

## ORIGINAL ARTICLE

# Long non-coding RNA ZEB2-AS1 expression is associated with disease progression and predicts outcome in gastric cancer patients

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## Summary

**Purpose:** To explore the correlation of expression of long non-coding RNA (lncRNA) zinc finger E-box binding homeobox 2 antisense RNA 1 (ZEB2-AS1) in gastric cancer tissues, analyze the correlations of its expression level with the clinicopathological features of gastric cancer and the malignant phenotype of tumor cells, and investigate the molecular mechanism of action of ZEB2-AS1 in gastric cancer.

**Methods:** The expression level of ZEB2-AS1 in gastric cancer tissues and cancer-adjacent tissues in 56 patients was detected by fluorescence real-time quantitative polymerase chain reaction (qRT-PCR). AGS human gastric cancer cells were transiently transfected with small interfering lncRNA (si-lncRNA) ZEB2-AS1 using RNA interference technique, and its effect on proliferation of gastric cancer cells was assessed via MTT assay. Hoechst 33342 staining and flow cytometry were performed to examine the effect of ZEB2-AS1 on the apoptosis of AGS cells and scratch and Transwell assay were applied to detect the effect of si-ZEB2-AS1 on the invasion and metastasis of AGS cells.

**Results:** qRT-PCR results showed that the expression of ZEB2-AS1 in cancer tissues was increased compared with

cancer-adjacent tissues. Cell Counting Kit-8 (CCK-8) results indicated that knockdown of ZEB2-AS1 could significantly inhibit the proliferation of AGS cells. Hoechst 33342 staining and flow cytometry demonstrated that knockdown of ZEB2-AS1 obviously promoted the apoptosis of AGS cells. According to scratch and Transwell assay, knocking down ZEB2-AS1 could remarkably inhibit the invasion and metastasis of AGS cells. Western blotting results revealed that knocking down ZEB2-AS1 could inhibit cell invasion and metastasis by suppressing the epithelial to mesenchymal transition (EMT) as well as the expressions of matrix metalloproteinase-2 (MMP-2) and MMP-9 in AGS cells.

**Conclusions:** The expression of lncRNA ZEB2-AS1 in gastric cancer tissues is significantly higher than in cancer-adjacent tissues. Patients with highly expressed lncRNA ZEB2-AS1 have a poor prognosis, and knockdown of lncRNA ZEB2-AS1 in AGS cells inhibits cell proliferation, invasion and metastasis, and promotes apoptosis.

**Key words:** apoptosis, gastric cancer, lncRNA ZEB2-AS1, molecular mechanism

## Introduction

The incidence rate of gastric cancer ranks fourth among malignant tumors, and its mortality rate ranks second [1]. Due to lack of specific symptoms in the early stage, patients are usually diagnosed in advanced disease stage. Despite great progress made in the diagnosis and treatment of gastric cancer in recent years, studies have revealed

that the prognosis of this disease has not been significantly improved [2]. The current evaluation of prognosis of gastric cancer patients is still based on the tumor histopathological classification. As the molecular mechanisms of the occurrence and development of gastric cancer are still not fully clear, it is necessary to conduct further in-depth

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Received: 05/07/2018; Accepted: 19/08/2018

investigations, so as to discover molecular markers for early detection and targeted treatment of this malignancy [3,4].

Recent studies have shown that lncRNAs can affect the occurrence and development of tumors [5]. For example, lncRNA HOX transcript antisense RNA (HOTAIR), maternally expressed 3 (MEG3), metastasis associated lung adenocarcinoma transcript 1 (MALAT-1) and H19 play important roles in the occurrence and development of tumors [6-9]. lncRNAs regulate gene expression at multiple levels through various pathways, and they can interfere with the binding of transcription factors to promoters, regulation of histone modifications, and chromatin remodeling, so as to inhibit the expression of adjacent protein encoding genes [10]. At the same time, lncRNAs can influence RNA processing and serve as structural components of micro RNA (miRNA) precursors, cytoskeletons, and mitotic spindles [11]. Relevant studies have indicated that ZEB2-AS1 has significant cancer-promoting effects in liver cancer, bladder cancer, and acute myeloid leukemia [12-14]. However, there has been no report on lncRNA ZEB2-AS1 in gastric cancer. Therefore, the expression level of ZEB2-AS1 in gastric cancer tissues and its relationship with the prognosis of patients were planned to be investigated, and its molecular mechanism of biological function was further explored.

## Methods

### *Gastric cancer sample collection*

In this study, 56 pairs of gastric cancer and corresponding cancer-adjacent tissue specimens were collected from 56 gastric cancer patients operated in our hospital from January 2012 to December 2015. All patients were definitely pathologically diagnosed and received no neoadjuvant chemotherapy, radiotherapy or any other special cancer treatment before surgery. Cases in this study were staged according to the American Cancer Society (AJCC) gastric cancer staging (8<sup>th</sup> Edn). After the specimens were surgically excised, they were immediately cryopreserved in liquid nitrogen and then transferred to a refrigerator at -80°C until assayed. All patients signed informed consent, and the study was approved by the Ethics Committee of the hospital.

### *Cell lines and culture conditions*

Human gastric cancer cell lines (AGS, MKN45, MGC803 and BGC823) and human gastric normal epithelial GES-1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cell lines were all cultured in 1640 medium containing 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 1% penicillin-streptomycin and 1% non-essential amino acids under 5% CO<sub>2</sub> and 95% humidity at a constant temperature of 37°C.

### *Quantitative reverse transcription-polymerase chain reaction (QRT-PCR)*

A total of 100 mg tissue or human gastric cell lines (AGS, MKN45, MGC803 and BGC823) and human normal gastric epithelial cell GES-1 were taken to extract the total RNA using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For further experiments, only AGS cells were chosen. RNA concentration and purity were measured by ultraviolet spectrophotometer. Complementary DNA (cDNA) was reversely transcribed, and fluorescence qRT-PCR was used to detect the relative quantity (RQ) value of lncRNA ZEB2-AS1. Reaction system: 10 µL SYBR Green/Rox Fluorescence qPCR mixed solution, 2 µL forward and 2 µL reverse primers including lncRNA ZEB2-AS1 specific primers (forward sequence: 5'-ATGAAGAAGCCGCGAAGTGT-3', and reverse sequence: 5'-CACACCCTAATACACATGCCCT3') and the internal reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers (forward sequence: 5'-GAAGAGAGAGACCCTCACGCTG', and reverse sequence: 5'-ACTGTGAGGAGGGGAGATTTCAGT-3'), and 2 µL template cDNA. The reaction was repeated 3 times for each specimen. Reaction conditions: at 50°C for 2 min, 95°C for 10 min, 95°C for 15 s and 58°C for 60 s, with 40 cycles in total. The gene-specific primers for ZEB2-AS1 and GAPDH were designed and synthesized by Sangon Biotech (Shanghai, China).

### *Transfection of gastric cancer cells*

AGS cells in the logarithmic growth phase were taken, inoculated into a 6-well plate at 2×10<sup>4</sup> cells/well, and incubated at 37°C overnight. The cells were co-cultured with Iofetamine 2000 (Invitrogen, Carlsbad, CA, USA) and si-ZEB2-AS1 sequence or si-normal control (NC) sequence for 6 hrs, and the medium was changed for further culturing for 48 hrs, followed by follow-up experiments. Both si-ZEB2-AS1 and si-NC were designed and synthesized by Genechem (Shanghai, China). Si-ZEB2-AS1 sequence: 5'-CAAAGGACACCTTTGGTTACTGAA-', and si-NC: 5'UUCUCCGAACGUGUCACGUTT-3'.

### *Cell proliferation assay*

MTT assay was used to detect cell proliferation. When AGS cells were in the logarithmic growth phase, cells in the si-ZEB2-AS1 interference group and the control group were seeded into a 96-well plate at 8×10<sup>3</sup> cells/well, respectively. The volume of the 1640 medium containing 10% FBS was 200 µL. After that, the MTT kit was applied to detect the proliferation degrees of the cells at 24, 48 and 72 hrs. The 450 nm absorbance wavelength was used to read the values by a microplate reader (Bio-RAD, Hercules, CA, USA) and the cell growth curve was plotted.

### *Hoechst 33342 staining assay*

AGS cells were transfected with si-NC and si-ZEB2-AS1, after which cells were collected and inoculated in a 12-well plate. After 24 hrs of culturing, the supernatant was discarded and the cells were washed with PBS twice, added with Hoechst 33242 fluorescent dye, and incubated at 37°C for 15 min, followed by the discard

of fluorescent dye. After washing with PBS, an inverted fluorescence microscope was applied for observation and photographing.

#### Flow cytometry

AGS cells were transfected with si-NC and si-ZEB2-AS1, followed by cell collection. The cell concentration in single cell suspension was adjusted to  $5 \times 10^5$  mL, and 1 mL cells were taken and centrifuged at 4°C and 1,000 rpm for 10 min. The supernatant was discarded and 1 mL cold PBS was added, followed by gently shaking and centrifugation at 4°C and 1,000 rpm for 10 min. Then the supernatant was discarded, and the cells were resuspended in Binding Buffer-200. Ten  $\mu$ L fluorescein isothiocyanate (FITC)-Annexin V and 5  $\mu$ L propidium iodide (PI) were added and gently mixed, and the reaction lasted 15 min at room temperature in the dark. Then, the cells were assessed by flow cytometer within 1 hr. The experiments were repeated three times, and the average was taken to calculate the apoptosis rate.

#### Western blotting analysis

Total protein was extracted with protein lysate, and its concentration was determined by means of bicinchoninic acid (BCA) protein quantification method. The whole protein gel was run on a Bio-Rad (Hercules, CA, USA) vertical electrophoresis system, and proteins were separated. Proteins in the polyacrylamide gel electrophoresis (PAGE) were transferred onto NC membrane (Sigma, St. Louis, MO, USA), sealed using 5% skim milk for 1 hr at room temperature and added with B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), Bcl-2, caspase-3, E-cadherin and other primary antibodies of target proteins (Cell Signaling Technology, Danvers, MA, USA) for overnight incubation. After that, the corresponding secondary antibody was added for incubation in the dark for 1 hr, enhanced chemiluminescence (ECL)

assay (Thermo Fisher Scientific, Waltham, MA, USA) was performed, and a gel imager was used for development. The gray value of each band was calculated using Photoshop software, and the gray ratio of the target protein = gray value of the target protein / gray value of the internal reference protein.

#### Scratch wound healing assay

Cells were inoculated into a 6-well plate, and the medium was changed to a serum-free culture medium after the cells were adherent to the wall. When confluence reached 90-100%, scratches were made slowly and evenly using a 10  $\mu$ L pipette tip perpendicular to the bottom of the 6-well plate, and then the cells were washed with PBS three times to remove scratched floating cells and cultured in an incubator. At 0 and 48 hrs after the scratch culturing, the metastasis distance of the cells in the scratch area was observed under a microscope, and a plurality of different fields of view were randomly selected for photographing.

#### Transwell metastasis and invasion assay

Two groups of cells treated differently were collected, and  $2 \times 10^5$  cells in each group were placed in the upper chamber (serum-free medium), which chamber was put in a well plate, in which 10% fetal bovine serum (FBS) was used as an induction factor. Cell motility was measured at 24 hrs after incubation (without matrigel), and cell invasion ability was detected at 48 hrs after incubation (chambers were paved with matrigel). Cells in the upper chamber were brushed off after incubation, while those in the lower chamber were stained with crystal violet, followed by photographing and observation. The number of the lower chamber cells was measured *via* elution with trypsin, so as to compare the cell invasion and metastasis abilities between two groups.

**Table 1.** Correlation between ZEB2-AS1 expression and clinicopathological characteristics of gastric cancer patients

Characteristics	ZEB2-AS1		p value
	High No. cases (26)	Low No. cases (30)	
Gender			0.938
Male	15	17	
Female	11	13	
Age (years)			0.536
$\leq 60$	16	16	
$> 60$	10	14	
TNM stage			0.008
I + II	9	21	
III+IV	17	9	
Lymph node metastasis			0.019
Negative	11	22	
Positive	15	8	
Histological grade			0.035
I+II	10	20	
III	16	10	

### Statistics

All experiments were performed in triplicate. Statistical analysis was performed SPSS 17.0 software (Chicago, IL, USA). The t-test was applied for count data. Kaplan-Meier method was used to calculate survival, which was compared using the log-rank test. Factors influencing the apoptosis were analyzed via Cox regression analysis. The significance level was set at  $\alpha=0.05$ .

## Results

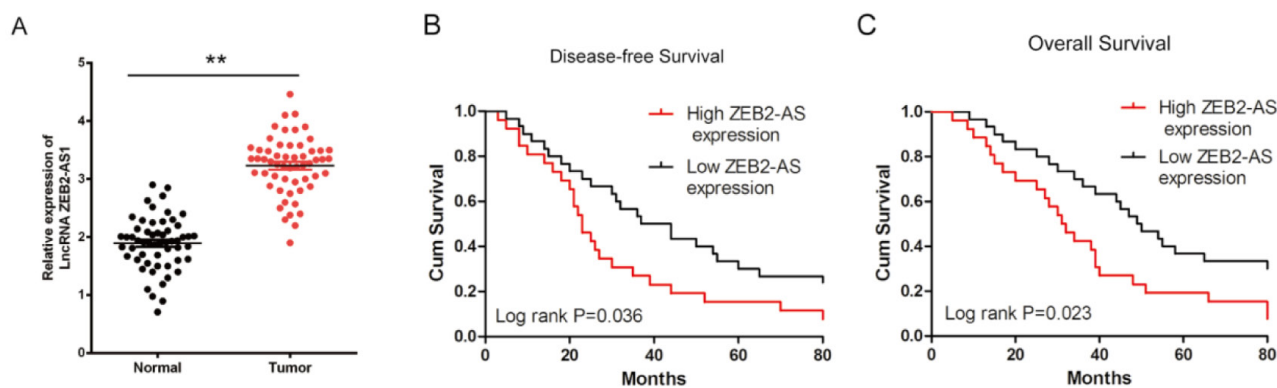
### *LncRNA ZEB2-AS1 was highly expressed in gastric cancer tissues and cells and correlated with poor prognosis*

To explore the roles of lncRNA ZEB2-AS1 in the occurrence and development of gastric cancer, qRT-PCR was firstly employed to detect the expression of lncRNA ZEB2-AS1 in gastric cancer tissue and the cancer-adjacent tissue. The results revealed that the expression of lncRNA ZEB2-AS1 in gastric cancer tissues was significantly higher than that in cancer-adjacent tissues ( $p<0.05$ ; Figure 1A), suggesting that lncRNA ZEB2-AS1 may play a certain promoting role in the occurrence and development of gastric cancer. Then, the mean expression level of lncRNA ZEB2-AS1 (3.23 relative to GAPDH) in 56 patients with gastric cancer was taken as the standard, and these patients were divided into lncRNA ZEB2-AS1 low expression group ( $n=26$ ) and lncRNA ZEB2-AS1 high expression group ( $n=30$ ). The expression level of lncRNA ZEB2-AS1 in gastric cancer tissues and clinicopathological data were further analyzed. Table 1 indicates that the high expression of lncRNA ZEB2-AS1 was associated with advanced TNM stage ( $p=0.008$ ), pathological grade ( $p=0.035$ ), and lymph node metastasis ( $p=0.019$ ), but was not correlated with gender and age of patients ( $p>0.05$ ). Interestingly,

Kaplan-Meier survival analysis manifested that the high expression of lncRNA ZEB2-AS1 was related to relatively poor disease-free survival (DFS) and overall survival (OS) of patients with gastric cancer. The median DFS in lncRNA ZEB2-AS1 high expression group was 40.5 months, while that in lncRNA ZEB2-AS1 low expression group was 23 months ( $p=0.036$ ). Furthermore, the median survival time in lncRNA ZEB2-AS1 high expression group was approximately 31.5 months, while that in lncRNA ZEB2-AS1 low expression group was about 49.5 months ( $p=0.023$ ).

### *Knockdown of lncRNA ZEB2-AS1 inhibited in vitro proliferation of gastric cancer AGS cell line*

The expression level of lncRNA ZEB2-AS1 in human gastric cancer cell lines (AGS, MKN45, MGC803 and BGC823) and human gastric normal epithelial GES-1 cells was detected via qRT-PCR. As shown in Figure 2A, the expression level of ZEB2-AS1 in AGS cells was the highest and these cells with the highest expression level of ZEB2-AS1 were selected for subsequent transfection. QRT-PCR validated the inhibitory efficiency of si-ZEB2-AS1 at 48 hrs after transfection. Figure 2B shows that si-ZEB2-AS1 is effective in suppressing the expression of lncRNA ZEB2-AS1 in AGS cells, which is statistically different from that in the negative control group ( $p<0.01$ ). In order to further investigate the effect of knockdown on cell biology, MTT assay was applied to verify the *in vitro* proliferation degree of AGS cells after knockdown of ZEB2-AS1 expression. AGS cells interfered by si-ZEB2-AS1 and Si-NC were cultured for 24, 48 and 72 hrs, respectively, and then MTT assay was performed. The results indicated that the growth rate of AGS cells was remarkably inhibited after knocking down ZEB2-AS1 (Figure 2C). The above



**Figure 1.** Relative ZEB2-AS1 expression in gastric cancer tissues and its clinical significance. **(A):** Relative expressions of ZEB2-AS1 in gastric cancer tissues ( $n=56$ ) and adjacent non-cancer tissues ( $n=56$ ) were examined by qRT-PCR and normalized to GAPDH expression. **(B,C):** Kaplan-Meier disease-free survival curves and overall survival according to ZEB2-AS1 expression level (\*\* $p<0.01$ ).

results suggest that knocking down LncRNA ZEB2-AS1 can inhibit the proliferation of AGS cells.

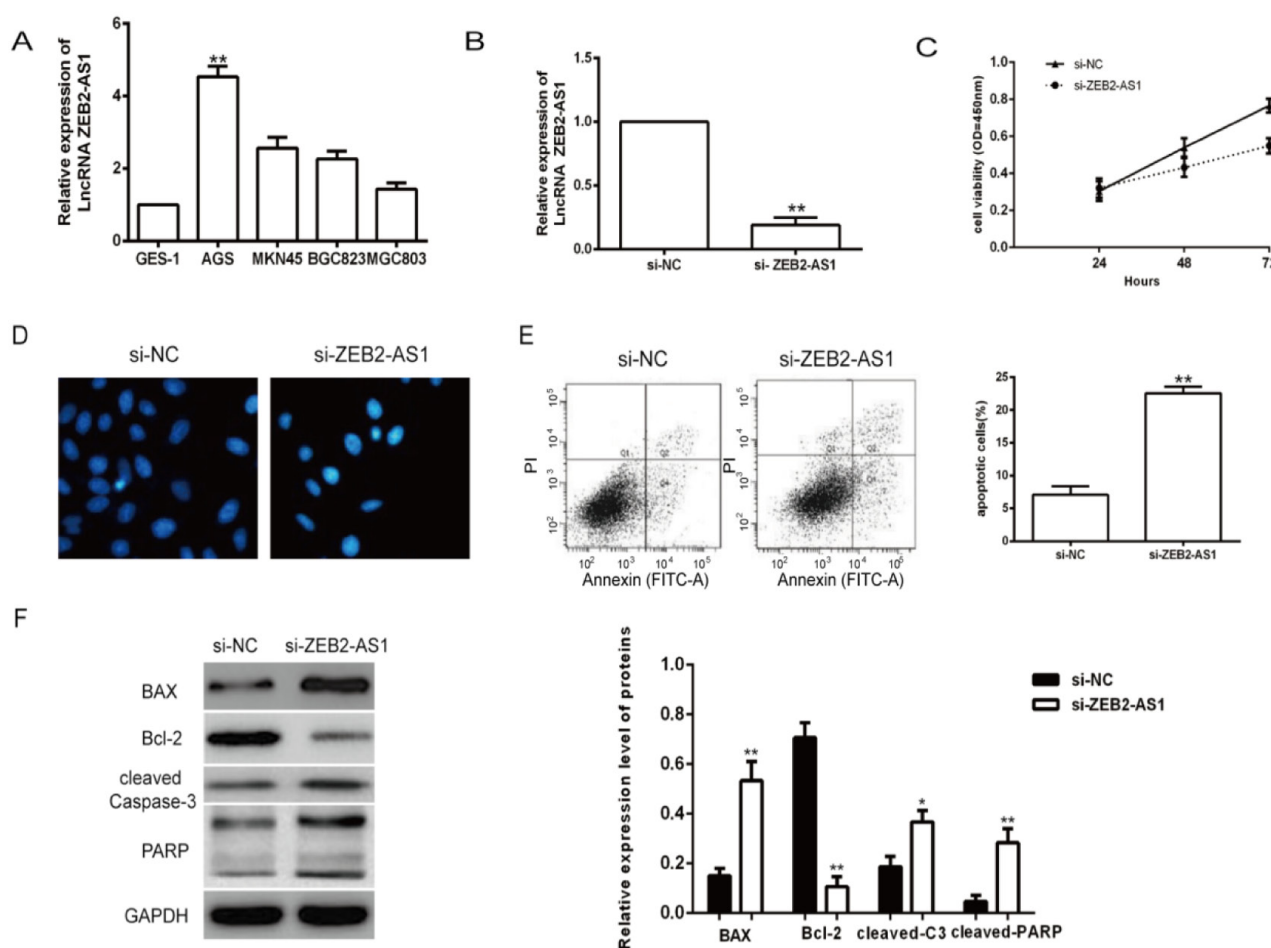
*Knockdown of lncRNA ZEB2-AS1 induced apoptosis of gastric cancer AGS cell line*

Hoechst 33342 staining results demonstrated that compared with that in the control group, the proportion of apoptotic cells in the lncRNA ZEB2-AS1 knockdown group was significantly increased (Figure 2D). Flow cytometry revealed that the total apoptosis rate in the knockdown group was significantly increased compared with that in the control group (Figure 2E,  $p < 0.05$ ). Consistent with the above results, Western blotting (Figure 2F) indicated that the expressions of cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP) were remarkably upregulated after knockdown of lncRNA ZEB2-AS1. The above results indicate that inhibiting the expression of lncRNA ZEB2-

AS1 can significantly upregulate the expressions of caspase-3 and cleaved PARP, initiate mitochondrial apoptosis pathway, and promote apoptosis of gastric cancer cells.

*Downregulation of ZEB2-AS1 inhibited the invasion and metastasis abilities of AGS cells*

To further verify the effects of ZEB2-AS1 on metastasis and invasion of gastric cancer cells *in vitro*, scratch and Transwell assays were conducted to explore the changes in metastasis and invasion abilities of gastric cancer cells after inhibiting the expression of lncRNA ZEB2-AS1. Scratch assay showed that 24 hrs later, the metastasis distance of cells in lncRNA ZEB2-AS1 knockdown group was significantly shorter than that in the control group ( $p < 0.05$ , Figure 3A and 3B). The results of transwell assay were consistent with those of scratch assay. After the knockdown, the number of cells passing



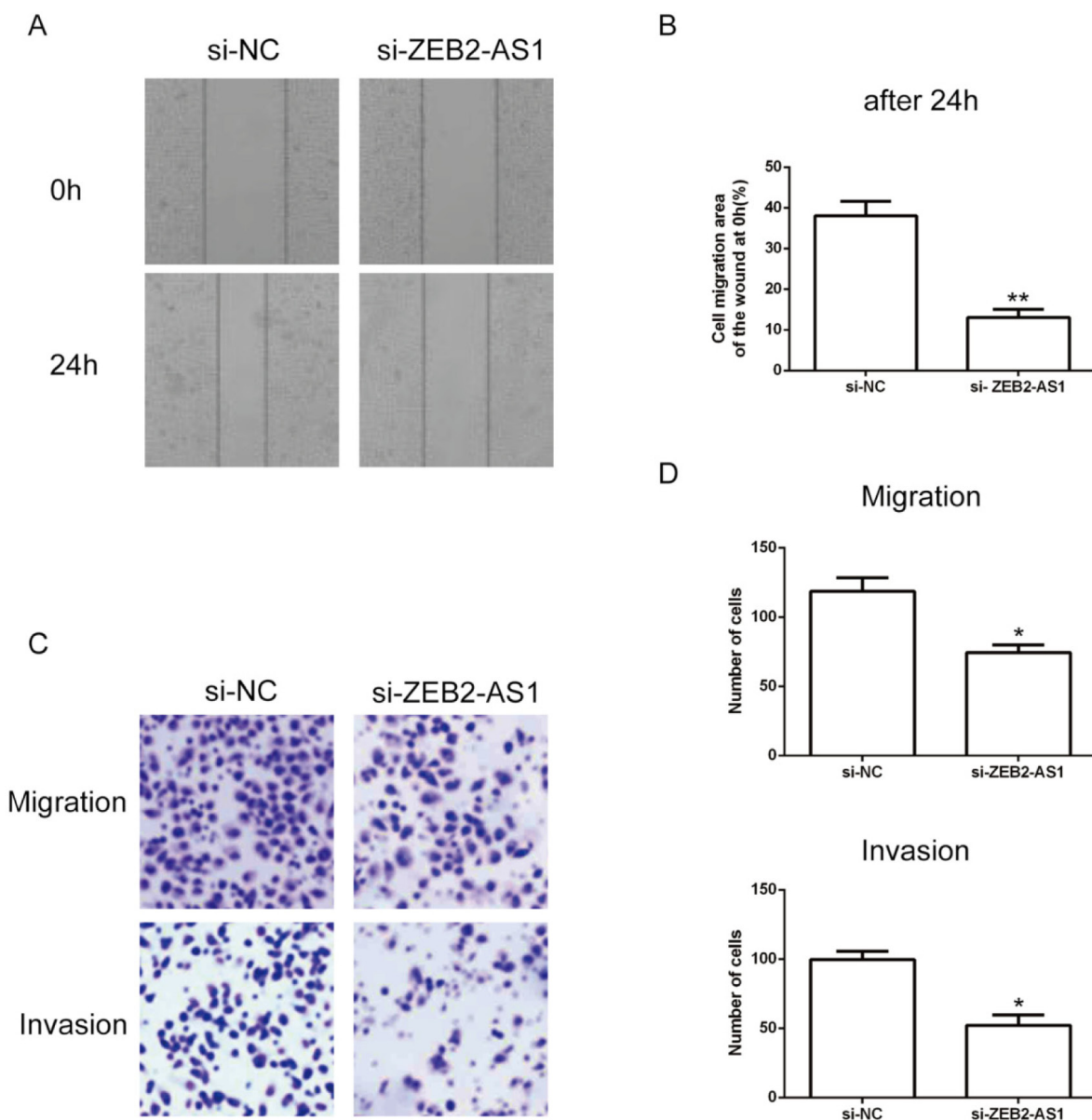
**Figure 2.** Effects of ZEB2-AS1 on AGS cells proliferation and apoptosis *in vitro*. (A): ZEB2-AS1 expression was assessed by qRT-PCR analysis in gastric cancer cell lines (AGS, MKN45, MGC803, BGC823) and human gastric normal epithelial cells (GES-1). (B): Effective knockdown of ZEB2-AS1 in AGS cells 48 hrs after siRNA treatment. (C): MTT assay was performed to determine the proliferation of si-ZEB2-AS1 and si-NC transfected AGS cells. (D,E): Hoechst 33342 staining and flow cytometry were conducted to determine the apoptosis of si-ZEB2-AS1-transfected AGS cells. (F): The proteins levels of BAX, Bcl-2, cleaved caspase-3, PARP and cleaved PARP were determined by western blotting in si-ZEB2-AS1 transfected AGS cells. The data represent the mean  $\pm$ SD from three independent experiments. (\* $p < 0.05$ , \*\* $p < 0.01$ ).

through the filter membrane in the lower chamber was significantly decreased ( $p < 0.05$ , Figure 3C and 3D). The above results suggest that lncRNA ZEB2-AS1 can promote the invasion and metastasis of gastric cancer AGS cells, and the targeted knockdown of lncRNA ZEB2-AS1 expression can impede these abilities.

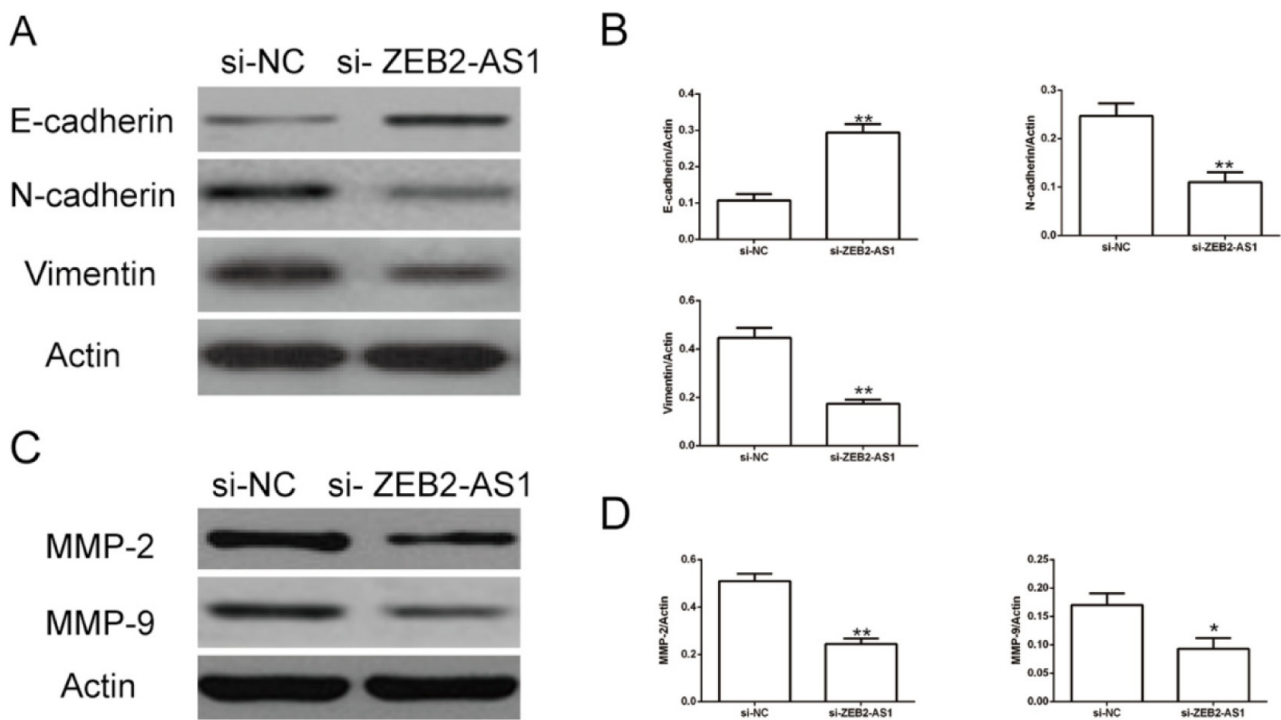
*Knockdown of lncRNA ZEB2-AS1 expression suppressed the epithelial to mesenchymal transition (EMT) process as well as the expressions of matrix metalloproteinase-2 (MMP-2) and MMP-9 in AGS cells*

In order to explore the mechanism of ZEB2-AS1 regulating the invasion and metastasis of AGS

cells, the expression of EMT-related molecules was further examined using Western blotting. Figure 4 indicates that after knocking down ZEB2-AS1, the expression of E-cadherin was significantly upregulated, while the expressions of N-cadherin and Vimentin were remarkably downregulated. To further examine whether lncRNA ZEB2-AS1 regulated the invasion and metastasis of gastric cancer cells through other mechanisms, Western blotting was applied again to detect the expression levels of MMP-2 and MMP-9 after knocking down ZEB2-AS1. The results evidenced that knocking down lncRNA ZEB2-AS1 also downregulated the expressions of MMP-2 and MMP-9 in AGS cells.



**Figure 3.** Effects of ZEB2-AS1 on AGS cells migration and invasion *in vitro*. **(A,B)**: AGS cells were treated with si-ZEB2-AS1 and si-NC, and the effects on cell migration were determined with cell scratch test. **(C,D)**: The effects on cell migration and invasion were determined with cell transwell assay. The data represent the mean  $\pm$ SD from three independent experiments (\* $p < 0.05$ , \*\* $p < 0.01$ ).



**Figure 4.** Effects of ZEB2-AS1 on the protein expression related to migration and invasion of AGS cells. **(A):** The expression levels of target proteins (E-cadherin, N-cadherin, Vimentin) were evaluated by western blotting in AGS cells transfected with si-ZEB2-AS1 or si-NC. **(B):** The data represent the mean $\pm$ SD from three independent experiments (E-cadherin, N-cadherin, Vimentin). **(C):** The expression levels of target proteins (MMP-2 and MMP-9) evaluated by western blotting in AGS cells transfected with si-ZEB2-AS1 or si-NC. **(D):** The data represents the mean $\pm$ SD from three independent experiments (MMP-2 and MMP-9) (\* $p$ <0.05, \*\* $p$ <0.01).

## Discussion

Despite the significant progress made in the treatment of gastric cancer in recent years, the survival rate of gastric cancer patients remains low [15,16]. lncRNAs are new members of ncRNAs. Recent studies have revealed that lncRNAs have a relatively long nucleotide chain with a specific and complex secondary spatial structure inside the molecule that can provide multiple sites for binding to proteins [4,8]. Moreover, lncRNAs can specifically and dynamically interact with DNA and RNA through the principle of complementary base pairing, thus forming a gene expression regulatory network involving lncRNA [17,18]. According to studies, lncRNAs participate in many biological processes in various organisms, including inactivation of X chromosome reactivation of pluripotent stem cells, and regulation of cell differentiation and apoptosis. More importantly, abnormal expression of lncRNA is related to many human diseases including cancer [19,20].

A large number of studies have shown that lncRNAs play important roles in the occurrence and development of gastric cancer [21]. Yang et al.[22] identified that the expression level of lncRNA H19 in gastric cancer tissues is significantly increased

compared with that in normal gastric tissue. *In vitro* cell experiments prove that H19 is correlated with cell proliferation and apoptosis, can bind to p53 and inhibit its activity, thereby reducing its downstream target gene Bax level, promoting proliferation and escape apoptosis. H19 serum level in patients with gastric cancer is also increased compared with that in normal people [22]. HOTAIR also plays an important role in gastric cancer. Compared with that in normal tissues, HOTAIR is remarkably highly expressed and significantly associated with lymph node metastasis, vascular invasion and survival [23].

Studies have verified that ZEB2-AS1 acts as an oncogene in hepatocellular carcinoma and bladder cancer [13,14]. However, ZEB2-AS1 in gastric cancer has not yet been reported. In this study, the expression of lncRNA ZEB2-AS1 in 56 pairs of gastric cancer and normal gastric tissue specimens was detected *via* qRT-PCR, and the correlations of lncRNA ZEB2-AS1 expression with clinicopathological features and prognosis were analyzed. The results revealed that, compared with normal gastric tissues, the expression level of lncRNA ZEB2-AS1 in gastric cancer tissues increased markedly, and the difference was statistically significant ( $p$ <0.05). The expression of lncRNA ZEB2-AS1 was not as-

sociated with gender and age of patients ( $p>0.05$ ), but with the TNM stage, lymph node metastasis, and tumor pathological grade ( $p<0.05$ ). The median PFS of patients with high expression of lncRNA ZEB2-AS1 (20 months) was significantly shortened compared with that in patients with low expression (34 months;  $p<0.05$ ). The median OS of patients with low expression of lncRNA ZEB2-AS1 was 70 months, which was obviously prolonged compared with that in patients with high expression (46 months;  $p=0.003$ ). These results suggest that lncRNA ZEB2-AS1 may be involved in the regulation of the occurrence and development of gastric cancer and can be used as a potential molecular marker for the diagnosis and prognosis of gastric cancer.

The biological function of lncRNA ZEB2-AS1 *in vitro* was further analyzed by siRNA-mediated silencing. MTT and cell colony formation experiments illustrated that knockdown of lncRNA ZEB2-AS1 significantly inhibited the proliferation ability of gastric cancer AGS cells. Hoechst 33342 staining and flow cytometry results demonstrated that inhibition of lncRNA ZEB2-AS1 greatly increased the proportion of apoptotic cells. Western blotting experiments further identified that knockdown of lncRNA ZEB2-AS1 could markedly upregulate the expressions of cleaved caspase-3 and cleaved PARP, initiate mitochondrial apoptosis pathway and promote apoptosis of gastric cancer cells.

Invasion and metastasis of tumors are the major causes of treatment failure and death in cancer patients. Invasion and metastasis experiments confirmed that knocking down lncRNA ZEB2-AS1 in AGS cells could significantly inhibit their invasion and metastasis abilities. EMT is one of the key steps in the invasion and metastasis of epithelial-derived malignant tumors, and it refers to a process

in which polar epithelial cells transform into cells that have the ability to move freely through the cell matrix. This process involves changes in the expressions of a variety of key molecules, such as loss of E-cadherin and expression of N-cadherin [24]. *In vitro* studies of Lan et al. [14] manifested that lncRNA ZEB2-AS1 can inhibit the metastatic ability of liver cancer Huh-7 cells by inhibiting their EMT.

In this study, Western blotting revealed that after knocking down lncRNA ZEB2-AS1, E-cadherin expression was significantly upregulated while Vimentin and N-cadherin expressions were downregulated, suggesting that knocking down lncRNA ZEB2-AS1 can inhibit EMT of gastric cancer AGS cells. Interestingly, it was further shown that knockdown of lncRNA ZEB2-AS1 also downregulated the expressions of MMP-2 and MMP-9 that are the most important enzymes in MMPs and play important roles in tumor invasion and metastasis. This result confirmed that lncRNA ZEB2-AS1 could promote the invasion and metastasis of gastric cancer by regulating the EMT process of tumors and the expression of MMPs.

## Conclusions

In conclusion, this study revealed that lncRNA ZEB2-AS1 could promote the occurrence and development of gastric cancer and functions as an oncogene. lncRNA ZEB2-AS1 can become a new and effective prognostic marker of gastric cancer, and can also provide a potential target for the treatment of gastric cancer.

## Conflict of interests

The authors declare no conflict of interests.

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