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Research article

ZC3H13 knockdown enhances the inhibitory effect of sevoflurane on gastric cancer cell malignancy by regulating the N6-methyladenosine modification of the lncRNA DLX6-AS1

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ABSTRACT

Sevoflurane, an inhalation anesthetic, has been shown to suppress cancer development. In this study, we investigated the specific mechanisms involving sevoflurane, zinc-finger CCCH-type containing 13 (ZC3H13), and lncRNA DLX6-AS1 in gastric cancer (GC) progression, focusing on the N6-methyladenosine (m^6A) modification of long non-coding RNAs (lncRNAs). We used quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot analyses to measure the levels of ZC3H13 and lncRNA DLX6-AS1 in GC tissues and cells. Furthermore, we conducted Cell Counting Kit-8, colony formation, Transwell, and tumor xenograft assays to evaluate changes in GC cell malignancy following cell transfection and sevoflurane treatment. Additionally, actinomycin D, methylated RNA immunoprecipitation, and qRT-PCR assays were performed to examine the regulatory effects of ZC3H13 on the DLX6-AS1 m^6A modification. We detected elevated levels of ZC3H13 in GC samples, while ZC3H13 silencing inhibited GC cell proliferation, migration, and invasion. Silencing ZC3H13 also enhanced the inhibitory effects of sevoflurane on GC cell malignancy. Moreover, we found that the increased expression of DLX6-AS1 in GC cells could be suppressed by ZC3H13 through the mediation of the m^6A modification of DLX6-AS1, thereby reducing DLX6-AS1 stability. In conclusion, ZC3H13 knockdown enhances the inhibitory effect of sevoflurane on GC cell malignancy by inducing DLX6-AS1 m^6A modification. Our findings may help identify potential therapeutic targets for the treatment of GC.

1. Introduction

As of 2020, the global incidence of gastric cancer (GC) was 11.1 per 100,000, with more than 760,000 deaths reported, accounting for approximately 7.8 % of all cancer-related deaths [1]. In China, GC became the second most common cancer after lung cancer in 2020, with over 0.47 million cases and 0.37 million deaths [1,2]. Despite these bleak statistics, considerable progress is being made in the treatment of GC. Sevoflurane, an inhalation anesthetic with strong anesthetic effects and high controllability, is routinely used for surgical procedures and has been proven to inhibit GC tumorigenesis [3–5]. For instance, sevoflurane induces apoptosis and inhibits the migration and invasion of GC cells in vitro by activating cancer-related genes [5]. Additionally, by upregulating the expression of

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the FOXP3 protein, sevoflurane has been found to inhibit the proliferation of GC cells and promote cell apoptosis [4]. However, the regulatory network of sevoflurane in GC progression is complex. Therefore, actively examining the mechanism of sevoflurane in GC development is deemed to be of considerable significance from the perspectives for enhancing the therapeutic efficacy of GC treatments.

N6-methyladenosine (m^6A) is a post-transcriptional mRNA modification mediated by methyltransferases, demethylases, and reading proteins [6]. By modifying the 5' and 3'UTRs of mRNAs, m^6A plays key roles in mRNA splicing, nucleation, and translation [7,8]. In recent years, m^6A modification has been recognized as a key regulatory mechanism in GC. For example, the m^6A reading protein IGF2BP1 is upregulated in GC, promoting cell migration and aerobic glycolysis by interacting with c-MYC mRNA in an m^6A-dependent manner [9]. Additionally, methyltransferase-like 3 (METTL3)-mediated m^6A modification has been shown to promote HDGF mRNA stability, leading to increased glycolysis, tumor growth, and liver metastasis in GC [10]. Bioinformatics analysis has revealed that the m^6A methyltransferase zinc-finger CCCH-type containing 13 (ZC3H13) is upregulated in GC. Previous studies have demonstrated that ZC3H13 can inhibit the DNA damage response in pancreatic cancer [11], and drive DNA replication in proliferating cervical cancer cells by mediating m^6A modification of CENPK mRNA [12]. However, the role of ZC3H13 in GC and its correlation with sevoflurane have yet to be established.

Genome sequencing studies have revealed that more than 80 % of the genome is transcribed into non-coding RNAs (ncRNAs) that do not encode proteins but instead have a wide range of regulatory roles [13]. Among these ncRNAs, long non-coding RNAs (lncRNAs), defined as being longer than 200 nucleotides, are involved in gene regulation at the transcriptional level and play key roles in various biological processes [14]. Recently, the m^6A modification of lncRNAs in GC has been frequently reported. For example, ALKBH5 reduces the stability of lncRNA TP53TG1 by regulating its m^6A modification, thereby inhibiting GC progression [15]. Similarly, the m^6A modification of another lncRNA THAP7-AS1, regulated by METTL3, can promote GC progression [16]. Bioinformatics analysis has identified a positive correlation between lncRNA DLX6-AS1, located on chromosome 7q21.3, and the m^6A methyltransferase ZC3H13 in GC. However, whether ZC3H13 can mediate the m^6A modification of DLX6-AS1 in GC has not been explored.

In this study, we aimed to determine the levels and roles of ZC3H13 by regulating the m^6A modification of DLX6-AS1 and its regulation by sevoflurane in GC malignancy. This research will enrich the regulatory network of sevoflurane in GC progression and provide a valuable theoretical basis for GC therapy.

2. Materials and methods

2.1. Clinical samples

This study was conducted with the approval from the Ethics Committee of Wuhan Fourth Hospital (approval number: KY2023-010-01). Tumor and adjacent non-cancerous tissue samples were collected from 50 patients with pathologically confirmed GC who were admitted to Wuhan Fourth Hospital. The inclusion criteria were: (1) patients diagnosed with GC; (2) patients who had not received any treatment prior to surgery; and (3) patients or a family member who had signed informed consent. The exclusion criteria included patients with diseases other than GC and those with a history of GC. The collected tissue samples were stored at −80 °C until analyses. Details of patient information are presented in Table 1.

2.2. Cell culture

All culture media and cell lines utilized in this study were procured from Procell (China), and authenticated by Short tandem repeat profiling. The GC cell lines AGS (Cat#: CL-0022) and HGC-27 (Cat#: CL-0107) were cultured in Ham's F-12 supplemented with 10 % fetal bovine serum (FBS) and RPMI-1640 medium supplemented with 20 % FBS, respectively. All media were supplemented with 1 % penicillin/streptomycin, and cells were maintained at 37 °C in a 5 % CO_2 atmosphere.

Table 1
Clinical characteristics of 50 gastric cancer patients.

Characteristics		No. (%)
Age, years	≤60	27 (54)
	>61	23 (46)
Gender	Female	19 (38)
	Male	31 (62)
Tumor size, cm	≤2	33 (66)
	>2	17 (44)
Tumor stage	I-II	30 (60)
	III-IV	20 (40)
Tumor differentiation	Well	1 (2)
	Moderate	26 (52)
	Poor	23 (46)
Lymph node metastasis	No	29 (58)
	Yes	21 (42)
Lymphovascular invasion	No	28 (56)
	Yes	22 (44)

2.3. Cell transfection and sevoflurane treatment

AGS and HGC-27 cells were cultured in antibiotic-free growth medium for 24 h. When reaching approximately 50 % confluence, cells were transfected with 100 nM ZC3H13 siRNA (si-ZC3H13#1 and si-ZC3H13#2) or negative control (si-NC) obtained from RiboBio (China) using Lipo6000 (Beyotime, China). After 48 h of transfection, ZC3H13 levels were assessed to determine transfection efficiency.

For sevoflurane treatment, AGS and HGC-27 cells were divided into blank and sevoflurane (Sev) groups. Cells in blank group were cultured in an incubator at 37 °C in a 5 % CO_2 atmosphere. Cells in Sev group were cultured in an incubator equipped with a sevoflurane vaporizer (Kent, China) set to deliver a continuous input of 3.4 % sevoflurane gas. After 6 h of incubation with 3.4 % sevoflurane, cells in the Sev groups were subsequently incubated under normal conditions for an additional 24 h, following previous protocols [17].

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from tissues and cells was extracted using RNA Extraction Kit (Generay Biotech, China). Then, 2 μg of total RNA was subsequently reverse transcribed to cDNA using a QuantiTect Reverse Transcription Kit (Qiagen, Germany). PCR analysis was performed using Fast SYBR Green Master Mix (Applied Biosystems, USA) with the following reaction conditions: 95 °C for 20 s, 40 cycles of 95 °C for 3 s and 60 °C for 30 s. GAPDH served as an internal control for determining the relative expression of ZC3H13 and DLX6-AS1 using the $2^{-\Delta\Delta Ct}$ method. The primers used for amplification are listed in Table 2.

2.5. Cell Counting Kit-8 (CCK-8) assays

AGS and HGC-27 cells (2×10^3 per well) were seeded in 96-well plates, and at 0, 24, 48, and 72 h, 10 μl of CCK-8 reagent (Beyotime, China) was added to each well followed by gentle mixing. The cells were thereafter incubated for a further 2 h at 37 °C, after which the absorbance of each well was determined at 450 nm using a microplate reader (DeTie, China).

2.6. Colony formation assays

AGS and HGC-27 cells (1×10^3 cells/well) were seeded in six-well plates and cultured for 14 days. Subsequently, the culture medium was aspirated, and the cell colonies were fixed with 4 % paraformaldehyde (Beyotime, China) for 15 min at 25 °C, followed by staining with 0.1 % crystal violet (Beyotime, China) for 20 min. The number of cell colonies were counted using a light microscope (Olympus, Japan).

2.7. Transwell assays

For the Transwell migration assays, 1×10^4 AGS and HGC-27 cells were seeded onto Transwell inserts (Corning, USA) in the upper chambers with serum-free medium, while the lower chambers contained 500 μl of complete medium with 10 % FBS. After incubation, the migrated cells were fixed with 4 % paraformaldehyde for 10 min, stained with 0.5 % crystal violet for 20 min, and then observed under a microscope. Invasion assays followed a similar procedure, except that the upper chambers were pre-coated with Matrigel (BioFroxx, Germany) at 4 °C overnight.

2.8. Methylated RNA immunoprecipitation (MeRIP)-qPCR

To analyze m^6A-methylation, an m6A MeRIP Kit (Cloud-seq Biotech., China) was used. AGS and HGC-27 cells (1×10^7) were lysed with lysis buffer to isolate RNA. The isolated RNA was then fragmented using fragmentation buffer. Subsequently, the fragmented RNA was incubated overnight at 4 °C with protein A/G beads (20 μL) conjugated with either anti-m^6A antibody (ab286164, Abcam, UK)/anti-IgG (ab133470, Abcam). Finally, the RNA was washed with RIP wash buffer, and the levels of DLX6-AS1 m^6A-methylation were determined using qRT-PCR analysis.

Table 2
List of primers used in this study.

Primer names	Sequences
DLX6-AS1	Forward:5′-AGTTTCTCTCTAGATTGCCTT-3′ Reverse:5′-ATTGACATGTTAGTGCCCTT-3′
ZC3H13	Forward:5′-TCTGATAGCACATCCCGAAGA-3′ Reverse:5′-CAGCCAGTTACGGCACTGT-3′
GAPDH	Forward:5′-CTGGGCTACACTGAGCACC-3′ Reverse:5′-AAGTGGTCGTTGAGGGCAATG-3′

2.9. Actinomycin D assays

The GC cells (1×10^7) transfected with either si-ZC3H13 or si-NC were treated with 2 μg/mL actinomycin D (Shanghai YSRIBIO industrial co., China) for 0, 3, and 6 h. Subsequently, total RNA was extracted using an RNA Extraction Kit (Generay Biotech, China), and the levels of DLX6-AS1 were assessed using qRT-PCR analysis.

2.10. Western blotting

Cellular proteins were extracted using RIPA buffer (Beyotime, China) and quantitated with a BCA kit (Beyotime). The extracted proteins (20 μg) were separated by electrophoresis on 10 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and subsequently transferred to PVDF membranes. The membranes were blocked with 5 % skim milk, then incubated overnight at 4 °C with primary antibodies [ZC3H13 (abs134925; Absin, China) and GAPDH (abs132004; Absin)]. After washing, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibodies (A0208; Beyotime, China). Protein signal intensity was analyzed using Quantity One software (Bio-Rad, USA) and detected with the BeyoECL Plus detection system (P0018S; Beyotime).

2.11. Tumor xenograft assays

Nine 4-week-old male BALB/C nude mice (Vital River Laboratory, China) were divided into three groups: blank, 3.4 % Sev, and 3.4 % Sev + sh-ZC3H13. To achieve stable ZC3H13 knockdown in AGS cells, ZC3H13 knockdown lentiviral vectors (sh-ZC3H13) constructed by OBiO Technology (China) were used for transfection. Mice in the blank and 3.4 % Sev groups were subcutaneously injected with 1×10^6 AGS cells incubated with sevoflurane for 6 h, while mice in the 3.4 % Sev + sh-ZC3H13 group received injections of 1×10^6 AGS cells transfected with sh-ZC3H13 (MOI = 30). Tumor volumes were measured weekly using a Vernier caliper with the formula: tumor volume = 0.5 × long diameter × short diameter^2. After 4 weeks, all mice were euthanized, and tumors were excised, weighed, and photographed. Animal procedures were approved by the Ethics Committee of Wuhan Fourth Hospital (approval number: 2023009).

2.12. Statistical analysis

Analyses were conducted in triplicate, and data are presented as means ± SD of the three treatments. Statistical significance was

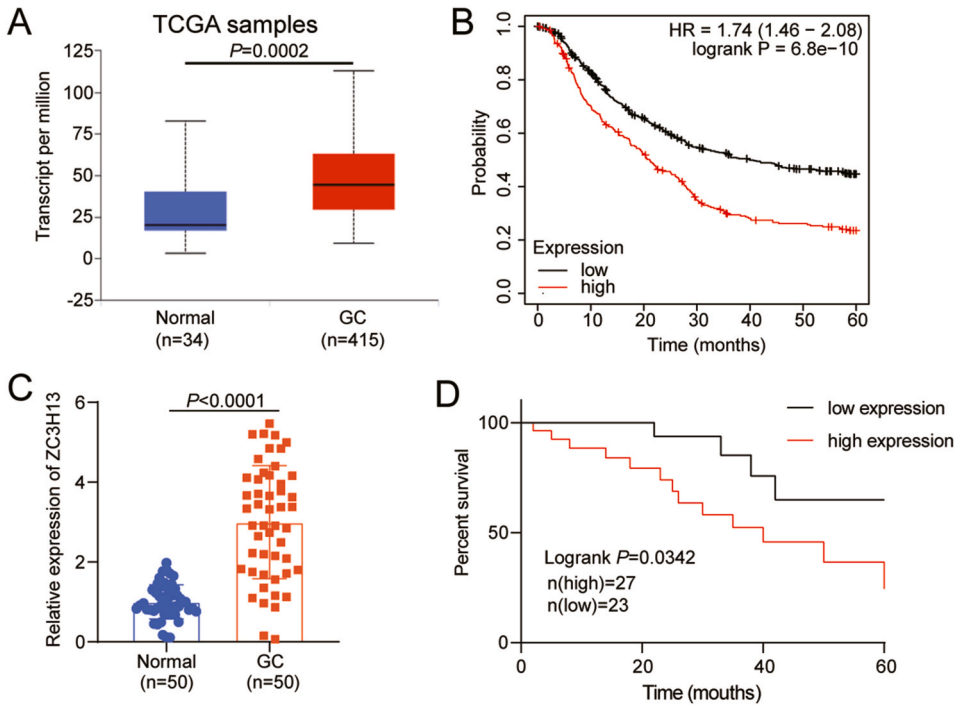

Fig. 1. ZC3H13 is upregulated in gastric cancer. (A) The levels of ZC3H13 in gastric cancer (GC) samples according to data from the TCGA database. (B) The association between ZC3H13 expression and prognosis of GC patients based on Kaplan–Meier Plotter analysis. (C) The levels of ZC3H13 in GC and normal tissues determined based on qRT-PCR analysis. (D) The association between ZC3H13 expression and the prognosis of GC patients revealed by Log-rank (Mantel–Cox) analysis.

performed using GraphPad Prism 8.0 software, with significance set at $P < 0.05$. Differences between groups were assessed by ANOVA followed by Dunnett's/Tukey's post hoc test for multiple comparisons or Student's *t*-test for two groups. Pearson correlation analysis was used to evaluate correlations between DLX6-AS1 and ZC3H13. The correlation between high and low expression of ZC3H13 and the prognosis of GC patients was assessed using a Log-rank (Mantel–Cox) test.

3. Results

3.1. ZC3H13 is upregulated in GC

Data analysis was conducted using the online portal UALCAN (https://ualcan.path.uab.edu/index.html), which accessed The Cancer Genome Atlas database comprising 415 GC samples and 34 adjacent non-cancerous samples. The results indicated upregulation of ZC3H13 in GC samples (Fig. 1A). Additionally, the Kaplan–Meier Plotter (https://kmplot.com/analysis/index.php?p=service&cancer=gastric#) categorized samples into low and high expression groups based on the mean ZC3G13 levels in GC

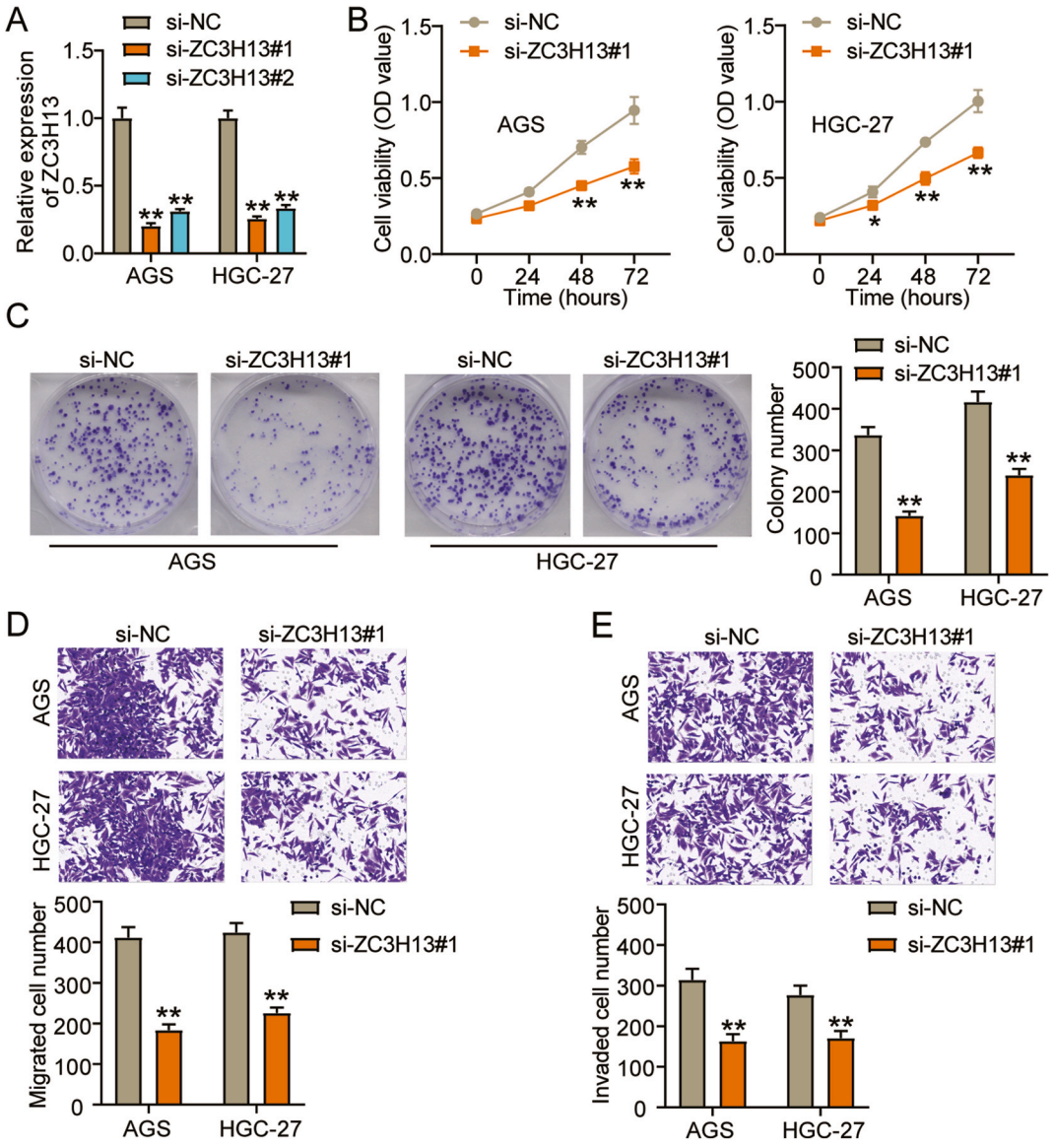

Fig. 2. A reduction in the expression of ZC3H13 suppresses gastric cancer cell malignancy. (A) The levels of ZC3H13 in AGS and HGC-27 cells transfected with si-ZC3H13#1, si-ZC3H13#2, or si-NC determined by qRT-PCR analysis. (B and C) The proliferation of AGS and HGC-27 cells transfected with si-ZC3H13#1 or si-NC determined by performing CCK-8 (B) and colony formation (C) assays. (D and E) The migration (D) and invasion (E) of AGS and HGC-27 cells transfected with si-ZC3H13#1 or si-NC determined using Transwell assays. *$P < 0.05$, **$P < 0.001$ vs. si-NC.

samples, revealing that higher ZC3H13 expression correlated with poor prognosis in GC patients (Fig. 1B). To validate these findings, 50 pairs of GC tissues and corresponding non-cancerous tissues were collected. qRT-PCR analyses demonstrated a 2.8-fold increase in ZC3H13 levels in GC tissues compared to non-cancerous tissues (Fig. 1C). Consistently, divided these GC samples into low and high expression groups based on mean ZC3G13 expression revealed through Log-rank (Mantel–Cox) analysis that elevated ZC3H13 expression was associated with poorer survival among GC patients (Fig. 1D). Collectively, these data confirm the elevated expression of ZC3H13 in GC.

3.2. A low expression of ZC3H13 is associated with a suppression GC cell malignancy

To investigate the role of ZC3H13 in GC cells, two ZC3H13 siRNAs were used to transfect two GC cell lines (AGS and HGC-27). qRT-PCR analysis showed approximately 80 % and 70 % reductions in ZC3H13 levels in the si-ZC3H13#1 and si-ZC3H13#2 groups,

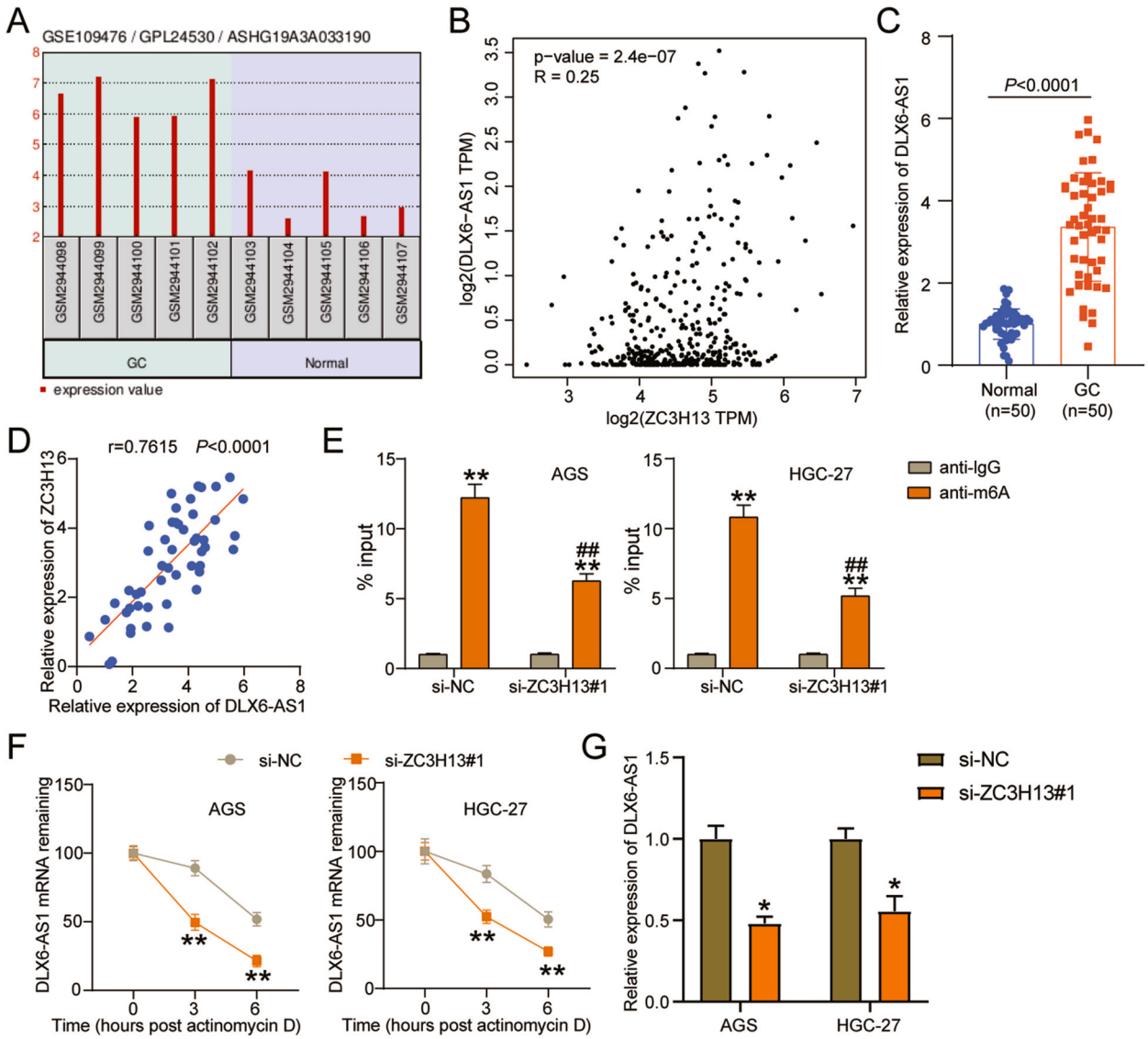

Fig. 3. ZC3H13 stabilizes DLX6-AS1 expression in gastric cancer by inducing m^{6}A modification. (A) The levels of DLX6-AS1 in gastric cancer (GC) samples according to the data obtained from GSE109476. (B) Correlations between DLX6-AS1 and ZC3H13 expression were determined by reference to the GEPIA database. (C) The levels of DLX6-AS1 in GC and normal tissues were determined by qRT-PCR analysis. (D) Correlations between the expression of DLX6-AS1 and ZC3H13 in GC cells were determined by Pearson correlation analysis. (E) m^{6}A modification of DLX6-AS1 in AGS and HGC-27 cells transfected with si-ZC3H13#1 or si-NC was assessed based of MeRIP-qRT-PCR assay. **P < 0.001 vs. anti-IgG; ##P < 0.001 vs. si-NC. (F) The levels of DLX6-AS1 in AGS and HGC-27 cells transfected with si-NC or si-ZC3H13#1 after treatment with actinomycin D were determined by qRT-PCR. **P < 0.001 vs. si-NC. (G) qRT-PCR analysis of the expression of DLX6-AS1 in AGS and HGC-27 cells transfected with si-NC or si-ZC3H13#1. *P < 0.05 vs. si-NC.

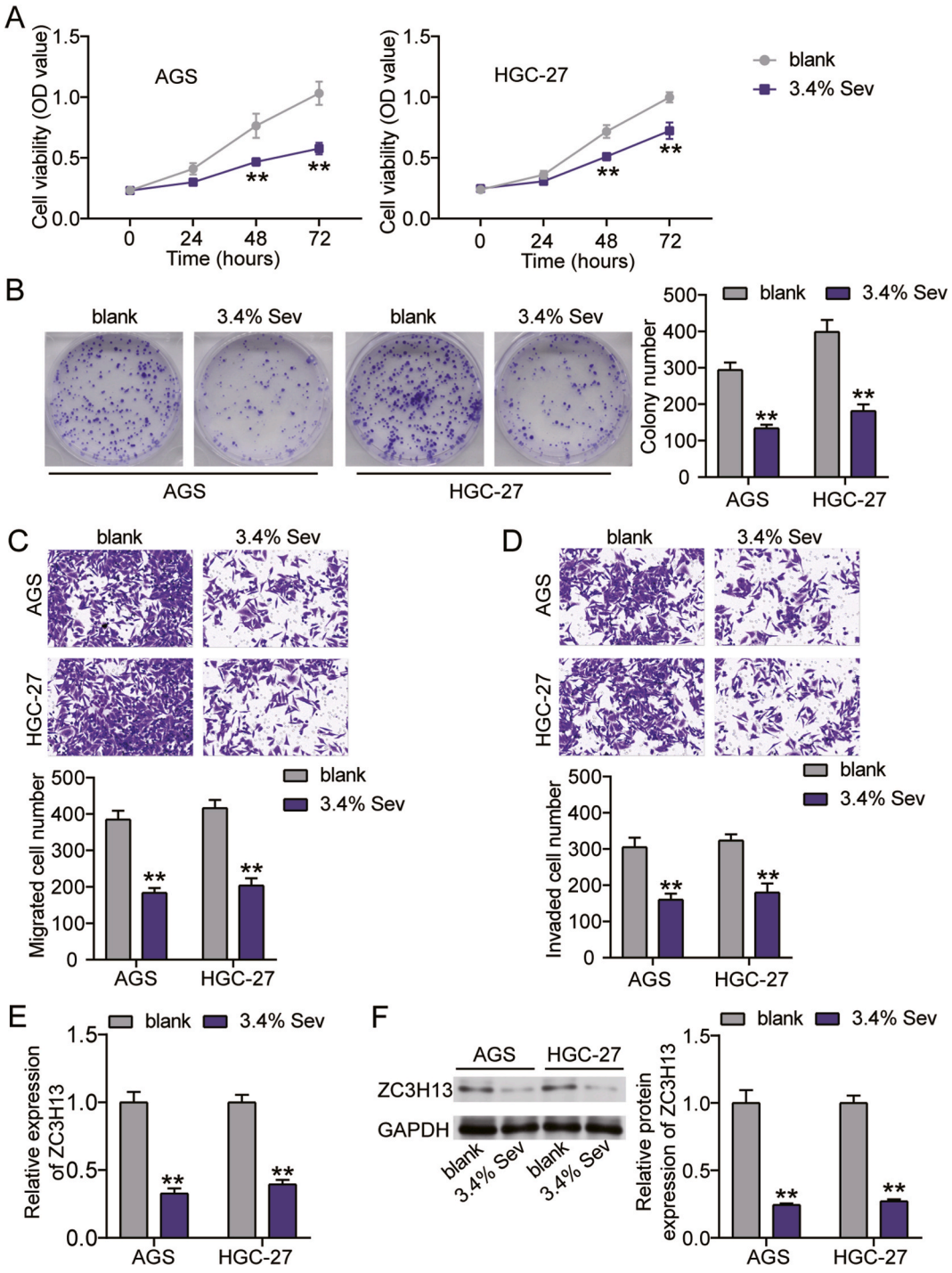

Fig. 4. Sevoflurane suppresses gastric cancer cell malignancy. (A and B) The proliferation of AGS and HGC-27 cells treated with or without 3.4 % sevoflurane determined based on CCK-8 (A) and colony formation (B) assays. (C and D) The migration (C) and invasion (D) of AGS and HGC-27 cells treated with or without 3.4 % sevoflurane determined based on Transwell assays. (E) The levels of ZC3H13 mRNA in AGS and HGC-27 cells treated with or without 3.4 % sevoflurane determined by qRT-PCR analysis. (F) The levels of ZC3H13 protein in AGS and HGC-27 cells treated with or without 3.4 % sevoflurane determined by western blotting. The images of gels and blots were shown in supplementary material. **P < 0.001 vs. blank. blank group: AGS and HGC-27 cells were treated without sevoflurane.

respectively, compared to si–NC–transfected cells (Fig. 2A). Due to the more pronounced reductions in ZC3H13 expression in the si-ZC3H13#1 group, this siRNA was selected for subsequent experiments. CCK-8 assays indicated lower OD values in GC cells transfected with si-ZC3H13#1 GC, suggesting that decreased ZC3H13 expression suppresses GC cell proliferation (Fig. 2B). Consistently, colony formation assays demonstrated a significant reduction in GC cell proliferation following si-ZC3H13#1 transfection (Fig. 2C). Moreover, Transwell assays revealed decreased cell migration and invasion abilities in cells transfected with si-ZC3H13#1 (Fig. 2D and E). These findings provide evidence that inhibiting ZC3H13 suppresses the development of a malignant phenotype in GC cells.

3.3. ZC3H13 stabilizes DLX6-AS1 expression via m^6A modification in GC

To elucidate the mechanism of ZC3H13 in GC, we first analyzed gene microarray GSE109476 from GEO DataSets, revealing upregulated DLX6-AS1 levels in five GC tissues compared to adjacent non-cancerous tissues (Fig. 3A). Subsequently, using the GEPIA database (http://gepia.cancer-pku.cn/index.html), we confirmed a positive correlation between DLX6-AS1 and ZC3H13 expression levels in GC samples (Fig. 3B). Further validation was conducted by qRT-PCR and Pearson correlation analyses using our collected GC tissues and adjacent non-cancerous tissues, demonstrating higher DLX6-AS1 expression levels in GC tissues compared to non-tumor tissues, and a positive correlation between DLX6-AS1 and ZC3H13 levels in GC tissues (Fig. 3C and D). Additionally, MeRIP-qPCR assays indicated revealed m^6A methylation of DLX6-AS1 following ZC3H13 knockdown (Fig. 3E). Consistently, actinomycin D assay showed decreased mRNA stability of DLX6-AS1 in GC cells with low ZC3H13 expression levels (Fig. 3F). qRT-PCR assays further confirmed that ZC3H13 knockdown suppressed DLX6-AS1 expression in GC cells (Fig. 3G). Together, these finding suggest that silencing ZC3H13 inhibits the m^6A methylation of DLX6-AS1, and consequently reducing its expression in GC.

3.4. Sevoflurane suppresses GC cells malignancy

Following treatment of AGS and HGC-27 cells with or without 3.4 % sevoflurane, both CCK8 and colony formation assays demonstrated reduced proliferation of GC cells exposed to 3.4 % sevoflurane (Fig. 4A and B). Similarly, Transwell assay indicated decreased migration and invasive capacity of these cells following exposure to 3.4 % sevoflurane (Fig. 4C and D). Furthermore, qRT-PCR analyses showed decreased levels of ZC3H13 mRNA in cells treated with 3.4 % sevoflurane compared to the blank group (Fig. 4E). Western blot analysis confirmed a reduction of approximately 70 % in ZC3H13 protein levels in sevoflurane-treated cells compared to blank cells (Fig. 4F). These findings provide evidence that sevoflurane decreases cellular levels of ZC3H13, thereby inhibiting the malignant development of GC cells.

3.5. ZC3H13 silencing enhances the suppressive effect of sevoflurane on GC cells in vitro via inhibiting m6A methylation of DLX6-AS1

To investigate the regulatory role of sevoflurane in interaction with ZC3H13, GC cells treated with 3.4 % sevoflurane were additionally transfected with si-ZC3H13#1. The CCK-8 assay showed that silencing ZC3H13 accelerated the sevoflurane-induced reduction in cell proliferation (Fig. 5A). Colony formation assay demonstrated that the decrease in colony formation induced by 3.4 % sevoflurane was further diminished upon transfection with si-ZC3H13#1 (Fig. 5B). Transwell assays revealed a synergistic reduction in migration and invasion of ZC3H13-silenced cells treated with 3.4 % sevoflurane (Fig. 5C and D). Moreover, MeRIP-aPCR assay revealed that m^6A methylation of DLX6-AS1 was inhibited by 3.4 % sevoflurane, and this inhibition was further enhanced by combining sevoflurane treatment with ZC3H13 silencing (Fig. 5E). These findings suggest that ZC3H13 silencing enhances the suppressive effects of sevoflurane on GC cells by inhibiting m^6A methylation of DLX6-AS1.

3.6. ZC3H13 silencing enhances the suppressive effect of sevoflurane on GC in vivo

To further investigate the effects of ZC3H13 and sevoflurane in GC in vivo, we conducted a tumor xenograft assay in mice treated with 3.4 % sevoflurane and subjected to ZC3H13 knockdown. As shown in Fig. 6A, exposure to 3.4 % sevoflurane led to a reduction in tumor volume, and this effect was further enhanced by ZC3H13 silencing. Consistent results were observed regarding tumor size and weight, showing that 3.4 % sevoflurane decreased tumor size and weight, with ZC3H13 knockdown enhancing these effects (Fig. 6B and C). Taken together, these in vivo findings confirm that silencing ZC3H13 can enhance the inhibitory effect of sevoflurane on GC cells.

4. Discussion

ZC3H13 is implicated in various cancer types. For instance, it is upregulated in laryngeal squamous-cell carcinoma, where its inhibition reduces cancer cell proliferation and induces iron depletion, a process linked to cell death [18]. Similarly, elevated ZC3H13 levels in cervical cancer correlate with increased malignancy [19]. In our study, we confirmed high ZC3H13 expression in GC tissues and, demonstrated that silencing ZC3H13 suppresses the migration, invasion, and proliferation of GC cells. These findings provide compelling evidence suggesting that ZC3H13 may play a role in promoting GC tumorigenesis.

Recent years have highlighted the role of m^6A modification in cancer development and drug resistance [20,21]. In GC, the demethylase ALKBH5 targets the m^6A site of lncRNA TP53TG1, reducing its stability and expression [15]. Conversely, METTL13-mediated m^6A modification upregulates lncRNA RPRD1B, promoting lymph node metastasis in GC [22]. Our study reveals, for the first time, m^6A modification of DLX6-AS1, suppressed in GC cells by ZC3H13 knockdown at both m^6A and mRNA levels.

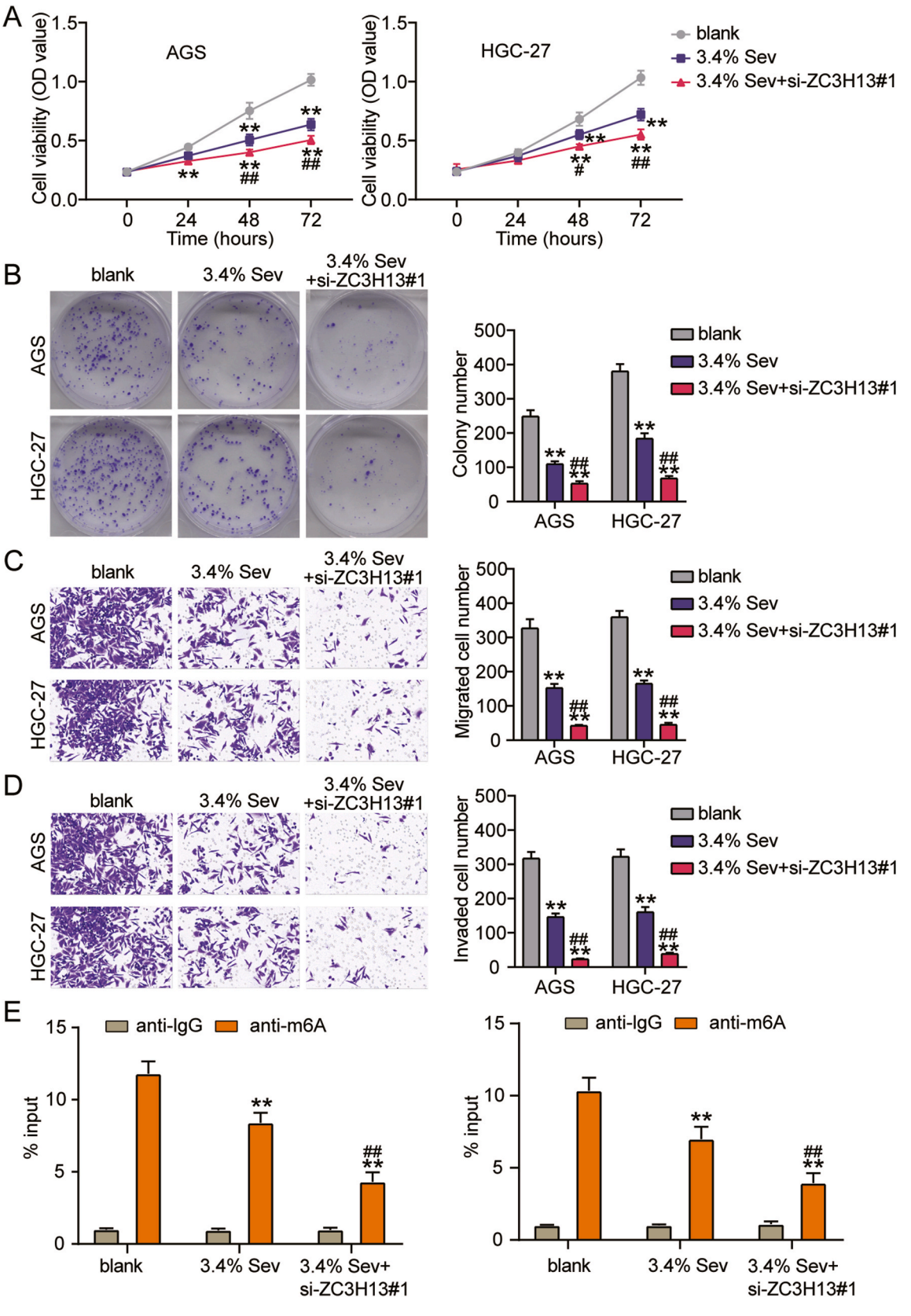

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Fig. 5. Silencing of ZC3H13 enhances the suppressive effect of sevoflurane on gastric cancer cells in vitro via inhibiting m6A methylation of DLX6-AS1. (A and B) The proliferation of AGS and HGC-27 cells transfected with si-ZC3H13#1 or 3.4 % sevoflurane was determined based on CCK-8 (A) and colony formation (B) assays. (C and D) The migration (C) and invasion (D) of AGS and HGC-27 cells treated with si-ZC3H13#1 or 3.4 % sevoflurane were determined using Transwell assays. (E) m^6A modification of DLX6-AS1 in AGS and HGC-27 cells treated with s si-ZC3H13#1 or 3.4 % sevoflurane was assessed based of MeRIP-qRT-PCR assay. **P < 0.001 vs. blank; #P < 0.05, ##P < 0.001 vs. 3.4 % Sev. blank group: AGS and HGC-27 cells were treated without sevoflurane.

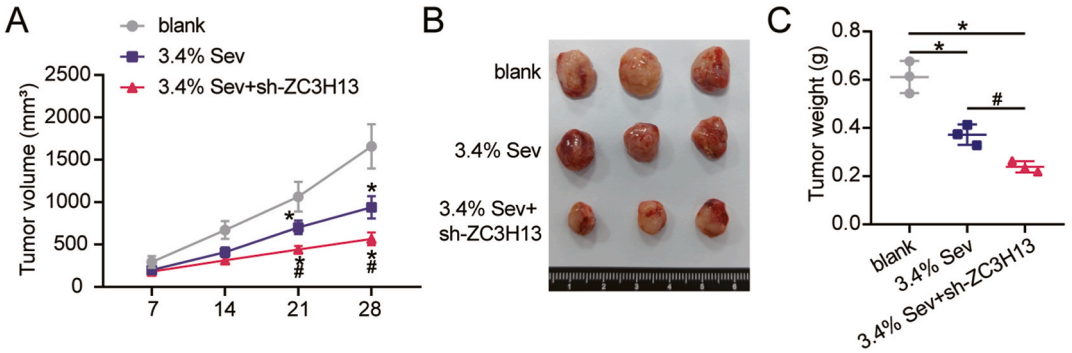

Fig. 6. Silencing of ZC3H13 enhances the suppressive effect of sevoflurane on gastric cancer in vivo. (A) Tumor volumes in mice was measured at weekly intervals. (B) Images of tumors resected from mice at 4 weeks after treatment. (C) The weight of tumors resected from mice after 4 weeks. *P < 0.05 vs. blank; #P < 0.05 vs. 3.4 % Sev. blank group: mice were injected with non-sevoflurane treated AGS cells.

DLX6-AS1, implicated in tumorigenesis across various cancers, is significantly upregulated in GC, where its inhibition stabilizes MAP4K1 expression via RNA-binding protein FUS [23]. Moreover, DLX6-AS1 enhances GC malignancy by upregulating OCT1 through miR-204-5p binding [24]. Our findings also show DLX6-AS1 upregulation in GC and its regulation by sevoflurane via ZC3H13-mediated m^6A modification. This study provides evidence that sevoflurane and ZC3H13 knockdown synergistically suppress GC malignancy by stabilizing DLX6-AS1.

Sevoflurane, an anesthetic agent, has been shown to dose- and time-dependently inhibit proliferation, and significantly reduce migrative capacity of GC cells at 3.4 % concentration [25]. It induces apoptosis and suppresses metastasis in GC by over activating forkhead box protein 3 (FOXP3) [5]. Consistent with previous findings, our study demonstrated that 3.4 % sevoflurane inhibits invasion, migration, and proliferation of GC cells in vitro, as well as tumor growth in vivo. However, this study is the first to show that silencing ZC3H13 enhances the sevoflurane-induced inhibition of malignant GC cell development by regulating m^6A modification of DLX6-AS1.

While this study provides invaluable insights, it does have certain limitations. It is well-established that GC progression involves multiple signaling pathways such as Hippo [26], Wnt/beta-catenin [27], and JNK [28]. Previous research has shown that sevoflurane can influence these pathways by regulating lncRNAs in other cancers like lung cancer [29] and neuroinflammation [30], but its specific impact on GC pathways remains unexplored. Further studies should aim to identify the key signaling pathway regulated by sevoflurane/ZC3H13/DLX6-AS1 in GC. DLX6-AS1, a lncRNA, which interacts with miR-204-5p in GC to promote tumorigenesis in GC [24]. It also stabilizes MAP4K1 expression by modulating the RNA-binding protein FUS, thereby enhancing GC cell malignancy [23]. Further investigation is needed to determine whether sevoflurane/ZC3H13/DLX6-AS1 regulates GC progression through miRNA or RNA-binding protein pathways. Moreover, the clinical relevance of sevoflurane and ZC3H13 in terms of patient prognosis requires further assessment.

5. Conclusion

Collectively, our findings reveal that silencing ZC3H13 in GC cells enhances the inhibitory effect of sevoflurane on cell malignancy by suppressing m^6A modification of the lncRNA DLX6-AS1. Based on these findings, we propose a novel regulatory mechanism for ZC3H13-mediated m^6A modification in GC, which may enhance our understanding of the role of ZC3H13 in tumor development. Furthermore, these findings provide a theoretical basis for developing effective diagnostic and therapeutic biomarkers for the treatment of GC.

Data availability statement

Data will be made available on request.

Funding statement

None.

Conflict of interest disclosure

The authors declared that there are no conflicts of interest.

Ethics approval statement

The ethics committee of Wuhan Fourth Hospital (Wuhan, China) granted approval to this research (approval number: KY2023-010-01). The processing of clinical tissue specimens had been accomplished in precise observance of the ethical standards of the Declaration of Helsinki. Each patient provided a signed consent form.

The animal experiment received authorization from the Ethics Committee of Wuhan Fourth Hospital in accordance with the ARRIVE guidelines (approval number: 2023009).

Patient consent statement

Informed consent forms have been achieved from all participants.

Consent for publication

All participants consented to the publication of this research.

CRediT authorship contribution statement

Chundong Liu: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Zeguang Chen:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35722.

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