## ORIGINAL RESEARCH LINC01436 Promotes the Progression of Gastric Cancer via Regulating miR-513a-5p/APE1 Axis

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**Background:** Gastric cancer (GC) is one of the deadliest the worldwid Multiple long non-coding RNAs (lncRNAs) are recently identified crucia. ctors or tumor ncogenic suppressors in GC. In this study, we aimed to preinto the effe NC01436 on GC of / progression.

GC tisses samples were measured Methods: LINC01436 and miR-513a-5p erress using quantitative real-time polymerase in reaction RT-P x). Western blot was used to detect the expression of apurinic/apy\_\_nide endodeoxy, onuclease 1 (APE1) expression. Human GC cell lines AGS and BGC-823 we employed to investigate the function and mechanism of LINC01436 in the counting k (CCK-8) assay was used to assess the effect of LINC01436 on p liferation. Flow cytometry was utilized to explore the effect of nd Transwee assay was conducted to detect the effect of LINC01436 on apoptosis, LINC01436 on the migratic and inversion. Colony formation assay was performed to UNC0145 evaluate the eff radioresistance of GC cells. Furthermore, luciferase RNA 1 oprecipitation assay were conducted to confirm the binding reporter assay and x-513a-5p and LINC01436. Additionally, Western blot was used to relationship betwee study nction of LINC01436 and miR-513a-5p on APE1. ne re, latory

sults: L C01436 pression of GC clinical samples was remarkably increased and o was conclated with unfavorable pathological indexes. LINC01436 high expres-Lhsion wassociated with shorter overall survival time. Its overexpression observably promoted the coliferation, metastasis and radioresistance of GC cells, and its knockdown ppressed the malignant phenotypes of GC cells. LINC01436 overexpression markedly ed the miR-513a-5p expression via sponging it and enhanced the APE1 expression. MiR-513a-5p overexpression or APE1 knockdown reversed the effects of LINC01436 on GC cells.

**Conclusion:** LINC01436 is a molecular sponge of tumor suppressor miR-513a-5p, which indirectly enhances the APE1 expression and functions as the oncogenic lncRNA in GC. Keywords: gastric cancer, LINC01436, miR-513a-5p, APE1, ceRNA

### Background

Gastric cancer (GC) is one of the most common cancers in the world, whose mortality rate ranks the second among cancers.<sup>1</sup> Due to the limited early diagnosis technique of GC, high recurrence rate and high metastasis rate, the prognosis of patients with GC is poor.<sup>2</sup> Therefore, to probe the potential biological mechanisms of GC is of great significance for the prevention and treatment of this disease.

Accumulating studies find that long non-coding RNAs (lncRNAs) are abnormally expressed in a variety of malignancies and can affect the progression of multiple cancers including GC.<sup>3,4</sup> For instance, abnormal upregulation of lncRNA PANDAR

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expression is a poor prognostic factor for predicting cervical cancer;<sup>5</sup> down-regulation of lncRNA MALAT1 expression suppresses the metastasis of breast cancer.<sup>6</sup> It is reported that LINC01436 accelerates the growth and metastasis of NSCLC cells, implying the oncogenic role of LINC01436 in cancer biology.<sup>7</sup> However, the specific regulatory mechanism of LINC01436 in GC is much less explored.

MicroRNAs (miRNAs) are a class of non-coding RNA (ncRNA) molecules of 21 to 25 nt in length, which are highly conserved and cannot encode proteins, and they are involved in the regulation of tumor progression through post-transcriptional regulation of genes.<sup>8–11</sup> MiR-513a-5p plays an essential role in modulating inflammatory response-mediated cell injury. It is reported that it is involved in TNF- $\alpha$  and LPS induced apoptosis via down-regulating the expression of X-linked inhibitors of apoptotic proteins in endothelial cells.<sup>12</sup> The dysregulation of miR-513a-5p expression is validated to be closely linked to breast cancer risk.<sup>13</sup> Nevertheless, the role of miR-513a-5p in GC progression remains to be studied.

Apurinic/apyrimidinic endodeoxyribonuclease 1/redox factor-1 (APE1/REF-1) is a multifunctional protein that is generally involved in repairing DNA injury through its endonuclease activity in base excision repair.<sup>14</sup> It controls is activity of diverse transcription factors such as STAT3, NF  $\kappa$ B, AP-1, p53 and hypoxia-inducible factor 1 $\alpha$  control  $\alpha$ ).<sup>15</sup> APE1 also plays a key role in cancer progression. For instance, the APE1 expression in triple-negative brance cer tissues is significantly higher than that in adjunct tissues, and APE1 is expected to be a progressic factor to breast cancer.<sup>16,17</sup> Particularly, APE1 is also involved in GC progression.<sup>18</sup> Nonetheless as upstream regatory mechanism in GC remains to bourther intestigated.

LncRNA can function and competitive endogenous RNA (ceRNA) interference IA, the eby inhibiting gene expression and function. In our reliminary research, we noticed that a R-51 and 1 d-potential binding sites with both 3'UTR of the I mRNA and LINC01436. Hence, we hypothesized that the GC, LINC01436 high expression could facilitate the GC progression by inhibiting miR-513a-5p expression and indirectly facilitating APE1 expression.

## Materials and Methods

### Clinical Tissues Collection

All GC tissues were obtained from GC patients at First Affiliated Hospital of Anhui Medical University from May 2017 to March 2018. Forty cases of GC tissues were randomly selected in this study. The specimens in the control group were resected from paracancerous tissues of the same patient (at least 3 cm from the surgical margin), and no cancer cells were found in the pathological examination. All specimens were placed in liquid nitrogen at  $-196^{\circ}$ C immediately after the removal. All patients involved gave informed consent and signed a written consent form. This study was ratified by the Ethics Review Committee of the First Affiliated Hospital of Anhui Medical University. All protocols were conducted in accordance with the principal of the Declaration of Helsinki.

## Cell Lines and Con Culture

The GC cell lines SGC × 01, MKN45, PGC-823, AGS and human normal extric epicelial con GES-1 were purchased from the coll Center come Chinese Academy of Sciences (Shanghai, China). Cells were cultured with RPML rec0 medium (Thermo Fisher Scientific, Cartoridge, MA, USA) containing 10% fetal bovine serue (FBS, Thomo Fisher Scientific, Cambridge, MA, USA) and 100 LomL penicillin, and 100 U/mL streptomyin (Invitrogen, Carlsbad, CA, USA) in an incubator at 37° Content SO<sub>2</sub>.

#### Cell Transfection

LINC01436 overexpression plasmid, LINC01436-siRNA (si-LINC01436), APE1 overexpression plasmid, APE1siRNA (si-APE1), miR-513a-5p mimics, miR-513a-5p inhibitors and their corresponding controls were constructed by GenePharma (Shanghai, China). Lipofectamine<sup>®</sup> 3000 (Invitrogen, Carlsbad, CA, USA) was used to perform the transfection in line with the supplier's instructions.

## Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA was extracted from tissues and cells with TRIzol reagent (Thermo Fisher Scientific, Shanghai, China), and then the RNA was reversely transcribed into cDNA using the TOYOBO Reverse Transcription Kit (TOYOBO, Osaka, Japan) following the manufacturer's instructions. qRT-RCR was performed with SYBR premix EX TAQ II (Takara, Dalian, China) on an ABI 7500 Real-Time PCR System (Applied Biological System, CA, USA). GAPDH and U6 were used as the internal references of LINC01436, and

Name	Sequence (5'–3')
LINC01436	Forward: 5'-AGATGCCAGGGGTAAAGTGG-3' Forward: 5'-GCATGGGTTCTAGAGTGGGC-3'
miR-513a-5p	Forward: 5'-GGAGGGTTCACAGGGAGGT-3' Reverse: 5'-GTGCGTGTCGTGGAGTCG-3'
APEI	Forward: 5'-GCTTGGATTGGGTAAAGGA-3' Reverse: 5'-GTCGAGGTAGTCTGTTTCTT-3'
U6	Forward: 5'-CTCGCTTCGGCAGCACATATACT-3 Reverse: 5'-ACGCTTCACGAATTTGCGTGTC-3'
GAPDH	Forward: 5'-CTCCTCCTGTTCGACAGTCAGC-3 Reverse: 5'-CCCAATACGACCAAATCCGTT-3'

#### Table I qRT-PCR Primer Sequences

APE1 and miR-513a-5p, respectively, and the relative expression levels were analyzed using the  $2^{-\Delta\Delta Ct}$  method. The primer sequences are shown in Table 1.

#### Western Blot

The cells were lysed with RIPA lysate (containing 1% PMSF), and the supernatant was collected after the highspeed centrifugation. Subsequently, the Bradford method was used for protein quantification. Then the sample was heated in a water bath at 100°C for 10 min. SDS-\GL was used to separate the proteins and then the proins were transferred onto polyvinylidene f JIR. (PVL membranes. After that, the membrar was becked room temperature with 5% skimmed ilk lowed by being added with nti-Anti-XE1 antibody (ab76472) (1:1000; Abcam an idge, UK) d Anti-βactin antibody (ab115777) (1:1000, bcam, Cambridge, UK) for incubation or might at 4°C and then rinsed with TBST solution. Sosequer , the membrane was incuntibodie (Beyotime, Shanghai, bated with seconda room mper ure before being washed China) for 11 3 times At last color redering was performed using hypersel tive LCL Jssci Biotechnology Co, Ltd., Wuhan, Ch. ).

## Cell Counting Kit-8 (CCK-8) Assay

The AGS and BGC-823 cells in the logarithmic growth phase were prepared as a single cell suspension by 0.25% trypsinization. After counting the cells, the cells were seeded in 96-well plates at about 2000 cells per well and cultured at 37°C in 5% CO<sub>2</sub>. After the culture for 0, 1, 2, 3, and 4 d in an incubator, respectively, 10  $\mu$ L of CCK-8 kit (Beyotime, Shanghai, China) was added to each well,

and the incubation was continued for 1 h. Then the absorbance of each well was measured at a wavelength of 450 nm with a microplate reader.

#### Migration and Invasion Analysis

The migration and invasion were detected using the Transwell cell assays. In migration assay, 2×10<sup>4</sup> cells/ well (without FBS) were seeded in the upper chamber of the Transwell chamber with an 8 µm pore size membrane (Corning, NY, USA), and the lower chamber was added with 500 µL of RPMI 1640 meaning polemented with 10% FBS. After being cultured for 24 h, e unmigrated cells were wiped off with a constant on swab Cells passing through the membrar were fixed the paraformaldehyde, stained with 1% c stal viole, photographed and avasion say, Matrigel (BD, San counted after ving. Jose, CA, (1) was used to can the Transwell membrane before we cell were inoculated. The other procedures wer migration assay. same as t

## uciferas Report Analysis

The lucifer c reporter assay was used to verify the targeting relationship between LINC01436 and miR-513a-5p. Accells were seeded at a density of  $5 \times 10^5$  cells in a 24well plate, and the luciferase reporter vectors containing wild type (WT) or mutant (Mut) LINC01436 sequence were cloned into pGL3 Basic vector (Promega, Madison, WI, USA), respectively, and then transfected into the cells. Following that, the miR-513a-5p mimics or negative control was co-transfected into the above cells, respectively. After 48 h of transfection, luciferase activity was determined using the dual-luciferase reporter assay system (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions.

#### RNA Immunoprecipitation (RIP) Assay

To pinpoint the relationship between LINC01436 and miR-513a-5p, RIP analysis was performed using the EZ-Magna RIP kit (Millipore, Billerica, MA, USA) complying with the manufacturer's instructions. AGS cells were lysed using RIP lysis buffer containing protease inhibitors and RNase inhibitors, and then antibody against Ago2 or mouse IgG coupled with magnetic beads was incubated with the lysate for 1 h at room temperature. Subsequently, after removing the protein with proteinase K, the immunoprecipitated RNA was extracted for qRT-PCR analysis.

## Flow Cytometry Analysis

The cells were trypsinized with trypsin and collected by centrifugation (1500 r/min, 3 min). Subsequently, the Annexin V-FTIC/Propidium iodide (PI) Apoptosis Detection Kit (Aladdin BioReagent Co., Ltd., Shanghai, China) was used to detect cellular apoptosis. In brief, after the cells were washed twice with PBS, 400  $\mu$ L of precooled PBS was added to resuspend the cells, followed by the addition of 10  $\mu$ L of Annexin V-FITC staining solution and 5  $\mu$ L of PI staining solution. Thereafter, the cells were incubated at 4°C in the dark. After 30 min, the percentage of apoptotic cells was detected by a flow cytometer (Becton-Dickinson, Fullerton, CA, USA) immediately.

### **Colony Formation Analysis**

First, 1000 cells in each group were seeded in a 35 mm culture dish. Then the cells were cultured for 24 h and then treated with different doses of irradiation (0 Gy, 4 Gy and 8 Gy). Subsequently, the cells were cultured for 2 weeks. Ultimately, the cells were stained with 0.1% crystal violet and washed with PBS, and the number of colonies was counted.

### Mice Assay

The procedures in animal experiments were approved by Ethics Review Committee of the First Affiliated Repital of Anhui Medical University. All the protocols *I* the animal experiments in this study mp. Ath the United Kingdom Coordinating Research (UKCCCR) guideline for elfare of imals in experimental neoplasia. welve six welve female BALB/c nude mice (SPI grade) were sele ed and randomly divided into o groups (si-NC group and si-LINC01436 group). 10 of A cell suspension (containing ab  $\pi^{2} \times 1^{6}$  cells, was subcutaneously inoculated to the right (si- NC0143) and left (si-NC) side of each mouse. We longest and shortest diameters of the tumor were mean red once a week using a caliper until the tumor was remued after 5 weeks. The formula was adopted to calculate the tumor volume: volume = (length  $\times$  width<sup>2</sup>  $\times$  0.5).

### Statistical Analysis

Statistical analysis was performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA), and the measurement data were expressed as mean  $\pm$  standard deviation (x  $\pm$  s). Differences between the different groups were analyzed by *t*-test or one-way ANOVA. A significant statistical difference was considered at P < 0.05.

### Results

## LINC01436 Was Highly Expressed in GC Tissues and Cells

expression characteristics То investigate the of LINC01436 in GC, we examined the LINC01436 expression in 40 cases of cancerous tissues from GC patients and compared it with the LINC01436 expression in corresponding adjacent tissues. qRT-PC, and vis revealed that the LINC01436 expression in tumor sues was observably higher than that not not not tissues (Figure 1A). Consistently, GEPL database tp://pia.cancerpku.cn/) unearthed the the LP 201436 pression was markedly up-regulated in S tissues comparison with the normal tises (Figure B) tata were from The Cancer Geome Atlas, TCA). Subsequently, the LINC01436 expression in each cell line was examined, dings of which demonstrated that the LINC01436 the f expession was markably increased in the GC cell lines (AC BGC-823 GC7901 and MKN45 cells) compared to in the porrect cell line GES-1 (Figure 1C). To further m the clinical significance of LINC01436 expression GC, we explored the association between the LINC01436 expression and the clinical features of GC atients. Forty patients were divided into LINC01436 high expression group and LINC01436 low expression group in accordance with the LINC01436 median expression. Our data demonstrated that LINC01436 high expression in GC tissues was significantly associated with T stage and differentiation status, but not with age, gender, tumor size, and lymphatic metastasis (Table 2). Importantly, TCGA data showed that the overall survival time of patients with high expression of LINC01436 was notably shorter than that of patients with low expression of LINC01436 (Figure 1D). From this, we could conclude that LINC01436 was highly expressed in GC tissues and might take part in promoting cancer progression.

## LINC01436 Regulated the Proliferation, Invasion and Radioresistance of GC Cells

To assess the effect of LINC01436 on GC cells, the LINC01436 low expression and overexpression models were established with AGS cells and BGC-823 cells, respectively (Figure 2A). CCK-8 assay implied that the transfection of LINC01436 siRNA markedly reduced the



**Figure 1** LINC01436 expression was up-regulated in GC. (**A**) qRT-PCR was used to the expression of LINC01436 in adjacent tissues and GC tissues. (**B**) The expression level of LINC01436 in GC tissues and gastric normal tissues was analyzed using the GA data with zEPIA database. (**C**) qRT-PCR was used to detect the expression of LINC01436 in GES-1, AGS, BGC-823, SGC7901 and MKN45 cell lines, which have been as used to analyze the association between GC patients' survival and LINC01436 expression using GEPIA database. \*,\*\* and \*\*\* represent P < 15, P < 100, respectively.

viability of GC cells, while LINC0143 over pressi notably increased the proliferation of C cells compare to NC group (Figure 2B). Furthemore # w cytometry analysis revealed that LINCO 6 knockden enhanced the apoptosis of GC cells, file NC01436 cerexpression exerted the opposite function (Figure 2C). To further LINCA1436 on Ge cell metastasis, elaborate the effect Transwell assay cared out, the results of which NCO 6 knor down reduced GC cell unveiled that LINC01436 overexpression migration and in sion, w enhance these (Figure 2D). Additionally, colassay implied that LINC01436 knockdown ony forma. increased the radiosensitivity of GC cells, whereas LINC01436 overexpression had the opposite effect (Figure 2E).

## LINC01436 Negatively Modulated miR-513a-5p Expression

To further probe the downstream mechanism of LINC01436, we turned to StarBase database (<u>http://star</u>base.sysu.edu.cn/) to predict potential downstream targets

of LINC01436 and found that miR-513a-5p was one of its potential targets (Figure 3A). Following that, gRT-PCR was employed to detect the miR-513a-5p expression in GC tissues and corresponding adjacent tissues. The results implied that miR-513a-5p expression was significantly decreased in GC tissues (Figure 3B). Importantly, there was a strong negative correlation between LINC01436 expression and miR-513a-5p expression in the 40 GC tissues mentioned above (Figure 3C). Subsequently, the miR-513a-5p expression in normal cell lines and GC cell lines was detected. As shown, in comparison with the normal cell line GES-1, miR-513a-5p expression was remarkably decreased in GC cell lines (Figure 3D). To further clarify the targeting relationship between LINC01436 and miR-513a-5p, the luciferase activity assay was performed and showed that miR-513a-5p mimics significantly repressed the luciferase activity of wild-type LINC01436 sequence, while overexpression of LINC01436 markedly reversed these effects (Figure 3E). In addition, there was no effect on the mutant LINC01436 sequence (Figure 3E). Consistently, RIP experiments showed that in AGS cells, LINC01436 and miR-513a-5p

Pathological Parameters	No. of Patients	LINC Expre	01436 ession	p-value
		Low	High	
All patients	40	20	20	
Age				0.1024
P < 60	15	10	5	
≥60	25	10	15	
Gender				0.5251
Male	22	12	10	
Female	18	8	10	
Tumor size (cm)				0.0565
<5	18	12	6	
≥5	22	8	14	
T stage				0.0006
I	7	5	2	
П	13	11	2	
Ш	20	4	16	
Differentiation				0.0039
Well and moderate	17	13	4	
Poor	23	7	16	
Lymphatic metastasis				0.0528
No	16	11	5	
Yes	24	9	15	

 $\begin{array}{cccc} \textbf{Table 2} & \text{Correlation Between LINC01436 and Pathological} \\ \text{Parameters in GC} \end{array}$ 

were significantly enriched in anti-Ago2 goup could al with anti-IgG group (Figure 3F). A diational agRT-PCR results revealed that LINC0142 expression, suppressed the miR-513a-5p expression, while LINC01436 knockdown facilitated the expression of LIR-513a-5p in GC cells (Figure 3G) eased on these results mentioned above, we identified use mic-513a-5p was a target of LINC01436.

## MiR-513a 5p over pression Notably Repressed the Proliferation, Invasion and Radioresistance of GC Cells

To delve into the effect of miR-513a-5p on GC cells, the low expression and overexpression models of miR-513a-5p were established (Figure 4A). As shown, miR-513a-5p inhibition enhanced the proliferation of AGS cells, whereas miR-513a-5p mimics restrained it in BGC823 cells (Figure 4B). Flow cytometry analysis indicated that miR-513a-5p inhibition repressed the apoptosis of GC cells, while miR-513a-5p overexpression induced the apoptosis of GC cells (Figure 4C). As we expected, miR-513a-5p inhibitors enhanced the migration and invasion of GC cells, while miR-513a-5p mimics had the opposite effects (Figure 4D). Additionally, colony formation assay revealed that miR-513a-5p inhibitors promoted the radioresistance of GC cells, whereas miR-513a-5p overexpression sensitized GC cell to radiation (Figure 4E). Based on these results, we concluded that miR-513a-5p was a tumor suppressor in GC.

## MiR-513a-5p Negatively Republed APE1 Expression

To investigate the APE1 expression in GC, qR PCR was employed to detect the AP' r expression in no mal tissues and GC tissues. The results uncovered the APE1 expression was markedly p-real at a in GC in comparison with the normal group (Figure 3. A provious study has confirmed a targe in the stationship ween miR-513a-5p and APE1.<sup>19</sup> Consistent the previous report, our results found that there was a gative correlation between the ssion levels of miR-513a-5p and APE1 in GC tissues exp (Figure 5B). Western blot was then adopted to detect the APE pressic after selective regulation of LINC01436 1 miR-513a-5p. The results revealed that miR-513a-5p or fexe ssion or LINC01436 knockdown suppressed the APE1 expression, while miR-513a-5p repression or INC01436 overexpression up-regulated APE1 expression (Figure 5C and D). Therefore, it could be concluded that APE1 expression was negatively regulated by miR-513a-5p, and could be indirectly and positively regulated by LINC01436.

## LINC01436/miR-513a-5p/APE1 Axis Modulated the Proliferation, Migration, Invasion and Radioresistance of GC Cells To figure out the interaction of the LINC01436/miR-513a-

5p/APE1 axis in GC, the miR-513a-5p mimics were transfected into BGC-823 cells overexpressing LINC01436. The LINC01436 and miR-513a-5p expressions were detected by qRT-PCR, and the APE1 expression was detected by Western blot. The results revealed that LINC01436 overexpression up-regulated APE1 protein expression and down-regulated miR-513a-5p expression in comparison with the NC group, and miR-513a-5p overexpression down-regulated APE1 expression (vs LINC01436 group) (Figure 6A–C). These results further indicated that LINC01436 could promote the expression of





Figure 3 LINC01436 targeted miR-513a-5p. (A) The downstream target of LINC01436 was predicted according to StarBase database, and the binding site between LINC01436 and miR-513a-5p was shown. (B) qRT-PCR was used to detect the expression of miR-513a-5p in adjacent tissues and GC tissues. (C) Pearson's correlation test was used to analyze the relationship between LINC01436 expression and miR-513a-5p expression in GC tissues. (D) qRT-PCR was used to detect the expression of miR-513a-5p in AGS, SGC7901, BGC-823 and MKN45 cell lines. (E) Luciferase activity experiments confirmed targeting relationship between LINC01436 and miR-513a-5p. (F) RIP experiments further verified the targeting relationship between LINC01436 and miR-513a-5p. (G) MiR-513a-5p expression was detected by qRT-PCR after LINC01436 expression was up-regulated or down-regulated in GC cells. \*\* and \*\*\* represent P < 0.01 and P < 0.001, respectively. ns represents no significance.



Figure 4 MiR-513a-5p inhibited the proliferation, migration, invasion and radionesistance of GC, (A) More 513a-5p inhibitors and mimics were transfected into AGS cells and BGC-823 cells, respectively, and then miR-513a-5p expression was device a gRT-PCR. (B) proliferation of GC cells was detected by CCK-8 method. (C) Flow cytometry was used to detect apoptosis of GC cells. (D) Transwell method has used to track the migration and invasion of GC cells. (E) The effect of miR-513a-5p on radiation resistance was examined by colony formation assay. \*,\*\* and \*\*\* reprinent P = 0.05, 0.01 and P < 0.001, respectively.

APE1 via repressing miR-513a-5p in GC conts. The sults of rescue experiments showed that co-transferries of miR-513a-5p or si-APE1 into BGC-82 cells partly reversed the promotive effects of LIN(0/143) on the margnant phenotypes of BGC-823 cons (Figure co-H). In brief, LINC01436 participated of GC cell proliferation, apoptosis, metastasis and ratioresistance via regulating miR-513a-5p and APE1 expressions.

## APE1 Singuine Tempekably Suppressed the Proliferction, Invasion and Radioresistance of GC Cells

To explore the biological roles of APE1 on GC cells, the overexpression and knockdown models of APE1 were established with AGS cells and BGC-823 cells, respectively (Figure 7A). Functional assays showed that APE1 overexpression significantly promoted GC cell proliferation but inhibited the cell apoptosis, whereas si-APE1 had the opposite effects in GC cells (Figure 7B and C). Additionally, APE1 overexpression facilitated GC cell

bigration and invasion, while silencing APE1 exerted opposite effects (Figure 7D). Besides, colony formation assay indicated that APE1 overexpression promoted the radioresistance of GC cells, whereas depleted APE1 sensitized GC cell to radiation (Figure 7E). Collectively, these data suggested that APE1 functioned as a tumor promoter in GC, which was consistent with the previous report.<sup>18</sup>

# Knockdown of LINC01436 Blocked the Growth of GC Tumors in vivo

In order to further verify the cancer-promoting effect of LINC01436 in GC in vivo, subcutaneous xenograft assay in nude mice was performed. As the result showed, the volume and weight of the tumor in LINC01436 knock-down group were remarkably lower than those in the control group (Figure 8A and B), and the knockdown of LINC01436 up-regulated the expression of miR-513a-5p in the tumor tissues (Figure 8C). Additionally, APE1 expression was detected by qRT-PCR and Western blot in the tumor tissues of mice. The results elucidated that the APE1 expression in tumor tissues of LINC01436



Figure 5 MiR-513a-5p could repress APEI expression. (A) qRT-PCR was used to depect the correlation test was used to analyze the relationship between APEI expression and mile 13a-expression of APEI after the selective regulation of LINC01436 and miR-513a-5p in C cells

It the expression of APEI in normal tissues and GC tissues. (B) The Pearson's 13a-5p expression in GC tissues. (C and D) Western blot was used to detect the cells. \* and \*\* receiver P < 0.05 and P < 0.01 respectively.

knockdown group was notably lower than that on the control group (Figure 8D). These results further valids the carcinogenic effects of LINC01436 i GC.

#### Discussion

tment strat Currently, standard clinical ies for GC include surgery, chemother, y, tar, ted therapy and radiotherapy. Due to current reatment line tions, most of the GC patients suffer from tumor recurrence, chemoresistance or radiores ance. Therefore, in order to find more effective treatments, identifying novel therapy taris of reat significance. Diverse IncRNAs are gets of G abnormally expressed in GC and are closely related to the proliferation and metastasis of GC, such as tumorigen IncRNA SNA 6, IncRNA H19, IncRNA DANCR and so on, and they are considered as potential biomarkers and therapy targets.<sup>20–22</sup> In this study, we observed that the LINC01436 expression in GC cells was markedly higher than that in the adjacent normal tissues, and the LINC01436 high expression was associated with several pathological features in GC (such as T stage and differentiation status). Importantly, LINC01436 high expression was correlated with poor prognosis of patients. Functional experiments revealed that LINC01436 overexpression

erkably enhanced the malignant phenotypes of GC cells, while knocking down LINC01436 showed the opposite effect. These results fully demonstrated that LINC01436 exerted a cancer-promoting effect in the GC development. To our best knowledge, this was the first study to explore the role of LINC01436 in GC.

In GC, miRNA is endowed with a remarkable regulatory function. For instance, the up-regulation of miR-93 expression facilitates GC cell proliferation, migration, invasion and epithelial-mesenchymal transition to accelerate cancer progression, and miR-532 promotes GC progression via targeting NKD1.<sup>23,24</sup> MiR-513a-5p is a miRNA that has been gradually recognized in recent years. Reportedly, miR-513a-5p expression is downregulated in osteosarcoma tissues and cells, and its overexpression can observably improve the radiosensitivity of osteosarcoma cells.<sup>19</sup> In this study, we found that miR-513a-5p expression was down-regulated in GC tissue in comparison with normal gastric tissue. Inhibition of miR-513a-5p enhanced the proliferation, migration and invasion of GC cells, while miR-513a-5p overexpression had the opposite effect, suggesting that miR-513a-5p exerted a tumor-suppressive effect during the GC development.



transfected with L 101/1 overexplane on plasmids and miR-513a-5p mimics, qRT-PCR was used to detect the expressions of LINC01436 and miR-513a-5p, respectively. (C) Western blot was not d to detect the expression of APE1. (D) MiR-513a-5p mimics or si-APE1 was transfected into BGC-823 cells, overexpressing LINC01436, and the proliferation of GC cells detected by CCK-8 method. (E) Flow cytometry was used to detect apoptosis of GC cells. (F and G) Transwell method was used to detect the migration and invasion of G cells. H. The radiosensitivity was examined by colony formation assay. \*, \*\* and \*\*\* represent P < 0.05, P < 0.01 and P < 0.001, respectively. Ns represents no significance.

Accumulating research verifies that lncRNAs act as competitive endogenous RNAs (ceRNAs) to modulate miRNA expression, and the latter regulates protein expression via binding to the 3'UTR of mRNAs. This interaction figures prominently in the GC progression.<sup>25</sup> For instance, lncRNA MALAT1 acts as a ceRNA to suppress autophagy-mediated chemotherapeutic resistance via inhibiting miR-23b-3p expression;<sup>26</sup> LINC01133 modulates APC expression and Wnt/ $\beta$ -catenin pathway via sponging miR-106a-3p to impede GC progression.<sup>27</sup> In this work, we found a negative correlation between the LINC01436 expression and miR-513a-5p expression in GC tissues.





qRT-PCR, lucite as reporter assay and RIP assay were employed to confirm that LINC01436 could target miR-513a-5p, and LINC01436 overexpression could repress the miR-513a-5p expression in GC cells. In addition, we validated that miR-513a-5p could partially reverse the malignant phenotypes of GC cells promoted by LINC01436 overexpression. Based on these results, we supposed that during GC progression, LINC01436 expression was upregulated, and then LINC01436, as a kind of ceRNA, suppressed the expression and function of miR-513a-5p, which acted as a tumor suppressor, thereby facilitating the GC progression.

Previous studies reveal that APE1 expression is modulated by microRNA.<sup>14,16</sup> For instance, miR-520g and miR-520h can reverse Bortezomib resistance in multiple myeloma via repressing APE1 expression; whereas miR-765 can enhance the anti-angiogenic effect of cisplatin in osteosarcoma via targeting APE1.<sup>28,29</sup> In this article, we



**Figure 8** LINC01436 knockdown inhibited the growth of GC in vivo. (**A** and **B**). The volume and weight of tumors in mice from different groups were shown. (**C**) The expression of miR-513a-5p in tumor tissues of mice was detected by qRT-PCR. (**D**) Western blot was used to detect the expression of APE1 in tumor tissues of mice. \*\* and \*\*\* represent P < 0.01 and P < 0.001, respectively.

found that APE1 expression was elevated in GC tissues and its expression was negatively correlated with mil 513a-5p. It is verified that miR-513a-5p can target th regulation of APE1 expression in GC.<sup>19</sup> so w also curious whether APE1 expression can be egulat by LINC01436/miR-513a-5p axis. We found that mj 5p overexpression or LINC01436 repressed Jckdo APE1 expression, whereas supported miR-5 5p or LINC01436 overexpression up egulate it. These results reflected that LINC01436 dulated GC ogression via modulating the miR-51 -5p/APT1 axis.

#### Conclusio ...

In summary LINCO 436 can adomete the proliferation, metastasis an aradi resistant. Of GC cells, suggesting that it can be used as a potential therapy target for GC treatment. Mechanism investigation confirms that LINC01436 can regulate GC progression via regulating miR-513a-5p/APE1 axis. Collectively, this study sheds light on the mechanisms of GC progression and provides a new theoretical basis for the diagnosis and treatment of GC.

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# Ethics Approval and Consent to Participate

This study was endorsed by the Ethics Review Committee of First Affiliated Hospital of Anhui Medical University and written informed consent was obtained from each patient.

## **Author Contributions**

All authors made great contributions to the experimental concept and design, data acquisition or data analysis and interpretation; participated in drafting the ended or strictly revised the content of important knowledge content; agreed on the journal to which the article will be submitted; gave final approval of the version to be published. All authors have agreed to be remonsible to all aspects of the work.

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