONSE
PROTEIN_SECRETION
ADIPOGENESIS
HEME_METABOLISM
Expression
−2 −1 0 1 2
Cluster:
C1
C2
C3


B
ImmuneScore
StromalScore
Cluster
CD274
PDCD1
CD247
PDCD1LG2
CTLA4
TNFRSF9
TNFRSF4
TLR9
T.cells.CD8
T.cells.regulatory..Tregs.
T.cells.CD4.naive
T.cells.follicular.helper
B.cells.naive
B.cells.memory
T.cells.gamma.delta
Dendritic.cells.activated
Macrophages.M1
NK.cells.activated
Plasma.cells
T.cells.CD4.memory.resting
T.cells.CD4.memory.activated
Mast.cells.activated
NK.cells.resting
Macrophages.M0
Macrophages.M2
Eosinophils
Monocytes
Dendritic.cells.resting
Mast.cells.resting
Neutrophils
Endothelial cells
Fibroblasts
ImmuneScore
2 1 0 −1 −2
StromalScore
2 1 0 −1 −2
Cluster
C1
C2
C3
ICI
2 1 0 −1 −2
TIME
1 0.5 0 −0.5 −1

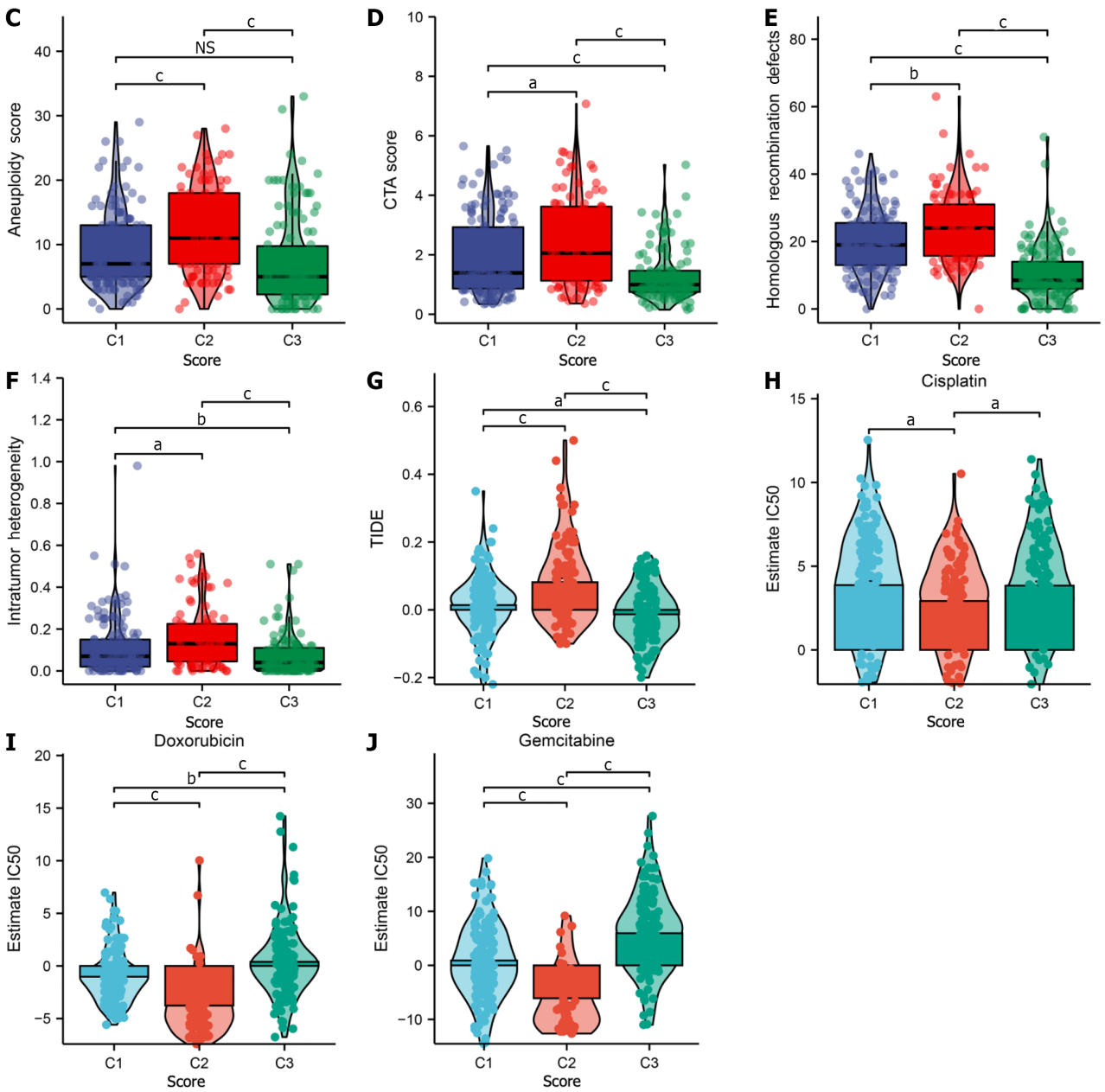
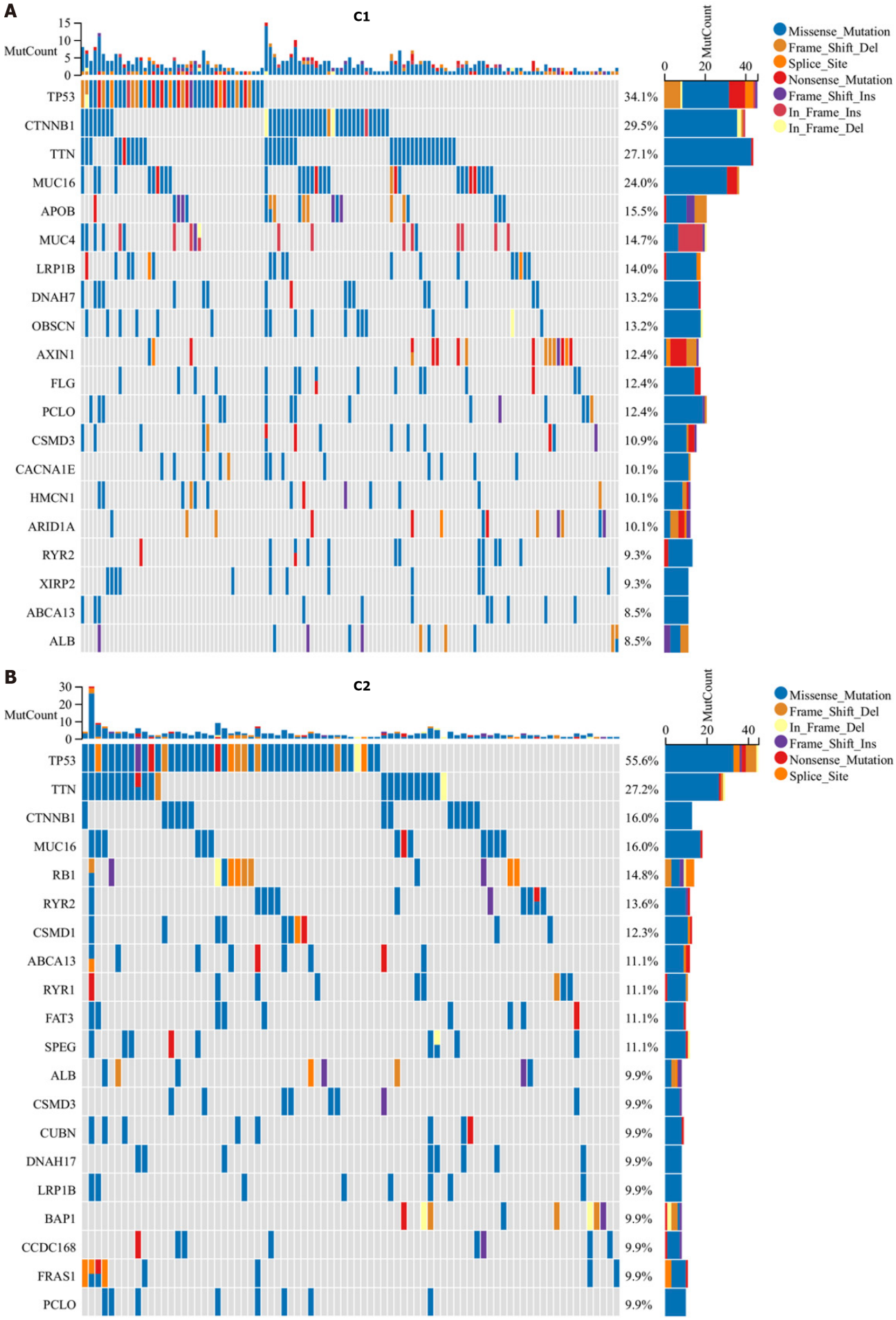
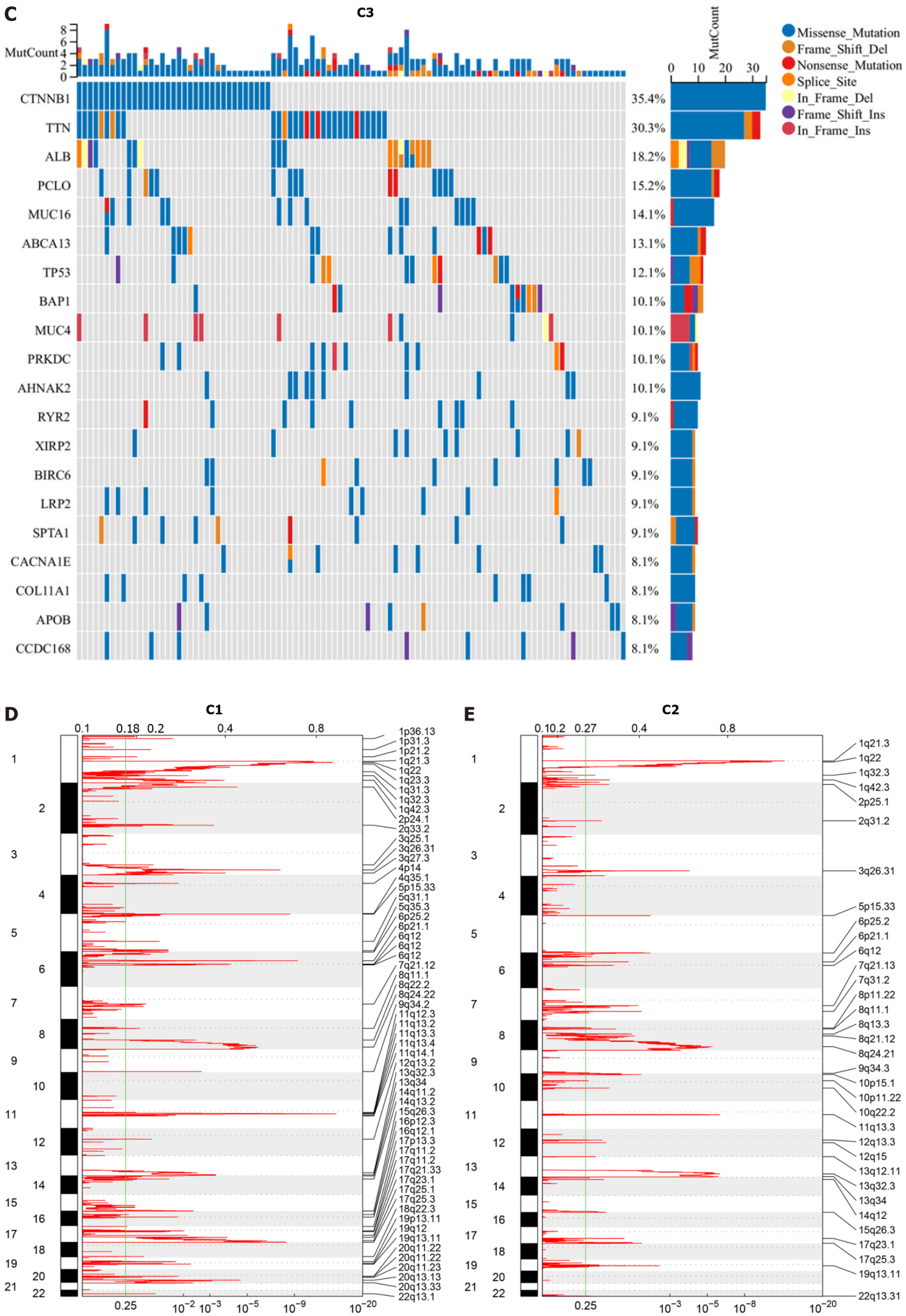


Figure 3 Immunogenomic landscape of three cellular senescence subtypes across TCGA-LIHC. A: The activity of 50 hallmark pathways in three subtypes; B: Abundance of the tumor microenvironment components, transcript levels of immune checkpoints, and stromal and immune scores across subtypes; C–F: Differences in aneuploidy score, cancer-testicular antigen score, homologous recombination defects, and intratumor heterogeneity between subtypes; G: Difference in TIDE score between subtypes; H–J: Differences in IC_{50} values of cisplatin, doxorubicin and gemcitabine between subtypes. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. NS: Not significant; TIDE: Tumor Immune Dysfunction and Exclusion.

progression[46]. G6PD weakens ferroptosis in HCC through targeting cytochrome P450 oxidoreductase[47]. PSRC1, a hypoxia- and immune-associated gene, associates with HCC survival[48]. The nonmetabolic role of UCK2 facilitates HCC metastasis *via* EGFR–AKT signaling activation[49]. TCOF1 coordinates oncogenic activation and rRNA generation as well as results in HCC initiation[50]. Clinical features of HCC patients are notably associated with survival outcomes. To better optimize the cellular-senescence-relevant gene signature and improve the prediction accuracy, we incorporated stage in combination with the cellular-senescence-relevant gene signature to build a nomogram that enabled us to generate the individual survival probability in HCC patients. High-RiskScore HCCs might respond to cisplatin, doxorubicin or gemcitabine, while low-RiskScore HCCs more possibly benefit from immunotherapy, proving the potential of the cellular-senescence-relevant gene signature in inferring therapeutic efficacy. Among the cellular-senescence-relevant genes, the expression and role of TRNP1 in HCC remain indistinct. Only bioinformatics evidence demonstrated the prognostic significance of TRNP1 in HCC[51]. Our study experimentally proved that TRNP1 was upregulated in HCC, and TRNP1 knockdown induced apoptosis and senescence of HCC cells and attenuated tumor growth. Thus, TRNP1 potentially participates in HCC senescence and progression, which might be a promising therapeutic target.


A
C1
MutCount
15
10
5
0
TP53 34.1%
CTNNB1 29.5%
TTN 27.1%
MUC16 24.0%
APOB 15.5%
MUC4 14.7%
LRP1B 14.0%
DNAH7 13.2%
OBSCN 13.2%
AXIN1 12.4%
FLG 12.4%
PCLO 12.4%
CSMD3 10.9%
CACNA1E 10.1%
HMCN1 10.1%
ARID1A 10.1%
RYR2 9.3%
XIRP2 9.3%
ABCA13 8.5%
ALB 8.5%
MutCount
0 20 40
Missense_Mutation
Frame_Shift_Del
Splice_Site
Nonsense_Mutation
Frame_Shift_Ins
In_Frame_Ins
In_Frame_Del
B
C2
MutCount
30
20
10
0
TP53 55.6%
TTN 27.2%
CTNNB1 16.0%
MUC16 16.0%
RB1 14.8%
RYR2 13.6%
CSMD1 12.3%
ABCA13 11.1%
RYR1 11.1%
FAT3 11.1%
SPEG 11.1%
ALB 9.9%
CSMD3 9.9%
CUBN 9.9%
DNAH17 9.9%
LRP1B 9.9%
BAP1 9.9%
CCDC168 9.9%
FRAS1 9.9%
PCLO 9.9%
MutCount
0 20 40
Missense_Mutation
Frame_Shift_Del
In_Frame_Del
Frame_Shift_Ins
Nonsense_Mutation
Splice_Site


C
C3
MutCount
CTNNB1 35.4%
TTN 30.3%
ALB 18.2%
PCLO 15.2%
MUC16 14.1%
ABCA13 13.1%
TP53 12.1%
BAP1 10.1%
MUC4 10.1%
PRKDC 10.1%
AHNAK2 10.1%
RYR2 9.1%
XIRP2 9.1%
BIRC6 9.1%
LRP2 9.1%
SPTA1 9.1%
CACNA1E 8.1%
COL11A1 8.1%
APOB 8.1%
CCDC168 8.1%
Missense_Mutation
Frame_Shift_Del
Nonsense_Mutation
Splice_Site
In_Frame_Del
Frame_Shift_Ins
In_Frame_Ins
D
C1
E
C2

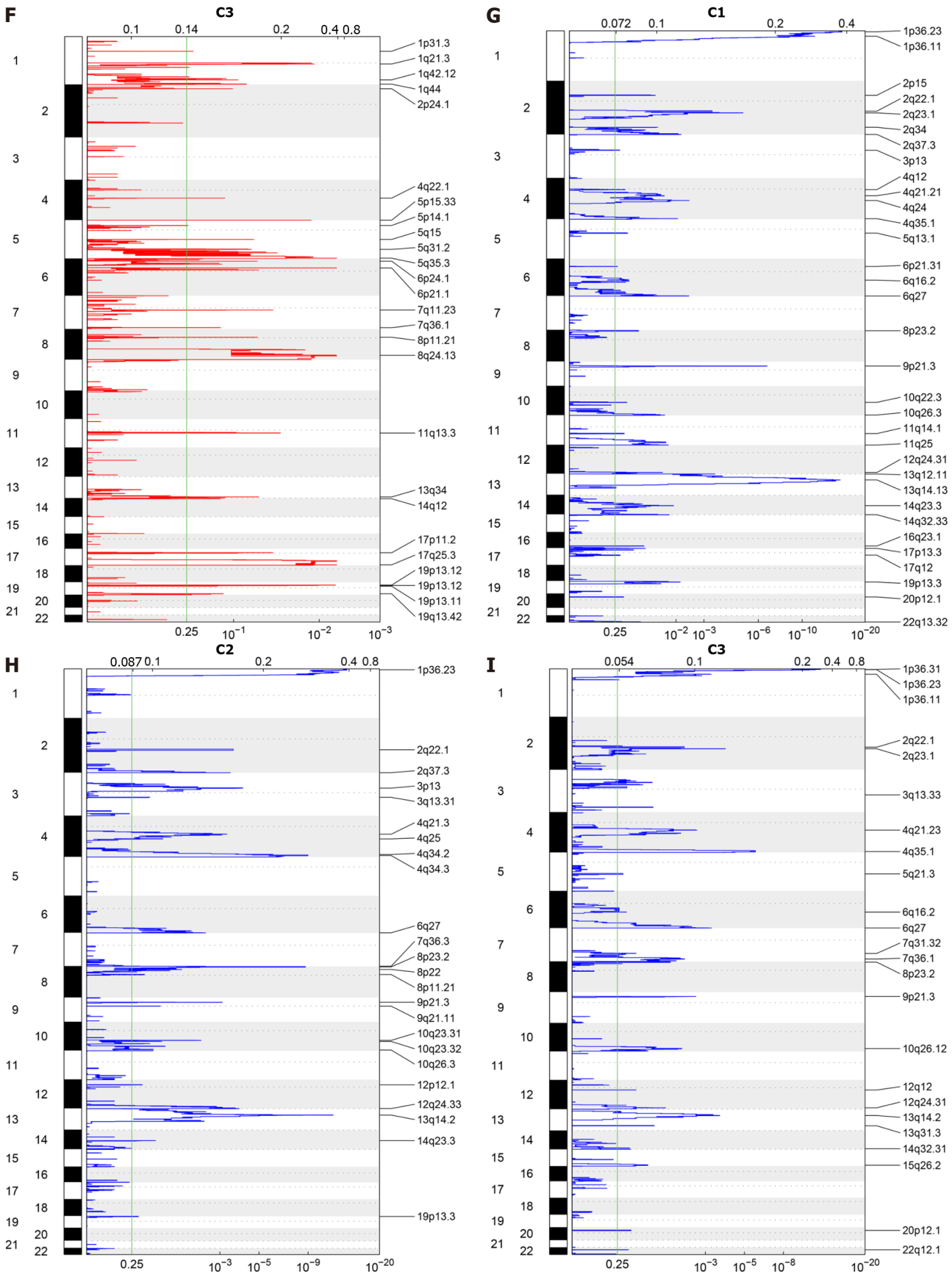


Figure 4 Genetic alterations of three cellular senescence subtypes. A–C: Significant mutated genes in The Cancer Genome Atlas hepatocellular carcinomas stratified by cellular senescence subtypes; D–F: Copy number amplification plots in three subtypes. The green line denotes the significance threshold (q = 0.25); G–I: Copy number deletion plots in three subtypes.

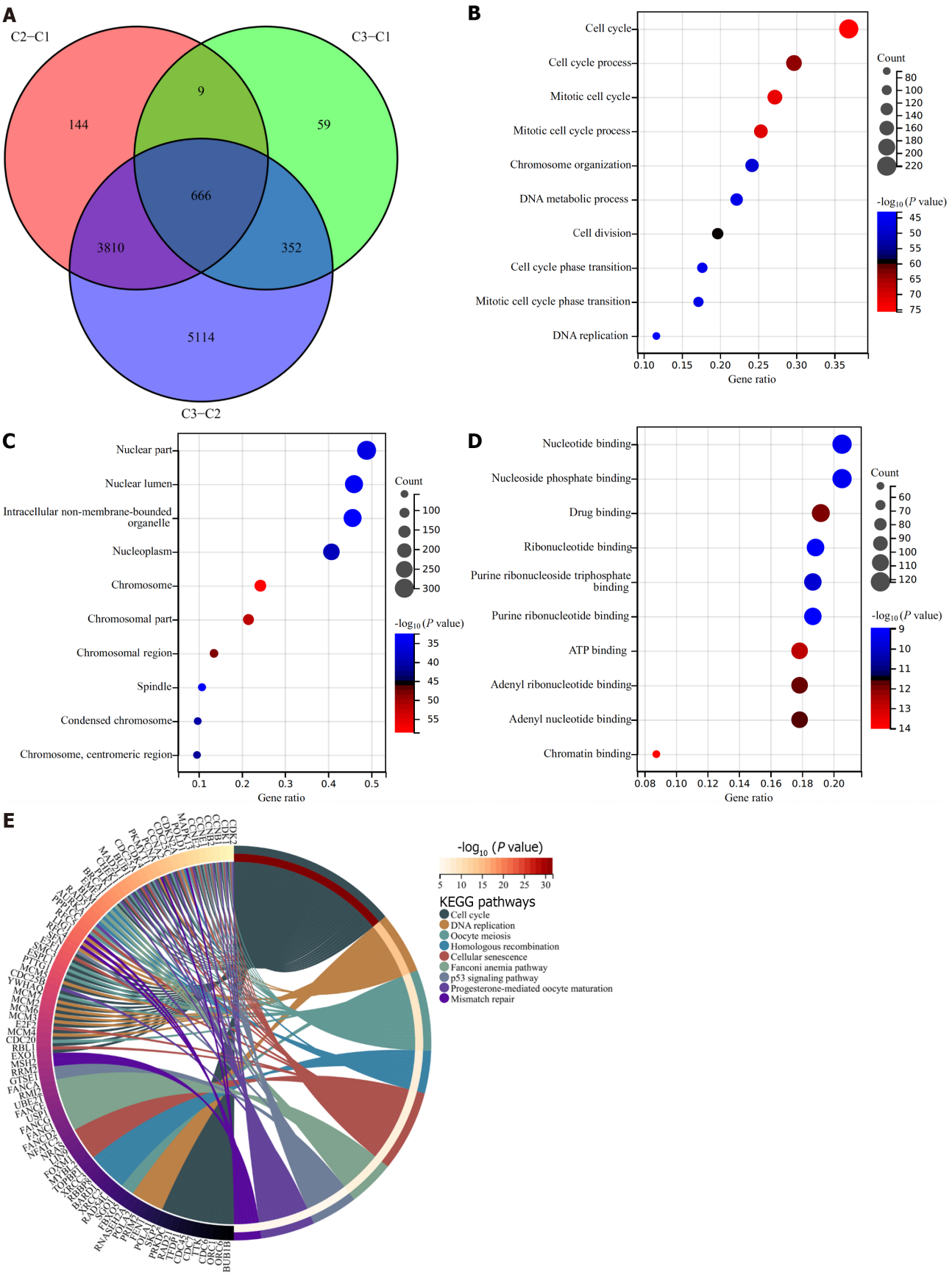
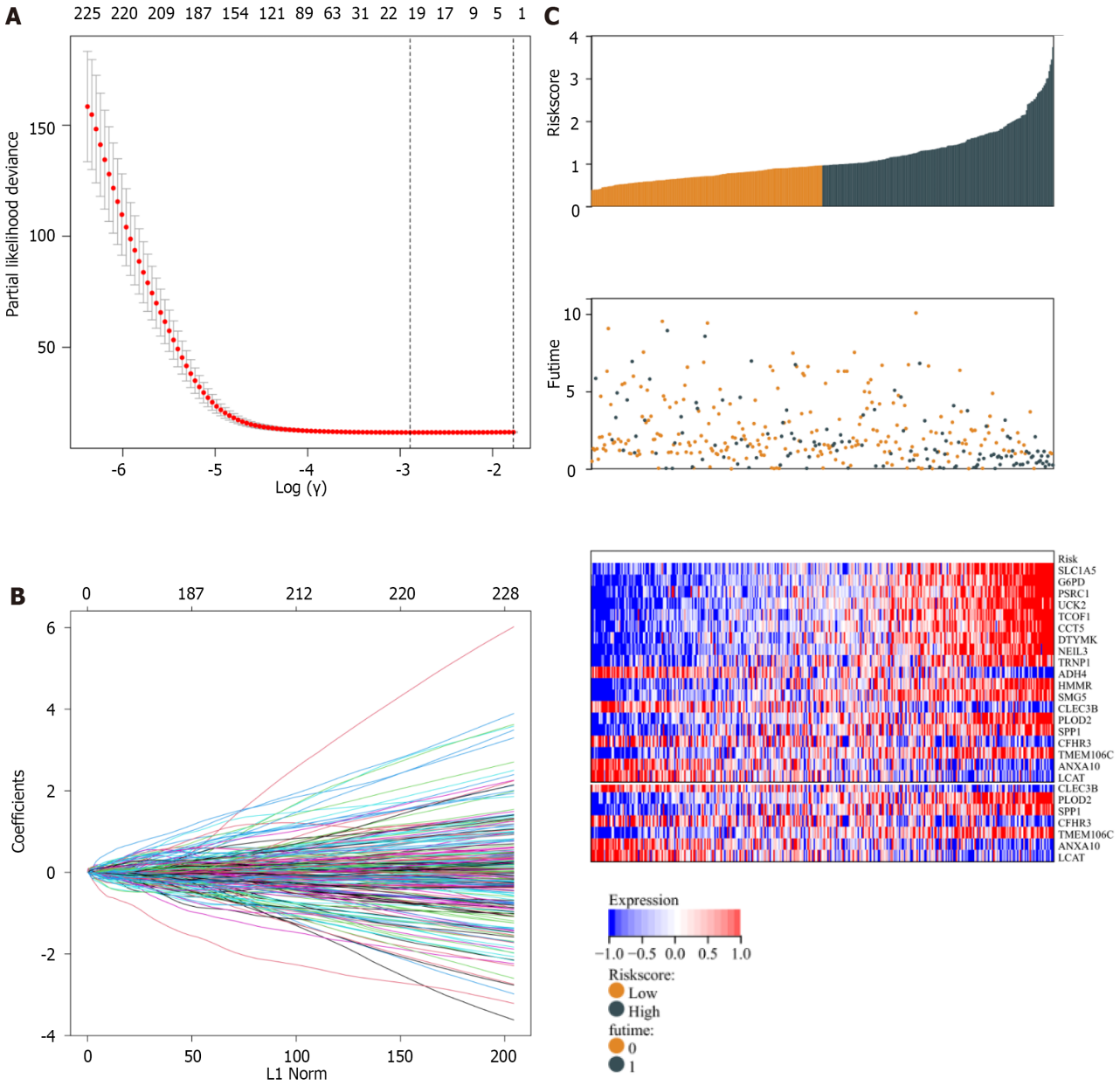


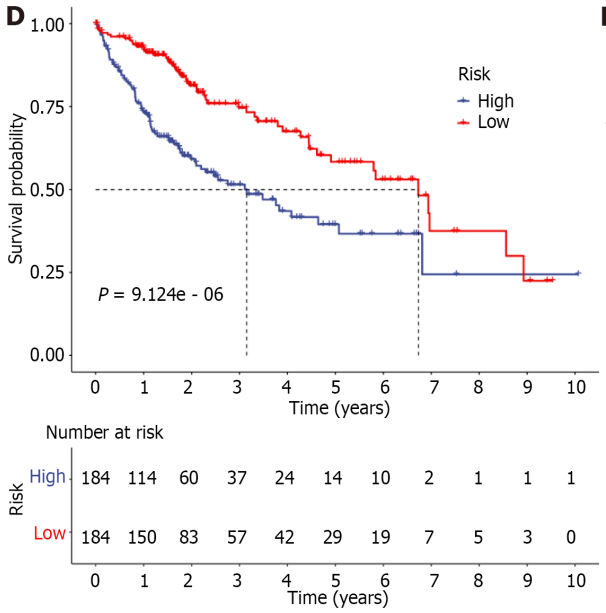
Figure 5 Identification of cellular senescence subtype-relevant genes and functional implications. A: Venn diagram of the intersected genes from differentially expressed genes between cellular senescence subtypes in TCGA-LIHC cohort; B–D: The top 10 biological processes, cellular components, together with molecular functions of cellular senescence subtype-relevant genes; E: Kyoto Encyclopedia of Genes and Genomes pathways significantly enriched by these genes.

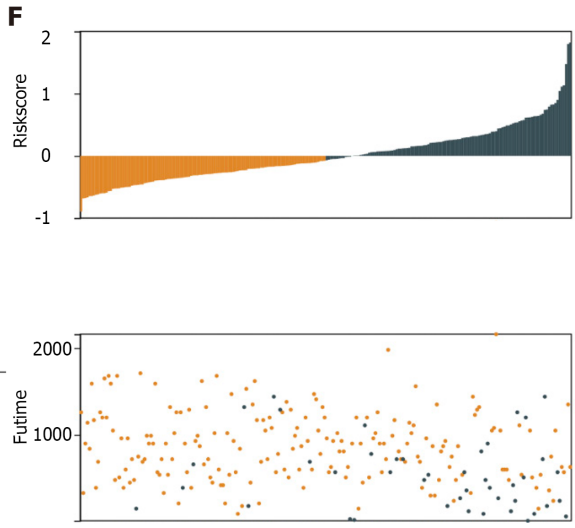
Our study had some limitations. Due to the lack of HCC patients who received neoadjuvant immunotherapy, the relationship between cellular senescence subtypes and relevant gene signature with immunotherapeutic response requires further verification in the immunotherapy cohorts. Despite the external verification in the ICGC dataset, the predictive efficacy of cellular senescence-relevant gene signature needs to be proven in prospective cohorts.

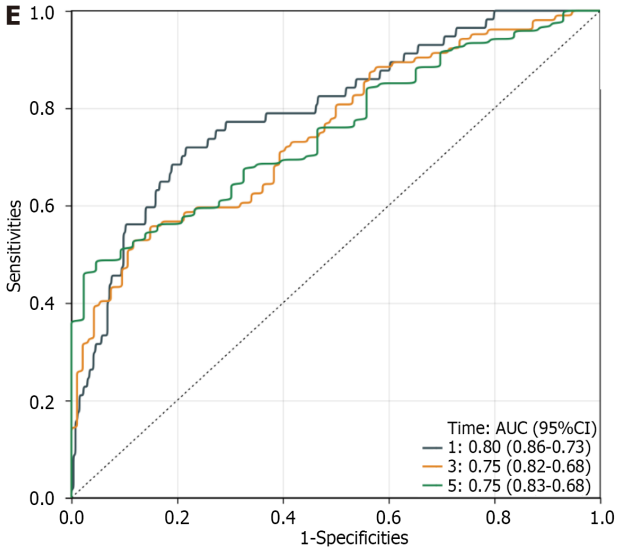
CONCLUSION

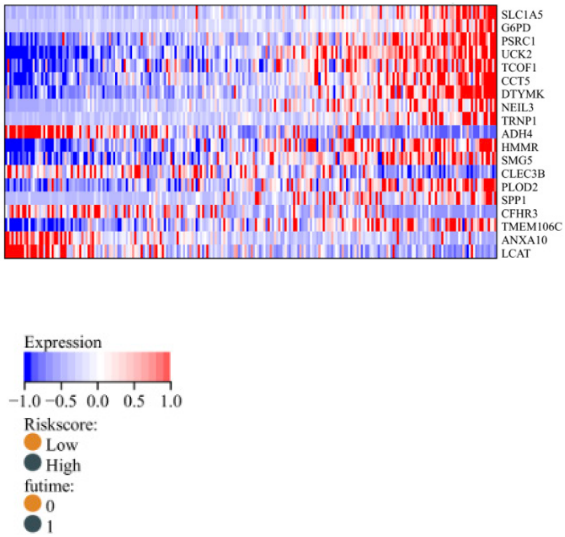
Our findings showed the importance of cellular senescence in HCC classification and pharmacological interventions for clinical management. Additionally, we defined a cellular-senescence-relevant scoring system that can infer patient survival and therapeutic efficacy. Considering the clinically relevant parameters were closely linked with HCC survival, we incorporated stage in combination with the cellular-senescence-relevant gene signature to build a nomogram. Our integrated analysis provides a valuable framework for comprehending cellular senescence in HCC, which sheds light on the senescence-associated biomarker discovery as well as therapeutic targets.

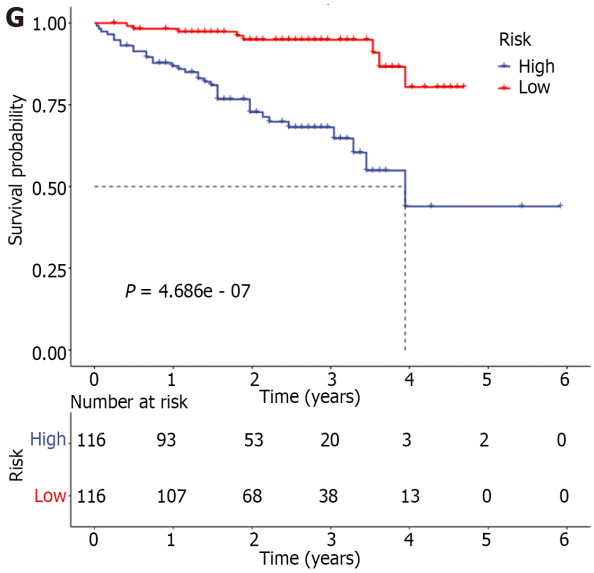


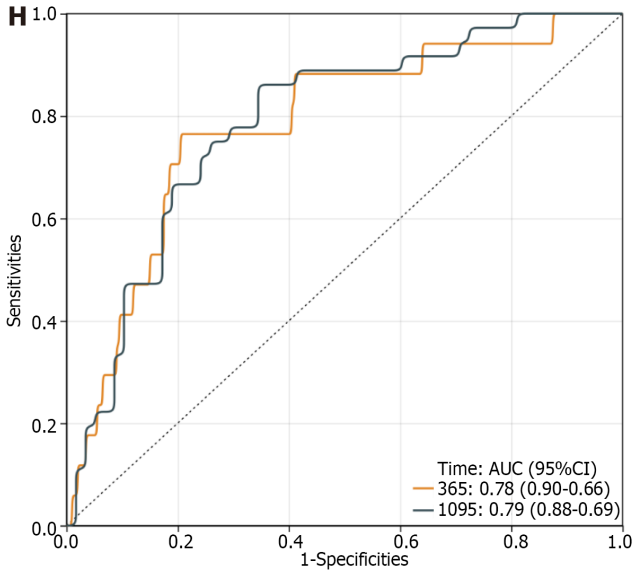

D
Survival probability
Risk
High
Low
P = 9.124e - 06
Time (years)
Number at risk
High 184 114 60 37 24 14 10 2 1 1 1
Low 184 150 83 57 42 29 19 7 5 3 0


F
Riskscore
Futime


E
Sensitivities
1-Specificities
Time: AUC (95%CI)
1: 0.80 (0.86-0.73)
3: 0.75 (0.82-0.68)
5: 0.75 (0.83-0.68)


SLC1A5
G6PD
PSRC1
UCK2
TCOF1
CCT5
DTYMK
NEIL3
TRNP1
ADH4
HMMR
SMG5
CLEC3B
PLOD2
SPP1
CFHR3
TMEM106C
ANXA10
LCAT
Expression
Riskscore:
Low
High
futime:
0
1


G
Survival probability
Risk
High
Low
P = 4.686e - 07
Time (years)
Number at risk
High 116 93 53 20 3 2 0
Low 116 107 68 38 13 0 0


H
Sensitivities
1-Specificities
Time: AUC (95%CI)
365: 0.78 (0.90-0.66)
1095: 0.79 (0.88-0.69)

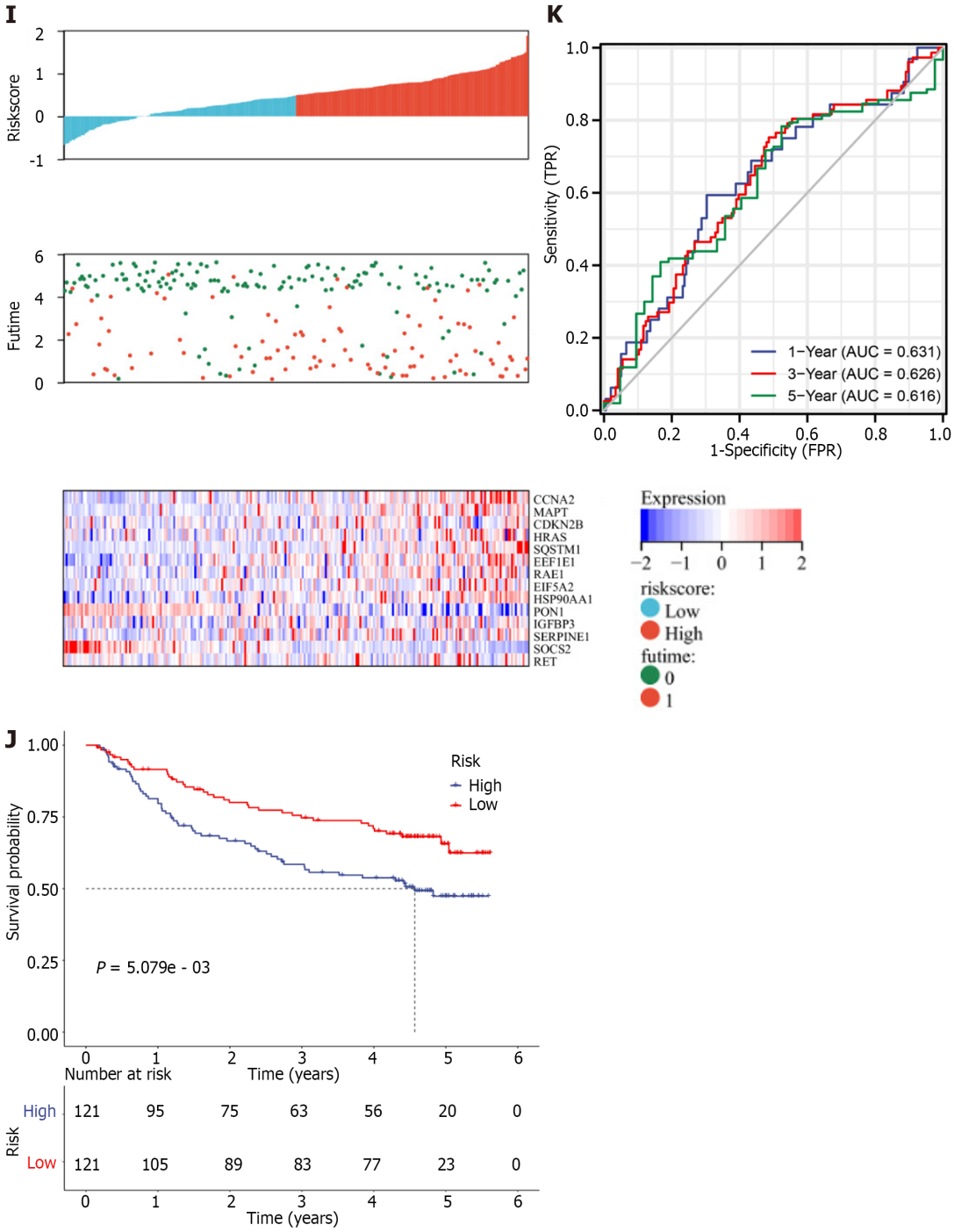


Figure 6 Definition and external verification of a cellular-senescence-relevant gene signature. A: Cross-validation for tuning the parameter selection in the least absolute shrinkage and selection operator (LASSO) analysis in TCGA-LIHC dataset; B: LASSO coefficient profiling; C: Distribution of RiskScore, survival status, and transcript levels of genes in the signature; D: Kaplan–Meier (curves of overall survival in low- and high-RiskScore hepatocellular carcinomas; E: Receiver operator characteristic curves (ROCs) at 1-, 3- and 5-year survival; F: External verification of distribution of RiskScore, survival status, and transcript levels of genes in the International Cancer Genome Consortium (ICGC) dataset; G: Kaplan–Meier curves of overall survival in two groups in the ICGC dataset; H: ROCs at 1- and 3-year survival based upon RiskScore in the ICGC dataset; I: External validation of distribution of RiskScore, survival status, and transcript levels of genes in the GSE14520 dataset; J: Kaplan–Meier curves of overall survival in two groups in the GSE14520 dataset; K: ROCs at 1- and 3-year survival in the GSE14520 dataset.

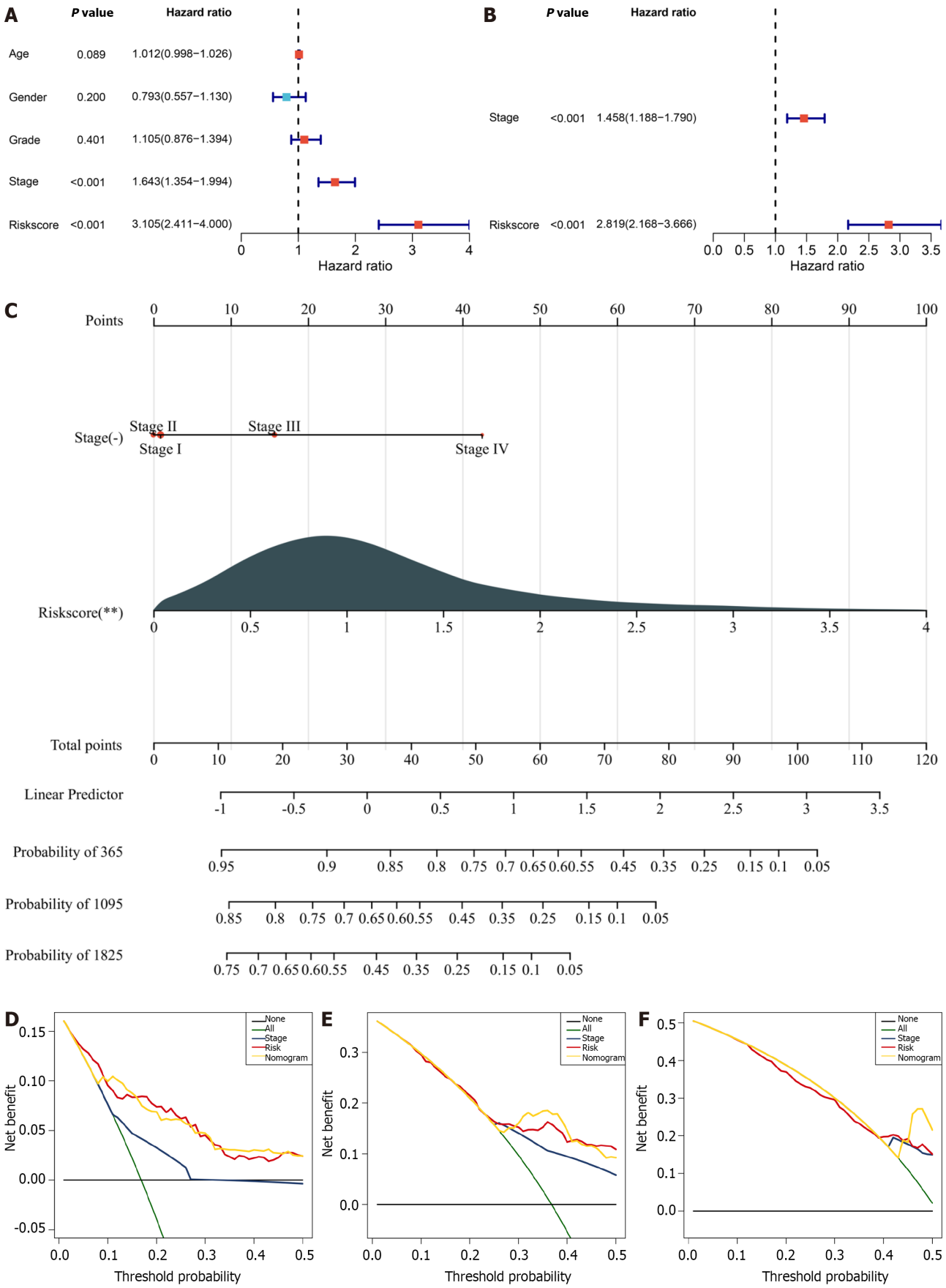


Figure 7 Establishment of a prognostic nomogram for clinical practice in the TCGA-LIHC dataset. A: Univariate-cox regression results of the cellular-senescence-relevant gene signature and conventional clinicopathological parameters with hepatocellular carcinoma prognosis; B: Multivariate Cox regression for selecting independent prognostic parameters; C: Generation of a nomogram based on stage and the cellular senescence-relevant RiskScore; D–F: Decision curve analysis at 1-, 1-, and 5-year survival.

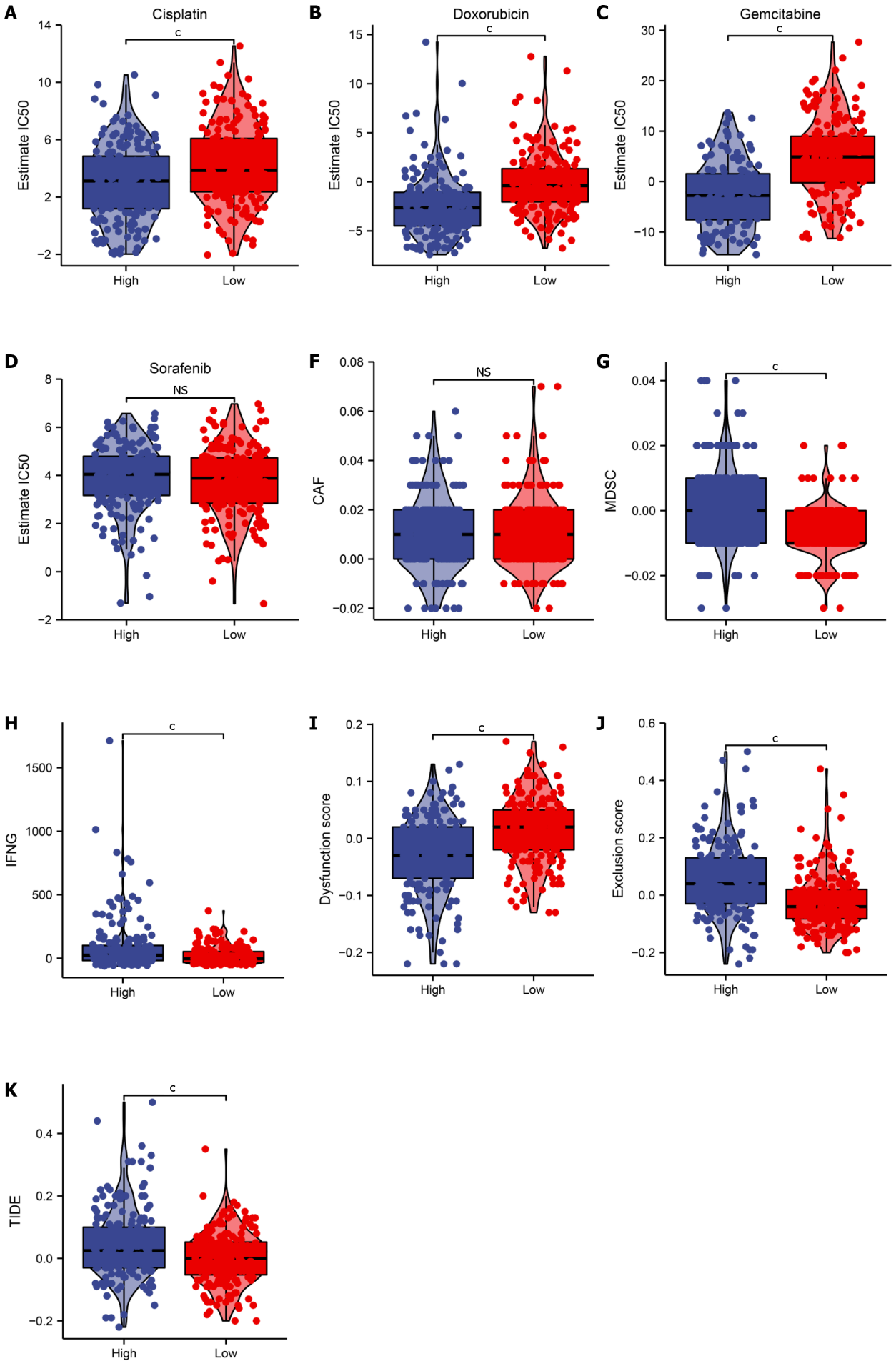

A
Cisplatin
Estimate IC50
c
High
Low
B
Doxorubicin
Estimate IC50
c
High
Low
C
Gemcitabine
Estimate IC50
c
High
Low
D
Sorafenib
Estimate IC50
NS
High
Low
F
CAF
NS
High
Low
G
MDSC
c
High
Low
H
IFNG
c
High
Low
I
Dysfunction score
c
High
Low
J
Exclusion score
c
High
Low
K
TIDE
c
High
Low

Figure 8 Assessment of the cellular senescence-relevant gene signature in predicting efficacy of pharmacological interventions in TCGA-LIHC dataset. A–D: IC_{50} value of cisplatin, doxorubicin, gemcitabine, and sorafenib in low- and high-RiskScore hepatocellular carcinomas (HCCs); E: Abundance of the tumor microenvironment components inferred by multiple algorithms; F–K: Comparison of carcinoma-associated fibroblast (CAF), myeloid-derived suppressor cell (MDSC), interferon gamma (IFNG), dysfunction score, exclusion score and Tumor Immune Dysfunction and Exclusion levels in low- and high-RiskScore HCCs. °P < 0.001. ns: No significant difference.

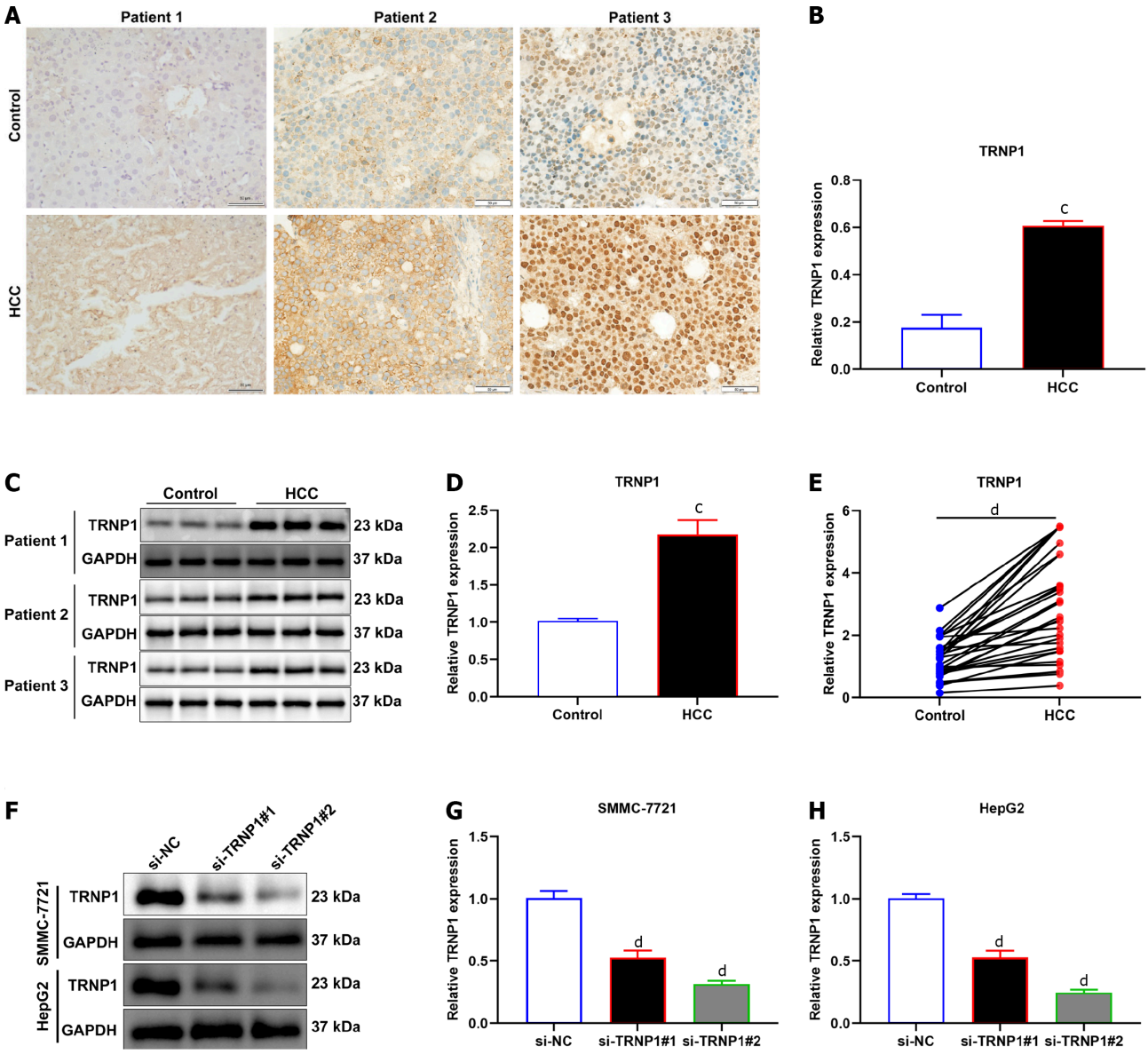
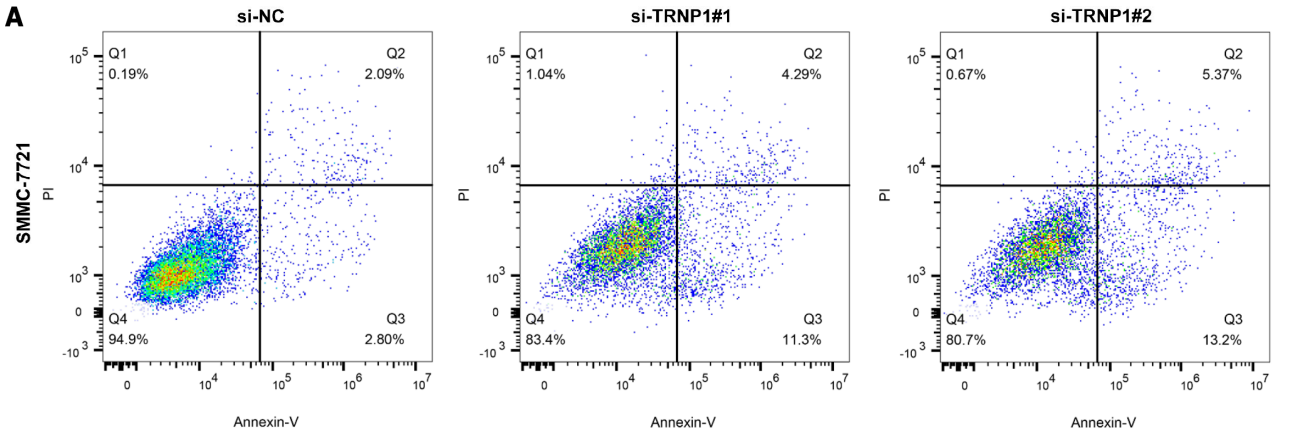
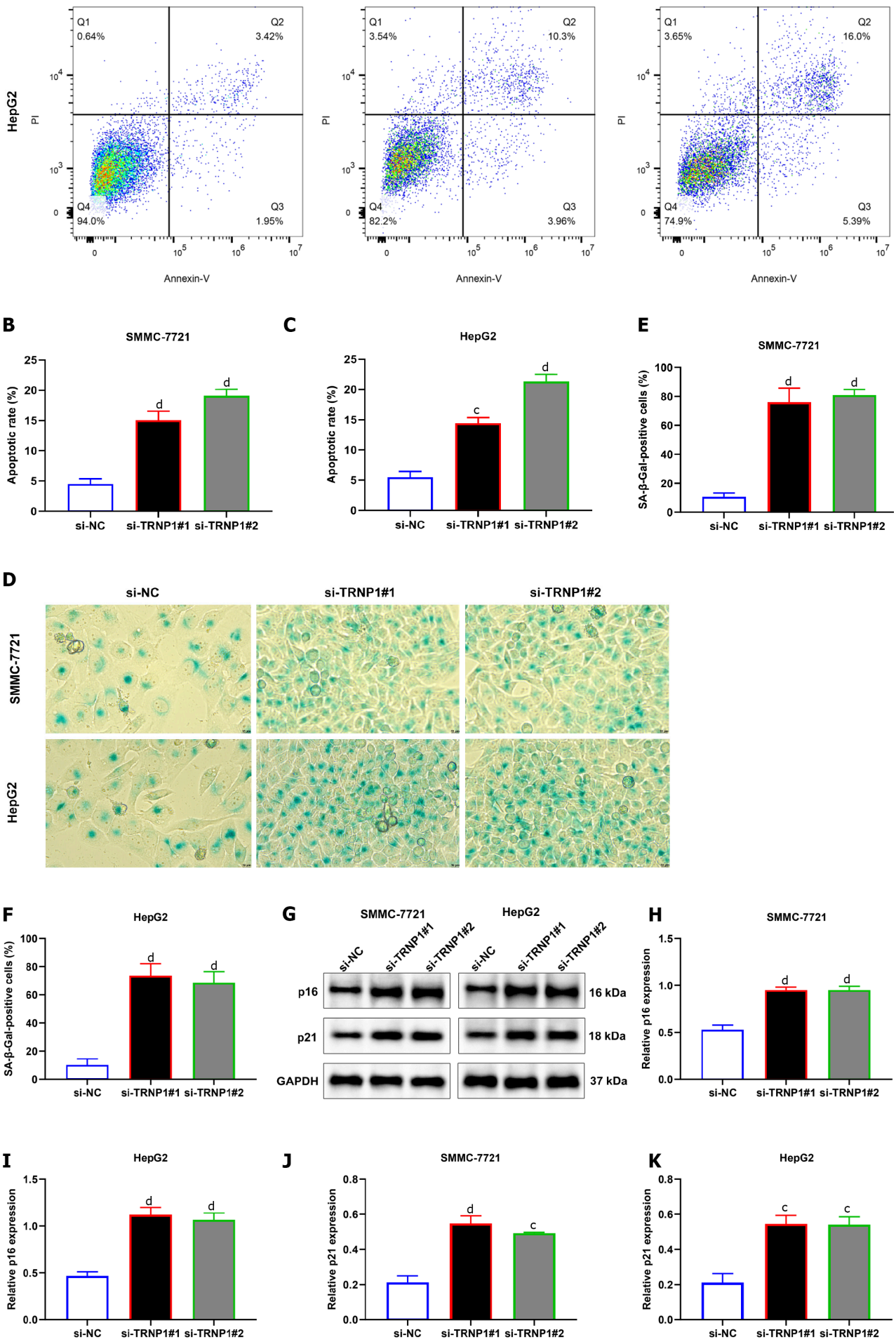


Figure 9 Experimental verification of expression of *TRNP1* in hepatocellular carcinomas. A and B: Representative immunohistochemistry of *TRNP1* expression in human hepatocellular carcinomas (HCCs) and normal tissues. Bar, 50 μm; C and D: Representative immunoblotting of *TRNP1* expression in human HCCs and normal tissues; E: Quantitative real-time polymerase chain reaction of *TRNP1* expression in 30 pairs of human HCCs and normal tissues; F–H: Immunoblotting of *TRNP1* expression in SMMC-7721 and HepG2 cells transfected with specific siRNAs of *TRNP1*. $^{c}P < 0.001$; $^{d}P < 0.0001$.




HepG2
Q1 0.64%
Q2 3.42%
Q4 94.0%
Q3 1.95%
Q1 3.54%
Q2 10.3%
Q4 82.2%
Q3 3.96%
Q1 3.65%
Q2 16.0%
Q4 74.9%
Q3 5.39%
PI
Annexin-V
B
SMMC-7721
Apoptotic rate (%)
si-NC
si-TRNP1#1
si-TRNP1#2
C
HepG2
E
SMMC-7721
SA-β-Gal-positive cells (%)
D
si-NC
si-TRNP1#1
si-TRNP1#2
SMMC-7721
HepG2
F
HepG2
G
SMMC-7721
HepG2
p16
p21
GAPDH
16 kDa
18 kDa
37 kDa
H
SMMC-7721
Relative p16 expression
I
HepG2
J
SMMC-7721
Relative p21 expression
K
HepG2

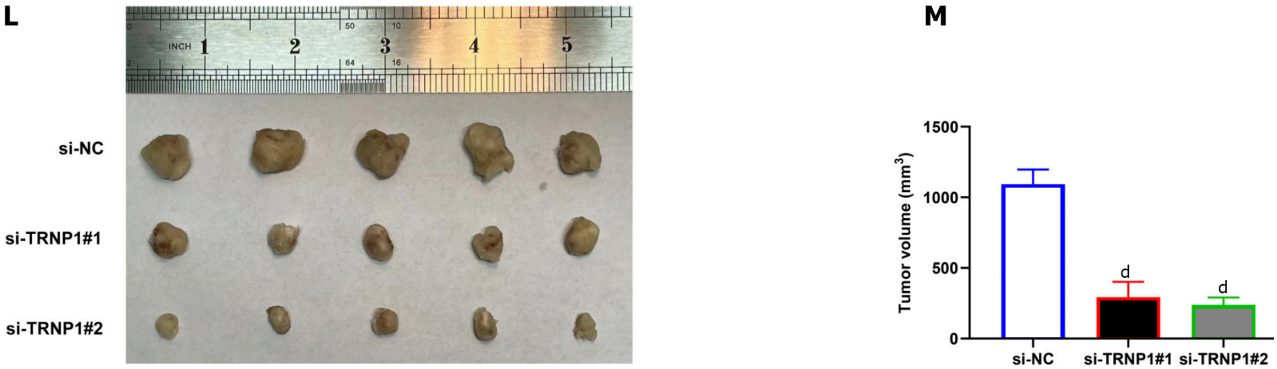


Figure 10 Suppression of TRNP1 induces apoptosis and senescence of hepatocellular carcinoma cells and attenuates tumor growth. A–C: Flow cytometry for measuring the apoptotic rate of in SMMC-7721 and HepG2 cells with transfection of specific siRNAs of TRNP1; D-F: SA-β-galactosidase (SA-β-Gal) staining for evaluating senescence of transfected hepatocellular carcinoma cells. Bar, 10 μm; G–K: Immunoblotting of p16 and p21 expression in transfected cells; L: Representative photographs of tumors from mice of si-NC, si-TRNP1#1 and si-TRNP1#2 groups; M: Calculation of tumor volume in above groups. $^{c}P < 0.001$; $^{d}P < 0.0001$.

ARTICLE HIGHLIGHTS

Research background

Cellular senescence, a state of stable growth arrest, is intertwined with human cancers. Due to the highly heterogeneous malignancy at the molecular and histological levels, characterization of cellular-senescence-based classification might facilitate the personalized treatment of hepatocellular carcinoma (HCC).

Research motivation

Nonetheless, the heterogeneity of cellular-senescence-related features makes the definition and targeting of treatment-induced senescent cells challenging.

Research objectives

This study aimed to characterize cellular-senescence-based phenotypes in HCC, and identify a novel cellular-senescence-related therapeutic target.

Research methods

We enrolled two HCC datasets, TCGA-LIHC and International Cancer Genome Consortium (ICGC). Unsupervised clustering was executed to probe tumor heterogeneity based upon cellular senescence genes. Least absolute shrinkage and selection operator algorithm was utilized to define a cellular-senescence-relevant scoring system. TRNP1 expression was measured in HCCs and normal tissues through immunohistochemistry, immunoblotting and quantitative real-time polymerase chain reaction. The influence of TRNP1 on HCC senescence and growth was proven *via* a series of experiments.

Research results

TCGA-LIHC patients were classified as three cellular senescence subtypes, named C1–3. The robustness and reproducibility of these subtypes were proven in the ICGC cohort. C2 had the worst overall survival, C1 the next, and C3 the best. C2 presented the highest levels of immune checkpoints, abundance of immune cells, and immunogenetic indicators. Thus, C2 might respond to immunotherapy. C2 had the lowest somatic mutation rate, while C1 presented the highest copy number variations. A cellular-senescence-relevant gene signature was generated, which can predict patient survival, and chemo- or immunotherapeutic response. Experimentally, it was proven that TRNP1 presented with remarkable upregulation in HCCs. TRNP1 knockdown induced apoptosis and senescence of HCC cells and attenuated tumor growth.

Research conclusions

These findings provide a systematic framework for assessing cellular senescence in HCC, which decode the tumor heterogeneity and tailor the pharmacological interventions to improve clinical management.

Research perspectives

Cellular senescence, a state of stable growth arrest, is implicated in human cancers. Nevertheless, characterization of cellular-senescence-associated phenotypes in HCC is still indistinct. Here, we proposed a novel cellular-senescence-based classification for HCC and identified TRNP1 as a novel therapeutic target.

FOOTNOTES

Author contributions: Wang HH and Chen WL contributed equally to this work; Li H conceived and designed the study; Wang HH and Chen WL conducted most of the experiments and data analysis, and wrote the manuscript; Cui YY and Gong HH participated in collecting data and helped to draft the manuscript; All authors reviewed and approved the manuscript.

Supported by Project of Bozhou Municipal Health Commission, No. bzwj2022A001; Project of Bozhou Science and Technology Bureau, No. bzzc2022008; and Scientific Research Fund of Bozhou Hospital, Anhui Medical University, No. by2022001.

Institutional review board statement: The study was approved by the Ethics Committee of The Affiliated Bozhou Hospital of Anhui Medical University, No. 2022-17.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: The data used to support the findings of this study are included within the supplementary information files.

Country/Territory of origin: China

ORCID number: Heng Li 0000-0002-6663-1046.

S-Editor: Fan JR
L-Editor: Kerr C
P-Editor: Zhang XD

REFERENCES

1 **Yamamoto-Imoto H**, Minami S, Shioda T, Yamashita Y, Sakai S, Maeda S, Yamamoto T, Oki S, Takashima M, Yamamuro T, Yanagawa K, Edahiro R, Iwatani M, So M, Tokumura A, Abe T, Imamura R, Nonomura N, Okada Y, Ayer DE, Ogawa H, Hara E, Takabatake Y, Isaka Y, Nakamura S, Yoshimori T. Age-associated decline of MondoA drives cellular senescence through impaired autophagy and mitochondrial homeostasis. *Cell Rep* 2022; **38**: 110444 [PMID: 35235784 DOI: 10.1016/j.celrep.2022.110444]

2 **Zhang D**, Liu Y, Zhu Y, Zhang Q, Guan H, Liu S, Chen S, Mei C, Chen C, Liao Z, Xi Y, Ouyang S, Feng XH, Liang T, Shen L, Xu P. A non-canonical cGAS-STING-PERK pathway facilitates the translational program critical for senescence and organ fibrosis. *Nat Cell Biol* 2022; **24**: 766-782 [PMID: 35501370 DOI: 10.1038/s41556-022-00894-z]

3 **Avelar RA**, Ortega JG, Tacutu R, Tyler EJ, Bennett D, Binetti P, Budovsky A, Chatsirisupachai K, Johnson E, Murray A, Shields S, Tejada-Martinez D, Thornton D, Fraifeld VE, Bishop CL, de Magalhães JP. A multidimensional systems biology analysis of cellular senescence in aging and disease. *Genome Biol* 2020; **21**: 91 [PMID: 32264951 DOI: 10.1186/s13059-020-01990-9]

4 **Chatsirisupachai K**, Palmer D, Ferreira S, de Magalhães JP. A human tissue-specific transcriptomic analysis reveals a complex relationship between aging, cancer, and cellular senescence. *Aging Cell* 2019; **18**: e13041 [PMID: 31560156 DOI: 10.1111/acel.13041]

5 **Laphanuwat P**, Gomes DCO, Akbar AN. Senescent T cells: Beneficial and detrimental roles. *Immunol Rev* 2023; **316**: 160-175 [PMID: 37098109 DOI: 10.1111/imr.13206]

6 **Lee DA**. Cellular therapy: Adoptive immunotherapy with expanded natural killer cells. *Immunol Rev* 2019; **290**: 85-99 [PMID: 31355489 DOI: 10.1111/imr.12793]

7 **Wang X**, Ma L, Pei X, Wang H, Tang X, Pei JF, Ding YN, Qu S, Wei ZY, Wang HY, Wang X, Wei GH, Liu DP, Chen HZ. Comprehensive assessment of cellular senescence in the tumor microenvironment. *Brief Bioinform* 2022; **23** [PMID: 35419596 DOI: 10.1093/bib/bbac118]

8 **Wang B**, Demaria M. The Quest to Define and Target Cellular Senescence in Cancer. *Cancer Res* 2021; **81**: 6087-6089 [PMID: 34911777 DOI: 10.1158/0008-5472.CAN-21-2032]

9 **Basu A**. The interplay between apoptosis and cellular senescence: Bcl-2 family proteins as targets for cancer therapy. *Pharmacol Ther* 2022; **230**: 107943 [PMID: 34182005 DOI: 10.1016/j.pharmthera.2021.107943]

10 **Wang L**, Lankhorst L, Bernards R. Exploiting senescence for the treatment of cancer. *Nat Rev Cancer* 2022; **22**: 340-355 [PMID: 35241831 DOI: 10.1038/s41568-022-00450-9]

11 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

12 **Yau T**, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, Melero I, Kudo M, Hou MM, Matilla A, Tovoli F, Knox JJ, Ruth He A, El-Rayes BF, Acosta-Rivera M, Lim HY, Neely J, Shen Y, Wisniewski T, Anderson J, Hsu C. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: e204564 [PMID: 33001135 DOI: 10.1001/jamaoncol.2020.4564]

13 **Krstic J**, Reinisch I, Schindlmaier K, Galhuber M, Riahi Z, Berger N, Kupper N, Moyschewitz E, Auer M, Michenthaler H, Nössing C,

Depaoli MR, Ramadani-Muja J, Usluer S, Stryeck S, Pichler M, Rinner B, Deutsch AJA, Reinisch A, Madl T, Chiozzi RZ, Heck AJR, Huch M, Malli R, Prokesch A. Fasting improves therapeutic response in hepatocellular carcinoma through p53-dependent metabolic synergism. *Sci Adv* 2022; **8**: eabh2635 [PMID: 35061544 DOI: 10.1126/sciadv.abh2635]

14 **Kang YK**, Chen LT, Ryu MH, Oh DY, Oh SC, Chung HC, Lee KW, Omori T, Shitara K, Sakuramoto S, Chung IJ, Yamaguchi K, Kato K, Sym SJ, Kadowaki S, Tsuji K, Chen JS, Bai LY, Oh SY, Choda Y, Yasui H, Takeuchi K, Hirashima Y, Hagihara S, Boku N. Nivolumab plus chemotherapy versus placebo plus chemotherapy in patients with HER2-negative, untreated, unresectable advanced or recurrent gastric or gastro-oesophageal junction cancer (ATTRACTION-4): a randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2022; **23**: 234-247 [PMID: 35030335 DOI: 10.1016/S1470-2045(21)00692-6]

15 **Liu X**, Niu X, Qiu Z. A Five-Gene Signature Based on Stromal/Immune Scores in the Tumor Microenvironment and Its Clinical Implications for Liver Cancer. *DNA Cell Biol* 2020; **39**: 1621-1638 [PMID: 32758021 DOI: 10.1089/dna.2020.5512]

16 **Xiang X**, Fu Y, Zhao K, Miao R, Zhang X, Ma X, Liu C, Zhang N, Qu K. Cellular senescence in hepatocellular carcinoma induced by a long non-coding RNA-encoded peptide PINT87aa by blocking FOXM1-mediated PHB2. *Theranostics* 2021; **11**: 4929-4944 [PMID: 33754036 DOI: 10.7150/thno.55672]

17 **Liu B**, Yi J, Yang X, Liu L, Lou X, Zhang Z, Qi H, Wang Z, Zou J, Zhu WG, Gu W, Luo J. MDM2-mediated degradation of WRN promotes cellular senescence in a p53-independent manner. *Oncogene* 2019; **38**: 2501-2515 [PMID: 30532073 DOI: 10.1038/s41388-018-0605-5]

18 **Paradis V**, Youssef N, Dargère D, Bâ N, Bonvoust F, Deschatrette J, Bedossa P. Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. *Hum Pathol* 2001; **32**: 327-332 [PMID: 11274643 DOI: 10.1053/hupa.2001.22747]

19 **Liu B**, Zhou Z, Jin Y, Lu J, Feng D, Peng R, Sun H, Mu X, Li C, Chen Y. Hepatic stellate cell activation and senescence induced by intrahepatic microbiota disturbances drive progression of liver cirrhosis toward hepatocellular carcinoma. *J Immunother Cancer* 2022; **10** [PMID: 34996812 DOI: 10.1136/jitc-2021-003069]

20 **Yildiz G**, Arslan-Ergul A, Bagislar S, Konu O, Yuzugullu H, Gursoy-Yuzugullu O, Ozturk N, Ozen C, Ozdag H, Erdal E, Karademir S, Sagol O, Mizrak D, Bozkaya H, Ilk HG, Ilk O, Bilen B, Cetin-Atalay R, Akar N, Ozturk M. Genome-wide transcriptional reorganization associated with senescence-to-immortality switch during human hepatocellular carcinogenesis. *PLoS One* 2013; **8**: e64016 [PMID: 23691139 DOI: 10.1371/journal.pone.0064016]

21 **Eggert T**, Wolter K, Ji J, Ma C, Yevsa T, Klotz S, Medina-Echeverz J, Longerich T, Forgues M, Reisinger F, Heikenwalder M, Wang XW, Zender L, Greten TF. Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. *Cancer Cell* 2016; **30**: 533-547 [PMID: 27728804 DOI: 10.1016/j.ccell.2016.09.003]

22 **Yu X**, Chen P, Yi W, Ruan W, Xiong X. Identification of cell senescence molecular subtypes in prediction of the prognosis and immunotherapy of hepatitis B virus-related hepatocellular carcinoma. *Front Immunol* 2022; **13**: 1029872 [PMID: 36275676 DOI: 10.3389/fimmu.2022.1029872]

23 **Luo Y**, Liu H, Fu H, Ding GS, Teng F. A cellular senescence-related classifier based on a tumorigenesis- and immune infiltration-guided strategy can predict prognosis, immunotherapy response, and candidate drugs in hepatocellular carcinoma. *Front Immunol* 2022; **13**: 974377 [PMID: 36458010 DOI: 10.3389/fimmu.2022.974377]

24 **Wang Z**, Yao J, Dong T, Niu X. Definition of a Novel Cuproptosis-Relevant lncRNA Signature for Uncovering Distinct Survival, Genomic Alterations, and Treatment Implications in Lung Adenocarcinoma. *J Immunol Res* 2022; **2022**: 2756611 [PMID: 36281357 DOI: 10.1155/2022/2756611]

25 **Yu G**, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012; **16**: 284-287 [PMID: 22455463 DOI: 10.1089/omi.2011.0118]

26 **Hänzelmann S**, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics* 2013; **14**: 7 [PMID: 23323831 DOI: 10.1186/1471-2105-14-7]

27 **Liberzon A**, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* 2015; **1**: 417-425 [PMID: 26771021 DOI: 10.1016/j.cels.2015.12.004]

28 **Wilkerson MD**, Hayes DN. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. *Bioinformatics* 2010; **26**: 1572-1573 [PMID: 20427518 DOI: 10.1093/bioinformatics/btq170]

29 **Eide PW**, Bruun J, Lothe RA, Sveen A. CMScaller: an R package for consensus molecular subtyping of colorectal cancer pre-clinical models. *Sci Rep* 2017; **7**: 16618 [PMID: 29192179 DOI: 10.1038/s41598-017-16747-x]

30 **Mayakonda A**, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res* 2018; **28**: 1747-1756 [PMID: 30341162 DOI: 10.1101/gr.239244.118]

31 **Mermel CH**, Schumacher SE, Hill B, Meyerson ML, Beroukhim R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol* 2011; **12**: R41 [PMID: 21527027 DOI: 10.1186/gb-2011-12-4-r41]

32 **Engebretsen S**, Bohlin J. Statistical predictions with glmnet. *Clin Epigenetics* 2019; **11**: 123 [PMID: 31443682 DOI: 10.1186/s13148-019-0730-1]

33 **Chen L**, Niu X, Qiao X, Liu S, Ma H, Shi X, He X, Zhong M. Characterization of Interplay Between Autophagy and Ferroptosis and Their Synergistical Roles on Manipulating Immunological Tumor Microenvironment in Squamous Cell Carcinomas. *Front Immunol* 2021; **12**: 739039 [PMID: 35185859 DOI: 10.3389/fimmu.2021.739039]

34 **Geeleher P**, Cox N, Huang RS. pRRophetic: an R package for prediction of clinical chemotherapeutic response from tumor gene expression levels. *PLoS One* 2014; **9**: e107468 [PMID: 25229481 DOI: 10.1371/journal.pone.0107468]

35 **Jiang P**, Gu S, Pan D, Fu J, Sahu A, Hu X, Li Z, Traugh N, Bu X, Li B, Liu J, Freeman GJ, Brown MA, Wucherpfennig KW, Liu XS. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med* 2018; **24**: 1550-1558 [PMID: 30127393 DOI: 10.1038/s41591-018-0136-1]

36 **Tao L**, Zhang W, Zhang Y, Zhang M, Niu X, Zhao Q, Liu Z, Li Y, Diao A. Caffeine promotes the expression of telomerase reverse transcriptase to regulate cellular senescence and aging. *Food Funct* 2021; **12**: 2914-2924 [PMID: 33720241 DOI: 10.1039/d0fo03246h]

37 **Chand V**, Liao X, Guzman G, Benevolenskaya E, Raychaudhuri P. Hepatocellular carcinoma evades RB1-induced senescence by activating the FOXM1-FOXO1 axis. *Oncogene* 2022; **41**: 3778-3790 [PMID: 35761036 DOI: 10.1038/s41388-022-02394-8]

38 **McGettigan SE**, Debes GF. Immunoregulation by antibody secreting cells in inflammation, infection, and cancer. *Immunol Rev* 2021; **303**: 103-118 [PMID: 34145601 DOI: 10.1111/imr.12991]

39 **Tian Y**, Xiao H, Yang Y, Zhang P, Yuan J, Zhang W, Chen L, Fan Y, Zhang J, Cheng H, Deng T, Yang L, Wang W, Chen G, Wang P, Gong P, Niu X, Zhang X. Crosstalk between 5-methylcytosine and N(6)-methyladenosine machinery defines disease progression, therapeutic

response and pharmacogenomic landscape in hepatocellular carcinoma. *Mol Cancer* 2023; **22**: 5 [PMID: 36627693 DOI: 10.1186/s12943-022-01706-6]

40 **Sperandio RC**, Pestana RC, Miyamura BV, Kaseb AO. Hepatocellular Carcinoma Immunotherapy. *Annu Rev Med* 2022; **73**: 267-278 [PMID: 34606324 DOI: 10.1146/annurev-med-042220-021121]

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

42 **Liu M**, Jiang L, Guan XY. The genetic and epigenetic alterations in human hepatocellular carcinoma: a recent update. *Protein Cell* 2014; **5**: 673-691 [PMID: 24916440 DOI: 10.1007/s13238-014-0065-9]

43 **Ren F**, Li W, Xiang A, Wang L, Li M, Guo Y. Distribution and difference of APOBEC-induced mutations in the TpCpW context of HBV DNA between HCC and non-HCC. *J Med Virol* 2020; **92**: 53-61 [PMID: 31429946 DOI: 10.1002/jmv.25572]

44 **Long J**, Wang A, Bai Y, Lin J, Yang X, Wang D, Jiang Y, Zhao H. Development and validation of a TP53-associated immune prognostic model for hepatocellular carcinoma. *EBioMedicine* 2019; **42**: 363-374 [PMID: 30885723 DOI: 10.1016/j.ebiom.2019.03.022]

45 **Wang S**, Shi H, Liu T, Li M, Zhou S, Qiu X, Wang Z, Hu W, Guo W, Chen X, Guo H, Shi X, Shi J, Zang Y, Cao J, Wu L. Mutation profile and its correlation with clinicopathology in Chinese hepatocellular carcinoma patients. *Hepatobiliary Surg Nutr* 2021; **10**: 172-179 [PMID: 33898558 DOI: 10.21037/hbsn.2019.09.17]

46 **Pan Y**, Han M, Zhang X, He Y, Yuan C, Xiong Y, Li X, Zeng C, Lu K, Zhu H, Lu X, Liu Q, Liang H, Liao Z, Ding Z, Zhang Z, Chen X, Zhang W, Zhang B. Discoidin domain receptor 1 promotes hepatocellular carcinoma progression through modulation of SLC1A5 and the mTORC1 signaling pathway. *Cell Oncol (Dordr)* 2022; **45**: 163-178 [PMID: 35089546 DOI: 10.1007/s13402-022-00659-8]

47 **Cao F**, Luo A, Yang C. G6PD inhibits ferroptosis in hepatocellular carcinoma by targeting cytochrome P450 oxidoreductase. *Cell Signal* 2021; **87**: 110098 [PMID: 34325001 DOI: 10.1016/j.cellsig.2021.110098]

48 **Hu B**, Yang XB, Sang XT. Development and Verification of the Hypoxia-Related and Immune-Associated Prognosis Signature for Hepatocellular Carcinoma. *J Hepatocell Carcinoma* 2020; **7**: 315-330 [PMID: 33204664 DOI: 10.2147/JHC.S272109]

49 **Cai J**, Sun X, Guo H, Qu X, Huang H, Yu C, Wu H, Gao Y, Kong X, Xia Q. Non-metabolic role of UCK2 links EGFR-AKT pathway activation to metastasis enhancement in hepatocellular carcinoma. *Oncogenesis* 2020; **9**: 103 [PMID: 33277463 DOI: 10.1038/s41389-020-00287-7]

50 **Wu C**, Xia D, Wang D, Wang S, Sun Z, Xu B, Zhang D. TCOF1 coordinates oncogenic activation and rRNA production and promotes tumorigenesis in HCC. *Cancer Sci* 2022; **113**: 553-564 [PMID: 34904330 DOI: 10.1111/cas.15242]

51 **Liu J**, Zhang SQ, Chen J, Li ZB, Chen JX, Lu QQ, Han YS, Dai W, Xie C, Li JC. Identifying Prognostic Significance of RCL1 and Four-Gene Signature as Novel Potential Biomarkers in HCC Patients. *J Oncol* 2021; **2021**: 5574150 [PMID: 34257652 DOI: 10.1155/2021/5574150]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1595-1604

DOI: 10.4251/wjgo.v15.i9.1595

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Case Control Study

Comparison of ethanol-soaked gelatin sponge and microspheres for hepatic arterioportal fistulas embolization in hepatic cellular carcinoma

Guang-Sheng Yuan, Li-Li Zhang, Zi-Tong Chen, Cun-Jing Zhang, Shu-Hui Tian, Ming-Xia Gong, Peng Wang, Lei Guo, Nan Shao, Bin Liu

Specialty type: Oncology

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

Peer-review model: Single-blind

Peer-review report's scientific quality classification

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

P-Reviewer: Lykoudis PM, United Kingdom; Ueda H, Japan

Received: June 1, 2023
Peer-review started: June 1, 2023
First decision: July 17, 2023
Revised: July 24, 2023
Accepted: August 18, 2023
Article in press: August 18, 2023
Published online: September 15, 2023

Guang-Sheng Yuan, Ming-Xia Gong, Nan Shao, Institute of Interventional Oncology, Shandong University, Jinan 250033, Shandong Province, China; Department of Radiology, Dongying Traditional Chinese Medicine Hospital, Dongying 257055, Shandong Province, China

Li-Li Zhang, Department of Gastroenterology, People's Hospital of Qihe County, Dezhou 251100, Shandong Province, China

Zi-Tong Chen, Shu-Hui Tian, Bin Liu, Department of Interventional Medicine, The Second Hospital of Shandong University, Jinan 250033, Shandong Province, China; Institute of Interventional Oncology, Shandong University, Jinan 250033, Shandong Province, China

Cun-Jing Zhang, Dean's Office, Jinan Vocational College of Nursing, Jinan 250100, Shandong Province, China

Peng Wang, Department of Interventional Medicine, People's Hospital of Zouping City, Binzhou 256299, Shandong Province, China

Lei Guo, Department of Vascular Anomalies and Interventional Radiology, Qilu Children's Hospital of Shandong University, Jinan 250022, Shandong Province, China

Corresponding author: Bin Liu, MD, Professor, Researcher, Department of Interventional Medicine, The Second Hospital of Shandong University, No. 247 Beiyuan Street, Jinan 250033, Shandong Province, China. gordon0221@sdu.edu.cn

Abstract

BACKGROUND

Hepatic arterioportal fistulas (APFs) are common in hepatocellular carcinoma (HCC). Moreover, correlated with poor prognosis, APFs often complicate anti-tumor treatments, including transarterial chemoembolization (TACE).

AIM

To compare the efficacy of ethanol-soaked gelatin sponges (ESG) and microspheres in the management of APFs and their impact on the prognosis of HCC.

METHODS

Data from patients diagnosed with HCC or hepatic APFs between June 2016 and December 2019 were retrospectively analyzed. Furthermore, APFs were embolized with ESG (group E) or microspheres (group M) during TACE. The primary outcomes were disease control rate (DCR) and objective response rate (ORR). The secondary outcomes included immediate and first follow-up APF improvement, overall survival (OS), and progression-free survival (PFS).

RESULTS

Altogether, 91 participants were enrolled in the study, comprising 46 in group E and 45 in group M. The DCR was 93.5% and 91.1% in groups E and M, respectively (P = 0.714). The ORRs were 91.3% and 66.7% in groups E and M, respectively (P = 0.004). The APFs improved immediately after the procedure in 43 (93.5%) patients in group E and 40 (88.9%) patients in group M (P = 0.485). After 2 mo, APF improvement was achieved in 37 (80.4%) and 33 (73.3%) participants in groups E and M, respectively (P = 0.421). The OS was 26.2 ± 1.4 and 20.6 ± 1.1 mo in groups E and M, respectively (P = 0.004), whereas the PFS was 16.6 ± 1.0 and 13.8 ± 0.7 mo in groups E and M, respectively (P = 0.012).

CONCLUSION

Compared with microspheres, ESG embolization demonstrated a higher ORR and longer OS and PFS in patients of HCC with hepatic APFs.

Key Words: Hepatocellular carcinoma; Arterioportal fistula; Ethanol; Gelatin sponge; Microsphere; Embolization

Core Tip: Hepatocellular carcinoma (HCC) was considered the seventh most common cancer and the second leading cause of cancer-related deaths worldwide in 2020. Hepatic arterioportal fistulas (APFs) are common in HCC and often complicate anti-tumor treatments, including transarterial chemoembolization. The ethanol-soaked gelatin sponge combined the advantages of alcohol and gelatin sponges, contributed to better local control of hepatic APFs, and improved the survival of patients with HCC.

Citation: Yuan GS, Zhang LL, Chen ZT, Zhang CJ, Tian SH, Gong MX, Wang P, Guo L, Shao N, Liu B. Comparison of ethanol-soaked gelatin sponge and microspheres for hepatic arterioportal fistulas embolization in hepatic cellular carcinoma. *World J Gastrointest Oncol* 2023; 15(9): 1595-1604

URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1595.htm

DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1595

INTRODUCTION

Hepatocellular carcinoma (HCC) was the seventh most common cancer and the second leading cause of cancer-related deaths worldwide in 2020, with 905677 new cases and 830180 deaths recorded annually[1]. Hepatic arterioportal fistulas (APFs), defined as fistulas between the hepatic artery and the neighboring portal vein[2,3], are common in HCC owing to tumor infiltration, vascular damage[4], or remodeling of the cirrhotic parenchyma.

Hepatic APFs may cause portal hypertension, ascites, and varices[5], which are strongly associated with poor prognosis[6]. The presence of hepatic APFs often complicates anti-tumor treatments, including transarterial chemoembolization (TACE). Chemotherapeutic agents and embolic materials run off through the fistulas, and tumor cells may detach from the hepatic artery, resulting in portal vein thrombosis[7].

Many materials have been used to treat hepatic APFs, including gelatin sponges[8], microspheres[9], coils[10], histoacryl[10], absolute ethanol[10], polyvinyl alcohol particles[10], and ethanol-soaked gelatin sponges (ESG)[11,12]. Additionally, ESG combines the advantages of alcohol and gelatin sponges and provides convincing results at different APF stages[12]. However, to the best of our knowledge, no study has compared the efficacies of ESG and microspheres. We conducted a retrospective study to evaluate the efficacy of ESGs and microspheres for the treatment of HCC with hepatic APF.

MATERIALS AND METHODS

Patients with HCC and hepatic APF treated with TACE and ESG (group E) or microspheres (group M) were enrolled between June 2016 and December 2019. The study protocol was approved by the ethics committee of the leading center. The requirement for written informed consent was waived owing to the retrospective nature of the study. All the experiments were performed in compliance with the Ethical Principles for Medical Research Involving Human Subjects

outlined in the 1975 Declaration of Helsinki (revised in 2000).

The inclusion criteria were as follows: (1) Confirmed diagnosis of HCC based on the American Association for the Study of Liver Diseases practice guidelines[13]; (2) Hypervascular tumor with Barcelona Clinic Liver Cancer (BCLC) Staging A-C; (3) Hepatic APF confirmed by angiography; (4) Predicted life span ≥ 1 year; and (5) Karnofsky score > 70.

The exclusion criteria were as follows: (1) Other malignancies within 5 years; (2) Child-Pugh score ≤ 10; and (3) Severe coagulopathy (prothrombin time > 17 s and/or platelet count ≤ 60 × 10^9/L).

Treatment of APF

For group E, an appropriate-sized gelatin sponge (Alicon Inc., Hangzhou, China) was mixed with 10 mL of iodixanol (Hengrui Co. Ltd, Lianyungang, China) and 10 mL of ethanol (Lingfeng Inc, Shanghai, China). For group M, appropriate-sized microspheres (Embosphere, Merit Medical, UT, United States) were mixed with 10 mL of iodixanol. Digital subtraction angiography (DSA) was performed after catheterization of the celiac or superior mesenteric artery to validate the location and size of the hepatic APFs (Figure 1). APFs were classified according to a previous study by Zhou *et al*[12] (Table 1). Each APF feeding artery was superselected using a 2.7-F microcatheter. ESG or the microspheres were injected under fluoroscopic guidance until the fistula was blocked. Coils were used if the fistula was not completely blocked. DSA was repeated to confirm the complete embolization of the APFs (Figure 2).

TACE procedure

After APF embolization, a microcatheter was advanced into each feeding artery of the HCC. An emulsion of poppy Lipiodol (Hengrui Co. Ltd., Lianyungang, China) and epirubicin (Qilu Co. Ltd., Jinan, China) was injected *via* a microcatheter until complete embolization of the tumor was achieved (Figure 3)[14].

Follow-up

Follow-up was conducted every 2 mo and included standard blood count, liver functional tests, alpha-fetoprotein (AFP), and abdominal contrast-enhanced computed tomography (CECT) or magnetic resonance imaging (MRI). The images were interpreted based on the consensus of three skilled interventional radiologists.

In case the tumor recurrence was detected on CECT or MRI, TACE was repeated. If APF recurrence with a grade ≥ 2 was observed, ESG or microsphere APF embolization was repeated; however, if APFs did not recur, TACE was the only procedure performed. Follow-up intervention was determined based on the tumor condition and general status.

Outcome measures

The modified Response Evaluation Criteria in Solid Tumors for HCC[15] were applied to assess tumor response after 4 mo. The primary outcomes were disease control rate (DCR) and objective response rate (ORR), and the secondary outcomes included immediate and first-time follow-up of APF improvement, overall survival (OS), and progression-free survival (PFS).

Immediate APF improvement was defined as a decrease in grade to 1 or 0. First-time follow-up APF improvement was defined as a decrease in at least two grades confirmed by angiography in the second session, whereas APF progression was defined as an increased grade on the first-time follow-up angiography. If the grade remained the same or decreased by one, the APFs were not considered to improve. Moreover, OS was defined as the time interval between the initial TACE and death or the last follow-up. Furthermore, PFS was defined as the time interval between initial TACE and disease progression or death.

Statistical analysis

Continuous variables were analyzed using Student's t-test to determine whether the variables were normally distributed; otherwise, the Mann–Whitney U test was used. Categorical variables were analyzed using the χ^2 or Fisher's exact tests.

Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. Statistical significance was defined as a two-tailed $P < 0.05$. All statistical analyses were conducted using the SPSS software (version 24.0; IBM Inc., Armonk, NY, United States).

RESULTS

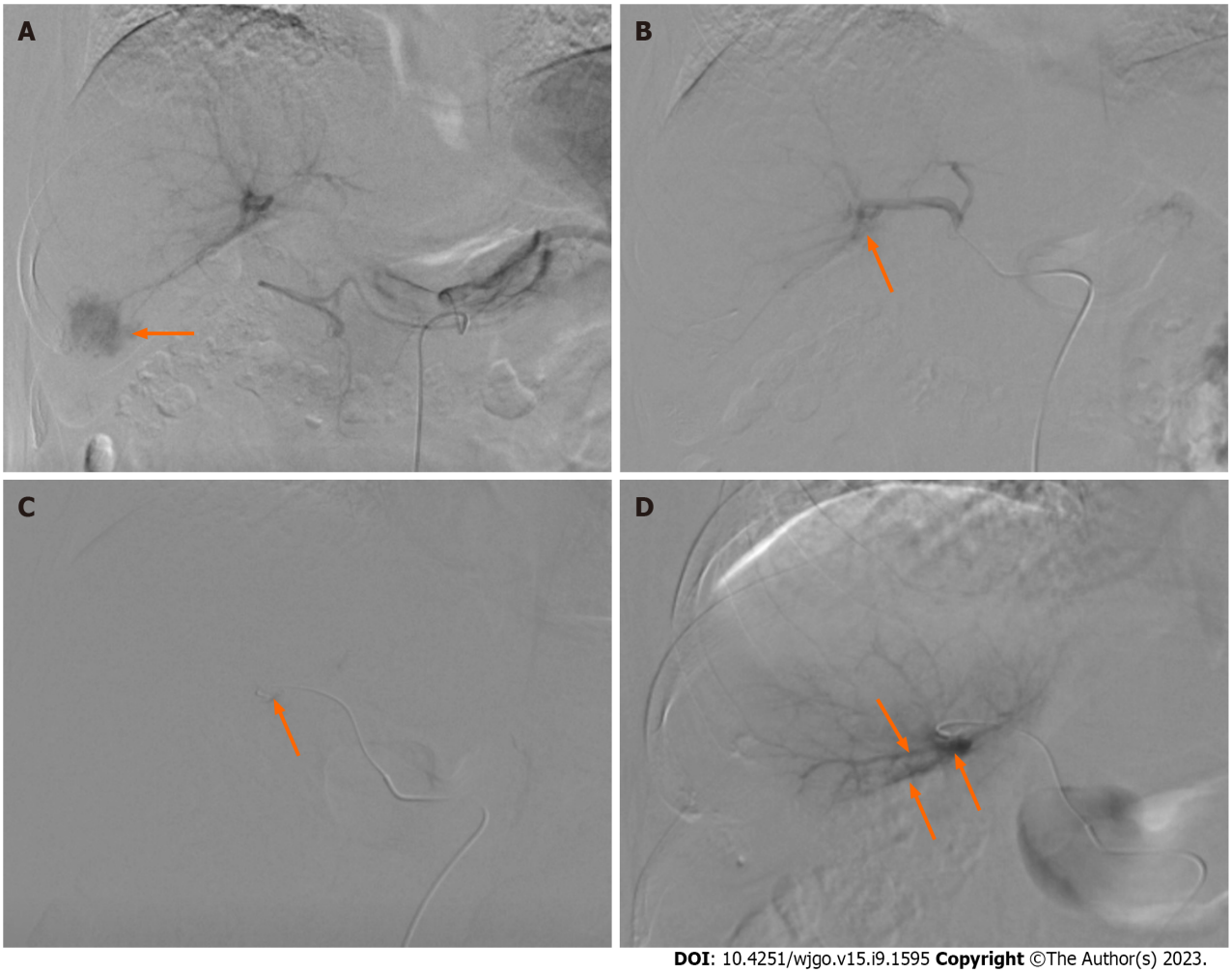
Participant characteristics

A consecutive series of 91 patients were enrolled in the study. During TACE, APFs were embolized using ESG in 46 participants and microspheres in 45 participants. The ratios of men to women were 33/13 in group E and 33/12 in group M ($\chi^2 = 0.029$, $P = 0.865$), with a mean age of 63.4 ± 8.5 and 58.4 ± 10.1 years ($P = 0.092$), respectively. The etiologies included hepatitis B virus (HBV) (38/46, 82.6% in group E; 39/45, 86.7% in group M), hepatitis C (4/46, 8.7% in group E; and 2/45, 4.4% in group M), HBV + hepatitis C virus (2/46, 4.3% in group E; 2/45, 4.4% in group M), and alcohol consumption (2/46, 4.3% in group E; 2/45, 4.4% in group M) ($P = 0.952$). No significant differences in the Child-Pugh stage, BCLC stage, or tumor location were observed between the two groups. The mean tumor diameters were 6.8 ± 2.9 mm and 7.1 ± 1.6 mm in groups E and M ($P = 0.765$), respectively. Portal vein thrombi were identified in 24 participants (24/46, 52.2%) in group E and 22 participants (22/45, 48.9%) in group M ($\chi^2 = 0.098$, $P = 0.754$), respectively. The treatments administered before TACE included surgery, microwave ablation (MWA), radiofrequency ablation (RFA), TACE, radiation, and TACE + MWA/RFA. We observed no significant differences in previous treatments between the

Table 1 Grading of arterioportal fistula

Grade	Definition	Class
0	APFs were not observed	-
1	APFs flow to the subsegmental portal branch	Mild
2	APFs flow to the segmental portal branch	Moderate
3	APFs flow into the main portal branch of the ipsilateral lobe	Moderate
4	APFs flow into the main portal branch of the contralateral lobe and/or the main portal vein	Severe
5	APFs flow into the main portal vein presenting with hepatofugal portal venous flow	Severe

APF: Arterioportal fistula.



DOI: 10.4251/wjgo.v15.i9.1595

Figure 1 Digital subtraction angiography of the tumor and shunt. A: Angiography of the celiac artery shows liver tumor staining (orange arrow) in segment VI; B: Angiography of the proper hepatic artery shows hepatic arterioportal shunt. The orange arrow indicates the branch of the portal vein; C: The feeding artery (orange arrow) of the shunt is super-selected with a microcatheter; D: Angiography with the microcatheter shows the branches of the portal vein (orange arrow).

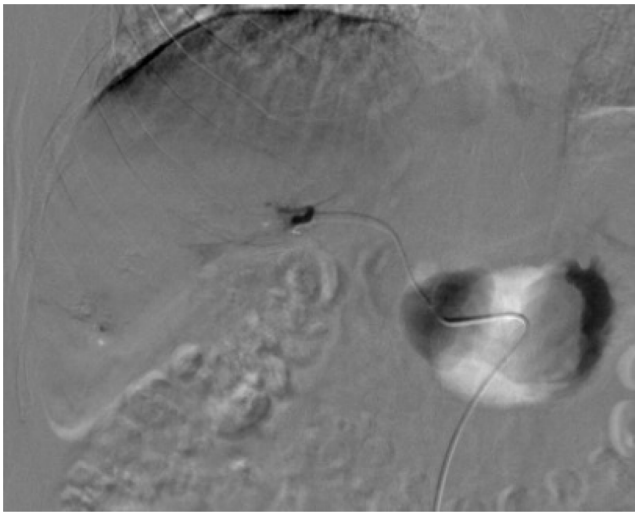
two groups (P = 0.925). The median levels of AFP were 137 [interquartile range (IQR): 9.8, 970.1] and 114.9 (IQR: 3.7, 725.7) ng/mL in groups E and M, respectively (P = 0.734). APF grades 1, 2, 3, 4, and 5 were recorded in 5 (5/46, 10.9%) and 6 (6/45, 13.3%); 15 (15/46, 32.6%) and 16 (16/45, 35.6%); 11 (11/46, 23.9%) and 14 (14/45, 31.1%); 9 (9/46, 19.6%), and 7 (7/45, 15.6%); and 6 (6/46, 13%) and 2 (2/45, 4.4%) participants in groups E and M, respectively (P = 0.636) (Table 2).

The mean follow-up period was 35.3 ± 2.7 mo in group E and 30.9 ± 3.8 mo in group M (P = 0.195). After 4 mo, complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were achieved in 18 (18/46, 39.1%) and 8 (8/45, 17.8%) patients; 21 (21/46, 45.7%) and 18 (18/45, 40%) patients; 4 (4/46, 8.7%) and 15 (15/45, 33.3%) patients; and 3 (3/46, 6.5%) and 4 (4/45, 8.9%) participants in groups E and M, respectively (P = 0.014). The DCR

Table 2 Demographic and baseline characteristics

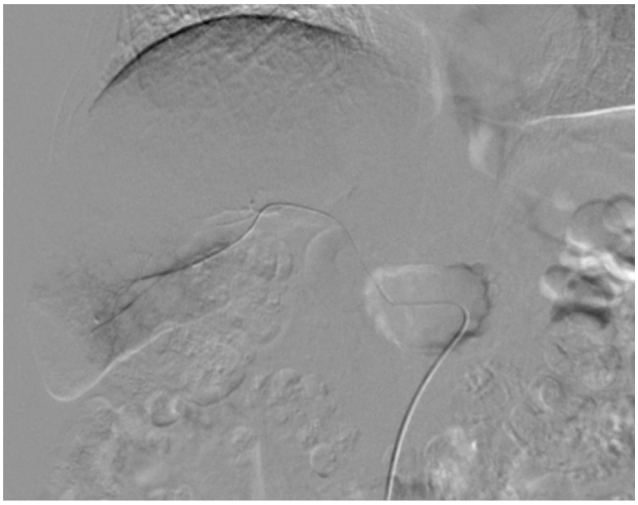
Characteristics	E group (*n* = 46)	M group (*n* = 45)	χ^2	*P* value
Sex, *n* (%)			0.029	0.865
Male	33 (71.7)	33 (73.3)		
Female	13 (28.3)	12 (26.7)		
Age (yr)	63.4 ± 8.5	58.4 ± 10.1	-	0.092
Etiology, *n* (%)			0.909	0.952
HBV	38 (82.7)	39 (86.8)		
HCV	4 (8.7)	2 (4.4)		
HBV + HCV	2 (4.3)	2 (4.4)		
Alcohol	2 (4.3)	2 (4.4)		
Child-Pugh stage, *n* (%)			0.297	0.586
A	25 (54.3)	27 (60)		
B	21 (45.7)	18 (40)		
BCLC stage, *n* (%)			0.271	0.873
A	7 (15.2)	6 (13.3)		
B	19 (41.3)	21 (45.7)		
C	20 (43.5)	18 (40)		
Tumor location			0.837	0.658
Right lobe	30 (65.2)	28 (62.2)		
Left lobe	9 (19.6)	10 (22.2)		
Right and left lobes	7 (15.2)	7.1 ± 1.6		
Mean tumor diameter (cm)	6.8 ± 2.9		-	0.765
Portal vein thrombus			0.098	0.754
Present	24 (52.2)	22 (48.9)		
Absent	22 (47.8)	23 (51.1)		
Previous treatment			1.639	0.925
Surgery	7 (15.2)	6 (13.3)		
MWA/RFA	9 (19.6)	7 (15.6)		
TACE	4 (8.7)	5 (11.1)		
Radiation	4 (8.7)	6 (13.3)		
TACE + MWA/RFA	2 (4.3)	3 (6.7)		
None	20 (43.5)	18 (40)		
AFP [ng/mL, median (IQR)]	137 (9.8, 970.1)	114.9 (3.7, 725.7)	-	0.734
APF grade, *n* (%)			2.689	0.636
1	5 (10.9)	6 (13.3)		
2	15 (32.6)	16 (35.6)		
3	11 (23.9)	14 (31.1)		
4	9 (19.6)	7 (15.6)		
5	6 (13)	2 (4.4)		

HCV: Hepatitis C virus; BCLC: Barcelona Clinic Liver Cancer; MWA: Microwave ablation; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemoembolization; AFP: Alpha-fetoprotein; IQR: Interquartile range; APFs: Arterioportal fistulas.



DOI: 10.4251/wjgo.v15.i9.1595 **Copyright** ©The Author(s) 2023.

Figure 2 Repeated angiography confirmed completed embolization of the shunt.



DOI: 10.4251/wjgo.v15.i9.1595 **Copyright** ©The Author(s) 2023.

Figure 3 Angiography performed to confirm complete embolization of the tumor.

was 93.5% (43/46) in group E and 91.1% (41/45) in group M (P = 0.714). The ORRs were 91.3% (42/46) and 66.7% (30/45) in groups E and M, respectively (P = 0.004).

The APFs immediately improved after the procedure in 43 (43/46, 93.5%) and 40 (40/45, 88.9%) participants in groups E and M, respectively (P = 0.485). After 2 mo, APF improvement was achieved in 37 (37/46, 80.4%) and 33 (33/45, 73.3%) participants in groups E and M, respectively (P = 0.421). The median AFP levels at 4 mo after the procedure were 28.48 (IQR: 4, 257.9) and 45.25 (IQR: 4.43, 359.5) ng/mL in groups E and M, respectively (P = 0.045). After 4 mo, the difference in Child-Pugh class distribution between the two groups was not significant (P = 0.083) (Table 3).

The OS was 26.2 ± 1.4 and 20.6 ± 1.1 mo in groups E and M, respectively (χ^2 = 10.3, P = 0.004; Figure 4A) (Table 3). The PFS was 16.6 ± 1.0 and 13.8 ± 0.7 mo in groups E and M, respectively (P = 0.012; Figure 4B) (Table 3).

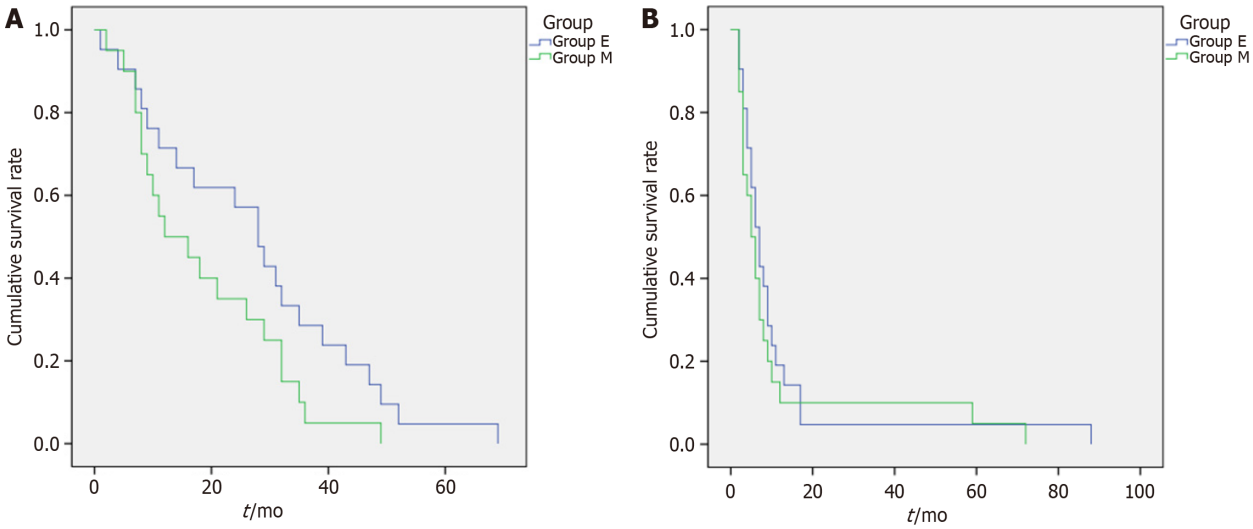
DISCUSSION

According to the updated BCLC prognosis and treatment strategy[16], TACE is recommended for intermediate-stage B HCC. With its tendency to infiltrate the portal and hepatic venous structures, HCC is often accompanied by APFs, which may reduce the therapeutic benefits of TACE[7]. Our study focused on comparing ESG and microspheres for the treatment of hepatic APFs. The DCRs were 93.5% (43/46) in group E and 91.1% (41/45) in group M (P = 0.714). The ORRs were 91.3% and 66.7% in groups E and M, respectively (P = 0.004). The OS was 26.2 ± 1.4 and 20.6 ± 1.1 mo in groups E and M, respectively (P = 0.004). The PFS was 16.6 ± 1.0 and 13.8 ± 0.7 mo in groups E and M, respectively (P = 0.012; Figure 4B) (Table 3).

Table 3 Outcome characteristics

Characteristics	E group (*n* = 46)	M group (*n* = 45)	χ^2	*P* value
Tumor response after four months (%)			10.578	0.014
CR	18 (39.1)	8 (17.8)		
PR	21 (45.7)	18 (40)		
SD	4 (8.7)	15 (33.3)		
PD	3 (6.5)	4 (8.9)		
DCR	43 (93.5)	41 (91.1)		0.714
ORR	42 (91.3)	30 (66.7)	8.358	0.004
Immediate improvement of APF (%)			-	0.485
Yes	43 (93.5)	40 (88.9)	-	
No	3 (6.5)	5 (11.1)		
First-time follow-up APF improvement (%)			0.646	0.421
Improved	37 (80.4)	33 (73.3)		
Not improved	9 (19.6)	12 (26.7)		
AFP after 4 mo [ng/mL, median (IQR)]	28.48 (4, 257.9)	45.25 (4.43, 359.5)		0.045
Child-Pugh score after 4 mo (%)			5.321	0.083
A	33 (71.7)	23 (51.1)		
B	10 (21.7)	20 (44.4)		
C	3 (6.6)	2 (4.5)		
OS, months (mean ± SD)	26.2 ± 1.4	20.6 ± 1.1	10.3	0.004
PFS, months (mean ± SD)	16.6 ± 1.0	13.8 ± 0.7	6.3	0.012

CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; DCR: Disease control rate; ORR: Objective response rate; APFs: Arterioportal fistulas; AFP: Alpha-fetoprotein; AP: Arterioportal; IQR: Interquartile range; OS: Overall survival; PFS: Progression-free survival.



DOI: 10.4251/wjgo.v15.i9.1595

Figure 4 The Kaplan-Meier curve. A: Overall survival; B: Progression-free survival.

Gelatin sponges and microspheres have several disadvantages in the treatment of hepatic APF. Gelatin sponges are absorbed 2-3 wk after the procedure, and APFs can be recanalized. Microspheres exerted a physical embolic effect without causing protein degradation in the vascular wall. Ethanol has been widely used in the embolization of arteriovenous malformations[17], which can denature blood proteins, dehydrate vascular endothelial cells, and cause segment fractures in the vascular wall[18-20]. Compared to gelatin sponges alone, ethanol demonstrated an improved long-term effect on hepatic APFs[21]. However, because of its liquid properties, ethanol alone is not suitable for shunts with high blood flow. ESG combines the advantages of ethanol and gelatin sponges, promoting local control of hepatic APFs and liver tumors[12].

In our study, the immediate improvement and first-time follow-up rates of APFs in group E were not significantly higher than those in group M (93.5% and 88.9%, P = 0.485, 80.4% and 73.3%, P = 0.421, respectively). Thus, ESG and microspheres may have similar short-term effects on the treatment of hepatic APFs. The immediate improvement rate in group E was comparable to the 97% reported by Zhou *et al*[12], whereas the first follow-up APF improvement rate was higher in both groups than that reported by Zhou *et al*[12] (54%). This discrepancy may be attributed to the higher proportion of patients with grades 1–3 APFs in our study.

Our study investigated tumor response 4 mo after the procedure and revealed that the CR, PR, SD, and PD rates were 39.1% and 17.8%, 45.7% and 40%, 8.7% and 33.3%, and 6.5% and 8.9% in groups E and M, respectively (P = 0.014). Moreover, the ORR was 84.8% and 57.8% in groups E and M, respectively (P = 0.004). Compared with microspheres, ESG led to complete long-term control of hepatic APF, including physical blockade and chemical destruction and yielded a significantly better local tumor response. Both the DCRs (93.5%) and ORRs (84.8%) in group E patients were higher than those reported in Zhou *et al*'s study (81.9% and 42.6%, respectively)[12]. This has three possible reasons. First, the tumor response in our study was evaluated 4 mo after the procedure, which provided an additional opportunity for tumor control. Second, the percentage of participants with portal vein thrombus (52.5%) was lower than that reported by Zhou *et al*'s study[12]. Third, the proportion of grade 1-3 APFs in our study was higher, resulting in a better embolic response.

The OS, PFS, and median AFP levels at 4 mo after the procedure in group E were significantly better than those in group M. The aforementioned outcome may be attributed to the complete blockage of hepatic APFs and well-controlled tumors. Compared with microspheres, ESG embolization demonstrated complete long-term blockade of hepatic APFs and therefore improved the local control of HCC and survival of patients with HCC.

Nevertheless, the study had some limitations. As this was a retrospective study, selection bias may have reduced the value of the results. However, further prospective studies are required to validate the findings.

CONCLUSION

Compared to microsphere embolization, ESG embolization resulted in a higher ORR and longer OS and PFS. The findings may contribute to the selection of embolic agents for treating hepatic APFs in patients with HCC.

ARTICLE HIGHLIGHTS

Research background

Hepatic arterioportal fistulas (APFs) are common in hepatocellular carcinoma (HCC) because of tumor infiltration, vascular damage, and remodeling of the cirrhotic parenchyma. The presence of hepatic APFs often complicates antitumor treatments, including transarterial chemoembolization (TACE).

Research motivation

Ethanol-soaked gelatin sponges (ESG) combine the advantages of alcohol and gelatin sponges, demonstrating a convincing effect at different stages of hepatic APFs. However, to date, no study has compared the efficacy of ESG and microspheres.

Research objectives

This retrospective study aimed to compare the efficacy of ESG and microspheres in the management of APFs, and their impact on the prognosis of HCC.

Research methods

The APFs were embolized using ESG (group E) or microspheres (group M) during TACE. The disease control rate (DCR) and objective response rate (ORR) were considered the primary outcomes. The secondary outcomes included immediate and first follow-up APF improvement, overall survival (OS), and progression-free survival (PFS).

Research results

The DCR was 93.5% and 91.1% in groups E and M, respectively (P = 0.714). The ORRs were 91.3% and 66.7% in groups E and M, respectively (P = 0.004). In 43 (93.5%) patients in group E and 40 (88.9%) patients in group M. the APFs improved immediately after the procedure (P = 0.485). After 2 mo, APF improvement was achieved in 37 (80.4%) and 33 (73.3%) participants in groups E and M, respectively (P = 0.421). The OS was 26.2 ± 1.4 and 20.6 ± 1.1 mo in groups E and M,

respectively (P = 0.004). The PFS was 16.6 ± 1.0 and 13.8 ± 0.7 mo in groups E and M, respectively (P = 0.012).

Research conclusions

Compared with microspheres, ESG embolization demonstrated a higher ORR and longer OS and PFS in patients with HCC with hepatic APFs.

Research perspectives

The findings may aid the selection of embolic agents for the treatment of hepatic APFs in patients with HCC.

FOOTNOTES

Author contributions: Yuan GS and Zhang LL have contributed equally to this work; Yuan GS and Liu B performed the conception and design; Guo L and Liu B contributed to the administrative support; Zhang LL, Chen ZT, and Zhang CJ performed the provision of study materials and patients; Chen ZT, Tian SH, Gong MX, Wang P, Guo L, and Shao N performed the data collection and assembly; Chen ZT and Zhang CJ contributed to the data analysis and interpretation; All authors wrote the manuscript and performed the final approval of manuscript.

Supported by National Natural Science Foundation of China, No. 12171285 and No. 11971269; Program for Integrated Traditional Chinese and Western Medicine in Shandong Province of China, No. YXH2019ZXY007; and Jinan New Support Projects for Universities (Talent Development Special Fund), No. 20228118.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of the Second Hospital of Shandong University (KYLL-2020 [LW] 23).

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: The authors declare no competing interests.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: China

ORCID number: Guang-Sheng Yuan 0009-0008-1787-1781; Li-Li Zhang 0009-0001-3906-3565; Zi-Tong Chen 0000-0002-9606-1654; Cun-Jing Zhang 0000-0003-1856-0543; Shu-Hui Tian 0009-0003-2712-9568; Ming-Xia Gong 0009-0005-2178-6246; Peng Wang 0009-0006-1343-7887; Lei Guo 0000-0002-7986-3786; Nan Shao 0009-0001-2916-6384; Bin Liu 0000-0003-1686-1553.

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

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Gülberg V**, Haag K, Rössle M, Gerbes AL. Hepatic arterial buffer response in patients with advanced cirrhosis. *Hepatology* 2002; **35**: 630-634 [PMID: 11870377 DOI: 10.1053/jhep.2002.31722]

3 **Richter S**, Mücke I, Menger MD, Vollmar B. Impact of intrinsic blood flow regulation in cirrhosis: maintenance of hepatic arterial buffer response. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G454-G462 [PMID: 10915656 DOI: 10.1152/ajpgi.2000.279.2.G454]

4 **Choi BI**, Lee KH, Han JK, Lee JM. Hepatic arterioportal shunts: dynamic CT and MR features. *Korean J Radiol* 2002; **3**: 1-15 [PMID: 11919473 DOI: 10.3348/kjr.2002.3.1.1]

5 **Kakati BR**, Pedersen MR, Chen SY, Hirsch KS, Berggreen PJ, Seetharam AB. Hepatic arterioportal fistula presenting as gastric variceal hemorrhage. *J Gastrointestin Liver Dis* 2014; **23**: 211-214 [PMID: 24949615 DOI: 10.15403/jgld.2014.1121.232.brk1]

6 **Wakamatsu T**, Ogasawara S, Chiba T, Yokoyama M, Inoue M, Kanogawa N, Saito T, Suzuki E, Ooka Y, Tawada A, Yokosuka O. Impact of

Radiofrequency Ablation-Induced Glisson's Capsule-Associated Complications in Patients with Hepatocellular Carcinoma. *PLoS One* 2017; **12**: e0170153 [PMID: 28099460 DOI: 10.1371/journal.pone.0170153]

7 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

8 **Kanogawa N**, Chiba T, Ogasawara S, Ooka Y, Suzuki E, Motoyama T, Saito T, Sekimoto T, Tawada A, Maruyama H, Yoshikawa M, Yokosuka O. Successful interventional treatment for arterioportal fistula caused by radiofrequency ablation for hepatocellular carcinoma. *Case Rep Oncol* 2014; **7**: 833-839 [PMID: 25685134 DOI: 10.1159/000370305]

9 **Cai L**, Li H, Guo J, Zhao W, Duan Y, Hou X, Cheng L, Du H, Shao X, Diao Z, Hao Y, Zheng X, Li C, Li W. Treatment efficacy and safety of drug-eluting beads transarterial chemoembolization versus conventional transarterial chemoembolization in hepatocellular carcinoma patients with arterioportal fistula. *Cancer Biol Ther* 2022; **23**: 89-95 [PMID: 35230928 DOI: 10.1080/15384047.2021.2020059]

10 **Chan WS**, Poon WL, Cho DH, Chiu SS, Luk SH. Transcatheter embolisation of intrahepatic arteriovenous shunts in patients with hepatocellular carcinoma. *Hong Kong Med J* 2010; **16**: 48-55 [PMID: 20124574]

11 **Li J**, Kang X, Guo L, Xiao J, Cheng J. Embolization of hepatic arterioportal shunt with ethanol-soaked gelatin sponge. *J Cancer Res Ther* 2019; **15**: 336-340 [PMID: 30964107 DOI: 10.4103/jcrt.JCRT_825_17]

12 **Zhou WZ**, Shi HB, Liu S, Yang ZQ, Zhou CG, Xia JG, Zhao LB, Li LS. Arterioportal shunts in patients with hepatocellular carcinoma treated using ethanol-soaked gelatin sponge: therapeutic effects and prognostic factors. *J Vasc Interv Radiol* 2015; **26**: 223-230 [PMID: 25645411 DOI: 10.1016/j.jvir.2014.11.002]

13 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

14 **Xie DY**, Ren ZG, Zhou J, Fan J, Gao Q. 2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights. *Hepatobiliary Surg Nutr* 2020; **9**: 452-463 [PMID: 32832496 DOI: 10.21037/hbsn-20-480]

15 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

16 **Reig M**, Forner A, Rimola J, Ferrer-Fábrega J, Burrel M, Garcia-Criado A, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C, Bruix J. BCLC strategy for prognosis prediction and treatment recommendation Barcelona Clinic Liver Cancer (BCLC) staging system. The 2022 update. *J Hepatol* 2021 [DOI: 10.1016/j.jhep.2021.11.018]

17 **Do YS**, Yakes WF, Shin SW, Lee BB, Kim DI, Liu WC, Shin BS, Kim DK, Choo SW, Choo IW. Ethanol embolization of arteriovenous malformations: interim results. *Radiology* 2005; **235**: 674-682 [PMID: 15858106 DOI: 10.1148/radiol.2352040449]

18 **Buchta K**, Sands J, Rosenkrantz H, Roche WD. Early mechanism of action of arterially infused alcohol U.S.P. in renal devitalization. *Radiology* 1982; **145**: 45-48 [PMID: 7122894 DOI: 10.1148/radiology.145.1.7122894]

19 **Hammer FD**, Boon LM, Mathurin P, Vanwijck RR. Ethanol sclerotherapy of venous malformations: evaluation of systemic ethanol contamination. *J Vasc Interv Radiol* 2001; **12**: 595-600 [PMID: 11340138 DOI: 10.1016/s1051-0443(07)61482-1]

20 **Takebayashi S**, Hosaka M, Kubota Y, Ishizuka E, Iwasaki A, Matsubara S. Transarterial embolization and ablation of renal arteriovenous malformations: efficacy and damages in 30 patients with long-term followup. *J Urol* 1998; **159**: 696-701 [PMID: 9474128 DOI: 10.1016/s0022-5347(01)63703-0]

21 **Huang MS**, Lin Q, Jiang ZB, Zhu KS, Guan SH, Li ZR, Shan H. Comparison of long-term effects between intra-arterially delivered ethanol and Gelfoam for the treatment of severe arterioportal shunt in patients with hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 825-829 [PMID: 15040025 DOI: 10.3748/wjg.v10.i6.825]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1605-1615

DOI: 10.4251/wjgo.v15.i9.1605

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Incorporation of perigastric tumor deposits into the TNM staging system for primary gastric cancer

Yang Li, Shuo Li, Lu Liu, Li-Yu Zhang, Di Wu, Tian-Yu Xie, Xin-Xin Wang

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Dambrauskas Z, Lithuania; Shang L, China; Win TT, Malaysia

Received: June 14, 2023
Peer-review started: June 14, 2023
First decision: July 7, 2023
Revised: July 15, 2023
Accepted: August 2, 2023
Article in press: August 2, 2023
Published online: September 15, 2023

Yang Li, Shuo Li, Lu Liu, Li-Yu Zhang, Di Wu, Medical School of Chinese People's Liberation Army, The First Medical Center, Chinese People's Liberation Army General Hospital, Beijing 100853, China

Tian-Yu Xie, Xin-Xin Wang, Department of General Surgery, The First Medical Center, Chinese People's Liberation Army General Hospital, Beijing 100853, China

Corresponding author: Xin-Xin Wang, MD, PhD, Assistant Professor, Chief Doctor, Department of General Surgery, The First Medical Center, Chinese People's Liberation Army General Hospital, No. 28 Fuxing Road, Haidian District, Beijing 100853, China.
301wxx@sina.com

Abstract

BACKGROUND

The current prognostic significance of perigastric tumor deposits (TDs) in gastric cancer (GC) remains unclear.

AIM

To assess the prognostic value of perigastric TDs and put forward a new TNM staging framework involving TDs for primary GC.

METHODS

This study retrospectively analyzed the pathological data of 6672 patients with GC who underwent gastrectomy or surgery for GC with other diseases from January 1, 2012 to December 31, 2017 at the Chinese PLA General Hospital. According to the presence of perigastric TDs or not, the patients were divided into TD-positive and TD-negative groups by using the method of propensity score matching. The differences between TD-positive and TD-negative patients were analyzed using binary logistic regression modeling. The Kaplan-Meier method was used to plot survival curves. Multivariate Cox regression modeling and the log-rank test were used to analyze the data.

RESULTS

Perigastric TDs were found to be positive in 339 (5.09%) of the 6672 patients with GC, among whom 237 were men (69.91%) and 102 were women (30.09%) (2.32:1). The median age was 59 years (range, 27 to 78 years). Univariate and multivariate survival analyses indicated that TD-positive GC patients had a poorer prognosis than TD-negative patients ($P < 0.05$). The 1-, 3-, and 5-year overall survival rates of

GC patients with TDs were 68.3%, 19.6%, and 11.2%, respectively, and these were significantly poorer than those without TDs of the same stages. There was significant variation in survival according to TD locations among the GC patients ($P < 0.05$). A new TNM staging framework for GC was formulated according to TD location. When TDs appear in the gastric body, the original stages T1, T2, and T3 are classified as T4a with the new framework, and the original stages T4a and T4b both are classified as T4b. When TDs appear in the lesser curvature, the previous stages N0, N1, N2, and N3 now both are classified as N3. When TDs appear in the greater curvature or the distant tissue, the patient should be categorized as having M1. With the new GC staging scheme including TDs, the survival curves of patients in the lower grade TNM stage with TDs were closer to those of patients in the higher grade TNM stage without TDs.

CONCLUSION

TDs are a poor prognostic factor for patients with primary GC. The location of TDs is associated with the prognosis of patients with primary GC. Accordingly, we developed a new TNM staging framework involving TDs that is more appropriate for patients with primary GC.

Key Words: Tumor deposits; Gastric cancer; Prognosis; Stage; Overall survival

Core Tip: The aim of this study was to assess the prognostic value of perigastric tumor deposits (TDs) and put forward a new TNM staging framework involving TDs for primary gastric cancer (GC). This study indicated that TDs serve as a bad prognostic factor in patients with primary GC and the new TNM staging system incorporating TDs is more suitable for patients with primary GC.

Citation: Li Y, Li S, Liu L, Zhang LY, Wu D, Xie TY, Wang XX. Incorporation of perigastric tumor deposits into the TNM staging system for primary gastric cancer. *World J Gastrointest Oncol* 2023; 15(9): 1605-1615
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1605.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1605

INTRODUCTION

Gastric cancer (GC) remains the fifth most common cancer worldwide. More than 70% of GC cases occur in developing countries, including Japan, Korea, and China. GC ranks as the fourth leading cause of cancer-related deaths both in men and women. It was estimated that in 2012, the GC mortality rate in East Asia was highest (24 male deaths and 9.8 female deaths per 100000 people) and lowest in North America (2.8 male deaths and 1.5 female deaths)[1].

The prognosis of GC varies according to pathological TNM (tumor, lymph node, and metastasis) stage, and the staging of GC is critical for its treatment and prognosis. GC pathological TNM stage is determined by the extent of primary tumor infiltration depth (T), the number of metastatic lymph nodes (N), and distant metastasis (M)[2]. In the past few years, some other predictors-for example, histological types, lymphatic vessel infiltration, and lymphatic wall carcinoma-have been identified as important or even independent predictors of survival[3].

Gabriel was the first clinician to discover tumor deposits (TDs) in 1935[4]. TDs were initially defined as peritumoral nodule clusters in the primary adipose tissue of GC, with no histological evidence of residual lymph nodes remaining in the nodules. It was speculated that TDs may reflect discontinuous spread, venous invasion, and extravascular spread, or complete replacement of lymph nodes[2]. The prognostic significance of TDs in colorectal cancer has been confirmed by several studies[5-9]. A series of studies has indicated that TDs are associated with other gastrointestinal tumors, including biliary tract cancer, GC, and pancreatic cancer[10,11].

The eighth edition of the American Joint Committee on Cancer (AJCC) Gastric Staging System considers all gastric metastatic nodules without residual lymph node tissue as regional lymph node metastases[5]. However, the AJCC TNM staging system for GC fails to distinguish between lymph node metastasis and TDs. The prognostic value of TDs in GC has not been extensively studied or confirmed. To date, no studies have investigated the prognostic significance of TDs in GC in detail[12-14]. In this study, we aimed to assess the prognostic value of TD location and to put forward a new TNM staging framework considering TDs for primary GC.

MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee of the Chinese PLA General Hospital (S2023-065-01). Patients provided written informed consent before being included. A retrospective cohort study was conducted to evaluate the clinicopathologic data of 6672 GC patients who underwent surgical procedures at the Chinese PLA General Hospital

between January 2012 and December 2017. According to the presence of TDs or not, the patients were divided into TD-positive and TD-negative groups by using the method of propensity score matching (PSM). The eighth edition of the AJCC TNM staging system for GC was adopted in this study. The following clinical data were collected: Sex, age, time of gastrectomy, histologic grade, location, T stage, number of lymph node metastases, N stage, and type of operation. Patients with or without TDs were compared in terms of overall survival rates. The survival curves associated with different pathological TNM stages were compared, including comparisons between TD-positive and TD-negative patients. An amendment to the eighth edition of the AJCC TNM staging system for GC was proposed and validated. A multivariate analysis included the following variables: Clinicopathological characteristics, including sex, age, operative method, histological grade, TNM stage, and TD status, and survival data.

In the previous research of colorectal cancer, TDs were defined as isolated tumor foci found in the pericolonic or perirectal fat or the adjacent mesentery (mesocolonic fat) away from the invasive margin of the tumor without evidence of residual lymphatic tissue[13,14]. In our study, TDs were defined as isolated tumor nodules located in the subserosal, perigastric adipose, or omental tissues away from the margin of primary tumor without histologic evidence of residual lymph nodes[15]. Postoperative adjuvant therapy was performed as needed according to the Japanese Gastric Cancer Treatment Guidelines.

In this study, TDs locations were categorized as the gastric body, lesser curvature, greater curvature, and distant tissue. When tumor nodules appeared in the subserosal tissue of the stomach but away from the primary tumor, we defined the location as the gastric body. When tumor nodules appeared in the perigastric adipose or omental tissues, we defined it as the lesser or greater curvature according to the two curvature sides of the stomach. When tumor nodules appeared in the adipose or omental tissues far away from stomach, we defined it as distant tissue.

GC patients who underwent gastrectomy were obligatorily followed every 6 mo during the first year and every 6 or 12 mo thereafter. Follow-up included physical examination, laboratory tests, chest X-ray, abdominal and pelvic ultrasonography, and computed tomography, as previously reported. Overall survival was calculated from the date of diagnosis to the last contact or the date of death.

All statistical analyses (and generation of graphics) were performed using SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, United States). For the comparisons of clinicopathologic characteristics between the two propensity-score-matched groups, logistic regression analysis was used for categorical variables as appropriate. Overall survival rates were determined using the Kaplan-Meier method. The log-rank test was used to identify differences between the survival curves of different patient groups. In the univariate and multivariate analyses, Cox proportional hazard modeling was used to identify independent factors correlated with prognosis. Confidence intervals (CIs) were used in the analysis of the predictive accuracy estimates for models that either included or did not include TDs. All P values were two-sided, with P values < 0.05 considered statistically significant.

RESULTS

Patient demographics

This study included 6672 patients with GC, among whom 339 (5.09%) had TDs detected. Among the patients with TDs, 237 were men, and 102 were women (P = 0.527). Among the patients without TDs, 256 were men, and 83 were women (P = 0.492). The clinical characteristics of the patients with and without TDs were comparable.

Survival analysis

Kaplan-Meier analysis was performed to calculate the overall survival rates for patients with and without TDs. The 1-, 3-, and 5-year survival rates of patients with TDs were 68.3%, 19.6%, and 11.2%, respectively, and those for patients without TDs were 81.7%, 56.3%, and 26.3%, respectively (Figure 1).

Univariate analysis indicated that survival was significantly correlated with age (P = 0.011), operative method (P ≤ 0.001), histologic grade (P = 0.003), depth of invasion (P ≤ 0.001), lymph node metastasis (P ≤ 0.001), distant metastasis (P ≤ 0.001), and TD status (P ≤ 0.001). Cox multivariate analysis revealed that age, depth of invasion, lymph node metastasis, distant metastasis, and the presence of TDs were independent prognostic factors for GC patients (P < 0.05) (Table 1).

Figures 2 and 3 show survival curves of GC patients with and without TDs according to pathological TNM stage. The Kaplan-Meier survival curves indicate significant differences among the TNM stages (except stage IV) between patients with and without TDs. Patients with TDs had poorer survival than those without in each stage.

Kaplan-Meier survival curves were plotted to assess the prognostic value of TDs. In Figure 4, patients with TDs and those with stage TNM, T, or M without TDs did not have similar survival rates, but patients with TDs and those with stage N3 without TDs had similar survival rates.

Figure 5 illustrates the comparative survival analysis among the locations. Figure 6 shows the survival curves of the patients with different TD locations, as well as the survival curves of patients without TDs with different TNM pathological stages.

We proposed an amendment to the eighth edition of the UICC/AJCC TNM staging system for GC. When TDs appear in the gastric body, the original T1, T2, and T3 stages correspond to T4a with the new system, and T4a changes to T4b. When TDs appear in the lesser curvature, the previous N0, N1, N2, N3 Labels correspond to N3 under the new system. When TDs appear both in the greater curvature and distant tissue, the patient should be categorized as having M1. Figure 7 shows Kaplan-Meier survival curves generated using the new pathological TNM staging framework for GC patients with TDs.

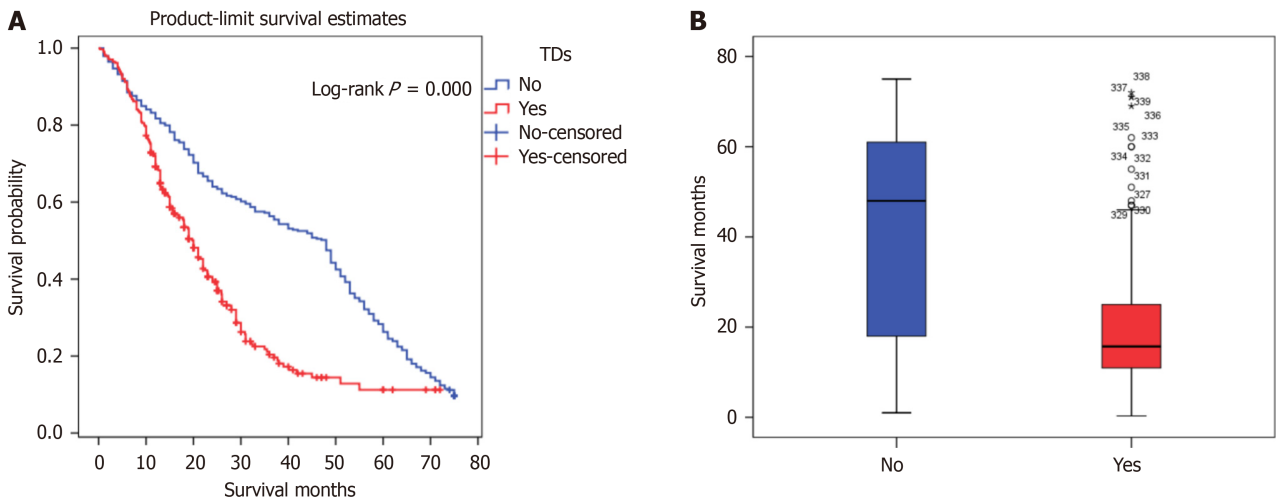
Table 1 Univariate and multivariate survival analysis of patients after operation for gastric cancer

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Sex		0.772		
Male	0.97 (0.80, 1.18)			
Female	1.00 (Reference)			
Age		0.011		0.009
≥ 65 yr	1.26 (1.05, 1.50)		1.27 (1.06, 1.53)	
< 65 yr	1.00 (Reference)		1.00 (Reference)	
Operation method		≤ 0.001		0.076
Proximal gastrectomy	0.56 (0.45, 0.70)	≤ 0.001	0.76 (0.60, 0.97)	0.026
Distal gastrectomy	0.57 (0.46, 0.70)	≤ 0.001	0.84 (0.67, 1.05)	0.118
Total gastrectomy	1.00 (Reference)		1.00 (Reference)	
Histologic grade		0.003		0.456
Low	1.41 (1.12, 1.76)		1.09 (0.86, 1.39)	
High	1.00 (Reference)		1.00 (Reference)	
AJCC 8 TNM T category		≤ 0.001		0.034
T4b	2.28 (1.58, 3.28)	≤ 0.001	1.19 (0.79, 1.78)	0.410
T4a	1.92 (1.34, 2.75)	≤ 0.001	0.92 (0.62, 1.37)	0.679
T3	1.65 (1.15, 2.36)	0.006	0.85 (0.58, 1.27)	0.429
T2	0.93 (0.59, 1.48)	0.766	0.70 (0.44, 1.12)	0.138
T1	1.00 (Reference)		1.00 (Reference)	
AJCC 8 TNM N category		≤ 0.001		≤ 0.001
N3	3.64 (2.86, 4.64)	≤ 0.001	2.72 (2.07, 3.59)	≤ 0.001
N2	2.01 (1.54, 2.63)	≤ 0.001	1.66 (1.24, 2.22)	0.001
N1	1.43 (1.06, 1.94)	0.019	1.37 (1.00, 1.89)	0.052
N0	1.00 (Reference)		1.00 (Reference)	
AJCC 8 TNM M category		≤ 0.001		≤ 0.001
M1	3.70 (2.89, 4.74)		2.78 (2.15, 3.60)	
M0	1.00 (Reference)		1.00 (Reference)	
Tumor Deposits		≤ 0.001		≤ 0.001
Yes	2.09 (1.72, 2.53)		1.64 (1.32, 2.03)	
No	1.00 (Reference)			

AJCC: American Joint Committee on Cancer; CI: Confidence interval.

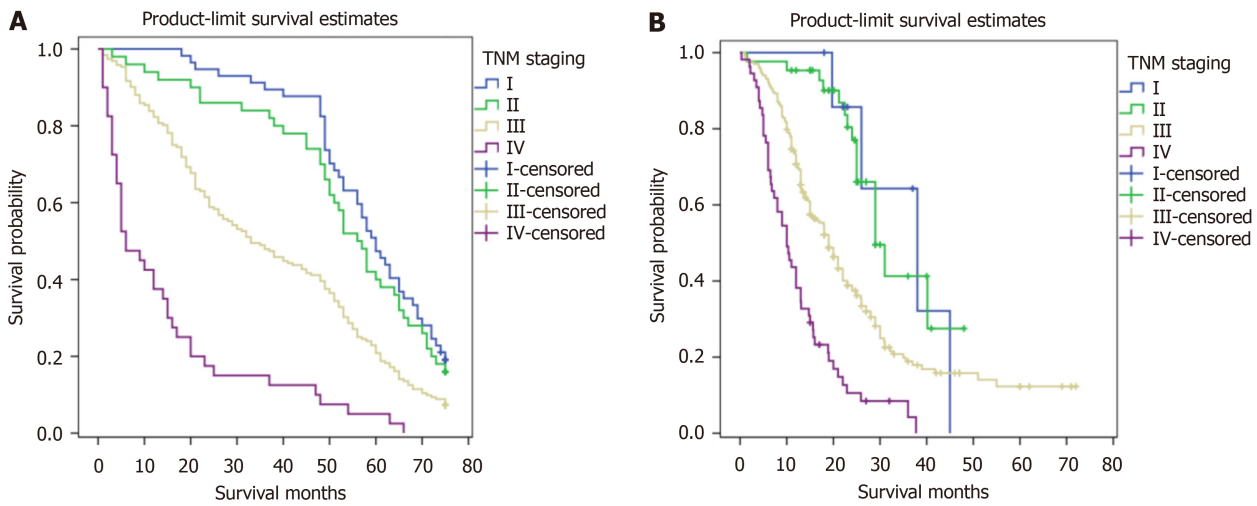
DISCUSSION

TDs first appeared in the fifth edition of UICC/AJCC Tumor Staging Guide for colorectal cancer staging in 1997, followed by the sixth and seventh editions of the colorectal cancer staging guide. However, the definitions of TDs vary between colorectal cancer stages. The criteria and histological features of TDs have been modified several times. In the seventh edition of the UICC/AJCC colorectal cancer staging guide, TDs are defined as non-contiguous with the primary tumor and as lacking evidence of lymphoid tissue structure; however, in the lymph node drainage area, TDs included as an indicator of stage N1c were considered an independent factor affecting the prognosis[16]. However, there is no evidence regarding how to best classify TDs with consideration of the actual survival of the patients. In the eighth edition of the UICC/AJCC guide, TDs are classified as regional lymph node metastasis without residual evidence of lymph node tissue [11].



DOI: 10.4251/wjgo.v15.i9.1605 **Copyright** ©The Author(s) 2023.

Figure 1 Comparison of survival between patients with and without tumor deposits. A: Kaplan-Meier survival curves of patients with and without tumor deposits (TDs); B: Survival months between patients with and without TDs. TDs: Tumor deposits.

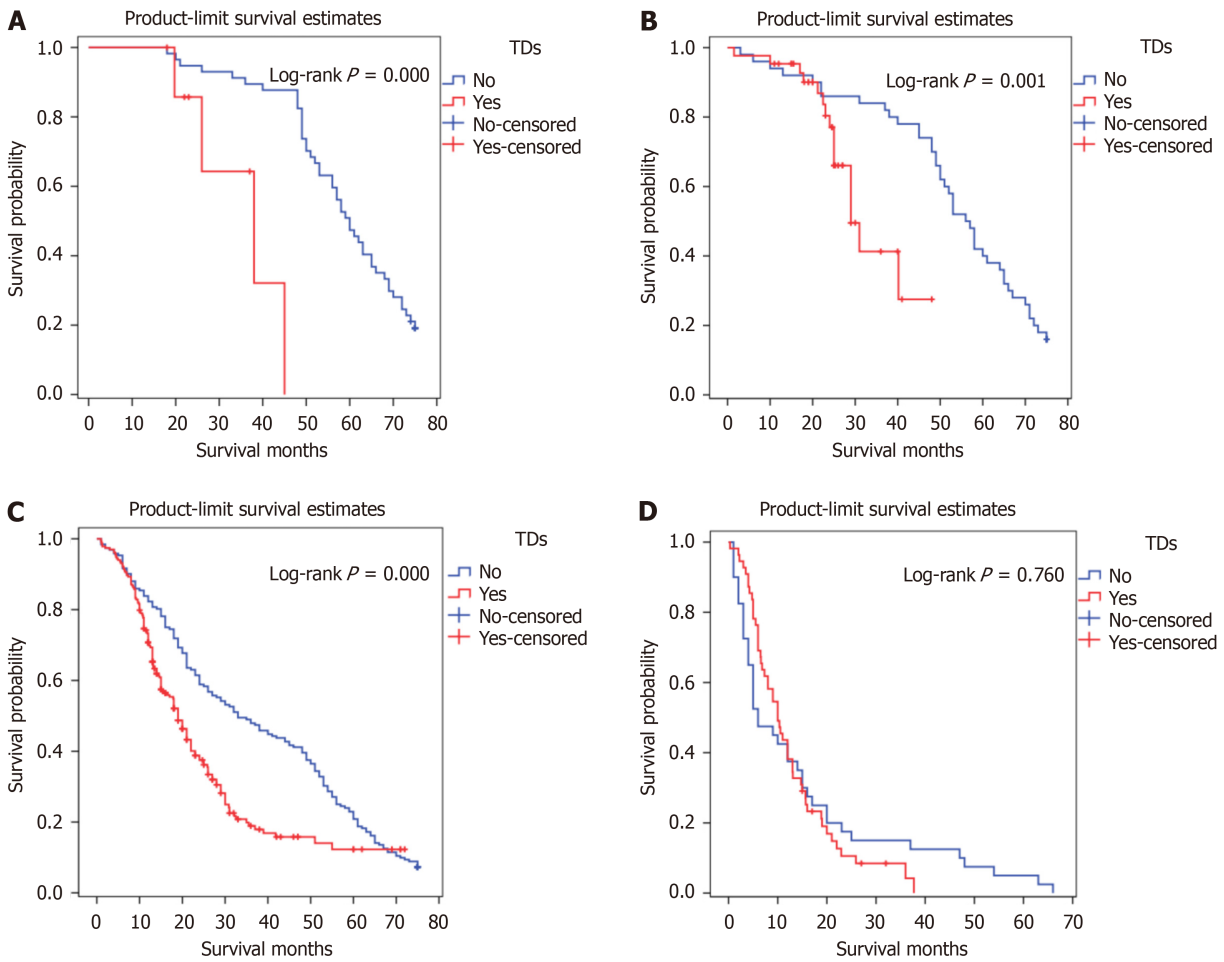


DOI: 10.4251/wjgo.v15.i9.1605 **Copyright** ©The Author(s) 2023.

Figure 2 Kaplan-Meier survival curves of patients with and without tumor deposits in different TNM stages. A: TNM stage–based survival analysis among postoperative patients without gastric cancer tumor deposits; B: TNM stage–based survival analysis among postoperative patients with gastric cancer tumor deposits.

The pathophysiological causes of TDs are still unclear, and most studies have shown that the presence of TDs is associated with lymph node metastasis, neurovascular invasion, and microvascular spread[5,8]. There is no credible evidence identifying the causes of TDs in GC. A colorectal cancer study observed four types of invasive non-continuous tumor infiltrations: Scattered, vascular, neurological, and nodular[17]. Subsequently, Goldstein and Turner[7] classified TDs into three types: The nerve disseminated type, the vascular disseminated type, and the intravascular tumor[7]. It has also been reported that when TDs appear in the mesorectum, they should be divided into intravascular, intratympanic, perineural, and isolated TDs[18]. Some studies have found that the formation of TDs may be related to the de-interstitialization of tumor cells[13]. Changes in the secretion of snail, twist, and epithelial cadherin promote the ability of the tumors to metastasize and spread through lymph nodes[19]. In summary, we found that the formation of TDs is associated with invasive tumor growth. In three previous reports, the probabilities of developing TDs in GC patients were 17.8%, 23.9%, and 24%, respectively (ordered according to publication date). It has been reported that the probability of TD development is associated with tumor size, Borrmann classification, the extent of tumor infiltration of lymphatic vessels, and lymphatic metastasis and expansion, and that the survival of GC patients is significantly correlated with TDs[20].

In this study, we analyzed the status of TDs and the clinicopathological characteristics of GC patients, and found that the presence of TDs was significantly associated with tumor infiltration (T), lymph node metastasis (N), tumor location, and neurovascular invasion. The associations of TD status with patient age (> 61 years), sex, body mass index, TNM pathological stage, and degree of differentiation were not statistically significant. This study demonstrated that TDs are associated with the invasive ability of tumor cells and that tumor cells in TDs are capable of migration, which may be *via*



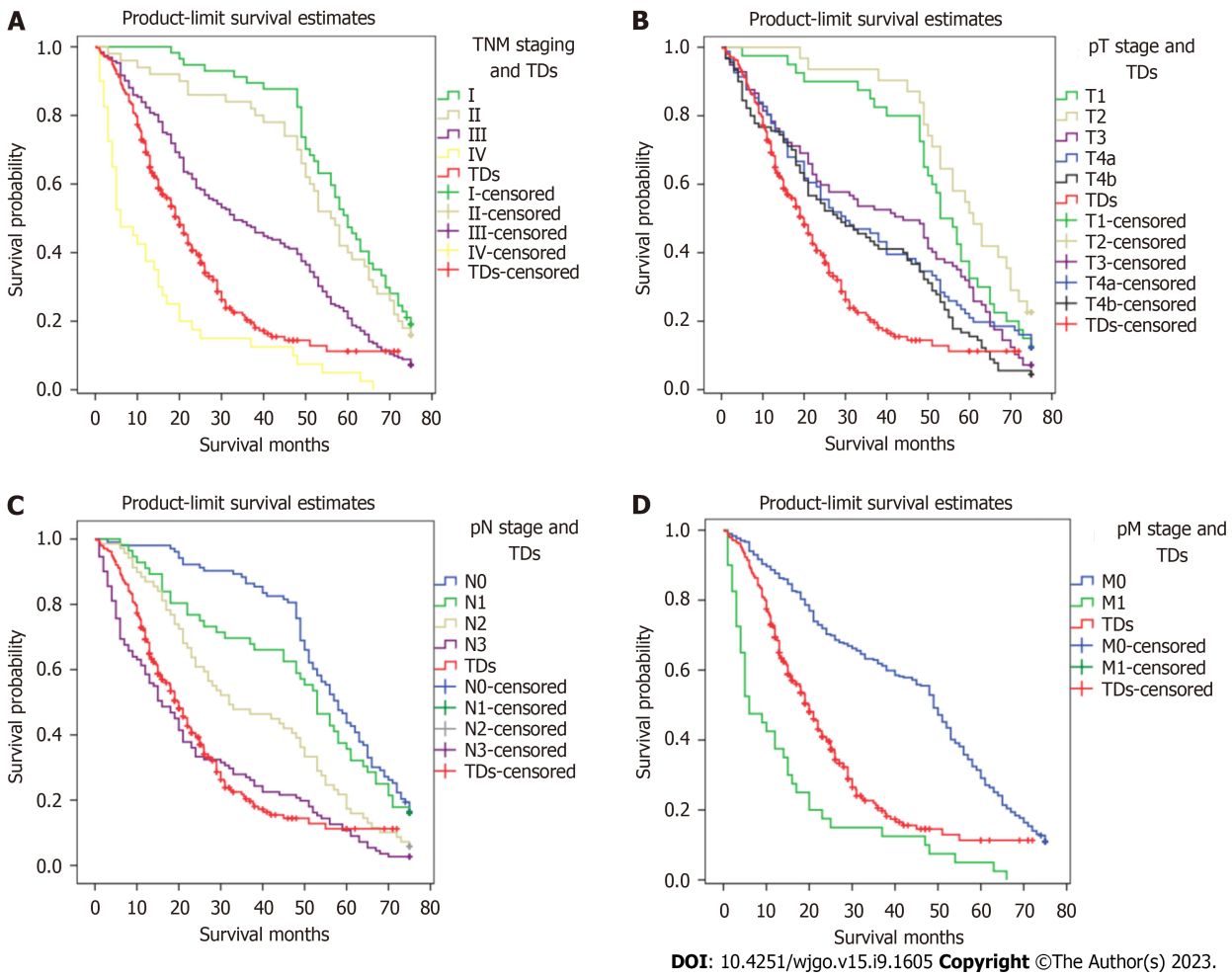
DOI: 10.4251/wjgo.v15.i9.1605

Figure 3 Overall survival of different TNM stages for patients with and without tumor deposits. A: Stage I patients with and without tumor deposits (TDs); B: Stage II patients with and without TDs; C: Stage III patients with and without TDs; D: Stage IV patients with and without TDs. TDs: Tumor deposits.

lymphatic pathways or the sudden infiltration of tumor cells of unknown causes.

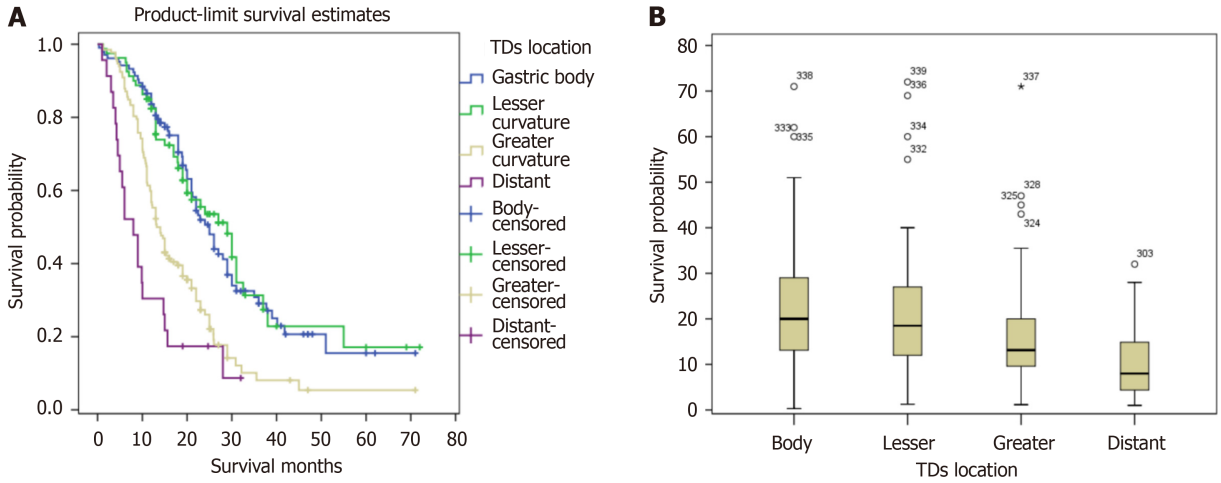
Although the overall survival of patients with GC has improved significantly over the past few decades, there are still many questions to be answered about histopathology and predictive factors. Studies have shown that TDs have independent prognostic value in colorectal cancer; however, few studies have investigated gastric TDs. Several studies have shown that the appearance of TDs predicts a poor prognosis, which is similar to our findings. Kaplan-Meier survival curves were used to plot the survival of patients with or without TDs, and the two groups were significantly different from one another. Multivariate Cox regression analysis revealed that TD positivity was not independently associated with survival in GC but that the presence of TDs may be associated with late-stage disease. Cox regression survival analysis of all patients revealed lymph node metastasis (N) and age (> 61 years old) as the only significant factors. One study (that also used Cox regression analysis) found that the presence or absence of TDs was not an independent predictor of survival[13]. The studies identified that perigastric TDs as an important prognostic indicator, and previous investigators hoped to incorporate TDs into the staging of lymph node metastasis. Additionally, they classified TDs as metastatic lymph nodes, restaged patients with colorectal cancer and advanced GC using the seventh edition of the UICC/AJCC guidelines, and found TDs in patients with the same stage of lymph node metastasis (N). The presence of TDs can lead to a worse prognosis. However, the study was unconvincing because there were only six patients with TDs with stage T1 or T2 disease[14]. In a recent study, the histological tumor type and the extent of vascular invasion were identified to be important causes of TDs, and TDs were more common in intestinal tumors. With all current research considered, there is insufficient evidence supporting TDs in GC as an independent prognostic factor.

This study included 6672 patients who underwent surgery for GC, and a total of 339 patients were TD-positive. The rate of TD positivity was 5.09%, lower than previously reported. After dividing the patients into two groups by using PSM, there were 193 patients with positive deposits and 297 with negative deposits. Among 193 patients with positive deposits, 5 (2.59%) were in stage I, 28 (14.51%) in stage II, 139 (72.02%) in stage III, and 21 (10.88%) in stage IV. The proportion of TD-positive GC patients with late-stage disease was high, suggesting that the presence of TDs may indicate later-stage disease and a worse prognosis. We found that the median survival of patients with the same TNM stage in the TD-positive group was lower than that in the TD-negative group, but there was no intergroup difference in survival among patients with stage IV disease. Kaplan-Meier survival curves were used to evaluate the survival of the two groups. The prognosis of patients in stages I, II, and III with TDs was lower than that of the TD-negative group ($P < 0.001$). The



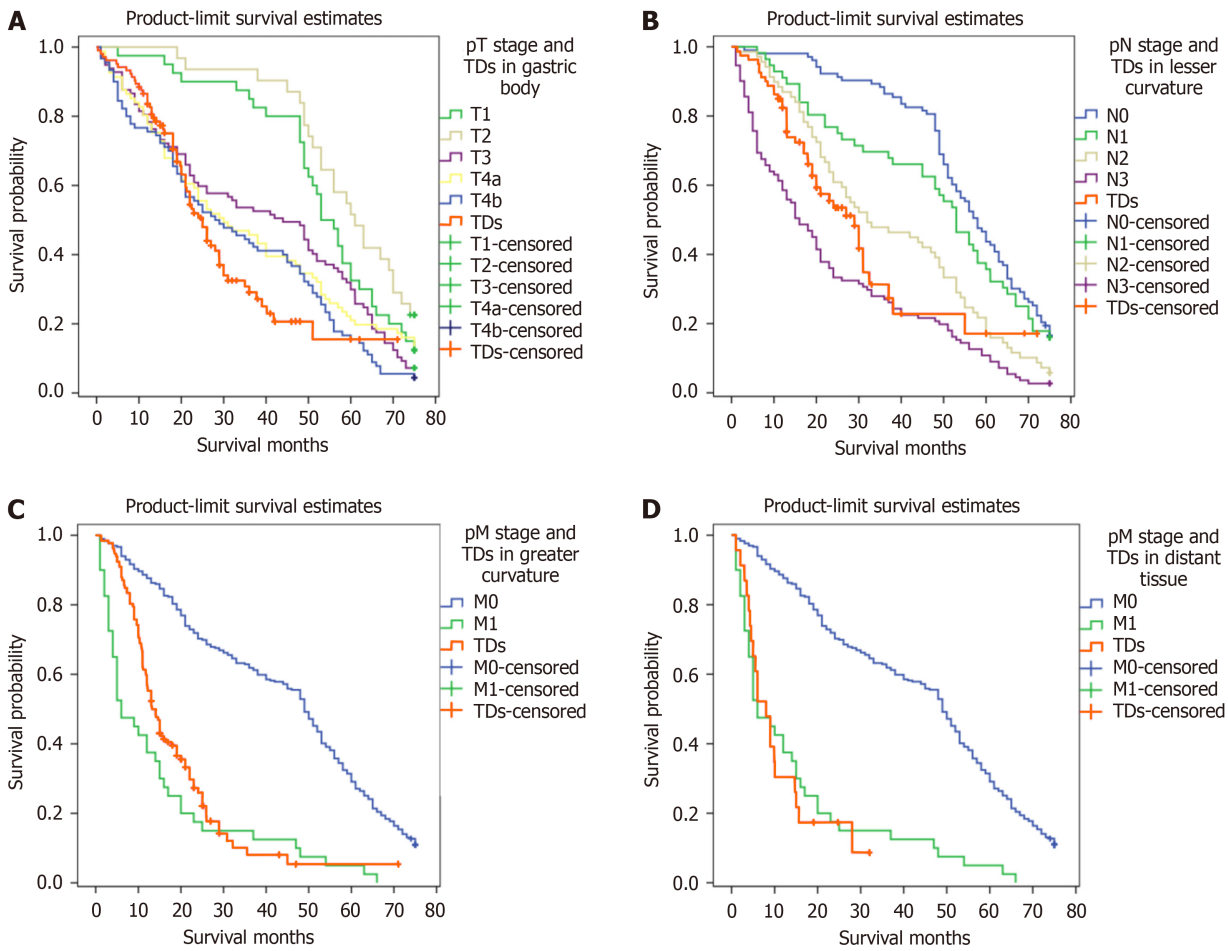
DOI: 10.4251/wjgo.v15.i9.1605 **Copyright** ©The Author(s) 2023.

Figure 4 Comparison of survival curves of patients with and without tumor deposits in different pathological TNM categories. A: Survival curves of patients with and without tumor deposits (TDs) in the category of pTNM; B: Survival curves of patients with and without TDs in the category of pathological stage of T; C: Survival curves of patients with and without TDs in the category of pathological stage of N; D: Survival curves of patients with and without TDs in the category of pathological stage of M. TDs: Tumor deposits; pT: Pathological stage of T; pN: Pathological stage of N; pM: Pathological stage of M.



DOI: 10.4251/wjgo.v15.i9.1605 **Copyright** ©The Author(s) 2023.

Figure 5 Comparison of survival outcomes associated with tumor deposits in different locations. A: Kaplan-Meier survival curves of patients with and without tumor deposits (TDs) in different locations; B: Survival months between patients with TDs in different locations. TDs: Tumor deposits.

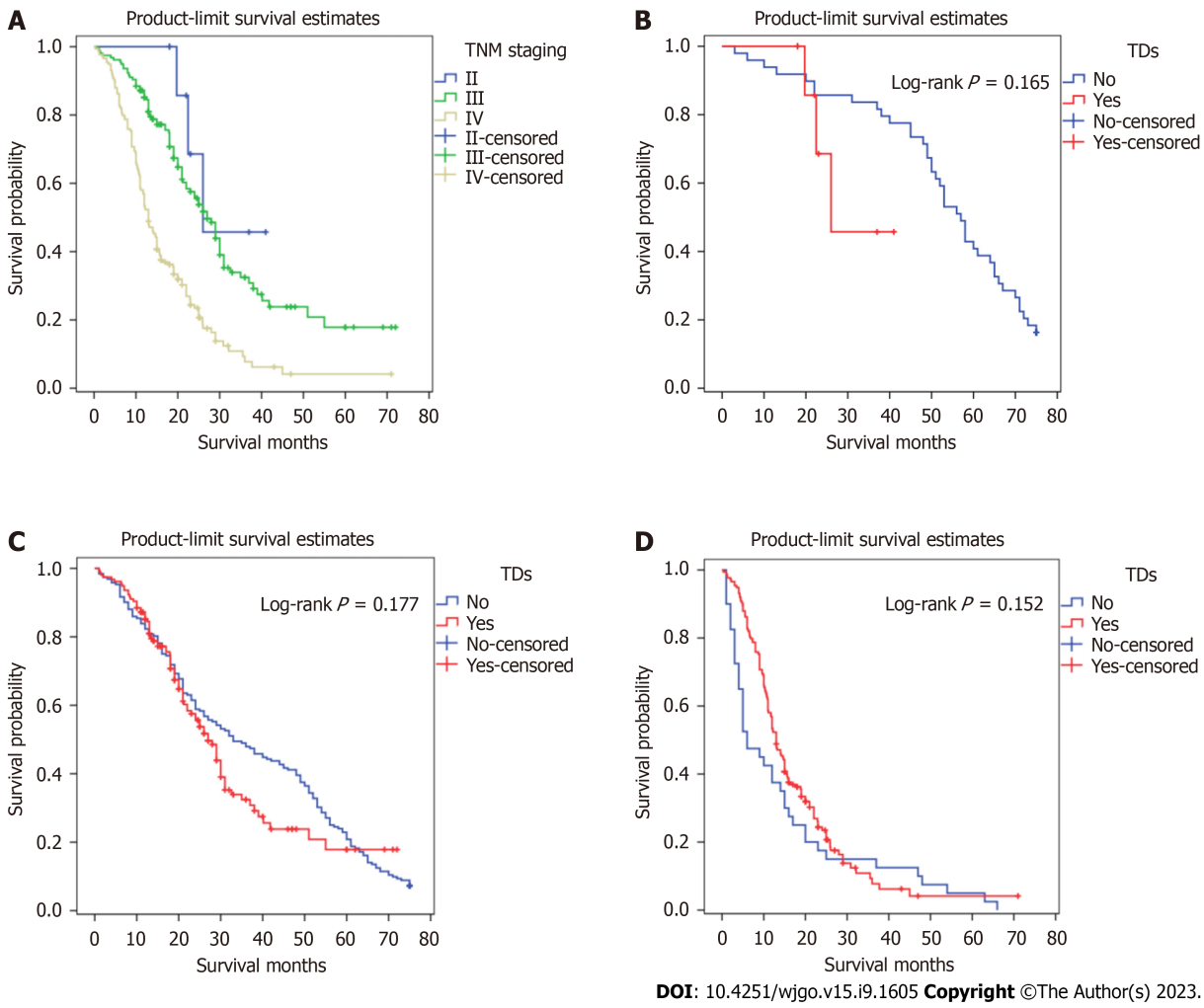


DOI: 10.4251/wjgo.v15.i9.1605

Figure 6 Comparison of survival curves of patients with different tumor deposit locations and with those of patients without tumor deposits with different TNM pathological stages. A: Comparison of Kaplan-Meier (K-M) curves of patients with tumor deposits (TDs) appearing in the gastric body with pathological stage of T without TDs; B: Comparison of K-M curves of patients with TDs appearing in the lesser curvature with pathological stage of N without TDs; C: Comparison of K-M curves of patients with TDs appearing in the greater curvature with pathological stage of M (pM) without TDs; D: Comparison of K-M curves of patients with TDs appearing in distant tissue with pM without TDs. TDs: Tumor deposits; pT: Pathological stage of T; pN: Pathological stage of N; pM: Pathological stage of M.

prognosis of the patients with TDs in stage IV was better than that of the TD-negative group, but this difference was not statistically significant. At the same time, the median overall survival durations among patients with TDs in the gastric body, lesser curvature, greater curvature, and distant tissue were 36.0 mo, 37.0 mo, 15.2 mo, and 9.9 mo, respectively; the variation among these four groups was statistically significant.

The significance of TD location in the prognosis of GC patients has not been studied. TD locations are not routinely included in histopathology reports. It was found that when TDs appeared in the greater curvature of the stomach or the omental fat connective tissue, patient survival was significantly decreased. When TDs appeared in the lesser curvature of the stomach or the gastric body, there was no significant difference in the survival between the two groups. Therefore, we speculated that when the TDs appear in the lesser curvature side of the stomach or the gastric body, the range of invasion may be limited by the anatomical positional relationship of the small omental sac and the lymphatic drainage pathway (which limits the possibility of distant metastasis). When TDs appear in the greater curvature of the stomach, the tumor cells may be transferred distally through the gastric colon ligament. When TDs appear in the distal fat connective tissue or lymph nodes, this should not be considered in N staging; rather, this scenario should be directly classified as M1. Among patients with TDs in different TNM stages, only the Kaplan-Meier survival curves of patients with stage III disease were significantly different, and the Kaplan-Meier survival curves of patients classified in the other three stages were not significantly different. In order to verify the influence of TD location on the staging of GC patients, and to find a staging framework that better aligns with the actual survival of TD-positive patients, we performed the following steps. We compared the T and N stages of TD-positive patients with TDs in the lesser curvature with those of the TD-negative group and found that the survival curve of the TD-positive patients with TDs in the lesser curvature was similar to those of the TD-negative patients classified as stages N1 and N2, respectively. Comparing the T and N stages of the TD-positive group with those of the negative group, we found that the survival curve of the TD-positive group was similar to those of the TD-negative patients classified as having T3, T4b, and N2, respectively. We determined that, when TDs appeared in the gastric body, the T stage should not be lower than T3, but there was no similar definitive conclusion that could be made for N stage. Comparing N stages of TD-positive patients with TDs in the greater curvature with those of TD-



DOI: 10.4251/wjgo.v15.i9.1605

Figure 7 Survival curves generated using the proposed TNM staging framework incorporating tumor deposits. A: Survival curves generated using the new TNM staging framework for patients with gastric cancer tumor deposits (TDs); B: Survival curves of stage II postoperative patients with and without TDs; C: Survival curves of stage III postoperative patients with and without TDs; D: Survival curves of stage IV postoperative patients with and without TDs. TDs: Tumor deposits.

negative patents, we found favorable survival among both the TD-positive patients with TDs in the greater curvature and the N3 patients in the TD-negative group. In this case, the N stage of patients with TDs was N3 regardless of the number of metastatic lymph nodes. The M stages of the TD-positive patients with TDs in the distant tissue were compared with those of the TD-negative patients, and the survival curve of the TD-positive patients with TDs in the distant tissue was similar to that of M1 patients in the TD-negative group. In summary, we believe that when the pathological report suggests that TDs appear in the gastric body, the T stage should be no less than T3; when TDs appear in the greater curvature of the stomach, the N stage should be no less than N3; when TDs are in the distant tissue, the M stage should be M1.

Although our study confirmed the adverse effects of TDs on the prognosis of GC and put forward a new TNM staging method containing TDs innovatively, the following limitations remained in this study. First, TDs were more common with diffuse histological type compared with intestinal type, but the results of Lauren typing were not used in the pathological report of our center, and it would be better if this part was added. Second, because of the single-center retrospective design, the number of cases was limited, and the follow-up time was insufficient. Third, the study was performed in Asian population, so the data might not be extrapolated to North American or European populations. Therefore, valid incorporation of TDs into the TNM staging system for primary GC requires multicenter and more large-scale clinical analyses including American and European data, as well as more in-depth basic research and exploration of the mechanism underlying TD development.

CONCLUSION

TDs are a poor prognostic factor in patients with primary GC, and a new TNM staging system combining TDs would be suitable for such patients.

ARTICLE HIGHLIGHTS

Research background

The current prognostic significance of perigastric tumor deposits (TDs) in gastric cancer (GC) remains unclear.

Research motivation

The prognostic value of TDs in GC has not been extensively studied or confirmed. To date, no studies have investigated the prognostic significance of TDs in GC in detail. This study aimed to assess the prognostic value of perigastric TDs and put forward a new and appropriate TNM staging framework involving TDs for primary GC.

Research objectives

This study aimed to assess the prognostic value of perigastric TDs and put forward a new and appropriate TNM staging framework involving TDs for primary GC.

Research methods

We retrospectively analyzed the pathological data of 6672 patients with GC who underwent gastrectomy or surgery for GC with other diseases from January 1, 2012 to December 31, 2017 at the Chinese PLA General Hospital. The patients were divided into TD-positive and TD-negative groups by using the method of propensity score matching. The differences between TD-positive and TD-negative patients were analyzed using binary logistic regression modeling. The Kaplan-Meier method was used to plot survival curves. Multivariate Cox regression modeling and the log-rank test were used to analyze the data.

Research results

With the new GC staging scheme including TDs, the survival curves of patients in the lower grade TNM stage with TDs were closer to those of patients in the higher grade TNM stage without TDs.

Research conclusions

TDs are a poor prognostic factor in patients with primary GC, and a new TNM staging system combining TDs would be suitable for such patients.

Research perspectives

From a clinical point of view, we found deficiencies in the current TNM staging system for GC and conducted this study.

FOOTNOTES

Author contributions: Li Y, Li S, and Liu L contributed equally to this work; Wang XX, Li Y, Li S, and Liu L designed the study; Li Y, Li S, and Liu L collected the sample data of the patients; Li S, Liu L, Zhang LY, Wu D, and Xie TY performed the statistical analysis; Li Y wrote the manuscript; all of the authors read and approved the final manuscript.

Institutional review board statement: This study was approved by the Medical Ethics Committee of the Chinese PLA General Hospital (No. S2023-065-01).

Informed consent statement: Due to the approval of the Medical Ethics Committee, the informed consent form has been waived.

Conflict-of-interest statement: All of the authors declare no interest in this study.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at [301wxx@sina.com]. Participants gave informed consent for data sharing.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: China

ORCID number: Yang Li 0000-0003-4024-8652; Shuo Li 0000-0002-1631-6654; Lu Liu 0000-0003-2593-3758; Li-Yu Zhang 0000-0001-8599-155X; Di Wu 0000-0003-1620-2224; Tian-Yu Xie 0000-0002-1745-221X; Xin-Xin Wang 0000-0001-2492-4932.

S-Editor: Fan JR
L-Editor: Wang TQ

P-Editor: Xu ZH

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **He X**, Wu W, Lin Z, Ding Y, Si J, Sun LM. Validation of the American Joint Committee on Cancer (AJCC) 8th edition stage system for gastric cancer patients: a population-based analysis. *Gastric Cancer* 2018; **21**: 391-400 [PMID: 29052053 DOI: 10.1007/s10120-017-0770-1]

3 **Gurzu S**, Orlowska J, Sugimura H, Bara T, Szentirmay Z, Januszewicz W, Bara T Jr, Szederjesi J, Jung I. Immunohistochemical features and staging of early gastric cancer. *Arch Med Sci* 2017; **13**: 1373-1382 [PMID: 29181068 DOI: 10.5114/aoms.2016.58665]

4 **Gabriel WB**, Dukes CE, Bussey HJ. Biopsy of the rectum. *Br J Surg* 1951; **38**: 401-411 [PMID: 14830775 DOI: 10.1002/bjs.18003815202]

5 **Ono C**, Yoshinaga K, Enomoto M, Sugihara K. Discontinuous rectal cancer spread in the mesorectum and the optimal distal clearance margin in situ. *Dis Colon Rectum* 2002; **45**: 744-9; discussion 742 [PMID: 12072624 DOI: 10.1007/s10350-004-6290-1]

6 **Tateishi S**, Arima S, Futami K, Kawahara K, Tachikawa D, Naritomi K, Iwashita A. A clinicopathological investigation of "tumor nodules" in colorectal cancer. *Surg Today* 2005; **35**: 377-384 [PMID: 15864419 DOI: 10.1007/s00595-004-2950-y]

7 **Goldstein NS**, Turner JR. Pericolonic tumor deposits in patients with T3N+MO colon adenocarcinomas: markers of reduced disease free survival and intra-abdominal metastases and their implications for TNM classification. *Cancer* 2000; **88**: 2228-2238 [PMID: 10820343]

8 **Belt EJ**, van Stijn MF, Bril H, de Lange-de Klerk ES, Meijer GA, Meijer S, Stockmann HB. Lymph node negative colorectal cancers with isolated tumor deposits should be classified and treated as stage III. *Ann Surg Oncol* 2010; **17**: 3203-3211 [PMID: 20625841 DOI: 10.1245/s10434-010-1152-7]

9 **Tong LL**, Gao P, Wang ZN, Song YX, Xu YY, Sun Z, Xing CZ, Xu HM. Is the seventh edition of the UICC/AJCC TNM staging system reasonable for patients with tumor deposits in colorectal cancer? *Ann Surg* 2012; **255**: 208-213 [PMID: 21527844 DOI: 10.1097/SLA.0b013e31821ad8a2]

10 **Luchini C**, Veronese N, Pea A, Sergi G, Manzato E, Nottegar A, Solmi M, Capelli P, Scarpa A. Extranodal extension in N1-adenocarcinoma of the pancreas and papilla of Vater: a systematic review and meta-analysis of its prognostic significance. *Eur J Gastroenterol Hepatol* 2016; **28**: 205-209 [PMID: 26566063 DOI: 10.1097/MEG.0000000000000520]

11 **Liang Y**, Wu L, Liu L, Ding X, Wang X, Liu H, Meng J, Xu R, He D, Liang H. Impact of extranodal tumor deposits on prognosis and N stage in gastric cancer. *Surgery* 2019; **166**: 305-313 [PMID: 31221435 DOI: 10.1016/j.surg.2019.04.027]

12 **Lee HS**, Lee HE, Yang HK, Kim WH. Perigastric tumor deposits in primary gastric cancer: implications for patient prognosis and staging. *Ann Surg Oncol* 2013; **20**: 1604-1613 [PMID: 23184289 DOI: 10.1245/s10434-012-2692-9]

13 **Sun Z**, Wang ZN, Xu YY, Zhu GL, Huang BJ, Xu Y, Liu FN, Zhu Z, Xu HM. Prognostic significance of tumor deposits in gastric cancer patients who underwent radical surgery. *Surgery* 2012; **151**: 871-881 [PMID: 22386276 DOI: 10.1016/j.surg.2011.12.027]

14 **Ersen A**, Unlu MS, Akman T, Sagol O, Oztop I, Atila K, Bora S, Ellidokuz H, Sarioglu S. Tumor deposits in gastric carcinomas. *Pathol Res Pract* 2014; **210**: 565-570 [PMID: 24726262 DOI: 10.1016/j.prp.2014.03.006]

15 **Nagtegaal ID**, Tot T, Jayne DG, McShane P, Nihlberg A, Marshall HC, Påhlman L, Brown JM, Guillou PJ, Quirke P. Lymph nodes, tumor deposits, and TNM: are we getting better? *J Clin Oncol* 2011; **29**: 2487-2492 [PMID: 21555695 DOI: 10.1200/JCO.2011.34.6429]

16 **Koelzer VH**, Lugli A, Dawson H, Hädrich M, Berger MD, Borner M, Mallaev M, Galván JA, Amsler J, Schnüriger B, Zlobec I, Inderbitzin D. CD8/CD45RO T-cell infiltration in endoscopic biopsies of colorectal cancer predicts nodal metastasis and survival. *J Transl Med* 2014; **12**: 81 [PMID: 24679169 DOI: 10.1186/1479-5876-12-81]

17 **Ueno H**, Mochizuki H. Clinical significance of extrabowel skipped cancer infiltration in rectal cancer. *Surg Today* 1997; **27**: 617-622 [PMID: 9306563 DOI: 10.1007/BF02388217]

18 **Ratto C**, Ricci R, Rossi C, Morelli U, Vecchio FM, Doglietto GB. Mesorectal microfoci adversely affect the prognosis of patients with rectal cancer. *Dis Colon Rectum* 2002; **45**: 733-42; discussion 742 [PMID: 12072622 DOI: 10.1007/s10350-004-6288-8]

19 **Fan XJ**, Wan XB, Yang ZL, Fu XH, Huang Y, Chen DK, Song SX, Liu Q, Xiao HY, Wang L, Wang JP. Snail promotes lymph node metastasis and Twist enhances tumor deposit formation through epithelial-mesenchymal transition in colorectal cancer. *Hum Pathol* 2013; **44**: 173-180 [PMID: 22974478 DOI: 10.1016/j.humpath.2012.03.029]

20 **Puppa G**, Ueno H, Kayahara M, Capelli P, Canzonieri V, Colombari R, Maisonneuve P, Pelosi G. Tumor deposits are encountered in advanced colorectal cancer and other adenocarcinomas: an expanded classification with implications for colorectal cancer staging system including a unifying concept of in-transit metastases. *Mod Pathol* 2009; **22**: 410-415 [PMID: 19136930 DOI: 10.1038/modpathol.2008.198]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1616-1625

DOI: 10.4251/wjgo.v15.i9.1616

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Multidisciplinary discussion and management of synchronous colorectal liver metastases: A single center study in China

Hao Li, Guo-Li Gu, Song-Yan Li, Yang Yan, Shi-Dong Hu, Ze Fu, Xiao-Hui Du

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Billeter A, Germany; Tanabe M, Japan

Received: June 30, 2023
Peer-review started: June 30, 2023
First decision: July 24, 2023
Revised: July 24, 2023
Accepted: August 4, 2023
Article in press: August 4, 2023
Published online: September 15, 2023

Hao Li, Ze Fu, Graduate School, Medical School of Chinese People's Liberation Army, Beijing 100039, China

Hao Li, Guo-Li Gu, Department of General Surgery, Air Force Medical Center, Air Force Medical University, Beijing 100142, China

Song-Yan Li, Yang Yan, Shi-Dong Hu, Xiao-Hui Du, Department of General Surgery, Chinese People's Liberation Army General Hospital, Beijing 100039, China

Corresponding author: Xiao-Hui Du, MD, PhD, Chief Doctor, Deputy Director, Professor, Surgeon, Department of General Surgery, Chinese People's Liberation Army General Hospital, No. 28 Fuxing Road, Haidian District, Beijing 100039, China. duxiaohui301@sina.com

Abstract

BACKGROUND

The multidisciplinary team (MDT) has been carried out in many large hospitals now. However, given the costs of time and money and with little strong evidence of MDT effectiveness being reported, critiques of MDTs persist.

AIM

To evaluate the effects of MDTs on patients with synchronous colorectal liver metastases and share our opinion on management of synchronous colorectal liver metastases.

METHODS

In this study we collected clinical data of patients with synchronous colorectal liver metastases from February 2014 to February 2017 in the Chinese People's Liberation Army General Hospital and subsequently divided them into an MDT+ group and an MDT- group. In total, 93 patients in MDT+ group and 169 patients in MDT- group were included totally.

RESULTS

Statistical increases in the rate of chest computed tomography examination (P = 0.001), abdomen magnetic resonance imaging examination (P = 0.000), and preoperative image staging (P = 0.0000) were observed in patients in MDT+ group. Additionally, the proportion of patients receiving chemotherapy (P = 0.019) and curative resection (P = 0.042) was also higher in MDT+ group. Multivariable analysis showed that the population of patients assessed by MDT meetings

had higher 1-year [hazard ratio (HR) = 0.608, 95% confidence interval (CI): 0.398-0.931, P = 0.022] and 5-year (HR = 0.694, 95%CI: 0.515-0.937, P = 0.017) overall survival.

CONCLUSION

These results proved that MDT management did bring patients with synchronous colorectal liver metastases more opportunities for comprehensive examination and treatment, resulting in better outcomes.

Key Words: Synchronous colorectal liver metastases; Multidisciplinary team; Imaging examination; Treatment strategy; Oncological outcome

Core Tip: Synchronous colorectal liver metastases usually predict a poor prognosis. Nevertheless, given the costs of time and money and with little strong evidence of multidisciplinary team (MDT) effectiveness being reported, critiques of MDTs still persist. This study demonstrates that MDT management brings patients more opportunities for aggressive examination and treatment. Retrospective clinical data shows that the population of patients assessed by MDT meetings has higher 1-year and 5-year overall survival.

Citation: Li H, Gu GL, Li SY, Yan Y, Hu SD, Fu Z, Du XH. Multidisciplinary discussion and management of synchronous colorectal liver metastases: A single center study in China. *World J Gastrointest Oncol* 2023; 15(9): 1616-1625
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1616.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1616

INTRODUCTION

Colorectal cancer is the second most commonly diagnosed cancer, with an estimated 1.78 million cases occurring in 2020 [1]. About 50% of patients with colorectal cancer will suffer distant metastases; the liver is the most common site. In particular synchronous liver metastases account for 15%-25% of colorectal liver metastases[2]. Synchronous colorectal liver metastases are usually defined as liver metastases detected at or before primary colorectal cancer. Curative resection is identified as the most effective method for curing synchronous colorectal liver metastases. However, data showed only 5%-15% patients with synchronous liver metastases were curable with resection[3,4], 5-year survival rates of patients with unresectable liver metastases were starkly lower, at less than 5% respectively[5].

The multidisciplinary team (MDT) originated in the United Kingdom in the 1960s and 1970s[6] and is defined as a regularly scheduled discussion of patients, especially those diagnosed with cancer, comprising professionals from different specialties[7]. After years of development, MDTs have been used in most large hospitals and are recommended by most guidelines on cancer therapy[8]. Nevertheless, given the costs of time and money and with little strong evidence of MDT effectiveness being reported, critiques of MDTs persist[9,10].

On a positive note, many retrospective and prospective studies have already provided clinical evidence in favor of MDT meetings with regard to diagnosis, tumor staging, treatment strategy, and oncological outcomes of cancer including colorectal cancers[11-13]. However, few reports have shown the impact of MDT meetings on synchronous colorectal liver metastases. In this study, we undertook a retrospective analysis of the impact of MDT meetings on the clinical data of patients with synchronous colorectal liver metastases, and we provide our insights on management of synchronous colorectal liver metastases in an MDT model.

MATERIALS AND METHODS

This retrospective study incorporated patients who were diagnosed with synchronous colorectal liver metastases from February 2014 to February 2017 in the Chinese People's Liberation Army General Hospital. All patients in the MDT group (MDT+) were discussed by the gastrointestinal cancer MDT of the Chinese People's Liberation Army General Hospital and had thorough records in minutes of the meetings. Patients without discussion at MDT meetings (MDT-) were treated by doctors with equivalent qualifications of the Chinese People's Liberation Army General Hospital. This study received approval from the ethics commission of the General Hospital of People's Liberation Army.

Data collection

Patients with uncertain diagnoses and medical records were excluded, as were patients suffering from extrahepatic metastases or other severe disease that might affect survival time seriously. These patients were followed up for 66 mo in this study. A total of 169 patients in MDT- group (80 men and 89 women; mean age: 60.15 years) and 93 patients in MDT+ group (53 men and 40 women; mean age: 59.19 years) were ultimately included in this study.

To analyze the impact of MDT on overall survival (OS), we compiled the following items in our data collection according to previous studies[14-17]: (1) Demographic data: Age, gender, body mass index; (2) Cancer characteristics: Site of primary tumor, primary lymph node (LN) involvement, multiple liver metastases, extrahepatic metastases; (3) Baseline examination including imaging data and serum carcinoembryonic antigen (CEA) levels; (4) Detailed data about chemotherapy and surgery; and (5) Clinical data of follow-up until patients' death or the end of the follow-up period (August 2022). Data were mainly collected from the Electronic Medical Record of the Chinese People's Liberation Army General Hospital, and those unavailable in the Electronic Medical Record were obtained from patients, in the form of copied records, imaging and laboratory data.

Statistical analysis

Continuous data are presented as median (range) unless indicated otherwise. Comparisons of differences in continuous variables between the two groups were performed with student's *t* test. Chi-square test and Fisher's exact method were carried out for categorical data. In the analysis of event-specific rates, patients were considered to be at risk of the studied event until death or the end of follow-up. Cumulative survival curves were plotted using the Kaplan-Meier method and statistically compared using the log-rank test. Univariate and multivariate survival analysis was performed using the Cox proportional hazards model, with results presented as a hazard ratio (HR) with a 95% confidence interval (CI). Univariate and multivariate logistic analysis was performed using the likelihood ratio test, with results presented as an odds ratio (OR) with a 95%CI. Multivariate analysis included items with univariate analysis results of $P < 0.20$. Statistical significance was set at $P < 0.05$. All analyses were performed using the Statistical Program for Social Sciences 26.0 software (SPSS, Inc., Chicago, IL, United States).

RESULTS

Patient

A total of 262 patients were included in this study. The clinical characteristics of patients are detailed in Table 1. In MDT+ group, a significant 80.65% of patients (75 out of 93) were diagnosed with liver metastases at more than one site. Interestingly, the proportion of patients in MDT- group was 79.88% ($P = 0.989$). No significant differences in demographic data and cancer characteristics were observed between these two groups.

Baseline imaging examination and radiological tumor-node-metastasis staging

The rate of chest computed tomography (CT) examination in patients in MDT+ group was significantly higher than that in MDT- group (100% *vs* 82.84%, $P = 0.001$). This trend was mirrored in the rate of abdomen magnetic resonance imaging (MRI) (100% *vs* 73.96%, $P = 0.000$). As radiological tumor-node-metastasis (TNM) staging was routinely required in our gastrointestinal cancer MDT meeting, all patients in MDT+ group had been diagnosed with TNM staging. However, only 20.12% of patients were evaluated with radiological TNM staging in MDT- group ($P = 0.000$). No significant difference in positron emission tomography-CT (PET-CT) between the two groups was observed ($P = 0.906$). Baseline imaging examination and radiological TNM staging results are represented in Table 2.

Oncology treatment and surgery

Of 17 patients in MDT+ group were diagnosed with initial resectable synchronous colorectal liver metastases. 77 patients in the MDT+ group and 116 patients in MDT- group received chemotherapy (82.80% *vs* 68.64%, $P = 0.0191$). Approximately 10% of these chemotherapy patients were successfully converted to be radically resectable after several chemotherapy cycles. At the end of the follow-up period, 30 patients in MDT+ group and 35 patients in MDT- group had undergone curative resection (32.29% *vs* 20.71%, $P = 0.0415$). Statistical differences were not observed in the proportion of initial resectable liver metastases, and successful conversion chemotherapy between the two groups. Oncology treatment and surgery is outlined in Table 3.

OS

The 1-year OS rate of all 262 patients was determined to be 54.58%. There was a significant difference between the two groups, with patients in MDT+ group demonstrating statistically higher 1-year OS rates than those in MDT- group (66.67% *vs* 47.93%; $P = 0.0036$, Figure 1). Univariate analysis employing the Cox proportional hazards model, age > 75 years, CEA > 5 ng/mL, primary LN involvement, multiple liver metastases, extrahepatic metastases, curative resection, MDT, and chemotherapy were associated with 1-year OS rates at $P < 0.20$ (Table 4).

Subsequent multivariate analysis illuminated that age > 75 years (HR = 2.276, 95%CI: 1.419-3.649, $P = 0.001$), CEA > 5 ng/mL (HR = 5.139, 95%CI: 3.093-8.539, $P = 0.000$), Primary LN involvement (HR = 1.828, 95%CI: 1.073-3.116, $P = 0.027$), multiple liver metastases (HR = 5.300, 95%CI: 1.627-17.262, $P = 0.006$), and extrahepatic metastases (HR = 6.187, 95%CI: 3.702-10.339, $P = 0.0001$) were high-risk factors. In contrast, MDT (HR = 0.608, 95%CI: 0.398-0.931, $P = 0.022$, Figure 1A) and curative resection (HR = 0.024, 95%CI: 0.003-0.177, $P = 0.000$) emerged as protective factors. During our analyses of 5-year OS rates, we found that despite the complexity of variables, MDT remained an independent protective factor (HR = 0.694, 95%CI: 0.515-0.937, $P = 0.017$, Table 5 and Figure 1B).

Table 1 Demographic and clinical characteristics of patients

Characteristics	MDT+ (*n* = 93)	MDT- (*n* = 169)	*P* value
Age (yr), mean (min-max)	59.19 (28.00-89.00)	60.15 (25.00-92.00)	0.605
Male/female, *n*	40/53	89/80	0.172
BMI (kg/m^2), mean (min-max)	24.83 (17.29-33.82)	23.62 (16.06-33.5)	0.221
KPs score ≥ 60	89/12	150/19	0.095
Adenocarcinoma/mucinous adenocarcinoma, *n*	83/10	143/26	0.393
Poor differentiation, *n* (%)	16 (17.20)	21 (12.43)	0.380
Primary tumor category ≥ T3, *n* (%)	67 (72.04)	135 (79.88)	0.197
Primary LN involvement, *n* (%)	60 (64.52)	118 (69.82)	0.458
Multiple liver metastases, *n* (%)	75 (80.65)	134 (79.29)	0.920

BMI: Body mass index; KPs: Karnofsky performance status; LN: Lymph node; MDT: Multidisciplinary team.

Table 2 Baseline imaging examination and radiological tumor-node-metastasis staging

	MDT+ (*n* = 93)	MDT- (*n* = 169)	*P* value
Chest CT, *n* (%)	93 (100)	140 (82.84)	0.001
Abdomen MRI, *n* (%)	89 (95.70)	125 (73.96)	0.000
PET-CT, *n* (%)	22 (23.66)	47 (27.81)	0.906
TNM staging, *n* (%)	93 (100)	34 (20.12)	0.000

CT: Computed tomography; MRI: Magnetic resonance imaging; PET: Positron emission tomography; TNM: Tumor-node-metastasis; MDT: Multidisciplinary team.

Table 3 Oncology treatment and surgery

	MDT+ (*n* = 93)	MDT- (*n* = 169)	*P* value
Initial resectable, *n* (%)	17(18.285)	21 (12.43)	0.270
Successful conversion chemotherapy, *n* (uninitial resectable, *n*)	13 (76[1])	14 (148[1])	0.148
Chemotherapy, *n* (%)	77 (82.80)	116 (68.64)	0.019
Curative resection, *n* (%)	30 (32.29)	35 (20.71)	0.042
Simultaneous resection, *n* (%)	29 (97.63)	19 (55.88)	0.001
RFA, *n* (%)	5 (16.67)	15 (44.12)	0.036

[1]Values in parentheses are numbers of patients with unresectable liver metastases.
RFA: Radiofrequency ablation; MDT: Multidisciplinary team.

DISCUSSION

In MDT+ group, a significant majority of patients underwent a chest CT examination (100% *vs* 82.84%, P = 0.001). A SEER-based study including 46027 colorectal cancer patients found that about 20% of patients with colorectal liver metastasis were diagnosed with lung metastases simultaneously[16]. Furthermore, resection of liver and lung metastases brings better oncological outcomes than resection of liver metastases only[18]. Thus, the high frequency of chest CT examinations observed in the MDT+ group aligns with the need for comprehensive diagnostics in the management of patients with synchronous colorectal liver cancer. Moreover, the rate of abdomen MRI examination was significantly higher in MDT+ group compared to the MDT- group (P = 0.000), indicating a greater focus on identifying patients with questionable or curatively resectable liver metastases[19,20]. Most cancer therapy guidelines and clinical research underscore the importance of TNM staging in informing treatment strategies, reinforcing the value of accurate preoperative radiological TNM staging in treatment planning and monitoring clinical efficacy. Moreover, researchers

Table 4 Univariate and multivariate analyses of risk factors associated with 1-year overall survival

	n (%)	Univariate			Multivariate		
		HR	95%CI	*P* value	HR	95%CI	*P* value
Age > 75	61 (23.28)	3.533	2.44-5.11	0.000	2.065	1.257-3.393	0.004
Sex (male)	133 (50.76)	0.845	0.590-1.211	0.358			
BMI > 28	46 (17.56)	0.765	0.468-1.250	0.285			
CEA > 5 ng/mL	125 (47.71)	7.296	4.674-11.391	0.000	5.308	3.262-8.638	0.000
Colon primary	118 (45.04)	1.283	0.896-1.838	0.174	1.058	0.724-1.544	0.772
Mucinous adenocarcinoma	36 (13.74)	0.863	0.502-1.482	0.593			
Poor differentiation	37 (14.12)	1.282	0.793-2.073	0.311			
Primary tumor category ≥ T3	202 (77.10)	1.284	0.820-2.009	0.274			
Primary LN involvement	178 (67.94)	3.336	2.061-5.400	0.000	1.948	1.156-3.281	0.012
Multiple liver metastases	210 (80.15)	13.97	4.44-43.97	0.000	4.747	1.470-15.333	0.009
MDT	93 (35.50)	0.53	0.353-0.801	0.003	0.572	0.374-0.874	0.010
chemotherapy	193 (73.66)	0.239	0.166-0.344	0.000	0.874	0.539-1.418	0.587
Curative resection	67 (25.57)	0.016	0.002-0.114	0.000	0.031	0.004-0.227	0.001

HR: Hazards ratio; CI: Confidence interval; BMI: Body mass index; CEA: Carcinoembryonic antigen; MDT: Multidisciplinary team; LN: Lymph node.

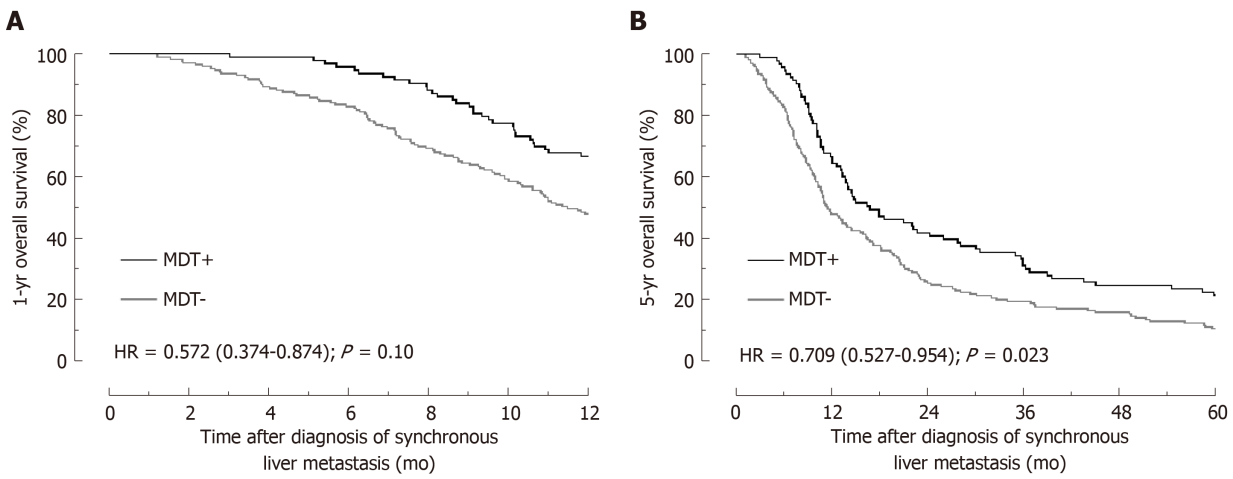
Table 5 Univariate and multivariate analyses of risk factors associated with 5-year overall survival

	n (%)	Univariate			Multivariate		
		HR	95%CI	*P* value	HR	95%CI	*P* value
Age > 75	61 (23.28)	3.471	2.532-4.758	0.000	2.040	1.322-3.149	0.001
Sex (male)	133 (50.76)	0.938	0.721-1.221	0.938			
BMI > 28	46 (17.56)	0.951	0.679-1.331	0.769			
CEA > 5 ng/mL	125 (47.71)	2.446	1.872-3.195	0.000	2.516	1.847-3.428	0.000
Colon primary	118 (45.04)	1.349	1.035-1.757	0.027	0.828	0.622-1.102	0.195
Mucinous adenocarcinoma	36 (13.74)	0.792	0.529-1.184	0.256			
Poor differentiation	37 (14.12)	1.102	0.758-1.603	0.611			
Primary tumor category ≥ T3	202 (77.10)	0.969	0.710-1.322	0.841			
Primary LN involvement	178 (67.94)	1.567	1.175-2.088	0.002	1.143	0.835-1.566	0.404
Multiple liver metastases	210 (80.15)	3.852	2.592-5.725	0.000	2.563	1.671-3.932	0.000
MDT	93 (35.50)	0.667	0.504-0.884	0.005	0.709	0.527-0.954	0.023
Chemotherapy	193 (73.66)	0.203	0.147-0.281	0.000	0.591	0.388-0.900	0.014
Curative resection	67 (25.57)	0.091	0.058-0.144	0.000	0.111	0.069-0.178	0.000

HR: Hazards ratio; CI: Confidence interval; BMI: Body mass index; CEA: Carcinoembryonic antigen; MDT: Multidisciplinary team; LN: Lymph node.

have also proved that preoperative tumor staging increased cancer-specific endpoints[21]. Therefore, the increased likelihood of comprehensive baseline examination in patients under the MDT model can significantly contribute to more effective cancer treatment planning. For patients with synchronous liver metastases, PET-CT examination was frequently selected as the diagnostic modality of choice[22]. Notably, a substantial 80% of patients in the MDT+ group received chemotherapy (P = 0.019). A study from Phelip *et al*[23] indicated that a multidisciplinary meeting was the only factor independently associated with administration of chemotherapy.

Within the MDT+ group, patients were categorized into two subgroups: Those initially deemed resectable and those considered potentially resectable. Despite ongoing controversies surrounding the use of neo-adjuvant therapy for patients



DOI: 10.4251/wjgo.v15.i9.1616

Figure 1 Overall survival comparison: Multidisciplinary team (+) group *versus* multidisciplinary team (-) group. A: Multidisciplinary team was a protective factor for 1-year overall survival rates; B: Multidisciplinary team was a protective factor for 5-year overall survival rates. MDT: Multidisciplinary team; HR: Hazard ratio.

with initially resectable synchronous liver metastases[24-28], several benefits of neo-adjuvant therapy can be identified. Firstly, neo-adjuvant chemotherapy provides a "window period" that allows for the observation of any new unresectable liver metastases, thereby preventing unnecessary operations[29]. Secondly, neo-adjuvant therapy can potentially increase the chances of R0 surgery and the volume of residual liver post-surgery[30,31]. Thirdly, combining neo-adjuvant chemotherapy with adjuvant chemotherapy may enhance the outcomes of patients undergoing curative surgery[32,33]. Given these benefits, we often advocate for neo-adjuvant therapy, especially for patients with large liver metastases and large number of liver metastases or suspicious LN metastases. However, the status of the primary tumor lesion, patient willingness, chemotherapy toxicity and risk of disease progression should still be considered[26].

Successful conversion is an important goal for potentially resectable patients, while the symptoms and tumor burden usually influence the treatment strategy for unresectable patients. Large clinical trials have reported that the rates of successful conversion of unresectable liver metastases were about 4%-15%[34,35]. We observed a similar proportion (17.11% in MDT+ group and 9.46% in MDT- group) in our study. Research showed that the resection margin width of liver metastases was independently associated with OS rates[36]. However, complete radiological response only contributed 15%-70% of complete pathological response, and even among patients with a complete pathological response, long-term remission occurred in only 20%-50% of those treated with systemic therapy[37]. For patients who convert to be curatively resectable, we advocate for immediate curative resection, given the hepatotoxicity and potential for decreased chemosensitivity associated with prolonged chemotherapy. As the macroscopic disease disappears on preoperative imaging, an excision extension according to the baseline imaging data is recommended.

Despite the significantly higher 5-year OS rates of resectable colorectal liver metastasis (37%-49%) in contrast to unresectable liver metastases(2%-4%)[5,38,39], only about 10% of patients in our study were diagnosed as initially resectable. Given these stark contrasts, the pursuit of resectability remains crucial. We typically discourage palliative excision of liver metastases, yet for patients who lose the opportunity for curative resection due to primary tumor complications, we do advocate for the R0 resection of liver metastases[40]. Over 90% of patients underwent simultaneous combined laparoscopic resection in MDT+ group. Simultaneous liver and colorectal resections for metastatic colorectal cancer are associated with similar long-term cancer outcomes compared with staged procedures[41,42]. Considering factors such as operation duration, blood loss, hospital stay, and morbidity[43,44], patients can benefit much more from simultaneous operations. While long-term outcomes like overall survival, progression-free survival, and local recurrence after excision radio frequency ablation (RFA) remain contentious[45,46], we usually prefer excision unless specialists in our MDT meeting agree that excision is a great risk or complete ablation of liver metastases with RFA is possible. In our MDT, intraoperative RFA was performed by doctors from the department of intraoperative ultrasound. And only 5 of 30 patients in MDT+ group received RFA.

In the last part of this study, after adjusting for variables like age, primary LN involvement, multiple liver metastases, extrahepatic metastases, and curative resection, we discovered that MDT meetings were a protective factor for 1-year OS (HR = 0.608, 95%CI: 0.398-0.931, P = 0.022, Table 4) and 5-year OS (HR = 0.694, 95%CI: 0.515-0.937, P = 0.017, Table 5). Patients may achieve this *via* the improvement of patients' treatment compliance, accurate radiological TNM staging, and an increased proportion of curative resection and systemic therapy in the MDT model.

CONCLUSION

The successful operation of a MDT necessitates fixed members, consistent meeting time, and location, an academic secretary with a medical background, and chat software enabling constant communication among team members. An

MDT can help mitigate incomplete decisions made by individual doctors. Nonetheless, further evidence is still needed to confirm these benefits and assess the clinical benefits in light of the time and financial costs.

ARTICLE HIGHLIGHTS

Research background

Multidisciplinary teams (MDTs) have been implemented in numerous large hospitals; however, critiques persist due to the high costs and limited strong evidence of their effectiveness.

Research motivation

The motivation behind this article is to provide further evidence on the application of MDTs in the field of colorectal liver metastasis. By conducting this research, we aim to contribute to the existing knowledge base and enhance the understanding of how MDTs can effectively improve patient outcomes in this specific context.

Research objectives

The objective of this study is to evaluate the effects of MDTs on patients with synchronous colorectal liver metastases and provide insights and recommendations on the management of synchronous colorectal liver metastases.

Research methods

This retrospective study investigated the influence of MDT involvement on clinical data of patients with synchronous colorectal liver metastases at the Chinese People's Liberation Army General Hospital.

Research results

The analysis revealed significant statistical increases in the rates of chest computed tomography examination (P = 0.001), abdomen magnetic resonance imaging examination (P = 0.000), and preoperative image staging (P = 0.0000) among patients in the MDT+ group. Furthermore, a higher proportion of patients in the MDT+ group received chemotherapy (P = 0.019) and underwent curative resection (P = 0.042). Multivariable analysis demonstrated that patients assessed through MDT meetings had higher 1-year overall survival [hazard ratio (HR) = 0.608, 95% confidence interval (CI): 0.398-0.931, P = 0.022] and 5-year overall survival (HR = 0.694, 95%CI: 0.515-0.937, P = 0.017).

Research conclusions

The findings of this study provide evidence that MDT management offers patients with synchronous colorectal liver metastases increased access to comprehensive examinations and treatments, ultimately leading to improved outcomes.

Research perspectives

This study conducted from the perspective of surgeons through a retrospective analysis of clinical records, observed that MDT management offers increased opportunities for comprehensive examinations and treatments in patients with synchronous colorectal liver metastases, consequently leading to improved treatment outcomes. This further validates the benefits of MDT management.

FOOTNOTES

Author contributions: Li H and Du XH were the guarantor of integrity of entire study, and contributed to the study concepts; Li H, Gu GL, Li SY, and Du XH designed the study; Li H, Gu GL, Li SY, and Hu SD involved in the literature research; Li H and Fu Z contributed to the data acquisition; Li H contributed to the statistical analysis/interpretation and manuscript preparation; Li H, Gu GL, Li SY, Hu SD, and Du XH contributed to the manuscript definition of intellectual content; Li H, Gu GL, and Du XH edited the manuscript.

Supported by National Natural Science Foundation of China, No. 81871317; and Military Medical Innovation Project, No. 18CXZ025.

Institutional review board statement: This study received approval from the ethics commission of the General Hospital of People's Liberation Army.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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

Data sharing statement: We are committed to promoting open data access. All research data generated as part of this study will be made available to the scientific community and interested parties, subject to legal, ethical, and privacy considerations.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Country/Territory of origin: China

ORCID number: Hao Li 0000-0002-5502-8786; Guo-Li Gu 0000-0002-9998-047X; Song-Yan Li 0000-0001-7094-5913; Yang Yan 0000-0001-8019-7314; Shi-Dong Hu 0000-0002-3820-2260; Ze Fu 0009-0002-0465-2009; Xiao-Hui Du 0000-0001-7083-2046.

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

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **McMillan DC**, McArdle CS. Epidemiology of colorectal liver metastases. *Surg Oncol* 2007; **16**: 3-5 [PMID: 17493802 DOI: 10.1016/j.suronc.2007.04.008]

3 **Manfredi S**, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. *Ann Surg* 2006; **244**: 254-259 [PMID: 16858188 DOI: 10.1097/01.sla.0000217629.94941.cf]

4 **Giannis D**, Sideris G, Kakos CD, Katsaros I, Ziogas IA. The role of liver transplantation for colorectal liver metastases: A systematic review and pooled analysis. *Transplant Rev (Orlando)* 2020; **34**: 100570 [PMID: 33002670 DOI: 10.1016/j.trre.2020.100570]

5 **Stewart CL**, Warner S, Ito K, Raoof M, Wu GX, Kessler J, Kim JY, Fong Y. Cytoreduction for colorectal metastases: liver, lung, peritoneum, lymph nodes, bone, brain. When does it palliate, prolong survival, and potentially cure? *Curr Probl Surg* 2018; **55**: 330-379 [PMID: 30526930 DOI: 10.1067/j.cpsurg.2018.08.004]

6 **Grass C**, Umansky R. Problems in promoting the growth of multi-disciplinary diagnostic and counseling clinics for mentally retarded children in nonmetropolitan areas. *Am J Public Health* 1971; **61**: 698-710 [PMID: 5139748 DOI: 10.2105/ajph.61.4.698]

7 **Kurpad R**, Kim W, Rathmell WK, Godley P, Whang Y, Fielding J, Smith L, Pettiford A, Schultz H, Nielsen M, Wallen EM, Pruthi RS. A multidisciplinary approach to the management of urologic malignancies: does it influence diagnostic and treatment decisions? *Urol Oncol* 2011; **29**: 378-382 [PMID: 19576797 DOI: 10.1016/j.urolonc.2009.04.008]

8 **Taylor C**, Munro AJ, Glynne-Jones R, Griffith C, Trevatt P, Richards M, Ramirez AJ. Multidisciplinary team working in cancer: what is the evidence? *BMJ* 2010; **340**: c951 [PMID: 20332315 DOI: 10.1136/bmj.c951]

9 **Thornton S**. Time to review utility of multidisciplinary team meetings. *BMJ* 2015; **351**: h5295 [PMID: 26446102 DOI: 10.1136/bmj.h5295]

10 **Chinai N**, Bintcliffe F, Armstrong EM, Teape J, Jones BM, Hosie KB. Does every patient need to be discussed at a multidisciplinary team meeting? *Clin Radiol* 2013; **68**: 780-784 [PMID: 23623261 DOI: 10.1016/j.crad.2013.02.011]

11 **Pillay B**, Wootten AC, Crowe H, Corcoran N, Tran B, Bowden P, Crowe J, Costello AJ. The impact of multidisciplinary team meetings on patient assessment, management and outcomes in oncology settings: A systematic review of the literature. *Cancer Treat Rev* 2016; **42**: 56-72 [PMID: 26643552 DOI: 10.1016/j.ctrv.2015.11.007]

12 **MacDermid E**, Hooton G, MacDonald M, McKay G, Grose D, Mohammed N, Porteous C. Improving patient survival with the colorectal cancer multi-disciplinary team. *Colorectal Dis* 2009; **11**: 291-295 [PMID: 18477019 DOI: 10.1111/j.1463-1318.2008.01580.x]

13 **Ye YJ**, Shen ZL, Sun XT, Wang ZF, Shen DH, Liu HJ, Zhang WL, Chen YL, Zhou J, Poston GJ, Wang S. Impact of multidisciplinary team working on the management of colorectal cancer. *Chin Med J (Engl)* 2012; **125**: 172-177 [PMID: 22340540]

14 **Gobbi PG**, Rossi S, Comelli M, Ravetta V, Rosa LL, Brugnatelli S, Corbella F, Delfanti S, Abumalouh I, Dionigi P. The Prognosis of Patients with Liver Metastases from Colorectal Cancer still Depends on Anatomical Presentation more than on Treatments. *Curr Cancer Drug Targets* 2015; **15**: 511-518 [PMID: 26282549 DOI: 10.2174/1568009615666150508094824]

15 **Schmidt T**, Strowitzki MJ, Reissfelder C, Rahbari NN, Nienhueser H, Bruckner T, Rahäuser C, Keppler U, Schneider M, Büchler MW, Ulrich A. Influence of age on resection of colorectal liver metastases. *J Surg Oncol* 2015; **111**: 729-739 [PMID: 25597497 DOI: 10.1002/jso.23872]

16 **Qiu M**, Hu J, Yang D, Cosgrove DP, Xu R. Pattern of distant metastases in colorectal cancer: a SEER based study. *Oncotarget* 2015; **6**: 38658-38666 [PMID: 26484417 DOI: 10.18632/oncotarget.6130]

17 **Lan YT**, Jiang JK, Chang SC, Yang SH, Lin CC, Lin HH, Wang HS, Chen WS, Lin TC, Lin JK. Improved outcomes of colorectal cancer patients with liver metastases in the era of the multidisciplinary teams. *Int J Colorectal Dis* 2016; **31**: 403-411 [PMID: 26662193 DOI: 10.1007/s00384-015-2459-4]

18 **Andres A**, Mentha G, Adam R, Gerstel E, Skipenko OG, Barroso E, Lopez-Ben S, Hubert C, Majno PE, Toso C. Surgical management of patients with colorectal cancer and simultaneous liver and lung metastases. *Br J Surg* 2015; **102**: 691-699 [PMID: 25789941 DOI: 10.1002/bjs.9783]

19 **Bipat S**, van Leeuwen MS, Ijzermans JN, Comans EF, Planting AS, Bossuyt PM, Greve JW, Stoker J. Evidence-base guideline on management of colorectal liver metastases in the Netherlands. *Neth J Med* 2007; **65**: 5-14 [PMID: 17293634]

20 **Zech CJ**, Korpraphong P, Huppertz A, Denecke T, Kim MJ, Tanomkiat W, Jonas E, Ba-Ssalamah A; VALUE study group. Randomized multicentre trial of gadoxetic acid-enhanced MRI versus conventional MRI or CT in the staging of colorectal cancer liver metastases. *Br J Surg* 2014; **101**: 613-621 [PMID: 24652690 DOI: 10.1002/bjs.9465]

21 **Palmer G**, Martling A, Cedermark B, Holm T. Preoperative tumour staging with multidisciplinary team assessment improves the outcome in locally advanced primary rectal cancer. *Colorectal Dis* 2011; **13**: 1361-1369 [PMID: 20958913 DOI: 10.1111/j.1463-1318.2010.02460.x]

22 **Moulton CA**, Gu CS, Law CH, Tandan VR, Hart R, Quan D, Fairfull Smith RJ, Jalink DW, Husien M, Serrano PE, Hendler AL, Haider MA, Ruo L, Gulenchyn KY, Finch T, Julian JA, Levine MN, Gallinger S. Effect of PET before liver resection on surgical management for colorectal adenocarcinoma metastases: a randomized clinical trial. *JAMA* 2014; **311**: 1863-1869 [PMID: 24825641 DOI: 10.1001/jama.2014.3740]

23 **Phelip JM**, Molinié F, Delafosse P, Launoy G, Trétarre B, Bara S, Buémi A, Velten M, Danzon A, Ganry O, Bouvier AM, Grosclaude P, Faivre J. A population-based study of adjuvant chemotherapy for stage-II and -III colon cancers. *Gastroenterol Clin Biol* 2010; **34**: 144-149 [PMID: 20079591 DOI: 10.1016/j.gcb.2009.08.012]

24 **Adam R**, de Gramont A, Figueras J, Kokudo N, Kunstlinger F, Loyer E, Poston G, Rougier P, Rubbia-Brandt L, Sobrero A, Teh C, Tejpar S, Van Cutsem E, Vauthey JN, Påhlman L; of the EGOSLIM (Expert Group on OncoSurgery management of LIver Metastases) group. Managing synchronous liver metastases from colorectal cancer: a multidisciplinary international consensus. *Cancer Treat Rev* 2015; **41**: 729-741 [PMID: 26417845 DOI: 10.1016/j.ctrv.2015.06.006]

25 **Gruenberger T**, Beets G, Van Laethem JL, Rougier P, Cervantes A, Douillard JY, Figueras J, Gruenberger B, Haller DG, Labianca R, Maleux G, Roth A, Ducreux M, Schmiegel W, Seufferlein T, Van Cutsem E. Treatment sequence of synchronously (liver) metastasized colon cancer. *Dig Liver Dis* 2016; **48**: 1119-1123 [PMID: 27375207 DOI: 10.1016/j.dld.2016.06.009]

26 **Zhu C**, Ren X, Liu D, Zhang C. Oxaliplatin-induced hepatic sinusoidal obstruction syndrome. *Toxicology* 2021; **460**: 152882 [PMID: 34352347 DOI: 10.1016/j.tox.2021.152882]

27 **Tepelenis K**, Pappas-Gogos G, Ntellas P, Tsimogiannis K, Dadouli K, Mauri D, Glantzounis GK. The Role of Preoperative Chemotherapy in the Management of Synchronous Resectable Colorectal Liver Metastases: A Meta-Analysis. *Curr Oncol* 2023; **30**: 4499-4511 [PMID: 37232798 DOI: 10.3390/curroncol30050340]

28 **Bonney GK**, Coldham C, Adam R, Kaiser G, Barroso E, Capussotti L, Laurent C, Verhoef C, Nuzzo G, Elias D, Lapointe R, Hubert C, Lopez-Ben S, Krawczyk M, Mirza DF; LiverMetSurvey International Registry Working Group. Role of neoadjuvant chemotherapy in resectable synchronous colorectal liver metastasis; An international multi-center data analysis using LiverMetSurvey. *J Surg Oncol* 2015; **111**: 716-724 [PMID: 25864987 DOI: 10.1002/jso.23899]

29 **Cleary JM**, Tanabe KT, Lauwers GY, Zhu AX. Hepatic toxicities associated with the use of preoperative systemic therapy in patients with metastatic colorectal adenocarcinoma to the liver. *Oncologist* 2009; **14**: 1095-1105 [PMID: 19880627 DOI: 10.1634/theoncologist.2009-0152]

30 **Tanaka K**, Adam R, Shimada H, Azoulay D, Lévi F, Bismuth H. Role of neoadjuvant chemotherapy in the treatment of multiple colorectal metastases to the liver. *Br J Surg* 2003; **90**: 963-969 [PMID: 12905549 DOI: 10.1002/bjs.4160]

31 **Leonard GD**, Brenner B, Kemeny NE. Neoadjuvant chemotherapy before liver resection for patients with unresectable liver metastases from colorectal carcinoma. *J Clin Oncol* 2005; **23**: 2038-2048 [PMID: 15774795 DOI: 10.1200/JCO.2005.00.349]

32 **Mentha G**, Majno P, Terraz S, Rubbia-Brandt L, Gervaz P, Andres A, Allal AS, Morel P, Roth AD. Treatment strategies for the management of advanced colorectal liver metastases detected synchronously with the primary tumour. *Eur J Surg Oncol* 2007; **33** Suppl 2: S76-S83 [PMID: 18006267 DOI: 10.1016/j.ejso.2007.09.016]

33 **Nordlinger B**, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T; EORTC Gastro-Intestinal Tract Cancer Group; Cancer Research UK; Arbeitsgruppe Lebermetastasen und-tumoren in der Chirurgischen Arbeitsgemeinschaft Onkologie (ALM-CAO); Australasian Gastro-Intestinal Trials Group (AGITG); Fédération Francophone de Cancérologie Digestive (FFCD). Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**: 1007-1016 [PMID: 18358928 DOI: 10.1016/S0140-6736(08)60455-9]

34 **Falcone A**, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, Crinò L, Benedetti G, Evangelista W, Fanchini L, Cortesi E, Picone V, Vitello S, Chiara S, Granetto C, Porcile G, Fioretto L, Orlandini C, Andreuccetti M, Masi G; Gruppo Oncologico Nord Ovest. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J Clin Oncol* 2007; **25**: 1670-1676 [PMID: 17470860 DOI: 10.1200/JCO.2006.09.0928]

35 **Souglakos J**, Androulakis N, Syrigos K, Polyzos A, Ziras N, Athanasiadis A, Kakolyris S, Tsousis S, Kouroussis Ch, Vamvakas L, Kalykaki A, Samonis G, Mavroudis D, Georgoulias V. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). *Br J Cancer* 2006; **94**: 798-805 [PMID: 16508637 DOI: 10.1038/sj.bjc.6603011]

36 **Sadot E**, Groot Koerkamp B, Leal JN, Shia J, Gonen M, Allen PJ, DeMatteo RP, Kingham TP, Kemeny N, Blumgart LH, Jarnagin WR, D'Angelica MI. Resection margin and survival in 2368 patients undergoing hepatic resection for metastatic colorectal cancer: surgical technique or biologic surrogate? *Ann Surg* 2015; **262**: 476-85; discussion 483 [PMID: 26258316 DOI: 10.1097/SLA.0000000000001427]

37 **Bischof DA**, Clary BM, Maithel SK, Pawlik TM. Surgical management of disappearing colorectal liver metastases. *Br J Surg* 2013; **100**: 1414-1420 [PMID: 24037559 DOI: 10.1002/bjs.9213]

38 **Valdimarsson VT**, Syk I, Lindell G, Sandström P, Isaksson B, Rizell M, Norén A, Ardnor B, Sturesson C. Outcomes of Simultaneous Resections and Classical Strategy for Synchronous Colorectal Liver Metastases in Sweden: A Nationwide Study with Special Reference to Major Liver Resections. *World J Surg* 2020; **44**: 2409-2417 [PMID: 32185455 DOI: 10.1007/s00268-020-05475-5]

39 **Lordan JT**, Karanjia ND, Quiney N, Fawcett WJ, Worthington TR. A 10-year study of outcome following hepatic resection for colorectal liver metastases - The effect of evaluation in a multidisciplinary team setting. *Eur J Surg Oncol* 2009; **35**: 302-306 [PMID: 18328668 DOI: 10.1016/j.ejso.2008.01.028]

40 **Hwang M**, Jayakrishnan TT, Green DE, George B, Thomas JP, Groeschl RT, Erickson B, Pappas SG, Gamblin TC, Turaga KK. Systematic review of outcomes of patients undergoing resection for colorectal liver metastases in the setting of extra hepatic disease. *Eur J Cancer* 2014; **50**: 1747-1757 [PMID: 24767470 DOI: 10.1016/j.ejca.2014.03.277]

41 **Lykoudis PM**, O'Reilly D, Nastos K, Fusai G. Systematic review of surgical management of synchronous colorectal liver metastases. *Br J Surg* 2014; **101**: 605-612 [PMID: 24652674 DOI: 10.1002/bjs.9449]

42 **Wang LJ**, Wang HW, Jin KM, Li J, Xing BC. Comparison of sequential, delayed and simultaneous resection strategies for synchronous colorectal liver metastases. *BMC Surg* 2020; **20**: 16 [PMID: 31952490 DOI: 10.1186/s12893-020-0681-7]

43 **Gavriilidis P**, Sutcliffe RP, Hodson J, Marudanayagam R, Isaac J, Azoulay D, Roberts KJ. Simultaneous versus delayed hepatectomy for synchronous colorectal liver metastases: a systematic review and meta-analysis. *HPB (Oxford)* 2018; **20**: 11-19 [PMID: 28888775 DOI: 10.1016/j.hpb.2017.08.008]

44 **Tian ZQ**, Su XF, Lin ZY, Wu MC, Wei LX, He J. Meta-analysis of laparoscopic versus open liver resection for colorectal liver metastases.

Oncotarget 2016; **7**: 84544-84555 [PMID: 27811369 DOI: 10.18632/oncotarget.13026]

45 **Lee WS**, Yun SH, Chun HK, Lee WY, Kim SJ, Choi SH, Heo JS, Joh JW, Choi D, Kim SH, Rhim H, Lim HK. Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. *J Clin Gastroenterol* 2008; **42**: 945-949 [PMID: 18438208 DOI: 10.1097/MCG.0b013e318064e752]

46 **Ko S**, Jo H, Yun S, Park E, Kim S, Seo HI. Comparative analysis of radiofrequency ablation and resection for resectable colorectal liver metastases. *World J Gastroenterol* 2014; **20**: 525-531 [PMID: 24574721 DOI: 10.3748/wjg.v20.i2.525]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1626-1635

DOI: 10.4251/wjgo.v15.i9.1626

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Hemoglobin, albumin, lymphocyte, and platelet score as a predictor of prognosis in metastatic gastric cancer

Yakup Duzkopru, Abdulkadir Kocanoglu, Ozlem Dogan, Hayriye Sahinli, Ebru Cilbir, Mustafa Altinbas

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Lin Q, China; MD UF, Italy; Shahid M, Pakistan

Received: April 26, 2023
Peer-review started: April 26, 2023
First decision: June 7, 2023
Revised: June 17, 2023
Accepted: July 29, 2023
Article in press: July 29, 2023
Published online: September 15, 2023

Yakup Duzkopru, Abdulkadir Kocanoglu, Ozlem Dogan, Hayriye Sahinli, Ebru Cilbir, Mustafa Altinbas, Department of Medical Oncology, Ankara Etlik City Hospital, Ankara 06170, Turkey

Corresponding author: Yakup Duzkopru, MD, Academic Fellow, Doctor, Department of Medical Oncology, Ankara Etlik City Hospital, Varlik Neighbourhood, Halil Sezai Erkut Street, Yenimahalle, Ankara 06170, Turkey. yakupduzkopru@gmail.com

Abstract

BACKGROUND

The hemoglobin, albumin, lymphocyte, and platelet (HALP) score, derived from a composite evaluation of markers reflecting the tumor-inflammation relationship and nutritional status, has been substantiated as a noteworthy prognostic determinant for diverse malignancies.

AIM

To investigate how the HALP score relates to prognosis in patients with metastatic gastric cancer.

METHODS

The cutoff values for the HALP score, neutrophil/lymphocyte ratio, and platelet/lymphocyte ratio were determined using receiver operating characteristic analysis. Low HALP scores were defined as those less than 24.79 and high HALP scores as those greater than 24.79.

RESULTS

The study cohort comprised 147 patients and 110 of them (74.8%) were male. The patients' median age was 63 (22-89) years. The median overall survival was significantly superior in the patients with high HALP scores than in those with low HALP scores (10.4 mo *vs* 7.5 mo, respectively; $P < 0.001$)

CONCLUSION

The HALP score was found to be a prognostic factor in patients with metastatic gastric cancer.

Key Words: Biomarker; Hemoglobin, albumin, lymphocyte, and platelet score; Gastric cancer; Nutritional index; Prognosis; Survival

Core Tip: Median overall survival (OS) was 10.4 mo in the high hemoglobin, albumin, lymphocyte, and platelet (HALP) group and 7.5 mo in the low HALP group. There was a statistically significant difference between the groups in terms of age ($P < 0.001$), second-line chemotherapy ($P < 0.001$), sex ($P = 0.035$), and HALP score ($P = 0.004$). The HALP score has been demonstrated to be useful as a prognostic factor in a variety of cancer types, including genitourinary and gastrointestinal malignancies. Our study is the first to investigate the HALP score in patients with metastatic gastric cancer. We found that patients with high HALP scores had longer OS. Given its simplicity and low cost, we think the HALP score can be utilized to manage patients with gastric cancer.

Citation: Duzkopru Y, Kocanoglu A, Dogan O, Sahinli H, Cilbir E, Altinbas M. Hemoglobin, albumin, lymphocyte, and platelet score as a predictor of prognosis in metastatic gastric cancer. *World J Gastrointest Oncol* 2023; 15(9): 1626-1635
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1626.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1626

INTRODUCTION

In Western countries, there has been a gradual decline in the prevalence of gastric cancer[1]. However, it remains a significant public health concern in certain regions of Eastern Asia[2]. Globally, gastric cancer ranks third in terms of cancer-related mortality and fifth in terms of overall prevalence[3]. Adenocarcinomas account for over 95% of all diagnosed cases of gastric cancer[4].

It is well known that gastric cancer has a poor prognosis. This is due to the disease usually being diagnosed at an advanced stage[5]. The most important factors in predicting the disease's prognosis are the stage of the tumor node metastasis (TNM), lymph node invasion, and the presence of distant metastases[6]. However, even among patients classified within the same stage, survival rates can significantly vary. Consequently, there is a pressing need for novel biomarkers to assist clinicians in accurately anticipating prognosis and making informed treatment decisions.

Numerous studies have demonstrated a significant association between systemic inflammation and the proliferation, invasion, and metastasis of the cancer[7]. At the same time, this inflammatory response around the tumor affects the formation and growth of the tumor[8]. Furthermore, blood cells trigger an adaptive immune response through the release of diverse cytokines, exerting an impact on tumor cells[9]. Based on the tumor inflammation relationship, biomarkers such as the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio, and prognostic nutritional index are utilized to predict disease prognosis[10]. Combining these parameters to generate scores is thought to enhance the predictive value of prognosis compared to using individual biomarkers alone. The integration of multiple biomarkers enables a more comprehensive assessment and potentially provides a more accurate prediction of disease outcomes. One of these combinations is the hemoglobin, albumin, lymphocyte, and platelet (HALP) score, calculated using the HALP counts. Together, the immune system and nutritional status are assessed by the HALP score. By evaluating both the immune system and nutritional status, the HALP score has shown utility as a prognostic factor in various cancer types, including genitourinary and gastrointestinal malignancies[11,12]. In the context of gastric cancer, some retrospective studies have demonstrated the predictive value of the HALP score in the preoperative stage, providing foresights into the disease prognosis prior to surgical intervention[13,14].

A scoring system incorporating clinical and laboratory data may be useful in determining the prognosis of gastric cancer. In the present study, we aimed to investigate the prognostic effect of the HALP score in patients with metastatic gastric cancer.

MATERIALS AND METHODS

Collection of material and follow-up of patients

A total of 158 patients were initially screened and, among them, 147 patients who met the inclusion criteria were included in the study. The data of patients diagnosed with metastatic gastric adenocarcinoma and followed up in the Medical Oncology Clinic of Diskapi Yildirim Beyazit Training and Research Hospital, Health Sciences University (Ankara, Turkey), between January 2010 and May 2021 were analyzed retrospectively. The inclusion criteria encompassed patients aged 18 years and older. However, those with heart failure, undergoing dialysis, having secondary malignancy, or suffering from any inflammatory disease were excluded.

Data for the study were obtained by collecting information from hospital records and patient files. Various variables were recorded and analyzed, including details regarding chemotherapy regimens, comorbidities, smoking and alcohol consumption histories, surgical procedures, pathological diagnoses, types of lymph node dissection, tumor sizes, metastasis sites, and patient survival durations. Overall survival (OS) was calculated as the time from the date of metastasis to the date of death or the last follow-up date. Also the HALP score was calculated using laboratory values at

the time of metastasis. It was calculated by multiplying the hemoglobin albumin and lymphocyte/platelet ratio [hemoglobin (g/L) × albumin (g/L) × lymphocyte count/thrombocyte count][15].

Approval for the study was granted by the Diskapi Yıldırım Beyazıt Training and Research Hospital ethics committee (number: 116/21, date: 26.07.2021). The protocol of the study was prepared in accordance with the 1964 Declaration of Helsinki.

Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics (version 22.0, IBM SPSS, United States). The clinical and demographic characteristics of the patients were subjected to descriptive analysis. Categorical and numerical variables were presented as numbers and percentages (*n*, %). Continuous data were expressed as means ± SD when the data were normally distributed; otherwise, they were expressed as median and range. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff values of NLR, PLR, and HALP score. Survival outcomes were compared using the Kaplan–Meier method with the log-rank test (univariate analysis) or the Cox proportional hazards regression model (multivariate analysis). Only the parameters that demonstrated statistical significance in the univariate analysis were included in the multivariate analysis. A P value < 0.05 was considered statistically significant in all analyses. The statistical methods used were reviewed by Yakup Duzkopru from Ankara Etlik City Hospital.

RESULTS

A total of 147 patients diagnosed with metastatic gastric cancer were included. The majority of the patients (74.8%) were male. The median age of the patients was 63 (22-89). Among the participants, 74 patients (50.3%) had no additional diseases, while 78 patients (53.1%) had a history of smoking. A total of 90 patients (61.2%) had never undergone any surgical procedures.

Surgical interventions were performed in a subset of the patients. Specifically, total gastrectomy was conducted in 41 patients (27.9%), while subtotal gastrectomy was performed in 16 (10.9%). The histopathological examination revealed adenocarcinoma in 128 patients (87.1%). Among these patients, 74 (50.3%) were classified as having moderately differentiated adenocarcinoma. For the majority of the patients (38.1%), the primary tumor was in the corpus. The number of de novo metastatic patients was 103 (70.1%). Table 1 provides an overview of the clinicopathological characteristics of the patients in the study.

Using ROC analysis, a cutoff value of 24.79 for the HALP score was determined, with 62.5% sensitivity and 62.3% specificity (AUC: 0.64, 95%CI: 0.48-0.80, P = 0.183). A HALP score of ≥ 24.79 was considered high and of < 24.79 low. The patients were divided into two groups: Those with low HALP scores (60.5%) and those with high HALP scores (39.5%).

The association between the HALP score and various characteristics of the patients was assessed, and the results indicate that there was no statistically significant relationship between the HALP score and sex (P = 0.816), smoking (P = 0.679), record of previous surgery (P = 0.804), type of operation performed (P = 0.783), pathological subtype (P = 0.18), presence of metastasis at the time of diagnosis (P = 0.894), and the tumor location (P = 0.142). However, statistically significant relationships were observed between the HALP score and other factors, specifically between the HALP score and the Eastern Cooperative Oncology Group Performance status (ECOG PS) (P = 0.02), the presence of additional diseases (P = 0.008), and the degree of differentiation (P = 0.045). The relevant patient characteristics related to the HALP score are summarized in Table 2.

The optimal cutoff values for NLR and PLR were determined by ROC analysis. NLR ≥ 2.88 was considered high (38.8% of patients) and < 2.88 low (61.2% of patients). PLR ≥ 166.1 was categorized as high (39.5% of patients) and < 166.1 low (60.5% of patients). The sensitivity and specificity for both NLR and PLR were 62.6% and 62.5%, respectively.

The median OS was 10.4 mo in the high HALP group and 7.5 mo in the low HALP group. The subgroups were further compared in terms of OS. In the univariate analysis, no statistically significant difference was observed in terms of NLR groups (P = 0.582), PLR groups (P = 0.350), differentiation groups (P = 0.06), and the presence of metastasis at the time of diagnosis (P = 0.754). However, statistically significant differences were found between the groups in terms of age (P < 0.001), second-line chemotherapy (P < 0.001), sex (P = 0.035), ECOG PS (P = 0.03), comorbidity (P = 0.004), and HALP score (P = 0.004) (Figure 1).

The multivariate analysis revealed that second-line chemotherapy (P < 0.001) and HALP score (P < 0.001) were statistically significant factors affecting OS. The HALP score was also statistically significant in the multivariate analysis (P = 0.001). The univariate and multivariate analyses of prognostic factors in terms of OS are presented in Table 3.

DISCUSSION

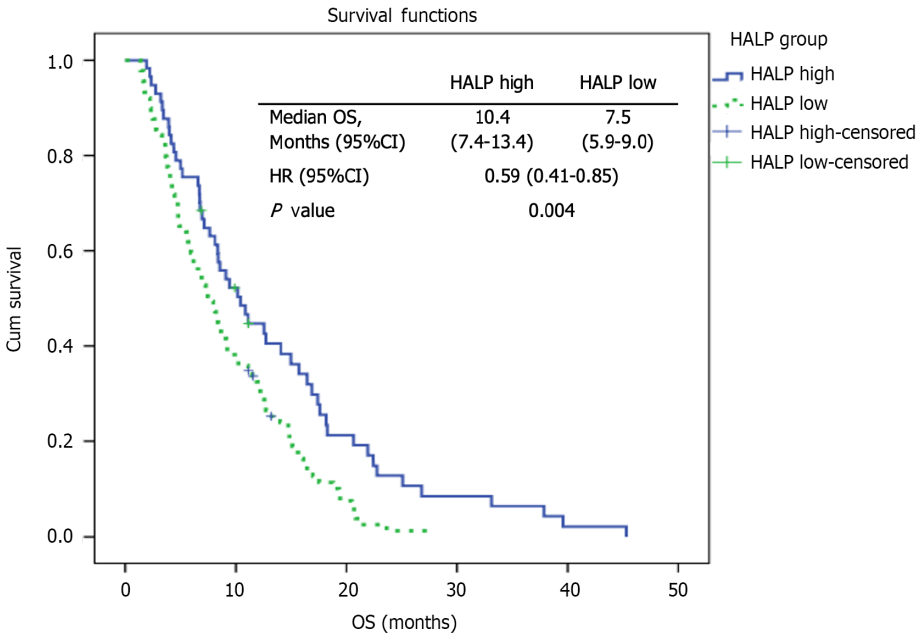
The HALP score, derived from the levels of hemoglobin, albumin, lymphocytes, and platelets, serves as an indicator of the patient's immunological and nutritional status. Anemia, commonly observed in cancer patients, particularly in gastric cancer, is recognized as a prevalent paraneoplastic syndrome. Chronic bleeding associated with gastric cancer often contributes to the development of anemia[16]. Anemia is thought to affect the performance status, chemotherapy tolerance, and course of the disease in patients with gastric cancer[17]. Additionally, hypoalbuminemia has been identified as an independent prognostic factor linked to poor outcomes in several studies[18]. It is known that immune system suppression raises the probability of cancer development[19]. Therefore, the HALP score, which encompasses

Table 1 Clinicopathological characteristics of 147 metastatic gastric cancer patients

Features	Frequency, *n* (%)
Gender	
Female	37 (25.2)
Male	110 (74.8)
ECOG PS	
0	32 (21.8)
1	83 (56.5)
2	32 (21.8)
Comorbidity	
No	74 (50.3)
Yes	73 (49.7)
Smoking	
No	69 (46.9)
Yes	78 (53.1)
Surgery	
No	90 (61.2)
Yes	57 (38.8)
Type of surgery	
No	90 (61.2)
Total gastrectomy	41 (27.9)
Subtotal gstrectomy	16 (10.9)
Patology	
Adenocarcinom	128 (87.1)
Signet ring cell carcinom	15 (10.2)
Musinoz adenocarcinom	2 (1.4)
Mix carcinom	2 (1.4)
Diferantiation	
Well	5 (3.4)
Moderate	74 (50.3)
Poorly	53 (36.1)
Signet ring cell carcinoma	15 (10.2)
Surgical margin	
No operation	90 (61.2)
Positive	6 (4.1)
Negative	51 (34.7)
Tumor location	
Fundus, cardia	48 (32.7)
Korpus	56 (38.1)
Antrum, pylor	43 (29.3)
De novo metastasis	
No	44 (29.9)
Yes	103 (70.1)

Age group	
≤ 63	74 (50.3)
> 63	73 (49.7)

ECOG PS: Eastern Cooperative Oncology Group Performance status.



DOI: 10.4251/wjgo.v15.i9.1626

Figure 1 Kaplan-meier plot according to hemoglobin, albumin, lymphocyte, and platelet score. HALP: Hemoglobin, albumin, lymphocyte and platelet; HR: Hazard ratio; CI: Confidence interval; OS: Overall survival.

both immunological and nutritional components, is promising as a valuable marker for predicting prognosis in patients with gastric cancer. Previous studies have demonstrated the predictive value of the HALP score in terms of lymph node involvement and the likelihood of recurrence in gastric cancer patients during the preoperative period[13,14]. In the present study, our objective was to explore the relationship between the HALP score and OS in patients diagnosed with metastatic gastric cancer.

In a study conducted by Chen *et al*[14], involving a cohort of 888 patients diagnosed with gastric cancer, a HALP score cutoff value of 56.8 was adopted. That study demonstrated that patients with high HALP scores had significantly longer OS times compared to those with low HALP scores. The authors also identified tumor size and T stage as independent factors associated with the HALP score. Subgroup analysis based on TNM stages revealed that there was no significant difference in survival between stage 4 patients with high HALP scores and those with low HALP scores. It is important to note that their study included a relatively small number of metastatic patients, with only 5 (1.9%) in the high HALP score group and 36 (6.1%) in the low HALP score group[14]. The lack of a difference in survival observed in the metastatic gastric cancer patients with high and low HALP scores in Chen's study could potentially be attributed to the small number of stage 4 patients and the imbalanced distribution of patients in the study cohort.

In a study conducted by Wang *et al*[13], the prognostic significance of the HALP score in the preoperative period was investigated in patients diagnosed with gastric cancer. A cutoff value of 35.3 was determined for the HALP score. Their study revealed that the HALP score, calculated prior to surgery, served as an effective marker for predicting lymph node status in gastric cancer patients. The authors emphasized that the HALP score could be utilized to personalize the surgical approach, providing valuable information for treatment planning and decision-making[13].

Sargın and Dusunceli[19] conducted a retrospective evaluation of 204 patients diagnosed with gastric cancer. Through the use of ROC analysis, they determined a cutoff of 23.8 for the HALP score. Their study revealed a significant difference in OS between patients with high HALP scores and those with low HALP scores (P = 0.05). Among the patient cohort, 136 individuals (66.7%) received adjuvant chemotherapy, while palliative chemotherapy was administered to 68 (33.3%). However, there was no statistically significant difference in OS between patients with high and low HALP scores in metastatic patients receiving palliative therapy. It is worth noting that the limited number of patients with metastatic disease in their study might have contributed to this lack of statistical significance[19].

In our study, which included 147 patients with metastatic gastric cancer, the patients with high HALP scores exhibited a significantly longer OS compared to those with low HALP scores. The median OS was 10.4 mo in the high HALP score group and 7.5 mo in the low HALP score group. These findings suggest that higher HALP scores are associated with

Table 2 Distribution of patients according to hemoglobin, albumin, lymphocyte, and platelet score in subgroups

Features	HALP low, *n* (%)	HALP high, *n* (%)	*P* value
Gender			0.816
Female	23 (25.8)	14 (24.2)	
Male	66 (74.2)	44 (75.9)	
ECOG PS			0.02
0	16 (18)	16 (27.6)	
1	47 (52.8)	36 (62.1)	
2	26 (29.2)	6 (10.3)	
Comorbidity			0.008
No	37 (41.6)	37 (63.8)	
Yes	52 (58.4)	21 (36.2)	
Smoking			0.679
No	43 (48.3)	26 (44.8)	
Yes	46 (51.7)	32 (55.2)	
Surgery			0.804
No	56 (62.9)	34 (58.6)	
Yes	33 (37.1)	24 (41.4)	
Type of surgery			0.783
No	55 (61.8)	34 (58.6)	
Total gastrectomy	23 (25.8)	18 (31)	
Subtotal gastrectomy	10 (11.2)	6 (10.3)	
Patology			0.18
Adenocarcinom	79 (88.8)	49 (84.5)	
Signet ring cell carcinom	8 (9)	7 (12.1)	
Musinoz adenocarcinom	2 (2.2)	0 (0)	
Mix carcinom	0 (0)	2 (3.4)	
Diferantiation			0.045
Well	1 (1.1)	4 (6.9)	
Moderate	42 (47.2)	32 (55.2)	
Poorly	38 (42.7)	14 (24.1)	
Signet ring cell carcinoma	8 (9)	8 (13.8)	
Tumor location			0.142
Fundus, cardia	25 (28.1)	23 (39.7)	
Corpus	33 (37.1)	23 (39.7)	
Antrum, pylor	31 (34.8)	12 (20.7)	
De novo metastasis			0.894
No	27 (30.3)	17 (29.3)	
Yes	62 (69.7)	41 (70.7)	

ECOG PS: Eastern Cooperative Oncology Group Performance status; HALP: Hemoglobin, albumin, lymphocyte and platelet.

Table 3 Analysis of prognostic factors in terms of overall survival

Features	Median (months)	Univariate analysis		Multivariate analysis	
		HR (95%CI)	*P* value	HR (95%CI)	*P* value
HALP groups					
HALP low	7.5	0.59 (0.41-0.85)	0.004	Reference	0.001
HALP high	10.4			0.53 (0.36-0.78)	
NLR groups					
NLR low	9.1	1.10 (0.78-1.56)	0.582		
NLR high	8.0				
PLR groups					
PLR low	8.6	1.18 (0.83-1.68)	0.350		
PLR high	8.4				
Age groups					
≤ 63	10.2	1.96 (1.37-2.8)	0.000	Reference	0.060
> 63	6.9			1.47 (0.98-2.19)	
ECOG PS					
0-1	9.1	1.57 (1.04-2.36)	0.030	Reference	0.850
2	6.9			1.04 (0.66-1.64)	
Comorbidity					
No	10.2	1.67 (1.18-2.36)	0.004	Reference	0.303
Yes	6.2			1.22 (0.84-1.78)	
Diferantiation					
Well-moderate	8.6	1.10 (0.78-1.53)	0.600		
Poorly-Signet ring cell	8.4				
Second line CT					
No	4.9	0.24 (0.16-0.35)	0.000	Reference	< 0.001
Yes	15.4			0.23 (0.16-0.34)	
De novo metastasis					
No	9.2	0.94 (0.65-1.37)	0.754		
Yes	8.2				
Gender					
Female	9.1	1.53 (1.03-2.28)	0.035	Reference	0.051
Male	8.2			1.52 (0.99-2.31)	
Tumor location					
Fundus, cardia	7.5		0.056		
Corpus	8.8			-	-
Antrum, pylor	9.2				

CI: Confidence interval; CT: Chemotherapy; HALP: Hemoglobin, albumin, lymphocyte and platelet; HR: Hazard ratio; NLR: Neutrophil/lenfocyte ratio; PLR: Platelet/lenfocyte ratio.

improved OS in patients with metastatic gastric cancer.

When reviewing previous studies on NLR and PLR, it is generally accepted that higher levels of NLR and PLR are associated with worse survival outcomes. However, the literature reveals conflicting results[7,20,21]. In the study conducted by Magdy *et al*[10], a borderline significant association was observed between NLR levels and OS, while no significant association was found with progression-free survival[10]. In the current study, there was no significant difference in OS between patients with high and low NLR, or between patients with high and low PLR. These findings support the hypothesis that the HALP score, which is obtained by combining nutritional and inflammatory markers, provides a better prognosis prediction for metastatic gastric cancer compared to other known inflammation-related markers.

Previous studies have consistently demonstrated that the HALP score, calculated based on preoperative values, serves as a valuable marker for predicting lymph node involvement, prognosis, and OS[13,14,19]. However, it did not reach statistical significance in the metastatic subgroups, which generally constitute a small portion of the patients in the studies. In the present study, our results indicate that the HALP score, determined using values obtained during the metastatic process, holds significant utility as a biomarker for predicting OS. By focusing specifically on patients with metastatic gastric cancer, we were able to evaluate the direct impact of the HALP score in this specific subgroup. Our findings highlight the prognostic value of the HALP score in the context of metastatic gastric cancer, further supporting its potential as a clinically useful biomarker in this setting.

The present study has some limitations. Firstly, the study was retrospective and conducted in a single-center setting, which inherently carries the risk of bias and compromises the generalizability of the findings. Secondly, the cutoff values from the ROC analysis did not exhibit the desired level of sensitivity and specificity, thus potentially affecting the accuracy of the results. Additionally, the exclusion of patients with missing records from the analyses reveals the possibility of bias. Hence, it is crucial to consider these limitations when interpreting the outcomes of our study, recognizing the need for further research with robust designs and larger, more diverse patient cohorts to enhance the validity and generalizability of the results.

CONCLUSION

HALP score is a biomarker that can be easily calculated by routine tests and is known to predict prognosis in many tumors. This is the first study to demonstrate the prognostic value of the HALP score in patients with metastatic gastric cancer. This score is a potential biomarker to utilize in the management of patients with metastatic gastric cancer. However, multicenter and prospective studies with more patients are required.

ARTICLE HIGHLIGHTS

Research background

The hemoglobin, albumin, lymphocyte, and platelet (HALP) score, derived from a composite evaluation of markers reflecting the tumor-inflammation relationship and nutritional status, has been substantiated as a noteworthy prognostic determinant for diverse malignancies. A scoring system incorporating clinical and laboratory data may hold utility in determining the prognosis of gastric cancer.

Research motivation

The need for healthcare professionals to utilize supportive tools in predicting prognosis and making treatment decisions in metastatic gastric cancer.

Research objectives

To investigate how the HALP score relates to prognosis in patients with metastatic gastric cancer.

Research methods

This retrospective study cohort comprised 147 patients with metastatic gastric cancer. The cutoff values for the HALP score, neutrophil/lymphocyte ratio, and platelet/lymphocyte ratio were determined using receiver operating characteristic analysis. Low HALP scores were defined as those less than 24.79 and high HALP scores as those greater than 24.79.

Research results

The median overall survival was significantly superior in patients with high HALP score than those with low HALP score (10.4 mo *vs* 7.5 mo, respectively; $P < 0.001$).

Research conclusions

The HALP score was found to be a prognostic factor in patients with metastatic gastric cancer.

Research perspectives

Given its simplicity and low cost, we think the HALP score can be utilized to manage patients with gastric cancer.

FOOTNOTES

Author contributions: Duzkopru Y and Kocanoglu A performed the study concept, study design, and statistical analysis; Dogan O and Cilbir E contributed to the data acquisition, data analysis and interpretation; Altinbas M and Sahinli H performed the manuscript editing; All authors contributed to the article and approved the submitted version.

Institutional review board statement: The ethics committee approval of the study was given by the Diskapi Yıldırım Beyazıt Training and Research Hospital ethics committee (date: 26.07.2021), No. 116/21.

Informed consent statement: The Institutional Review Board waived the need for informed consent due to the study's retrospective methodology.

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

Data sharing statement: The raw data supporting the conclusions of this article will be made available without restriction by the authors.

Country/Territory of origin: Turkey

ORCID number: Yakup Duzkopru 0000-0003-2314-5870; Abdulkadir Kocanoglu 0000-0002-5579-2135; Ozlem Dogan 0000-0003-1023-8410; Hayriye Sahinli 0000-0002-1561-9346; Ebru Cilbir 0000-0001-8967-2505; Mustafa Altinbas 0000-0002-8024-2362.

S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Zhang XD

REFERENCES

1 **National Cancer Institute**. Surveillance, Epidemiology, and End Results (SEER) Program. Cancer Stat Facts: Stomach Cancer. Surveillance, Epidemiology, and End Results (SEER) Program Cancer Stat Facts: Stomach Cancer, 2021

2 **GBD 2017 Stomach Cancer Collaborators**. The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 42-54 [PMID: 31648970 DOI: 10.1016/S2468-1253(19)30328-0]

3 **World Health Organization**. International Agency for Research on Cancer. GLOBOCAN 2020: Stomach cancer fact sheet, 2020

4 **Ma J**, Shen H, Kapesa L, Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. *Oncol Lett* 2016; **11**: 2959-2964 [PMID: 27123046 DOI: 10.3892/ol.2016.4337]

5 **Dicken BJ**, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: review and considerations for future directions. *Ann Surg* 2005; **241**: 27-39 [PMID: 15621988 DOI: 10.1097/01.sla.0000149300.28588.23]

6 **Sano T**, Coit DG, Kim HH, Roviello F, Kassab P, Wittekind C, Yamamoto Y, Ohashi Y. Proposal of a new stage grouping of gastric cancer for TNM classification: International Gastric Cancer Association staging project. *Gastric Cancer* 2017; **20**: 217-225 [PMID: 26897166 DOI: 10.1007/s10120-016-0601-9]

7 **Zhang X**, Zhao W, Yu Y, Qi X, Song L, Zhang C, Li G, Yang L. Clinicopathological and prognostic significance of platelet-lymphocyte ratio (PLR) in gastric cancer: an updated meta-analysis. *World J Surg Oncol* 2020; **18**: 191 [PMID: 32731872 DOI: 10.1186/s12957-020-01952-2]

8 **Colotta F**, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 2009; **30**: 1073-1081 [PMID: 19468060 DOI: 10.1093/carcin/bgp127]

9 **Varga G**, Foell D. Anti-inflammatory monocytes-interplay of innate and adaptive immunity. *Mol Cell Pediatr* 2018; **5**: 5 [PMID: 29616417 DOI: 10.1186/s40348-018-0083-4]

10 **Magdy M**, Hussein T, Ezzat A, Gaballah A. Pre-treatment Peripheral Neutrophil-Lymphocyte Ratio as a Prognostic Marker in Gastric Cancer. *J Gastrointest Cancer* 2019; **50**: 763-768 [PMID: 30058031 DOI: 10.1007/s12029-018-0144-x]

11 **Jiang H**, Li H, Li A, Tang E, Xu D, Chen Y, Zhang Y, Tang M, Zhang Z, Deng X, Lin M. Preoperative combined hemoglobin, albumin, lymphocyte and platelet levels predict survival in patients with locally advanced colorectal cancer. *Oncotarget* 2016; **7**: 72076-72083 [PMID: 27765916 DOI: 10.18632/oncotarget.12271]

12 **Peng D**, Zhang CJ, Gong YQ, Hao H, Guan B, Li XS, Zhou LQ. Prognostic significance of HALP (hemoglobin, albumin, lymphocyte and platelet) in patients with bladder cancer after radical cystectomy. *Sci Rep* 2018; **8**: 794 [PMID: 29335609 DOI: 10.1038/s41598-018-19146-y]

13 **Wang X**, He Q, Liang H, Liu J, Xu X, Jiang K, Zhang J. A novel robust nomogram based on preoperative hemoglobin and albumin levels and lymphocyte and platelet counts (HALP) for predicting lymph node metastasis of gastric cancer. *J Gastrointest Oncol* 2021; **12**: 2706-2718 [PMID: 35070400 DOI: 10.21037/jgo-21-507]

14 **Chen XL**, Xue L, Wang W, Chen HN, Zhang WH, Liu K, Chen XZ, Yang K, Zhang B, Chen ZX, Chen JP, Zhou ZG, Hu JK. Prognostic significance of the combination of preoperative hemoglobin, albumin, lymphocyte and platelet in patients with gastric carcinoma: a retrospective cohort study. *Oncotarget* 2015; **6**: 41370-41382 [PMID: 26497995 DOI: 10.18632/oncotarget.5629]

15 **Hasselmann M**, Alix E. Tools and procedures for screening for malnutrition and its associated in risks in hospital. *Nutrition Clinique et Metabolisme* 2003; **17**: 218-226 [DOI: 10.1016/j.nupar.2003.09.004]

16 **Knight K**, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *Am J Med* 2004; **116** Suppl 7A: 11S-26S [PMID: 15050883 DOI: 10.1016/j.amjmed.2003.12.008]

17 **Tomita M**, Shimizu T, Hara M, Ayabe T, Onitsuka T. Impact of preoperative hemoglobin level on survival of non-small cell lung cancer patients. *Anticancer Res* 2008; **28**: 1947-1950 [PMID: 18630486]

18 **Crumley AB**, Stuart RC, McKernan M, McMillan DC. Is hypoalbuminemia an independent prognostic factor in patients with gastric cancer? *World J Surg* 2010; **34**: 2393-2398 [PMID: 20602101 DOI: 10.1007/s00268-010-0641-y]

19 **Sargin ZG**, Dusunceli I. The Effect of HALP Score on the Prognosis of Gastric Adenocarcinoma. *J Coll Physicians Surg Pak* 2022; **32**: 1154-1159 [PMID: 36089712 DOI: 10.29271/jcpsp.2022.09.1154]

20 **Wang W**, Tong Y, Sun S, Tan Y, Shan Z, Sun F, Jiang C, Zhu Y, Zhang J. Predictive value of NLR and PLR in response to preoperative chemotherapy and prognosis in locally advanced gastric cancer. *Front Oncol* 2022; **12**: 936206 [PMID: 36110962 DOI: 10.3389/fonc.2022.936206]

21 **Gunaldi M**, Goksu S, Erdem D, Gunduz S, Okuturlar Y, Tiken E, Kahraman S, Inan YO, Genc TB, Yildirim M. Prognostic impact of platelet/lymphocyte and neutrophil/lymphocyte ratios in patients with gastric cancer: a multicenter study. *Int J Clin Exp Med* 2015; **8**: 5937-5942 [PMID: 26131188]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1636-1643

DOI: 10.4251/wjgo.v15.i9.1636

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Efficacy of multi-slice spiral computed tomography in evaluating gastric cancer recurrence after endoscopic submucosal dissection

Jian-Jun Yin, Xiao Hu, Sen Hu, Guo-Hong Sheng

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Banach M, Poland; Trapani D, Italy

Received: July 4, 2023
Peer-review started: July 4, 2023
First decision: July 19, 2023
Revised: July 25, 2023
Accepted: August 8, 2023
Article in press: August 8, 2023
Published online: September 15, 2023

Jian-Jun Yin, Sen Hu, Guo-Hong Sheng, Department of Radiology, Huangshi Maternity and Children's health Hospital, Affiliated Maternity and Children's Health Hospital of Hubei Polytechnic University, Huangshi 435000, Hubei Province, China

Xiao Hu, Department of Geriatrics, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Huangshi 435000, Hubei Province, China

Corresponding author: Xiao Hu, RN, Associate Chief Nurse, Department of Geriatrics, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, No. 19 Floor, Building 6, Huangshi 435000, Hubei Province, China. 11786356@qq.com

Abstract

BACKGROUND

Recurrence is the major challenge facing endoscopic submucosal dissection (ESD)-based treatment therapies for early gastric cancer (EGC). Urgent development of simple and easy surveillance approaches will enhance clinical treatment of the disease.

AIM

To explore the role of computed tomography (CT) recurrence in evaluating EGC after ESD treatment.

METHODS

We retrospectively recruited patients from our endoscopy department, between January 2002 and December 2015, and analyzed their basic characteristics, including symptoms, CT results, and results of endoscopy with biopsy, among others.

RESULTS

Among a total of 2150 patients EGC patients surveyed, 1362 met our inclusion and exclusion criteria and were therefore enrolled in our study. The cohort's sensitivity of CT for recurrent GC and specificity were 44.22% and 43.86%, respectively, with negative and positive predictive values of 40.15% (275/685) and 48.01% (325/677), respectively. The area under the curve of arterial and venous CT values for recurrent EGC were 0.545, and 0.604, respectively. Receiver operating characteristic curve revealed no statistically significant differences between arterial and venous CT values for recurrent EGC.

CONCLUSION
Enhanced CT has superior diagnostic efficacy, but less accuracy, compared to gold standard techniques in patients with recurrent EGC.

Key Words: Computed tomography; Early gastric cancer; Gastric cancer; Multi-slice spiral computed tomography

Core Tip: Development of a simple and easy approach to detect recurrence of early gastric cancer (EGC) treated with endoscopic mucosal exfoliation is imperative to effective clinical therapy. Here, we report the feasibility of multi-slice spiral computed tomography (CT), a quick and convenient auxiliary examination with sensitivity and specificity values of 44.22% and 43.86%, respectively, in evaluating arterial and venous CT values for recurrent EGC. Area under the curve value of arterial and venous CT values for recurrent EGC respectively were 0.55 and 0.60, indicating that enhanced CT can accurately predict EGC, although with low accuracy.

Citation: Yin JJ, Hu X, Hu S, Sheng GH. Efficacy of multi-slice spiral computed tomography in evaluating gastric cancer recurrence after endoscopic submucosal dissection. *World J Gastrointest Oncol* 2023; 15(9): 1636-1643
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1636.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1636

INTRODUCTION

Gastric cancer (GC), a disease ranked 5th and 4th with regards to incidence and mortality rates, respectively worldwide as of 2020, is associated with a heavy economic burden[1]. In some Asian countries, such as Korea, China, and Japan, GC is the most prevalent type of cancer and the leading cause of cancer-related deaths[2]. Early detection and treatment is considered the most effective way for reducing GC-related mortalities. Early GC (EGC) is a key stage of GC, characterized by invasion of cancer cells no deeper than the submucosa, regardless of lymph node metastasis. Studies have shown that the 5-year survival rate of EGC operative treatment is approximately 90%[3].

Various operative therapies, such as subtotal gastrectomy, endoscopic submucosal dissection (ESD), and endoscopic mucosal resection (EMR), have been developed for treatment of GC. Among them, ESD, which was developed by Japan in the late 1990s, is the current standard technique in most East Asian countries[4]. ESD, which is based on EMR, is a minimally invasive procedure that has been used for treatment of early gastrointestinal swelling tumor for long. Although this technique is minimally invasive, it has a lower recurrence rate, lower risk, and faster recovery compared to traditional surgical procedures[5,6]. Tanabe *et al*[7] conducted a long-term multicenter collaborative study, and found that ESD was efficacious against EGC. However, ESD application is significantly limited by recurrence, with studies reporting local recurrence rates was between 2.8% and 38.5% based on studies with a median follow-up of 15-36 mo[8,9]. Therefore, urgent development of simple and easy approaches to detect recurrence of EGC is imperative to effective treatment of the disease. Several "gold standard" diagnostic techniques for GC have been developed and applied, including endoscopy with biopsy, computed tomography (CT), endoscopic ultrasonography and sometimes diagnostic laparoscopy[10]. Based on patient acceptance, CT has shown excellent promise, whereas enhanced spiral CT scan has a high resolution thus can provide a basis for the identification of GC lesions. To date, however, the role of enhanced spiral CT scan in recurrence assessment of GC patients after ESD remains unclear.

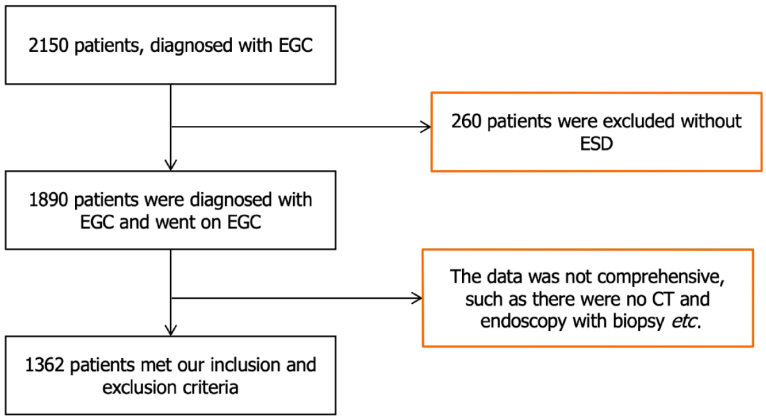
In the present study, we explored this role with the aim of generating insights to guide future development of diagnostic and treatment approaches.

MATERIALS AND METHODS

This retrospective study was approved by the department of Radiology, Huangshi Maternity and Children's health Hospital, Affiliated Maternity. This study was approved by the Ethics committee of our hospital. And all patients signed an informed consent form prior to inclusion in the study.

Patient recruitment and selection criteria

We searched our endoscopy department database for patients who were diagnosed with EGS and received ESD treatment between January 2000 and December 2015. The inclusion criteria were as follows: (1) Patients with EGC who received ESD treatment; (2) The patient has been returned regularly for more than 5 years; (3) No other cancer disease occurred after ESD treatment; (4) Signed informed consent to participate in our study; and (5) CT and endoscopy with biopsy was chosen as the tool to detect EGC recurrence. Patients who met the following criteria were excluded from the study: (1) EGS was diagnosed as other type of cancer; and (2) Had missing data.



DOI: 10.4251/wjgo.v15.i9.1636

Figure 1 A flow chart showing the screening process. CT: Computed tomography; EGC: Early gastric cancer; ESD: Endoscopic submucosal dissection.

Study design

This was a retrospective cohort study. We searched for eligible patients in the database at our endoscopy department from January 2002 to December 2015. In cases where CT or the patients' symptoms indicated there was any chance of EGC recurrence, then endoscopy with biopsy were checked for definitive diagnosis. We collected each patient's basic characteristics, including their symptoms, CT results, and results of endoscopy with biopsy, among others.

CT imaging and analysis

All subjects were asked to fast for 8 h, then given water to fill their stomachs and bowel. The patients were placed in supine position and scanned using the Siemens 64-slice spiral CT system under the following parameters: Tube current 250 mA, voltage 120 kV, layer thickness 5-10 mm, layer distance 0.5 mm, and pitch 1. Briefly, a plain scan was first performed from the top of diaphragm to the iliac crest. Next, 300 mg/mL of the contrast agent iodophenyl (plant Home: Shanghai Yuanye Biotechnology Co., LTD), injection rate was 3.5 mL/s, Gastrointestinal arterial phase (delay time 20-25 s), portal venous phase (delay time 40-45 s), delay period (delay time 120-180 s) to implement enhanced scanning CT values of the arterial and portal venous phases were described and measured.

Histopathological examination

We conducted gastroscopic biopsies to procure 1362 specimens from patients diagnosed with recurrent EGC. These specimens were then meticulously processed by fixing them in formaldehyde, embedding them in paraffin, and finally, sectioning and staining them with hematoxylin and eosin. Visible dilated lymphatic vessels were distributed in the lamina propria of the intestinal mucosa, submucosa, muscular layer, and the serosal layer. The stained sections were evaluated by two physicians with wide experience in pathological diagnosis.

Statistical analysis

Data were statistically analyzed using packages implemented in R 4.1.0 software, unless otherwise indicated. Descriptive statistics were used to report patients, patients' symptoms, CT results, and results of endoscopy with biopsy. Continuous variables were presented as means and SD. Diagnostic efficacy was based on receiver operating characteristic curve (ROC), and the roc.test function in pROC package used to compare CT values between the arterial stage and portal stage groups during EGC.

RESULTS

Patient characteristics

We initially recruited a total of 2150 patients, who were diagnosed with EGC at our department, of which 1890 underwent ESD. A total of 1362 patients met our inclusion criteria and therefore included in the final analysis (Figure 1). Patient characteristics and recurrent EGC parameters are presented in Table 1. In summary, 49.71% (677/1362) of the patients exhibited EGC recurrence, with the condition found to be highly occur in the lower place of stomach. The most TNM stage of recurrent EGC was T1b.

Diagnostic value

CT sensitivity and specificity for recurrent GC were 44.22% (325/735), and 43.86% (275/627), respectively, with negative and positive predictive values of 40.15% (275/685), and 48.01% (325/677), respectively, which was seen in Table 2. The Youden's index was -0.12.

Table 1 Characteristics of patients with recurrent gastric cancer

Variables	No. (%)
Relapsed patients	735 (53.96)
Non-relapsed patients	627 (46.04)
Sex	
Male	695 (51.03)
Female	667 (48.98)
Age (year), mean ± SD	64.7 ± 7.7
Location	
Upper	66 (9.75)
Middle	13 (1.92)
Lower	598 (88.33)
Size (cm), mean ± SD	2.8 ± 0.5
Tumor depth	
T1a	65 (9.60)
T1b	612 (90.40)
Lymphovascular invasion	
Absent	636 (93.94)
Present	41 (6.06)

Table 2 Diagnostic value of electromagnetic navigation bronchoscopy combined with computed tomography in recurrent early gastric cancer

CT	Tissue pathology (Gold standard)		Total
	Positive	Negative	
Positive	325	352	677
Negative	410	275	685
Total	735	627	1362

CT: Computed tomography.

CT enhancement characteristics for recurrent EGC

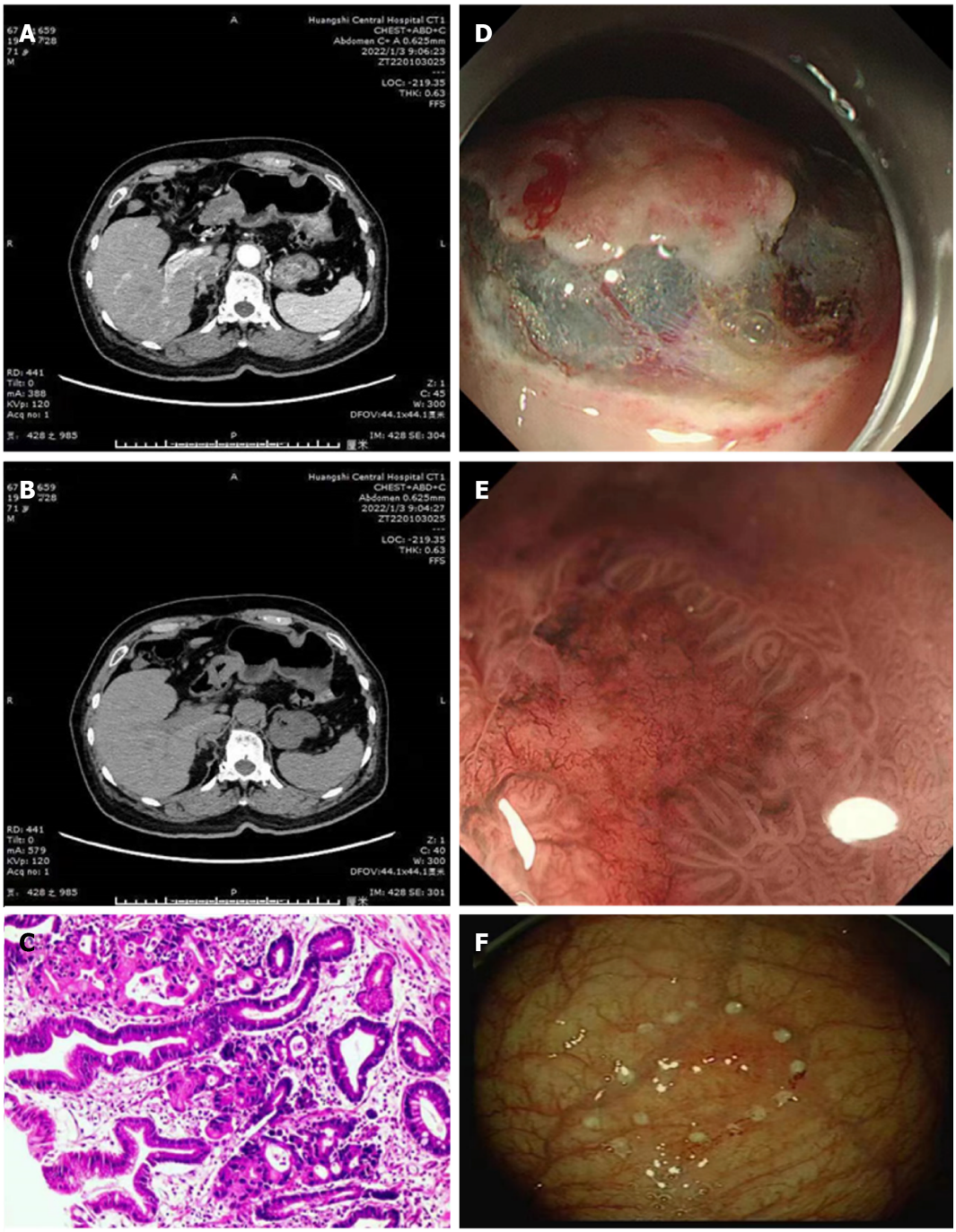
CT, gastroscopic, and histopathological examination results for patients with recurrent EGC are presented in Figure 2. Notably, patients without recurrent EGC had a mean arterial CT and venous CT values of 60.77, and 42.67, respectively. Patients with recurrent EGC had a mean arterial CT and venous CT values of 69.52, and 62.21, respectively. Predictive efficacy of arterial and venous CT values for EGC are summarized in Figure 2. A total of 473 (69.87%) and 204 (30.13%) cases exhibited obvious enhancement in the arterial and portal vein phase of the lesions, respectively. The enhancement ranged between 40-70 hu. The enhanced lesions had a slightly rough surface, which could also be accompanied by mild nodular or indentation changes. The gastric wall was also slightly stiff.

ROC curve reveal recurrent EGC

The predictive efficacy of arterial and venous CT values for patients with recurrent EGC is presented in Figure 3. The area under the curve (AUC) values for arterial and venous CT values for recurrent EGC were 0.545, and 0.604, respectively. Resulting ROC curves revealed no statistically significant differences between arterial and venous CT values for recurrent EGC (P = 0.001).

DISCUSSION

The symptoms of EGC are nonspecific, easy to be confused with other benign lesions, and often have entered the late

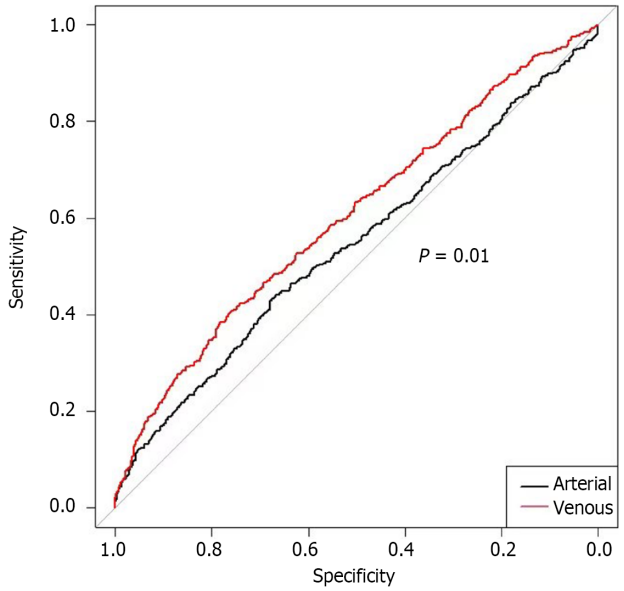


DOI: 10.4251/wjgo.v15.i9.1636 **Copyright** ©The Author(s) 2023.

Figure 2 Computed tomography, gastroscope, and histopathological examination results characteristics for recurrent early gastric cancer. A and B: Computed tomography; C: Histopathological examination; D-F: Gastroscope.

stage when diagnosed, a phenomenon that leads to poor clinical treatment effect. Therefore, early screening and diagnosis of GC is imperative to prolonging the life of patients. Studies have shown that EGC patients who were treated with ESD are at risk of recurrence, thus should be subjected to early screening[11-13]. Although gastroscopy biopsy is the gold standard technique for GC diagnosis, it has poor acceptability among patients due to various shortcomings, key among them complicated examination and painful procedures, as well as high costs[14].

Enhanced spiral CT scan generates high resolution images, thus can provide a basis for identification of GC lesions. Studies have shown that application of phase III enhanced multi-slice spiral CT and window technique can increase its diagnostic efficacy in patients with EGC[15-17]. In this study, we found that Multi-layer helical CT enhanced imaging with narrow window + raised window has reduced image layers, with less display content, but revealed clear details, which offers critical advantages in the discovery and detection of subtle lesions. In addition, the lesions exhibited morphology and enhancement characteristics that were in sharp contrast with those of the adjacent normal gastric wall. In cases where the lesion exhibited a single-layer structure, we observed a noteworthy enhancement in the non-permeability of the gastric wall. This enhancement was characterized by either focal thickening of the gastric wall or a



DOI: 10.4251/wjgo.v15.i9.1636

Figure 3 Receiver operating characteristic curve for screening for early gastric cancer.

significant increase in enhancement without accompanying thickening of the gastric wall. When the lesion showed a multi-layer structure, the gastric wall was thickened and significantly enhanced without sudden disappearance of the middle and outer layers. At the lesion site, mucosal enhancement was obvious in arterial and portal vein stages, and basically subsided at the equilibrium stage. Some studies have reported the value of enhanced CT in the diagnosis of gastrointestinal neoplasms[18-21]. The standard window setting is suitable for general diagnostic purposes. However, a narrow window width offers advantages such as reduced layering and display content, which results in clearer details, increased image contrast, and improved resolution of both lesions and surrounding tissues. Additionally, using a narrow window width can enhance the darkening of images displayed on the window screen, further aiding in the diagnostic process. Moreover, enhanced lesion tissues appear on a good background due to the high CT value, whereas obvious superior substandard can be shown for local subtle enhanced lesions in the stomach wall.

In this study, most positive patients exhibited enhancement in both arterial and venous phases. However, there were still cases of missed diagnosis (55.78%). The technique used herein had sensitivity and specificity rates of 44.22% and 43.86%, respectively, which are far from satisfactory. However, the AUC of arterial and venous CT values for recurrent EGC was greater than 0.5, indicating that enhanced CT can predict EGC, albeit with low accuracy.

This study had some shortcomings. Firstly, this was a retrospective study. Secondly, some patients' data records were not detailed, which necessitated their elimination from the study, thus affecting the sample size.

CONCLUSION

Enhanced CT has superior diagnostic efficacy but lower accuracy in patients with recurrent EGC, compared to gold-standard techniques. Application value of CT in recurrent GC needs more extensive research.

ARTICLE HIGHLIGHTS

Research background

There is an urgent need to develop a simple and easy approach for screening for early gastric cancer (EGC) recurrence in patients treated with endoscopic submucosal dissection (ESD).

Research motivation

Multi-slice spiral computed tomography (CT) is a quick, convenient and promising auxiliary examination. Enhanced spiral CT scan generates high resolution images, thus can provide a basis for the identification of gastric cancer lesions.

Research objectives

To explore the role of CT recurrence assessment in EGC patients who were treated with ESD.

Research methods

This retrospective study recruited patients from the endoscopy department between January 2002 and December 2015. Basic characteristics, symptoms, CT results, and endoscopy with biopsy findings were analyzed. Sensitivity, specificity, negative and positive predictive values of CT for recurrent gastric cancer were calculated. Arterial and venous CT values were evaluated using area under the curve (AUC) analysis, and receiver operating characteristic curve analysis compared their performance for detecting recurrent EGC. The diagnostic efficacy and accuracy of enhanced CT were assessed in comparison to gold standard techniques for detecting recurrent EGC.

Research results

The approach had sensitivity and specificity rates of 44.22% and 43.86%, respectively, which are far from satisfactory. AUC value of arterial and venous CT values for recurrent EGC was greater than 0.5, indicating that enhanced CT can predict EGC, albeit at low accuracy.

Research conclusions

Enhanced CT has superior diagnostic efficacy but lower accuracy than gold standard techniques in patients with recurrent EGC.

Research perspectives

Multi-slice spiral CT is valuable in EGC screening.

FOOTNOTES

Author contributions: Yin JJ and Hu X contributed equally to this work; Hu S designed the study; Sheng GH contributed to the analysis of the manuscript; Yin JJ and Hu X were involved in the data and writing of this article; and all authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Huangshi Maternity and Children's health Hospital, Affiliated Maternity and Children's health Hospital of Hubei Polytechnic University, Huangshi, China, Institutional Review Board.

Informed consent statement: All study participants and their legal guardians provide informed written consent before the study recruitment.

Conflict-of-interest statement: All authors have no conflicts of interest.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Jian-Jun Yin 0009-0004-9211-2444; Xiao Hu 0009-0004-6062-7048.

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

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Xia C**, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N, Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. *Chin Med J (Engl)* 2022; **135**: 584-590 [PMID: 35143424 DOI: 10.1097/CM9.0000000000002108]

3 **Yang K**, Lu L, Liu H, Wang X, Gao Y, Yang L, Li Y, Su M, Jin M, Khan S. A comprehensive update on early gastric cancer: defining terms, etiology, and alarming risk factors. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 255-273 [PMID: 33121300 DOI: 10.1080/17474124.2021.1845140]

4 **Landin MD**, Guerrón AD. Endoscopic Mucosal Resection and Endoscopic Submucosal Dissection. *Surg Clin North Am* 2020; **100**: 1069-1078 [PMID: 33128880 DOI: 10.1016/j.suc.2020.07.004]

5 **Gong SD**, Li H, Xie YB, Wang XH. Construction and analysis of an ulcer risk prediction model after endoscopic submucosal dissection for early gastric cancer. *World J Gastrointest Oncol* 2022; **14**: 1823-1832 [PMID: 36187385 DOI: 10.4251/wjgo.v14.i9.1823]

6 **Ning B**, Abdelfatah MM, Othman MO. Endoscopic submucosal dissection and endoscopic mucosal resection for early stage esophageal cancer. *Ann Cardiothorac Surg* 2017; **6**: 88-98 [PMID: 28446997 DOI: 10.21037/acs.2017.03.15]

7 **Tanabe S**, Ishido K, Matsumoto T, Kosaka T, Oda I, Suzuki H, Fujisaki J, Ono H, Kawata N, Oyama T, Takahashi A, Doyama H, Kobayashi M, Uedo N, Hamada K, Toyonaga T, Kawara F, Tanaka S, Yoshifuku Y. Long-term outcomes of endoscopic submucosal dissection for early gastric cancer: a multicenter collaborative study. *Gastric Cancer* 2017; **20**: 45-52 [PMID: 27807641 DOI: 10.1007/s10120-016-0664-7]

8 **Domper Arnal MJ**, Ferrández Arenas Á, Lanas Arbeloa Á. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. *World J Gastroenterol* 2015; **21**: 7933-7943 [PMID: 26185366 DOI: 10.3748/wjg.v21.i26.7933]

9 **Ono H**, Yao K, Fujishiro M, Oda I, Uedo N, Nimura S, Yahagi N, Iishi H, Oka M, Ajioka Y, Fujimoto K. Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer (second edition). *Dig Endosc* 2021; **33**: 4-20 [PMID: 33107115 DOI: 10.1111/den.13883]

10 **Lim JS**, Kim MJ, Yun MJ, Oh YT, Kim JH, Hwang HS, Park MS, Cha SW, Lee JD, Noh SH, Yoo HS, Kim KW. Comparison of CT and 18F-FDG pet for detecting peritoneal metastasis on the preoperative evaluation for gastric carcinoma. *Korean J Radiol* 2006; **7**: 249-256 [PMID: 17143028 DOI: 10.3348/kjr.2006.7.4.249]

11 **Douda L**, Cyrany J, Tachecí I. Early gastric cancer. *Vnitr Lek* 2022; **68**: 371-375 [PMID: 36316198 DOI: 10.36290/vnl.2022.077]

12 **Gillen S**. Advancing early gastric cancer detection. *FEBS Open Bio* 2021; **11**: 1812-1813 [PMID: 34081843 DOI: 10.1002/2211-5463.13217]

13 **Shimizu S**, Tada M, Kawai K. Early gastric cancer: its surveillance and natural course. *Endoscopy* 1995; **27**: 27-31 [PMID: 7601031 DOI: 10.1055/s-2007-1005628]

14 **Karimi P**, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 700-713 [PMID: 24618998 DOI: 10.1158/1055-9965.EPI-13-1057]

15 **Wani AH**, Parry AH, Feroz I, Choh NA. Preoperative Staging of Gastric Cancer Using Computed Tomography and Its Correlation with Histopathology with Emphasis on Multi-planar Reformations and Virtual Gastroscopy. *J Gastrointest Cancer* 2021; **52**: 606-615 [PMID: 32535756 DOI: 10.1007/s12029-020-00436-6]

16 **Wang J**, Zhong L, Zhou X, Chen D, Li R. Value of multiphase contrast-enhanced CT with three-dimensional reconstruction in detecting depth of infiltration, lymph node metastasis, and extramural vascular invasion of gastric cancer. *J Gastrointest Oncol* 2021; **12**: 1351-1362 [PMID: 34532093 DOI: 10.21037/jgo-21-276]

17 **Li CF**, Zheng J, Xue YW. The value of contrast-enhanced computed tomography in predicting gastric cancer recurrence and metastasis. *Cancer Biomark* 2017; **19**: 327-333 [PMID: 28482620 DOI: 10.3233/CBM-160528]

18 **Kim EY**, Lee WJ, Choi D, Lee SJ, Choi JY, Kim BT, Kim HS. The value of PET/CT for preoperative staging of advanced gastric cancer: comparison with contrast-enhanced CT. *Eur J Radiol* 2011; **79**: 183-188 [PMID: 20226612 DOI: 10.1016/j.ejrad.2010.02.005]

19 **Cheng J**, Wu J, Ye Y, Zhang C, Zhang Y, Wang Y. The prognostic significance of extramural venous invasion detected by multiple-row detector computed tomography in stage III gastric cancer. *Abdom Radiol (NY)* 2016; **41**: 1219-1226 [PMID: 27315092 DOI: 10.1007/s00261-015-0627-1]

20 **Lee IJ**, Lee JM, Kim SH, Chang S, Han JK, Choi BI, Lee HJ, Yang HK, Lee KU. Helical CT evaluation of the preoperative staging of gastric cancer in the remnant stomach. *AJR Am J Roentgenol* 2009; **192**: 902-908 [PMID: 19304693 DOI: 10.2214/AJR.07.3520]

21 **Wang D**, Zhang GB, Yan L, Wei XE, Zhang YZ, Li WB. CT and enhanced CT in diagnosis of gastrointestinal neuroendocrine carcinomas. *Abdom Imaging* 2012; **37**: 738-745 [PMID: 22310902 DOI: 10.1007/s00261-011-9836-4]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1644-1652

DOI: 10.4251/wjgo.v15.i9.1644

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Factors associated with heterochronic gastric cancer development post-endoscopic mucosal dissection in early gastric cancer patients

Bing Xie, Yun Xia, Xia Wang, Yan Xiong, Shao-Bo Chen, Jie Zhang, Wei-Wei He

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Fujiyoshi Y, Japan; Thiem S, Australia

Received: July 12, 2023
Peer-review started: July 12, 2023
First decision: July 31, 2023
Revised: August 9, 2023
Accepted: August 21, 2023
Article in press: August 21, 2023
Published online: September 15, 2023

Bing Xie, Yun Xia, Xia Wang, Jie Zhang, Department of Spleen and Stomach, Nanjing Pu Kou District Hospital of Traditional Chinese Medicine, Pukou 210000, Jiangsu Province, China

Yan Xiong, Science and Education Section, Nanjing Pu Kou District Hospital of Traditional Chinese Medicine, Pukou 210000, Jiangsu Province, China

Shao-Bo Chen, Anesthesiology Department, Nanjing Pu Kou District Hospital of Traditional Chinese Medicine, Pukou 210000, Jiangsu Province, China

Wei-Wei He, Department of Oncology, Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing 210022, Jiangsu Province, China

Corresponding author: Wei-Wei He, PhD, Associate Chief Physician, Department of Oncology, Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, No. 157 Daming Road, Qinhuai District, Nanjing 210022, Jiangsu Province, China. heweiwei1983@hotmail.com

Abstract

BACKGROUND

Endoscopic mucosal resection is an innovative method for treating early gastric cancer and has been widely used in clinical practice.

AIM

To analyze the factors associated with the development of heterochronic gastric cancer in patients with early gastric cancer who had undergone endoscopic mucosal dissection (EMD).

METHODS

A cohort of patients with early gastric cancer treated using EMD was retrospectively analyzed, and patients who developed heterochronic gastric cancer after the surgery were compared with those who did not. The effects of patient age, sex, tumor size, pathological type, and surgical technique on the development of heterochronic gastric cancer were assessed using statistical analysis.

RESULTS

Of the 300 patients with early gastric cancer, 150 patients developed heterochronic gastric cancer after EMD. Statistical analysis revealed that patient age (*P* value =

XX), sex (*P* value = XX), tumor size (*P* value = XX), pathological type (*P* value = XX), and surgical technique (*P* value = XX) were significantly associated with the occurrence of heterochronic gastric cancer.

CONCLUSION

Age, sex, tumor size, pathological type, and surgical technique are key factors influencing the occurrence of heterochronic gastric cancer after EMD in patients with early gastric cancer. To address these factors, postoperative follow-up and management should be strengthened to improve the prognosis and survival rate of patients.

Key Words: Early gastric cancer; Endoscopic mucosal dissection; Heterochronic gastric cancer; Associated factors; Statistical analysis

Core Tip: Factors affecting heterochronic gastric cancer after endoscopic mucosal dissection for early gastric cancer include age, gender, tumor size, pathological type, and surgical technique. Postoperative follow-up and management should be strengthened to improve the patient's prognosis and survival rate.

Citation: Xie B, Xia Y, Wang X, Xiong Y, Chen SB, Zhang J, He WW. Factors associated with heterochronic gastric cancer development post-endoscopic mucosal dissection in early gastric cancer patients. *World J Gastrointest Oncol* 2023; 15(9): 1644-1652
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1644.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1644

INTRODUCTION

Endoscopic mucosal resection (EMR) is an innovative method for treating early gastric cancer and has been widely applied in clinical practice. EMR allows the local excision of early gastric cancer *via* endoscopic techniques while maximizing the preservation of the normal gastric wall. Thus, the treatment goal is achieved, and at the same time, the trauma and adverse effects are minimized[1-15].

Different technical approaches for endoscopic mucosal dissection (EMD), including the typical EMR and the large endoscopic submucosal dissection (ESD), have been described. Their indications, operational difficulties, and risks of complications have been compared and analyzed. Furthermore, the advantages of ESD in treating early gastric cancer have been discussed. Compared with conventional surgical resection, EMD has the advantages of less trauma, faster recovery, and shorter hospital stays. Several clinical studies and retrospective analyses have evaluated the treatment outcomes and survival rates of EMD. In addition, complications and risk management of EMD have been examined. Although EMD is a relatively safe technique, complications such as bleeding, perforation, and infection can occur. Relevant preventive strategies and treatments to reduce the occurrence of complications have been presented[16-20]. The future direction of EMD has also been explored. With advances in technology and equipment, the application of EMD in treating early gastric cancer is expected to become more promising. Directions for further research, including postoperative follow-up and prognostic evaluation, application of new instruments and techniques, and exploration of individualized treatment strategies, have also been proposed.

Globally, gastric cancer is the fifth most common malignancy and has the third highest mortality rate[21-30]. With the improvements in diagnostic techniques and the popularization of endoscopic screening, the diagnosis rate of early gastric cancer has gradually increased. Early gastric cancer is defined as gastric cancer confined to the mucosa or submucosa, with or without regional lymph node metastasis. Several guidelines recommend endoscopic resection as the first-line treatment for early gastric cancer[31,32]. Unlike the surgical approach, endoscopic resection preserves a large portion of the gastric mucosa and is associated with an increased risk of metachronous gastric cancer (MGC) in the remaining gastric mucosa[33]. However, an increasing number of patients with early gastric cancer are treated *via* endoscopic resection. Identifying the risk factors for the development of MGC is therefore important to devise an appropriate surveillance strategy.

Endoscopic resection is extensively employed for treating superficial gastrointestinal tumors and has become the treatment of choice for patients with early gastric cancer without the risk of lymph node metastasis. EMR, ESD, and endoscopic submucosal tunnel dissection are the major endoscopic resection methods for early gastric cancer. The absolute indications for endoscopic dissection of early gastric cancer include the following: (1) Differentiated intramucosal carcinoma (cT1a) without ulcers; (2) differentiated intramucosal carcinoma (cT1a) with ulcers ≤ 3 cm in size; and (3) high-grade gastric intraepithelial neoplasia. Expanded indications include undifferentiated intramucosal carcinoma (cT1a) with a lesion size of ≤ 2 cm and no ulceration. The morphology, extent, nature, and depth of infiltration of the lesion must be accurately diagnosed preoperatively so that appropriate therapy can be selected according to the indication.

EMR can be grouped into two main categories: (1) Nonattractive methods: Submucosal injection-loop resection, submucosal injection-presection-excision, *etc*.; and (2) attractive methods: Transparent cap method and ligature method. EMR is suitable for the resection of lesions ≤ 2 cm in diameter with no surface ulceration and can also be used to obtain large histological specimens of superficial malignancies and provide accurate pathological staging[34,35]. Although endoscopic piecemeal mucosal resection can be performed on larger lesions, it may not be possible to obtain the entire lesion for accurate pathological assessment and the risk of local recurrence may be exacerbated.

Heterochronous gastric cancer refers to the progressive development of inflammatory mucosa outside the primary lesion in the direction of "atrophy-enterosis-heterogeneous hyperplasia". This process is more prolonged than concurrent gastric cancer and takes at least V1 years. There are few studies on concurrent or heterochronic gastric cancer. Therefore, this study investigated the risk factors affecting the development of concurrent and heterochronic gastric cancer after ESD and serves as a reference for the clinical management of this condition.

MATERIALS AND METHODS

Case selection and general information

A total of 300 patients diagnosed with early gastric cancer and treated using ESD at our gastrointestinal endoscopy center from 2016 to 2023 were selected for this study. The inclusion criteria were as follows: (1) Preoperative evaluation meeting the indications for ESD surgery (differentiated intramucosal carcinoma without combined ulceration, differentiated intramucosal carcinoma of < 3 cm with ulceration, or high-grade intraepithelial neoplasia of gastric mucosa); (2) postoperative pathology suggestive of curative or relatively curative resection of differentiated intramucosal carcinoma of < 3 cm with combined ulceration or differentiated carcinoma of < 3 cm with a submucosal infiltration depth of < 500 μm; (3) repeat gastroscopy at 3, 6, 12, 18, 24, 30, 36, 42, and 48 mo after ESD, with complete results; and (4) a follow-up period of 18 mo, and availability of complete clinical records. The exclusion criteria were as follows: (1) Additional surgery, radiotherapy, or chemotherapy after the surgery; and (2) patients lost to follow-up. Clinical data, such as age, smoking history, family history, sex, degree of postoperative pathological differentiation, depth of tumor infiltration, first multifocal lesion, tumor size, initial lesion location, and degree of background mucosal atrophy and intestinalization, were retrospectively collected from patients who met the various inclusion criteria. Pathological staging was performed according to the Vienna classification criteria for epithelial tumors of the gastrointestinal tract[36], and histological staging and depth of infiltration were determined as per the criteria of the Japanese Gastric Cancer Society.

Follow-up visits

Gastroscopy was repeated at 3, 6, 12, 18, 24, 30, 36, 42, and 48 mo postoperatively, and the findings were documented. A lesion detected at ≤ 12 mo and 1 cm from the original lesion was considered concurrent gastric cancer, whereas a new lesion detected at > 12 mo was considered heterochronic gastric cancer. The occurrence of concurrent or heterochronous gastric cancer during follow-up was collectively referred to as multiple gastric cancers, whereas the absence of concurrent and heterochronous gastric cancer signified single gastric cancer.

Statistical analysis

SPSS 26.0 was used for the statistical analysis of the data. Quantitative data that conformed to a normal distribution were expressed as mean ± SD, and a *t*-test was used for the comparison of means between groups. Statistical data were expressed as percentages, and the χ^2 test was used for comparison between groups. The influential factors associated with tumor recurrence in the univariate analysis were substituted in the multifactor dichotomous logistic regression model for the analysis of independent risk factors. The test level was α = 0.05 (two-tailed).

RESULTS

Clinical characteristics of the patients

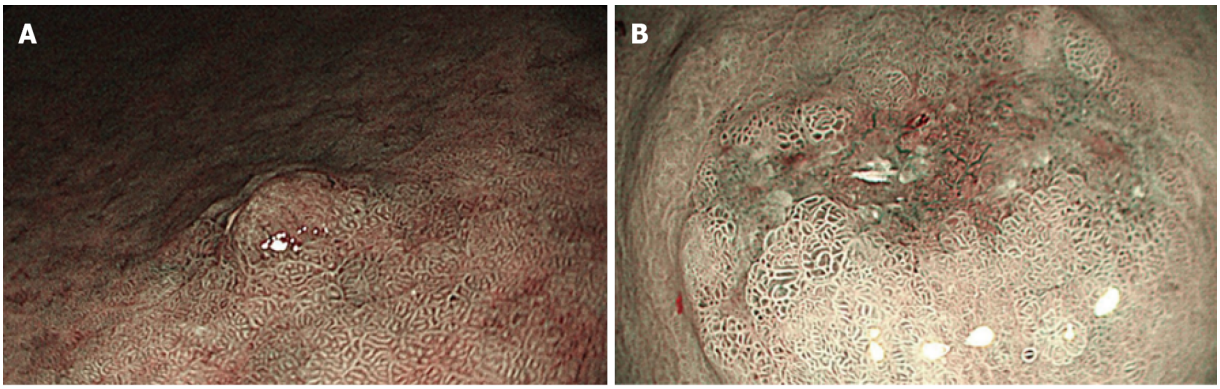
Of the 300 patients included in this study, 170 (56.7%) were men, 66 (22.0%) had a history of heavy smoking (BIW400), 15 (5.0%) had a family history of gastric cancer, and 10 (3.33%) were initially diagnosed with multiple early carcinoma lesions. The median age of the patients was 63 years, and the mean diameter of the initial lesions was 1.92 cm ± 0.89 cm. Furthermore, 58% (76/331) of the initial lesions were located in the lower third of the stomach, and 43.5% of the patients demonstrated severe intestinalization. In addition, of the 300 patients (331 lesions in total) with early gastric cancer, 265 had single (304 lesions), 74 had heterochronous (86 lesions) and 51 had concurrent (51 lesions) gastric cancer (Figure 1).

Analysis of risk factors for multiple gastric cancers after ESD surgery

The results of the single factor analysis of multiple gastric cancers indicated that age W65 years, being a male, heavy smoking, initial lesion in the lower third of the stomach, O-shaped atrophy of the background mucosa, severe enterosis, and the pathology of differentiated gastric cancer were the factors that influenced the occurrence of multiple gastric cancers. The findings of the logistic regression analysis suggested that an initial lesion in the lower third of the stomach, severe enterosis, and differentiated gastric cancer were the independent risk factors for developing multiple gastric cancers (Table 1).

Table 1 Basic information about the study patients

	Age (yr)	Body mass index (kg/m^2)	White blood cell count (× 10^9/L)	Platelet count (× 10^9/L)	Admission creatinine (mg/dL)
Patients	61.31 ± 9.60	24.68 ± 3.36	14.57 ± 3.40	169.55 ± 49.70	0.94 (0.70, 1.20)
$t/Z/\chi^2$ values	0.78	0.82	0.41	2.09	1.50
P value	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05



DOI: 10.4251/wjgo.v15.i9.1644 **Copyright** ©The Author(s) 2023.

Figure 1 Imaging results of gastric cancer. A: Imaging results of metachronous gastric cancer; B: Imaging results of synchronous gastric cancer.

Independent risk factors for simultaneous and heterochronous gastric cancer after ESD

Univariate analysis of concurrent gastric cancer signified that age ≥ 65 years and severe intestinalization were the risk factors for developing concurrent gastric cancer (Table 2). Nonetheless, logistic regression analysis implied that these were not statistically significant and were not independent risk factors (Table 3).

In the case of heterochronous gastric cancer, univariate analysis showed that age ≥ 65 years, being a male, initial lesion in the lower third of the stomach, and severe intestinal and differentiated gastric cancer were the possible risk factors for developing heterochronous gastric cancer. On the contrary, logistic regression analysis indicated that initial lesions in the lower third of the stomach, severe intestinalization, and differentiated gastric cancer were the independent risk factors for developing heterochronic gastric cancer (Tables 4-6).

DISCUSSION

The results of this study showed that the incidence rates of heterochronic and simultaneous gastric cancer were 11.7% and 9.2%, respectively, which agrees with the findings of previous studies. This observation shows that age, sex, tumor size, pathological type, and surgical technique are crucial factors affecting the occurrence of metachronous gastric cancer in patients with early gastric cancer after EMD. Older men are more likely to suffer from this disease. Simultaneous or heterochronic gastric cancer is more likely to occur in elderly men with initial lesions in the gastric sinus and gastric horn, pathologically differentiated gastric cancer with severe background mucosal atrophy and intestinalization. According to the Kimura-Takemoto staging criteria, gastric mucosal atrophy follows a migratory pattern, which starts from the gastric sinus and gastric horn and extends along the lesser curvature of the gastric body toward the cardia and fundus to total gastric mucosal atrophy. Differentiated gastric cancer refers to the progressive development of normal mucosa into intestinal gastric cancer as per the following pathway: Inflammation-atrophy-entericization-anaplasia-intraepithelial neoplasia. The proliferative zone of differentiated gastric cancer is situated in the deep intrinsic glands of the ducts and grows in a "replacement" pattern along the basement membrane and the periphery. Furthermore, the adjacent atrophic intestinal mucosa of differentiated gastric cancer may receive the "replacement signal" from the margins of the lesion and progress to differentiated gastric cancer over time. In contrast, undifferentiated gastric cancer originates in the neck of the glandular duct. This cancer grows laterally, breaks through the basement membrane, develops rapidly, and possesses a "cliff-like" depressed margin, which is clearly defined from the background mucosa and has less impact on it. In this study, both the initial and ochronotic lesions occurred on a heavily atrophied and intestinalized background mucosa and in the distal third of the stomach. Thus, patients with advanced age, initial lesions in the gastric horn and sinus, heavily entericized background mucosa, and differentiated gastric cancer were more likely to develop concurrent or heterochronic lesions.

This study further confirmed that a heavily intestinalized background mucosa, with an initial lesion in the gastric sinus and gastric horn and a differentiated pathology, was an independent risk factor for the development of ochronous gastric

Table 2 Logistics regression analysis of risk factors for multiple gastric cancers after endoscopic submucosal dissection

Clinical and lesion characteristics	OR	95%CI	*P* value
Age ≥ 65 yr	1.902	0.435-8.328	0.393
Male	1.435	0.383-5.382	0.592
Smoking (BI ≥ 400)	2.697	0.707-10.290	0.146
Lesion in the lower third of the stomach	11.280	2.720-46.775	0.001
O-shaped atrophy	1.547	0.372-6.442	0.549
Severe intestinalization	6.206	1.667-23.109	0.006
Divergent	9.178	1.642-51.305	0.012

95%CI: 95% confidence interval; OR: Odds ratio; BI: Brinkman index.

Table 3 Univariate analysis of risk factors for developing concurrent gastric cancer

Clinicopathological features	OR	95%CI	*P* value
Age ≥ 65 yr	5.679	1.164-27.701	0.025
Male	2.400	0.600-9.604	0.343
Smoking (BI ≥ 400)	2.622	0.689-9.971	0.291
Family history of stomach cancer	3.067	0.291-32.329	0.359
Initial multiple foci	4.547	0.101-19.960	0.912
Lesion ≥ 2 cm	2.042	0.563-7.399	0.348
Lesion in the lower third of the stomach	2.469	0.620-9.830	0.220
O-shaped atrophy	2.115	0.531-8.425	0.447
Severe intestinalization	4.632	1.159-18.514	0.045
Divergent	6.25	0.771-50.695	0.109
Depth of submucosal infiltration < 500 μm	4.4	0.745-25.991	0.134

95%CI: 95% confidence interval; OR: Odds ratio; BI: Brinkman index.

Table 4 Logistics regression analysis of risk factors for the development of concurrent gastric cancer

Influencing factors	OR	95%CI	*P* value
Age ≥ 65 yr	2.458	0.404-14.958	0.329
Severe intestinalization	4.711	0.969-22.896	0.055

95%CI: 95% confidence interval; OR: Odds ratio.

carcinoma. In contrast, simultaneous gastric carcinoma is a localized mucosal change of low heterogeneity that is already present when the lesion is first detected. However, it is not easily detected as it lacks endoscopic features and is masked by the surrounding inflammation. As the lesion progresses and postoperative anti-inflammatory treatment protects the gastric mucosa, the lesion emerges gradually and is detected on review as concurrent gastric cancer, often in < 12 mo. Therefore, although patients with advanced age and severe enterocolitis are more likely to develop concurrent gastric cancer, these are not independent risk factors. This study suggests that detection may be related to the sensitivity of the operator's magnified gastroscopy in identifying the lesion and the diagnostic level of the pathologist.

Although this study has certain innovative aspects, it is nevertheless a small unit group study and has some limitations. Hence, follow-up studies should be performed with a larger sample size. Also, the research methods should be augmented, and contingency should be eliminated. Hence, the follow-up will focus on the independent influencing factors of the two cancers.

Table 5 Univariate analysis of risk factors for the development of heterochronic gastric cancer

Clinicopathological features	OR	95%CI	*P* value
Age ≥ 65 yr	7.571	1.606-35.699	0.004
Male	5.400	1.146-25.446	0.022
Smoking (BI ≥ 400)	3.441	1.056-11.214	0.074
Family history of stomach cancer	5.111	0.774-33.752	0.123
Initial multiple foci	1.813	0.344-9.560	0.831
Lesion ≥ 2 cm	1.167	0.407-3.344	0.794
Lesion in the lower third of the stomach	7.778	1.690-35.795	0.034
O-shaped atrophy	3.437	0.924-12.784	0.061
Severe intestinalization	3.821	1.234-11.828	0.047
Divergent	9.375	1.192-73.735	0.037
Depth of submucosal infiltration < 500 μm	1.320	0.144-12.089	0.585

95%CI: 95% confidence interval; OR: Odds ratio; BI: Brinkman index.

Table 6 Logistics regression analysis of risk factors for the development of heterochronic gastric cancer

Influencing factors	OR	95%CI	*P* value
Age ≥ 65 yr	4.119	0.696-24.358	0.119
Male	4.205	0.882-20.057	0.072
Lesion in the lower third of the stomach	14.87	2.508-88.166	0.003
Severe intestinalization	4.484	1.029-19.536	0.046
Divergent	12.644	1.303-122.714	0.029

95%CI: 95% confidence interval; OR: Odds ratio.

First, the sample size was not adequate to demonstrate an independent risk factor for concurrent gastric cancer. Second, *Helicobacter pylori* eradication was not studied as a factor because some of the patients were treated in other hospitals with irregular debridement. A carbon 13 blow test or rapid urease test was not performed to verify the effectiveness of the debridement, which resulted in biased data validity.

CONCLUSION

Based on the study findings, it could be concluded that older men with initial lesions in the sinus angle, differentiated gastric cancer pathology, severe background mucosal atrophy, and enterosis are more likely to develop multiple gastric cancers. Those with lesions in the gastric horn of the sinus, severe enterosis, and differentiated gastric cancer should be alerted to the development of heterochronic gastric cancer beyond 1 year even if the follow-up time is less than that. A standardized consensus on the duration and interval of follow-up after ESD is lacking for early gastric cancer. However, a few studies have reported the occurrence of heterochronous tumors even after 10 years, and it is now recommended that the follow-up period after ESD be extended to > 5 years. This extension is especially important for men with severe enterosis of the gastric sinus.

ARTICLE HIGHLIGHTS

Research background

Endoscopic mucosal resection is an innovative method for treating early gastric cancer and has been extensively applied in clinical practice.

Research motivation

This study aimed to analyze the factors associated with the development of heterochronic gastric cancer in patients with early gastric cancer who had undergone endoscopic mucosal dissection (EMD).

Research objectives

This research sheds light on the future direction of EMD. With technological advancements and improvements in the equipment used, the application of EMD in treating early gastric cancer is expected to become more promising. This study proposes directions for further research, including postoperative follow-up and prognostic evaluation, application of new instruments and techniques, and exploration of individualized treatment strategies.

Research methods

A cohort of patients with early gastric cancer treated using EMD was retrospectively analyzed, and patients who developed heterochronic gastric cancer after the surgery were compared with those who did not. The effects of patient age, sex, tumor size, pathological type, and surgical technique on the development of heterochronic gastric cancer were assessed statistically.

Research results

Of the 300 patients with early gastric cancer, 150 developed heterochronic gastric cancer after EMD. Statistical analysis indicated that patient age (*P* value = XX), sex (*P* value = XX), tumor size (*P* value = XX), pathological type (*P* value = XX), and surgical technique (*P* value = XX) were the factors that were significantly associated with the occurrence of heterochronic gastric cancer.

Research conclusions

In patients with early gastric cancer, age, sex, tumor size, pathological type, and surgical technique are the key factors influencing the occurrence of heterochronic gastric cancer after EMD. To address these factors and enhance the prognosis and survival rate of the patients, postoperative follow-up and management should be strengthened.

Research perspectives

For patients with early gastric cancer, factors affecting the development of heterochronic gastric cancer after EMD include age, sex, tumor size, pathological type, and surgical technique.

FOOTNOTES

Author contributions: Xie B and He WW contributed equally to this work; Xie B, Xia YX, Wang X, Xiong Y, Chen SB, Zhang J, and He WW designed the research study; Xie B, Xia YX ,Wang X, Xiong Y, Chen SB, Zhang J, and He WW performed the research; Xie B, Xia Y, and Wang X contributed new reagents and analytic tools; Xie B and He WW analyzed the data and wrote the manuscript; and all authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Nanjing Pu Kou District Hospital of Traditional Chinese Medicine Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors declare that there are no conflicts of interest

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Bing Xie 0009-0002-2560-7282; Wei-Wei He 0009-0005-9850-8076.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Ferlay J**, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; **144**: 1941-1953 [PMID: 30350310 DOI: 10.1002/ijc.31937]

2 **Feng RM**, Zong YN, Cao SM, Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun (Lond)* 2019; **39**: 22 [PMID: 31030667 DOI: 10.1186/s40880-019-0368-6]

3 **Ono H**, Yao K, Fujishiro M, Oda I, Uedo N, Nimura S, Yahagi N, Iishi H, Oka M, Ajioka Y, Fujimoto K. Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer (second edition). *Dig Endosc* 2021; **33**: 4-20 [PMID: 33107115 DOI: 10.1111/den.13883]

4 **Choi JM**, Kim SG, Choi J, Park JY, Oh S, Yang HJ, Lim JH, Im JP, Kim JS, Jung HC. Effects of Helicobacter pylori eradication for metachronous gastric cancer prevention: a randomized controlled trial. *Gastrointest Endosc* 2018; **88**: 475-485.e2 [PMID: 29800546 DOI: 10.1016/j.gie.2018.05.009]

5 **Kim JH**, Jeong SH, Yeo J, Lee WK, Chung DH, Kim KO, Chung JW, Kim YJ, Kwon KA, Park DK. Clinicopathologic Similarities of the Main and Minor Lesions of Synchronous Multiple Early Gastric Cancer. *J Korean Med Sci* 2016; **31**: 873-878 [PMID: 27247495 DOI: 10.3346/jkms.2016.31.6.873]

6 **Michalinos A**, Constantinidou A, Kontos M. Gastric collision tumors: an insight into their origin and clinical significance. *Gastroenterol Res Pract* 2015; **2015**: 314158 [PMID: 25767509 DOI: 10.1155/2015/314158]

7 **Yang HJ**, Kim SG, Lim JH, Choi JM, Oh S, Park JY, Han SJ, Kim J, Chung H, Jung HC. Novel risk stratification for metachronous recurrence after curative endoscopic submucosal dissection for early gastric cancer. *Gastrointest Endosc* 2018; **87**: 419-428.e3 [PMID: 28713064 DOI: 10.1016/j.gie.2017.07.005]

8 **Moon HS**, Yun GY, Kim JS, Eun HS, Kang SH, Sung JK, Jeong HY, Song KS. Risk factors for metachronous gastric carcinoma development after endoscopic resection of gastric dysplasia: Retrospective, single-center study. *World J Gastroenterol* 2017; **23**: 4407-4415 [PMID: 28706423 DOI: 10.3748/wjg.v23.i24.4407]

9 **Jiang HY**, Chen J, Xia CC, Cao LK, Duan T, Song B. Noninvasive imaging of hepatocellular carcinoma: From diagnosis to prognosis. *World J Gastroenterol* 2018; **24**: 2348-2362 [PMID: 29904242 DOI: 10.3748/wjg.v24.i22.2348]

10 **Nakata R**, Nagami Y, Hashimoto A, Sakai T, Ominami M, Fukunaga S, Otani K, Hosomi S, Tanaka F, Ohira M, Taira K, Yamagami H, Tanigawa T, Watanabe T, Fujiwara Y. Successful Eradication of Helicobacter pylori Could Prevent Metachronous Gastric Cancer: A Propensity Matching Analysis. *Digestion* 2021; **102**: 236-245 [PMID: 31678978 DOI: 10.1159/000504132]

11 **Kato M**. Metachronous gastric cancer risk after endoscopic resection of early gastric cancer and H. pylori status. *J Gastroenterol* 2019; **54**: 478-479 [PMID: 30770974 DOI: 10.1007/s00535-019-01560-2]

12 **Stolte M**, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol* 2001; **15**: 591-598 [PMID: 11573102 DOI: 10.1155/2001/367832]

13 **Quach DT**, Hiyama T. Assessment of Endoscopic Gastric Atrophy according to the Kimura-Takemoto Classification and Its Potential Application in Daily Practice. *Clin Endosc* 2019; **52**: 321-327 [PMID: 31327182 DOI: 10.5946/ce.2019.072]

14 **Nomura A**, Grove JS, Stemmermann GN, Severson RK. A prospective study of stomach cancer and its relation to diet, cigarettes, and alcohol consumption. *Cancer Res* 1990; **50**: 627-631 [PMID: 2297702]

15 **Cho CJ**, Ahn JY, Jung HY, Jung K, Oh HY, Na HK, Jung KW, Lee JH, Kim DH, Choi KD, Song HJ, Lee GH, Kim JH, Kim SO. The incidence and locational predilection of metachronous tumors after endoscopic resection of high-grade dysplasia and early gastric cancer. *Surg Endosc* 2017; **31**: 389-397 [PMID: 27444840 DOI: 10.1007/s00464-016-4985-8]

16 **Kotelevets SM**, Chekh SA, Chukov SZ. Updated Kimura-Takemoto classification of atrophic gastritis. *World J Clin Cases* 2021; **9**: 3014-3023 [PMID: 33969087 DOI: 10.12998/wjcc.v9.i13.3014]

17 **Yoon H**, Kim N, Shin CM, Lee HS, Kim BK, Kang GH, Kim JM, Kim JS, Lee DH, Jung HC. Risk Factors for Metachronous Gastric Neoplasms in Patients Who Underwent Endoscopic Resection of a Gastric Neoplasm. *Gut Liver* 2016; **10**: 228-236 [PMID: 26087797 DOI: 10.5009/gnl14472]

18 **Leung WK**, Sung JJ. Review article: intestinal metaplasia and gastric carcinogenesis. *Aliment Pharmacol Ther* 2002; **16**: 1209-1216 [PMID: 12144569 DOI: 10.1046/j.1365-2036.2002.01300.x]

19 **Xiao W**, Ma ZS. Influences of Helicobacter pylori infection on diversity, heterogeneity, and composition of human gastric microbiomes across stages of gastric cancer development. *Helicobacter* 2022; **27**: e12899 [PMID: 35678078 DOI: 10.1111/hel.12899]

20 **Kishino M**, Nakamura S, Shiratori K. Clinical and Endoscopic Features of Undifferentiated Gastric Cancer in Patients with Severe Atrophic Gastritis. *Intern Med* 2016; **55**: 857-862 [PMID: 27086796 DOI: 10.2169/internalmedicine.55.4841]

21 **Lee A**, Chung H. Endoscopic Resection of Undifferentiated-type Early Gastric Cancer. *J Gastric Cancer* 2020; **20**: 345-354 [PMID: 33425437 DOI: 10.5230/jgc.2020.20.e37]

22 **Ryu DG**, Choi CW, Kim SJ, Kang DH, Kim HW, Park SB, Nam HS. Possible indication of endoscopic resection in undifferentiated early gastric cancer. *Sci Rep* 2019; **9**: 16869 [PMID: 31728024 DOI: 10.1038/s41598-019-53374-0]

23 **Hahn KY**, Park JC, Kim EH, Shin S, Park CH, Chung H, Shin SK, Lee SK, Lee YC. Incidence and impact of scheduled endoscopic surveillance on recurrence after curative endoscopic resection for early gastric cancer. *Gastrointest Endosc* 2016; **84**: 628-638.e1 [PMID: 26996290 DOI: 10.1016/j.gie.2016.03.1404]

24 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

25 **Nashimoto A**, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, Tsujitani S, Seto Y, Furukawa H, Oda I, Ono H, Tanabe S, Kaminishi M. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. *Gastric Cancer* 2013; **16**: 1-27 [PMID: 22729699 DOI: 10.1007/s10120-012-0163-4]

26 **Gao Y**, Xi H, Mattsson F, Liang W, Xie SH, Chen L, Lagergren J. Surgical starting time of the day and survival in gastric cancer. *Sci Rep* 2023; **13**: 6955 [PMID: 37117226 DOI: 10.1038/s41598-023-33692-0]

27 **Min BH**, Kim ER, Kim KM, Park CK, Lee JH, Rhee PL, Kim JJ. Surveillance strategy based on the incidence and patterns of recurrence after curative endoscopic submucosal dissection for early gastric cancer. *Endoscopy* 2015; **47**: 784-793 [PMID: 26111362 DOI: 10.1055/s-0034-1392249]

28 **Abe S**, Oda I, Suzuki H, Nonaka S, Yoshinaga S, Nakajima T, Sekiguchi M, Mori G, Taniguchi H, Sekine S, Katai H, Saito Y. Long-term surveillance and treatment outcomes of metachronous gastric cancer occurring after curative endoscopic submucosal dissection. *Endoscopy* 2015; **47**: 1113-1118 [PMID: 26165734 DOI: 10.1055/s-0034-1392484]

29 **Gotoda T**, Ho KY, Soetikno R, Kaltenbach T, Draganov P. Gastric ESD: current status and future directions of devices and training. *Gastrointest Endosc Clin N Am* 2014; **24**: 213-233 [PMID: 24679233 DOI: 10.1016/j.giec.2013.11.009]

30 **Kim N**. Chemoprevention of gastric cancer by Helicobacter pylori eradication and its underlying mechanism. *J Gastroenterol Hepatol* 2019; **34**: 1287-1295 [PMID: 30828872 DOI: 10.1111/jgh.14646]

31 **Li L**, Yu C. Helicobacter pylori Infection following Endoscopic Resection of Early Gastric Cancer. *Biomed Res Int* 2019; **2019**: 9824964 [PMID: 31737682 DOI: 10.1155/2019/9824964]

32 **Chung CS**, Woo HS, Chung JW, Jeong SH, Kwon KA, Kim YJ, Kim KO, Park DK. Risk Factors for Metachronous Recurrence after Endoscopic Submucosal Dissection of Early Gastric Cancer. *J Korean Med Sci* 2017; **32**: 421-426 [PMID: 28145644 DOI: 10.3346/jkms.2017.32.3.421]

33 **Watari J**, Tomita T, Tozawa K, Oshima T, Fukui H, Miwa H. Preventing Metachronous Gastric Cancer after the Endoscopic Resection of Gastric Epithelial Neoplasia: Roles of Helicobacter pylori Eradication and Aspirin. *Gut Liver* 2020; **14**: 281-290 [PMID: 31547640 DOI: 10.5009/gnl19079]

34 **Song Z**, Chen Y, Lu H, Zeng Z, Wang W, Liu X, Zhang G, Du Q, Xia X, Li C, Jiang S, Wu T, Li P, He S, Zhu Y, Xu J, Li Y, Huo L, Lan C, Miao Y, Jiang H, Chen P, Shi L, Tuo B, Zhang D, Jiang K, Wang J, Yao P, Huang X, Yang S, Wang X, Zhou L. Diagnosis and treatment of Helicobacter pylori infection by physicians in China: A nationwide cross-sectional study. *Helicobacter* 2022; **27**: e12889 [PMID: 35363917 DOI: 10.1111/hel.12889]

35 **Mamori S**, Higashida A, Kawara F, Ohnishi K, Takeda A, Senda E, Ashida C, Yamada H. Age-dependent eradication of Helicobacter pylori in Japanese patients. *World J Gastroenterol* 2010; **16**: 4176-4179 [PMID: 20806435 DOI: 10.3748/wjg.v16.i33.4176]

36 **Argueta EA**, Moss SF. The prevention of gastric cancer by Helicobacter pylori eradication. *Curr Opin Gastroenterol* 2021; **37**: 625-630 [PMID: 34411037 DOI: 10.1097/MOG.0000000000000777]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1653-1661

DOI: 10.4251/wjgo.v15.i9.1653

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Utilization of access to colorectal cancer screening modalities in low-income populations after medicaid expansion

Gerald Fletcher, Joan Culpepper-Morgan, Alvaro Genao, Eric Alatevi

Specialty type: Health care sciences and services

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Bustamante-Lopez LA, Brazil; Sano W, Japan

Received: May 29, 2023
Peer-review started: May 29, 2023
First decision: July 23, 2023
Revised: July 31, 2023
Accepted: August 15, 2023
Article in press: August 15, 2023
Published online: September 15, 2023

Gerald Fletcher, Joan Culpepper-Morgan, Alvaro Genao, Eric Alatevi, Department of Gastroenterology, NYC Health + Hospitals/Harlem, New York, NY 10037, United States

Gerald Fletcher, College of Public Health/Health Policy and Management, University of Arizona, Tucson, AZ 85006, United States

Corresponding author: Gerald Fletcher, MD, Doctor, Department of Gastroenterology, NYC Health + Hospitals/Harlem, 506 Lenox Ave, New York, NY 10037, United States. pkfletcher@gmail.com

Abstract

BACKGROUND

Colorectal cancer (CRC) remains a relevant public health problem. Current research suggests that racial, economic and geographic disparities impact access. Despite the expansion of Medicaid eligibility as a key component of the Affordable Care Act (ACA), there is a dearth of information on the utilization of newly gained access to CRC screening by low-income individuals. This study investigates the impact of the ACA's Medicaid expansion on utilization of the various CRC screening modalities by low-income participants. Our working hypothesis is that Medicaid expansion will increase access and utilization of CRC screening by low-income participants.

AIM

To investigate the impact of the Affordable Care Act and in particular the effect of Medicaid expansion on access and utilization of CRC screening modalities by Medicaid state expansion status across the United States.

METHODS

This was a quasi-experimental study design using data from the Behavioral Risk Factor Surveillance System, a large health system survey for participants across the United States and with over 2.8 million responses. The period of the study was from 2011 to 2016 which was dichotomized as pre-ACA Medicaid expansion (2011-2013) and post-ACA Medicaid expansion (2014-2016). The change in utilization of access to CRC screening strategies between the expansion periods were analyzed as the dependent variables. Secondary analyses included stratification of the access by ethnicity/race, income, and education status.

RESULTS

A greater increase in utilization of access to CRC screening was observed in Medicaid expansion states than in non-expansion states [+2.9%; 95% confidence interval (95%CI): 2.12, 3.69]. Low-income participants showed a +4.02% (95%CI: 2.96, 5.07) change between the expansion periods compared with higher income groups +3.19% (1.70, 4.67). Non-Hispanic Whites and Hispanics [+3.01% (95%CI: 2.16, 3.85) *vs* +5.51% (95%CI: 2.81, 8.20)] showed a statistically significant increase in utilization of access but not in Non-Hispanic Blacks, or Multiracial. There was an increase in utilization across all educational levels. This was significant among those who reported having a high school graduate degree or more +4.26 % (95%CI: 3.16, 5.35) compared to some high school or less +1.59% (95%CI: -1.37, 4.55).

CONCLUSION

Medicaid expansion under the Affordable Care Act led to an overall increase in self-reported use of CRC screening tests by adults aged 50-64 years in the United States. This finding was consistent across all low-income populations, but not all races or levels of education.

Key Words: Medicaid expansion; Colorectal cancer screening; Low-income; Disparities; Minorities; Affordable care act

Core Tip: While many researchers have shown and studied how the Affordable Care Act through its Medicaid's expansion increased healthcare access to different categories of potential beneficiaries, little is known about actual utilization of this "newly gained" access. Our paper focuses specifically on examining this specific question.

Citation: Fletcher G, Culpepper-Morgan J, Genao A, Alatevi E. Utilization of access to colorectal cancer screening modalities in low-income populations after medicaid expansion. *World J Gastrointest Oncol* 2023; 15(9): 1653-1661

URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1653.htm

DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1653

INTRODUCTION

Colorectal cancer (CRC) remains a relevant public health problem in the United States being the second leading cause of cancer-related death in the United States and the third most common cancer in men and in women[1]. The screening guidelines based on the 2016 recommendations by the United States Preventive Services Task Force (USPSTF) for adults aged 50-75 years included one of three modalities: An annual high-sensitivity fecal occult blood test (FOBT), sigmoidoscopy every 5 years with high-sensitivity FOBT every 3 years, or colonoscopy every 10 years[1].

Despite these recommendations, there are wide disparities in CRC screening rates where only 61% of adults in the 50-75-year group report recent CRC screening[1]. Racial and geographic disparities impact access to screening and cancer-related outcomes. The incidence of and mortality from CRC is higher for Blacks than for Whites as well as for lower-income populations than for higher-income groups[2,3]. Several barriers to equitable access to screening have been identified including affordability, a lack of a doctor's recommendation for screening, as well as a lack of a usual care primary care provider.

A key component of the Affordable Care Act (ACA) was an expansion of Medicaid eligibility to adults earning up to 138% of the federal poverty level (FPL). To support this expanded coverage, effective January 1, 2014, states would receive 100 percent federal funding for the first 3 years phasing to 90 percent federal funding in subsequent years[4-6]. Although this provision was originally intended to be enacted in all states, a United States Supreme Court decision gave states the option not to adopt it[7,8]. As of August 2018, only 34 states including the District of Columbia had expanded Medicaid[7]. Furthermore, under the ACA, private health plans are required to cover a range of preventive services including cancer screening at no cost to beneficiaries and at intervals defined by the USPSTF.

While elements of the ACA have remained politically controversial[8], the literature on the ACA and healthcare is growing but remains limited in depth of query[9-15]. On cancers in the United States specifically, recent studies have suggested that the ACA has resulted in an increase in cervical and CRC screening rates and the diagnosis of cancers at an earlier more treatable stage.

The purpose of this study was to investigate the impact of the ACA and in particular the effect of Medicaid expansion on access and utilization of the colorectal screening services by Medicaid state expansion status. The central hypothesis was that the ACA's Medicaid expansion would reduce healthcare access disparities and thereby increase access to CRC screening services by individuals across all socio-economic strata thereby increasing the likelihood of utilization of the access.

MATERIALS AND METHODS

Our study used a quasi-experimental design. Data source was the Behavioral Risk Factor Surveillance System (BRFSS) for the period 2011 to 2016. Data from 2011-13 BRFSS was the pre-Medicaid expansion period while that from 2014-2016, post-Medicaid expansion period. BRFSS is an annual state-wide survey of adults aged 18 years and older. Details of the methodology of the survey can be found elsewhere[16]. BRFSS completes more than 400000 adult interviews each year, making it the largest continuously conducted health survey system in the world. Adjusted response rates vary by state and in 2009 ranged between 39% and 67%; unadjusted response rates ranged between 19% and 62%, depending on state and survey year.

Compared to the analysis from previous researchers[14], we used data from all 6 years available. Although ACA was passed into law in 2010, coverage under the Medicaid expansion became effective January 1, 2014, in all states that had adopted the Medicaid expansion. We thus used the period beginning 2014 as the expansion period. At the time of this analysis, the most complete dataset was for the year 2016.

To be included in the study, participants must be adult non-elderly United States citizens, 50-64 years, who took the survey in the years 2011-2016. They also must have completed at least one of the screening modalities and be eligible for Medicaid. Persons who were less than 50 years of age or older than 64 years of age, did not complete a CRC screening modality in the surveyed years were excluded. Although BRFSS reported annual household income in eight categories, in our analysis, we dichotomized this variable into incomes less than $25000 and more than $25000. Our Medicaid eligible population was based on household income adjusted for by household size. This was approximately $25000 per annum, a value used as proxy for Medicaid eligibility, and which corresponded to approximately 138% of the FPL during the period of study.

The treatment variable for this study was Medicaid expansion status. A detailed list of the states by expansion status is available in the references[7]. CRC screening eligibility was based on the 2015 USPSTF recommendations as follows: Eligible for CRC screening if not had a colonoscopy within the past 10 years or a sigmoidoscopy with FOBT within the past 5 years. Participants who had had reported screening using only FOBT were still screen-eligible during the survey year, since FOBT is an annual test.

A difference-in-differences technique was used to analyze the effect of Medicaid expansion status on the utilization of access to colorectal screening. Here, we compared rates of colonoscopy, sigmoidoscopy, and FOBT among screen eligible adults who responded to the survey. The binary outcome of change in utilization of access to CRC screening strategies pre and post ACA were analyzed as the dependent variables. Other secondary analysis included stratification of the access by ethnicity/race, income, and education status.

Descriptive and demographic variables were analyzed by stratification of the respondents' socioeconomic characteristics: Self-reported household income, educational attainment, employment status, and race/ethnicity, age, and gender. Educational status was treated as binary characteristic: Whether the respondent had at least graduated from high school or not.

We used the stratification, primary sampling unit and final weight variables through a survey analysis to account for collection design of the BRFSS as recommended by the CDC. The results were generated using SAS software version 9.4.

RESULTS

In total, 2.86 million responses in the BRFSS database were considered during the period of study, 893004 were adults aged between 50 and 64 who had utilized at least one colorectal screening modality. This was considered our "screening cohort". We used a complete case analysis because less than 1.65% of the data had missing values. There were 179734 respondents included from Medicaid non-expansion states in the pre-ACA era while 285589 were in Medicaid expansion states in the pre-ACA period. In the post-ACA period, 159169 were in Medicaid non-expansion states and 268512 in Medicaid expansion states. In our analysis, 33 states were considered as expansion states during the period of study 2014-2016.

The proportion of individuals across the three age groups (50-54 years, 55-59 years, and 60-64 years) were 39%, 32%, and 27%, respectively across all states in the pre-ACA era while in the post-ACA period, these were 37%, 32%, and 31% respectively. Other demographic characteristics are shown in Table 1.

Responses to the type of screening modality used by adults aged 50-64 years is shown in Table 2. All 893004 eligible adults had had at least one type of CRC screening done. Out of this number, screening with colonoscopy was reported by 838694, sigmoidoscopy 41459 and FOBT 467428 which corresponded to weighted percentages as follows: 62% had at least a colonoscopy, 35% had had a sigmoidoscopy and 3% a fecal occult blood test. A summary of responses to the type of screening completed during the period of study in our screening cohort is shown in Table 2.

In general, while there was an overall increase in utilization of access to CRC screening reported across all states, the rate of increase was significantly greater in expansion states than non-expansion states [+2.9%; 95% confidence interval (95%CI): 2.12, 3.69]. Amongst the three screening modalities only colonoscopy and FOBT were significant statistically when utilization in expansion states were compared to non-expansion states. Utilization of colonoscopies increased by 2.4% (95%CI: 1.64, 3.15) in the Medicaid expansion states while that for FOBT increased by 0.88% (95%CI: 0.50, 1.26).

Table 3 shows a difference-in-difference analysis of expansion *vs* non-expansion states by income level using $25000 as a proxy for Medicaid eligibility. Across all income levels, the effect of Medicaid expansion due to ACA was statistically significant when the pre-ACA and post-ACA eras were compared. Low-income participants with incomes < $25000, showed a 4.02% (95%CI: 2.96, 5.07) change between the pre-ACA and post-ACA periods while higher income groups had

Table 1 Demographic variables, *n* (%)

	All states (*n* = 893004)		Expansion states (*n* = 554101)		Non-expansion states (*n* = 338903)	
	Pre-ACA	Post-ACA	Pre-ACA	Post-ACA	Pre-ACA	Post-ACA
Age (yr)						
50-54	144633 (39.15)	125057 (36.68)	89366 (39.16)	78752 (36.63)	55267 (39.13)	46305 (36.78)
55-59	158010 (31.18)	145440 (31.86)	97145 (31.49)	91564 (32.07)	60865 (30.61)	53876 (31.48)
60-64	162680 (29.67)	157184 (31.46)	99078 (29.35)	98196 (31.30)	63602 (30.25)	58988 (31.74)
Gender						
Male	189909 (48.59)	183869 (48.49)	117729 (48.72)	116204 (48.69)	72180 (48.35)	67665 (48.13)
Race						
Non-Hispanic white	367535 (70.94)	335133 (69.10)	227986 (72.30)	212968 (70.46)	139549 (68.47)	122165 (66.67)
Non-Hispanic black	40005 (11.05)	35498 (11.80)	21748 (9.64)	19925 (10.17)	18257 (13.64)	15573 (14.73)
Other non-Hispanic	17151 (4.95)	16381 (5.23)	11524 (6.16)	11230 (6.60)	5627 (2.73)	5151 (2.78)
Multiracial non-Hispanic	8075 (1.25)	7433 (1.14)	5445 (1.36)	5174 (1.22)	2630 (1.05)	2259 (1.01)
Hispanic	26999 (11.80)	27038 (12.73)	15400 (10.54)	15079 (11.56)	11599 (14.11)	11959 (14.82)
Income						
< $25000	108872 (26.91)	90810 (26.19)	62264 (24.56)	53270 (24.04)	46608 (31.27)	37540 (30.07)
Employment status						
Employed for wages	229742 (49.78)	214339 (50.54)	143761 (51.16)	137816 (52.12)	85981 (47.27)	76523 (47.70)
Self-employed	51031 (10.74)	48916 (11.28)	31149 (10.79)	30137 (11.23)	19882 (10.65)	18779 (11.37)
Out of work > 1 yr	19335 (5.04)	13208 (3.67)	12399 (5.18)	8569 (3.70)	6936 (4.79)	4639 (3.62)
Out of work < 1 yr	12272 (3.12)	9159 (2.50)	7854 (3.19)	6055 (2.60)	4418 (3.01)	3104 (2.33)
Homemaker	22567 (5.44)	20059 (5.82)	12528 (4.99)	11537 (5.22)	10039 (6.26)	8522 (6.06)
Student	1248 (0.28)	1022 (0.24)	788 (0.28)	692 (0.25)	460 (0.28)	330 (0.12)
Retired	67400 (12.91)	60896 (12.66)	41744 (12.83)	37970 (12.43)	25656 (13.05)	22926 (13.07)
Unable to work	59643 (12.69)	56912 (13.61)	33900 (11.58)	33669 (12.46)	25743 (14.70)	23243 (15.65)
Education						
Some high school or less	32774 (13.35)	28992 (13.71)	18155 (15.34)	16686 (12.80)	14619 (15.23)	12306 (12.32)
At least a high school graduate	431169 (86.65)	397159 (86.29)	266416 (84.66)	250815 (87.20)	164753 (84.77)	146344 (87.68)

ACA: Affordable care act.

Table 2 Difference-in-difference analysis of the three colorectal screening strategies (fobt only, sigmoidoscopy only or colonoscopy only) or at least any colorectal screening, *n* = 893004

Screening modality	Percentage point change		Difference-in- difference estimate	*P* value
	Expansion states	Non-expansion states		
Colonoscopy	16.88 (16.44, 17.32)	14.49 (13.87, 15.11)	2.40 (1.64, 3.15)	< 0.001
Sigmoidoscopy	0.31 (0.20, 0.44)	0.15 (0.001, 0.280)	0.17 (-0.01, 0.35)	0.060
FOBT	2.52 (2.28, 2.76)	1.64 (1.35, 1.94)	0.88 (0.50, 1.26)	< 0.001
CRC screen	18.34 (17.89, 18.80)	15.44 (14.80, 16.08)	2.90 (2.12, 3.69)	< 0.001

Colorectal cancer screen: At least one colorectal screening strategy during the study period. FOBT: Fecal occult blood test; CRC: Colorectal cancer.

Table 3 Difference-in-difference analysis comparing utilization of access to colorectal cancer screening services by income - expansion states *versus* non-expansion

Annual income	Percentage point change		Difference-in-difference estimate	
	Expansion states	Non-expansion states	%	95%CI
< $25000	18.94	14.92	4.02	(2.96, 5.07)
> $25000	19.84	16.65	3.19	(1.70, 4.67)

a difference of 3.19% (95%CI: 1.70, 4.67) because of Medicaid expansion.

Utilization of access to screening by Medicaid eligible respondents was stratified by race/ethnicity and education (Table 4). There was a statistically significant increase in utilization of access to screening in Non-Hispanic Whites and Hispanics. This translated to 3.01% (95%CI: 2.16, 3.85) in Non-Hispanic Whites and 5.51% (95%CI: 2.81, 8.20) in Hispanics. This difference was however not significant statistically in Non-Hispanic Blacks, Non-Hispanic multiracial and others.

Table 4 also summarizes utilization of access to screening in the Medicaid eligibility population stratified by education status. While there was an increase in access across all educational levels, it was only statistically significant in the population who reported having high school graduate or more+4.26 % (95%CI: 3.16, 5.35) compared to some high school or less 1.59% (95%CI: -1.37, 4.55).

DISCUSSION

In our analysis, self-reported utilization of CRC screening modalities according to 2015 USPSTF by adults aged 50-64 years increased more for residents of Medicaid expansion states than for those in non-expansion states (Table 2). The increased uptake of colonoscopy was double that of FOBT. The increased uptake of flexible sigmoidoscopy was minimal and failed to reach statistical significance. With the increased access to health care provided by the ACA, colonoscopy remains the preferred screening strategy. The change in screening rates was consistent across both low and high-income residents in states that expanded Medicaid compared to non-expansion states but this was inconsistent across Race/Ethnicity and education status. Across different races and ethnic groups, Non-Hispanic Whites and Hispanics, from our analysis, were positively impacted by Medicaid expansion while there was no statistically significant difference in colorectal screening rates for Non-Hispanic Blacks, Multiracial groups, and others. Lastly, a subgroup analysis of low income (< $25000/year) subjects revealed that education status was significantly associated with uptake of CRC screening in expansion states over non-expansion states.

Our study was designed to determine the impact of the ACA Medicaid expansion on the access to and utilization of CRC screening modalities. We presented our preliminary analysis in 2019 at an international conference[18]. Other studies have used the BRFSS database to explore aspects of our study question. In 2018, Hendryx and Luo examined the effect of ACA expansion on screening for cervical, breast, and colon cancers. They concluded that the ACA increased screening for cervical and colon but not for breast cancer in low-income adults. Their analysis used $20000 as an income cutoff instead of $25000 and excluded households with dependent children. They also limited colonoscopies to the last 2 years of ACA expansion. Because of these study design choices especially limiting their analysis to low-income adults only, we believe that they may have under-estimated the effect of the ACA on uptake of CRC screening due to loss of power from a smaller sample size.

It is reasonable to expect that increased access to care through Medicaid expansion would result in cancers diagnosed at an earlier stage. Han *et al*[17], examined cancer registry data in expansion and non-expansion states comparing the percentage of uninsured with a diagnosis of cancer from 2010-2013 to the percentage in the first year of expansion 2014. They found the greatest decrease in the number of uninsured new cancer diagnoses among low-income patients in the expansion states. As a secondary outcome they evaluated the increase in early-stage cancer diagnosis. Overall, there was a small (0.4%) but statistically significant increase in diagnosis of CRC at an early stage for patients who resided in expansion states. This trend is consistent with our previous analysis of our own cancer registry data in which we were able to show a highly significant shift in the diagnosis of CRC at an earlier stage with aggressive NY State Medicaid expansion from 2000 to 2012. The change in percentage of uninsured was much larger, 50%, and the comparison was over two decades[16]. It is likely that the Han analysis was too short a time period to confirm a difference.

Zerhouni *et al*[14], chose to parse the BRFSS database into 3 groups: Early expansion (2012), Expansion (2014 and 2016), and non-expansion states. Data from 2013 and 2015 were excluded. Their analysis concentrated on the differences in uptake over time. They also noted that each time period included different states that likely implemented the expansion in different ways. We chose to dichotomize the data and avoid any bias that may have been inadvertently created by the exclusion of specific years. These differences in design may explain why our results and conclusions diverge from Zerhouni. We found that Whites and Hispanics had greater uptake of CRC screening than Blacks. They concluded that Non-Hispanic Whites and Blacks benefited but that Hispanics did not. We certainly agree that the overall screening rate for Hispanics still lags Non-Hispanic Blacks and Whites. It is possible that other factors were needed to improve uptake of screening. Both studies evaluated the effect of income. Significant differences between expansion and non-expansion states occurred in each income group. Education level did reveal a difference, with subjects having graduated high school or more, showing more uptake of screening modalities.

Table 4 Difference-in-difference analysis comparing utilization of access to colorectal cancer screening in the Medicaid eligible population

Race/ethnicity	Percentage point change		Difference-in- difference estimate	
	Expansion states	Non-expansion states	%	95%CI
[1]Non-Hispanic White	19.92	16.91	3.01	(2.16, 3.85)
[1]Hispanic	13.74	8.23	5.51	(2.81, 8.20)
Non-Hispanic Black	18.32	16.60	1.72	(-0.83, 4.26)
Non-Hispanic multiracial	17.34	13.20	4.15	(-2.93, 11.22)
Other	12.39	13.93	-1.54	(-6.84, 3.76)
Education level				
Some high school or less	14.63	13.42	1.59	(-1.37, 4.55)
[1]High school grad or more	18.83	15.77	4.26	(3.16, 5.35)

[1]Statistically significant.

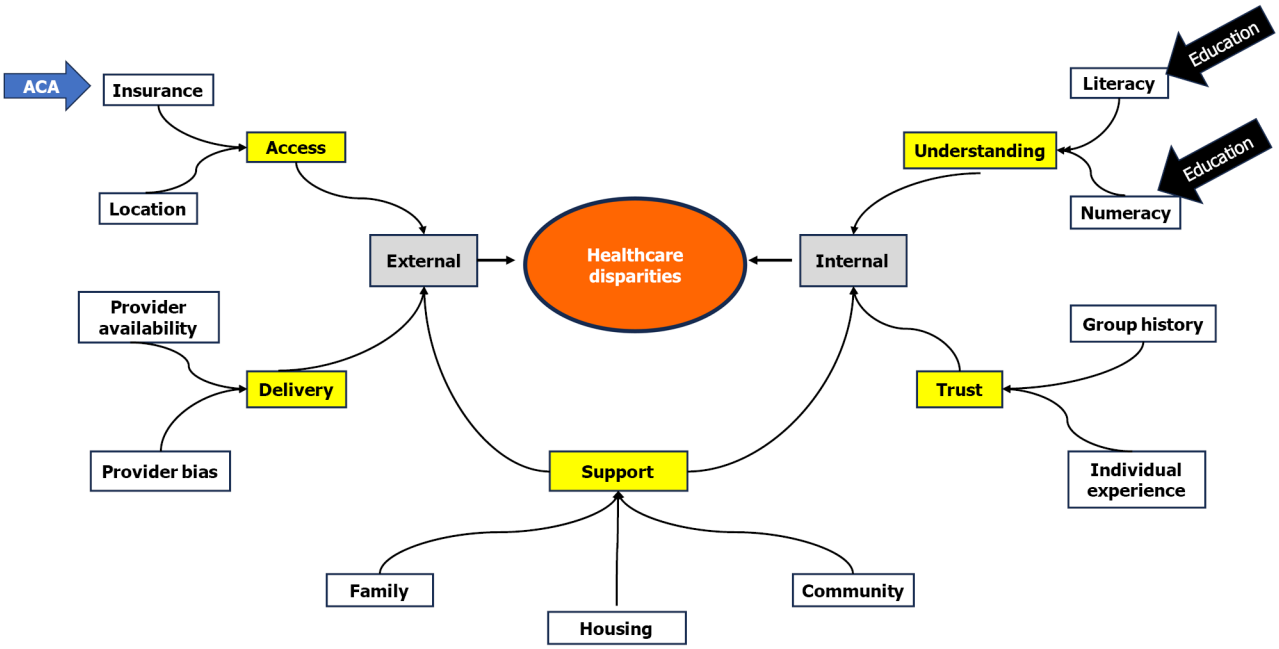
It has been shown that low-income adults in Medicaid non expansion states are disproportionately Black and rural. This group is less likely to have a primary care provider or utilize preventive services. They are more likely to have out of pocket medical expenses and fill fewer prescriptions. These racial and geographic disparities strongly impact access to screening and cancer related outcomes. CRC screening specifically has been shown to be affected by the affordability of health insurance, associated cost-sharing by beneficiaries as well a lack of a recommendation for screening by a primary care provider[5-7]. With the passage of the ACA, there has been an increase in the number of individuals with a primary care provider and a decrease in the number of individuals who defer care due to cost[5]. Primary care providers are therefore able to have established relationships with their patients and refer them for preventive healthcare screenings including colonoscopies. It was thus surprising to note that this did not translate into an increase in utilization of screening services consistently across all socio-demographic groups including race/ethnicity, education status and income.

In our analysis, although Medicaid expansion had positively impacted Non-Hispanic Whites and Hispanics there was not enough evidence to conclude its effect on Non-Hispanic Blacks and other races. Even though our analysis covered most of the roll out of the ACA, this difference may be partly due to the limited period of analysis and perhaps there may be some delayed effect on Blacks and other races. However, the psychosocial determinants of healthcare represent a complex interplay of issues. Figure 1 summarizes factors we suggest may promote or inhibit healthcare disparities. Insurance coverage is the major external barrier to healthcare access. The ACA Medicaid expansion clearly addresses the issue of insurance coverage. However, there are internal patient factors including health care literacy, for which education level may be a surrogate indicator that can result in patients not taking advantage of increased access to care. It is also important to note that the impact of education on uptake of CRC screening was only significant in the low-income subgroup.

Our study has several strengths. Firstly, being an analytic study with a quasi-experimental design, we were able to provide moderately compelling evidence to establish cause and effect. This differentiates our study from others. The lack of randomization, however, prevents the establishment of causality. We limited the potential for confounders (including variations due to trends and controlling for exposure) by our use of the differences-in-differences statistical technique. In addition, this study design is particularly suitable for assessing the early effects of a policy change and not later effects as it is exposed to selection-maturation interaction which poses a threat to its internal validity. Our study utilized data from all states in the United States from randomly selected participants and this makes this generalizable to the US population. Other limitations of the study include the fact that the data had already been collected and thus restrictive on the inferences and assessments that can be made as the variables were already pre-determined. In addition to this, recall bias may be a concern as responses were dependent on subjects being able to recall details from the past year. Also, selection bias is possible as people who respond to surveys may be different from those people who chose to ignore the survey. Both of which may lead to a loss of internal validity.

CONCLUSION

In conclusion, Medicaid expansion under the ACA has led to an overall increase in self-reported use of CRC screening tests by adults aged 50-64 years in the United States. This finding was consistent across low-income populations, but not across all races or levels of education. Further analysis is needed to investigate other barriers to CRC screening that exist in Black and other Non-Hispanic multiracial groups including psychosocial and economic determinants of CRC screening choices.



DOI: 10.4251/wjgo.v15.i9.1653 **Copyright** ©The Author(s) 2023.

Figure 1 Factors that may be promoters and/or inhibitors of healthcare disparities. ACA: Affordable care act.

ARTICLE HIGHLIGHTS

Research background

Wide disparities exist in access to screening, management, treatment and outcomes of colorectal cancer (CRC) in the United States. With many barriers previously described, various health policies and interventions have been designed to address these disparities. With the passage of the Affordable Care Act about a decade ago, many researchers have shown that Medicaid expansion has led to an increase in insurance coverage but the actual utlization of this newly gained access especially by low-income populations and minority groups remain poorly described in the era of Medicaid expansion.

Research motivation

There are many factors at play in understanding healthcare disparities and outcomes including the interplay between individual and societal factors.

Research objectives

To investigate the effect of Medicaid expansion on low-income populations and minorities on utilization of access to various colon cancer screening modalities. Understanding utilization after Medicaid expansion is key in further decreasing gaps and barriers in CRC screening in the United States.

Research methods

Our study used a quasi-experimental design (a "natural" experiment) given that only some states expanded Medicaid while others did not. Data was from the Behavioral Risk Factor Surveillance System for the period 2011 to 2016. The treatment variable for this study was Medicaid expansion status. A difference-in-differences technique was used to analyze the effect of Medicaid expansion status on the utilization of access to colorectal screening. Other secondary analysis included stratification of the access by ethnicity/race, income, and education status.

Research results

States that expanded Medicaid showed a greater increase in utilization of access to CRC screening. Among minority populations, our analysis revealed that Hispanics showed a greater statistically significant increase in utilization of access but not Non-Hispanic Blacks, or Multiracial. Low-income participants showed a higher change in access and utilization between the expansion periods compared with higher income groups. There was an increase in utilization across all educational levels particularly among those who reported having a high school graduate degree or more.

Research conclusions

We conclude that Medicaid expansion under the ACA was associated with an overall increase in self-reported use of CRC screening tests by adults aged 50-64 years in the United States. This finding was consistent across low-income populations, but not across all races or levels of education. We suggest that despite equally gained access by low-income populations in expansion states, there may be other barriers to CRC screening that exist in Black and other Non-Hispanic

multiracial groups including psychosocial and economic determinants of CRC screening choices.

Research perspectives

Future studies should consider investigating economic determinants of CRC screening choices in minority populations.

FOOTNOTES

Author contributions: Fletcher G designed the study and performed the analysis; Fletcher G, Culpepper-Morgan J, Genao A, and Alatevi E were involved in the initial draft and final version of the manuscript.

Institutional review board statement: This study was deemed by our institution's IRB as exempt. The data used is from the Behavioral Risk Factor Surveillance System (BRFSS) which is a de-identified and a publicly available dataset.

Informed consent statement: Not applicable given that data is de-identified and publically available. Study is deemed IRB exempt.

Conflict-of-interest statement: Disclosures of financial arrangements by authors: No conflicts of interest exist. Funding sources, funding sources or institutional or corporate affiliations by authors: None; Grant support: None Writing Assistance: None.

Data sharing statement: Technical appendix, statistical code and dataset available from corresponding author at pkfletcher@gmail.com.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: United States

ORCID number: Gerald Fletcher 0009-0005-0463-6211.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **US Preventive Services Task Force**, Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, Donahue KE, Doubeni CA, Krist AH, Kubik M, Li L, Ogedegbe G, Owens DK, Pbert L, Silverstein M, Stevermer J, Tseng CW, Wong JB. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2021; **325**: 1965-1977 [PMID: 34003218 DOI: 10.1001/jama.2021.6238]

2 **Jones RM**, Devers KJ, Kuzel AJ, Woolf SH. Patient-reported barriers to colorectal cancer screening: a mixed-methods analysis. *Am J Prev Med* 2010; **38**: 508-516 [PMID: 20409499 DOI: 10.1016/j.amepre.2010.01.021]

3 **Wilkins T**, Gillies RA, Harbuck S, Garren J, Looney SW, Schade RR. Racial disparities and barriers to colorectal cancer screening in rural areas. *J Am Board Fam Med* 2012; **25**: 308-317 [PMID: 22570394 DOI: 10.3122/jabfm.2012.03.100307]

4 **Forum on Medical and Public Health Preparedness for Catastrophic Events**; Board on Health Sciences Policy; Board on Health Care Services; Institute of Medicine. The Impacts of the Affordable Care Act on Preparedness Resources and Programs: Workshop Summary. Washington (DC): National Academies Press, 2014. Available from: https://www.ncbi.nlm.nih.gov/books/NBK241401/

5 **Manchikanti L**, Hirsch JA. Patient Protection and Affordable Care Act of 2010: a primer for neurointerventionalists. *J Neurointerv Surg* 2012; **4**: 141-146 [PMID: 21990501 DOI: 10.1136/neurintsurg-2011-010036]

6 **Rosenbaum S**, Westmoreland TM. The Supreme Court's surprising decision on the Medicaid expansion: how will the federal government and states proceed? *Health Aff (Millwood)* 2012; **31**: 1663-1672 [PMID: 22869643 DOI: 10.1377/hlthaff.2012.0766]

7 **Current Status of State Medicaid Expansion Decisions**. The Henry J. Kaiser Family Foundation. [cited 20 July 2019]. Available from: https://www.kff.org/health-reform/slide/current-status-of-the-medicaid-expansion-decision/

8 **Elwood TW**. Aftermath of the Supreme Court ruling on health reform. *Int Q Community Health Educ***32**: 267-281 [PMID: 23376755 DOI: 10.2190/IQ.32.4.b]

9 **Zerhouni YA**, Scott JW, Ta C, Hsu PC, Crandall M, Gale SC, Schoenfeld AJ, Bottiggi AJ, Cornwell EE 3rd, Eastman A, Davis JK, Joseph B, Robinson BRH, Shafi S, White CQ, Williams BH, Haut ER, Haider AH. Impact of the Affordable Care Act on trauma and emergency general surgery: An Eastern Association for the Surgery of Trauma systematic review and meta-analysis. *J Trauma Acute Care Surg* 2019; **87**: 491-501 [PMID: 31095067 DOI: 10.1097/TA.0000000000002368]

10 **Kamermayer AK**, Leasure AR, Anderson L. The Effectiveness of Transitions-of-Care Interventions in Reducing Hospital Readmissions and Mortality: A Systematic Review. *Dimens Crit Care Nurs* 2017; **36**: 311-316 [PMID: 28976480 DOI: 10.1097/DCC.0000000000000266]

11 **Sterbenz JM**, Chung KC. The Affordable Care Act and Its Effects on Physician Leadership: A Qualitative Systematic Review. *Qual Manag*

Health Care 2017; **26**: 177-183 [PMID: 28991812 DOI: 10.1097/QMH.0000000000000146]

12 **Hendryx M**, Luo J. Increased Cancer Screening for Low-income Adults Under the Affordable Care Act Medicaid Expansion. *Med Care* 2018; **56**: 944-949 [PMID: 30199428 DOI: 10.1097/MLR.0000000000000984]

13 **Han X**, Yabroff KR, Ward E, Brawley OW, Jemal A. Comparison of Insurance Status and Diagnosis Stage Among Patients With Newly Diagnosed Cancer Before vs After Implementation of the Patient Protection and Affordable Care Act. *JAMA Oncol* 2018; **4**: 1713-1720 [PMID: 30422152 DOI: 10.1001/jamaoncol.2018.3467]

14 **Zerhouni YA**, Trinh QD, Lipsitz S, Goldberg J, Irani J, Bleday R, Haider AH, Melnitchouk N. Effect of Medicaid Expansion on Colorectal Cancer Screening Rates. *Dis Colon Rectum* 2019; **62**: 97-103 [PMID: 30407931 DOI: 10.1097/DCR.0000000000001260]

15 **Centers for Disease Control and Prevention**. Behavioral Risk Factor Surveillance System Questionnaire Behavioral Risk Factor Surveillance System 2012 Questionnaire Table of Contents. 2012. Available from: https://www.cdc.gov/brfss/index.html

16 **Siba Y**, Culpepper-Morgan J, Schechter M, Alatevi E, Jallow S, Onaghise J, Sey A, Ozick L, Sabbagh R. A decade of improved access to screening is associated with fewer colorectal cancer deaths in African Americans: a single-center retrospective study. *Ann Gastroenterol* 2017; **30**: 518-525 [PMID: 28845107 DOI: 10.20524/aog.2017.0155]

17 **Han X**, Nguyen BT, Drope J, Jemal A. Health-Related Outcomes among the Poor: Medicaid Expansion vs. Non-Expansion States. *PLoS One* 2015; **10**: e0144429 [PMID: 26720311 DOI: 10.1371/journal.pone.0144429]

18 **Fletcher G**, Culpepper-Morgan J, Genao A, Alatevi E. Disparities in colorectal cancer screening: the effect of Affordable Care Act's (ACA's) Medicaid expansion on Minorities and Low-income populations. *Gastroenterology* 2019; **156**: S-183 [DOI: 10.1016/S0016-5085(19)37248-8]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1662-1672

DOI: 10.4251/wjgo.v15.i9.1662

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Fibrinogen-to-albumin ratio predicts overall survival of hepatocellular carcinoma

Hao Sun, Jie Ma, Jian Lu, Zhi-Hong Yao, Hai-Liang Ran, Hai Zhou, Zhong-Qin Yuan, Yun-Chao Huang, Yuan-Yuan Xiao

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Ding H, China; Ker CG, Taiwan

Received: May 31, 2023
Peer-review started: May 31, 2023
First decision: July 23, 2023
Revised: August 4, 2023
Accepted: August 18, 2023
Article in press: August 18, 2023
Published online: September 15, 2023

Hao Sun, Hai-Liang Ran, Yuan-Yuan Xiao, NHC Key Laboratory of Drug Addiction Medicine, School of Public Health, Kunming Medical University, Kunming 650500, Yunnan Province, China

Jie Ma, Jian Lu, Zhi-Hong Yao, Hai Zhou, Zhong-Qin Yuan, Yun-Chao Huang, The Third Affiliated Hospital, Kunming Medical University, Kunming 650500, Yunnan Province, China

Corresponding author: Yuan-Yuan Xiao, PhD, Professor, NHC Key Laboratory of Drug Addiction Medicine, School of Public Health, Kunming Medical University, No.1168 West Chunrong Road, Yuhua Street, Chenggong District, Kunming 650500, Yunnan Province, China. 33225647@qq.com

Abstract

BACKGROUND

Fibrinogen-to-albumin ratio (FAR) has been found to be of prognostic significance for several types of malignant tumors. However, less is known about the association between FAR and survival outcomes in hepatocellular carcinoma (HCC) patients.

AIM

To explore the association between FAR and prognosis and survival in patients with HCC.

METHODS

A total of 366 histologically confirmed HCC patients diagnosed between 2013 and 2018 in a provincial cancer hospital in southwestern China were retrospectively selected. Relevant data were extracted from the hospital information system. The optimal cutoff for baseline serum FAR measured upon disease diagnosis was established using the receiver operating characteristic (ROC) curve. Univariate and multivariate Cox proportional hazards models were used to determine the crude and adjusted associations between FAR and the overall survival (OS) of the HCC patients while controlling for various covariates. The restricted cubic spline (RCS) was applied to estimate the dose-response trend in the FAR-OS association.

RESULTS

The optimal cutoff value for baseline FAR determined by the ROC was 0.081. Multivariate Cox proportional hazards model revealed that a lower baseline

serum FAR level was associated with an adjusted hazard ratio of 2.43 (95% confidence interval: 1.87–3.15) in the OS of HCC patients, with identifiable dose-response trend in the RCS. Subgroup analysis showed that this FAR-OS association was more prominent in HCC patients with a lower baseline serum aspartate aminotransferase or carbohydrate antigen 125 level.

CONCLUSION

Serum FAR is a prominent prognostic indicator for HCC. Intervention measures aimed at reducing FAR might result in survival benefit for HCC patients.

Key Words: Fibrinogen-to-albumin ratio; Hepatocellular carcinoma; Overall survival; Survival analysis; Cox proportional hazards model

Core Tip: It is important to explore the affecting factors of survival for hepatocellular carcinoma (HCC) patients. A receiver operating characteristic curve was used to establish the optimal cutoff value for baseline serum fibrinogen-to-albumin ratio (FAR) in disease diagnosis. Univariate and multivariate Cox proportional risk models were employed to determine the correlation between FAR and overall survival (OS) in HCC patients. Restricted cubic spline was used to estimate dose-response trends in FAR-OS associations. Serum FAR is an important prognostic index of HCC. Effective FAR reduction may benefit HCC patient survival.

Citation: Sun H, Ma J, Lu J, Yao ZH, Ran HL, Zhou H, Yuan ZQ, Huang YC, Xiao YY. Fibrinogen-to-albumin ratio predicts overall survival of hepatocellular carcinoma. *World J Gastrointest Oncol* 2023; 15(9): 1662-1672

URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1662.htm

DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1662

INTRODUCTION

As one of the most common malignant tumors of the digestive system, liver cancer is a burden on human health and social economy due to its insidious onset and poor prognosis. The latest global cancer data released by the International Agency for Research on Cancer in 2020 showed that liver cancer accounted for 4.7% of all new cancer cases and 8.3% of all cancer-related deaths worldwide[1]. In China, liver cancer accounted for 9.0% and 13.0% of new cancer cases and cancer deaths in 2020, respectively[1]. Early diagnosis and treatment of cancer patients are critical. However, since patients with liver cancer only experience minor clinical symptoms in the early stages of the disease, only a minority of them can achieve early diagnosis. A 2018 study noted that the one, three, and five-year survival rates of liver cancer patients in Asian countries were 34.8%, 19%, and 18.1%, respectively, which were comparatively lower than those in the European and North American countries[2].

Considering the less optimistic survival rates of liver cancer patients, it is imperative to explore its potential prognostic factors, especially those that are easy to monitor and allow for a possible intervention. Hepatocellular carcinoma (HCC) is the predominant histological type of liver cancer and accounts for 75%–85% of all liver cancer cases[3,4]. Major risk factors for HCC include viral hepatitis B or C, consumption of aflatoxin-contaminated food, alcohol abuse, and metabolic and endocrine diseases[5,6]. HCC prognosis is related to various factors, such as clinical stage, tumor size, portal vein invasion, and non-alcoholic fatty liver disease[7-9].

In recent years, serum markers and their significance in cancer survival have gradually attracted considerable research interest in the field of cancer epidemiology, probably due to their easy accessibility and low cost. Many serum indicators had been found to be associated with HCC prognosis, such as blood profiling indexes [total bilirubin (TBIL), platelets, and albumin][10], serum enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl aminotransferase, and alkalinephosphatase][11], systemic inflammatory indicators (neutrophil-to-lymphocyte ratio, systemic immune-inflammatory index)[12], and tumor biomarkers [carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), and carbohydrate antigen 19-9 (CA19-9)][10,13-15].

Fibrinogen is a glycoprotein synthesized by hepatic epithelial cells. Elevated plasma fibrinogen level has been shown to be associated with poorer survival in various malignancies[16]. Albumin is also produced by the liver and is generally used to assess nutritional status. In the advanced stage of cancer, malnutrition and inflammation jointly inhibit albumin synthesis[17]. Therefore, a significant decrease in serum albumin level can predict compromised cancer prognosis[18]. It has been reported that the composite index of fibrinogen and albumin, fibrinogen-to-albumin ratio (FAR), is an independent prognostic factor for progression-free-survival and overall survival (OS) in patients with head and neck squamous cell carcinoma[19]. In another newly published study, Yang *et al*[20] have found that FAR was also related to disease-free survival (DFS) and OS in triple-negative breast cancer patients, with longer median DFS and OS observed in patients with a lower FAR.

In two previously published studies, scholars have evaluated the association between FAR and survival outcomes in early-stage HCC patients who underwent curative resection and found that an elevated FAR was significantly associated with poorer survival and higher risk of recurrence[21]. Nevertheless, the general prognostic significance of serum FAR measured upon disease diagnosis in HCC patients remains unknown. The present study explores this issue in a large sample of HCC patients. The independent association between serum FAR and OS in HCC patients was analyzed and its dose-response trend was estimated.

MATERIALS AND METHODS

Study design

Patients with histologically confirmed HCC between January 1, 2013 and December 31, 2018 at the Third Affiliated Hospital of Kunming Medical University (Yunnan Provincial Cancer Hospital) were retrospectively identified. The hospital had a well-established hospital information system, including a digital clinical information system and a computer-assisted telephone interview follow-up system. In the digital clinical information system, all data related to the clinical practice of inpatients and outpatients are recorded and updated on a daily basis. The following information was extracted from the clinical information system in the present study: Gender, age at diagnosis, ethnicity, cigarette smoking status, alcohol drinking status, body mass index (BMI), clinical stage, and serum indicator levels measured at diagnosis (fibrinogen, albumin, AST, ALT, alpha-fetoprotein (AFP), TBIL, neutrophil-to-lymphocyte ratio (NLR), CEA, CA125, and CA19-9). Information about patient death, such as date and cause, was obtained from the follow-up system. HCC patients with complete required information were included in the final study analysis. The study protocol was approved by the Ethics Review Committee of Kunming Medical University. The requirement for informed consent was waived by the committee due to the retrospective study design.

Variables and definitions

The OS served as the study outcome. Survival interval was defined as the time from the histological diagnosis to the date of death from any cause or the end of follow-up (December 31, 2018), whichever came first. Baseline serum FAR was calculated as serum fibrinogen level divided by serum albumin level measured around the date of disease diagnosis (within seven days prior to or post diagnosis). Because no commonly used cutoffs have been proposed for serum FAR, the receiver operating characteristic (ROC) curve was used to establish the best cutoff value. Other baseline blood indicators to be controlled for, including AST, ALT, AFP, TBIL, NLR, CEA, CA125, and CA19-9, were also extracted from the system using the same time requirements as those for serum FAR. The most commonly adopted cutoffs were employed to dichotomize these blood indicators as follows: 40 U/L for AST and ALT, 17.1 μmol/L for TBIL, 400 ng/mL for AFP[22,23], 5 U/L for CEA, 35 U/L for CA125, and 37 U/L for CA19-9. BMI and serum NLR were dichotomized using their medians.

Statistical analysis

Descriptive statistics were used to illustrate and compare the general characteristics of the participants. The Kaplan-Meier survival curves were plotted for HCC patients based on different baseline FAR levels and compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were employed to evaluate the crude and adjusted associations between baseline serum FAR and the OS of HCC patients. Variables that achieved a less strict significance level ($P < 0.10$) in univariate analyses were incorporated into the subsequent multivariate model. Considering the quantitative nature of FAR, the dose-response trend in its association with the OS of HCC patients was subsequently estimated using the restricted cubic spline (RCS). A two-tailed probability of < 0.05 was deemed statistically significant. Subgroup analyses based on clinical stage, AST level, and CA125 level were further performed. All statistical analyses were carried out in R software (version 4.2.2).

RESULTS

Patient characteristics

A total of 366 histologically confirmed HCC patients were retrospectively identified between 2013 and 2018. The ROC curve determined an optimal cutoff of 0.081 for baseline FAR (Supplementary Figure 1). Therefore, the patients were dichotomized into higher (FAR ≥ 0.081) and lower (FAR < 0.081) FAR groups. General characteristics of the HCC patients are represented and compared in Table 1. Except for age at diagnosis, alcohol drinking status, BMI, and baseline CEA, all other characteristics were statistically different in HCC patients with different baseline serum FAR levels. The median survival time for all patients was 645.00 d (interquartile range: 757.25 d). Compared to patients with a lower baseline FAR level, HCC patients with higher baseline FAR levels (FAR ≥ 0.081) had a significantly shorter median survival length (947.50 *vs*. 530.50 d).

Baseline serum FAR and OS in HCC patients

Figure 1 represents an overview of the OS for HCC patients with higher (≥ 0.081) and lower (< 0.081) baseline FAR levels. Patients with a lower baseline FAR had a superior OS (log-rank = 47.8, $P < 0.01$). The results of univariate and multivariate Cox proportional hazard model fitting results are shown in Table 2. Baseline FAR remained a significant

Table 1 General characteristics of the 366 hepatocellular carcinoma patients

Characteristics	All patients (*n* = 366)			*P* value
Gender				
Male	312 (85.24)[c]	113 (81.88)[c]	199 (87.28)[c]	0.16
Female	54 (14.76)[c]	25 (18.12)[c]	29 (12.72)[c]	
Age at diagnosis	54.59 (10.60)[a]	52.98 (10.83)[a]	55.56 (10.36)[a]	0.02
Cigarette smoking				
No	129 (35.24)[c]	54 (39.19)[c]	75 (32.89)[c]	0.23
Yes	237 (64.76)[c]	84 (60.81)[c]	153 (67.11)[c]	
Alcohol drinking				
No	161 (43.99)[c]	64 (46.38)[c]	97 (42.54)[c]	0.47
Yes	205 (56.01)[c]	74 (53.62)[c]	131 (57.46)[c]	
BMI (kg/m^2)	21.55 (2.71)[b]	21.93 (2.70)[b]	21.32 (2.70)[b]	0.04
Stage				< 0.01
I-II	68 (18.58)[c]	53 (38.41)[c]	15 (6.58)[c]	
III	166 (45.35)[c]	52 (37.68)[c]	114 (50.00)[c]	
IV	132 (36.07)[c]	33 (23.91)[c]	99 (43.42)[c]	
Survival length (d)	645.00 (757.25)[b]	947.50 (1002.25)[b]	530.50 (683.50)[b]	< 0.01
AST (U/L)	66.35 (75.22)[b]	46.50 (48.08)[b]	80.15 (81.30)[b]	< 0.01
ALT (U/L)	43.80 (38.68)[b]	38.95 (28.10)[b]	50.05 (40.93)[b]	0.01
AFP (ng/mL)	593.35 (9018.82)[b]	315.20 (483.18)[b]	743.25 (10910.73)[b]	0.53
TBIL (μmol/L)	17.40 (14.00)[b]	16.35 (13.55)[b]	19.45 (14.13)[b]	0.81
NLR (unit free)	2.75 (2.09)[b]	1.93 (1.29)[b]	3.30 (2.18)[b]	< 0.01
CEA (U/L)	2.80 (3.10)[b]	2.90 (3.11)[b]	2.73 (3.14)[b]	0.56
CA125 (U/L)	34.77 (83.51)[b]	19.63 (30.11)[b]	55.96 (104.58)[b]	0.01
CA19-9 (U/L)	29.50 (67.38)[b]	21.56 (36.71)[b]	36.56 (81.11)[b]	0.09
FAR (unit free)	0.09 (0.05)[b]		-	

[a]mean ± SD;
[b]Median with interquarti le range (IQR);
[c]Frequency with proportion (%).
AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CA125: Carbohydrate antigen 125; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; FAR: Fibrinogen-to-albumin ratio; NLR: Neutrophil-to-lymphocyte ratio; TBIL: Total bilirubin.

prognostic factor after adjusting for possible covariates, while HCC patients with higher FAR levels showed a hazard ratio (HR) of 2.43 [95% confidence interval (95%CI): 1.87–3.15].

To verify the reliability and determine the trend of this association, HCC patients were further divided into four groups based on their baseline serum FAR quartiles as follows: Group 1 (FAR < 0.071), group 2 (0.071 ≤ FAR < 0.093), group 3 (0.093 ≤ FAR < 0.131), and group 4 (FAR ≥ 0.131). After adjustment for potential covariates identified in the previous univariate model, the adjusted HRs for groups 2 to 4 compared to group 1 were 1.07 (95%CI: 0.73–1.57), 1.62 (95%CI: 1.10–2.39), and 1.37 (95%CI: 0.92–2.04), respectively (Figure 2). The RCS fitting results showed that after controlling for the same potential confounders as those in the multivariate Cox proportional hazards model, a nonlinear relationship between baseline FAR levels and HR was observed, with the overall risk of death in HCC patients arising with an increasing baseline FAR. Furthermore, the risk of death was significantly higher when the baseline FAR exceeded 0.093 (Figure 3).

Subgroup analysis

A series of subgroup analyses were performed separately using three important characteristics of the HCC patients, including baseline serum AST, CA125, and clinical stage (Figure 4). Among the three stratified factors, baseline serum AST and CA125 levels presented as noticeable effect modifiers. Specifically, the FAR-OS association tended to be much

Table 2 Univariate and multivariate Cox proportional hazards model fitting results

Covariates	Univariate Cox model		Multivariate Cox model	
	Crude HR (90%CI)	*P* value	Adjusted HR (95%CI)	*P* value
Sex: Male	1.74 (1.30, 2.35)	< 0.01		
Age at diagnosis: + 5 yr	1.01 (0.96, 1.06)	0.72		
Cigarette smoking: Yes	1.51 (1.23, 1.87)	< 0.01		
Alcohol drinking: Yes	1.23 (1.01, 1.50)	0.08		
BMI: + 1 kg/m^2	0.97 (0.93, 1.00)	0.14		
Stage (Ref: I-II)				
III	3.49 (2.46, 4.96)	< 0.01	2.14 (1.37, 3.35)	< 0.01
IV	4.86 (3.40, 6.95)	< 0.01	2.33 (1.45, 3.74)	< 0.01
AST: 40 U/L	2.74 (2.12, 3.53)	< 0.01	1.50 (1.01, 2.22)	0.04
ALT: 40 U/L	1.72 (1.41, 2.10)	< 0.01		
AFP: 400 ng/mL	1.93 (1.58, 2.36)	< 0.01	1.53 (1.19, 1.96)	< 0.01
TBIL: 17.1 μmo/L	1.81 (1.49, 2.20)	< 0.01		
NLR: + 5	1.72 (1.45, 2.05)	< 0.01		
CEA: 5 U/L	1.26 (1.01, 1.57)	< 0.01		
CA125: 35 U/L	2.89 (2.36, 3.55)	< 0.01	1.72 (1.30, 2.27)	< 0.01
CA19-9: > 37 U/L	1.95 (1.60, 2.37)	< 0.01		
FAR: 0.081	2.43 (1.95, 3.02)	< 0.01	1.39 (1.05, 1.83)	< 0.01

90%CI: 90% confidence interval; 95%CI: 95% confidence interval; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CA125: Carbohydrate antigen 125; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; FAR: Fibrinogen-to-albumin ratio; HR: Hazard ratio; NLR: Neutrophil-to-lymphocyte ratio; TBIL: Total bilirubin.

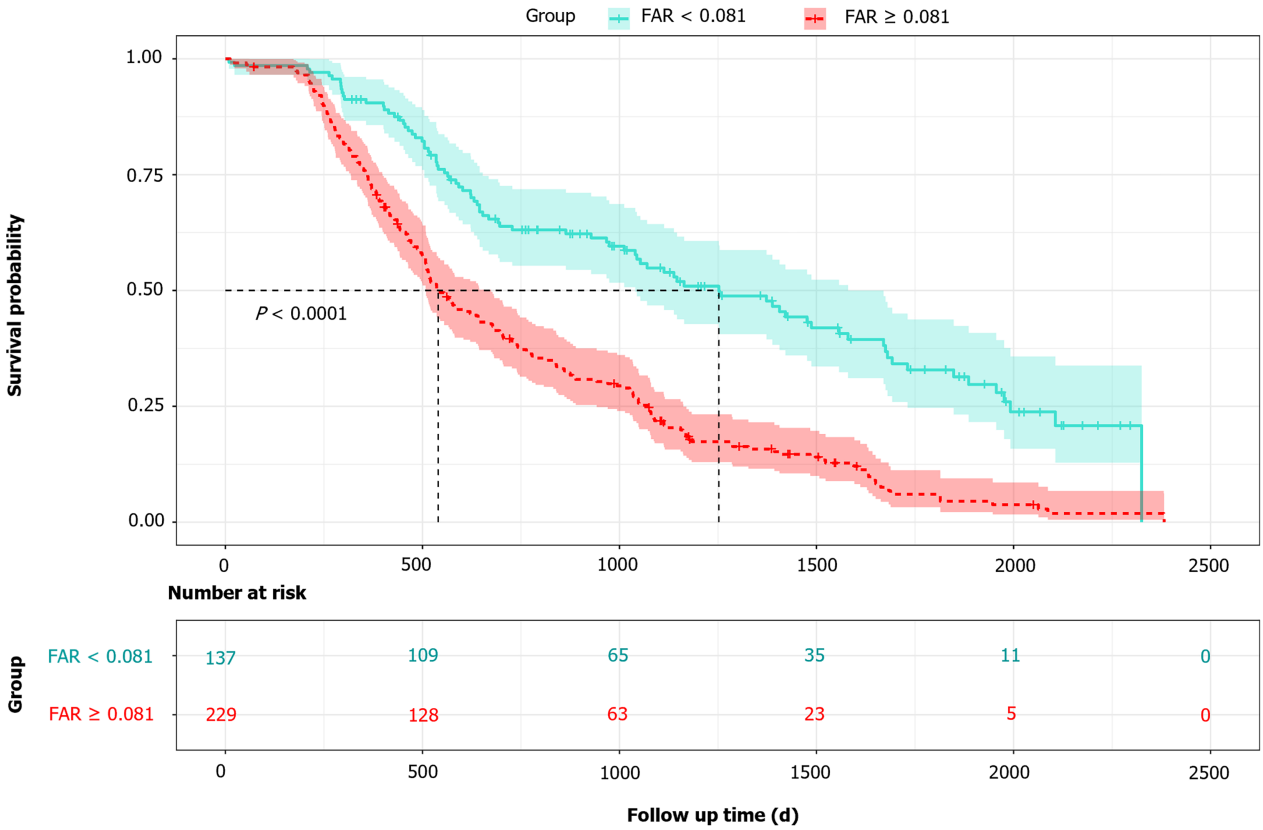
stronger for HCC patients with lower baseline serum AST or CA125 levels.

DISCUSSION

The present retrospective study examined the prognostic role of serum FAR measured upon diagnosis for the OS in 366 HCC patients. As a result, a higher baseline FAR was related to significantly inferior OS in HCC. Further analysis using either FAR quartiles or the RCS revealed a prominent dose-response trend in this association. Subgroup analysis revealed that the FAR-OS association was more significant in patients with lower baseline serum AST or CA125 Levels. These findings may have potential clinical significance in guiding the HCC treatment.

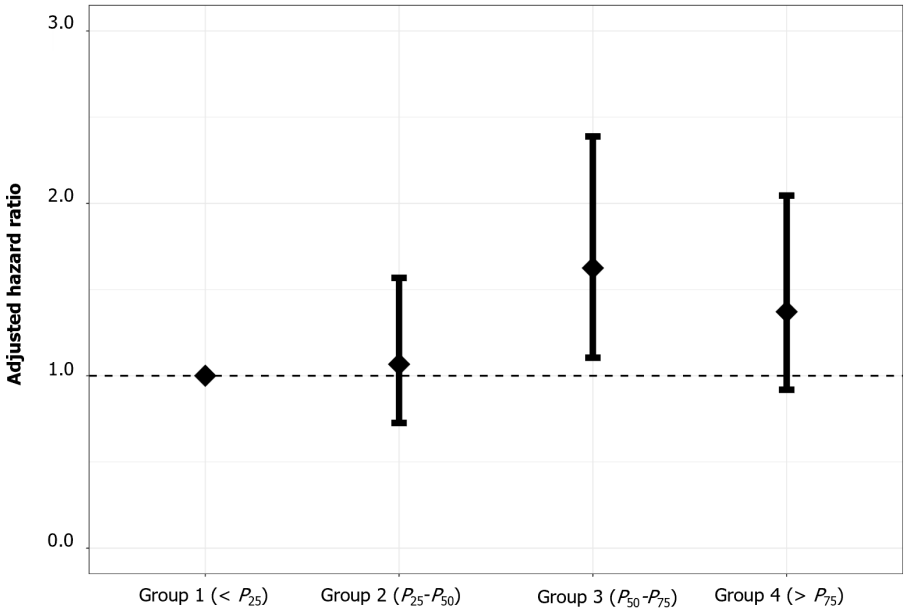
FAR is a composite index. It increases along with an increase in serum fibrinogen level or a decrease in serum albumin level. Elevated serum fibrinogen level may be associated with increased fibrinogen deposition in tumor tissue[24]. It has been found that fibrinogen has strong adhesion to tumor cells. With the help of thrombin, fibrinogen is converted into fibrin, which forms a physical barrier around tumor cells, helps them survive, and plays an important role in cancer progression[25]. Serum albumin has been recognized as not only an important indicator of nutritional status in cancer patients[26,27], but also a tumor suppressor that inhibits matrix metalloproteinase (MMP)-induced invasion and metastasis of HCC by modulating urokinase plasminogen activator surface receptor signaling[28]. In the advanced stages of cancer, albumin level also decreases with the increase in concentrations of other acute phase proteins[29]. These mechanisms can justify the positive connection between elevated serum FAR and compromised OS in HCC patients identified in the present study.

Subgroup analysis suggested that the FAR-OS association appeared to be stronger in patients with lower serum AST or CA125 Levels. AST is an enzyme that reflects liver damage and is often used to evaluate the progression of liver-related diseases. The clearance of serum AST decreases as liver function declines, causing elevated serum AST levels[30]. Elevated serum AST levels have been found to be independently related to inferior survival rates of HCC patients[31]. CA125 is a macromolecular glycoprotein synthesized and stored in the somatic cavity of epithelial cells and is not normally accessible to the circulation. Elevation in this indicator reflects various tumor behaviors that may be associated with severe cell damage, angiogenesis, vascular invasion, and destruction[32]. In some previously published studies, a higher serum CA125 level has been demonstrated to be prominently related to compromised prognosis of liver cancer patients[33]. These findings suggest that even if the reduction in serum FAR can be manipulated, little survival benefit



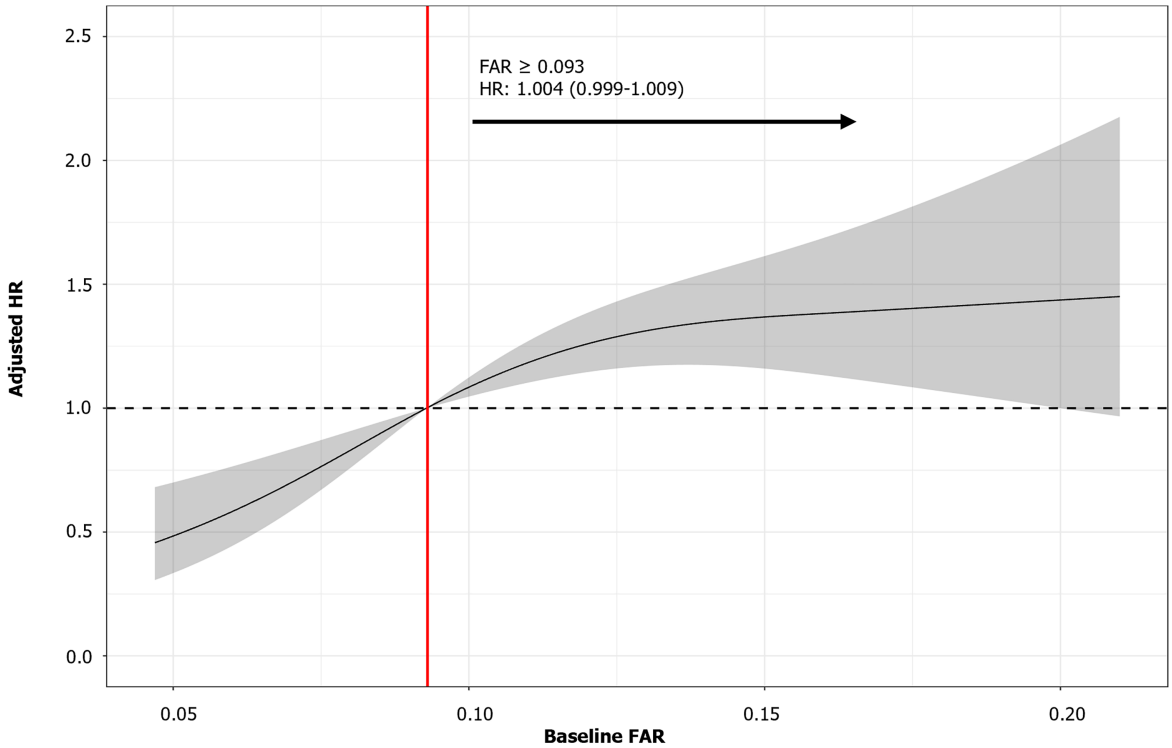
DOI: 10.4251/wjgo.v15.i9.1662 **Copyright** ©The Author(s) 2023.

Figure 1 Kaplan-Meier survival curves for hepatocellular carcinoma patients with different baseline fibrinogen-to-albumin ratio levels. FAR: Fibrinogen-to-albumin ratio.



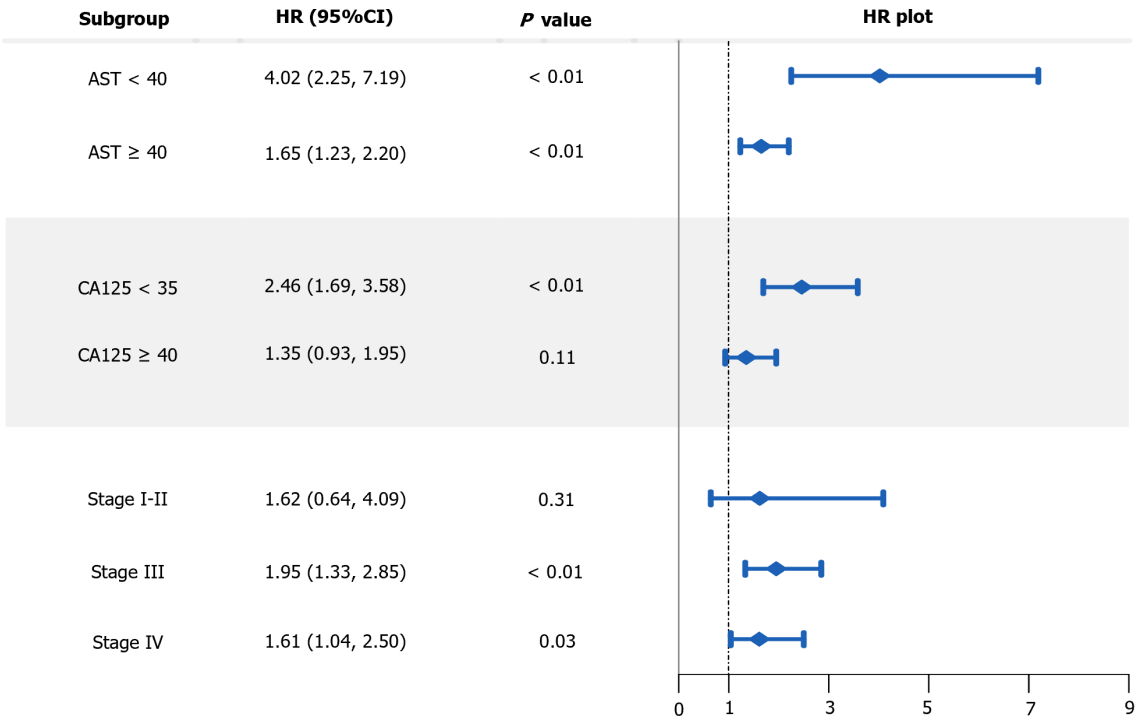
DOI: 10.4251/wjgo.v15.i9.1662 **Copyright** ©The Author(s) 2023.

Figure 2 Dose-response association between baseline fibrinogen-to-albumin ratio and the overall survival of hepatocellular carcinoma by using the quartiles.



DOI: 10.4251/wjgo.v15.i9.1662 **Copyright** ©The Author(s) 2023.

Figure 3 Dose-response relationship between quantitative baseline fibrinogen-to-albumin ratio and the overall survival of hepatocellular carcinoma patients by using the restricted cubic spline. FAR: Fibrinogen-to-albumin ratio; HR: Hazard ratio.



DOI: 10.4251/wjgo.v15.i9.1662 **Copyright** ©The Author(s) 2023.

Figure 4 Subgroup analysis stratified by aspartate aminotransferase, carbohydrate antigen 125 and clinical stage. 95%CI: 95% confidence interval; AST: Aspartate aminotransferase; CA125: Carbohydrate antigen 125; HR: Hazard ratio.

can be achieved in HCC patients with higher AST or CA125 levels.

The present study's major findings highlight the prognostic significance of serum FAR in HCC patients. The decrease in fibrinogen level or increase in albumin level, which result in reduced serum FAR, might be related to improved survival. Many drugs that lower serum fibrinogen level are currently available. For instance, fibrates directly reduce fibrinogen mRNA transcription *in vitro* and *in vivo* through their effect on peroxisome proliferator-activated receptor alpha[34]. It has been observed that benzofibrate significantly reduces serum fibrinogen level, which, in turn, is associated with the anti-tumor effect of benzofibrate as a biomarker or potential mediator[35]. In addition, MMPs and serine proteases contained in snake venom drugs also have a good fibrinogen-lowering effect[36]. Moreover, it has been reported that the median survival of HCC patients can be extended by taking low doses of aspirin[37]. Branched-chain amino acid (BCAA) supplementation is commonly used for correcting malnutrition issues in cancer patients, and it significantly increases serum albumin concentration[38]. It has been reported that BCAA supplementation can also alleviate the impairment of liver function in HCC patients after transarterial chemoembolization[39]. Some literature has reported that the administration of n-3 polyunsaturated fatty acids, especially eicosa-pentaenoic, can also help to maintain serum albumin level in cancer patients[40]. Nonetheless, the effect of either fibrinogen reduction or albumin supplementation aimed at improving the survival outcomes in HCC patients should be further validated by clinical experimental studies.

The main strengths of the present study include its comparatively large sample size. In addition, many potential confounders were simultaneously controlled for when analyzing the FAR-OS association. Furthermore, a dose-response trend revealed by the RCS further corroborated the stability of this FAR-OS association. However, some study limitations were present. First, analytical data were collected retrospectively, introducing a risk of information bias. Second, all HCC patients were from a single institution, which limits the ability to generalize the study results. Multi-centered longitudinal studies are needed to further validate the present study's major findings.

CONCLUSION

In this retrospective study of 366 histologically confirmed HCC patients, serum FAR measured at disease diagnosis was found to be significantly associated with the OS. Patients with a higher baseline FAR had an increased death hazard with a prominent dose-response trend. This FAR-OS association was more prominent in HCC patients with lower serum AST or CA125 levels. The study findings have important implications for clinical treatment of HCC, suggesting that intervention measures aiming at reducing FAR might provide a survival benefit for this group of patients. Prospective studies with more representative samples should be carried out.

ARTICLE HIGHLIGHTS

Research background

Fibrinogen-to-albumin ratio (FAR) has been found significantly associated with survival of some types of cancer. Less is known regarding to its association with prognosis for hepatocellular carcinoma (HCC) patients.

Research motivation

We intend to thoroughly discuss the association between baseline serum FAR and the overall survival (OS) for HCC patients.

Research objectives

To provide estimation for the association between baseline FAR and the OS of HCC patients, and to discuss potential effect modification by some important characteristics of the patients.

Research methods

Retrospective study design was used to identify qualified HCC patients from a provincial cancer hospital in China. Relevant information was extracted from the Hospital Information System. Kaplan-Meier survival curves were plotted to compare the OS of HCC patients with different baseline serum FAR levels. Cox proportional hazards models were applied to estimate the adjusted association between FAR and the OS of HCC patients. The Restricted Cubic Spline was used to further delineate the dose-response association.

Research results

A lower baseline serum FAR level was associated with an adjusted hazard ratio of 2.43 (95% confidence interval: 1.87–3.15) in the OS of HCC patients, with identifiable dose-response trend. The FAR-OS association was more prominent in HCC patients with a lower baseline serum aspartate aminotransferase or carbohydrate antigen 125 level.

Research conclusions

Serum FAR is a prominent prognostic indicator for HCC.

Research perspectives

Intervention measures which aiming at regulating serum FAR might of clinical interest for treating HCC patients.

FOOTNOTES

Author contributions: All authors contributed to the conception and design of the study; the data extraction conditions were determined by Huang YC and Xiao YY; data extraction was performed by Sun H, Ma J, Lu J, Yao ZH, Zhou H, Yuan ZQ; data analysis plan was determined by Xiao YY, and data analysis was performed by Sun H, Ma J, Tan HL; the first draft of the manuscript was written by Sun H and Ma J, and all the authors commented on the previous versions of the manuscript; all authors have read and approved the final manuscript.

Supported by the Basic Research Program of Yunnan, No.202201AT070200; the Top Young Talents of Yunnan Ten Thousand Talents Plan, No. YNWR-QNBJ-2018-286.

Institutional review board statement: The study protocol was approved by the Ethics Review Committee of Kunming Medical University.

Conflict-of-interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data sharing statement: Database of this manuscript can be available from the corresponding author under reasonable request.

Country/Territory of origin: China

ORCID number: Hao Sun 0000-0002-3235-3615; Hai-Liang Ran 0000-0001-7290-3880; Yuan-Yuan Xiao 0000-0003-2441-7209.

S-Editor: Lin C
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Hassanipour S**, Vali M, Gaffari-Fam S, Nikbakht HA, Abdzadeh E, Joukar F, Pourshams A, Shafaghi A, Malakoutikhah M, Arab-Zozani M, Salehiniya H, Mansour-Ghanaei F. The survival rate of hepatocellular carcinoma in Asian countries: a systematic review and meta-analysis. *EXCLI J* 2020; **19**: 108-130 [PMID: 32038120 DOI: 10.17179/excli2019-1842]

3 **Mohammadian M**, Mahdavifar N, Mohammadian-Hafshejani A, Salehiniya H. Liver cancer in the world: epidemiology, incidence, mortality and risk factors. *World Cancer Res J* 2018; **5**: e1082

4 **Karaoğullarından Ü**, Üsküdar O, Odabaş E, Ak N, Kuran S. Hepatocellular Carcinoma in Cirrhotic Versus Noncirrhotic Livers: Clinicomorphologic Findings and Prognostic Factors. *Turk J Gastroenterol* 2023; **34**: 262-269 [PMID: 36688381 DOI: 10.5152/tjg.2023.21791]

5 **Zhang CH**, Cheng Y, Zhang S, Fan J, Gao Q. Changing epidemiology of hepatocellular carcinoma in Asia. *Liver Int* 2022; **42**: 2029-2041 [PMID: 35319165 DOI: 10.1111/liv.15251]

6 **Volc-Platzer B**, Hanak H, Weiss W, Denk H. [Immunohistochemical studies on the association between hepatitis B surface antigen and primary hepatocellular carcinoma (author's transl)]. *Wien Klin Wochenschr* 1982; **94**: 108-114 [PMID: 6281995]

7 **Petrelli F**, Manara M, Colombo S, De Santi G, Ghidini M, Mariani M, Iaculli A, Rausa E, Rampulla V, Arru M, Viti M, Lonati V, Ghidini A, Luciani A, Facciorusso A. Hepatocellular carcinoma in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis: HCC and Steatosis or Steatohepatitis. *Neoplasia* 2022; **30**: 100809 [PMID: 35636146 DOI: 10.1016/j.neo.2022.100809]

8 **Kim TH**, Kim BH, Park JW, Cho YR, Koh YH, Chun JW, Oh ES, Lee DY, Lee SU, Suh YG, Woo SM, Moon SH, Kim SS, Lee WJ. Proton Beam Therapy for Treatment-Naïve Hepatocellular Carcinoma and Prognostic Significance of Albumin-Bilirubin (ALBI) Grade. *Cancers (Basel)* 2022; **14** [PMID: 36139604 DOI: 10.3390/cancers14184445]

9 **Qiu Y**, Yang Y, Wang T, Shen S, Wang W. Efficacy of Postoperative Adjuvant Transcatheter Arterial Chemoembolization in Hepatocellular Carcinoma Patients With Microscopic Portal Vein Invasion. *Front Oncol* 2022; **12**: 831614 [PMID: 35795039 DOI: 10.3389/fonc.2022.831614]

10 **Wu B**, Wu Y, Guo X, Liu Y, Yue Y, Zhao W, Liu J, Wu X, Shen A, Zhang S. Prognostic Significance of Preoperative Integrated Liver Inflammatory Score in Patients with Hepatocellular Carcinoma. *Med Sci Monit* 2022; **28**: e937005 [PMID: 35726168 DOI: 10.12659/MSM.937005]

11 **Chen Y**, Liu H, Zhang J, Wu Y, Zhou W, Cheng Z, Lou J, Zheng S, Bi X, Wang J, Guo W, Li F, Zheng Y, Li J, Cheng S, Zeng Y, Liu J. Prognostic value and predication model of microvascular invasion in patients with intrahepatic cholangiocarcinoma: a multicenter study from

China. *BMC Cancer* 2021; **21**: 1299 [PMID: 34863147 DOI: 10.1186/s12885-021-09035-5]
12 **Li YN**, Qin J, Li WW. Systemic immune-inflammation index in hepatocellular carcinoma patients survival outcomes: a meta-analysis. *Acta Medica Mediterr* 2021; **37**: 129-134 [DOI: 10.19193/0393-6384_2021_1_18]
13 **Huang Y**, Zeng J, Liu T, Lin X, Guo P, Zhou W, Liu J. Prognostic Significance of Elevated Preoperative Serum CA125 Levels After Curative Hepatectomy for Hepatocellular Carcinoma. *Onco Targets Ther* 2020; **13**: 4559-4567 [PMID: 32547086 DOI: 10.2147/OTT.S236475]
14 **Lee CW**, Lin SE, Yu MC, Kou HW, Lee CH, Kuo T, Lee KC, Tsai HI. Does Neutrophil to Lymphocyte Ratio Have a Role in Identifying Cytokeratin 19-Expressing Hepatocellular Carcinoma? *J Pers Med* 2021; **11** [PMID: 34834430 DOI: 10.3390/jpm11111078]
15 **Li H**, Liu R, Qiu H, Huang Y, Liu W, Li J, Wu H, Wang G, Li D. Tumor Burden Score Stratifies Prognosis of Patients With Intrahepatic Cholangiocarcinoma After Hepatic Resection: A Retrospective, Multi-Institutional Study. *Front Oncol* 2022; **12**: 829407 [PMID: 35330712 DOI: 10.3389/fonc.2022.829407]
16 **Zhao G**. Albumin/fibrinogen ratio, a predictor of chemotherapy resistance and prognostic factor for advanced gastric cancer patients following radical gastrectomy. *BMC Surg* 2022; **22**: 207 [PMID: 35643493 DOI: 10.1186/s12893-022-01657-1]
17 **Wu M**, Pan Y, Jia Z, Wang Y, Yang N, Mu J, Zhou T, Guo Y, Jiang J, Cao X. Preoperative Plasma Fibrinogen and Serum Albumin Score Is an Independent Prognostic Factor for Resectable Stage II-III Gastric Cancer. *Dis Markers* 2019; **2019**: 9060845 [PMID: 31781312 DOI: 10.1155/2019/9060845]
18 **Gan W**, Yi Y, Fu Y, Huang J, Lu Z, Jing C, Fan J, Zhou J, Qiu S. Fibrinogen and C-reactive protein score is a prognostic index for patients with hepatocellular carcinoma undergoing curative resection: a prognostic nomogram study. *J Cancer* 2018; **9**: 148-156 [PMID: 29290780 DOI: 10.7150/jca.22246]
19 **Wang S**, Feng Y, Xie Y, Zhao X, Ma J, Liu X, Hu C, Hou T. High fibrinogen-albumin ratio index (FARI) predicts poor survival in head and neck squamous cell carcinoma patients treated with surgical resection. *Eur Arch Otorhinolaryngol* 2022; **279**: 4541-4548 [PMID: 35462579 DOI: 10.1007/s00405-022-07391-x]
20 **Yang Q**, Liang D, Yu Y, Lv F. The Prognostic Significance of the Fibrinogen-to-Albumin Ratio in Patients With Triple-Negative Breast Cancer: A Retrospective Study. *Front Surg* 2022; **9**: 916298 [PMID: 35774393 DOI: 10.3389/fsurg.2022.916298]
21 **Xu Q**, Yan Y, Gu S, Mao K, Zhang J, Huang P, Zhou Z, Chen Z, Zheng S, Liang J, Lin Z, Wang J, Yan J, Xiao Z. A Novel Inflammation-Based Prognostic Score: The Fibrinogen/Albumin Ratio Predicts Prognoses of Patients after Curative Resection for Hepatocellular Carcinoma. *J Immunol Res* 2018; **2018**: 4925498 [PMID: 30027102 DOI: 10.1155/2018/4925498]
22 **Liang L**, Wang MD, Zhang YM, Zhang WG, Zhang CW, Lau WY, Shen F, Pawlik TM, Huang DS, Yang T. Association of Postoperative Biomarker Response with Recurrence and Survival in Patients with Hepatocellular Carcinoma and High Alpha-Fetoprotein Expressions (>400 ng/ml). *J Hepatocell Carcinoma* 2021; **8**: 103-118 [PMID: 33748017 DOI: 10.2147/JHC.S289840]
23 **Zhang J**, Qin SD, Li Y, Lu F, Gong WF, Zhong JH, Ma L, Zhao JF, Zhan GH, Li PZ, Song B, De Xiang B. Prognostic significance of combined α-fetoprotein and CA19-9 for hepatocellular carcinoma after hepatectomy. *World J Surg Oncol* 2022; **20**: 346 [PMID: 36258212 DOI: 10.1186/s12957-022-02806-9]
24 **Garcia MG**, Bayo J, Bolontrade MF, Sganga L, Malvicini M, Alaniz L, Aquino JB, Fiore E, Rizzo MM, Rodriguez A, Lorenti A, Andriani O, Podhajcer O, Mazzolini G. Hepatocellular carcinoma cells and their fibrotic microenvironment modulate bone marrow-derived mesenchymal stromal cell migration in vitro and in vivo. *Mol Pharm* 2011; **8**: 1538-1548 [PMID: 21770423 DOI: 10.1021/mp200137c]
25 **Sharma BK**, Mureb D, Murab S, Rosenfeldt L, Francisco B, Cantrell R, Karns R, Romick-Rosendale L, Watanabe-Chailland M, Mast J, Flick MJ, Whitlock PW, Palumbo JS. Fibrinogen activates focal adhesion kinase (FAK) promoting colorectal adenocarcinoma growth. *J Thromb Haemost* 2021; **19**: 2480-2494 [PMID: 34192410 DOI: 10.1111/jth.15440]
26 **Yang M**, Liu Z, Li G, Li B, Li C, Xiao L, Zhou J. Geriatric Nutritional Risk Index as a Prognostic Factor of Patients with Non-Small Cell Lung Cancer: A Meta-Analysis. *Horm Metab Res* 2022; **54**: 604-612 [PMID: 36070746 DOI: 10.1055/a-1903-1943]
27 **Harimoto N**, Muranushi R, Hoshino K, Yamanaka T, Hagiwara K, Ishii N, Tsukagoshi M, Igarashi T, Watanabe A, Kubo N, Araki K, Shirabe K. Albumin-Indocyanine Green Evaluation (ALICE) grade predicts bile leakage after hepatic resection. *Surg Today* 2020; **50**: 849-854 [PMID: 31938831 DOI: 10.1007/s00595-020-01955-2]
28 **Fu X**, Yang Y, Zhang D. Molecular mechanism of albumin in suppressing invasion and metastasis of hepatocellular carcinoma. *Liver Int* 2022; **42**: 696-709 [PMID: 34854209 DOI: 10.1111/liv.15115]
29 **Almasaudi AS**, Dolan RD, Edwards CA, McMillan DC. Hypoalbuminemia Reflects Nutritional Risk, Body Composition and Systemic Inflammation and Is Independently Associated with Survival in Patients with Colorectal Cancer. *Cancers (Basel)* 2020; **12** [PMID: 32708140 DOI: 10.3390/cancers12071986]
30 **Li M**, Kong YD, Zou JX, Wu XQ, Yin Z, Niu XT, Wang GQ. Transcriptome and physiological analyses reveal the mechanism of the liver injury and pathological alterations in northern snakehead (Channa argus) after dietary exposure to aflatoxin B1. *Aquaculture* 2022; **561**: e738727 [DOI: 10.1016/j.aquaculture.2022.738727]
31 **Zhang LX**, Lv Y, Xu AM, Wang HZ. The prognostic significance of serum gamma-glutamyltransferase levels and AST/ALT in primary hepatic carcinoma. *BMC Cancer* 2019; **19**: 841 [PMID: 31455253 DOI: 10.1186/s12885-019-6011-8]
32 **Zhang M**, Zhang Y, Fu J, Zhang L. Serum CA125 levels are decreased in rectal cancer but increased in fibrosis-associated diseases and in most types of cancers. *Prog Mol Biol Transl Sci* 2019; **162**: 241-252 [PMID: 30905453 DOI: 10.1016/bs.pmbts.2018.12.012]
33 **Meng Q**, Shi S, Liang C, Xiang J, Liang D, Zhang B, Qin Y, Ji S, Xu W, Xu J, Ni Q, Yu X. Diagnostic Accuracy of a CA125-Based Biomarker Panel in Patients with Pancreatic Cancer: A Systematic Review and Meta-Analysis. *J Cancer* 2017; **8**: 3615-3622 [PMID: 29151947 DOI: 10.7150/jca.18901]
34 **Bordbar M**, de Mutsert R, Cevval M, Rosendaal FR, Jukema JW, Lijfering WM. Differential effect of statin use on coagulation markers: an active comparative analysis in the NEO study. *Thromb J* 2021; **19**: 45 [PMID: 34176487 DOI: 10.1186/s12959-021-00299-2]
35 **Iakobishvili Z**, Hasin T, Klempfner R, Shlomo N, Goldenberg I, Brenner R, Kornowski R, Gerber Y. Association of Bezafibrate Treatment With Reduced Risk of Cancer in Patients With Coronary Artery Disease. *Mayo Clin Proc* 2019; **94**: 1171-1179 [PMID: 31272567 DOI: 10.1016/j.mayocp.2018.10.026]
36 **Op den Brouw B**, Ghezellou P, Casewell NR, Ali SA, Fathinia B, Fry BG, Bos MHA, Ikonomopoulou MP. Pharmacological Characterisation of Pseudocerastes and Eristicophis Viper Venoms Reveal Anticancer (Melanoma) Properties and a Potentially Novel Mode of Fibrinogenolysis. *Int J Mol Sci* 2021; **22** [PMID: 34199017 DOI: 10.3390/ijms22136896]
37 **Sitia G**, Iannacone M, Guidotti LG. Anti-platelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1135-1138 [PMID: 23742914 DOI: 10.1016/j.jhep.2013.05.040]

38 **Zeid SA**, Rabiee A, El Refaey FA, Sherif N. Effect of branched chain amino acid supplementation on dialysis adequacy and nutritional parameters in hemodialysis patients. *Saudi J Kidney Dis Transpl* 2020; **31**: 1361-1365 [PMID: 33565448 DOI: 10.4103/1319-2442.308346]

39 **Shiozawa S**, Usui T, Kuhara K, Tsuchiya A, Miyauchi T, Kono T, Asaka S, Yamaguchi K, Yokomizo H, Shimakawa T, Yoshimatsu K, Katsube T, Naritaka Y. Impact of Branched-Chain Amino Acid-Enriched Nutrient on liver Cirrhosis with Hepatocellular Carcinoma Undergoing Transcatheter Arterial Chemoembolization in Barcelona Clinic Liver Cancer Stage B: A Prospective Study. *J Nippon Med Sch* 2016; **83**: 248-256 [PMID: 28133005 DOI: 10.1272/jnms.83.248]

40 **Mocellin MC**, Fernandes R, Chagas TR, Trindade EBSM. A meta-analysis of n-3 polyunsaturated fatty acids effects on circulating acute-phase protein and cytokines in gastric cancer. *Clin Nutr* 2018; **37**: 840-850 [PMID: 28666598 DOI: 10.1016/j.clnu.2017.05.008]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2023 September 15; 15(9): 1673-1674

DOI: 10.4251/wjgo.v15.i9.1673

ISSN 1948-5204 (online)

CORRECTION

Correction to "Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells"

Chuan-Hong Li, Zhang-Ming Chen, Pei-Feng Chen, Lei Meng, Wan-Nian Sui, Song-Cheng Ying, A-Man Xu, Wen-Xiu Han

Specialty type: Oncology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Senchukova M, Russia; Sheykhhasan M, Iran; Yildiz K, Turkey

Received: May 16, 2023
Peer-review started: May 16, 2023
First decision: June 26, 2023
Revised: July 7, 2023
Accepted: July 31, 2023
Article in press: July 31, 2023
Published online: September 15, 2023

Chuan-Hong Li, Zhang-Ming Chen, Pei-Feng Chen, Lei Meng, Wan-Nian Sui, A-Man Xu, Wen-Xiu Han, Department of General Surgery, First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China

Song-Cheng Ying, Department of Immunology, College of Basic Medicine, Anhui Medical University, Hefei 230022, Anhui Province, China

Corresponding author: Wen-Xiu Han, MD, PhD, Professor, Department of General Surgery, First Affiliated Hospital of Anhui Medical University, No. 218 Jixi Avenue, Shushan District, Hefei 230022, Anhui Province, China. hwxhbh@126.com

Abstract

Correction to "Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells". In this article, we found the following error in Figure 3A: The panel image "24 h, sh-RNA1" in the AGS cells wound healing assay was incorrectly inserted during the preparation of the submission; the correct figure is provided in this correction.

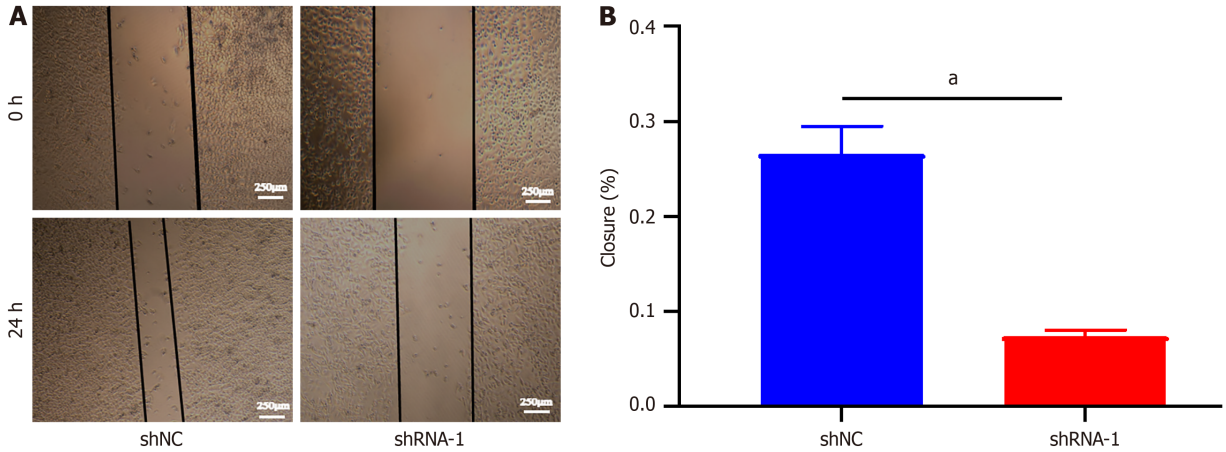
Key Words: Correction; Gastric cancer; Interleukin-34; Proliferation; Epithelial-mesenchymal transition; Metastasis

Core Tip: The aim of this manuscript is to correct the image in Figure 3A of "Li CH, Chen ZM, Chen PF, Meng L, Sui WN, Ying SC, Xu AM, Han WX. Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells. *World J Gastrointest Oncol* 2022; 14: 1968-1980 [PMID: 36310707 DOI: 10.4251/wjgo.v14.i10.1968].

Citation: Li CH, Chen ZM, Chen PF, Meng L, Sui WN, Ying SC, Xu AM, Han WX. Correction to "Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells". *World J Gastrointest Oncol* 2023; 15(9): 1673-1674
URL: https://www.wjgnet.com/1948-5204/full/v15/i9/1673.htm
DOI: https://dx.doi.org/10.4251/wjgo.v15.i9.1673

TO THE EDITOR

Correction to: Li CH, Chen ZM, Chen PF, Meng L, Sui WN, Ying SC, Xu AM, Han WX. Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells. *World J Gastrointest Oncol* 2022; 14: 1968-1980 [PMID: 36310707 DOI: 10.4251/wjgo.v14.i10.1968]. We identified the following error in Figure 3A of the original version of the published article: The image relative to "24 h, sh-RNA1" in the AGS cell wound healing assay was inserted incorrectly during the preparation of the submission. The correct Figure is provided in this correction (Figure 1). This correction will have no influence on the interpretation of the results and conclusion in this study. We apologize for any inconvenience this may have caused.



DOI: 10.4251/wjgo.v15.i9.1673 **Copyright** ©The Author(s) 2023.

Figure 1 Interleukin-34 regulates the migration and invasiveness of AGS cells. A and B: Wound-healing assay revealed that downregulation of endogenous interleukin-34 significantly reduced the migration rate. Data derived from three independent experiments performed in triplicate and expressed as mean ± SD, and $^{a}P < 0.05$ was considered statistically significant.

FOOTNOTES

Author contributions: Li CH wrote the manuscript; All authors approved the submitted version.

Conflict-of-interest statement: There is no conflict of interest in this study.

Country/Territory of origin: China

ORCID number: Chuan-Hong Li 0000-0002-3945-972X; Zhang-Ming Chen 0000-0002-4830-0745; Pei-Feng Chen 0000-0003-2843-425X; Lei Meng 0000-0002-1089-1228; Wan-Nian Sui 0000-0002-4337-5238; Song-Cheng Ying 0000-0002-3446-4482; A-Man Xu 0000-0002-7171-5295; Wen-Xiu Han 0000-0002-5335-9645.

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

REFERENCES

1 **Li CH**, Chen ZM, Chen PF, Meng L, Sui WN, Ying SC, Xu AM, Han WX. Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric cancer cells. *World J Gastrointest Oncol* 2022; **14**: 1968-1980 [PMID: 36310707 DOI: 10.4251/wjgo.v14.i10.1968]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com