**Clinical and Translational Research**

**METTL5 promotes gastric cancer progression *via* sphingomyelin metabolism**

Introduction Methods Result Discussion Conclusion

Zhang Yaqiong, Li Jian, Qin Zhe, Li Deming, Ye Fangzhou, Bei Songhua, Zhang Xiaohong, Li Feng

**Abstract**

**BACKGROUND**

The treatment of gastric cancer (GC) has caused an enormous social burden worldwide. Accumulating studies have reported that m6A is closely related to tumor progression. METTL5 is a m6A methyltransferase that plays a pivotal role in maintaining the metabolic stability of cells. However, its aberrant regulation in GC has not been fully elucidated.

**AIM**

To excavate the role of METTL5 in the development of GC.

**METHODS**

METTL5 expression and clinicopathological characteristics were analyzed *via* The Cancer Genome Atlas (TCGA) dataset and further verified *via* immunohistochemistry, western blotting and real-time quantitative polymerase chain reaction in tissue microarrays and clinical samples. The tumor-promoting effect of METTL5 on HGC-27 and AGS cells was explored *in vitro* by Cell Counting Kit-8 assays, colony formation assays, scratch healing
assays, Transwell assays and flow cytometry. The tumor-promoting role of METTL5 in vivo was evaluated in a xenograft tumor model. The EpiQuik m6A RNA Methylation Quantification Kit was used for m6A quantification. Next, liquid chromatography–mass spectrometry (LC–MS) was used to evaluate the association between METTL5 and sphingomyelin metabolism, which was confirmed by ELISA and rescue tests. In addition, we investigated whether METTL5 affects the sensitivity of GC cells to cisplatin via colony formation and Transwell experiments.

RESULTS
Our research revealed substantial upregulation of METTL5, which suggested a poor prognosis of GC patients. Increased METTL5 expression indicated distant lymph node metastasis, advanced cancer stage and pathological grade. An increased level of METTL5 correlated with a high degree of m6A methylation. METTL5 markedly promotes the proliferation, migration, and invasion of GC cells in vitro. METTL5 also promotes the growth of GC in animal models. METTL5 knockdown resulted in significant changes in sphingomyelin metabolism, which implies that METTL5 may impact the development of GC via sphingomyelin metabolism. In addition, high METTL5 expression led to cisplatin resistance.

CONCLUSION
METTL5 was found to be an oncogenic driver of GC and may be a new target for therapy since it facilitates GC carcinogenesis through sphingomyelin metabolism and cisplatin resistance.

Key Words: Gastric cancer; METTL5; sphingomyelin metabolism; cisplatin

Yaqiong Z, Jian L, Zhe Q, Deming L, Fangzhou Y, Songhua B, Xiaohong Z, Li F. METTL5 promotes gastric cancer progression via sphingomyelin metabolism. World J Gastrointest Oncol 2024; In press
Core Tip: This study revealed that increased METTL5 expression indicates an unfavorable prognosis and advanced clinical stage in patients with GC. METTL5 markedly promotes the proliferation, migration, and invasion of GC cells. METTL5 stimulated the metabolism of sphingomyelin to induce GC tumorigenesis. In addition, METTL5 weakened the sensitivity of GC cells to cisplatin, serving as a novel target for GC treatment.

INTRODUCTION

Gastric cancer (GC) has the fifth-highest number of new cases globally and a high mortality rate due to its high malignancy. One million newly diagnosed cases of GC were reported in 2020 [1,2]. Although excellent progress has been made in terms of treatment strategies, the overall survival of patients with advanced GC has remained unsatisfactory. Chemotherapy resistance continues to be a barrier to the long-term survival of GC patients. Therefore, a detailed characterization of GC carcinogenesis is crucial for identifying effective therapeutic targets.

N6-methyladenosine (m6A) is one of the more than 170 types of RNA modifications that have been identified to date [3,4]. Methyltransferases, demethylases, and methylated reading proteins are the three distinct functional enzymes that carry out the dynamically convertible chemical change known as m6A modification [5]. Mounting evidence has suggested that m6A disorders are involved in different physiological activities, including cell proliferation, cell death [5-9], developmental defects [10], impaired self-renewal ability [11,12], and immune regulatory ability [13-15]. As an important m6A writer, METTL5 affects the metabolic stability of cells by forming a heterodimeric complex with TRMT112 [16,17]. In addition, METTL5 promotes the m6A modification of 18S rRNA, which occurs at position A1832 and plays a vital role in various biological processes [16]. In a METTL5-cKO mouse model, METTL5 was shown to regulate the translation of SUZ12 mRNA, which contributes to the disruption of cardiac cell shape and function induced by pressure overload [18]. METTL5 also plays a fundamental role in mesenchymal stem cell
differentiation by regulating translation \[^{19}\]. Additionally, METTL5 plays a role in the malignant phenotypes of tumors. In uterine corpus endometrial carcinoma, METTL5 promotes tumor development by mediating MMR protein levels \[^{20}\]. Rong et al. reported that METTL5 knockout reduces the proliferative capacity of breast cancer cells by impairing translation initiation \via\ p70-S6K activation \[^{21}\]. Additionally, the loss of METTL5 impairs the assembly of 80S ribosomes, which accelerates the development of hepatocellular carcinoma \[^{22}\]. METTL5 can regulate the translation of USP5, thereby promoting its stability by modulating the ubiquitination of c-Myc, ultimately activating the expression of glycolysis-related genes and driving the progression of liver cancer \[^{23}\]. In addition, METTL5 facilitates the propagation of pancreatic cancer by increasing the translation of c-myc \[^{24}\]. Similarly, upregulation of METTL5 is associated with poor tumor survival in patients with cholangiocarcinoma. The depletion of METTL5 leads to reduced methylation of 18S RNA, which in turn affects the transforming growth factor (TGF)-\(\beta\) pathway, promoting intrahepatic cholangiocarcinoma progression \[^{28}\]. These studies confirmed that METTL5 may serve as a supporter of various tumors. However, the possible function and mode of action of METTL5 in GC are still unknown.

Lipids are significant biomolecules that are extensively distributed in cells. They are involved in a number of physiological activities, including signal transmission, cell motility, division, and membrane composition. Lipids also play unique roles in the mechanism underlying inflammation \[^{26,27}\], autoimmune disease \[^{28,29}\], malignant disease \[^{30}\], and neurodegenerative diseases \[^{31}\]. Recent studies have shown that sphingomyelin metabolic pathway-related products are key regulators of malignant tumor progression. Sphingosine is a fundamental component of the plasma membrane lipid raft domain and has a structural role in cell apoptosis and/or cell proliferation. Sphingomyelin metabolism is important for drug resistance and tumor immune escape \[^{32}\]. SPTLC1 hinders cell growth by coordinating the Akt/FOXO1 axis in renal cell carcinoma \[^{33}\]. C24 ceramide promotes the activation of the mammalian target sirolimus signal, thus promoting the progression of gallbladder cancer \[^{34}\]. Repression of SPT and SPHK1/2 reduces Merkel cell cancer growth \[^{35}\]. SPHK1 enhances cisplatin resistance in bladder
cancer cells through the NONO/STAT3 pathway [37]. In addition, SPHK1 promotes immune escape in the tumor microenvironment by modulating the MTA3-PD-L1 pathway [37]. The regulation of ceramide glycosylation and sphingomyelin metabolism can facilitate the progression of colorectal cancer [38]. However, the fundamental connections between GC incidence and sphingomyelin metabolism are still unknown.

According to our research, METTL5 expression is noticeably greater in GC tissues than in normal tissues, and increased METTL5 expression obviously indicates a poor prognosis in patients with GC. METTL5 deficiency reduces the growth and mobility of GC cells in vitro. METTL5 may support GC via sphingomyelin metabolism. The cisplatin sensitivity of GC cells was enhanced by METTL5 knockdown. To the best of our knowledge, this in-depth study is the first to identify METTL5 as a tumor supporter in GC, and the results may offer new insights into the development of innovative treatments for GC.

MATERIALS AND METHODS

GC tissue collection
The fresh GC tissues and adjacent normal samples (five pairs) used in our study were persevered from June 2022 to December 2023. The patients involved in our study never underwent anticancer treatment. This study was approved from the Ethical Review Board of the Shanghai Minhang Hospital. All the patients involved in our study signed informed consent forms.

Cell lines
GES-1, HGC-27, AGS, BGC-823, and MGC-803 cells were purchased from Shanghai Jinyuan Biotechnology, and 293T cells were obtained from Dong Laboratory. GES-1, HGC-27, AGS, BGC-823, MGC-803, and 293T cells were cultured in DMEM, while MKN-45 cells were cultured in 1640 medium. All cells were cultured in an incubator at 37 °C and 5% CO₂.

Cellular transfection
To create METTL5 overexpression plasmids, full-length METTL5 cDNA was generated and subsequently subcloned and inserted into pTSBX-MCS vectors (Transheep, Shanghai, China). Using an annealing technique, shRNAs targeting METTL5 were created and subcloned and inserted into pLKO.1 (Addgene, Cambridge, MA, USA) to knock down METTL5 (shMETTL5: GTCATTGCATACAGTAATTAT).

**RNA extraction and Q-PCR**

An RNA isolation kit was used for RNA extraction from GC tissues and cells. The procedures for RNA extraction were carried out according to the manufacturer's instructions (Vazyme, Nanjing, China). A PrimeScript RT–PCR kit (Vazyme, Nanjing, China) was used for reverse transcription of the mRNAs. The PCR primers used were as follows (Table 1):

**Table 1 Primer sequences**

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METTL5</td>
<td>AAGGGAGAAGGTTGGGGGTA</td>
<td>GTTCAGGCGACTCTCTTAGT</td>
</tr>
<tr>
<td>TRMT112</td>
<td>GCCACCGAGGTCCGTATCTG</td>
<td>TTTCCACTCACGACGGCAAA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AAGAGGCTTGACAGTG</td>
<td>TTCTCCGAAACGTTGCACG</td>
</tr>
</tbody>
</table>

SYBR Green Master Mix (YEASEN, China) was used for Q-PCR, and the 2^–ΔΔCt method was used to calculate the relative gene expression.

**m6A quantification**

Total RNA was extracted from GC tissues and cells for m6A quantification using the EpiQuik m6A RNA Methylation Quantification Kit (Colorimetric) (EpiGentek, New York, USA) following the manufacturer's instructions. Two hundred nanograms of purified RNA was used in each assay.

**Cell Counting Kit-8 assay**

A total of 3×10^3 cells were seeded in a 96-well plate. Cell viability was assessed using a CCK-8 reagent (Beyotime, China). A mixture of CCK-8 reagent (10 μl) and 90 μl of culture medium was added to each well. The absorbance at 450 nm was recorded every 24 h (0, 24, 48, and 72 h).
Scratch-healing and Transwell assays
For the scratch healing assay, a scratch was created using a 10 μl sterile pipette when the cell density was 80%. Images of the scratches were recorded at 24-h intervals. Invasion assays were completed with Matrigel-covered chambers (Corning, USA) in 24-well plates. A total of $5 \times 10^4$ cells were seeded in the upper chamber with serum-free medium, whereas the lower chamber was placed in medium supplemented with 10% FBS. The traversed cells were fixed with 4% PFA, and images were captured after the cells had incubated for 48 h.

Apoptosis analysis
After digestion, the cells were washed repeatedly with PBS until they reached 80-90% confluence. The cells were tested via flow cytometry following staining with propidium iodide, and FlowJo software (version 10.0.7) was used to evaluate the results.

Liquid chromatography–mass spectrometry (LC/MS)
The lipid metabolism profile of HGC-27 cells was evaluated using ultra-performance liquid chromatography assisted with tandem mass spectrometry (UPLC–MS/MS).

Immunohistochemistry
The sample slides were dewaxed for antigen retrieval, after which endogenous peroxidase activity was blocked. Then, the slides were incubated with a METTL5 (1:50; 16791-1AP; Proteintech) antibody in a 4 °C shaker overnight. Then, horseradish peroxidase and DAB-conjugated secondary antibodies were added to the slides, and the nuclei were stained with hematoxylin. The images were obtained by confocal laser scanning microscopy (Zeiss). The IHC staining procedure for the tissue slices was performed as previously described.

Xenograft tumor model
The xenograft tumor model was established with the approval and guidance of the Institutional Animal Care and Use Committee of Fudan University. HGC-27 cells ($1 \times 10^7$) were subcutaneously administered to 4-week-old BALB/c nude mice. Tumors were weighed and recorded after the mice were euthanized.

Statistical analysis
Continuous and categorical variables were analyzed using Student’s t test and Fisher’s exact test, respectively. The Kaplan–Meier method was used to construct survival curves. All the statistical analyses were performed using GraphPad Prism software (GraphPad Prism, USA). $P < 0.05$ (*) was regarded as statistically significant, and $P$ values $< 0.01$ and $< 0.001$ are marked with $^b$ and $^c$, respectively.

**RESULTS**

METTL5 was significantly upregulated in patients with GC and correlated with unfavorable prognosis.

To investigate the potential influence of METTL5 in GC, we first analyzed METTL5 expression profiles in tumors using the Cancer Genome Atlas (TCGA) database. As shown, we observed that METTL5 was markedly upregulated in diverse cancer tissues compared to normal tissues, particularly in GC samples (Figures 1A, B). As a structural protein that interacts with METTL5, TRMT112 plays an indispensable role in enhancing the function of METTL5 [16]. We further analyzed TRMT112 expression in the TCGA database and discovered that TRMT112 was obviously elevated in various tumor tissues ($P < 0.001$) (Figures 1C, D). Next, we investigated the correlation between METTL5 and TRMT112 in GC. As expected, the correlation coefficient between METTL5 and TRMT112 was 0.4 according to the TCGA database (Figure 1E). Kaplan-Meier analysis demonstrated that elevated METTL5 and TRMT112 mRNA expression was significantly associated with unfavorable clinical outcomes in patients with GC (METTL5, $P < 0.001$; TRMT112, $P < 0.01$) (Figures 1F-G). We also analyzed the potential predictive value of METTL5 and TRMT112 expression in clinical features. The increased expression of METTL5 and TRMT112 implied advanced cancer stage, distant lymph node metastasis, and advanced pathological grade (Figures 1H, I, G; Supplementary Figures 1A, B, C). Next, we verified the expression of METTL5 by a GC tissue microarray, which included 85 pairs of GC and adjacent normal gastric tissues. Immunohistochemistry analysis was also conducted to determine the potential relationship between METTL5 expression and the clinical features of patients with GC (Figure 2A). We found that METTL5 expression
was greater in tumor tissue than in adjacent normal tissue (P < 0.001) (Figure 2B), and elevated METTL5 expression in GC was closely associated with an unfavorable prognosis (Figure 2C). Moreover, the protein levels of METTL5 and TRMT112 in the five pairs of GC tissues were dramatically greater than those in the precancerous tissues, as confirmed by western blot and RT–PCR (Figures 2D, E, F). Furthermore, we also evaluated METTL5 protein levels in gastric epithelial cells (GES-1) and 5 different GC cell lines. Elevated METTL5 Levels were notably observed in HGC-27, AGS, MKN-45, MGC-803, and BGC-823 cells compared to those in GSE-1 cells (Figure 2G). We chose HGC-27 and AGS cells for subsequent experiments. We also detected the degree of m6A methylation in 5 pairs of GC and paracancerous normal tissues. The m6A level in GC tissues was markedly elevated compared with that in precancerous tissues, as detected by the EpiQuik m6A RNA methylation kit (Figure 2H). Overall, METTL5 might support the progression of GC.

**Knockdown of METTL5 represses the malignant phenotype in vitro**

Because of the overexpression of METTL5 in GC tissue, we inferred that METTL5 may facilitate the progression of GC. To explore the possible function of METTL5 in GC, we silenced METTL5 expression in HGC-27 and AGS cells via shRNA. The knockdown efficiency of METTL5 was verified by western blotting and RT–PCR (Figures 3A, B, C). In line with our previous study, the RNA levels of TRMT112 were also decreased after METTL5 deletion (Figures 3D, E). The proliferative ability of cells with METTL5 knockdown was significantly decreased, as detected by CCK8 and colony formation assays (Figures 3F, G, H, I). As expected, we observed marked suppression of invasion with METTL5 knockdown (Figures 3J, M). In addition, the scratch healing assay indicated decreased migration ability in METTL5-knockdown cells (Figures 3K, L). The percentage of apoptotic cells was significantly lower in the METTL5-silenced group than in the control group (Figures 3N, O). Collectively, these results show that METTL5, which serves as a tumor promotor, is involved in GC proliferation, migration, and invasion.

**METTL5 overexpression facilitates the malignant phenotype of GC in vitro**
To determine the oncogenic role of METTL5 in GC, we created a METTL5 overexpression vector and explored the carcinogenic effect of METTL5 overexpression on AGS cells. The efficiency of METTL5 overexpression was validated (Figure 4A), and the mRNA level of METTL5 did not increase (Figure 4B). To our surprise, we observed a corresponding marked increase in the RNA level of TRMT112 with METTL5 overexpression (Figure 4C). Functionally, we observed a dramatic increase in proliferation, as characterized by an accelerated cell growth rate and increased cell colony formation (Figures 4D, E). Cell migration was significantly enhanced by METTL5 overexpression, as reflected by the scratch healing and Transwell assays (Figures 4F, G), of which the results were consistent with previous results. Overall, METTL5 overexpression promoted the malignant phenotype of GC, suggesting that METTL5 plays an indispensable role in GC progression.

**METTL5 inhibits the progression of GC through sphingomyelin metabolism**

To further determine the comprehensive mechanism of METTL5 involvement in GC, we first performed KEGG analysis, in which the patients were divided into high and low groups on the basis of the median METTL5 expression in the TCGA database. The results showed that the sphingomyelin metabolic pathway was considerably enriched in patients with elevated METTL5 expression (Figure 5A). To further verify whether sphingomyelin metabolism participates in the regulatory effect of METTL5 on GC progression, an untargeted liquid chromatography–mass spectrometry (LC–MS) assay was performed. As expected, the lipid metabolite profiles exhibited obvious differences in response to METTL5 knockdown, as did the lipid metabolite profiles, which included sphingosine, an intermediate metabolite of sphingomyelin metabolism (Figure 5B). Bubble diagrams showed that sphingomyelin metabolism was enriched in cells with METTL5 knockdown (Figures 5C, D). The sphingosine concentration was further verified by ELISA and was shown to be reduced in METTL5-knockdown cells and increased in METTL5-overexpressing cells (P < 0.001) (Figure 6A). To gain a deep understanding of the impact of disordered sphingomyelin metabolism on the malignant proliferation of GC cells, we conducted a sphingosine rescue experiment. Rescue experiments were
performed by supplementing METTL5 knockdown GC cells with sphingosine 1 phosphate (s1p). S1p partially restored the proliferative potential of cells caused by METTL5 knockdown, as revealed by CCK-8 and colony formation experiments (Figures 6B, C). We used scratch healing and Transwell experiments to further investigate the effect of supplementing s1p on the migrative and invasive abilities of GC cells. We observed that supplementation with s1p increased the migration area of METTL5-silenced GC cells via a scratch healing motility assay. The number of cells that passed through the Transwell also increased (Figures 6D, E). Various important enzymes are involved in SM metabolism, including CERS6, ACER2, ACER3, CERS2, DEGS1, SGPP2, SMPD1, SPHK1, SPTLC1, SPTLC2, and UGCG. We explored the correlation between METTL5 and sphingomyelin metabolic enzymes in GC patients in the TCGA database (Supplementary Figures 2A-M), and the correlation coefficient between METTL5 and CERS6 or SPTLC1 reached 0.39 (Supplementary Figures 2A, M), suggesting that METTL5 may affect the expression of CERS6 and SPTLC1 in sphingomyelin metabolism. Overall, our results revealed that METTL5 participates closely in the malignant development of GC via sphingolipid metabolism.

METTL5 promotes the progression of GC in vivo

To investigate the carcinogenic functions of METTL5 in GC, we conducted animal experiments in vivo. METTL5-knockdown cells were subcutaneously implanted into male BALB/c nude mice to establish tumor xenografts. After 21 days of normal feeding, METTL5 knockdown inhibited tumor growth, as indicated by a decreased tumor formation rate and decreased tumor volume (Figures 7A, B, C). In contrast, overexpression of METTL5 promoted tumor growth, resulting in significantly greater tumor volume and weight (Figures 7D, E, F). In summary, our experiments demonstrated that METTL5 expression enhances the progression of GC.

METTL5 reduces the sensitivity of GC cells to cisplatin

To gain insight into the potential relationships between chemotherapy resistance and METTL5 expression, our study assessed the proliferation of cisplatin-treated HGC-27 cells. Surprisingly, the cell proliferation rate and colony formation ability were decreased
in HGC-27 cells treated with cisplatin, particularly in HGC-27 cells with METTL5 knockdown (Figure 8A). The migration capacity was also obviously reduced in cisplatin-treated HGC-27 and AGS cells. Furthermore, we revealed that METTL5 deletion combined with cisplatin treatment markedly decreased the migration of GC cells (Figure 8B). Overall, the knockdown of METTL5 enhanced the sensitivity of GC cells to cisplatin, suggesting that METTL5 may be a significant factor affecting the chemotherapeutic resistance of GC cells, further playing a crucial role in the clinical outcomes of patients with GC.

DISCUSSION

Mammalian m6A is the most common RNA modification and is essential for many aspects of life, including mRNA splicing, stability, degradation, nuclear export, translation efficiency, and preservation of mRNA structural stability [39,40,41]. Moreover, 18S rRNA is associated with protein translation and has an irreplaceable role in biological activities [21]. However, the role of METTL5, a methylase of 18S rRNA, in GC has not been determined.

According to our research, METTL5 expression is clearly elevated in GC tissues [42]. A growing body of evidence has shown that m6A participates in the development and occurrence of GC. The downregulation of ALKBH5 suppresses the distant metastasis of GC through PKMYT1 and IGF2BP3 [42]. However, Yuan Fang et al showed that ALKBH5 was upregulated in GC tissues, and a high level of ALKBH5 elevated JAK1 mRNA expression, which activated the JAK1/STAT3 axis to accelerate GC progression [43]. The CBSLR/YTHDF2/CBS signaling pathway may be established by hypoxia-induced increases in CBSLR expression, which could exacerbate GC patients' reactions to chemotherapy [44]. METTL3 contributes to the development of GC by elevating the m6A level of NDUFA4 and enhancing glycolysis and mitochondrial fission [45]. METTL3 also interacts with HDGF mRNA via IGF2BP3 to promote tumor angiogenesis and glycolysis, contributing to tumor growth and liver metastasis [46]. METTL3 also binds to the E-cadherin promoter through the zinc finger protein MYM-type, thereby promoting EMT
and metastasis in GC [47]. Stable binding of METTL3 to PBX1 mRNA increases BH4 expression and accelerates tumor growth [48]. Additionally, YTHDF1 can target engineered small extracellular vesicles for GC therapy via epigenetic and immune regulation [49]. In addition, the downregulation of METTL14 facilitated the growth and migration of GC cells through circORC5 [50]. Elevated METTL16 Levels upregulate cyclin D1 and promote GC [51]. In summary, these investigations demonstrated that the malignant development of GC is significantly influenced by m6A imbalance.

The identification of METTL5 as a methylase that functions on the ribosomal A1832 site is widely acknowledged [16]. METTL5 can activate p70-S6K and initiate translation; the quantity of polymeric ribosomes is clearly decreased when METTL5 is deleted [21]. Mounting evidence has demonstrated that METTL5 plays an instrumental role in various tumors. Loss of METTL5 Leads to impaired 80S ribosomal assembly, which reduces the translation of the ACSL4 mRNA involved in fatty acid metabolism and restrains tumorigenesis in liver cancer [22]. Furthermore, by managing the translation process, METTL5 controls the ubiquitination of c-Myc, which in turn activates a number of downstream genes involved in glycolysis, thereby promoting the malignant development of liver cancer [23]. METTL5 increases HSF4b translation in nasopharyngeal cancer to accelerate carcinogenesis [52]. Our work is the first to demonstrate the effect of METTL5 in GC. We discovered that METTL5 expression was significantly upregulated in various tumors, especially in GC, through the TCGA database. In addition, high METTL5 expression was associated with an unfavorable prognosis in patients with GC. METTL5 is also associated with clinical grade, lymph node metastasis, and pathological grade in patients with GC. In our study, METTL5 was markedly elevated in GC tissues and cell lines. The expression profile of TRMT112, a structural protein that forms a complex with METTL5, was also strongly correlated with the other proteins (r = 0.4). In line with METTL5, TRMT112 was elevated in various tumors and related to a worse prognosis in patients with GC. Gain-and-loss experiments were subsequently performed to determine the oncogenic effects of METTL5 on the growth, migration and invasion abilities of GC cells. Our results showed that proliferation and colony formation were
decreased in METTL5-depleted GC cells. In addition, we found an increased proportion of apoptotic cells following METTL5 knockdown, which is consistent with the findings of previous reports on breast and liver cancer cells\textsuperscript{[22,23,53]}.

One of the most notable metabolic alterations observed in malignancies is a disturbance of lipid metabolism. Tumor progression may be influenced by abnormal lipid metabolism. Lipids are complex molecules that are closely related to the structure, signaling molecules, and energy sources of cell biofilms. In the past few years, numerous studies have investigated the effect of lipid metabolism on tumor progression and metastasis\textsuperscript{[54,55]}, including in pancreatic cancer\textsuperscript{[56]}, breast cancer\textsuperscript{[53,57]}, and colorectal cancer\textsuperscript{[38,59]}. Sphingomyelin, an important lipid component, also has physiological significance. Several reports have shown that sphingomyelin metabolites participate in the malignant behavior of tumors\textsuperscript{[33,34]}. In addition, sphingomyelin metabolism also regulates drug resistance and tumor immune escape\textsuperscript{[60]}. In melanoma, neutral sphingomyelinase 2 improves the effects of anti-immune and anti-PD-1 therapy\textsuperscript{[60]}. In our study, an untargeted LC-MS assay showed that sphingomyelin metabolism was obviously altered in METTL5-deleted GC cells. ELISA verified that sphingosine, a sphingomyelin metabolite, was reduced in METTL5-knockdown cells, whereas the opposite effect was observed in METTL5-overexpressing cells. Furthermore, supplementation with s1p, a product of sphingomyelin, prevented the malignant phenotype caused by METTL5 knockdown. The process of sphingomyelin metabolism requires the involvement of various metabolic enzymes, including CERS6, ACER2, ACER3, CERS2, DEGS1, SGPP2, SMPD1, SPHK1, SPTLC1, SPTLC2, and UGCG. We analyzed the expression of METTL5 and its correlation with the expression of these metabolic enzymes in the TCGA database. Surprisingly, the correlation coefficient between CERS6 and SPTLC1 and between CERS6 and METTL5 reached 0.39. CerS6 was reported to prevent obesity and insulin resistance\textsuperscript{[61]}. The decreased expression of CerS6 caused by downregulation of FTO can promote the progression of ulcerative colitis\textsuperscript{[62]}. Relevant reports on the role of CerS6 in GC are rare. In renal cell carcinoma, decreased SPTLC1 expression was associated with a poorer prognosis\textsuperscript{[63]}. We have not yet published
any research reports on SPTLC1 in other tumors, which means that SPTLC1 is expected to attract increased amounts of attention in tumor research. Our future research will investigate the role of SPTLC1 and explore the mechanism by which METTL5 promotes GC.

Currently, advanced GC is identified in a growing number of individuals. Chemotherapy, targeted therapy, and immunotherapy are the main tools used by patients with advanced GC or surgically removed tumors to slow the growth of the cancer and stop it from spreading to other parts of the body. However, most patients with GC do not benefit from immunotherapy \cite{64,65}. The effect varies depending on the targeted medicine because of the substantial tumor heterogeneity of GC. Chemotherapy remains the mainstay of care for GC patients, as the disease has a recurrence rate of over 50%. Currently, the main chemotherapeutic regimen is platinum-based chemotherapy, which includes cisplatin and oxaliplatin. However, resistance to chemotherapeutic drugs leads to tumor recurrence and metastasis. There are numerous pathways for cisplatin resistance, including decreased intracellular platinum accumulation, increased platinum inactivation, DNA damage repair, and apoptosis inhibition \cite{66}. Chen et al have shown that METTL5 is associated with chemotherapy resistance in nasopharyngeal carcinoma \cite{53}. However, no research has focused on the relationship between METTL5 and cisplatin resistance in GC cells. Our findings indicated that METTL5 knockdown enhanced cisplatin sensitivity, as indicated by a decrease in cell proliferation and migration ability. In our next study, we investigated the potential mechanism of METTL5-mediated changes in the cisplatin sensitivity of GC cells.

There were several limitations in our study. First, additional clinical samples should be included to confirm the associations between METTL5 expression and clinical variables such as TNM stage, pathological stage, lymph node metastasis, and prognosis. Second, the correlation between METTL5 and sphingomyelin levels needs to be verified in clinical samples. Finally, the exact mechanism by which METTL5 affects sphingomyelin metabolism remains elusive. METTL5 was reported to affect protein translation processes \cite{21}. We hypothesized that METTL5 is involved in synthesizing
sphingomyelin metabolism-related enzymes, leading to a reduction in intracellular sphingolipid levels and thereby affecting the malignant phenotype of GC. Ribosome profiling and ribo-seq are required for subsequent exploration. Furthermore, it is important to compare METTL5 expression between cisplatin-resistant and cisplatin-sensitive samples. However, the mechanism by which METTL5 reduces the sensitivity of GC cells to cisplatin requires further investigation. In addition, the efficacy of METTL5 in combination with other chemotherapeutic drugs and immunotherapy for GC is also a very interesting topic.

**CONCLUSION**

In summary, our findings showed that METTL5 promotes the progression of GC. The upregulation of METTL5 implied an unfavorable prognosis and advanced clinical stage in GC patients. METTL5 stimulated the metabolism of sphingomyelin to cause GC tumorigenesis. In addition, METTL5 blunted the sensitivity of GC cells to cisplatin. Therefore, METTL5 is a novel target for GC treatment.

**ARTICLE HIGHLIGHTS**

**Research background**

Previous study reported that METTL5 plays a pivotal role in maintaining the metabolic stability of cells. However, its aberrant regulation in gastric cancer (GC) has not been fully elucidated.

**Research motivation**

To find new targets for the therapy of GC.

**Research objectives**

To excavate the role and potential mechanism of METTL5 in the development of GC.

**Research methods**
The Cancer Genome Atlas (TCGA) dataset, immunohistochemistry, western blotting and real-time quantitative polymerase chain reaction were used to analyse the expression of METTL5. The Cell Counting Kit-8 assays, colony formation assays, scratch healing assays, transwell assays and flow cytometry and xenograft tumor model were used to explore effect of METTL5 on GC. Next, liquid chromatography– mass spectrometry (LC–MS) was used to evaluate the association between METTL5 and sphingomyelin metabolism.

**Research results**

The Cancer Genome Atlas (TCGA) dataset, immunohistochemistry, western blotting and real-time quantitative polymerase chain reaction were used to analyse the expression of METTL5. The Cell Counting Kit-8 assays, colony formation assays, scratch healing assays, transwell assays and flow cytometry and xenograft tumor model were used to explore effect of METTL5 on GC. Next, liquid chromatography– mass spectrometry (LC–MS) was used to evaluate the association between METTL5 and sphingomyelin metabolism.

**Research conclusions**

The upregulation of METTL5 suggested a poor prognosis of GC patients. METTL5 markedly promotes the proliferation, migration, and invasion of GC cells in vitro. METTL5 also promotes the growth of GC in animal models. METTL5 knockdown resulted in significant changes in sphingomyelin metabolism. In addition, high METTL5 expression led to cisplatin resistance.

**Research perspectives**

The mechanism between METTL5 and sphingophospholipid metabolism in gastric cancer is worth further research.
<table>
<thead>
<tr>
<th>Place</th>
<th>Source</th>
<th>Excluded Words</th>
<th>Excluded %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td><a href="http://www.science.gov">science.gov</a></td>
<td>50</td>
<td>1%</td>
</tr>
<tr>
<td>3</td>
<td>Weilong Qu, Xuedong Wei, Haoyu Zhang, Jianquan Hou. &quot;FOXD1-AS1 promotes malignant behaviours of prostate cancer cells via the miR-3167/YWHAZ axis&quot;, <em>Andrologia</em>, 2021</td>
<td>30</td>
<td>1%</td>
</tr>
<tr>
<td>4</td>
<td>Jinming Xu, Yan Wang, Jiahao Jiang, Cong Yin, Bentao Shi. &quot;ADAM12 promotes clear cell renal cell carcinoma progression and triggers EMT via EGFR/ERK signaling pathway&quot;, <em>Journal of Translational Medicine</em>, 2023</td>
<td>13</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>5</td>
<td><a href="http://boris.unibe.ch">boris.unibe.ch</a></td>
<td>13</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>