Clinical and Translational Research

METTL5 promotes gastric cancer progression via sphingomyelin metabolism

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Abstract

BACKGROUND
The treatment of gastric cancer (GC) has caused an enormous social burden worldwide. Accumulating studies have reported that N6-methyladenosine (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 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 was used to evaluate the association between METTL5 and sphingomyelin metabolism, which was confirmed by Enzyme-linked immunosorbent assay 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

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**Core Tip:** This study revealed that increased METTL5 expression indicates an unfavorable prognosis and advanced clinical stage in patients with gastric cancer (GC). METTL5 markedly promoted 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.

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**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 RNA, 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[21] reported that METTL5 knockout reduces the proliferative capacity of breast cancer cells by impairing translation initiation via p70-S6K activation. 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-β pathway, promoting intrahepatic cholangiocarcinoma progression[25]. 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[36]. 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, MKN-45, 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, United States) to knock down METTL5 (shMETTL5: GTCATTCGATACAGTAATTAT).

RNA extraction and quantitative 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 Reverse-transcription PCR (RT-PCR) kit (Vazyme, Nanjing, China) was used for reverse transcription of the mRNAs. The PCR primers used were as follows (Table 1). SYBR Green Master Mix (YEASEN, China) was used for quantitative PCR, and the $2^{-\Delta\Delta Ct}$ 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, United States) following the manufacturer’s instructions. Two hundred nanograms of purified RNA was used in each assay.

Cell Counting Kit-8 assay
A total of $3 \times 10^5$ cells were seeded in a 96-well plate. Cell viability was assessed using a Cell Counting Kit-8 assay (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, United States) 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.
Table 1 Primer sequences

<table>
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<th>Name</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
</tr>
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<tr>
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<td>AAGGGAGAAGGTTGGGGGTA</td>
<td>GTTGCAAGGGCAGCTTCTGTAGT</td>
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<tr>
<td>TRMT112</td>
<td>GCCACCCAGGGTCCGTATCTTG</td>
<td>TTTCACTCTACGAGCCGCAA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AAGGAGCTGAGCAAGTGC</td>
<td>TCTCGAAGATGTCCAGG</td>
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**Liquid chromatography-mass spectrometry**
The lipid metabolism profile of HGC-27shNC and HGC-27shM5 cells was evaluated using ultra-performance liquid chromatography assisted with tandem mass spectrometry.

**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 immunohistochemistry (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 and AGS cells (1 × 10⁶) 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, United States). *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 (Figure 1A and 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) (Figure 1C and 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) (Figure 1F and 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 (Figure 1H-1; Supplementary Figure 1). Next, we verified the expression of METTL5 by a GC tissue microarray, which included 85 pairs of GC and adjacent normal gastric tissues. IHC 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-4). 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 2C). 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 greater than those in the precancerous tissues, as detected by the EpiQuik m6A RNA methylation kit (Figure 2H). Overall, METTL5 might support the progression of GC.

**Knockdown of METTL5 restrains 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 (Figure 3A-C). In line with our previous study, the RNA levels of TRMT112 were also decreased after METTL5 deletion (Figure 3D and E). The proliferative ability of cells with METTL5 knockdown was significantly decreased, as detected by CCK8 and colony formation assays (Figure 3F-I). As expected, we observed marked suppression of invasion with METTL5 knockdown (Figure 3J and K). In addition, the scratch healing assay indicated decreased migration ability in METTL5-knockdown cells (Figure 3L and M). The percentage of apoptotic cells was significantly lower in the METTL5-silenced group than in the control group (Figure 3N and O). Collectively, these results show that METTL5, which serves as a tumor promoter, 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 (Figure 4D and E). Cell migration was significantly enhanced by METTL5 overexpression, as reflected by the scratch healing and Transwell assays (Figure 4F and 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 Kyoto Encyclopedia of Genes and Genomes 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 (Figure 5C and D). The sphingosine concentration was further verified by Enzyme-linked immunosorbent assay (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 (Figure 6B and 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 and E). Various important enzymes are involved in SM metabolism, including CERS6, ACER2, ACER3, CERS2, DEGS1, SGPP2, SMDP1, SPH1, and SPTLC1.
Figure 2 Elevated METTL5 expression were exhibited in patients with gastric cancer. A: Representative immunohistochemistry staining showing METTL5 protein expression in gastric cancer (GC) and paracancerous tissue; B: Staining intensity score of METTL5 in GC tissue microarray; C: Higher expression of METTL5 implied poorer prognosis; D: Western blot of METTL5 and TRMT112 expression in 5 pairs of GC and paracancerous tissue; E and F: The RNA expression of METTL5 and TRMT112 in 5 pairs of GC and paracancerous tissue; G: Western blot of METTL5 expression in gastric mucosal epithelial cells as well as 5 types of GC cells; H: The m6A methylation was measured by m6A RNA Methylation Assay in 5 pairs of GC and paracancerous tissue. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 3 METTL5 knockdown inhibited the malignant phenotype of gastric cancer cells. A: Western blot of METTL5 knockdown efficiency in HGC-27 and AGS cells; B and C: Reverse-transcription PCR of the knockout efficiency of METTL5 in HGC-27 and AGS cells; D and E: RNA expression of TRMT112 in HGC-27 and AGS after METTL5 knockdown; F and G: Cell Counting Kit-8 assay experiment assay to detect the proliferation ability of HGC-27 and AGS cells after METTL5 knockdown; H and I: Clone formation assay to detect the proliferation ability of HGC-27 and AGS cells after METTL5 knockdown; J and K: Tranwell assay to detect invasion ability of HGC-27 and AGS cells after METTL5 knockdown; L and M: A wound-healing motility assay was used to detect the migration ability of HGC-27 and AGS cells after METTL5 knockdown; N and O: Flow cytometry was used to detect the apoptosis of HGC-27 and AGS cells after METTL5 knockdown.

SPTLC2, and UGCG. We explored the correlation between METTL5 and sphingomyelin metabolic enzymes in GC patients in the TCGA database (Supplementary Figure 2), and the correlation coefficient between METTL5 and CERS6 or SPTLC1 reached 0.39 (Supplementary Figure 2), 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 (Figure 7A-C). In contrast, overexpression of METTL5 promoted tumor growth, resulting in significantly greater tumor volume and weight (Figure 7D-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
Figure 4 METTL5 overexpression promoted malignant phenotype in gastric cancer cells. A: Western blot verified the overexpression efficiency of
METTL5 in AGS cells; B: The RNA expression of METTL5 in AGS cell with METTL5 overexpression; C: The RNA expression of TRMT112 in AGS cell with METTL5 overexpression; D: The Cell Counting Kit-8 assay detected the proliferation ability of AGS cell with METTL5 overexpression; E: Clone formation assay to detect the clone formation ability of AGS cell with METTL5 overexpression; F: The wound-healing motility assay to detect the migration ability of AGS cell with METTL5 overexpression; G: Transwell assay to detect the invasion ability of AGS cells with METTL5 overexpression. *P < 0.05, **P < 0.01, ***P < 0.001.

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[39-41]. 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, Fang et al.[43] showed that ALKBH5 was upregulated in GC tissues, and a high level of ALKBH5 elevated AK1 mRNA expression, which activated the AK1/STAT3 axis to accelerate GC progression. 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 NDUF4A4 and enhancing glycosylation and mitochondrial fission[45]. METTL3 also interacts with HDGF mRNA via IGF2BP3 to promote tumor angiogenesis and glycosylation, 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 progression[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 glycosylation, thereby promoting the malignant development of liver cancer[23]. METTL5 increases HSF4α 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[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[54,55], including in pancreatic cancer[36], breast cancer[33,57], and colorectal cancer[58,59]. Sphingomyelin, an important lipid component, also has physiological significance. Several reports have shown that sphingomyelin metabolites participate in the malignant behavior of tumors [33,34]. In addition, sphingomyelin metabolism also regulates drug resistance and tumor immune escape[60]. In melanoma, neutral sphingomyelinase 2 improves the effects of anti-immune and anti-PD-1 therapy[60]. In our study, an untargeted LC-MS assay showed that sphingomyelin metabolism was obviously altered in METTL5-depleted 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, ACERS, CERS2, DEGS1, SGP2, SMPD1, SPHK1, SPTLC1, SPTLC2, and UGCC. 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.
Figure 5 METTL5 promoted gastric cancer progression via sphingomyelin metabolism. A: Kyoto Encyclopedia of Genes and Genomes demonstrated that METTL5 was associated with sphingolipid metabolism; B: Heatmap of differential metabolites in HGC-27shNC and HGC-27shM5 cell; C: Changes in the relative content of differential metabolites in HGC-27shNC and HGC-27shM5 cell; D: Metabolic pathways of differential metabolites in HGC-27shNC and HGC-27shM5 cell.

[61]. The decreased expression of CerS6 caused by downregulation of FTO can promote the progression of ulcerative colitis[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[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[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[66]. Chen et al[52] have shown that METTL5 is associated with chemotherapist resistance in nasopharyngeal carcinoma. 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 tumor-node-metastasis 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[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.
Zhang YQ et al. Introduction methods result discussion conclusion

D

![Images showing comparison of migration rates and cell morphology](image)

E

![Images showing cell morphology](image)
Figure 6 Supplementing s1p could partially rescue the phenotype caused by METTL5 knockdown. A: Enzyme-linked immunosorbent assay to detect the content of sphingosine in HGC-27, AGS cells after METTL5 knockdown and METTL5 overexpression; B: Cell Counting Kit-8 assay to detect cell proliferation ability after addition of s1p in HGC-27 and AGS cells with METTL5 knockdown; C: Clone formation assay to detect cell clone formation ability after the addition of s1p in HGC-27 and AGS cells with METTL5 knockdown; D: The wound-healing motility assay to detect migration formation ability after the addition of s1p in HGC-27 and AGS cells with METTL5 knockdown; E: Transwell assay to detect invasion formation ability after the addition of s1p in HGC-27 and AGS cells with METTL5 knockdown. \( \* P < 0.05, \odot P < 0.01, \odot \odot P < 0.001. \)

Figure 7 Knockdown and overexpression of METTL5 influenced cell growth in vivo. A: METTL5 knockout inhibited the growth of subcutaneous tumors in nude mice (\( n = 5 \)); B and C: The tumor growth volume and weight were measured; D: METTL5 overexpression promoted the growth of subcutaneous tumors in nude mice (\( n = 5 \)); E and F: The tumor growth volume and weight were measured every 3 days. \( \* P < 0.05, \odot P < 0.01, \odot \odot P < 0.001. \)

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.
Figure 8 METTL5 blunted the sensitivity of gastric cancer cells to cisplatin. A: Clone formation assay to detect the effect of cisplatin on the clone formation ability of gastric cancer cells after METTL5 knockdown; B: Transwell assay to detect the effect of cisplatin on the migration ability of gastric cancer cells after METTL5 knockdown. *P < 0.05, **P < 0.01, ***P < 0.001.

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 (IHC), western blotting and real-time quantitative polymerase chain reaction were used to analyze 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
An upregulation of METTL5 suggested a poor prognosis of GC patients. Increased METTL5 expression implied distant lymph node metastasis, advanced cancer stage, pathological grade and a high degree of m6A methylation. METTL5 promotes the proliferation, migration, and invasion of GC cells in vitro. METTL5 knockdown resulted in significant changes in sphingomyelin metabolism. In addition, high METTL5 expression led to cisplatin resistance.

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 sphingomyelin metabolism in GC is worth further research.

FOOTNOTES
Co-corresponding authors: Xiao-Hong Zhang and Li Feng.

Author contributions: Zhang YQ and Feng L designed the framework of the entire article; Zhang XH and Feng L provided funding support; Zhang YQ completed the entire manuscript; and Qin Z, Li DM, Ye FZ, Bei SH completed the experimental part of the article. All authors were involved in the critical review of the results and have contributed to, read, and approved the final manuscript. Zhang XH and Feng L contributed equally to this work as co-corresponding authors. The reasons for designating Zhang XH and Feng L as co-corresponding authors are as followed. Firstly, the research funding was jointly provided by Zhang XH and Feng L; Secondly, Feng L provided extensive guidance on the epidemiology and clinical treatment status of gastric cancer at the beginning of the project design; Zhang XH provided extensive guidance in analyzing experimental data and answering questions. So, Zhang XH and Feng L can serve as co-corresponding authors of this manuscript.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Minhang Hospital Affiliated to Fudan University.

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

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Chen B, Huang Y, He S, Yu P, Wu L, Peng H. N(6)-methyladenosine modification in 18S rRNA promotes tumorigenesis and chemoresistance


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