European Review for Medical and Pharmacological Sciences 2019; 23: 9651-9659

A study of effect of lncRNA MVIH on sensitivity of gastric cancer cells to gemcitabine

H.-F. XU, D.-M. SHI, X.-Q. ZHU

Department of Pharmacy, The First Hospital of Fuyang, Hangzhou, China

Abstract. – OBJECTIVE: To study the effect of expression of long non-coding ribonucleic acid (lncRNA) associated with microvascular invasion in hepatocellular carcinoma (MVIH) on the sensitivity of gastric cancer cells to gemcitabine.

MATERIALS AND METHODS: BGC-823 cell lines were divided into control group (no treatment), low expression group (lentiviral transfection with sh-lncRNA MVIH), and high expression group (lentiviral transfection with lncRNA MVIH). The expression of lncRNA MVIH, the protein expressions of E-cadherin and Vimentin, and the differences in proliferation, migration, invasion, and apoptosis of gastric cancer cells were detected *via* Reverse Transcription-Polymerase Chain Reaction (RT-PCR), Western blotting, Cell counting kit-8 (CCK-8) assay, transwell assay, wound healing assay, methyl thiazolyl tetrazolium (MTT) assay, and flow cytometry.

RESULTS: The results of RT-PCR revealed that compared with that in control group, the mRNA expression of lncRNA MVIH was significantly decreased in low expression group and markedly increased in high expression group. Also, there were statistically significant differences ($p<0.05$). The results of Western blotting showed that compared with those in control group and low expression group, the protein expression of E-cadherin was significantly decreased, while the protein expression of Vimentin was markedly increased in high expression group ($p<0.05$). The results of transwell assay manifested that the number of invading gastric cancer cells was the largest in high expression group at 48 h ($p<0.05$), significantly larger than that in control group and low expression group ($p<0.05$), while it was the smaller in low expression group. It was found through the wound healing assay that the migration ability of gastric cancer cells was enhanced in high expression group, markedly stronger than in control group and low expression group, while it significantly declined in low expression group compared to control group. Besides, the results of CCK-8 assay showed that compared with that in control group, the sensitivity of gastric cancer cells to gemcitabine was remarkably increased in low expression group ($p<0.05$), while it significantly declined in high expression group ($p<0.05$). Finally, according to the flow cytometry, the apoptosis rate of gastric cancer cells was markedly higher in low expression group than that in control group and high expression group ($p<0.05$), indicating that the low expression of lncRNA MVIH can promote the apoptosis of gastric cancer cells.

CONCLUSIONS: Reducing the expression of lncRNA MVIH can significantly lower the migration and invasion ability of gastric cancer cells and raise the sensitivity of gastric cancer cells to gemcitabine.

Key Words:
LncRNA MVIH, Gemcitabine, Gastric cancer cells, Sensitivity.

Introduction

Food is the foundation of human existence and the unhealthy dietary environment will lead to diseases, where gastric cancer is the most common digestive system tumor[1,2]. It is known that the morbidity and mortality rates of gastric cancer are among the top worldwide[3]. China has been listed as the most severely affected area of gastric cancer, in which the morbidity rate of gastric cancer ranks second in malignant tumors. There are about 700,000 new cases and about 500,000 deaths every year, and the detection rate of early gastric cancer is very low. Therefore, most patients are diagnosed with advanced gastric cancer in the initial treatment, seriously affecting their prognosis and survival rate[4]. With the development and improvement of medical technology, the patient's lifetime has been prolonged, but there are still approximately 350,000 deaths from gastric cancer every year. The early prevention, diagnosis, and anti-tumor comprehensive treatment of patients attract much

Corresponding Author: Haofeng Xu, BM; e-mail: 1374009687@qq.com

attention[5]. Therefore, to further understand the molecular mechanism of the occurrence and development of gastric cancer, it is urgent to search for new biomarkers and therapeutic targets[6].

Gemcitabine, a kind of anticancer drug, was approved by the Food and Drug Administration, USA as early as 1996 in the first-line treatment of advanced pancreatic cancer[7]. Gemcitabine, like cytarabine, is activated by deoxycytidine kinase and metabolized by cytidine deaminase in the human body. Its main metabolites are incorporated with DNA in cells to act in the G1/S phase. Gemcitabine is also able to inhibit ribonucleotide reductase, leading to a decrease in intracellular deoxynucleoside triphosphates, which is effective for a variety of tumors according to clinical verifications[8]. In recent years, there have been several studies[9] on the sensitivity of cancer cells to gemcitabine in China, and it has been found that the objective response and survival benefit are promoted and the quality of life of patients is also improved after its sensitivity is increased.

Long non-coding ribonucleic acids (lncRNAs) play important roles in the regulation of gene expression and regulatory roles in tumor formation, proliferation, invasion, and metastasis[10]. During the invasion and metastasis of cancer cells, the epithelial phenotype is lost and the mesenchymal phenotype is obtained in cells. The expressions of Vimentin and E-cadherin have been considered as the most prominent features of the epithelial-mesenchymal transition. Recently, scholars[11] have found that the expression of lncRNA associated with microvascular invasion in hepatocellular carcinoma (MVIH) is abnormally elevated in various cancer cells and gastric cancer tissues, but its clinical significance has not been fully clarified. In this report, therefore, the gastric cancer model was established to further analyze the expression of lncRNA MVIH in gastric cancer tissues in patients, and explore its correlation with the sensitivity to gemcitabine.

Materials and Methods

Main Experimental Materials

LncRNA MVIH (GenePharma, Shanghai, China), human gastric cancer BGC-823 cell lines (YY Biotechnology, Shanghai, China), Roswell Park Memorial Institute-1640 (RPMI-1640, BIOSUN, Shanghai, China), Cell counting kit-8 (CCK-8) assay kit, and bicinchoninic acid (BCA) protein concentration assay kit (Affandi, Shanghai, China), Real Time Polymerase Chain Reaction (PCR) instrument (Beijing Zhongke Keer Instrument, Beijing, China), automatic gel imaging analyzer (Shanghai Clinx Science Instrument Co., Ltd., Shanghai, China), and 5415D centrifuge (Eppendorf, Hamburg, Germany).

Cell Culture, Transfection, and Grouping

Human gastric cancer BGC-823 cell lines purchased from the American type culture collection (ATCC, Manassas, VA, USA) were cultured in the Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) in an incubator with 5% CO_2 at 37°C. The cells were uniformly inoculated into a 24-well plate (1.2×10^6/well), cultured in 1 mL of DMEM containing 10% FBS and divided into control group, low expression group (lentiviral transfection with sh-lncRNA MVIH), and high expression group (lentiviral transfection with lncRNA MVIH). 1 μg of lncRNAs (50 pmol) were diluted with a certain amount of serum-free diluent and mixed evenly to prepare the RNA diluent at a final volume of 25 μL. Then 1.5 μL of Entranster™-R4000 was added with 24 μL of serum-free diluent and mixed evenly to prepare the Entranster™-R4000 diluent at a final volume of 25 μL. After being placed at room temperature for 5 min, Entranster™-R4000 diluent and RNA diluent were mixed evenly (shaken using a shaker or aspirated using a pipette 10 times or more) and placed at room temperature for 15 min to prepare the transfection complex. Then, 50 μL of transfection complex was added dropwise into cells in 0.45 mL of complete medium containing 10% serum and antibiotics, the medium was moved back and forth and they were mixed evenly. After transfection for 6 h, the cell state was observed. If the cells were in a good state, the medium could be retained, and the cells were cultured for another 72 h for further investigations.

Detection of MRNA Expression Via Reverse Transcription-Quantitative PCR (RT-qPCR)

The expression of lncRNA MVIH in the three groups was detected *via* RT and qPCR. The chloroform and TRIzol (Invitrogen, Carlsbad, CA, USA) were added (200 μL of chloroform/mL TRIzol) to prepare the chloroform/TRIzol reagent, shaken and mixed evenly in the 24-well plate, placed at room temperature for 15 min, transferred into the Eppendorf (EP) tube and cen-

trifuged at 12,000 rpm and 4°C for 15 min. Next, the supernatant was aspirated into another centrifuge tube, added with isopropanol (0.7-1-fold volume of the supernatant), placed at room temperature for 10-30 min and centrifuged at 12,000 rpm for 10 min. The supernatant was discarded, and RNAs were precipitated to the bottom of the tube. Then, 75% ethanol (1 mL of 75% ethanol/mL TRIzol) was added into the centrifuge tube, gently shaken to suspend the precipitates, centrifuged at 12,000 rpm and 4°C for 5 min. The supernatant was discarded as far as possible, and the precipitates were blown dry on a super clean bench for 10-20 min and dissolved using 10-50 μL of diethyl pyrocarbonate-treated (ddH_2O). The concentration was detected using OneDrop micro-spectrophotometer. Reverse transcription (RT) reaction was performed using 4.5 μL of RNase-free ddH_2O, 2 μL of 5×RT reaction buffer, 0.5 μL of random primers, 0.5 μL of Oligo dT, 0.5 μL of reverse transcriptase and 2 μL of RNAs. The complementary deoxyribonucleic acids (cDNAs) samples were divided into three pieces (diluted at 1:20), and 3 μL of cDNA was taken for PCR amplification. The amplification level of the target gene was verified *via* 5% agarose gel electrophoresis. Then, the LabWorks 4.0 image acquisition and analysis software was used for quantification and data processing. To obtain reliable data, the experiment was performed for three times in each group. In the present study, the changes in the relative expression levels of target genes were analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are shown in Table I.

Detection of Changes in Protein Expressions of E-cadherin and Vimentin Via Western Blotting

The cells were added with an appropriate amount of radioimmunoprecipitation assay (RIPA) lysis buffer and the protease inhibitor phenylmethylsulfonyl difluoride (PMSF) (RIPA: PMSF = 100:1) (Beyotime, Shanghai, China) and mixed evenly. After the cells were digested with 0.25% trypsin, they were centrifuged and the supernatant was discarded. The lysis buffer was added, followed by centrifugation at 14,000 rpm and 4°C for 30 min using the refrigerated high-speed centrifuge. Then, the protein supernatant was collected and subjected to a heating bath at 95°C for 10 min for protein denaturation. The protein samples prepared were placed in a refrigerator at -80°C for later use, and the protein was quantified using the BCA kit. After that, the dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared, and the protein samples were loaded into the gel loading well for electrophoresis under the constant pressure of 80 V for 2.5 h. Then, the protein was transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) using a semi-dry transfer method. The PVDF membrane was immersed in Tris-Buffered Saline with Tween®20 (TBST) containing 5% skim milk powder and shaken slowly for 1 h on a shaking table to be sealed. Next, the protein was incubated with the primary antibody diluted with 5% skim milk powder, rinsed with TBST for 3 times (10 min/time), incubated again with the secondary antibody at room temperature for 2 h and rinsed again with TBST twice and with TBS once (10 min/time). Finally, the protein was detected using the enhanced chemiluminescence (ECL) reagent, followed by exposure in a dark room. The relative expression of the protein was analyzed using Image-Pro Plus v6 (Media Cybernetics, Silver Spring, MD, USA). The protein expression was detected with β-actin as an internal reference.

Detection of Cell Migration Ability Via Wound Healing Assay

The cells were routinely digested with trypsin, inoculated into the 24-well plate at a density of 10×10^5 cells/mL (500 μL/well) and cultured in the DMEM containing 10% FBS for 24 h, forming the monolayer cells. Then, the monolayer cells were scratched using a 10 μL pipette tip (or sterile toothpick) in a straight line and washed with phosphate-buffered saline (PBS) for 3 times. The above operations were repeated 3 times in each

Table I. Primer sequences.

Primer	Gene	Primer sequence
LncRNA MVIH	Forward	5'-AATTTTGCACATCTGAACAGCC-3'
	Reverse	5'-TTCAAAATCCCACTACGCCCA-3'
GAPDH	Forward	5'-GTCAACGGATTTGGTCTGTATT-3'
	Reverse	5'-AGTCTTCTGGGTGGCAGTGAT-3'

sample. After incubation for 24 h, the original medium was replaced with the DMEM containing 10% FBS, followed by incubation for another 24 h. Then, the medium was aspirated, and the cells were washed with PBS for 3 times, observed and photographed under an inverted fluorescence microscope.

Detection of Cell Invasion Ability Via Transwell Assay

The transwell chamber was taken out from a sterile bench and placed in the 24-well plate. A small amount of serum-free medium was added into the chamber and wells in which the chamber was placed for moistening for 15 min. The cells were routinely digested with trypsin in the three groups, added with a pre-cooled serum-free medium at 4°C, gently blown and beaten, and collected into the 15 mL centrifuge tube, followed by centrifugation at 700 rpm for 5 min. After the supernatant was discarded, the cells were washed with an appropriate amount of serum-free medium and centrifuged. After the supernatant was discarded, the cells were resuspended with an appropriate amount of serum-free medium. After counting, a certain volume of cell suspension was resuspended with serum-free medium, and the cell density was adjusted to 1.25×10^5/mL. The transwell chamber was placed in the 24-well plate (BD, Franklin Lakes, NJ, USA) and added dropwise with 400 μL of the above cell suspension containing 5×10^4 cells. 500 μL of medium containing 10% serum was added into the wells along the gap between the side wall of the chamber and the wall of well, with no bubbles between the chamber membrane and medium. After the medium in the wells was discarded, the chamber was taken out and washed with PBS 3 times. Subsequently, PBS was quickly sucked clean to avoid making the chamber membrane too dry. Then, the chamber was placed in the well with 500 μL of fixative, and 200 μL of fixative was added into the upper chamber and fixed for about 10 min, during which the culture plate should be covered with a cap to avoid evaporation. After the fixative in the wells was discarded, the chamber was washed with PBS for 3 times, and then PBS was sucked clean. After that, the chamber was placed in the well with 500 μL of hematoxylin dye, and 200 μL of dye was added into the upper chamber for staining for 10-15 min. The chamber was transferred to a new well and washed till appropriate color, and the cells in the upper chamber that did not pass through the upper chamber membrane were wiped off with cotton swabs. Then 100 μL of PBS was added into the chamber, and the cells passing through the membrane were observed under an inverted microscope (200×). The number of invading cells in 10 fields was counted, and the average was taken. The relative number of invading cells indicated the invasion ability of tumor cells.

Detection of Cell Proliferation Via CCK-8 Assay

The cells in the logarithmic growth phase in the three groups were uniformly inoculated into a 96-well plate (1×10^4/well) and gemcitabine diluted at different concentrations was added into the wells, with 6 replicate wells in each concentration gradient. The cells were cultured in the incubator for 72 h, the original medium was discarded, and 20 μL of CCK-8 solution (Dojindo, Laboratories, Kumamoto, Japan) and 170 μL of cell culture medium were added, followed by culture in a dark place at 37°C for 2 h. Then the cells were shaken on a micro-vibrator for 3 min. Finally, the absorbance was detected at a wavelength of 450 nm using a microplate reader.

Detection of Apoptosis Via Flow Cytometry

The cells in the three groups were digested with trypsin, and the cell density was adjusted to 1.2×10^6/L. Then, the cells were uniformly inoculated into the 6-well plate (total volume of 2 mL in each well), and added with an equal volume of gemcitabine at the same concentration, making the final concentration 100 μmoL, followed by culture in a carbon dioxide incubator for 48 h. The cells were digested with trypsin, and the changes in apoptosis after gemcitabine treatment were detected using the Annexin V/propidium iodide (PI) double-staining kit. After the cells were rinsed twice with PBS at 4°C, the cells centrifuged were resuspended in 500 μL of staining buffer and added with 5 μL of Annexin V-FITC and 5 μL of PI dye, followed by staining in a dark place at 37°C for 15 min. Finally, the cells were detected using the Guava flow cytometer.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (SPSS, Chicago, IL, USA) was used for data processing. The measurement data were expressed as mean ± standard deviation. Chi-square test was performed for the

comparison among the three groups, and *t*-test for the pairwise comparison. $p<0.05$ suggested statistically significant differences.

Results

MRNA Expression of LncRNA MVIH in Gastric Cancer Cells Detected Via RT-PCR

The expression of lncRNA MVIH in BGC-823 cells was detected *via* RT-PCR. Compared with that in control group, the mRNA expression of lncRNA MVIH was significantly decreased in low expression group and significantly increased in high expression group, and there were statistically significant differences ($p<0.05$) (Figure 1).

Protein Expression Detected Via Western Blotting

The protein expression was detected *via* Western blotting in the three groups. The results showed that compared with those in control group and low expression group, the protein expression of E-cadherin was significantly decreased, while the protein expression of Vimentin was significantly increased in gastric cancer cells in high expression group ($p<0.05$). Compared with those in control group, the protein expression of E-cadherin was significantly increased, while the protein expression of Vimentin was significantly decreased in gastric cancer cells in low expression group, displaying statistically significant differences ($p<0.05$) (Figure 2).

Cell Invasion Ability Detected Via Transwell Assay

The effect of lncRNA MVIH on the invasion of gastric cancer cells was detected *via* transwell assay. It was found that the number of invading gastric cancer cells was the largest in high expression group at 48 h, markedly larger than that in control group and low expression group, while it declined significantly in low expression group compared with that in control group (Figure 3).

Cell Migration Ability Detected Via Wound Healing Assay

The effect of lncRNA MVIH on the migration of gastric cancer cells was detected *via* wound healing assay. The results revealed that the migration ability of gastric cancer cells was enhanced in high expression group at 24 h significantly stronger than that in control group and low expression group, while it markedly declined in low expression group compared with that in control group (Figure 4).

Sensitivity of Gastric Cancer Cells to Gemcitabine Detected Via CCK-8 Assay

The inhibition rate on the proliferation of gastric cancer cells was detected *via* CCK-8 assay. After treatment with gemcitabine at different concentrations for 24 h, compared with that in control group, the sensitivity of gastric cancer cells to gemcitabine was remarkably increased in low expression group, showing statistically significant differences ($p<0.05$), while it remark-

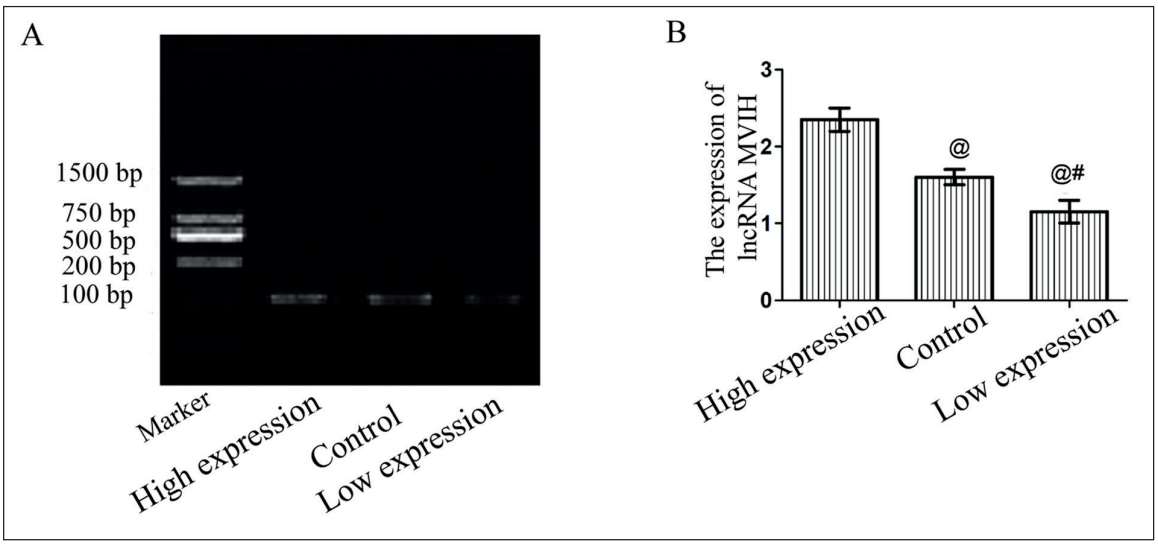

Figure 1. MRNA expression of lncRNA MVIH in gastric cancer cells in each group. **A,** Electrophoresis map of lncRNA MVIH, **B,** Quantitative detection results of lncRNA MVIH. $^{@}p<0.05$ *vs.* high expression group, $^{\#}p<0.05$ *vs.* control group.

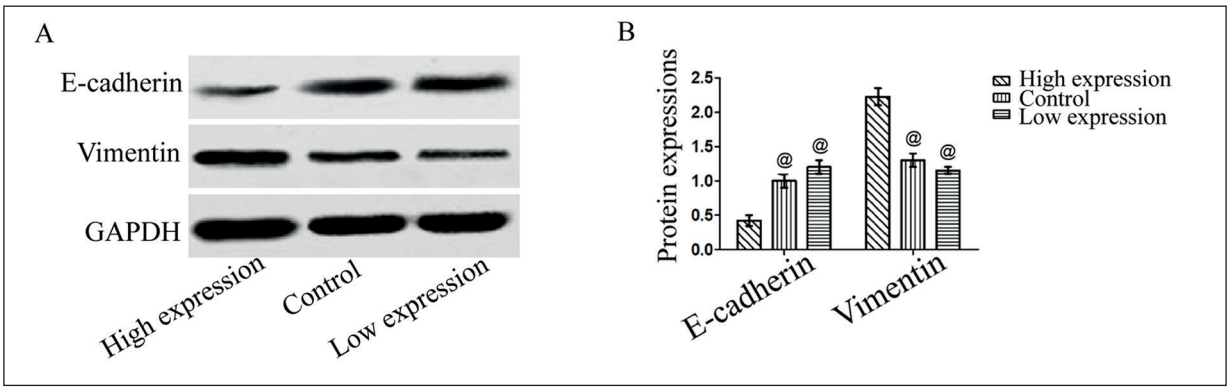

Figure 2. Protein expressions of E-cadherin and Vimentin in gastric cancer cells in each group. **A,** Protein expression detected *via* Western blotting, **B,** Quantitative protein expressions of E-cadherin and Vimentin. $^{@}p<0.05$ *vs.* high expression group.

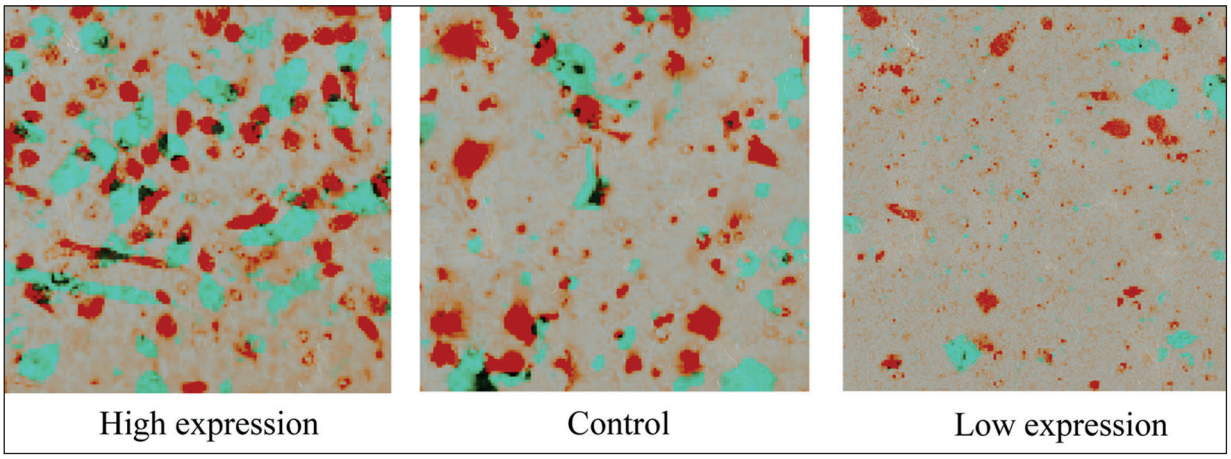

Figure 3. Cell invasion ability detected *via* transwell assay (magnification: 40×).

ably declined in high expression group, showing statistically significant differences ($p<0.05$). The above results indicate that reducing lncRNA MVIH can increase the sensitivity of BGC-823 cells to gemcitabine (Figure 5).

Effect of Gemcitabine on Apoptosis of Gastric Cancer Cells Detected Via Flow Cytometry

According to the flow cytometry, after treatment with 100 nM gemcitabine for 24 h, the

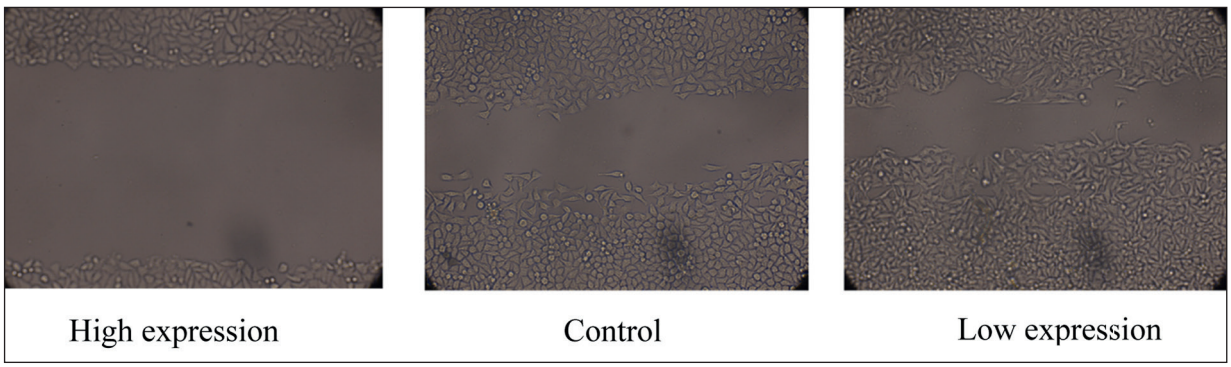

Figure 4. Cell migration ability detected *via* wound healing assay (magnification: 10×).

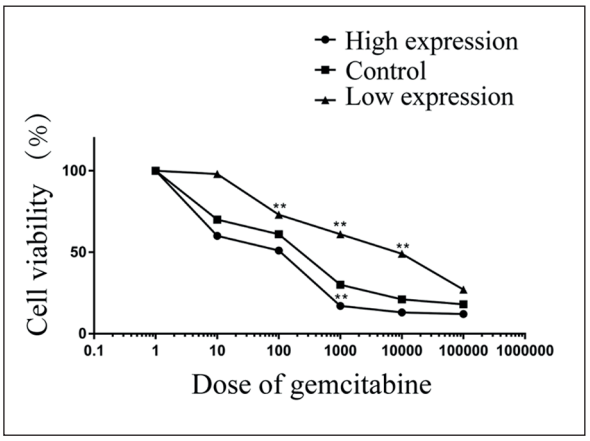

Figure 5. Sensitivity of gastric cancer cells to gemcitabine detected *via* CCK-8 assay. **$p<0.01$ *vs.* control group.

apoptosis rate of gastric cancer BGC-823 cells was significantly higher in low expression group [(37.73 ± 2.9)%] than that in control group [(21.45 ± 3.1)%] and high expression group [(27.83 ± 2.7)%] ($p<0.05$), indicating that the low expression of lncRNA MVIH can promote the apoptosis of gastric cancer cells (Figure 6).

Discussion

There are many causes of gastric cancer, and it is related to the human living environment, dietary environment, genetic inheritance, etc., but its specific pathogenesis remains unclear[12]. The patients with early gastric cancer suffer from upper abdominal discomfort and similar responses of dyspepsia, and anorexia and inappetence will further occur, and even emaciation in severe cases. These patients can be treated with operation, chemotherapy and targeted therapy[13]. Targeted therapy has become a hotspot in the research on malignant tumors in recent years, which is also a new and highly targeted anti-tumor method. With the continuous development of technology and the maturity of gene chips and high-throughput measurement technology, researchers have discovered a large number of important ncRNAs[14], which can regulate the gene expression, cell differentiation, proliferation and apoptosis, closely related to life activities[15]. Scholars[16] have found that lncRNAs can regulate such biological processes as proliferation, apoptosis, migration, and invasion of a variety of tumor cells, playing key roles in the molecular mechanism of tumorigenesis, which are new targets for cancer therapy. LncRNA MVIH was first discovered in the detection of liver cancer tissues, and then its abnormally high expression was found in various cancer cells in in-depth studies, which may intervene in the sensitivity of cancer cells to drugs[17].

The results of RT-PCR revealed that compared with that in control group, the mRNA expression of lncRNA MVIH was significantly decreased in low expression group and significantly increased in high expression group, and there were statistically significant differences ($p<0.05$). The detection results of protein expression in the three groups *via* Western blotting showed that compared with those in control group and low expression group, the protein expression of E-cadherin was significantly decreased, while the protein expression of Vimentin was significantly increased in high expression group ($p<0.05$), indicating that lncRNA MVIH can inhibit the protein expression

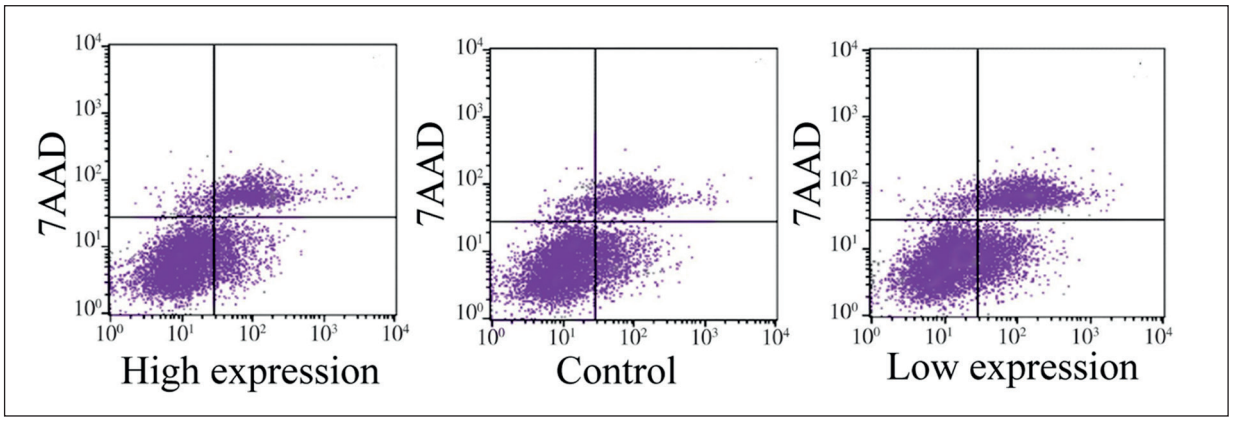

Figure 6. Effect of gemcitabine on apoptosis of gastric cancer cells detected *via* flow cytometry.

of E-cadherin and enhance the protein expression of Vimentin. Experiments have demonstrated that the high expression of MVIH can directly affect the abnormal expression of genes in cells, promote the decrease in protein expression of E-cadherin and increase in protein expression of Vimentin, and facilitate the epithelial-mesenchymal transition (EMT), ultimately accelerating the proliferation of cancer cells[18]. According to Hagiwara et al[19], lncRNA MVIH can regulate the activity of cancer biomarkers E-cadherin and Vimentin in cancer cells. In the present work, the effects of lncRNA MVIH on the migration and invasion of gastric cancer cells were detected *via* transwell assay. It was found that the number of invading gastric cancer cells was the largest in high expression group at 48 h ($p<0.05$), significantly larger than that in control group and low expression group, while it declined significantly in low expression group compared with that in control group, suggesting that the high expression of lncRNA MVIH can enhance the migration and invasion ability of gastric cancer cells. Research has found that the low expression of MVIH can inhibit the migration and invasion of tumor cells, and directly affect the cell proliferation, apoptosis, and differentiation[20]. Moreover, Okada et al[21] has shown that lncRNA MVIH can be involved in the whole self-renewal process of liver cancer cells, which is of great significance for the treatment of liver cancer, and its mechanism is related to the migration and invasion ability of liver cancer cells.

In addition, the inhibition rate on the proliferation of gastric cancer cells was detected *via* CCK-8 assay. After treatment with gemcitabine at different concentrations for 24 h, compared with that in control group, the sensitivity of gastric cancer cells to gemcitabine was remarkably increased in low expression group, showing statistically significant differences ($p<0.05$), while it remarkably declined in high expression group, also showing statistically significant differences ($p<0.05$). The above results indicate that reducing lncRNA MVIH can increase the sensitivity of BGC-823 cells to gemcitabine. The apoptosis rate of gastric cancer cells was significantly higher in low expression group than that in control group and high expression group ($p<0.05$), indicating that the low expression of lncRNA MVIH can promote the apoptosis of gastric cancer cells. Investigations have confirmed that gemcitabine can effectively inhibit the proliferation and increase the apoptosis rate of cancer cells. Gemcitabine is an anti-tumor drug, and it has efficacy in a single administration, but it is often combined with cisplatin, carboplatin, and oxaliplatin in clinical application to improve the therapeutic effect[22]. The main metabolites of gemcitabine are incorporated with DNA in cells to suppress ribonucleotide reductase and proliferation of cancer cells, ultimately improving the disease in patients. Both resistance and sensitivity of cancer cells to drugs are related to EMT in cells, during which the protein expression is lost. Teng et al showed[23] that gemcitabine can effectively promote apoptosis and reduce migration and invasion of cancer cells, while a study has found that the level of lncRNA MVIH in non-small cell lung cancer tissues is associated with resistance to gemcitabine, indicating that lncRNA MVIH can serve as a predictive marker for resistance to gemcitabine.

Conclusions

We found that lowly expressed lncRNA MVIH can activate the protein expression of E-cadherin, inhibit the protein expression of Vimentin, increase the sensitivity of gastric cancer cells to gemcitabine, suppress the proliferation and differentiation, and promote the apoptosis of gastric cancer cells, thereby improving the quality of life of patients, which provides a new target for the clinical treatment of gastric cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Chen S, Li T, Zhao Q, Xiao B, Guo J. Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer. Clin Chim Acta 2017; 466: 167-171.
2) Zou Y, Li Q, Xu Y, Yu X, Zuo Q, Huang S, Chu Y, Jiang Z, Sun L. Promotion of trophoblast invasion by lncRNA MVIH through inducing Jun-B. J Cell Mol Med 2018; 22: 1214-1223.
3) Japanese Gastric Cancer Treatment Guidelines 2014 (ver. 4). Gastric Cancer 2017; 20: 1-19.
4) Cao Q, Liu F, Ji K, Liu N, He Y, Zhang W, Wang L. MicroRNA-381 inhibits the metastasis of gastric cancer by targeting TMEM16A expression. J Exp Clin Cancer Res 2017; 36: 29.

5) Li P, Chen H, Chen S, Mo X, Li T, Xiao B, Yu R, Guo J. Circular RNA 0000096 affects cell growth and migration in gastric cancer. Br J Cancer 2017; 116: 626-633.

6) Zheng X, Song X, Shao Y, Xu B, Chen L, Zhou Q, Hu W, Zhang D, Wu C, Tao M, Zhu Y, Jiang J. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: a meta-analysis. Oncotarget 2017; 8: 57386-57398.

7) Imaoka H, Kou T, Tanaka M, Egawa S, Mizuno N, Hijioka S, Hara K, Yazumi S, Shimizu Y, Yamao K. Clinical outcome of elderly patients with unresectable pancreatic cancer treated with gemcitabine plus S-1, S-1 alone, or gemcitabine alone: subgroup analysis of a randomised phase III trial, GEST study. Eur J Cancer 2016; 54: 96-103.

8) Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, Faluyi O, O'Reilly DA, Cunningham D, Wadsley J, Darby S, Meyer T, Gillmore R, Anthoney A, Lind P, Glimelius B, Falk S, Izbicki JR, Middleton GW, Cummins S, Ross PJ, Wasan H, McDonald A, Crosby T, Ma YT, Patel K, Sherriff D, Soomal R, Borg D, Sothi S, Hammel P, Hackert T, Jackson R, Büchler MW; European Study Group for Pancreatic Cancer. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet 2017; 389: 1011-1024.

9) Ottaiano A, Capozzi M, De Divitiis C, De Stefano A, Botti G, Avallone A, Tafuto S. Gemcitabine mono-therapy versus gemcitabine plus targeted therapy in advanced pancreatic cancer: a meta-analysis of randomized phase III trials. Acta Oncol 2017; 56: 377-383.

10) Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. Oncogene 2017; 36: 5661-5667.

11) Cheng S, Wang L, Deng CH, Du SC, Han ZG. ARID1A represses hepatocellular carcinoma cell proliferation and migration through lncRNA MVIH. Biochem Biophys Res Commun 2017; 491: 178-182.

12) Pan L, Liang W, Fu M, Huang ZH, Li X, Zhang W, Zhang P, Qian H, Jiang PC, Xu WR, Zhang X. Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression. J Cancer Res Clin Oncol 2017; 143: 991-1004.

13) Hao NB, He YF, Li XQ, Wang K, Wang RL. The role of miRNA and lncRNA in gastric cancer. Oncotarget 2017; 8: 81572-81582.

14) Zou Y, Li Q, Xu Y, Yu X, Zuo Q, Huang S, Chu Y, Jiang Z, Sun L. Promotion of trophoblast invasion by lncRNA MVIH through inducing Jun-B. J Cell Mol Med 2018; 22: 1214-1223.

15) Nie FQ, Zhu Q, Xu TP, Zou YF, Xie M, Sun M, Xia R, Lu KH. Long non-coding RNA MVIH indicates a poor prognosis for non-small cell lung cancer and promotes cell proliferation and invasion. Tumour Biol 2014; 35: 7587-7594.

16) Lei B, Xu SP, Liang XS, Li YW, Zhang JF, Zhang GQ, Pang D. Long non-coding RNA MVIH is associated with poor prognosis and malignant biological behavior in breast cancer. Tumour Biol 2016; 37: 5257-5264.

17) Yuan SX, Yang F, Yang Y, Tao QF, Zhang J, Huang G, Yang Y, Wang RY, Yang S, Huo XS, Zhang L, Wang F, Sun SH, Zhou WP. Long noncoding RNA associated with microvascular invasion in hepatocellular carcinoma promotes angiogenesis and serves as a predictor for hepatocellular carcinoma patients' poor recurrence-free survival after hepatectomy. Hepatology 2012; 56: 2231-2241.

18) Han H, Valdepérez D, Jin Q, Yang B, Li Z, Wu Y, Pelaz B, Parak WJ, Ji J. Dual enzymatic reaction-assisted gemcitabine delivery systems for programmed pancreatic cancer therapy. ACS Nano 2017; 11: 1281-1291.

19) Hagiwara Y, Ohashi Y, Uesaka K, Boku N, Fukutomi A, Okamura Y, Konishi M, Matsumoto I, Kaneoka Y, Shimizu Y, Nakamori S, Sakamoto H11, Morinaga S, Kainuma O, Imai K, Sata N, Hishinuma S, Ojima H, Yamaguchi R, Hirano S, Sudo T; JASPAC 01 Study Group. Health-related quality of life of adjuvant chemotherapy with S-1 versus gemcitabine for resected pancreatic cancer: Results from a randomised phase III trial (JASPAC 01). Eur J Cancer 2018; 93: 79-88.

20) Xia G, Wang H, Song Z, Meng Q, Huang X, Huang X. Gambogic acid sensitizes gemcitabine efficacy in pancreatic cancer by reducing the expression of ribonucleotide reductase subunit-M2 (RRM2). J Exp Clin Cancer Res 2017; 36: 107.

21) Okada KI, Hirono S, Kawai M, Miyazawa M, Shimizu A, Kitahata Y, Ueno M, Hayami S, Yamaue H. Phase I study of nab-paclitaxel plus gemcitabine as neoadjuvant therapy for borderline resectable pancreatic cancer. Anticancer Res 2017; 37: 853-858.

22) Zhao H, Duan Q, Zhang Z, Li H, Wu H, Shen Q, Wang C, Yin T. Up-regulation of glycolysis promotes the stemness and EMT phenotypes in gemcitabine-resistant pancreatic cancer cells. J Cell Mol Med 2017; 21: 2055-2067.

23) Teng JP, Yang ZY, Zhu YM, Ni D, Zhu ZJ, Li XQ. Gemcitabine and cisplatin for treatment of lung cancer in vitro and vivo. Eur Rev Med Pharmacol Sci 2018; 22: 3819-3825.