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Values of long noncoding RNA SAMMSON in the clinicopathologic features and the prognostic implications of human gastric cancer

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Abstract. – OBJECTIVE: The aim of this study was to explore the clinical significance of lncRNA-survival associated mitochondrial melanoma-specific oncogenic non-coding RNA (lncRNA-SAMMSON) in the development and clinicopathological parameters of gastric cancer (GC).

PATIENTS AND METHODS: Tissue specimens were collected from GC patients who received treatment in our hospital. Real-time quantitative polymerase chain reaction (QRT-PCR) was used to determine lncRNA-SAMMSON expression. Small interfering RNA (siRNA) was transfected to suppress the expression of lncRNA-SAMMSON *in vitro*. Pearson's χ^2-test was used to investigate the interaction of lncRNA-SAMMSON with clinicopathological parameters of GC patients. Kaplan-Meier method and Log rank analysis were used to analyze the progression-free survival time and overall time of GC patients. Furthermore, transwell assay and wound healing assay were conducted to determine the invasion and migration abilities of GC cells, respectively.

RESULTS: QRT-PCR results showed that lncRNA-SAMMSON was abnormally overexpressed in GC tissues and cells ($p<0.05$). Pearson's χ^2-test illustrated that clinical stage, distant metastasis and lymph node metastasis were closely related to lncRNA-SAMMSON expression in GC patients ($p<0.05$). Kaplan-Meier survival analysis represented that GC patients with high lncRNA-SAMMSON expression had significantly shorter progression-free survival time and overall survival time ($p<0.05$). Transwell assay and wound healing assay proved that inhibition of lncRNA-SAMMSON in GC cells dramatically reduced the invasion and migration abilities of GC cells, respectively ($p<0.05$).

CONCLUSIONS: LncRNA-SAMMSON played an important role in the development of GC, which might be regarded as a new target for the diagnosis and treatment of GC.

Key Words:
Gastric cancer (GC), LncRNA-SAMMSON, Clinicopathological parameters.

Introduction

Gastric cancer (GC), a kind of disease seriously threatening human health, has extremely high morbidity and mortality in the world[1,2]. Due to easily neglect related inconspicuous discomforts in the early stage and the lack of treatment in time, GC patients have already in the intermediate and advanced stage when first diagnosed[3]. With the improvement in diagnostic methods in recent years, the early detection rate of GC is rising. Meanwhile, certain progress has been made in combination therapies such as surgery, chemoradiotherapy and immunotherapy[4-7]. However, metastasis still occurs in most patients, resulting in undesirable prognosis of GC patients and unnoticeable increase in overall survival rate[2,8]. Hence, deeply understanding and exploring the molecular mechanism of GC metastasis and searching for molecular markers and therapeutic targets related to GC metastasis are of important clinical significance for the prevention and treatment of GC, as well as the improvement of survival rate of GC patients in the advanced stage.

Long non-coding ribonucleic acids (lncRNAs) are a kind of mature RNA molecules with over 200 bases in length, without protein-coding products[9]. They are widely distributed in eukaryote cells and implicated in many physiological and pathological processes[10,11]. Therefore, it is very

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important to investigate the pro-oncogenic or anti-cancer effects of lncRNAs in the occurrence and development of tumors. All these findings may further help to reveal the potential molecular regulatory mechanism *in vitro* and *in vivo*.

LncRNA-survival associated mitochondrial melanoma-specific oncogenic non-coding RNA (lncRNA-SAMMSON) has been found widely involved in tumor development[12-14]. However, whether lncRNA-SAMMSON is abnormally expressed in GC and whether it can affect GC metastasis remain unclear. In this study, therefore, the expression of lncRNA-SAMMSON in GC tissue specimens was detected. The influences of lncRNA-SAMMSON on the migratory and invasive abilities of GC cells were analyzed through *in-vitro* experiments. Our findings might help to lay a solid foundation for further verification of the molecular regulatory mechanism of lncRNA-SAMMSON in GC.

Patients and Methods

Species of GC Tissues

Paired GC tissues and corresponding normal tissues were obtained from patients who received treatment in Weihai Municipal Hospital from June 2017 to June 2019. The selection of patients was based on the guideline proposed by the Union for International Cancer Control (UICC). None of these patients received any other anti-tumor therapies such as surgery and chemotherapy before operation. Collected tissue samples were made into frozen sections after operation and definitely diagnosed with GC by virtue of pathological examinations. Subsequently, resected specimens were immediately frozen in liquid nitrogen for use. This investigation was approved by the Research Ethics Committee of Weihai Municipal Hospital.

Culture of Cells

Human GC cell lines (SGC-7901, HCG-27, AGS and MGC803) were cultured in complete Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS) (HyClone, South Logan, UT, USA) and 1% penicillin-streptomycin mixture. Meanwhile, normal human gastric mucosal cells (GES) were cultured in DEME (high glucose) base medium (Gibco, Rockville, MD, USA) supplemented with 10% FBS and 1% penicillin-streptomycin mixture. All these cells were maintained in an incubator with 5% CO_2 at 37°C under sterile conditions. When cell density reached about 90%, they were added with approximately 0.5 mL of tryptic digest containing EDTA (ethylenediaminetetraacetic acid), placed in a culture dish at 37°C for about 1 min and observed under an inverted microscope. If the majority of cells were detached, 2 mL of relevant complete medium was added immediately to terminate the digestion. Next, the suspension was collected into 15 mL of centrifuge tubes, followed by centrifugation at 800 rpm for 5 min. After discarding the supernatant, the cells were pipetted into cell suspension using fresh complete medium. Finally, the cells were diluted into a suitable concentration based on the results of cell count and cultured again with a culture flask in the sterile incubator with CO_2 at 37°C.

Cell Transfection

Cell transfection was performed according to the reagent manufacturer's instructions. In brief, when cell density reached 50%, scramble siRNA or lncRNA-SAMMSON si-RNA, together with lipo3000 were first mixed with FBS free medium for 5 min. Subsequently, the above two mixtures were gently mixed together, followed by standing for 20 min. Thereafter, the mixed medium was added into the plate and changed with fresh medium 6 h later. All the procedures were conducted according to the instructions of the manufacturer. Cells with different treatment were divided into two groups, including si-NC group (transfected with scramble siRNA) and si-lncRNA-SAMMSON group (transfected with lncRNA-SAMMSON siRNA).

Wound Healing Assay

After transfection, cells in each group were quickly crossed using a 100 μL tip perpendicular to the plate. Then, the cells were washed with phosphate-buffered saline (PBS) for three times and cultured with FBS-free medium in a 37°C, 5% CO_2 incubator for 24 h. Finally, the plate was observed under a microscope and photographed.

Transwell Assay

A total of 20 μL of Matrigel (Corning, Corning, NY, USA) was first evenly smeared on the inner surface of transwell chambers. Subsequently, they were put into a well plate and placed in an incubator with 5% CO_2 at 37°C for 30 min for the purpose of solidification into gel. Next, cells in each group were digested into single-cell sus-

pension. After cell density was adjusted to 1×10^4 cells/mL, 200 μL of cell suspension was evenly added in drops into the upper transwell chambers, without causing air bubbles. Meanwhile, 500 μL of complete medium containing FBS was added to lower chambers. Then, the cells were cultured at 37°C under 5% CO_2 for 24 h. After fixing with 1% paraformaldehyde for 15 min, the cells were stained with an appropriate amount of crystal violet for 10 min. After that, cells on the upper surface of the chambers were gently wiped out using wet cotton swabs. Invasive cells were observed under a microscope, and the number of invasive cells was counted. 3 fields of view were randomly selected for each sample. The changes in the invasive ability of cells were compared between experimental group and control group.

Real-Time Quantitative Polymerase Chain Reaction (QRT-PCR) Analysis

Total RNA was extracted from tissues and cells according to the instructions of extraction kit (TIANGEN, Beijing, China). The concentration and A_{260}/A_{280} value of extracted RNA were determined. After that, reverse transcription was performed on a PCR instrument as follows: 42°C for 60 min and 85°C for 5 min, so as to eliminate the activity of reverse transcriptase. Next, the product (cDNA) was used for PCR as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 60°C for 45 s and extension at 72°C for 45 s for 35 cycles, followed by final extension at 72°C for 7 min and preservation at 4°C. Primers used in this study were GGGGATTTCAGGCAAAATCAGAACTGATCCGATGCATATG.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was utilized for statistical analysis. GraphPad Prism 5 software employed for plotting. The correlations of lncRNA-SAMMSON expression level in GC tissues with clinicopathological data were analyzed *via* Pearson's χ^2-test. Kaplan-Meier survival analysis was evaluated by log-rank test. The *t*-test was adopted for the comparison of mean values between two groups. $p<0.05$ was considered statistically significant.

Results

Expression Levels of LncRNA-SAMMSON In GC Tissues

We first collected GC tissues and normal adjacent tissues from patients and performed qRT-PCR to determine the expression level of lncRNA-SAMMSON. As shown in Figure 1A, the expression of lncRNA-SAMMSON was nearly 2.7-fold in GC tissues compared with corresponding adjacent tissues ($p<0.05$). Besides, we detected the expression of lncRNA-SAMMSON in GES cell line and four different GC cell lines, including SGC-7901, HCG-27, AGS and MGC-803. The results indicated that compared with normal GES cell line, lncRNA-SAMMSON was significantly up-regulated in the above GC cell lines, especially in HCG-27 cell line and AGS cell line ($p<0.05$; Figure 1B). These findings suggested that lncRNA-SAMMSON was involved in the development of GC.

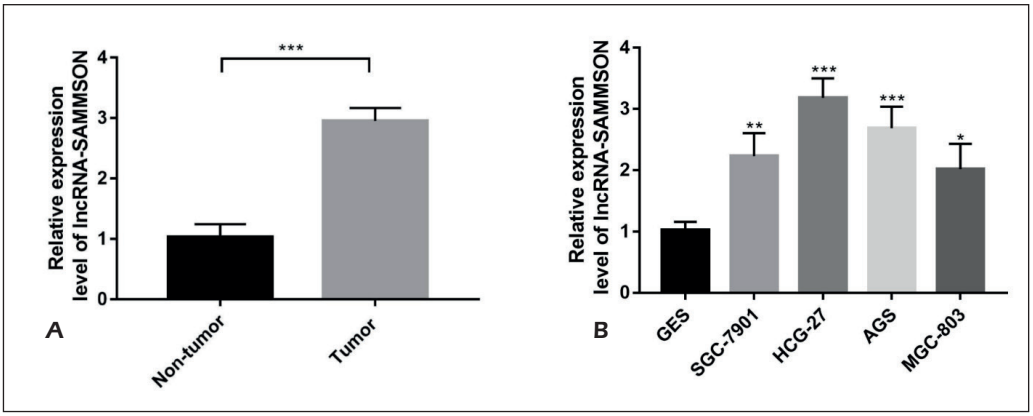

Figure 1. Expression levels of lncRNA-SAMMSON in GC tissues and cells. QRT-PCR results showed that LncRNA-SAMMSON was significantly up-regulated in GC tissues and cell lines (SGC-7901, HCG-27, AGS and MGC-803) compared with adjacent normal tissues (**A**) and GE cell line (**B**), respectively. (**$p<0.01$, ***$p<0.001$).

Table I. Correlations between lncRNA-SAMMSON expression and different clinicopathological characteristics of patients with breast cancer.

Characteristics	Cases n = 126	lncRNA-SAMMSON expression		*p*-value
		High (n =70)	Low (n =56)	
Age (years)				
< 60	62	29	33	0.720
≥ 60	64	27	37	
Gender				
Male	66	26	40	0.282
Female	60	30	30	
Stage				
I-II	63	21	42	0.012*
III-IV	63	35	28	
Differentiation				
Well-moderate	69	33	36	0.472
Poor	57	23	34	
Distant metastasis				
No	69	37	32	0.030*
Yes	57	19	38	
Lymph node metastasis				
Negative	66	35	31	0.049*
Positive	60	21	39	

LncRNA-SAMMSON Was Associated With Clinicopathological Characteristics of GC Patients

According to the median expression of lncRNA-SAMMSON, GC patients were divided into two groups, including low lncRNA-SAMMSON group and high lncRNA-SAMMSON group. Subsequently, we analyzed the association between the clinicopathological characteristics of GC patients and lncRNA-SAMMSON expression. The results (shown in Table I) found that clinical stage, distant metastasis and lymph node metastasis were closely correlated with lncRNA-SAMMSON expression ($p<0.05$). However, no significant differences were observed in age, gender and differentiation ($p>0.05$). Further, Kaplan-Meier survival analysis showed that GC patients with high lncRNA-SAMMSON expression had significantly shorter progression-free survival time and overall survival time ($p<0.05$; Figure 2A, 2B).

Inhibition of LncRNA-SAMMSON Decreased the Invasion Ability of GC Cells

After confirming that transfection of siRNA inhibited lncRNA-SAMMSON expression (data not shown), transwell assay was performed to better clarify the role of lncRNA-SAMMSON on the in-

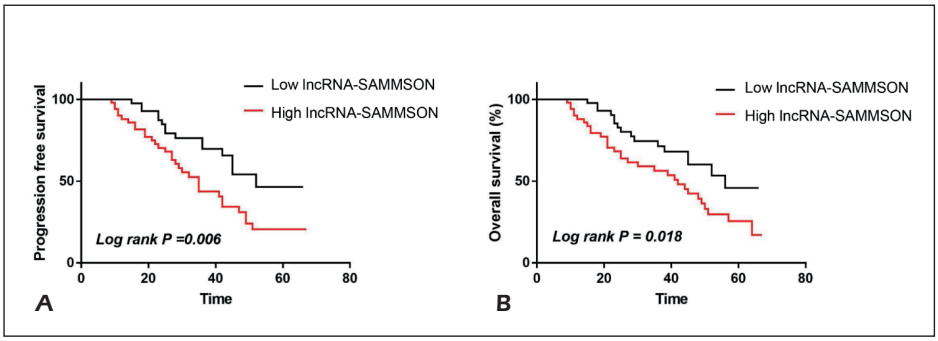

Figure 2. Significance of lncRNA-SAMMSON expression in GC patients. GC patients with high expression of lncRNA-SAMMSON had significantly shorter progression-free survival time (**A**) and overall survival time (**B**) in comparison with those with low expression of lncRNA-SAMMSON.

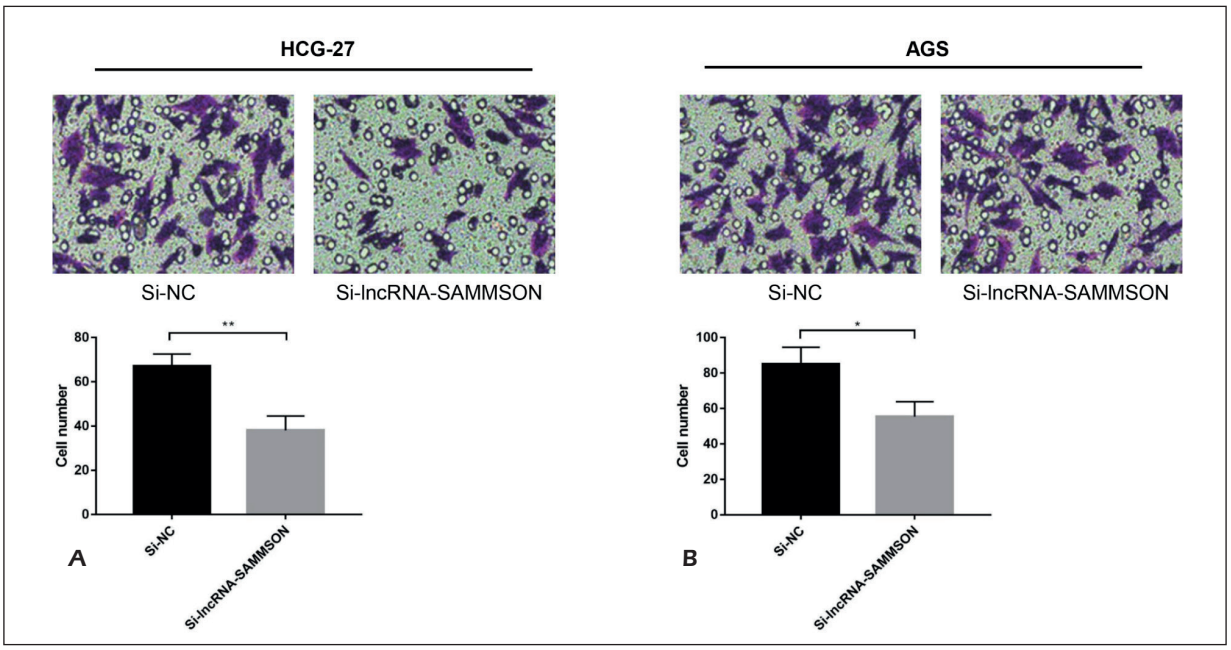

Figure 3. Inhibition of lncRNA-SAMMSON decreased the invasion ability of GC cells. HCG-27 cells (**A**) and AGS cells (**B**) in si-lncRNA-SAMMSON group represented decreased invasion ability compared with HCG-27 cells and AGS cells in si-NC group, magnification: 10×. (*p<0.05, *p<0.01).

vasion ability of GC cells. As shown in Figure 3A, 3B, when the expression of lncRNA-SAMMSON was both inhibited in HCG-27 cells and AGS cells, the number of invasive cells decreased remarkably in si-NC group (p<0.05). This implied that GC cells with low-expression of lncRNA-SAMMSON had impaired invasion ability.

Suppression of LncRNA-SAMMSON Reduced the Migration Ability of GC Cells

Later, wound healing assay was applied to detect the role of lncRNA-SAMMSON on the migration ability of GC cells. Compared with cells transfected with scramble siRNA, both HCG-27 cells and AGS cells treated with lncRNA-SAMMSON siRNA showed decreased migration ability, as the wound healing rate in Si-lncRNA-SAMMSON group decreased remarkably compared with Si-NC group (p<0.05; Figure 4A, 4B). These results indicated that inhibition of lncRNA-SAMMSON in GC cells significantly reduced the migration ability of cells.

Discussion

As an invasive cancer, the metastasis of GC is the leading cause of death and recurrence. Meanwhile, lymph node metastasis or distant metastasis is a crucial factor influencing the prognosis and survival of GC patients[15-17]. Large quantities of molecules have been proven to be involved in the occurrence and development of GC[18-20]. Nevertheless, the postoperative survival rate of the patients is still far from satisfactory[21,22].

LncRNAs are a category of non-coding RNA molecules with more than 200 nucleotides in length. They are produced by intracellular encoding genes during normal transcription. Besides, their structure is relatively conserved and modulated by complex networks in cells[23]. LncRNAs have been found closely associated with the incidence and progression of multiple diseases. They also play essential roles in varying biological processes, such as cell proliferation, metabolism, differentiation and organogenesis[24,25]. In addition, lncRNAs account for a large part of genomic transcription, which exert critical carcinogenic effects by modulating the expression of target genes[26]. Moreover, the correlations of aberrant expression of lncRNAs with the occurrence and progression of tumor have become one of the hotspots of cancer research so far[27].

LncRNA-SAMMSON exerts significant roles in the development and progression of several tumors. Abnormal expression of lncRNA-SAMMSON in melanoma may be related to the development of tumor cells. Knocking down the expression of SAMMSON can

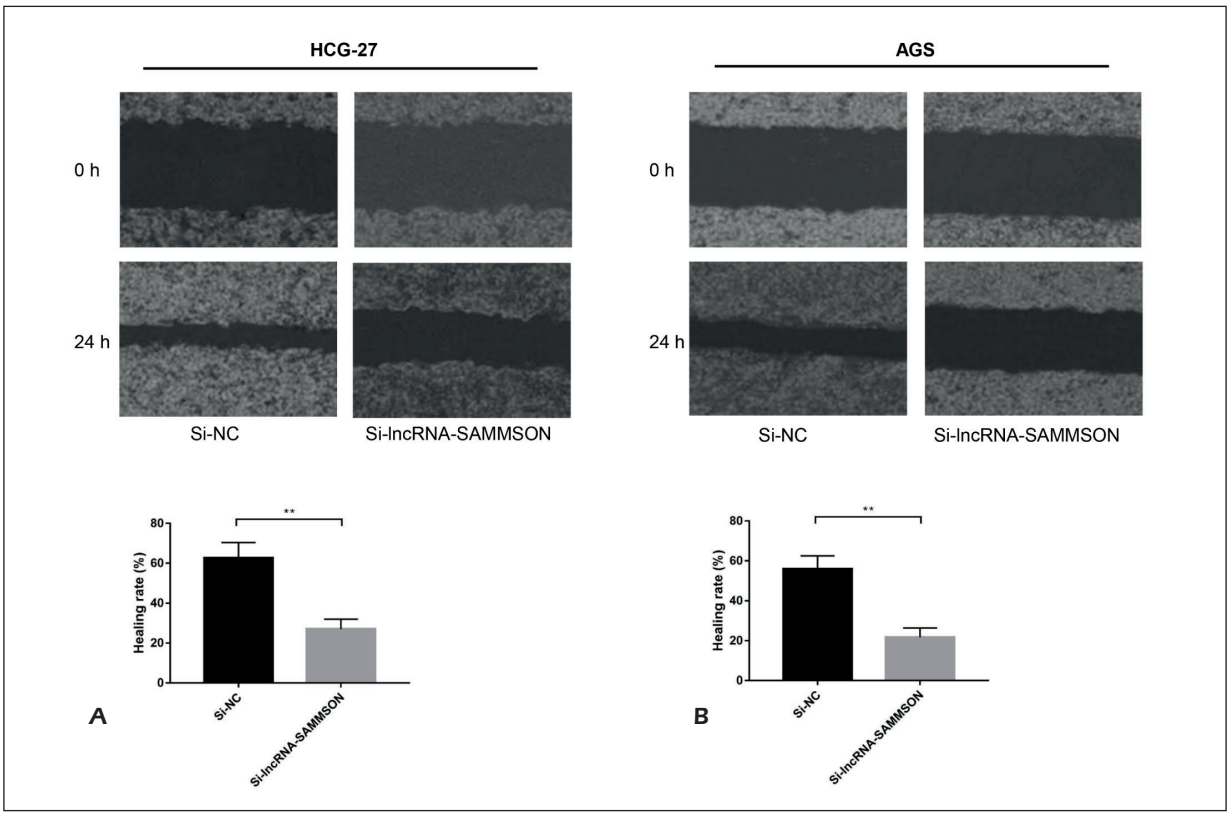

Figure 4. Suppression of LncRNA-SAMMSON reduced the migration ability of GC cells. HCG-27 cells (**A**) and AGS cells (**B**) under transection of lncRNA-SAMMSON siRNA showed reduced migration ability compared with HCG-27 cells and AGS cells under scramble siRNA treatment, magnification: 10×. (**p<0.01).

reduce the viability of melanoma cells[28]. Li et al[29] have reported that lncRNA SAMMSON is highly expressed in liver cancer and liver tumor initiating cells. Cells with low expression of lncRNA SAMMSON show impaired self-renewal capacity. However, overexpression of lncRNA SAMMSON in cells induces enhanced self-renewal.

In this study, we aimed to explore the clinical significance of lncRNA-SAMMSON in patients with GC. We firstly collected paired GC tissues and adjacent normal tissues. Subsequently, total RNA was extracted from tissues to examine the expression level of lncRNA-SAMMSON *in vitro*. The results showed that lncRNA-SAMMSON was highly expressed in GC tissues than adjacent normal tissues. Similarly, up-regulated expression of lncRNA-SAMMSON was observed in different GC cell lines (SGC-7901, HCG-27, AGS and MGC803) in comparison with GES cells. These findings suggested that lncRNA-SAMMSON was closely correlated with GC development.

By analyzing the clinical data of patients, we found that GC patients with high expression of lncRNA-SAMMSON had significantly shorter progression-free survival time and overall survival time compared with those with low expression of lncRNA-SAMMSON. Kaplan-Meier survival analysis further revealed that the expression level of lncRNA-SAMMSON was closely associated with clinical stage, distant metastasis and lymph node metastasis. These results strongly support the clinical significance of lncRNA-SAMMSON in patients with GC.

Since the expression levels of lncRNA-SAMMSON in HCG-27 cells and AGS cells were relatively highest, the two cell lines were transfected with siRNA to exogenously inhibit the expression of lncRNA-SAMMSON. Later, we performed *in vitro* experiments such as transwell assay and wound healing assay to investigate the effect of lncRNA-SAMMSON on the bio-behavior of GC cells. In response to the above results, we observed that inhibition of

lncRNA-SAMMSON in HCG-27 and AGS cells significantly impaired its invasion and migration characteristics. All these findings further proved the important effect of lncRNA-SAMMSON on the development of GC.

Conclusions

Our findings revealed an interaction of lncRNA-SAMMSON expression with GC development. LncRNA-SAMMSON was abnormally overexpressed in GC tissues and cells. LncRNA-SAMMSON was tightly associated with the clinicopathological characteristics of GC patients. Meanwhile, inhibition of lncRNA-SAMMSON dramatically reduced the invasion and migration abilities of GC cells. Altogether, the novelty of this study was that that lncRNA-SAMMSON might be a new target for the diagnosis and treatment of GC.

Conflict of Interests

The authors declare that they have no conflict of interests.

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