

Overexpression of long non-coding RNA RP11-363E7.4 inhibits proliferation and invasion in gastric cancer

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LncRNA RP11-363E7.4 has been shown to be downregulated in gastric cancer (GC), while the effect of lncRNA RP11-363E7.4 on GC and its potential molecular mechanisms is unclear. The purpose of this study was to explore the functional role and underlying molecular mechanisms of lncRNA RP11-363E7.4 involved in GC progress.

To address the question, quantitative real-time PCR assay was performed to confirm lncRNA RP11-363E7.4 expression levels in GC tissues and cell lines. Cell proliferation, apoptosis, migration and invasion were estimated using Cell Counting Kit-8, colony formation, scratch wound healing and Transwell assays. Potential molecular mechanisms were evaluated using western blot assay. The results showed that lncRNA RP11-363E7.4 was significantly downregulated in GC cell lines and 82 paired tissues. The correlation between expression and clinicopathological features indicated that low expression of lncRNA RP11-363E7.4 was associated with T stage ($P = .010$). Functional experiments showed that overexpression of lncRNA RP11-363E7.4 prevented proliferation, migration, and invasion and induced apoptosis of GC cells. Western blot assay revealed that lncRNA RP11-363E7.4 functioned via the p53, Bax/Bcl-2, β -catenin pathway. In summary, this study revealed that lncRNA RP11-363E7.4 functioned as a tumour suppressor by inhibiting proliferation, migration, and invasion and inducing apoptosis of GC cells.

Significance of the study:

LncRNA RP11-363E7.4 has been shown to be downregulated in GC, while the effect of lncRNA RP11-363E7.4 on GC and its potential molecular mechanism is unclear. We revealed that lncRNA RP11-363E7.4 functioned as a tumour suppressor by inhibiting proliferation, migration, and invasion and inducing apoptosis of GC cells. LncRNA RP11-363E7.4 might become an attractive diagnostic and prognostic biomarker of GC and a promising target for GC treatment.

KEYWORDS

gastric cancer, long non-coding RNA, proliferation, RP11-363E7.4, invasion

Chao Chen and Xin Wang contributed equally to this work.

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1 | INTRODUCTION

In the past decades, gastric cancer (GC) has become the fifth most prevalent malignant tumour and the third leading cause of cancer-related death around the world.¹ In China, the incidence rate of GC ranks third among malignant tumours, while its mortality rate ranks second.² Although development of surgical resection and chemotherapy has improved the 5-year overall survival rate in early-stage GC patients, prognosis for patients with late-stage GC remains poor due to uncontrolled proliferation and migration of tumour cells.³ Many GC patients are diagnosed at advanced stages of disease due to lack of specific symptoms early on, leading to disappointing outcomes.⁴ Therefore, it is essential to investigate molecular mechanisms that are associated with occurrence and development of GC and discover novel molecular biomarkers for early diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are non-coding RNAs more than 200 nucleotides in length.⁵ They are always transcribed by RNA polymerase II and lack opening reading frames.⁶ In recent years, lncRNAs have been reported to be involved in many biological functions, including regulating gene expression and controlling gene transcription, by interacting with DNA, RNA and proteins, although they are not able to encode proteins.⁷⁻⁹ Emerging evidence has revealed that lncRNAs play important roles in regulating cell occurrence, proliferation, migration and invasion, including in GC cells, suggesting that lncRNAs could be used as diagnostic and prognostic markers, as well as therapeutic targets.^{10,11} For example, lncRNA DANCR has a higher expression level in GC tissues than in normal adjacent tissues. Patients with increased lncRNA DANCR always have a poor prognosis. Knockdown of lncRNA DANCR was able to suppress proliferation, metastasis and invasion of GC cells.^{12,13} Knockdown of lncRNA HOTAIR inhibited proliferation of GC cells by promoting miR-454-3p expression and decreasing STAT3/cyclin D1 activity.¹⁴ Overexpression of lncRNA MALAT1 promoted tumorigenicity and metastasis of GC cells by regulating vasculogenic mimicry and angiogenesis. Silencing of lncRNA MALAT1 decreased the expression level of vimentin and increased the expression of E-cadherin, thus suppressing epithelial mesenchymal transition (EMT) and inhibiting migration and invasion of GC cells.^{15,16} Based on these results, it is important to thoroughly study the role of lncRNAs in GC.

lncRNA RP11-363E.74 is a recently discovered novel lncRNA. It has been reported to be dysregulated in papillary thyroid carcinoma, in hepatocellular carcinoma after cisplatin exposure, and in patients with atrial fibrillation and myocardial infarction.¹⁷⁻²⁰ As previously reported, it was downregulated in GC tissues according to the tissue microarrays results, while high expression of lncRNA RP11-363E.74 was associated with better overall survival in GC patients.²¹ However, these results lack further validation in large clinical samples. The functional role of lncRNA RP11-363E.74 and its potential molecular mechanisms in GC remain unclear. To provide potential targets for prognosis and GC therapy, this study was performed to investigate the role of abnormal expression of lncRNA RP11-363E.74 in GC and explore the underlying molecular mechanisms.

2 | MATERIALS AND METHODS

2.1 | GC clinical specimens

A total of 82 paired GC tissues and corresponding adjacent non-tumour samples were obtained between 2016 and 2018 from the Department of Gastrointestinal Nutrition and Hernia Surgery, the Second Hospital of Jilin University, Changchun, China. All samples were obtained during the operation and stored by RNA later (Invitrogen, MA, USA). Patients did not receive any treatment before the operation. The postoperative pathology in all patients confirmed GC. This study was approved by the Ethics Review Board of the Second Hospital of Jilin University. Informed consent was obtained from all patients.

2.2 | Cell lines and culture

Human GC cell lines including SGC-7901, BGC-823 and MGC-803 and normal gastric epithelial GES-1 cells were purchased from the Institute of Basic Medicine at the Chinese Academy of Medical Science. Cells were cultured in RPMI-1640 (HyClone, Victoria, Australia) medium supplemented with 10% fetal bovine serum (FBS, Gibco, Gaithersburg, USA) at 37°C in a humidified atmosphere containing 5% CO₂.

2.3 | RNA extraction and quantitative real-time PCR

Total RNA was extracted from the GC tissues using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instructions. For quantitative real-time PCR (qRT-PCR) detection, RNA was reverse transcribed into complementary DNA (cDNA) using a Reverse Transcription Kit (Takara, Dalian, China). A Roche system was used to perform qPCR detection with SYBR II Premix Taq (Takara, Dalian, China). L13 was used as an internal reference. The specific primers were as follows: RP11-363E.74 forward primer: ATGCTGAAGCTCACC CAGTC, RP11-363E.74 reverse primer: ATCCCAATCTGGCCCA AGAC, L13 forward primer: CCAACGTGCAGCGGCTGAA, L13 reverse primer: CGTGGCCATCTTGAGTTCT. Each sample was detected three times. Relative expression levels of RP11-363E.74 compared to those of L13 were calculated using the 2^{-ΔΔCt} method.

2.4 | Lentivirus transfection

SGC7901 and MGC803 cells (1 × 10⁵) were plated in 6-well plates and cultured overnight. The culture medium was removed on the next day and cells were washed twice with PBS. A total of 1 mL of culture medium with 5 μL of polybrene (5 μg/mL) was added to each well. Then, 2 μL of RP11-363E.74-overexpressing lentiviral expression vector (titre: 1 × 10⁵, GenePharma, Suzhou, China) and 2 μL of negative control viral suspension (titre: 1 × 10⁵ of titre, GenePharma, Suzhou, China) were added to the appropriate plates and cells were cultured for

24 hours at 37°C. The culture medium was then replaced with 2 mL of culture medium and 1 μ L of puromycin in each well and cells were cultured for two generations to screen for stable expression. After stable growth was established with passage of transfected cells, their fluorescent expression was observed under a microscope. When fluorescent rate reached ~90% of total cells, cells were collected for further analyses. The qRT-PCR was used to confirm transfection efficiency, which was considered to be of practical significance when the expression increased by more than 30 times.

2.5 | Cell proliferation assay

Cell proliferation was measured using a Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). Briefly, 3000 transfected cells were seeded in a 96-well microplate and cultured overnight. A total of 10 μ L of CCK-8 reagent were added to each well after 24, 48, 72 or 96 hours of incubation and the cells were further incubated for 2 hours at 37°C. The absorbance value of each well was detected using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 450 nm. The experiments were performed independently three times.

2.6 | Colony formation assay

Briefly, after the establishment of stable transfected cells, cells were seeded in six-well plates at a density of 400 cells/well and cultured at 37°C for 2 weeks. When visible colonies were formed, the culture medium was removed and cells were washed twice with PBS. Then, cells were fixed with 4% paraformaldehyde for 20 minutes and washed with PBS again. The cells were then stained using a Giemsa stain kit (Solarbio, Beijing, China) for 30 minutes. Finally, cells were washed twice with PBS and positive colonies containing more than 50 cells were counted under an ordinary optical microscope.

2.7 | Apoptosis detection

Cell apoptosis was detected using a FITC/annexin V Apoptosis Detection Kit (BD Biosciences, New Jersey, USA) according to the manufacturer's protocol. Briefly, cells were cultured in six-well plates overnight at a density of 4×10^5 cells/well and then harvested and washed twice with PBS. Then, 5 μ L of annexin

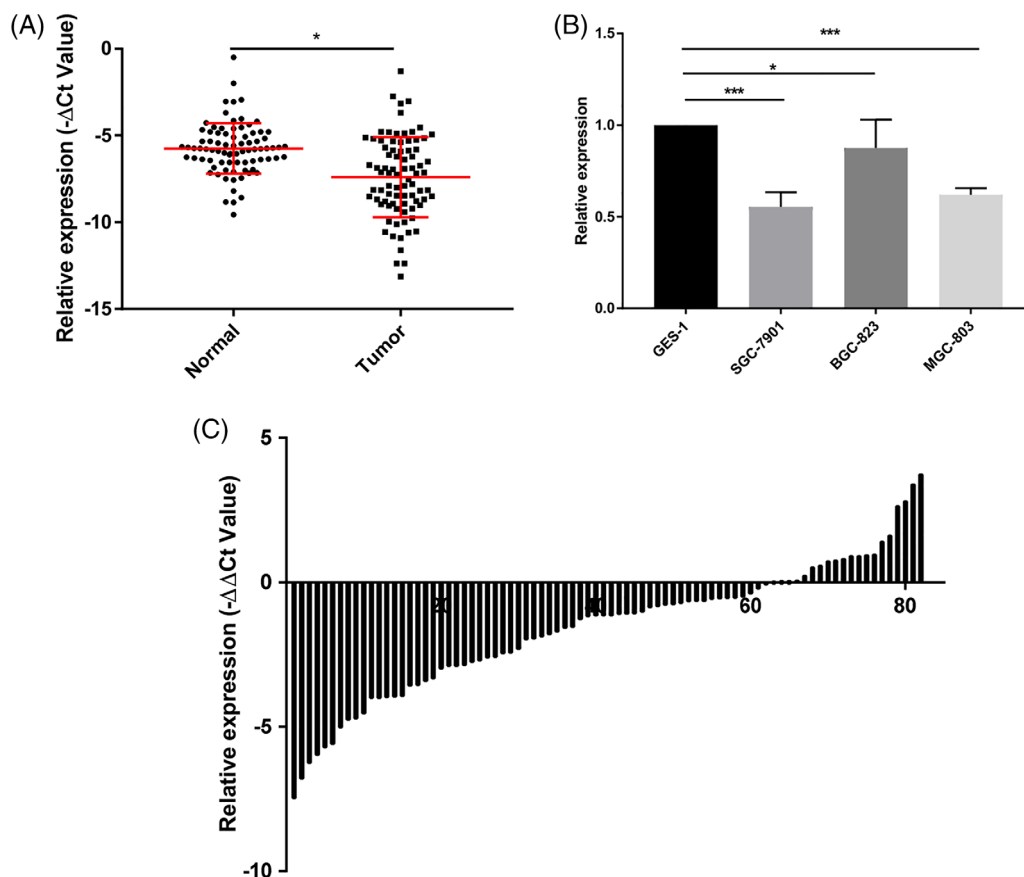


FIGURE 1 The expression level of lncRNA RP11-363E7.4 was downregulated in GC tissues and cell lines. (A) RP11-363E7.4 expression in 82 paired GC tissues and adjacent normal tissues was examined by qRT-PCR. (B) The fold change of RP11-363E7.4 expression in GC tissues and adjacent normal tissues for each patient (n = 82) was detected by qRT-PCR. (C) Relative expression of RP11-363E7.4 in GC cell lines and immortalised normal gastric epithelial cell line (GES-1) was examined by qRT-PCR. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$

TABLE 1 Relationship of lncRNA RP11-363E7.4 expression in tissue samples with clinicopathological factors of gastric cancer patients

Characteristics	Number of cases (%)	RP11-363E7.4	P value
Ages (year):			0.889
≤61	41 (50.0)	0.331 (0.131,0.998)	
>61	41 (50.0)	0.439 (0.122,0.655)	
Gender:			0.205
Male	52 (63.4)	0.419 (0.089,0.666)	
Female	30 (36.6)	0.447 (0.178,1.165)	
Borrmann:			0.565
I	22 (26.8)	0.581 (0.139,1.719)	
II	33 (40.2)	0.299 (0.123,0.689)	
III	19 (23.2)	0.439 (0.142,0.660)	
IV	8 (9.8)	0.291 (0.103,0.598)	
Maximum tumour size (cm):			0.073
≤4.0	55 (67.1)	0.456 (0.142,0.994)	
>4.0	27 (32.9)	0.265 (0.051,0.606)	
Tumour invasion:			0.010
T1 & T2	35 (42.7)	0.618 (0.169,1.586)	
T3 & T4	47 (57.3)	0.331 (0.062,0.596)	
Lymphatic metastasis:			0.734
N0	37 (45.1)	0.331 (0.159,0.743)	
N1 & N2 & N3	45 (54.9)	0.439 (0.091,0.833)	
Distal metastasis:			0.163
M0	75 (91.5)	0.439 (0.132,0.949)	
M1	7 (8.5)	0.131 (0.067,0.408)	
pTNM stage:			0.152
0 & I	47 (57.3)	0.438 (0.132,0.876)	
II	14 (17.1)	0.569 (0.173,1.156)	
III	18 (22.0)	0.435 (0.145,0.653)	
IV	3 (3.6)	0.067 (0.054,0.079)	

Notes: Median of relative expression, with 25th to 75th percentile in parenthesis. Statistical significance is marked by bold type ($p < 0.05$).

Abbreviation: pTNM, pathological Tumour Node Metastasis.

V-FITC and PI were successively added to cells resuspended in 400 μ L of binding buffer. The cells were then incubated for 15 minutes in the dark at 37°C. After that, the samples were analysed by flow cytometry (Beckman Coulter, Brea, CA, USA). Q1 and Q4 referred to dead and viable cells, respectively, while Q2 and Q3 referred to early apoptotic and late apoptotic cells, respectively. Total apoptotic cells were represented as the sum of early and late stages of apoptosis.

2.8 | Scratch wound healing assay

The transfected cells were seeded into 6-well plates and cultured until cell density reached approximately 90%. A straight line was then drawn using a 200- μ L pipette tip on the bottom of the 6-well plate. Cells were washed three times with PBS to remove unattached cells. The cells were then cultured in serum-free medium in the incubator at 37°C for

48 hours. Photographs were taken at 0 and 48 hours under a microscope to observe the metastatic distance of cells in the scratch area.

2.9 | Transwell migration and invasion assay

Migration and invasion assay was performed in 24-well Transwell chambers with an 8- μ m/L pore size (Corning, New York, USA). After collecting the transfected cells, 1×10^5 cells from each group were seeded in the upper chamber with 200 μ L of serum-free medium with or without Matrigel (BD, Beijing, China). The chambers were placed in a 24-well plate and 500 μ L of medium with 10% FBS, which served as a chemo-attractant, were added to each well. After 48 hours of incubation, several cells migrated to and invaded into the lower surface of each chamber. Cells on the upper surface were brushed, while cells on the lower surface were fixed with 4% paraformaldehyde and then stained with haematoxylin and eosin. The permeable membrane was removed, placed

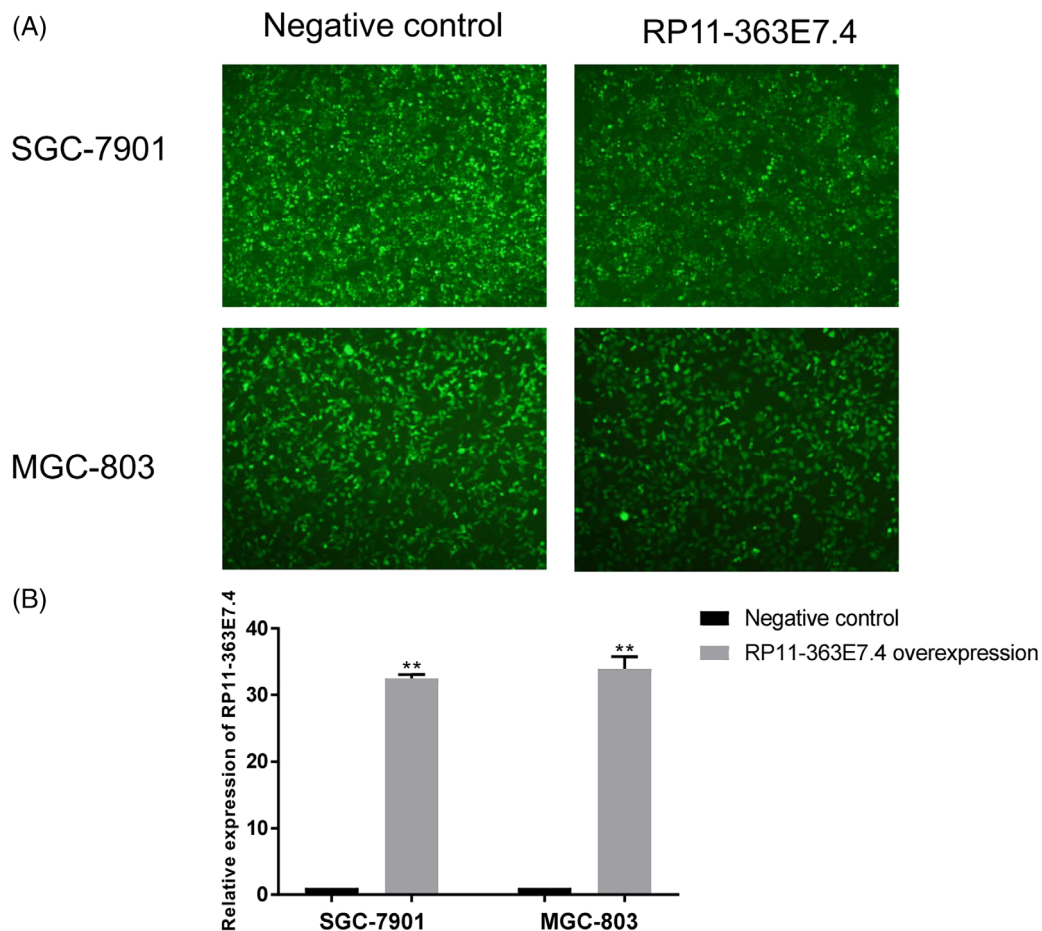


FIGURE 2 (A) Lentivirus stabilisation transfected SGC-7901 and MGC-803 cells observed by fluorescence microscope. (B) The relative expression level of lncRNA RP11-363E7.4 in stable transfected cell lines tested by qRT-PCR. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$

on the slide and sealed with neutral resin. Cell numbers in randomly selected fields of view were counted and photographed.

2.10 | Western blot assay

Protein expression was measured using western blotting. Transfected cells were cultured at a density of 1×10^6 cells in 60-mm dishes. The cells were then collected and washed with PBS three times. According to the manufacturer's instructions, total protein was extracted from cells using radio immunoprecipitation assay lysis buffer (KeyGen Bio-Tech, Nanjing, China) and protein concentration was determined using a bicinchoninic acid protein assay kit (KeyGen BioTech, Nanjing, China). Subsequently, 30 μ g of protein were separated using 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis at 120 V for 1.5 hours. Then, proteins were transferred onto a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA) at 300 mA for 1 hour. The membranes were incubated in 5% skim milk for 1 h at room temperature and then overnight at 4°C with the primary antibodies. Primary antibodies against β -actin (1:2000), Bax (1:1000), caspase-3 (1:500), P53 (1:1000), β -catenin (1:1000), E-cadherin (1:500),

N-cadherin (1:1000), vimentin (1:1000) and Slug (1:1000) were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against Bcl-2 (1:1000) were purchased from Abcam (Cambridge, MA). On the next day, after washing with tris-buffered saline Tween 20 (TBST) for 30 minutes (10 minutes \times three times), the membranes were further incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. Following three 10-minute washes with TBST, the signals were detected using an enhanced chemiluminescence detection kit (Thermo Fisher, Waltham, USA).

2.11 | Statistical analysis

All data were analysed with SPSS v19.0 software (IBM, Chicago, IL, USA) and expressed as means \pm SD. Unpaired Student's *t* test was performed to analyse significant differences between groups. Association between expression level of lncRNA RP11-363E7.4 and clinicopathological data for GC patients was analysed by non-parametric test. Mann-Whitney *U* test was used for comparison between two groups and Kruskal-Wallis *H* test was used for comparison among

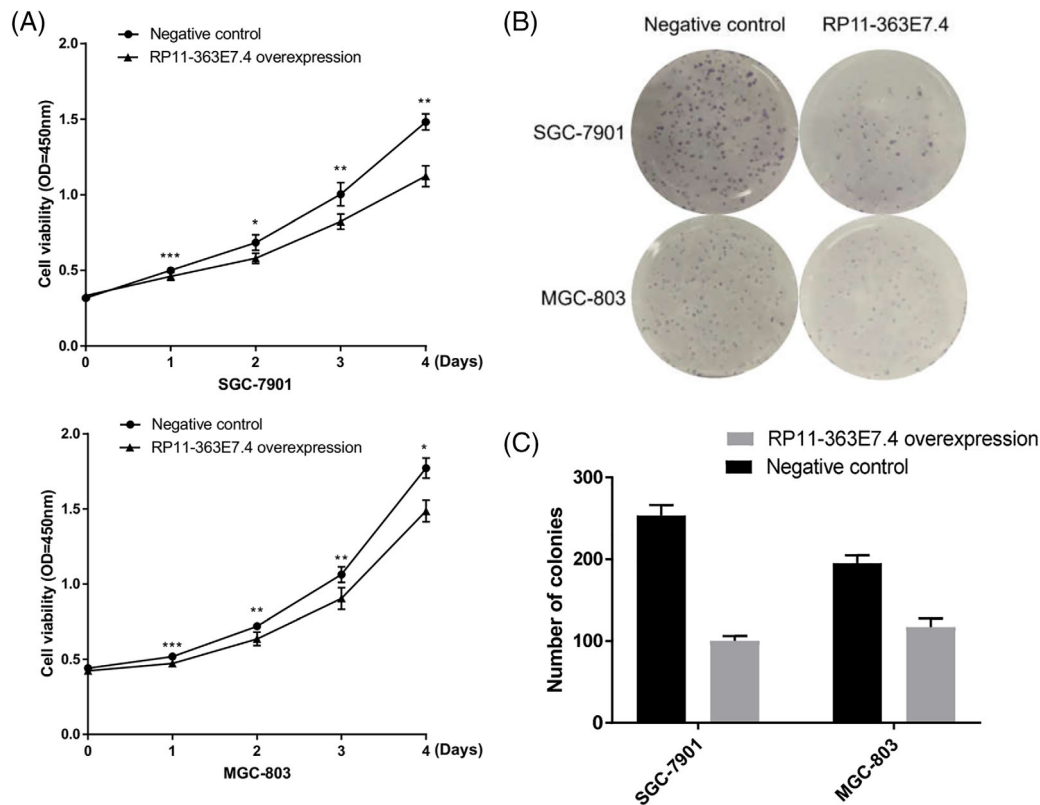


FIGURE 3 Overexpression of lncRNA RP11-363E7.4 prevents proliferation of GC cells. (A and B) The role of RP11-363E7.4 on proliferation of GC cells were examined by cell counting kit-8 assay and colony formation assay. (C) Number of colonies in colony formation assays. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$

multiple groups, where $P < .05$ was considered to indicate a statistically significant difference.

3 | RESULTS

3.1 | LncRNA RP11-363E7.4 had a low expression in GC tissues and cell lines

The expression level of lncRNA RP11-363E7.4 was analysed in 82 paired GC and adjacent normal tissues by qRT-PCR. The resulting lncRNA RP11-363E7.4 expression was significantly lower in GC tissues compared to adjacent normal tissues ($P < .05$, Figure 1A, B). Then, lncRNA RP11-363E7.4 expression level in human GC cell lines (SGC-7901, MGC-803 and BGC-823) and human normal gastric epithelial GES-1 cells was tested by qRT-PCR. The expression level of lncRNA RP11-363E7.4 was decreased in three GC cell lines (SGC-7901, BGC-823 and MGC-803) compared to GES-1 (Figure 1C). Clinicopathological data for GC patients were also analysed. It was shown that low expression of lncRNA RP11-363E7.4 was associated with tumour invasion ($P = 0.010$), but not with age, gender, tumour size, Borrmann type, lymphatic metastasis, distal metastasis and tumour node metastasis (TNM) stage (all $P > 0.05$, Table 1).

3.2 | Overexpression of lncRNA RP11-363E7.4 prevents proliferation of GC cells

SGC-7901 and MGC-803 cells were transfected by RP11-363E7.4-overexpressing lentiviral expression vector. The stable transfected cell lines were observed by fluorescence microscopy after screened by puromycin, and the expression level of lncRNA RP11-363E7.4 in transfected cell lines were detected by qRT-PCR (Figure 2A, B). Then CCK-8 and colony formation assays were performed to detect the effect of lncRNA RP11-363E7.4 on proliferation of GC cells. CCK-8 results showed that the growth rate of SGC-7901 and MGC-803 cells were decreased compared to controls after overexpression of lncRNA RP11-363E7.4 (Figure 3A). The colony formation assay showed that compared to controls, colony numbers in SGC-7901 and MGC-803 cells were decreased after overexpression of lncRNA RP11-363E7.4 (Figure 3B, C). These results indicated that lncRNA RP11-363E7.4 overexpression can suppress proliferation of GC cells *in vitro*.

3.3 | Overexpression of lncRNA RP11-363E7.4 induces apoptosis of GC cells

Flow cytometry was subsequently performed to examine the apoptosis of transfected cells. Results revealed that the total apoptosis rate

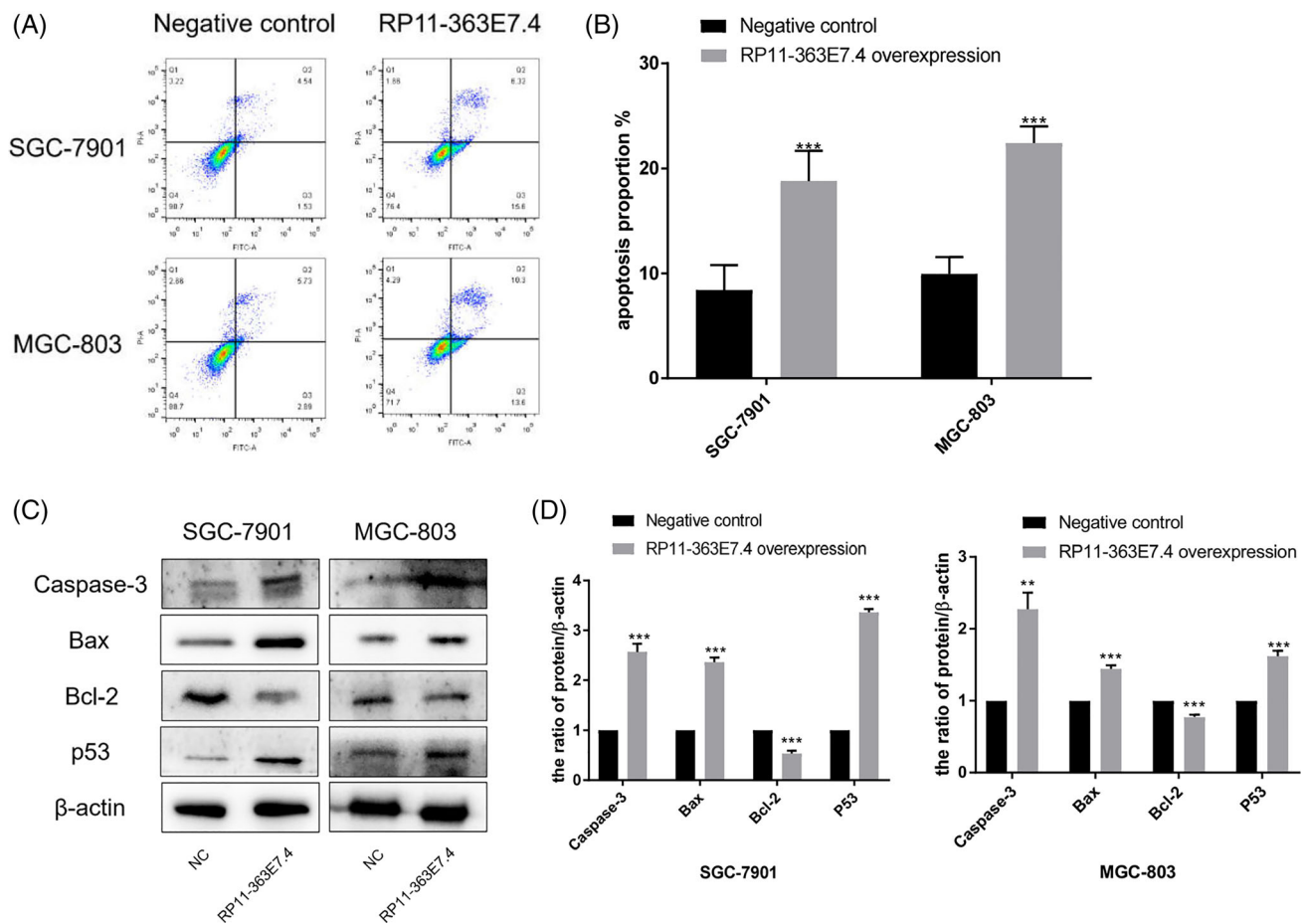


FIGURE 4 The role of lncRNA RP11-363E7.4 on apoptosis of GC cells and protein related with apoptosis. (A and B) The apoptosis of GC cells was examined by flow cytometry. (C and D) The expression level of protein related with apoptosis was examined by western blot analysis. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$

in the overexpressed group was higher than that in the control group (increased apoptosis proportion in SGC-7901 cells was 10.38%, $P = .008$ and 12.45%, $P < 0.005$ in MGC-803 cells, Figure 4A, B). Then, western blot assays were performed to explore the potential molecular mechanisms. Expression levels of several proteins associated with apoptosis, including Bax, Bcl-2, caspase-3 and P53, were detected. The results showed that after overexpression of lncRNA RP11-363E7.4, Bax, caspase-3 and P53 protein expression increased, while Bcl-2 protein expression decreased in GC cell lines (Figure 4C, D). These results demonstrated that overexpression of lncRNA RP11-363E7.4 can likely induce apoptosis of GC cell lines *in vitro* via the P53, Bax/Bcl-2 pathway.

3.4 | Overexpression of lncRNA RP11-363E7.4 inhibits EMT to prevent migration and invasion of GC cells

To further verify the effects of lncRNA RP11-363E7.4 on migration and invasion of GC cells *in vitro*, scratch wound healing and Transwell assays were performed. The scratch wound healing assay results

showed that migration in the group with overexpressed lncRNA RP11-363E7.4 was significantly decreased than in the control group (Figure 5A and B). After overexpression of lncRNA RP11-363E7.4, the number of GC cells that passed into the lower chamber was decreased compared to that in the control group as demonstrated by the Transwell assays (Figure 5C, D). Then, western blot assays were performed to explore the potential molecular mechanisms. Expression levels of several proteins related to EMT, such as E-cadherin, N-cadherin, vimentin, Slug and β -catenin, were detected. Results showed that expression of E-cadherin increased significantly, while that of N-cadherin, vimentin, Slug and β -catenin decreased (Figure 6A, B). These results indicated that lncRNA RP11-363E7.4 might change the expression of Slug and β -catenin to suppress the EMT process and inhibit migration and invasion ability of GC cells *in vitro*.

4 | DISCUSSION

Emerging evidence has demonstrated that dysregulated lncRNAs play a critical role in many biological processes in GC cells *in vitro*, including occurrence, development, proliferation, invasion and migration,

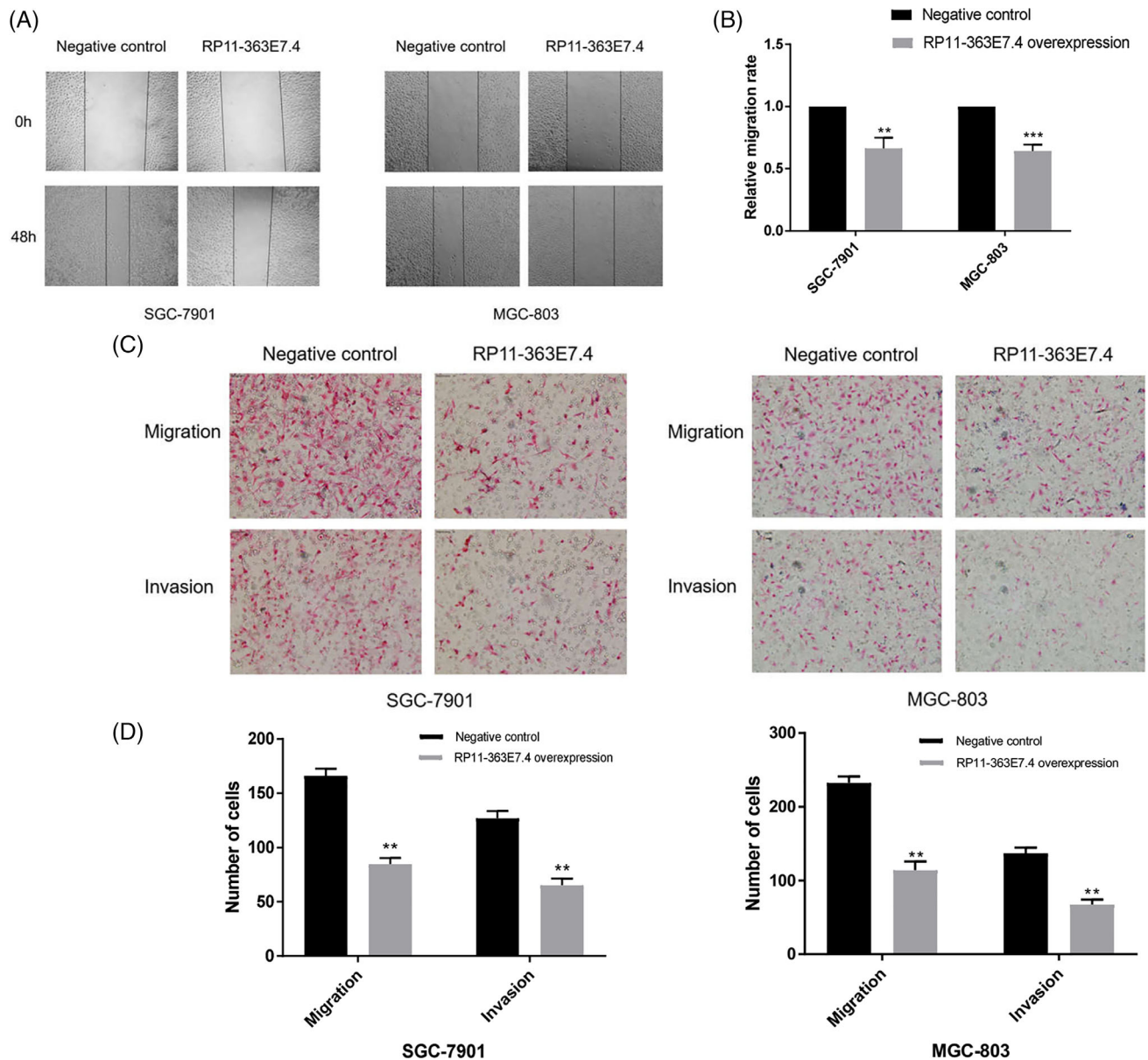


FIGURE 5 Overexpression of lncRNA RP11-363E7.4 prevents migration and invasion of GC cells. (A and B) The role of lncRNA RP11-363E7.4 on migration ability of GC cells was examined by wound healing assay. (C and D) The role of lncRNA RP11-363E7.4 on migration and invasion ability of GC cells was examined by Transwell assay. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$

indicating that lncRNAs may have the potential to be novel diagnostic markers and therapeutic targets of GC.²²⁻³¹ Recent research has also suggested that lncRNAs can influence different functions in tumours including GC in the following ways: (a) promoting or silencing the expression of targeted genes on an epigenetic level by DNA methylation, DNA hydroxymethylation and other methods; (b) regulating transcription factor activity on a transcriptional level; (c) modulating pre-mRNA splicing process, functioning as competing endogenous RNAs and interfering in post-translational protein modification on a post-transcriptional level.^{32,33} Studies on the mechanisms of lncRNA function in tumorigenesis have made great progress, more specific research is needed to explain specific mechanism details, which may

be helpful for the early diagnosis of GC and establishing therapeutic strategies for different GC patients.

lncRNA RP11-363E.74 is a recently discovered novel lncRNA. As reported previously, lncRNA RP11-363E7.4 has a significantly low expression level in GC tissues, while high lncRNA RP11-363E7.4 expression has been related to better overall survival of GC patients.²¹ However, these reports have not revealed an association between lncRNA RP11-363E7.4 expression level, GC clinicopathological characteristics and lncRNA RP11-363E7.4 functional mechanism in GC. It is thus necessary to understand the role of lncRNA RP11-363E7.4 in occurrence and development of GC.

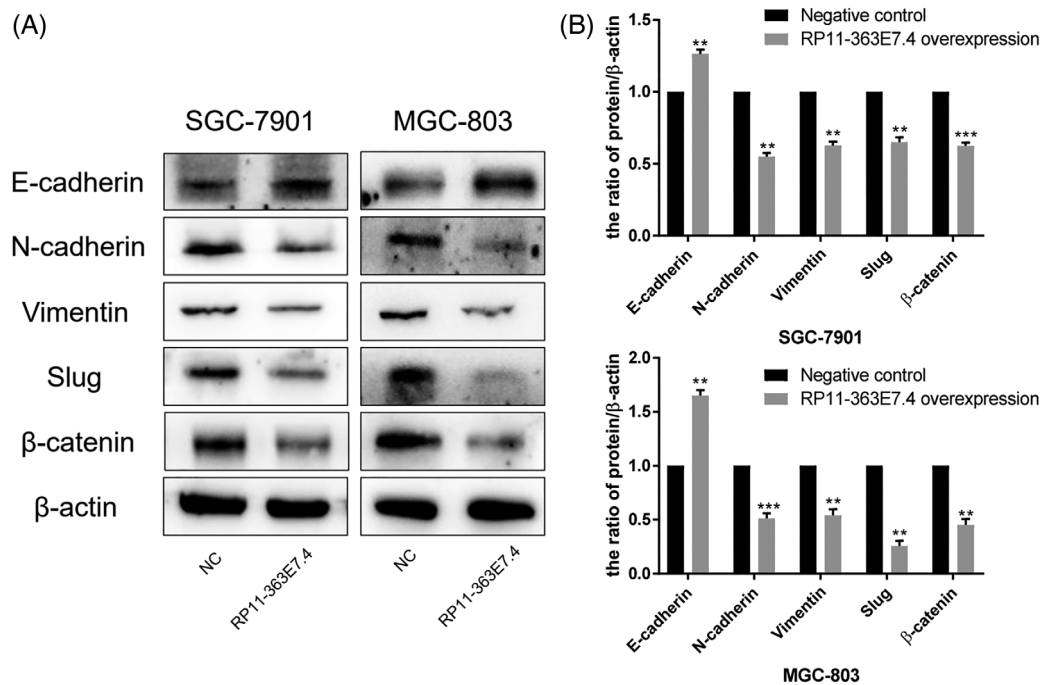


FIGURE 6 The role of lncRNA RP11-363E7.4 on protein EMT related with EMT. (A and B) The expression level of protein related with EMT was examined by western blot analysis. Data are presented as the mean \pm SD of three independent experiments. * $P < .05$, ** $P < .01$, *** $P < .005$. EMT: epithelial mesenchymal transition

The present study used qRT-PCR to demonstrate that lncRNA RP11-363E7.4 expression level was significantly downregulated in 82 pairs of GC, adjacent normal tissues and three cell lines. The correlation between clinicopathological characteristics and lncRNA RP11-363E7.4 expression was also analysed. The results indicated that lncRNA RP11-363E7.4 expression level was associated with tumour invasion ($P < 0.05$), but not with age, gender, tumour size, Borrmann type, lymphatic metastasis, distal metastasis and TNM stage. Subsequently, a series of molecular and cellular experiments *in vitro* was performed to explore the biological function of lncRNA RP11-363E7.4 in GC. The results revealed that overexpression of lncRNA RP11-363E7.4 inhibited cell proliferation, colony formation, migration, and invasion and induced apoptosis in GC cells *in vitro*. Finally, western blot assays were performed to investigate potential lncRNA RP11-363E7.4 mechanisms in GC cells. Overexpression of lncRNA RP11-363E7.4 was found to increase caspase-3, Bax, and E-cadherin expression and decrease Bcl-2, N-cadherin, and vimentin expression. In addition, it changed the expression level of P53, Slug and β -catenin. These results confirmed that lncRNA RP11-363E7.4 can inhibit proliferation, migration and invasion of GC cells by regulating apoptosis and the EMT process via P53, Bcl-2/Bax ratio and β -catenin signalling pathway.

The mechanism of apoptosis in tumour cells is quite complex. Bcl-2 family proteins, which include pro-apoptotic and anti-apoptotic proteins, play an important role in apoptosis of tumour cells. Bcl-2 can inhibit the intrinsic apoptotic pathway by preventing the release of cytochrome C, while Bax can allow its release from mitochondria to

caspase-3 in the cytoplasm to induce cell death.³⁴ On the other hand, increased P53 protein level, which is the most studied tumour suppressor, can lead to upregulation of its downstream target gene Bax and downregulation of Bcl-2.³⁵ Because increased levels of caspase-3, Bax and P53 and decreased levels of Bcl-2 were observed, it was suggested that lncRNA RP11-363E7.4 can induce apoptosis in GC via the p53, Bax/Bcl-2 pathway.

The EMT process, which switches the epithelial phenotype to mesenchymal phenotype, has been reported by many researches to be associated with tumour metastasis.³⁶ The loss of cell-cell junctions and reorganisation of cytoskeleton are two classic characteristics of EMT.³⁷ It can also facilitate tumour cell migration to distant organ sites through the matrix.³⁸ β -catenin and transcription factor Slug have been reported to have a positive correlation with the EMT process.^{39,40} In the present study, the expression level of β -catenin and Slug both decreased, which might indicate that lncRNA RP11-363E7.4 might influence the EMT process via the β -catenin pathway, changing cancer cell metastasis. This conjecture needs more experimental verification in future study.

Some limitations were present in this study. First, more GC and adjacent normal tissue samples should be used to confirm the lncRNA RP11-363E7.4 expression level in GC. The number of GC samples was insufficient in the present study. Second, the expression level of lncRNA RP11-363E7.4 in blood samples of GC patients should be examined to estimate its significance as a diagnostic marker of GC. Third, lncRNA RP11-363E7.4 silencing in GC cells should be performed and subsequent changes in biological behaviours of GC cells

must be examined. Fourth, since Wang et al speculated that there may be a regulatory network between lncRNA RP11-363E7.4 and some particular miRNAs, including miR-106a-5p, miR-17-5p and miR-19b-3p lncRNA RP11-363E7.4, target genes and target miRNAs that could influence progression and metastasis of GC should be determined and validated.²¹

In summary, this study revealed that lncRNA RP11-363E7.4 can function as a tumour suppressor by inhibiting proliferation, migration, and invasion and inducing apoptosis of GC cells. Therefore, lncRNA RP11-363E7.4 might become an attractive diagnostic and prognostic biomarker of GC and a promising target for GC treatment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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