HDAC3-Mediated Repression of LncRNA-LET Regulates Gastric Cancer Cell Growth Proliferation, Invasion, Migration, and Apoptosis via MiR-548k

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ABSTRACT: Emerging studies have indicated the aberrant expression of histone deacetylases (HDACs) is closely associated with the development of tumors. However, the regulatory roles of HDACs-regulated long noncoding RNAs (lncRNA) in gastric cancer (GC) remain largely unknown. In this study, the effects of HDAC3 and HDAC3-mediated lncRNA-LET on the progression of GC were investigated. The expressions of HDAC3, lncRNA-LET, and miR-548k in GC cell lines were analyzed. The biological functions of HDAC3 and lncRNA-LET were measured by CCK-8 assay, Transwell assay, Western blot analysis, and cell apoptosis assays. Chromatin immunoprecipitation and luciferase reporter assay verified the regulatory relationship between HDAC3 and lncRNA-LET, and lncRNA-LET and miR-548 in GC cells. HDAC3 was significantly overexpressed in GC cell lines compared to GES-1. Knockdown of HDAC3 suppressed the proliferation, invasion, and migration of AGS and SGC-7901 cells, while cell apoptosis was promoted. Silenced HDAC3 promoted histone acetylation in the promoter region of lncRNA-LET, subsequently upregulating the expression of lncRNA-LET in AGS and SGC-7901 cells. In addition, overexpressed lncRNA-LET notably inhibited the proliferation, invasion, and migration of GC cells, whereas apoptosis was enhanced. LncRNA-LET could function as the sponge of miR-548k. HDAC3 was able to regulate the progression of GC cells via the lncRNA-LET/miR-548k signaling pathway. We confirmed that the HDAC3/lncRNA-LET/miR-548k signal axis mediated the occurrence and development of GC, and HDAC3 could be a novel therapeutic target for the treatments of GC.

KEY WORDS: HDAC3, lncRNA-LET, miR-548k, proliferation, invasion, migration, apoptosis, gastric cancer

I. INTRODUCTION

Gastric cancer (GC) is one of the most common malignant tumors due to its high morbidity and mortality.[1,2] GC occurs when cells in the lining of the stomach grow uncontrollably and form tumors that are able to invade normal tissues and spread to other organs. It can cause eating disorders and is life-threatening.[3,4]

Histone acetylation is a type of epigenetic modification.[5] Histone deacetylases (HDACs) can remove the acetyl group from lysine residues in the N-terminal tail of histone, which results in the formation of positively charged histone and tighter wrapping of DNA around the histone core. Consequently, the binding of transcription factors to DNA promoter regions is inhibited and gene expression can be affected.[6] Previous studies suggested that HDACs were abnormally expressed in numerous types of tumors, which is associated with tumor development.[7–9] In addition, HDACs and their inhibitors are considered as putative biomarkers in cancer research, and they can also be novel targets for cancer treatments.[8–11]

LncRNAs are a group of non-coding RNAs longer than 200 nucleotides.[12] Previous studies suggested that lncRNAs could function as both oncogenic factors and tumor suppressors in GC.[13–15] For

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instance, lncRNA H19 suppresses the expression of CANN1 through targeting miR-675, subsequently affecting the malignant progression of GC.[14] Additionally, lncRNA DANCR promotes the development of GC by activating the β-catenin pathway.[13] LncRNA DNM30S functions as an oncogenic factor that enhances the migration and invasion of GC cells through regulating snail-mediated epithelial-to-mesenchymal transition (EMT).[16] Furthermore, lncRNA MEG3 and lncRNA TUSC7 serve as tumor suppressors in GC.[15,17] However, the detailed functions of lncRNAs in GC and the underlying mechanisms remain largely unknown.

Recent studies have suggested that HDACs could be upstream regulators of lncRNAs and affect tumor progression by regulating lncRNAs. For instance, Yang et al.[18] have revealed that histone deacetylase 3 (HDAC3) significantly suppresses the expression of lncRNAs. Briefly, HDAC3 could mediate the deacetylation of histone at the promotor region of lncRNAs. Wang et al.[19] have also indicated that the levels of histone H3, H4 acetylation at the transcription initiation site of lncRNA-HULC were significantly upregulated in hepatocellular carcinoma cells, while the levels of repressive histone marker H3K9me3 were remarkably downregulated in normal hepatocytes. However, the effects of histone acetylation on lncRNAs in GC are still unclear and require further investigation.

HDAC3 is a member of the HDACs superfamily.[20] Previous studies have revealed upregulation of HDAC3 in GC tissues and cells.[21,22] A previous study has also indicated that HDAC3 is involved in the metastasis of cancer cells by regulating lncRNA-low expression in tumor (lncRNA-LET).[23] Based on the abovementioned findings, our study aimed to elucidate the roles of HDAC3 in GC and explore the underlying mechanisms of HDAC3-regulated expression of lncRNA-LET. In the present study, the expression levels of HDAC3 were examined in GC cell lines, and the effects of HDAC3 and lncRNA-LET on the proliferation, invasion, migration, and apoptosis of GC cells were determined. In summary, the HDAC3/lncRNA-LET/miR-548k signaling was able to regulate the development of GC. More importantly, HDAC3 could be a novel candidate for the targeted therapy of GC.

II. MATERIALS AND METHODS

A. Cell Cultures

Normal human gastric mucosal epithelial cell line (GES-1) and human GC cell lines (AGS, SGC-7901, and HGC-27) were purchased from the cell bank of the Chinese Academy of Sciences. Cells were cultured using DMEM/RPMI-1640 medium (Haoran Biotechnology, Shanghai, China) supplemented with 10% fetal bovine serum (FBS, Lianshuo Biotechnology, Shanghai, China). Cells were maintained at 37°C in a humidified incubator supplied with 5% CO_2.

B. Cell Transfection

To generate the knockdown and overexpression models of lncRNA-LET and HDAC3, si- HDAC3, si-lncRNA-LET and LV-lncRNA-LET vectors together with the negative controls (si-NC and LV-NC) were purchased from GenePharma (Shanghai, China). miR-548k mimics and the respective control, as well as miR-548k inhibitor and its control were synthesized by GenePharma. siRNAs, miR mimics, miR inhibitors, and their NC oligonucleotides (50 nM) were transfected using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol.

C. qRT-PCR

TRIzol® reagent (BeiNuo Biotechnology, Shanghai, China) was used to extract total RNAs from AGS, SGC-7901, HGC-27, and GES-1 cells. The quality and concentration of RNAs were measured using NanoDrop 2000 (Quawell, San Jose, CA, USA). First Strand cDNA Synthesis Kit (Takara, Dalian, China) was used to generate cDNAs using 200 ng of extracted RNAs. Subsequently, qPCR was performed using Power SYBR Green PCR master Mix (Unicom Biotechnology, Beijing, China). The data were calculated using a $2^{-\Delta\Delta Ct}$ method, and β-actin was used as internal control. All primers used in our study were as follows: HDAC3 forward, 5'-TTGCCATTCTGTAATTGCT-3' and reverse, 5'-CTGGCGTCGT-

CAGTGGG-3'; lncRNA LET forward, 5'-GGCTC TGTGGGATCAGTTATG-3' and reverse, 5'-GGCT GAGGAAGGTGGTATTG-3'; miR-548k forward, 5'-CTCCAAAGCCTGAACTGATAGC-3' and reverse, 5'-AAATGAACTACTCCCTCCCAGA-3'; Ki-67 forward, 5'-TGTCACGGCAGAGAGTTCTT-3' andreverse,5'-CGGAGCACTTTGAAACACCA-3'; β-actin forward, 5'-TGCAGCGACTAAGCAGGA-3' and reverse, 5'-TCACCAGCACGAAGGACA-3'.

D. Cell Counting Kit-8 (CCK-8) Assays

Cell proliferation was determined by CCK-8 assay (CK04, Dojindo Molecular Technologies, Shanghai, China). AGS and SGC-7901 cells were seeded on 96-well plates, and 10 μL of CCK-8 solution was added into each well. Cells were incubated at 37°for 2 h. The microplate reader (9200; Bio-Rad Laboratories, Hercules, CA, USA) was used to measure the absorbance (wavelength = 450 nm) at different time points (0, 24, 48, and 72 h).

E. Chromatin Immunoprecipitation (ChIP) Assay

Upstate Biotechnology ChIP kit (Upstate Biotechnology, Lake Placid, NY, USA) was used to evaluate the enrichment of HDAC3 in the promoter region of lncRNA-LET. Briefly, cells or tissues were subjected to an ultrasonic homogenizer. The sizes of chromatins were sheared to 100–1000 bp using sonication. Chromatin extracts were immunoprecipitated using anti-histone acetylase H3 antibody (AmyJet Scientific, Wuhan, China). Normal mouse IgG antibody (Syntek, Wuhan, China) was used as negative control.

F. Transwell Assay

The transwell chambers (pore size = 8 mm, 24-well; BD Biosciences, Franklin Lakes, NJ, USA) were used to determine the invasion and migration of GC cells. For cell migration, cells in serum-free culture medium were seeded onto the upper chamber, which was then placed in a 24-well plate containing 600 μL culture medium containing 10% FBS. Cells were incubated for 48 h and then fixed with ice-cold methanol for 30 min. Subsequently, cells were stained with 0.5% crystal violet for 20 min. Five random fields were selected, and the numbers of migrated cells were counted. For cell invasion, the assay was carried out in a similar manner, but the Transwell chambers were pre-coated with Matrigel (Suobao Biotechnology, Shanghai, China).

G. Western Blotting

RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) was used for cell lysis. Then, BCA protein assay kit (Pierce, Rockford, IL, USA) was utilized to determine the concentrations of extracted protein samples. Equal amount of samples were loaded onto a 10% SDS-PAGE gel. Subsequently, separated protein samples were transferred onto a PVDF membrane (Yubo Biotechnology, Shanghai, China). The membranes were then blocked with 5% skimmed milk at room temperature for 1 h, followed by the incubation with primary antibodies against HDAC3, Ki-67, pro-caspase-3, and cleaved-caspase-3 or β-actin (Abcam, Cambridge, UK) at 4°C overnight. The following day, the membranes were incubated with HRP-conjugated goat anti-rabbit or sheep anti-mouse IgG (CST, Danvers, MA, USA). ECL reagent (Pierce, Rockford, IL, USA) was used to visualize the protein bands, and densitometry was performed using ImageJ software (NIH, Bethesda, MD, USA).

H. Evaluation of Cell Apoptosis

PE Annexin V apoptosis detection kit (BD Pharmingen, Franklin Lakes, NJ, USA) was used to determine the apoptotic rate of GC cells, according to the manufacturer's protocols. Data were analyzed using CellQuest software (Becton-Dickinson, Franklin Lakes, NJ, USA). Experiments were performed in triplicate.

I. Statistical Analysis

Student *t*-test was conducted to compare the difference between two groups. ANOVA was performed for comparison among multiple groups. Data were presented as mean ± standard deviation. Assays were carried out in three independent replicate experiments. Statistical

analyses was performed using SPSS15.0 (SPSS IBM, Armonk, NY, USA), and $P < 0.05$ was considered to indicate a statistically significant difference.

III. RESUITS

A. Expression Levels of HDAC3 Expression were Significantly Up-Regulated in GC Cells

The levels of HDAC3 were examined in AGS, SGC-7901 and HGC-27 cells, and data were normalized to GES-1 cells. The results indicated that the expression levels of HDAC3 were significantly increased in GC cell lines compared to GES-1 cells (Fig. 1A). The relative expression of HDAC3 was measured in GC cells treated with si-HDAC3 using quantitative real time-polymerase chain reaction (qRT-PCR). Knockdown of HDAC3 in AGS and SGC-7901 cells induced significant down-regulation of HDAC3 in AGS and SGC-7901 cells (Fig. 1B).

B. Knockdown of HDAC3 Inhibited GC Cell Proliferation, Invasion, and Migration, While Cell Apoptosis was Promoted

To investigate the biological behavior changes caused by siRNA-HDAC3 (si-HDAC3) in GC cells,

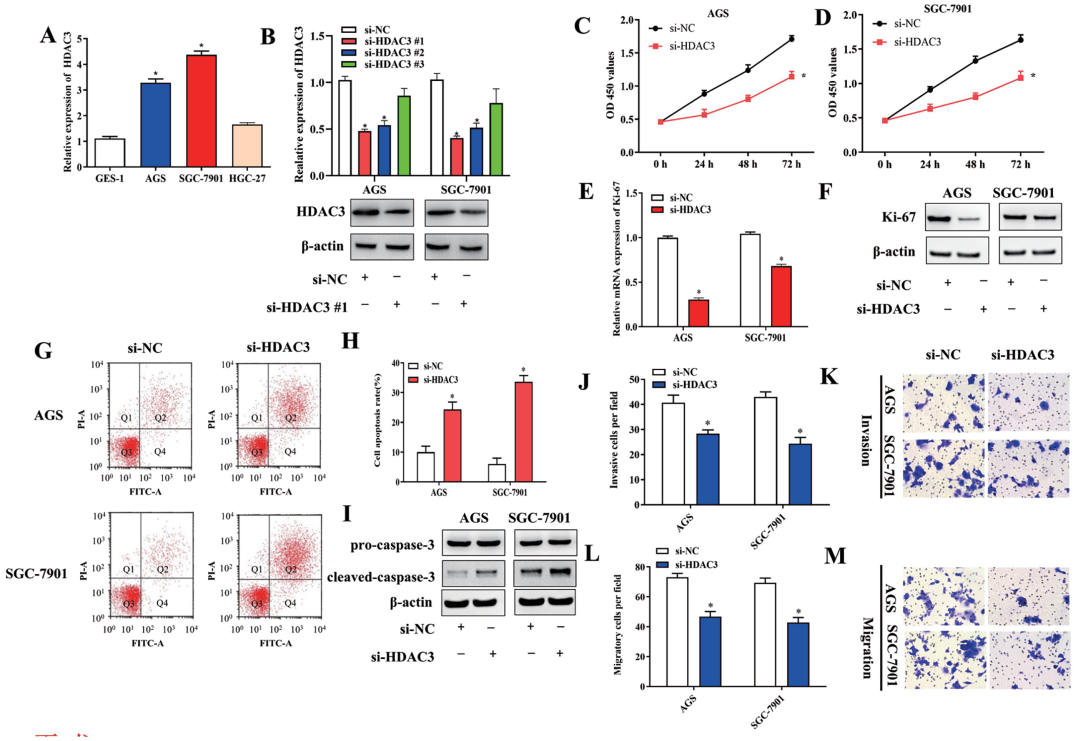

FIG. 1: The expression of HDAC3 was significantly up-regulated in GC cells, and knockdown of HDAC3 inhibited GC cell growth. (A) Relative expression of HDAC3 in human GC cell lines (AGS, SGC-7901, and HGC-27) was determined by qRT-PCR, and data were normalized to gastric mucosal epithelial cells (GES-1). (B) The levels of HDAC3 were examined in AGS and SGC-7901 cells transfected with si-HDAC3 using qRT-PCR and Western blot. (C and D) CCK-8 assay was used to evaluate the proliferation of GC cells transfected with si-HDAC3. (E and F) The mRNA and protein levels of Ki-67 were measured in AGS and SGC-7901 cells after the transfection with si-HDAC3. (G and H) Flow cytometry was used to the apoptotic rate of GC cells treated with si-HDAC3. (I) The levels of pro-caspase-3 and cleaved-caspase-3 in transfected GC cells were measured by Western blot analysis. (J–M) Transwell assay was used to determine the invasion and migration of AGS and SGC-7901 cells after the transfection with si-HDAC3. *$P < 0.05$. GC, gastric cancer; qRT-PCR, reverse transcription-quantitative polymerase chain reaction.

CCK-8 assay was used to examine the proliferation of GC cells transfected with si-HDAC3. The results suggested that the proliferative ability of AGS and SGC-7901 cells was remarkably reduced following the transfection with si-HDAC (Fig. 1C and 1D). Furthermore, Western blotting and qRT-PCR were conducted to evaluate the expression of proliferation-associated marker Ki-67 in transfected cells. The results indicated that the expression of Ki-67 was notably decreased in AGS and SGC-7901 transfected with si-HDAC3 (Fig. 1E and 1F). In addition, the data of flow cytometry revealed that the apoptotic rates of AGS and SGC-7901 cells were remarkably elevated after the treatment with si-HDAC3 (Fig. 1G and 1H). Moreover, the levels of apoptosis-related molecule cleaved-caspase-3 were also increased (Fig. 1I). Additionally, Transwell assay was used to examine the invasion and migration of AGS and SGC-7901 cells transfected with si-HDAC3. The number of invasive cells was significantly decreased in GC cells following the treatment with si-HDAC3 (Fig. 1J and 1K). Similarly, the migration of AGS and SGC-7901 cells was remarkably inhibited after the transfection with si-HDAC3 (Fig. 1L and 1M).

C. HDAC3 Suppressed Expression of LncRNA-LET by Reducing Histone Acetylation Level of LncRNA-LET Promoter

To further investigate the underlying mechanisms of HDAC3-mediated biological behavior alterations in GC cells, the expression levels of lncRNA-LET were examined. The results revealed significant downregulation of lncRNA-LET in GC cells compared to GES-1 cells (Fig. 2A). Following the transfection with si-lncRNA-LET, the levels of lncRNA-LET were notably reduced in AGS and SGC-7901 cells (Fig. 2B). In addition, the expression levels of lncRNA-LET were remarkably increased in GC cells transfected with LV-lncRNA-LET (Fig. 2C). The results of ChIP assay also indicated that the levels of HDAC3 in lncRNA-LET promoter region were notably elevated after the transfection with si-HDAC3 (Fig. 2D and 2E). These findings suggested that knockdown of HDAC3 increased the levels of histone acetylation in the promoter region of lncRNA-LET. In order to confirm the interaction of HDAC3 and lncRNA-LET in GC cells, further experiments were conducted. The expression of lncRNA-LET was significantly elevated in AGS and SGC-7901 cells transfected with si-HDAC3 (Fig. 2F), and vice versa, the levels of lncRNA-LET were decreased in GC cells following the treatment with LV-HDAC3 (Fig. 2G).

D. Overexpression of LncRNA-LET Inhibited Proliferation, Invasion, and Migration of GC Cells, While Cell Apoptosis was Promoted

To further study the effects of lncRNA-LET on GC cells, AGS and SGC-7901 cells were transfected with LV-lncRNA-LET and further cultured for 3 days. The results indicated that the proliferation ability of AGS and SGC-7901 cells were significantly reduced after the transfecting with LV-lncRNA-LET (Fig. 3A and 3B). In addition, the levels of Ki-67 were remarkably decreased in GC cells treated with LV-lncRNA-LET (Fig. 3C and 3D). The number of invasive/migratory cells was also reduced in after the transfection with LV-lncRNA-LET (Fig. 3E–3H). Furthermore, the apoptotic rate of AGS and SGC-7901 cells was notably elevated after the treatment with LV-lncRNA-LET (Fig. 3I). Additionally, the expression of cleaved-caspase-3 was increased in GC cells transfected with LV-lncRNA-LET (Fig. 3J and 3K).

E. LncRNA-LET Functioned as Sponge of MiR-548k in GC Cells

To explore the putative downstream signaling of lncRNA-LET in GC cells, further experiments were conducted. The potential binding site of lncRNA-LET on miR-548k was revealed (Fig. 4A). The results of luciferase reporter assay indicated that luciferase activity was significantly reduced in AGS and SGC-7901 cells co-transfected with miR-548k mimics and lncRNA-LET-WT, but not in the mutant control (Fig. 4B). The expression levels of miR-548k in GC cells were also examined. The results revealed that the expression of miR-548k was remarkably increased in GC cells compared to GES-1 cells (Fig. 4C). After the transfection with miR-548k mimics, the levels

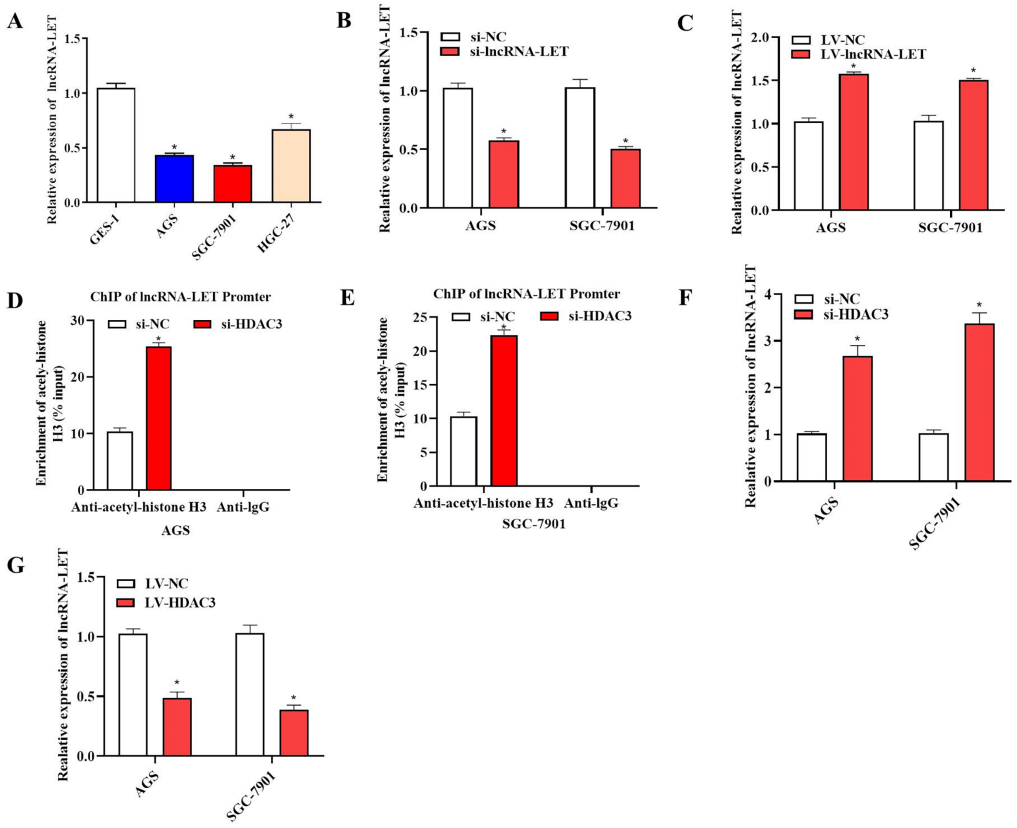

FIG. 2: HDAC3 suppressed the expression of lncRNA-LET by reducing the histone acetylation levels of lncRNA-LET promoter. (A) The expression of lncRNA-LET was measured in AGS, SGC-7901, SGC-7901, and GES-1 cells. (B) The levels of lncRNA-LET were determined in GC cells transfected with si-lncRNA-LET. (C) The expression of lncRNA-LET was examined in AGS and SGC-7901 cells after treatment with LV- lncRNA-LET. (D and E) Chromatin immunoprecipitation (ChIP) was used to evaluate the enrichment of HDAC3 in AGS and SGC-7901 cells after the transfection with si-HDAC3. (F and G) The expression of lncRNA-LET was determined in AGS and SGC-7901 cells transfected with si-HDAC3 and LV-HDAC3, respectively. *$P < 0.05$. GC, gastric cancer.

of miR-548k were significantly elevated in AGS and SGC-7901 cells. Furthermore, the expression of miR-548k was reduced in GC cells transfected with miR-548k inhibitors (Fig. 4D). In addition, the levels of miR-548k were elevated in AGS and SGC-7901 cells after the transfection with si-lncRNA-LET (Fig. 4E). Expression of miR-548k was reduced after the transfection with LV-lncRNA-LET (Fig. 4F).

F. LncRNA-LET was Involved in HDAC3-Mediated GC Cell Growth

To further confirm the involvement of lncRNA-LET in HDAC3-regulated GC cell growth, AGS and SGC-7901 cells were co-transfected with si-HDAC3 and si-lncRNA-LET or LV-lncRNA-LET, respectively. The proliferation of AGS and SGC-7901 cells were reduced after the transfection with si-HDAC3 alone, which was abolished by the treatment with si-HDAC3 and enhanced by the transfection with LV-lncRNA-LET, respectively (Fig. 5A and 5B). In addition, the apoptotic rate was increased in AGS and SGC-7901 cells transfected with si-HDAC3, and these effects were abrogated by si-lncRNA-LET and strengthened by LV-lncRNA-LET, respectively (Fig. 5C and 5D). The invasive/migratory ability of AGS and SGC-7901 cells was inhibited after the transfection with si-HDAC3, which was reversed by

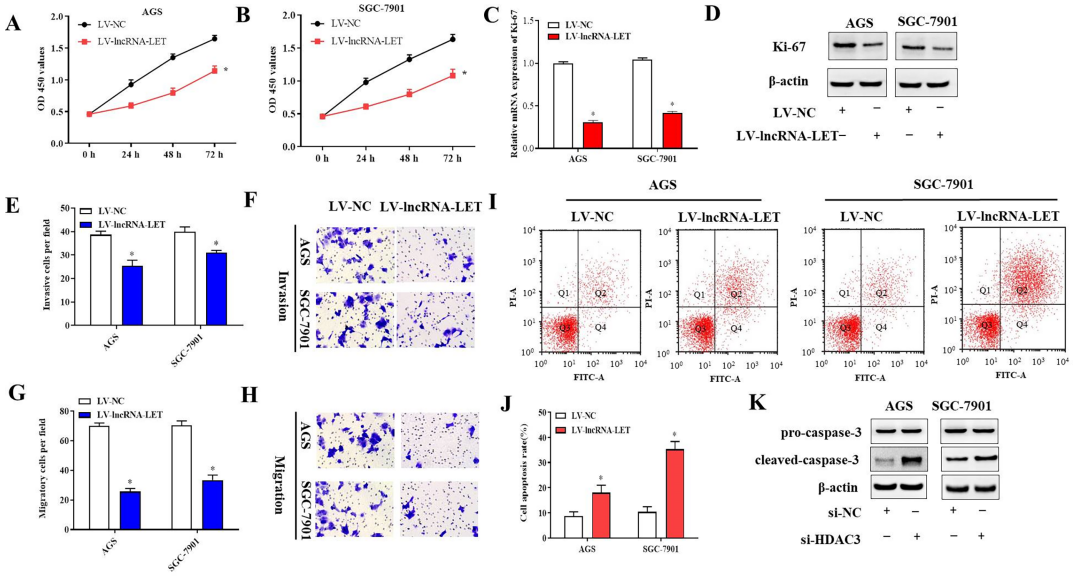

FIG. 3: Overexpression of lncRNA-LET inhibited the proliferation, invasion and migration of GC cells, whereas cell apoptosis was enhanced. (A and B) CCK-8 assay was conducted to measure the proliferation ability of AGS and SGC-7901 cells transfected with LV-lncRNA-LET. (C and D) The expression of Ki-67 was determined by qRT-PCR and Western blotting in transfected GC cells. (E–H) Transwell assay was performed to determine the invasive/migratory capacity of AGS and SGC-7901 cells. (I and J) Flow cytometry was carried out to evaluate the apoptosis of AGS and SGC-7901 cells after the transfection with LV-lncRNA-LET. (K) The protein levels of pro-caspase-3 and cleaved-caspase-3 were assessed by Western blotting. *$P < 0.05$. GC, gastric cancer.

si-lncRNA-LET and enhanced by LV-lncRNA-LET, respectively (Fig. 5E–5H).

G. LncRNA-LET Inhibited Growth of GC Cells through Targeting MiR-548k

To further confirm that miR-548k was involved in lncRNA-LET-modulated GC cell growth, AGS and SGC-7901 cells were co-transfected with LV-lncRNA-LET and miR-548k mimics or inhibitors, respectively. The results suggested that the proliferative ability of AGS cells and SGC-7901 was reduced after the transfection with LV-lncRNA-LET alone, which was reversed by miR-548k mimics and enhanced by miR-548k inhibitors, respectively (Fig. 6A and 6B). Flow cytometry also indicated that the apoptosis rate of AGS and SGC-7901 cells were elevated after the treatment with LV-lncRNA-LET, and these effects were abrogated by miR-548k mimics and strengthened by miR-548k inhibitors, respectively (Fig. 6C and 6D). The invasive/migratory ability of GC cells were inhibited by LV-lncRNA-LET, which was reversed by the treatment with miR-548k mimics and enhanced by the transfection with miR-548k inhibitors, respectively (Fig. 6E–6H).

H. HDAC3/LncRNA LET/MiR-548k Axis Regulated Proliferation, Invasion, Migration, and Apoptosis of GC Cells

HDAC3 was significantly overexpressed in GC cells, which suppressed the expression of lncRNA LET by reducing the acetylation level of its promoter. These effects triggered the up-regulation of miR-548k, consequently promoting the growth of GC cells (Fig. 7).

IV. DISCUSSION

GC is a type of malignant tumor, originating from gastric mucosa. It is a leading cause of cancer-related death worldwide and one of the most common

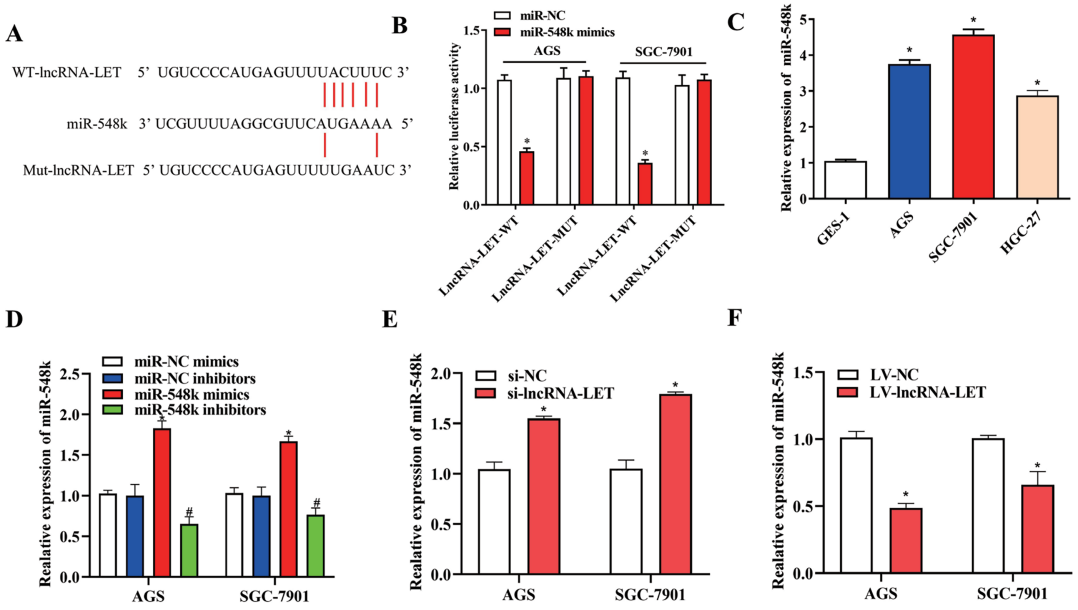

FIG. 4: LncRNA-LET functioned as the sponge of miR-548k in GC cells. (A) The predicted binding sites between miR-548k and lncRNA-LET. (B) Luciferase assay was used to confirm the interaction of lncRNA-LET and miR-548k. (C) The expression of miR-548k was measured in AGS, SGC-7901, SGC-7901, and GES-1 cells. (D) The levels of miR-548k were determined in GC cells transfected with miR-548k mimics and inhibitors, respectively. (E) The expression of miR-548k was evaluated in AGS and SGC-7901 after the transfection with si-lncRNA-LET. (F) The expression of miR-548k was decreased in AGS and SGC-7901 after the transfection with LV-lncRNA-LET, respectively. $^{*}P < 0.05$, $^{\#}P < 0.05$. GC, gastric cancer.

malignancies in China. The mortality rate of GC is extremely high due to its high malignancy and poor prognosis, and it is difficult to diagnose GC at an early stage. Therefore, this disease poses a great threat to human health.[24]

Histone acetylation, a type of epigenic modification, refers to acetylation of histone N-terminal tails.[25–27] The levels of histone acetylation are regulated by both histone acetylase (HATs) and deacetylase (HDACs). HATs and HDACs work together to maintain the dynamic balance of histone acetylation. The disruption of the balance between HATs and HDACs activities can affect cell proliferation and apoptosis, consequently leading to uncontrolled cell growth and tumor.[28,29] Recent studies have indicated that HDACs serve essential roles on tumorigenesis.[30,31] In addition, Alzoubi et al.[32] have suggested that the expression levels of HDAC2 are significantly increased in colorectal cancer (CRC) tissues/cells. Up-regulation of HDAC2 is able to inhibit the apoptosis of CRC cells through targeting the p53 signaling pathway. Furthermore, HDAC4 can promote the proliferation, invasion, and migration of CRC cells by suppressing the transcription of key result areas (KRAs).[33] A recent study by Huang et al. has revealed that knockdown of HDAC2 induces the up-regulation of KRAs, subsequently promoting the apoptosis of CRC cells.[34] In the present study, our data suggested that the expression levels of HDAC3 were remarkably elevated in GC cell lines, as compared to normal human gastric mucosal epithelial cells, which indicated that HDAC3 was aberrantly expressed in GC cells. Further experiments revealed that knockdown of HDAC3 inhibited the proliferation of AGS and SGC-7901 cells, and the expression levels of proliferation-associated gene Ki-67 were also reduced. Moreover, si-HDAC3 was also able to suppress the invasive and migratory abilities of AGS and SGC-7901 cell, while cell apoptosis was notably promoted. Consistent with these findings, the levels of apoptosis-related molecule caspase-3

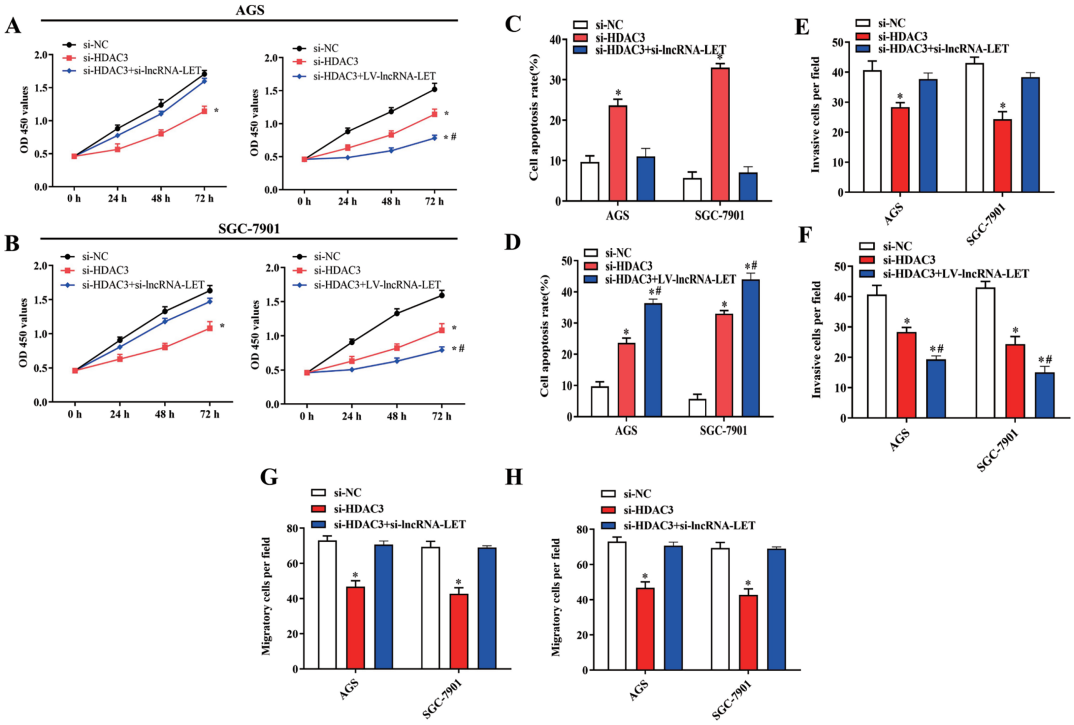

FIG. 5: LncRNA-LET was involved in HDAC3-mediated GC cell growth. (A and B) CCK-8 assay was performed to examine the proliferative ability of AGS and SGC-7901 cells co-transfected with si-HDAC3 and si-lncRNA-LET or LV-lncRNA-LET, respectively. (C and D) Flow cytometry was conducted to determine the apoptotic rate of GC cells. (E–H) Transwell assay was carried out to evaluated the invasion/migration capacity of transfected GC cells. **P* < 0.05, #*P* < 0.05. GC, gastric cancer.

were remarkably increased in GC cells treated with si-HDAC3. These results indicated that HDAC3 was significantly up-regulated in GC cells, which could promote cancer cell growth, consequently facilitating tumor progression in GC. However, the detailed regulatory mechanisms are not completely understood and require further investigation.

Accumulating evidence have revealed abnormal expression of lncRNAs in numerous types of cancers, which could be associated with tumor progression. Recent studies have suggested that HDACs are involved in the regulation of lncRNAs in tumor cells. Yang et al.[18] have indicated that the expression levels of lncRNA-LET were significantly reduced in liver cancer cells and tissues in a hypoxic environment. These findings have suggested that hypoxia could cause significant down-regulation of HDAC3, which lead to the reduced levels of histone acetylation in the promoter region of lncRNA-LET and consequently suppress the expression of lncRNA-LET. Huang et al.[35] have indicated that HDAC3 could regulate the expression of Xist and AKT in breast cancer cells through targeting lncRNA-PHLPP1. In summary, increased levels of histone acetylation could indicate the activation of transcription. Histone acetylation results in the formation of positively charged histone and open chromatin structure, subsequently promoting the transcription of related genes. In our study, the results of chIP assay indicated that knockdown of HDAC3 could significantly increase the levels of histone H3 acetylation in the promoter region of lncRNA-LET. Furthermore, si-HDAC3 was able to suppress the expression levels of lncRNA-LET. Overexpression of HDAC3 promoted the expression of lncRNA-LET expression in GC cells. Taken all together, HDAC3 could suppress the transcription of lncRNA-LET by reducing the

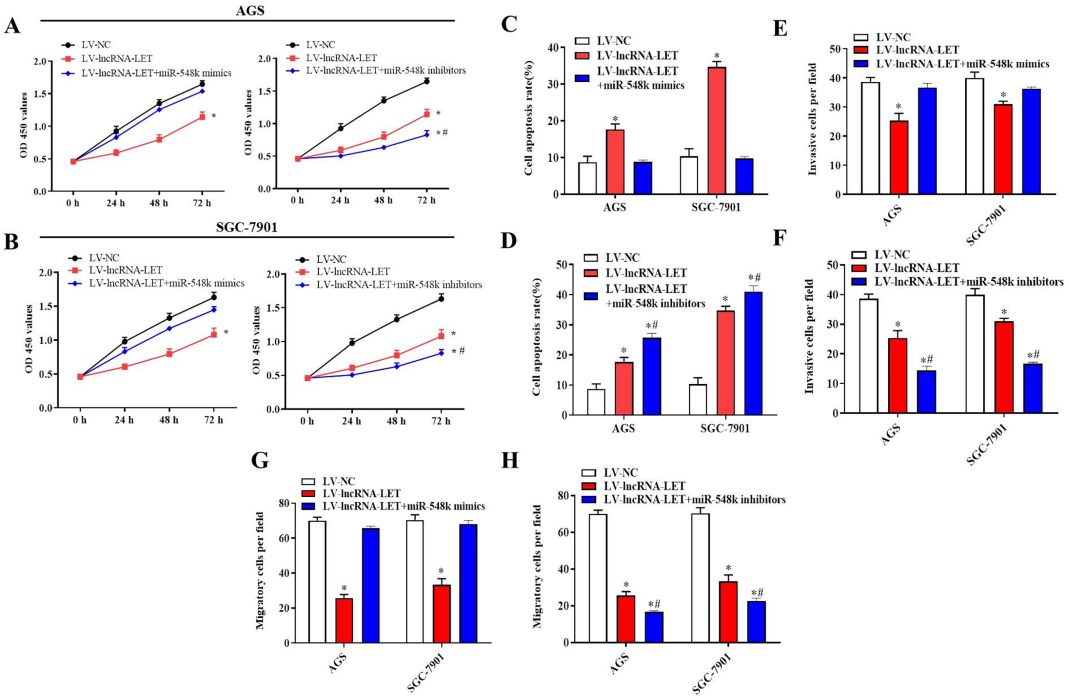

FIG. 6: LncRNA-LET suppressed the growth of GC cells through targeting miR-548k. (A and B) CCK-8 assay was performed to measure the proliferation of GC cells co-transfected with LV-lncRNA-LET and miR-548k mimics or inhibitors, respectively. (C and D) Flow cytometry was conducted to determine the apoptotic rate of transfected AGS and SGC-7901 cells. (E–H) Transwell assay was carried out to evaluate the invasive/migratory activity of transfected GC cells. $^{*}P < 0.05$, $^{\#}P < 0.05$. GC, gastric cancer.

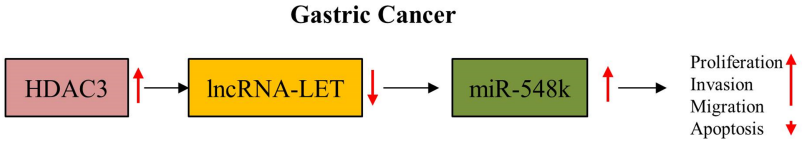

FIG. 7: The HDAC3/lncRNA LET/ miR-548k regulated the growth of GC cells. GC, gastric cancer.

levels of histone acetylation in its promoter region. Furthermore, the results of luciferase reporter assay revealed the interaction of lncRNA-LET and miR-548k in AGS and SGC-7901 cells. Our data also indicated that miR-548k was significantly overexpressed in GC cells. Knockdown of lncRNA-LET induced the expression of miR-548k expression, and overexpression of lncRNA-LET decreased the levels of miR-548k in GC cells. The results of further function experiments indicated the biological behavior changes in GC cells caused by si-HDAC3 were reversed by the knockdown of lncRNA-LET, and vice versa, the effects induced by si-HDAC3 were enhanced by the transfection with LV-lncRNA-LET. The alterations on the proliferation, invasion, migration, and apoptosis of GC cells caused by LV-lncRNA-LET were abrogated by miR-548k mimics, and vice versa, the above mentioned behavior changes induced by LV-lncRNA-LET were strengthened by miR-548k inhibitors. These findings suggested that HDAC3 was able to promote the proliferation, invasion, and migration, and to suppress the apoptosis of GC cells through the lncRNA/miR-548k signaling pathway.

V. CONCLUSIONS

In summary, our findings suggested that HDAC3 was significantly overexpressed in GC cells, which suppressed the expression of lncRNA-LET by reducing the levels of histone acetylation in the promoter region of lncRNA-LET. Furthermore, lncRNA-LET functioned as the sponge of miR-548k in GC cells. However, there were still some limitations in this study. For example, in order to verify the regulatory roles of HDAC3 on tumor progression in GC, *in vivo* experiments and EMT-related molecules should be performed to confirm existing findings. In addition, apart from HDAC3, the transcription of lncRNA-LET could be affected by other acetylases or deacetylases, which should be considered in future study. In conclusion, the HDAC3/lncRNA-LET/miR-548k axis could affect the proliferation, invasion, migration, and apoptosis of GC cells. Therefore, HDAC3 may be a novel candidate for the targeted therapies of GC.

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