

RESEARCH ARTICLE

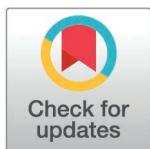
The METTL3 inhibitor STM2457 suppresses gastric cancer progression by modulating m⁶A RNA modification

Hang Sun¹✉, Haozhi Xu^{1,2}✉, Junying Li³✉, Xiaoman Xie¹, Junmei Zhang¹, Hongjie Dong¹, Huanhuan Xie¹, Qi Wang¹, Guihua Zhao¹, Kun Yin¹✉, Jingyu Yang², Jianwei Zhou⁴*, Ruili Wu²*, Chao Xu¹*

1 Shandong Institute of Parasitic Diseases, Shandong First Medical University & Shandong Academy of Medical Sciences, Jining, Shandong, China, **2** Digestive Disease Hospital of Shandong First Medical University, Shandong First Medical University & Shandong Academy of Medical Sciences, Jining, Shandong, China, **3** Instrumentation and Service Center for Science and Technology, Beijing Normal University, Zhuhai, Guangdong, China, **4** Clinical Laboratory, Affiliated Hospital of Jining Medical University, Jining, Shandong, China

✉ These authors contributed equally to this work.

* immunolife@126.com (JZ); wrl3602@hotmail.com (RW); xu0927@hotmail.com (CX)



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Abstract

Gastric cancer (GC) is one of the most common and lethal cancers globally. methyltransferase-like 3 (METTL3)-mediated N⁶-methyladenosine (m⁶A) RNA methylation plays a crucial role in tumor initiation and progression by regulating RNA function. STM2457, a highly efficient METTL3 inhibitor, can inhibit METTL3 activity and may serve as a potential therapeutic strategy in cancers. However, the role of STM2457 for GC cells is still unknown. In this study, we analyzed the expression profile data of GC in TCGA and GEO databases, and further explored the expression involvement of METTL3 in GC cell line, investigated the therapeutic effect of STM2457 targeted inhibition of METTL3 in GC both *in vitro* and *in vivo* experiments. The results indicated that STM2457 could suppress GC cell proliferation and migration by inhibiting METTL3, and also promoted cell apoptosis and arrest the cell cycle in S phase. In addition, STM2457 could inhibit tumor growth in subcutaneous xenotransplantation mouse model. Our findings suggested that STM2457 had great potential for the treatment of GC and could serve as a foundation for future clinical applications.

Introduction

Gastric cancer is a malignant tumor originating from gastric mucosal epithelium. Its incidence rate ranks fifth and mortality ranks third worldwide [1–3]. Due to the difficulty in early detection of gastric cancer, patients in the middle and late stages often experience invasion and metastasis, resulting in poor treatment outcomes and a low 5-year survival rate. Therefore, searching for biomarkers and therapeutic targets

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for early diagnosis of gastric cancer has been a hot research topic in recent years. Despite advancements in diagnosis and treatment over the past few decades, the overall survival rate for GC remains suboptimal. Particularly, for patients diagnosed with distant metastases, the five-year survival rate is still below 5% [4–6]. Currently, the treatment for gastric cancer primarily involves monoclonal antibodies, chimeric antigen receptor T-cell (CAR-T) therapy, bispecific antibodies, and antibody-drug conjugates. Additionally, cancer genes play an essential role in the treatment of gastric cancer. Moreover, epigenetics also influences the onset and progression of GC. Thus, it is crucial to investigate targeted therapies that focus on epigenetics-related molecules in the development of GC.

N⁶-methyladenine (m⁶A) modification is a dynamic and reversible post-transcriptional modification at the RNA level, playing a crucial role in gene expression regulation and various biological processes such as cellular immunity [7,8]. When epigenetic regulation within the biological body becomes dysregulated, it may impact cell growth, life cycle, apoptosis, and other aspects, potentially leading to the activation or inhibition of oncogenes and ultimately resulting in cancer [9–11]. Emerging evidence suggests that m⁶A methyltransferase can lead to various types of cancer, such as pancreatic ductal adenocarcinoma, by altering the morphology of RNA [12,13]. Meanwhile, studies have found that the inactivation of three m⁶A methyltransferases, namely methyltransferase like 3 (METTL3), methyltransferase class 14 (METTL14), and Wilms tumor associated protein 1 (WTAP), can affect tumor progression [14,15]. As the most critical factor in the m⁶A methylation process, METTL3 has been continuously linked to various cancers and exhibits overexpression, playing an important role in regulating tumor occurrence and development [16–18]. Previous studies have explored various treatment strategies for gastric cancer from different perspectives, including using natural products [19] (such as p-hydroxycinnamic aldehyde) to regulate multiple pathways, and activating the TP53 signaling through bioactive peptides to induce apoptosis [20], etc. These efforts suggest that targeting key regulatory nodes is an important direction for intervention in gastric cancer. Based on this, this study focuses on the global regulatory mechanism of METTL3-mediated m⁶A RNA modification at the level of the epigenetic transcriptome, aiming to provide new evidence for expanding targeted treatment for gastric cancer.

STM2457 is a newly discovered small molecule compound, acting as a METTL3 inhibitor with *in vivo* activity. Structural analysis and enzyme activity experiments have revealed that STM2457 can directly bind to the S-adenosylmethionine (SAM) binding site of METTL3, resulting in the inhibition of METTL3's methyltransferase activity without affecting other methyltransferases. As a result, the m⁶A level is reduced, which affects the translation of m⁶A-positive genes. This selective effect may provide new possibilities for the development of drugs to treat related diseases. The clinical heterogeneity of gastric cancer [21] and the heterogeneity of the tumor microenvironment [22] jointly highlight the complexity and treatment challenges of this disease. Therefore, targeting METTL3-m⁶A, a key mechanism that plays extensive regulatory roles in post-transcriptional modification, may provide new synergistic

strategies for intervening at different levels of gastric cancer progression. Previous studies have demonstrated that STM2457 can intervene the progression of acute myeloid leukemia and have authenticated its activity *in vivo* experiments [23]. Additionally, it has been found that STM2457 can inhibit the proliferation of non-small cell lung cancer and reverse chemotherapy resistance [24]. Due to these promising findings, STM2457 has attracted significant attention in the field of RNA methylation modification. However, there is no relative report on whether it can be used as a treatment for other cancer cells including GC.

The present study aimed to analyze the expression profile of METTL3 in GC, and explore the pathways involved in METTL3 in GC. Based on the analysis, we have detected the therapeutic effect of STM2457 both *in vivo* and *in vitro*, in order to provide evidence for the future clinical applications of the specific METTL3 inhibitor STM2457.

Materials and methods

Cell line and culture

Human gastric epithelial GES-1 cells, human gastric cancer AGS, BGC-823, and HGC-27 cells were obtained from the central laboratory of the Shandong Institute of Parasitic Diseases. The GSE-1, AGS, HGC-27, and BGC-823 cells were cultured in RPMI-1640 complete medium (Gibco; Thermo Fisher Scientific, Inc.). The medium used for GSE-1, AGS, and BGC-823 cells were supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.), compared to HGC-27 with 20% FBS and 1% penicillin/streptomycin. The cells were incubated at 37°C with 5% CO₂.

Reverse transcription-quantitative (RT-q) PCR

After 24 hours of cell culture, Trizol reagent (AG, China) was added to extract total RNA from the cells, and mRNA was reverse transcribed into cDNA, followed by PCR reaction. Using GAPDH as an internal reference gene and 2^{-ΔΔCt} method calculates the relative expression level of the target gene. The primer sequences were as follows: METTL3 sense, 5'-TTGTCTCCAACCTTCGCTAG-3' and antisense, 5'-CCAGATCAGAGAGGTGGTGTAG-3'; GAPDH sense, 5'-TTGG-TATCGTGAAGGACTCA-3' and antisense, 5'-GGATGATGTTCTGGAGAGC-3'.

Cell transfection

For shRNA or plasmid transfection, cells were seeded in appropriate culture plates and grown to 70–80% confluence. Transfection complexes were prepared by diluting the indicated siRNA (e.g., shMETTL3, shControl) or plasmid DNA (e.g., METTL3 overexpression vector, empty vector) in serum-free medium, which was then mixed gently with an appropriate volume of Lipofectamine® 3000 reagent (Invitrogen) according to the manufacturer's protocol. After incubating at room temperature for 15–20 minutes to allow complex formation, the mixture was added dropwise to the cells. The culture medium was replaced with complete growth medium 6 hours post-transfection. Cells were harvested for subsequent functional assays or RNA/protein extraction at 48–72 hours after transfection.

Cell viability assay

Initially, the cells were counted and seeded into the central region of a 96-well plate, with 100 μL per well. The cell count was 1 × 10⁵, and sterile PBS was used to fill the edge wells to prevent evaporation. After 12 hours of cell culture, the old medium was discarded and replaced with a medium without fetal bovine serum; After a 4-hour starvation period, STM2457 (MCE, USA) was added at 0, 24, 48, and 72 hours, followed by the addition of 10 μL of CCK-8 (Beyotime Institute of Biotechnology) solution to each well and continued cultivation for 4 hours; The optical density (OD) value was measured at a wavelength of 450 nm using an enzyme-linked immunosorbent assay reader. Prism software was utilized to calculate and plot the curve graph.

Flow cytometry assay

Apoptosis and cell cycle were detected using the flow cytometry assay. The cells were seeded in 6-well plates, and different concentrations of STM2457 were added to each well. After 36 hours, the cells were collected and centrifuged at 1500 rpm, then resuspended with 200 μ L of Annexin V-FITC (Beyotime Institute of Biotechnology, China), and stained with 10 μ L of PI staining solution (Beyotime Institute of Biotechnology, China) in the dark. For cell cycle detection, the cells were dyed by PI, then cells were detected using flow cytometry.

Cell scratch experiment

The cells were cultured in 6-well plates to 80%. Then, a 200 μ L of volume sterile pointed straw was used to vertically draw several equidistant linear gaps at the cell monolayer. After that, the cells were washed twice with PBS, and the corresponding serum-free medium was added. For the control, STM2457 and DMSO solvent were added according to the experimental grouping. The cell image at the scratch was immediately taken and the reference data at time point 0 were recorded. After 48 hours of culturing, the cell image was taken again, to measure the changes before and after the scratch, as well as the cell migration rate.

Bioinformatics analysis

The expression level of METTL3 was analyzed using the TCGA database (<https://www.cancer.gov/aboutnci/organization/ccg/research/structural-genomics/tcga>). Additionally, the UALCAN website (<http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl>) was used to further analyze the expression level of METTL3 in GC. For survival analysis, the Kaplan-Meier Plotter (<http://kmplot.com/analysis/index.php?p=service&cancer=gastric>) was employed. Initially, the genes were ranked according to their absolute mean values, from largest to smallest. The top 5,000 genes were then selected to construct a weighted gene co-expression network (WGCNA) for METTL3 expression. The independence and average connectivity of the co-expressed modules were calculated using the gradient method, with a soft threshold from 1 to 20. The optimal soft threshold, corresponding to an independence of 0.9, was chosen to construct a scale-free topological network. The adjacency matrix was subsequently converted into a topological overlay matrix, which was used to measure the connectivity of the gene network. Next, the dissTOM function was used to generate a hierarchical clustering tree diagram of genes, and genes with comparable expression patterns are grouped into a module comprising at least 50 genes through the dynamic cut-tree function. Efficient combination of modules with a similarity of more than 0.75 was conducted. For KEGG enrichment analysis, using the R package clusterprofiler was performed. A P value less than 0.05 was considered statistically significant.

Animal model

Nude mice were purchased from Jinan Xingkang Biotechnology Co., Ltd. Female nude mice (4–5 weeks old, approximately 20 g) were used in the experiment. A total of 12 mice were randomly divided into treatment and control groups. All research staff involved in animal handling, monitoring, and euthanasia received specialized training in rodent techniques, humane endpoint recognition, and aseptic procedures. The mice were housed under specific pathogen-free conditions in a laminar airflow cabinet, with temperature maintained at 23 ± 2 °C and humidity at 40%–70%, and were provided with free access to food and water. The mice were anesthetized with isoflurane (4% for induction and 1.5–2% for maintenance in oxygen) and subcutaneously injected in the armpit with 5×10^6 cells resuspended in 100 μ L PBS. Health and behavior were monitored daily throughout the study. To assess the impact of STM2457 on the overall health of mice, we monitored the body weight every 3 days throughout the 25-day experimental period. The data were presented as mean $\pm$ standard deviation, and the comparisons between groups were conducted using the t-test. The complete data can be found in the [S1 Table](#). Tumor volume was measured every 4 days starting from the day when tumors became visible. The treatment group received intraperitoneal injections of STM2457 (10 mg/kg), while the control group received an equal volume of

physiological saline. The experiment lasted 28 days. Humane endpoints were defined as follows: severe lethargy, inability to access food or water, weight loss exceeding 20% of baseline. Once an animal met any endpoint criterion, euthanasia was performed within 4 hours to prevent unnecessary suffering. No animals died spontaneously before the endpoint. On day 28, all remaining mice were deeply anesthetized with an overdose of sodium pentobarbital (150 mg/kg, i.p.) and euthanized by cervical dislocation. All procedures were approved by the Animal Care and Use Committee of Shandong First Medical University (approval number: w202103030088). Tumors were harvested and weighed for further analysis.

Statistical analysis

All statistical data were analyzed using GraphPad Prism 7.0 or R 4.2.1. T-test and one-way ANOVA were used to test the data in the experiment. Three independent replicates were performed for each experiment. P value less than 0.05 was considered statistically significant.

Result

METTL3 was highly expressed in gastric cancer

To explore the potential significance of METTL3 in gastric cancer, we investigated its expression level in gastric cancer. Our analysis of the TCGA database revealed that METTL3 is expressed at higher levels in tumors such as gastric cancer, colon cancer, and esophageal cancer compared to normal tissues. However, there was no significant difference in pancreatic cancer (Fig 1A). Next, we compared non-paired (Fig 1B) and paired (Fig 1C) samples of gastric cancer and found that METTL3 expression in tumor tissue was significantly higher than that in normal tissue, which was consistent with the findings of UALCAN analysis (Fig 1D). Moreover, the Kaplan-Meier Plotter survival analysis demonstrated that high expression of METTL3 was associated with a poor prognosis in gastric cancer patients (Fig 1E). Furthermore, we examined the mRNA expression of METTL3 in gastric cancer AGS, BGC-823, HGC-27 cell lines and gastric normal epithelial GES-1 cells. The qPCR results indicated that compared to gastric epithelial GES-1 cells, the expression of METTL3 was increased in gastric cancer cell lines, with the AGS cells displaying the highest expression (Fig 1F). Therefore, we choose AGS cells to conduct the subsequent studies.

METTL3 was involved in pathways related to tumor proliferation

To explore the role of METTL3 and its potential association with gastric cancer progression, we constructed a gene co-expression network for METTL3 expression by using the R package “WGCNA”. We choose the Asian gastric cancer tumor data GSE62254 to construct a scale-free topological network. We selected the optimal power value of 5, which was corresponded to an independence of 0.85. We combined modules with a similarity greater than 0.75, and finally obtained 10 modules. It was evident that the yellow module ranked first in terms of its correlation with METTL3 expression, suggesting genes within the yellow module might have a strong correlation with METTL3 expression (Fig 2A and B). Then, we conducted KEGG enrichment analysis on the genes within the yellow module and found that the ranked first and second pathways were cell cycle and DNA replication pathways, respectively (Fig 2C). We conducted KEGG enrichment analysis on genes related to METTL3 expression in TCGA, the activation of cell cycle and DNA replication pathways were also highly enriched in the front row (Fig 2D). These findings suggest that METTL3 overexpression may participate in the proliferation of tumor cells through influencing cell cycle progression and DNA replication, thereby affecting the progression of gastric cancer.

STM2457 inhibited cell proliferation and migration by inhibition of METTL3 activity

First, we measured the IC_{50} of STM2457 on gastric cancer AGS cells using the CCK-8 assay, and obtained an IC_{50} value of 14.997 μ M (Fig 3A). To evaluate the m⁶A level in cells treated with STM2457, we conducted an m⁶A dot blot assay in the gastric cancer cell line AGS after 48-hour STM2457 treatment. The results indicated that the global m⁶A modification level in the STM2457 treatment group was significantly lower than that in the control group (S1A Fig). Based on

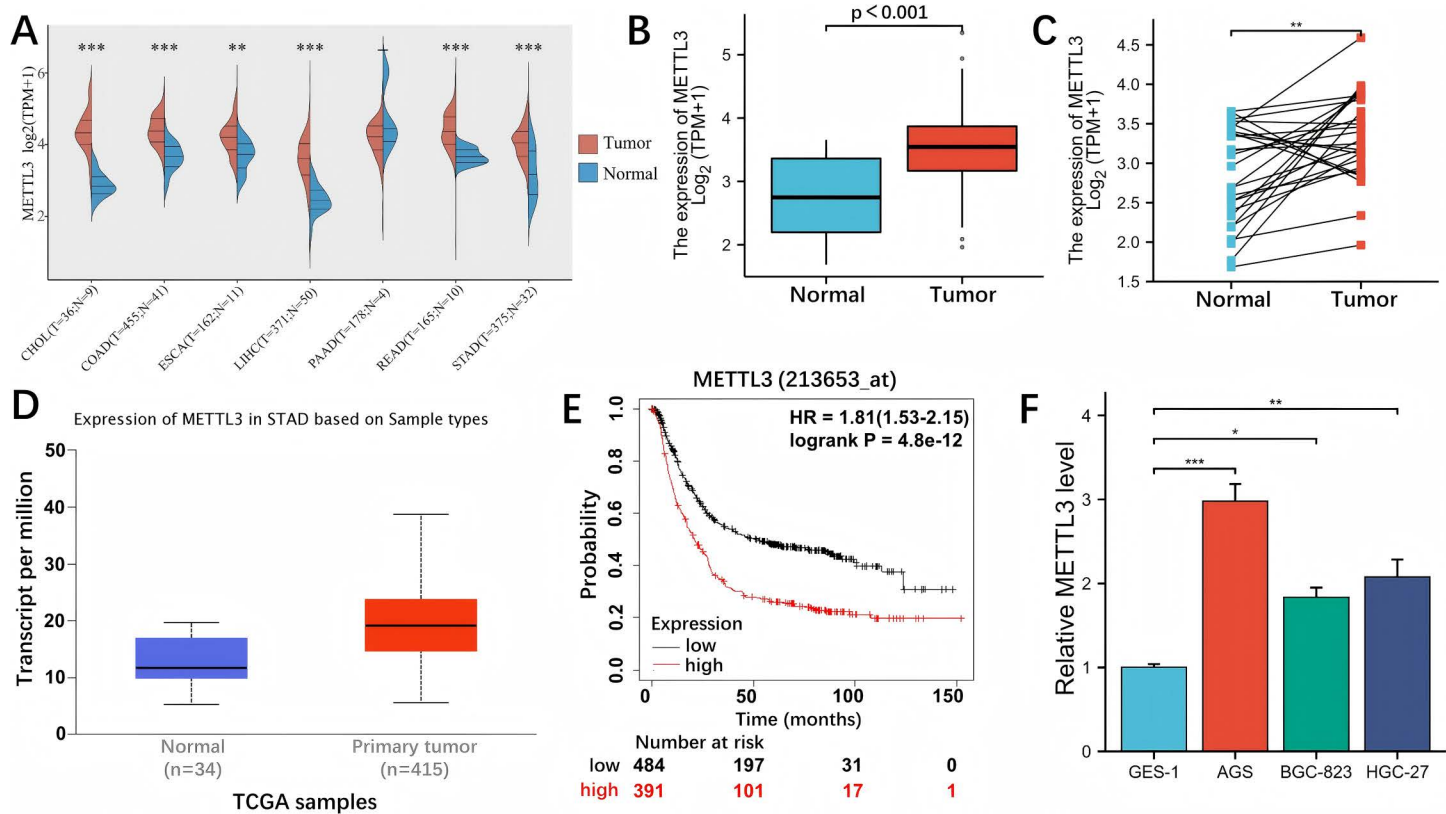


Fig 1. METTL3 was highly expressed in gastric cancer (GC). (A) The expression levels of METTL3 in GC, colon cancer, esophageal cancer and other tumors in TCGA database. (B-C) The expression of METTL3 in non-paired (B) and paired (C) samples of gastric cancer. (D) METTL3 expression was assessed on the UALCAN website. (E) High expression of METTL3 is associated with poor prognosis in gastric cancer patients. (F) The expression of METTL3 in three GC cell lines using qRT-PCR.

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the literature and reagent configuration conditions, we selected 10 μ M and 25 μ M as the concentrations for subsequent experiments. To determine whether METTL3 can inhibit the proliferation of gastric cancer cells, we assessed the biological activity of STM2457 on AGS cells using the CCK8 method. A difference in cell proliferation became apparent at 48 hours after treatment, with slower cell proliferation observed at higher concentrations of STM2457 (Fig 3B). Additionally, we conducted a cell scratch assay to evaluate cell migration. The results revealed a decrease in migration rate with increasing concentration of STM2457 (Fig 3C-D).

To confirm whether the anti-tumor effect of STM2457 is exerted through the inhibition of METTL3, we co-transfected METTL3 overexpression plasmids and treated with STM2457 in gastric cancer cells. We first verified by qRT-PCR that the METTL3 overexpression plasmid significantly upregulated METTL3 expression (S1B Fig). Then, CCK-8 assay results showed that treatment with STM2457 alone significantly inhibited cell proliferation compared with the control group. However, co-treatment with METTL3 overexpression plasmids largely restored cell proliferation to a level comparable to that of the control group (S1C Fig). This key finding indicates that the anti-proliferative effect of STM2457 can be effectively reversed by restoring METTL3 expression. We transfected shMETTL3 into the cells and confirmed its knockdown effect (S1D Fig). At the same time, we found that consistent with the pharmacological inhibitory effect of STM2457, inhibition of METTL3 significantly suppressed cell proliferation and migration in the wound healing assay (S1E-F Fig). These findings indicated that STM2457 can restrain cell proliferation and migration likely by inhibiting METTL3 activity.

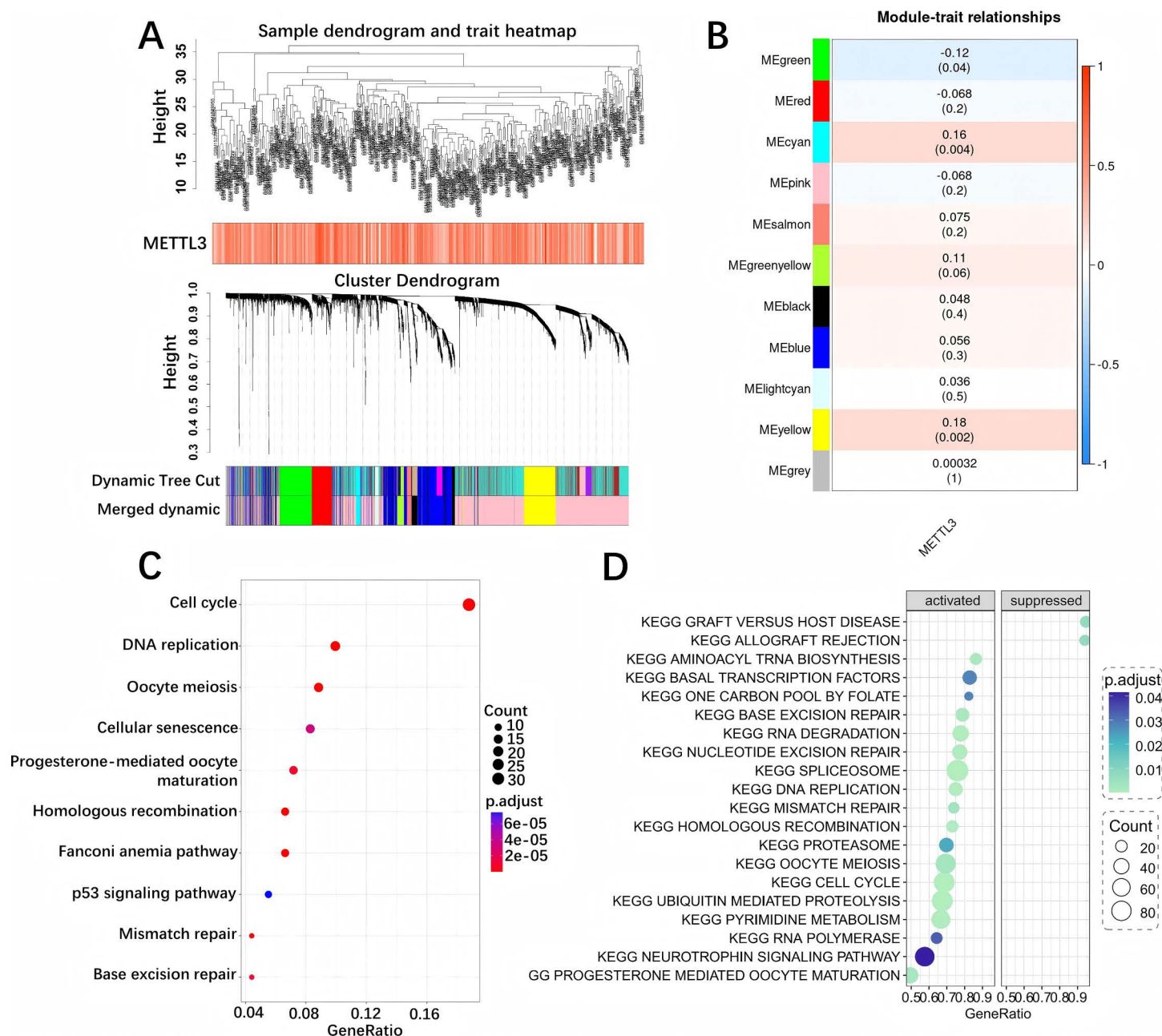


Fig 2. METTL3 was involved in pathways associated with tumor proliferation. (A) The sample clustering dendrogram. **(B)** A heatmap of correlation between modular signature genes and METTL3 expression. **(C)** KEGG enrichment analysis of METTL3 related module genes. **(D)** KEGG enrichment analysis of METTL3-related genes specific to GC in TCGA database.

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STM2457 promoted AGS cells apoptosis by inhibiting METTL3 activity and arrested the cell cycle in the S phase

To investigate the changes of apoptosis after METTL3 inhibition, AGS cells were treated with STM2457 at concentrations of 10 μ M and 25 μ M for 36 hours, respectively. The results revealed that as the concentration of STM2457 increased, apoptosis also increased (Fig 4A and B). Consistent with the pharmacological inhibitory effect of STM2457,

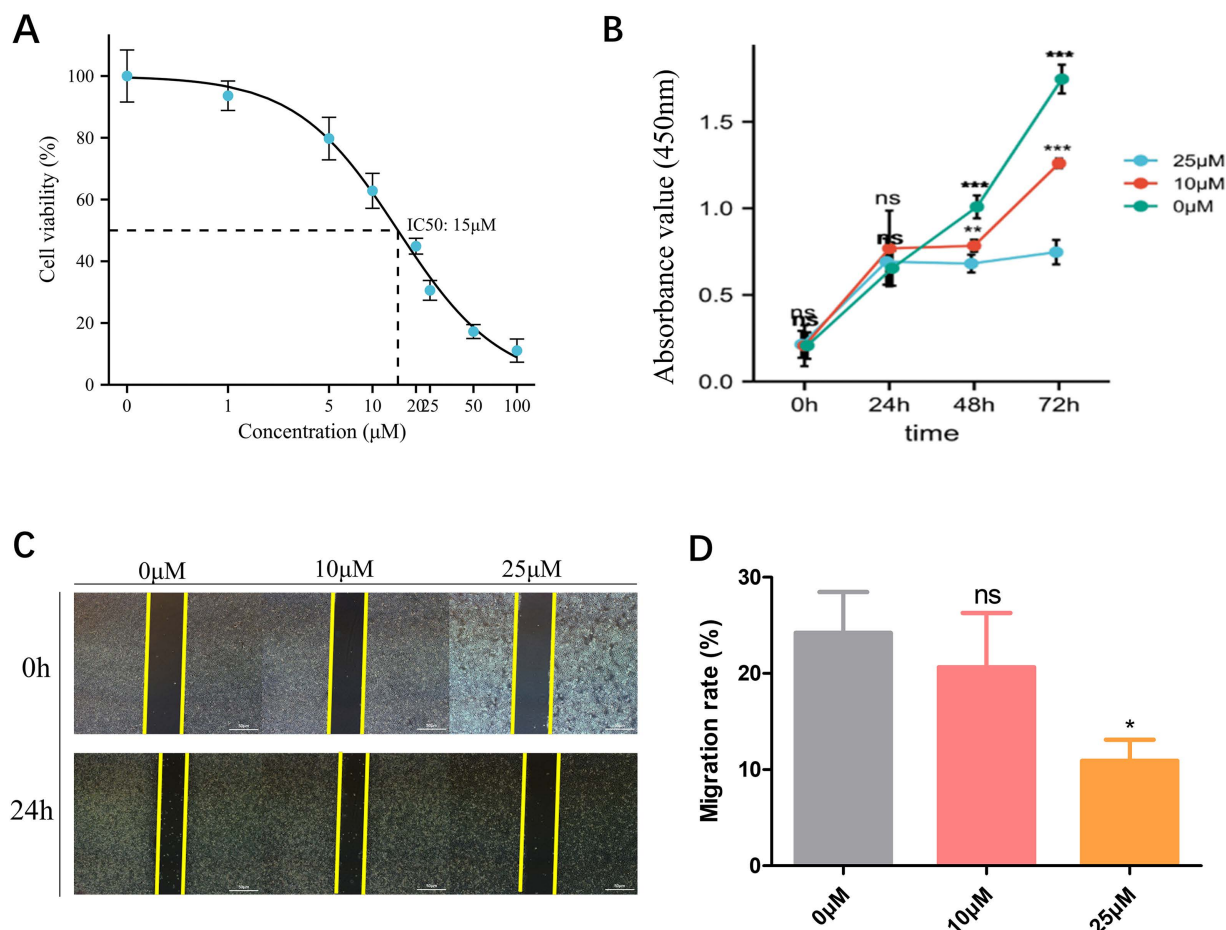


Fig 3. STM2457 down-regulating cell proliferation and migration by inhibiting METTL3. (A) The IC_{50} of STM2457 in AGS cells. (B) The proliferation of AGS cells treated with different concentrations of STM2457 detected by CCK8 assay. (C) The migration of AGS cells treated with different concentrations of STM2457 evaluated using scratch assay (100×). (D) The percentage of cell migration that treated with different concentrations of STM2457.

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the inhibition of METTL3 significantly increased the apoptosis rate of AGS cells (S1G Fig). Additionally, the changes in cell cycle were examined following the same treatment. It was observed that the percentage of cells in the G0 and G2/M phase decreased, while the percentage of cells in the S phase increased (Fig 4C and D). These findings suggested that STM2457 could promote apoptosis by inhibiting METTL3 activity and arrest cells in the S phase.

STM2457 inhibited tumor growth in a mouse model of subcutaneous xenograft

To simulate the effect of the microenvironment *in vivo* on the anti-gastric cancer effect of STM2457 and to evaluate the impact of STM2457 on AGS subcutaneous xenograft mice, we constructed a subcutaneous xenograft model of AGS cells using nude mice. The tumor-bearing mice were divided into a control group and a STM2457 treatment group, with 6 mice in each group and a treatment dose of 10 mg/kg. We observed that after intraperitoneal injection of STM2457, the tumor growth in the mice of treatment group significantly slowed down and their weight also reduced (Fig 5A and B). The tumor growth curve showed that compared to the control group mice, the treatment group mice exhibited slower tumor volume growth (Fig 5C). These findings suggested that STM2457 can inhibit cell proliferation *in vivo* and thus has *in vivo* activity.

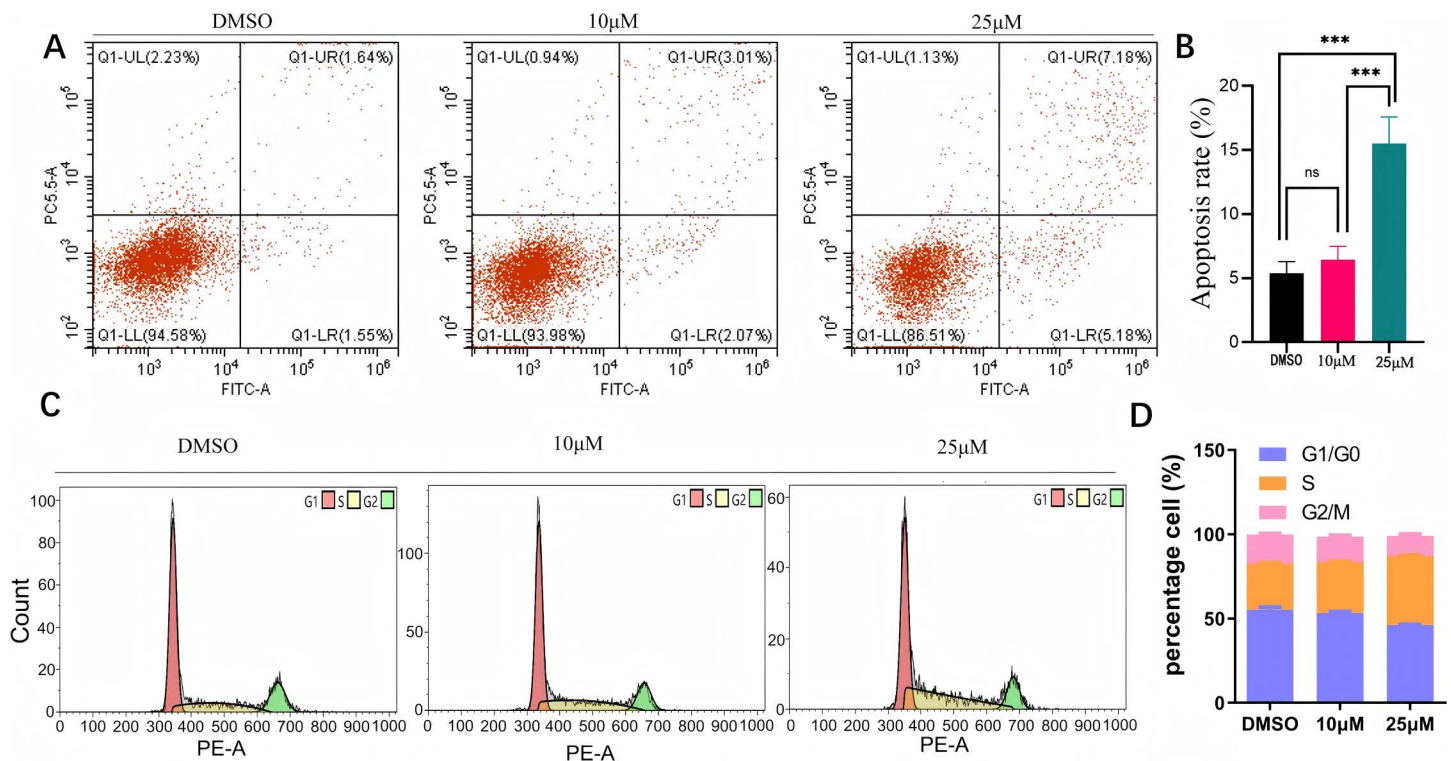


Fig 4. STM2457 arrested AGS cell cycle in the S phase and promoted apoptosis by inhibiting METTL3 activity. (A) The apoptosis analysis results of AGS cells treated with different concentrations of STM2457. (B) The percentage of apoptosis that treated with different concentrations of STM2457. (C) AGS cell cycle results detected by flow cytometry after treated with different concentrations of STM2457. (D) The percentage of the cell cycle that treated with different concentrations of STM2457.

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Discussion

GC is a commonly occurring malignant tumor. Its treatment typically involves surgical resection, radiotherapy, chemotherapy and targeted therapy. At present, chemotherapy is one of the main methods used to treat GC. The commonly used chemotherapy drugs, such as 5-fluorouracil (5-FU), cisplatin, and docetaxel, can inhibit the proliferation and spread of cancer cells through various mechanisms [25–27]. Additionally, targeted therapy is a novel treatment approach that can specifically target tumor cells through specific targets. For instance, trastuzumab, a drug targeting HER2, has been used to treat HER2-positive GC [28]. Although these drugs have played an active role in the treatment of GC, there are still challenges, including drug resistance and limited treatment effectiveness. Consequently, the discovery of new therapeutic drugs and techniques holds significant clinical importance.

METTL3 plays an essential role in the occurrence and development of cancer. Many previous studies have revealed that METTL3 promotes translation in human cancer cells, thus promoting the progression of cancer [29–31]. Wang *et al* evaluated the emerging role of METTL3 in gastrointestinal cancer and discovered that METTL3 was significantly expressed in gastrointestinal tumors, and played a crucial role in driving the progression of gastrointestinal cancer [32]. For instance, METTL3 promoted tumor progression through the m⁶A-IGF2 BP2-dependent mechanism in the colorectal cancer [33]. Regarding to the liver cancer, METTL3 facilitated cancer progression by the m⁶A-YTHDF2-dependent mechanism [18]. In addition, Yang *et al* and Huo *et al* revealed a high expression of METTL3 and demonstrated its capacity to facilitate the GC progression [34,35].

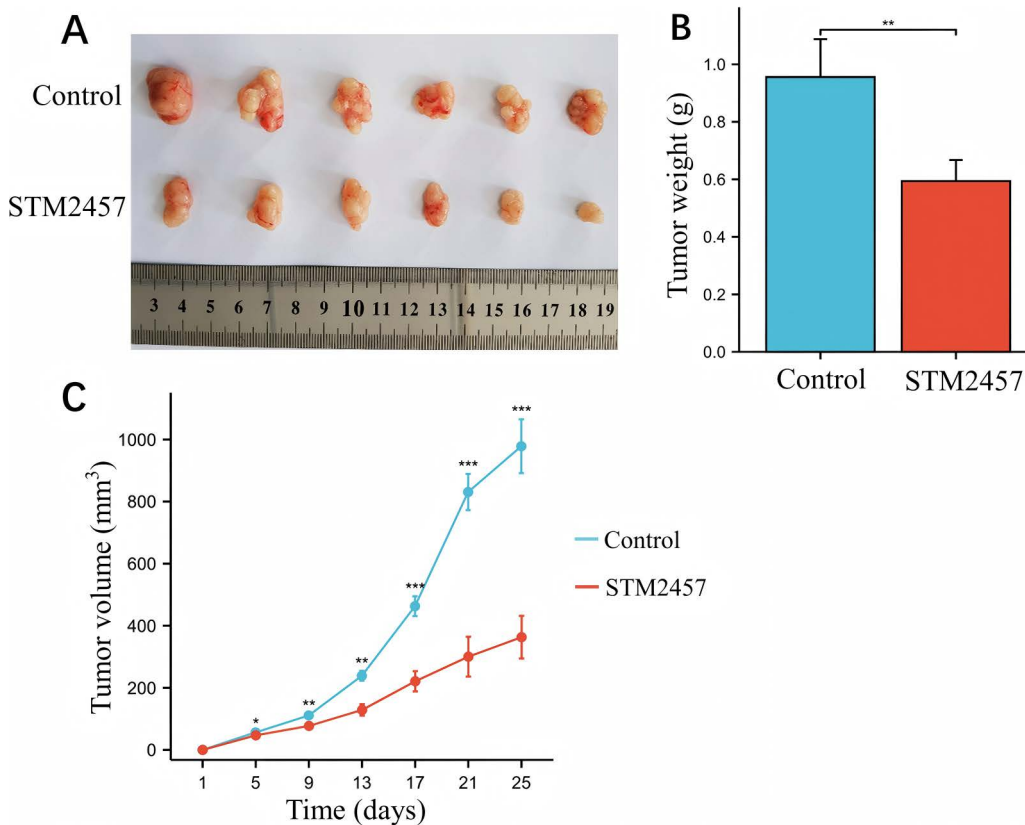


Fig 5. STM2457 inhibited tumor growth in a subcutaneous xenograft mouse model. (A-B) The tumor size and weight in tumor-bearing nude mice treated with or without STM2457. **(C)** The tumor volume growth rate of tumor-bearing nude mice treated with or without STM2457.

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It has been observed that METTL3 can bind to Pbx1 mRNA, stabilize its expression, and regulate the transcriptional activity of the GCH1 gene through Pbx1. This ultimately increased the level of BH4 in GC cells and promoted tumor progression [36]. Yang *et al* [37] discovered that METTL3 enhanced m⁶A modification of myc mRNA, leading to the increased myc translation and promotion of proliferation, migration and invasion of GC cells. This was consistent with our results. Previous studies and our findings together suggested that METTL3 was capable of being a potential target with clinical therapeutic effect. Furthermore, other m⁶A modification proteins, apart from METTL3, also was observed to play a crucial role in GC. For example, the demethylase ALKBH5 inhibited GC invasion through PKMYT1 m⁶A modification [38], and METTL14-facilitated circORC5 m⁶A modification suppressed the progression of GC by regulating the miR-30c-2-3p/AKT1S1 axis [39]. These findings indicated that the m⁶A regulatory pathway played a crucial role in the occurrence and development of GC, and suggested that METTL3 may serve as a potential biomarker and therapeutic target for the prognosis of GC patients.

METTL3 plays a crucial role in various types of cancers. It is necessary for mining for METTL3 inhibitors to suppress cancer [40]. The STM2457 is widely accepted as a novel inhibitor of METTL3 that exerts anti-tumor effects in cancers. For instance, Eliza *et al* used the STM2457 as a treatment strategy against myeloid leukemia [23]. Xu *et al* discovered that the STM2457 also exhibited anti-tumor effects in intrahepatic cholangiocarcinoma [41]. Furthermore, Sun *et al* found that STM2457 could reverse the chemoresistance of small cell lung cancer [42]. However, the role of STM2457 for GC is still unknown. In our study, it was found that STM2457 inhibits the proliferation of

GC cells *in vitro*, promotes the apoptosis and S-phase arrest, and slows down GC cell migration. This led to restrict proliferation and cell cycle progression of GC cells, as well as increased apoptosis. In addition, we also included the effect of STM2457 on another gastric cancer cell line, HGC-27. Our results consistently demonstrated that STM2457 significantly inhibited the proliferation and migration of HGC-27 cells and induced their apoptosis (S2 Fig), further confirming its potent and broad anti-tumor activity in the context of gastric cancer. Our results suggested that STM2457 has a certain inhibitory effect on the progression of GC, and highlighted the potential value of STM2457 as a treatment for GC.

In addition, the experimental results of our study also showed that STM2457 effectively inhibited the growth of GC cell xenografts *in vivo*. This was consistent with our *in vitro* experiments and previous studies, which demonstrated that STM2457 has antitumor activity *in vivo*. Nevertheless, our study only conducted *in vivo* experiments in nude mice. Further verification in humanized mice still need to be conducted. Moreover, since the mechanism of action of STM2457 involved the inhibition of METTL3's methyltransferase activity, reduced the m⁶A levels, and impacted the translation of m⁶A-positive genes [43,44], it is plausible that this compound may also influence some signaling pathways associated with m⁶A methylation modification. This potential mechanism could potentially clarify its inhibitory effect on tumor growth, presenting STM2457 as a promising candidate drug for GC treatment.

In conclusion, our experiments showed that STM2457 could inhibit the proliferation of gastric cancer cells *in vitro*, arrest gastric cancer cells in S phase, and promote cell apoptosis by inhibiting the methylation modification function of METTL3. At the same time, it can inhibit the growth of subcutaneous transplanted tumor *in vivo*. Our results proved that STM2457 had the potential for antitumor activity in GC treatment. A limitation of this study is the use of a subcutaneous xenograft model, which does not fully recapitulate the complex tumor microenvironment and metastatic process of gastric cancer. Furthermore, the clinical relevance of our findings requires further validation, as the correlation between METTL3 expression levels in primary patient tumors and sensitivity to STM2457 remains to be established. Future studies employing orthotopic or metastatic models, alongside analyses of clinical specimens, will be valuable to further validate the therapeutic potential of STM2457 and its biomarker utility. Meanwhile, this study has not yet fully elucidated the specific downstream target genes that are regulated by the m⁶A modifications that mediate the effects of STM2457. This is a limitation of the current work. Future research will integrate transcriptome and epigenetic transcriptome analyses to systematically identify and functionally validate these key targets, in order to fully reveal the molecular map of the action of STM2457. Even though more research is still required, our findings provided favorable support for the clinical development of STM2457.

Supporting information

S1 Fig. STM2457 exerts an anti-tumor effect by inhibiting METTL3. (A) The m⁶A methylation modification level in the STM2457 treatment group decreased. (B) The overexpression effect of the METTL3 plasmid was verified by qRT-PCR. (C) The anti-proliferative effect of STM2457 can be effectively reversed by restoring the expression of METTL3. (D) The knockdown effect of shMETTL3 was verified by qRT-PCR. (E) The proliferation of cells treated with shMETTL3 was detected by the CCK8 assay. (F) The migration of cells treated with shMETTL3 was evaluated by scratch assay (100×). (G) The results of apoptosis analysis of shMETTL3 treated cells. (TIF)

S2 Fig. STM2457 inhibits the proliferation and migration of HGC cells and promotes cell apoptosis. (A) The proliferation of HGC-27 cells treated with different concentrations of STM2457 detected by CCK8 assay. (B) The migration of HGC-27 cells treated with different concentrations of STM2457 evaluated using scratch assay (100×). (C) The apoptosis analysis results of HGC-27 cells treated with different concentrations of STM2457. (TIF)

S1 Table. Body weight monitoring of nude mice during the 25-day experimental period. Data are presented as individual values and group mean $\pm$ SD (n = 6). P-values from comparisons between the Control and STM2457-treated groups at each time point are shown.

(DOCX)

Author contributions

Conceptualization: Haozhi Xu, Qi Wang, Jianwei Zhou.

Formal analysis: Junying Li, Hongjie Dong.

Funding acquisition: Hang Sun, Kun Yin, Chao Xu.

Investigation: Hongjie Dong, Guihua Zhao.

Methodology: Xiaoman Xie, Qi Wang, Guihua Zhao, Jianwei Zhou, Ruili Wu.

Project administration: Xiaoman Xie.

Software: Junying Li.

Supervision: Junmei Zhang, Huanhuan Xie, Jingyu Yang.

Visualization: Huanhuan Xie.

Writing – original draft: Hang Sun, Haozhi Xu.

Writing – review & editing: Hang Sun, Kun Yin, Chao Xu.

References

1. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet*. 2020;396(10251):635–48. [https://doi.org/10.1016/S0140-6736\(20\)31288-5](https://doi.org/10.1016/S0140-6736(20)31288-5) PMID: [32861308](#)
2. Kim M, Seo AN. Molecular pathology of gastric cancer. *J Gastric Cancer*. 2022;22(4):273–305. <https://doi.org/10.5230/jgc.2022.22.e35> PMID: [36316106](#)
3. Businello G, Angerilli V, Parente P, Realdon S, Savarino E, Farinati F. Molecular landscapes of gastric pre-neoplastic and pre-invasive lesions. *Int J Mol Sci*. 2021;22(18).
4. Yang D, Hendifar A, Lenz C, Togawa K, Lenz F, Lurje G, et al. Survival of metastatic gastric cancer: Significance of age, sex and race/ethnicity. *J Gastrointest Oncol*. 2011;2(2):77–84. <https://doi.org/10.3978/j.issn.2078-6891.2010.025> PMID: [22811834](#)
5. Riihimäki M, Hemminki A, Sundquist K, Sundquist J, Hemminki K. Metastatic spread in patients with gastric cancer. *Oncotarget*. 2016;7(32):52307–16. <https://doi.org/10.18632/oncotarget.10740> PMID: [27447571](#)
6. Zhang Y, Lin Y, Duan J, Xu K, Mao M, Wang X. A population-based analysis of distant metastasis in Stage IV Gastric Cancer. *Med Sci Monit*. 2020;26:e923867. <https://doi.org/10.12659/MSM.923867> PMID: [32409630](#)
7. He L, Li H, Wu A, Peng Y, Shu G, Yin G. Functions of N6-methyladenosine and its role in cancer. *Mol Cancer*. 2019;18(1):176. <https://doi.org/10.1186/s12943-019-1109-9> PMID: [31801551](#)
8. Yang X, Hu X, Liu J, Wang R, Zhang C, Han F, et al. N6-methyladenine modification in noncoding RNAs and its function in cancer. *Biomark Res*. 2020;8(1):61. <https://doi.org/10.1186/s40364-020-00244-x> PMID: [33292652](#)
9. Hu F-F, Chen H, Duan Y, Lan B, Liu C-J, Hu H, et al. CBX2 and EZH2 cooperatively promote the growth and metastasis of lung adenocarcinoma. *Mol Ther Nucleic Acids*. 2021;27:670–84. <https://doi.org/10.1016/j.omtn.2021.12.032> PMID: [35070495](#)
10. Gimeno-Valiente F, Lopez-Rodas G, Castillo J, Franco L. Alternative splicing, epigenetic modifications and cancer: A dangerous triangle, or a hopeful one?. *Cancers (Basel)*. 2022;14(3).
11. Liu L-Q, Li H-S, Nie S-P, Shen M-Y, Hu J-L, Xie M-Y. Tea polysaccharide prevents colitis-associated carcinogenesis in mice by inhibiting the proliferation and invasion of tumor cells. *Int J Mol Sci*. 2018;19(2):506. <https://doi.org/10.3390/ijms19020506> PMID: [29419740](#)
12. Yi D, Wang R, Shi X, Xu L, Yilihamu Y, Sang J. METTL14 promotes the migration and invasion of breast cancer cells by modulating N6-methyladenosine and hsa-miR-146a-5p expression. *Oncol Rep*. 2020;43(5):1375–86. <https://doi.org/10.3892/or.2020.7515> PMID: [32323801](#)
13. Hua Y-Q, Zhang K, Sheng J, Ning Z-Y, Li Y, Shi W-D, et al. NUCB1 suppresses growth and shows additive effects with gemcitabine in pancreatic ductal adenocarcinoma via the unfolded protein response. *Front Cell Dev Biol*. 2021;9:641836. <https://doi.org/10.3389/fcell.2021.641836> PMID: [33855021](#)

14. Zheng W, Dong X, Zhao Y, Wang S, Jiang H, Zhang M, et al. Multiple functions and mechanisms underlying the role of METTL3 in human cancers. *Front Oncol*. 2019;9:1403. <https://doi.org/10.3389/fonc.2019.01403> PMID: [31921660](#)
15. Meyer KD, Jaffrey SR. Rethinking m6A readers, writers, and erasers. *Annu Rev Cell Dev Biol*. 2017;33:319–42. <https://doi.org/10.1146/annurev-cellbio-100616-060758> PMID: [28759256](#)
16. Zhang Y, Liu S, Zhao T, Dang C. METTL3-mediated m6A modification of Bcl-2 mRNA promotes non-small cell lung cancer progression. *Oncol Rep*. 2021;46(2):163. <https://doi.org/10.3892/or.2021.8114> PMID: [34132367](#)
17. Wang Q, Chen C, Ding Q, Zhao Y, Wang Z, Chen J, et al. METTL3-mediated m6A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance. *Gut*. 2020;69(7):1193–205. <https://doi.org/10.1136/gutjnl-2019-319639> PMID: [31582403](#)
18. Chen M, Wei L, Law C-T, Tsang FH-C, Shen J, Cheng CL-H, et al. RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2. *Hepatology*. 2018;67(6):2254–70. <https://doi.org/10.1002/hep.29683> PMID: [29171881](#)
19. Fatima S, Song Y, Zhang Z, Fu Y, Zhao R, Malik K, et al. Exploring the pharmacological mechanisms of P-hydroxycinnamaldehyde for Treating Gastric Cancer: A pharmacological perspective with experimental confirmation. *Curr Mol Pharmacol*. 2024;17:e18761429322420. <https://doi.org/10.2174/0118761429322420241112051105> PMID: [39660529](#)
20. Suyila Q, Li X, Su X. Anti-cancer bioactive peptide induces apoptosis in gastric cancer cells through TP53 signaling cascade. *Protein Pept Lett*. 2025;32(3):194–205. <https://doi.org/10.2174/0109298665350654250111144722> PMID: [39950267](#)
21. Wang K, Yu Y, Zhao J, Meng Q, Xu C, Ren J, et al. A Retrospective analysis of the lauren classification in the choice of XELOX or SOX as an adjuvant chemotherapy for gastric cancer. *Curr Gene Ther*. 2024;24(2):147–58. <https://doi.org/10.2174/0115665232247694230921060213> PMID: [37767800](#)
22. Peng Z, Fang C, Tong Z, Rao Q, Ren Z, Hu K. Crosstalk between cancer-associated fibroblasts and myeloid cells shapes the heterogeneous microenvironment of gastric cancer. *Curr Genomics*. 2024;25(5):390–411. <https://doi.org/10.2174/0113892029300608240531111743> PMID: [39323622](#)
23. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. *Nature*. 2021;593(7860):597–601. <https://doi.org/10.1038/s41586-021-03536-w> PMID: [33902106](#)
24. Xiao H, Zhao R, Meng W, Liao Y. Effects and translational characteristics of a small-molecule inhibitor of METTL3 against non-small cell lung cancer. *J Pharm Anal*. 2023;13(6):625–39. <https://doi.org/10.1016/j.jpha.2023.04.009> PMID: [37440912](#)
25. Wu N, Huang Y, Zou Z, Gimenez-Capitan A, Yu L, Hu W, et al. High BIM mRNA levels are associated with longer survival in advanced gastric cancer. *Oncol Lett*. 2017;13(3):1826–34. <https://doi.org/10.3892/ol.2017.5660> PMID: [28454330](#)
26. Meng C, Yin H, Sun Z, Zhou J, Chen S, Bai C, et al. Adjuvant Chemotherapy with Docetaxel, Cisplatin, and Continuous-Infusion 5-Fluorouracil for Gastric Cancer: A Phase II Study. *Transl Oncol*. 2014;7(2):277–83. <https://doi.org/10.1016/j.tranon.2014.02.014> PMID: [24704535](#)
27. Park JB, Lee JS, Lee MS, Cha EY, Kim S, Sul JY. Corosolic acid reduces 5-FU chemoresistance in human gastric cancer cells by activating AMPK. *Mol Med Rep*. 2018;18(3):2880–8. <https://doi.org/10.3892/mmr.2018.9244> PMID: [30015846](#)
28. Liang J, Dai W, Li Z, Liang X, Xiao M, Xie C, et al. Evaluating the efficacy and microenvironment changes of HER2+ gastric cancer during HLX02 and Endostar treatment using quantitative MRI. *BMC Cancer*. 2022;22(1):1033. <https://doi.org/10.1186/s12885-022-10136-y> PMID: [36192709](#)
29. Lin S, Choe J, Du P, Triboulet R, Gregory RI. The m(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. *Mol Cell*. 2016;62(3):335–45. <https://doi.org/10.1016/j.molcel.2016.03.021> PMID: [27117702](#)
30. Meng W, Xiao H, Mei P, Chen J, Wang Y, Zhao R, et al. Critical Roles of METTL3 in translation regulation of cancer. *Biomolecules*. 2023;13(2).
31. Han J, Wang J-Z, Yang X, Yu H, Zhou R, Lu H-C, et al. METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner. *Mol Cancer*. 2019;18(1):110. <https://doi.org/10.1186/s12943-019-1036-9> PMID: [31228940](#)
32. Wang Q, Geng W, Guo H, Wang Z, Xu K, Chen C, et al. Emerging role of RNA methyltransferase METTL3 in gastrointestinal cancer. *J Hematol Oncol*. 2020;13(1):57. <https://doi.org/10.1186/s13045-020-00895-1> PMID: [32429972](#)
33. Li T, Hu P-S, Zuo Z, Lin J-F, Li X, Wu Q-N, et al. METTL3 facilitates tumor progression via an m6A-IGF2BP2-dependent mechanism in colorectal carcinoma. *Mol Cancer*. 2019;18(1):112. <https://doi.org/10.1186/s12943-019-1038-7> PMID: [31230592](#)
34. Yang Z, Jiang X, Li D, Jiang X. HBXIP promotes gastric cancer via METTL3-mediated MYC mRNA m6A modification. *Aging (Albany NY)*. 2020;12(24):24967–82. <https://doi.org/10.18632/aging.103767> PMID: [33048840](#)
35. Huo F-C, Zhu Z-M, Zhu W-T, Du Q-Y, Liang J, Mou J. METTL3-mediated m6A methylation of SPHK2 promotes gastric cancer progression by targeting KLF2. *Oncogene*. 2021;40(16):2968–81. <https://doi.org/10.1038/s41388-021-01753-1> PMID: [33758320](#)
36. Liu Y, Zhai E, Chen J, Qian Y, Zhao R, Ma Y, et al. m6A-mediated regulation of PBX1-GCH1 axis promotes gastric cancer proliferation and metastasis by elevating tetrahydrobiopterin levels. *Cancer Commun (Lond)*. 2022;42(4):327–44. <https://doi.org/10.1002/cac2.12281> PMID: [35261206](#)
37. Yang D-D, Chen Z-H, Yu K, Lu J-H, Wu Q-N, Wang Y, et al. METTL3 Promotes the Progression of Gastric Cancer via Targeting the MYC Pathway. *Front Oncol*. 2020;10:115. <https://doi.org/10.3389/fonc.2020.00115> PMID: [32175271](#)
38. Hu Y, Gong C, Li Z, Liu J, Chen Y, Huang Y, et al. Demethylase ALKBH5 suppresses invasion of gastric cancer via PKMYT1 m6A modification. *Mol Cancer*. 2022;21(1):34. <https://doi.org/10.1186/s12943-022-01522-y> PMID: [35114989](#)

39. Fan H-N, Chen Z-Y, Chen X-Y, Chen M, Yi Y-C, Zhu J-S, et al. METTL14-mediated m6A modification of circORC5 suppresses gastric cancer progression by regulating miR-30c-2-3p/AKT1S1 axis. *Mol Cancer*. 2022;21(1):51. <https://doi.org/10.1186/s12943-022-01521-z> PMID: [35164771](https://pubmed.ncbi.nlm.nih.gov/35164771/)
40. Li J, Gregory RI. Mining for METTL3 inhibitors to suppress cancer. *Nat Struct Mol Biol*. 2021;28(6):460–2. <https://doi.org/10.1038/s41594-021-00606-5> PMID: [34040230](https://pubmed.ncbi.nlm.nih.gov/34040230/)
41. Xu Q-C, Tien Y-C, Shi Y-H, Chen S, Zhu Y-Q, Huang X-T, et al. METTL3 promotes intrahepatic cholangiocarcinoma progression by regulating IFIT2 expression in an m6A-YTHDF2-dependent manner. *Oncogene*. 2022;41(11):1622–33. <https://doi.org/10.1038/s41388-022-02185-1> PMID: [35094011](https://pubmed.ncbi.nlm.nih.gov/35094011/)
42. Sun Y, Shen W, Hu S, Lyu Q, Wang Q, Wei T, et al. METTL3 promotes chemoresistance in small cell lung cancer by inducing mitophagy. *J Exp Clin Cancer Res*. 2023;42(1):65. <https://doi.org/10.1186/s13046-023-02638-9> PMID: [36932427](https://pubmed.ncbi.nlm.nih.gov/36932427/)
43. Zhang Z-W, Teng X, Zhao F, Ma C, Zhang J, Xiao L-F, et al. METTL3 regulates m6A methylation of PTCH1 and GLI2 in Sonic hedgehog signaling to promote tumor progression in SHH-medulloblastoma. *Cell Rep*. 2022;41(4):111530. <https://doi.org/10.1016/j.celrep.2022.111530> PMID: [36288719](https://pubmed.ncbi.nlm.nih.gov/36288719/)
44. Li S, Zou D, Chen W, Britz GW, Liu Z, Weng Y-L. METTL3 inhibition reduces N6 -methyladenosine levels and prevents allogeneic CD4+ T-cell responses. *Immunol Cell Biol*. 2022;100(9):718–30. <https://doi.org/10.1111/imcb.12581> PMID: [36005900](https://pubmed.ncbi.nlm.nih.gov/36005900/)