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LncRNA CCHE1 in the proliferation and apoptosis of gastric cancer cells

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Abstract. – OBJECTIVE: We aimed at investigating the effects of long non-coding RNA (lncRNA) CCHE1 on proliferation and apoptosis of gastric cancer cells.

MATERIALS AND METHODS: qRT-PCR method was used to detect lncRNA CCHE1 in cancer cell lines. Cells with relative expression were selected to change the expression by transfecting with corresponding lentiviral vector, and nonsense transfection group and blank control group were also constructed; cell counting kit-8 (CCK-8) method was used to assess cell proliferation activity; colony formation assay was used to evaluate the rate of cell cloning; flow cytometry assay was used to determine the apoptosis rate and cell cycle. Western blot was utilized to detect the expressions of Bax and Bcl-2.

RESULTS: Expression of lncRNA CCHE1 in the tumor cell lines was increased ($p<0.05$). Up-regulated lncRNA CCHE1 increased proliferative activity and colony formation capacity. However, down-regulated lncRNA CCHE1 decreased proliferative activity and colony formation capacity. Bcl-2 protein expression increased, but Bax protein expression decreased in lncRNA CCHE1 upregulation group ($p<0.05$); the effect was reversed in lncRNA CCHE1 down-regulation group ($p<0.05$).

CONCLUSIONS: lncRNA CCHE1 may promote the proliferation of gastric cancer cells and inhibit cell apoptosis.

Key Words:

Gastric cancer, Long non-coding RNA, lncRNA CCHE1, Cell proliferation, Apoptosis.

Introduction

Long non-coding RNA (LncRNA) refers to a kind of RNA with the length of more than 200 nucleotides, which does not have a protein-coding function[1]. Researches showed that lncRNA was a "dark matter" or "noise" after transcription[2,3], but recent investigations have confirmed that lncRNA, although not coding protein, can affect it[4]; thus, lncRNA is involved in the regulation of cell differentiation, cell growth, apoptosis and tumor cell migration[5,6]. It plays an important role in the occurrence and development of cancer, such as the lncRNA BRAF, lncRNA (BANCR), lncRNA HOTAIR, lncRNA MALAT1, lncRNA CCAT1[7-10]. LncRNA-SNHG7 expression is increased in lung cancer, and is able to promote cell proliferation, migration and invasion. It suppresses cell apoptosis via up-regulating FAIM2 expression[11]; for gastric cancer, up-regulation of lncRNA-SNHG7 can promote cell proliferation and inhibit apoptosis via inhibiting expressions of P15 and P16[12].

We mainly discussed lncRNA CCHE1 expression in gastric cancer cell lines, as well as the effect of lncRNA CCHE1 on the proliferation and apoptosis of cells, which provide a theoretical basis for the diagnosis and treatment of gastric cancer targeted on lncRNA in clinical practice.

Materials and Methods

Materials

Human gastric cancer cell lines and normal human epithelial cells were purchased from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China). Roswell Park Memorial Institute-1640 (RPMI-1640) and fetal bovine serum (FBS) were purchased from the Gibco (Rockville, MD, USA). LncRNA CCHE1 primer sequences and internal reference GAPDH primers were purchased from Shanghai Biotech., (Shanghai, China). CCK-8 reagent Box was purchased from Japan colleagues; PE kit was purchased from BD Company (Franklin Lakes, NJ, USA); Cell cycle, apoptosis kits and BCA protein concentration test kit were purchased from Beyotime (Shanghai, China); Bax, Bcl-2 antibody (rabbit anti-human)

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were purchased from Abcam Company (Cambridge, MA, USA), β-actin internal reference (mouse anti-human) and anti-rabbit, anti-mouse secondary antibodies were purchased from Beyotime (Shanghai, China). BioRad CFX96 Touch Fluorescence Quantitative polymerase chain reaction (PCR) instrument was purchased from BioRad (Hercules, CA, USA); electrophoresis apparatus and electrophoresis tank were purchased from Brilliant Scientific Instruments (Shanghai, China); chemiluminescence imaging system was purchased from China Gene, Co., Ltd., (Shanghai, China).

Cell Culture

Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% of inactivated fetal bovine serum (FBS) and 1% penicillin was used to culture cancer cell lines. According to the color of the medium, culture medium was replaced every 2-3 d, when cell density was up to about 90%, cells were passaged for the subsequent experiments.

qRT-PCR Method

Total RNA was extracted by TRIzol method and synthesized by reverse transcription of cDNA. Reaction conditions: 2 min at 95°C for pre-denaturation, then 30 s at 95°C, 30 s at 55°C (collected fluorescence signal), 30 s at 72°C, for a total of 45 cycles. The primer sequences were: CCHE1: 5′-AAGGTCCCAGGATACTCGC-3′ (forward) and 5′-GTGTCGTGGACTGGCAAAAT-3′ (reverse). The internal reference GAPDH primer: 5’-GACTCATGACCACAAGTCCATGC-3’ (forward), 5-’AGAGGCAGGGATGATGTTCTG-3 ‘ (reverse).

Stable Cell Lines Construction

Overexpression and knockdown of lncRNA CCHE1, as well as nonsense sequence stability cell lines, were constructed through transfecting with lentiviral vectors. The relative expression of lncRNA CCHE1 was confirmed by qRT-PCR.

Cell Proliferation Assay

Cells proliferation was tested by CCK-8 method. Cells were digested by trypsinized, resuspended and adjusted up to 10^4/mL by medium. 100 μL of medium containing cells were seeded into 96-well plates to culture for 24 h. Each group contained three repeated wells, and a negative control well was set up (with only medium and CCK-8 solution, without cells). The absorbance (OD) value of each well was measured at a wavelength of 450 nm on a microplate reader.

Colony formation assay

Cells were digested using trypsin and were cultured in 6-well plates. An average of 300 cells with 10% fetal bovine serum (FBS) was seeded per well. After culturing for 2-3 weeks, the colony formation was observed. Clone formation rate was calculated using the following formula: Clone formation rate = number of clones/number of seeded cells × 100%.

Apoptosis Rate and Cell Cycle Detection

$1\times10^5 \sim 5\times10^5$ cells were washed twice with phosphate-buffered saline (PBS) (centrifuged at 1000 r/min for 5 min); 100 μL of binding buffer were added to resuspend cells and 400 μL of binding buffer containing 5 μL of PE and 5 μL of dye were added into each tube. At the same time, a blank control was set-up (without dye), single dye tube (5 μL of PE and 5 μL of dye, respectively), and the reaction was performed in the dark, at room temperature condition for 15 min. Flow cytometry was used to detect the total apoptosis expressed as a percentage.

Cell cycle test: $1\times10^5 \sim 5\times10^5$ cells were washed twice with phosphate-buffered saline (PBS) (centrifuged at 1000 r/min for 5 min). Supernatant was removed, washed with 0.5 mL of PBS, fixed with 5 mL of 70% B alcohol, and incubated at 4°C overnight. Supernatant was centrifuged for 15 min (1000 r/min), and 500 μL of buffer were added with 10 μL of RNase at 37°C water bath for 30 min. Finally, 25 μL of PI were added in the dark reaction for 30 min.

Bcl-2 and Bax Potein Expressions

Total proteins were obtained from cell lines, and the concentrations were detected by bicinchoninic acid (BCA) method. After that, they were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After transferred to the polyvinylidene fluoride (PVDF) membrane, all the proteins were incubated with antibodies. Image J software was used to detect the protein expression.

Statistical Analysis

Statistical product and service solutions (SPSS19.0, Armonk, NY, USA) statistical software were used. The two samples were compared by *t*-test. $p<0.05$ indicated the difference was statistically significant.

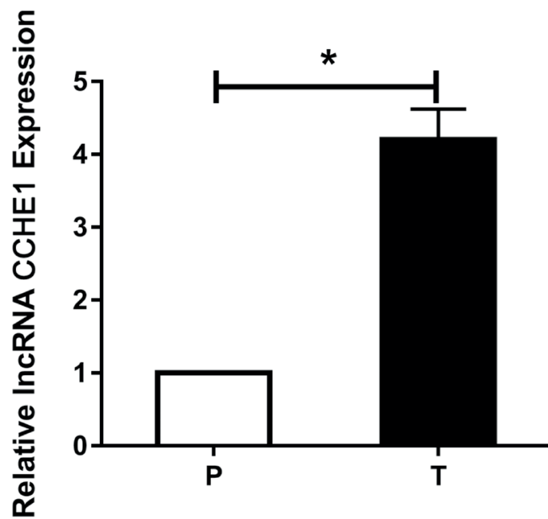

Figure 1. Relative lncRNA CCHE1 expression was detected by RT-PCR. P refers to adjacent tissues, T refers to tumor tissues. *$p<0.05$.

Results

Increased lncRNA CCHE1 was Related to the Clinical Factors of Patients with Gastric Cancer

qRT-PCR was used to examine lncRNA CCHE1 expression level in gastric cancer samples and adjacent normal tissues, and normalized to GAPDH. lncRNA CCHE1 level was significantly up-regulated in gastric cancer tissues compared with corresponding adjacent non-tumorous tissues. It had a significant correlation with clinical factors (Table I), which indicated that abnormal lncRNA CCHE1 expression may be related to gastric cancer progression (Figure 1).

LncRNA CCHE1 mRNA was Elevated in Gastric Cancer Cell Lines

The expression of LncRNA CCHE1 was elevated in gastric cancer cell lines (Figure 2A).

Table I. LncRNA-CCHE1 expression and clinical characteristics of patients with gastric cancer.

Factor		NO.	LncRNA-CCHE1 high	LncRNA-CCHE1 low	p
NO.		77	39	38	
Gender	Male	40	17	23	0.137
	Female	37	22	15	
Age (years)	< 50	35	21	14	0.134
	≥ 50	42	18	24	
Size (cm)	< 5	40	14	26	0.004
	≥ 5	37	25	12	
Lymph nodes metastasis	Negative	45	14	31	0.000
	Positive	32	25	7	
TNM stage	I-II	38	13	25	0.004
	III-IV	39	26	13	

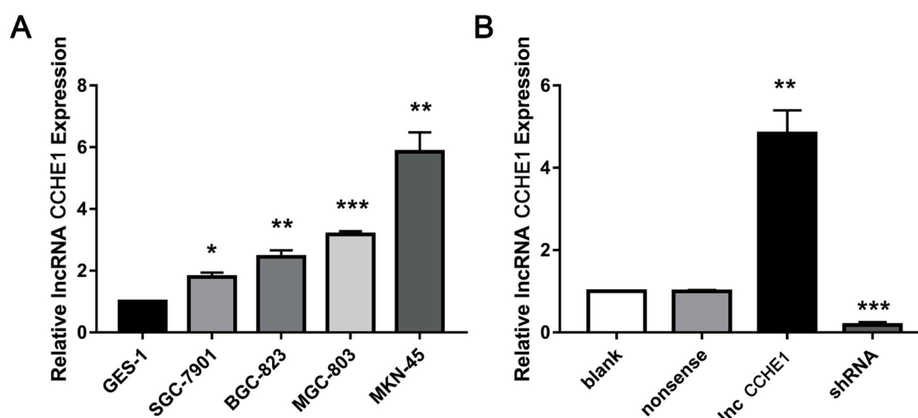

Figure 2. ***A,*** Relative lncRNA CCHE1 expression among gastric cancer cell lines (SGC-7901, BGC-823, MGC-803, MKN-45) and GES-1 were detected by RT-PCR. ***B,*** Relative lncRNA CCHE1 expression was identified by RT-PCR among blank, nonsense, lncRNA CCHE1 and shRNA groups. *$p<0.05$.

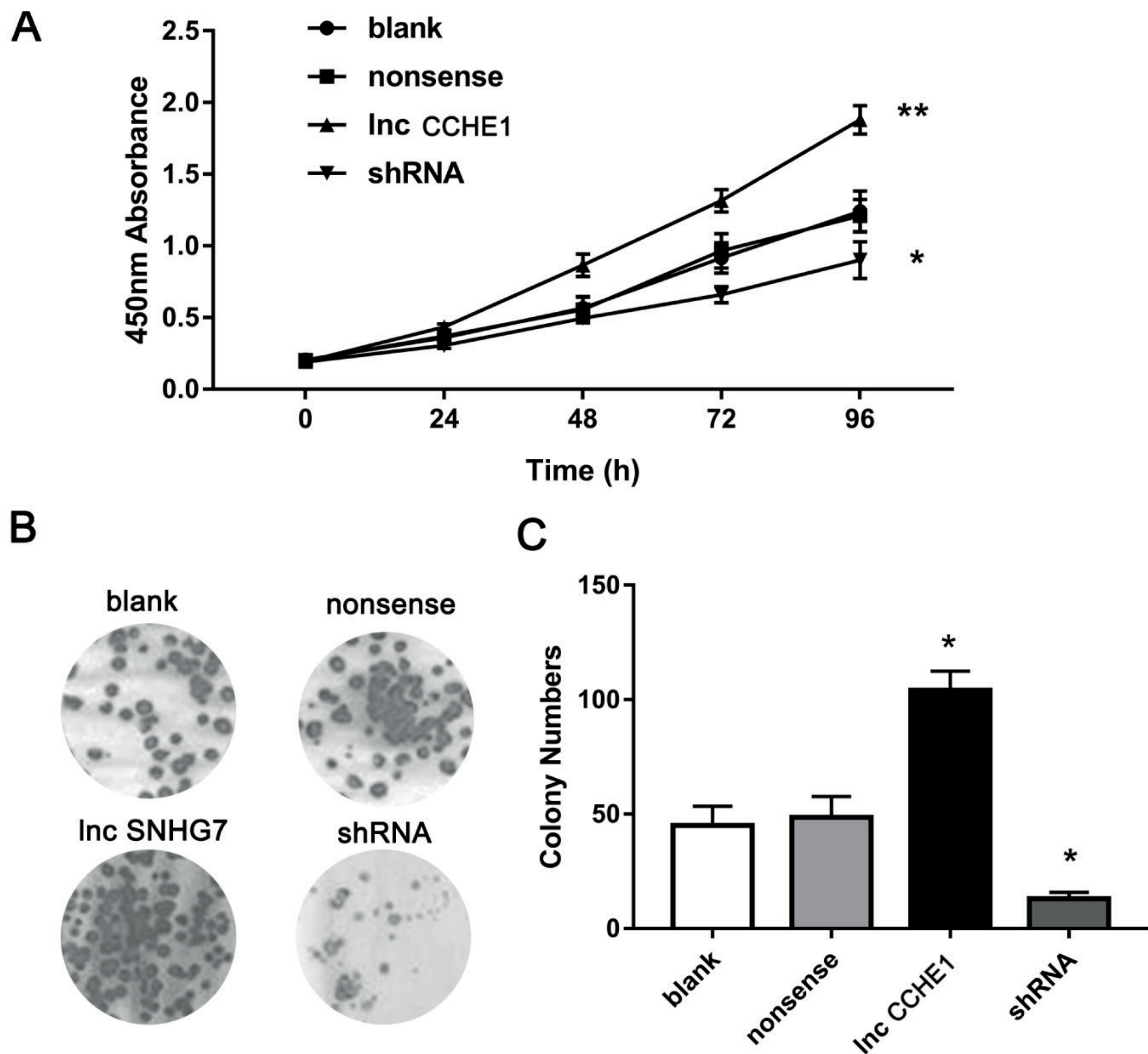

Figure 3. ***A,*** CCK-8 assay was used to detect cells proliferation among blank, nonsense, lncRNA CCHE1 and shRNA groups. *$p<0.05$ ***B-C,*** Cells clonal formation was detected among blank, nonsense, lncRNA CCHE1 and shRNA groups. *$p<0.05$.

The mRNA expression of lncRNA CCHE1 was increased in the up-regulation group, which was decreased in down regulation group ($p<0.05$) when comparing to non-sense transfected group and blank control group, respectively (Figure 2B).

LncRNA CCHE1 Regulated Cell Proliferation and Clonal Formation

Firstly, to detect cell proliferation, we performed CCK-8 method. As compared with the non-sense transfected group, the blank control group, proliferative activity increased in lncRNA CCHE1 up-regulation group, but decreased in lncRNA CCHE1 down-regulation group ($p<0.05$) (Figure 3A).

In addition, the colony formation assay showed that colony formation rate increased in lncRNA CCHE1 up-regulation group, but decreased in lncRNA CCHE1 down-regulation group compared with non-sense transfected group, blank control group, respectively ($p<0.05$) (Figure 3B-C). To sum up, the above findings indicated that lncRNA CCHE1 regulates cell proliferation and colony formation.

LncRNA CCHE1 Regulated Cells Apoptosis and Cell Cycle

To investigate cell apoptosis and cell cycle, we also conducted flow cytometry assay. The analysis results demonstrated that compared to non-sense transfected group and the blank control group, up-regulated lncRNA CCHE1 could inhibit cell apoptosis rate; reversely, down-regu-

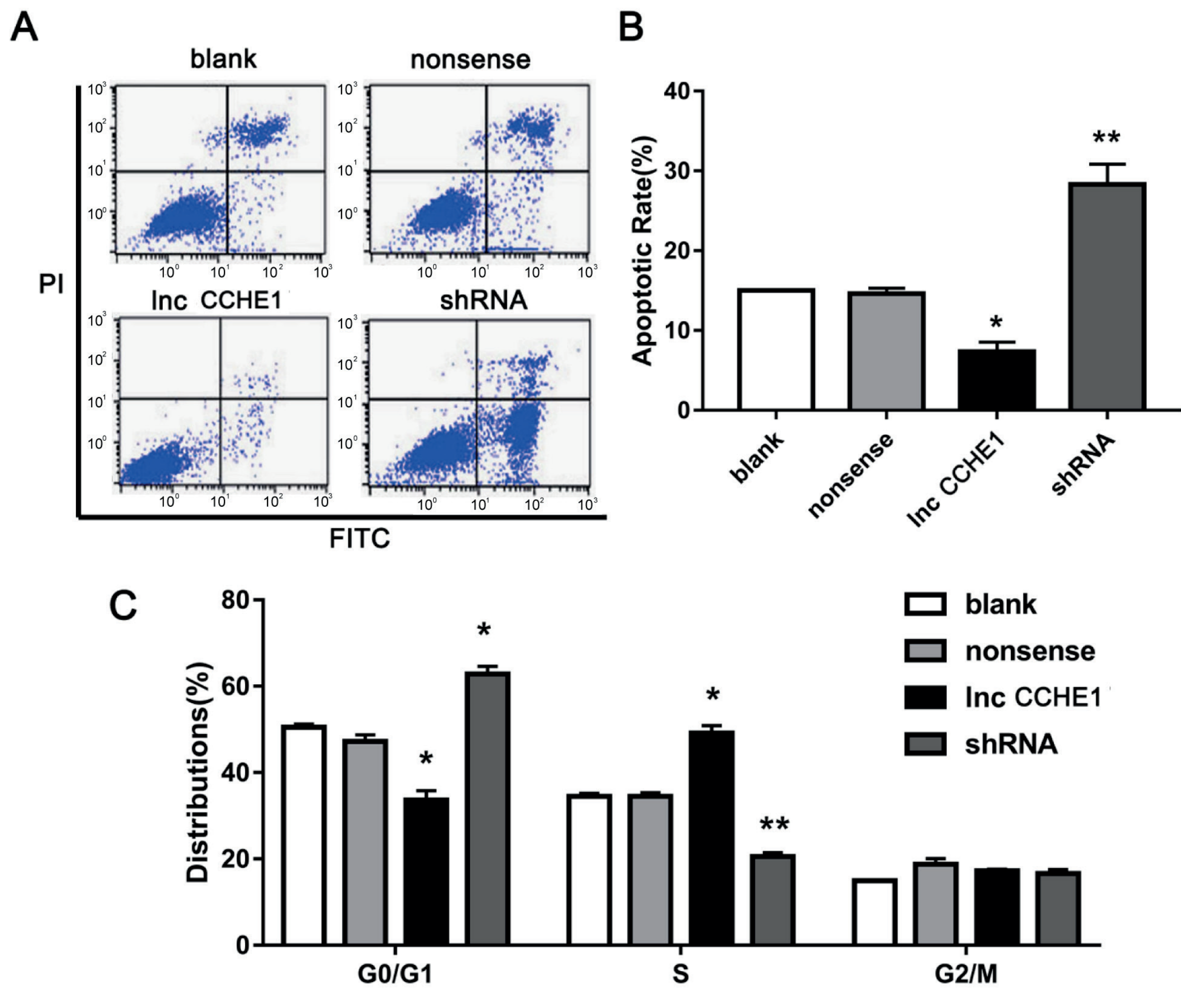

Figure 4. A-B, Cells apoptosis was detected among blank, nonsense, lncRNA CCHE1 and shRNA groups. *$p<0.05$ C, Cell cycle was detected among blank, nonsense, lncRNA CCHE1 and shRNA groups. *$p<0.05$.

lated lncRNA CCHE1 could enhance cell apoptosis rate ($p<0.05$) (Figure 4A and B). Meanwhile, compared with non-sense transfected group and the blank control group, cell cycle was promoted in lncRNA CCHE1 up-regulation group, but inhibited in lncRNA CCHE1 down-regulation group ($p<0.05$) (Figure 4C). All the data suggested that lncRNA CCHE1 could regulate cell apoptosis and cell cycle.

LncRNA CCHE1 Could Regulate Bcl -2, Bax Protein Expressions

Finally, the protein expression levels of Bcl-2 and Bax were detected by Western blot. Relative to the blank control group and non-sense transfected group, Bcl-2 protein expression was increased, and Bax protein expression was decreased in lncRNA CCHE1 up-regulation group ($p<0.05$). However, Bcl-2 protein expression was decreased, and the protein expression of Bax was increased in lncRNA CCHE1 down-regulation group ($p<0.05$) (Figure 5A and B). In conclusion, lncRNA CCHE1 could regulate Bcl-2, Bax protein expressions in gastric cancer cell lines.

Discussion

Many researches[13-15] found that lncRNAs are involved in the occurrence, development, treatment and prognosis of tumors, and they could regulate the proliferation, migration and apoptosis process of tumor cells, but other potential biological functions of lncRNAs are still not very clear and need of further investigation. In cervical cancer, high expression of lncRNA

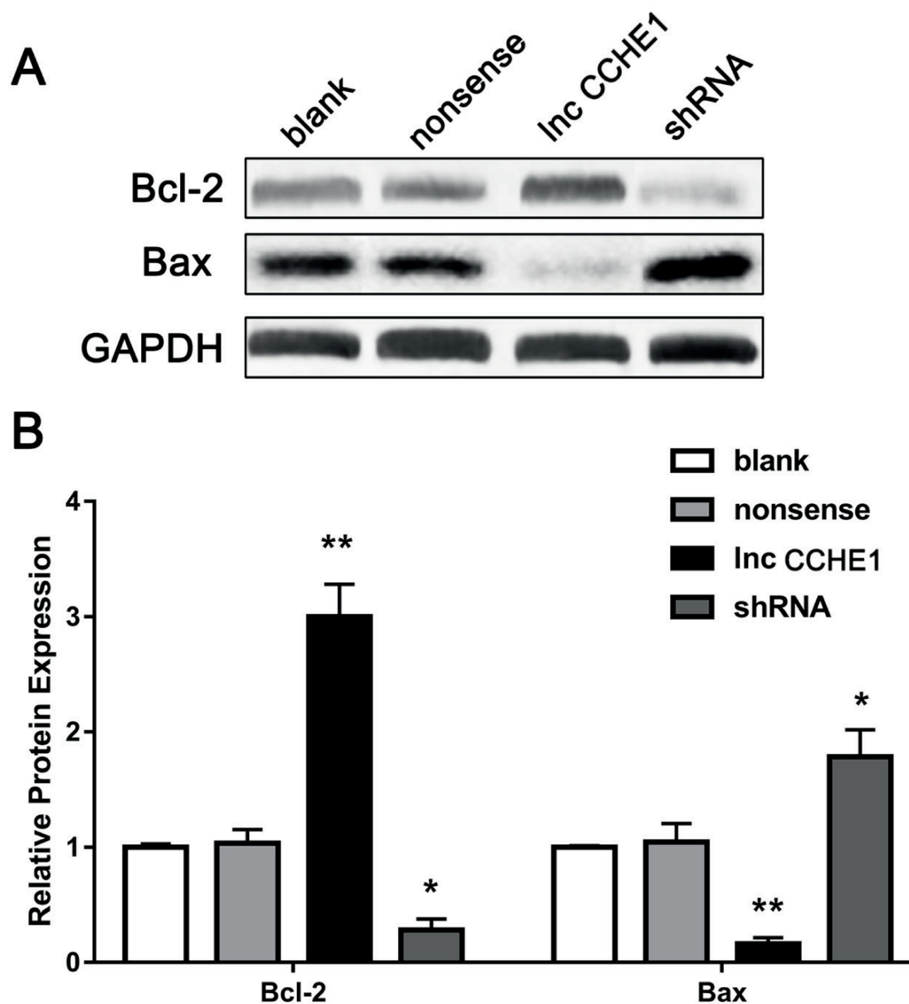

Figure 5. ***A-B,*** LncRNA CCHE1 could regulate Bcl -2, Bax protein expression by Western blot assay. *$p<0.05$.

CCHE1 might serve as a marker for predicting a poor prognosis[16]. Moreover, in hepatocellular carcinoma, lncRNA CCHE1 can also predict a poor prognosis, and activate the ERK/MAPK pathway to enhance cancer progression[17]. LncRNA CCHE1 expression level is elevated in cervical cancer; then, high expression of lncRNA CCHE1 could influence PCNA so as to promote proliferative ability[18].

We analyzed that the relative expression of LncRNA CCHE1 was higher in gastric cancer cell line. Up-regulation of lncRNA CCHE1 could promote cell proliferative ability and colony formation, inhibiting cell apoptosis; however, low-expression of lncRNA CCHE1 could inhibit cell proliferation and colony formation, whilst promoting cell apoptosis.

B lymphoma-2 gene, referred to as Bcl-2, can inhibit apoptosis. It can enhance cell damage against most of the DNA resistance. Bax is another protein related to cell apoptosis, which promotes cell apoptosis. Increased lncRNA CCHE1 expression could promote the expression of Bcl-2, and inhibit Bax expression, thereby inhibiting cell apoptosis; on the contrary, decreased lncRNA CCHE1 expression could inhibit Bcl-2 expression and promote Bax expression, thus promoting cells apoptosis.

Conclusions

We demonstrated lncRNA CCHE1 in gastric cancer cells exerted oncogene properties, so as to

provide a theoretical basis for clinical diagnosis and treatment of gastric cancer. LncRNA CCHE1 may become a molecular therapy targeted on cancer in future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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