IncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway

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Abstract. – OBJECTIVE: IncRNA MEG3 has been reported as a tumor suppressor gene in many different kinds of cancer, but its role in gastric cancer has not been fully understood. Then, we would like to explore its mechanism in gastric cancer.

PATIENTS AND METHODS: We first used qRT-PCR to detect the expression of IncRNA MEG3, then CCK8 and wound healing assay were used to detect the effect of IncRNA MEG3 on gastric cancer cells. Western blot assay was used to measure the expression of p53 when IncRNA MEG3 was overexpressed.

RESULTS: IncRNA MEG3 was highly expressed in the adjacent tissue, compared to the one in gastric cancer tissue. What’s more, we also found that overexpression of IncRNA MEG3 could decrease the proliferation and metastasis of gastric cancer cells. Finally, overexpression of IncRNA MEG3 could also increase the expression of p53.

CONCLUSIONS: IncRNA MEG3 could inhibit the proliferation and metastasis of gastric cancer.

Key Words: MEG3, IncRNA, Gastric cancer, Metastasis.

Introduction

According to a recent study¹, there were 951600 new gastric cancer cases and 723,100 deaths due to gastric cancer occurred in 2012. The incidence of gastric cancer is the highest in Eastern Asia, such as China, Japan and Korea. Torre et al¹ showed that the incidence of gastric cancer in China has ranked the first in the whole world. What’s more, gastric cancer has caused 498,000 deaths in China². These investigations indicated that gastric cancer has become a serious threat to the whole world. Despite great advance has been made in the clinical treatment of gastric cancer, the effects were far from satisfaction, suggesting that new therapy or target should be found in the treatment of gastric cancer.

IncRNAs are a kind of non-coding RNA which are more than 200 nt. Recent works³-⁷ have shown that IncRNA played as crucial regulators in various kinds of cancer. Zhang et al⁸ found that IncRNA Linpl was overexpressed in human triple-negative breast cancer, which could enhance repair of DNA double-strand breaks by serving as a scaffold linking Ku80 and DNA-PKcs, thereby coordinating the non homologous end joining pathway, promoting metastasis of breast cancer. Similarly, Wan et al⁹ also observed that IncRNA Jade could transcriptionally activate the expression of Jade1, a key component of human histone acetylation complex, inducing histone H4 acetylation in DNA damage response, promoting breast tumorigenesis. Peter et al¹⁰ showed that IncRNA was crucial to corresponding more balanced changes for mRNAs in urothelial cancer; they identified 32 IncRNAs with potential roles in disease progression. Especially, they found IncRNA AB074278 was upregulated and maintained to a pro-proliferative state in cancer through potential interaction with EMP1. Takahashi et al¹¹ reported that IncRNA ROR was highly expressed in malignant liver cancer cells, especially in hypoxic regions within tumor cell xenografts in vivo, and suppression of IncRNA ROR could lead to the decreased phosphorylation of p70S6K1, PDK1 and HIF-1α, suggesting that IncRNA ROR was a hypoxia-responsive IncRNA that is functionally related to hypoxia signaling in liver cancer. These studies have shown that IncRNA played various roles in the progression of different kinds of tumors, but the mechanism of IncRNA in cancer are far from being clear yet. Accumulating evidence has shown that IncRNA could play as either oncogenic or tumor suppressor gene¹². Among these tumor suppressor IncRNAs, IncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway.
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(maternally expressed gene 3) has attracted a lot of attention13. Scholars have shown that lncRNA MEG3 could regulate as tumor suppressor gene via regulating the expression of p53. Lu et al14 reported that MEG3 could regulate cell proliferation and apoptosis by activating p53 in meningioma and NSCLC. Zhu et al15 also showed that lncRNA MEG3 could inhibit the proliferation of liver cancer via regulating the expression of p53. However, investigations have found that MEG3 could act as tumor suppressor gene in gastric cancer, but the relation between lncRNA MEG3 and p53 remained largely unclear in gastric cancer. In our paper, we focused on the role of lncRNA MEG3 in gastric cancer. We showed that the expression of lncRNA MEG3 was lower in gastric cancer tissue. Importantly, the expression of lncRNA MEG3 was related to the tumor size of gastric cancer tissue. Suppression of lncRNA MEG3 could reduce the proliferation and metastasis ability of gastric cancer cells. At last, we found that lncRNA MEG3 could induce suppression in gastric cancer by regulating the expression of p53.

Patients and Methods

Patients, Specimens and Clinical Data Collection

Clinical samples (gastric cancer tissue and adjacent tissue) were collected from the General Surgery Department, the First Affiliated Hospital of Jinzhou Medical University (Jinzhou City, Liaoning Province) from Feb 2013 to Oct 2014 (n=31). None of these patients received any surgery operation, chemical or radiation treatment before. Their clinical data, such as gender, age, tumor size, tumor location, clinical stage and distant metastasis, were collected for future analysis. The specimens were divided into small pieces and stored in the liquid nitrogen for further experiments. All of the patients were well informed about the use of samples and informed consents were also signed. This study followed institutional ethical guidelines which were reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University.

Total RNA Preparation and qRT-PCR Reactions

Total RNA samples from cell and tissue were obtained by using RNAiso Plus (TaKaRa, Dalian, China) following the manufacturer’s protocol. The concentration of RNA was detected and reverse transcription polymerase chain reaction was performed using PrimeScript™ RT reagent Kit according to the manufacturer’s instructions. The levels of mRNA expression were quantified by real-time PCR with SYBR Premix Ex Taq (TaKaRa, Dalian, China).

Cell Culture

Human gastric cancer cell lines (SGC7901, BGC823, MKN45, HGC27) and one normal gastric epithelial cell line (GES) were obtained from the Institute of Biochemistry and Cell Biology at
the Chinese Academy of Sciences (Shanghai, China). SGC7901, GES and HGC27 were cultured in Dulbecco minimum essential medium (DMEM) (Hyclone, CA, America), while BGC823 and MKN45 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone, South Logan, UT, USA). The medium contained 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 2.5% penicillin and streptomycin (Beyotime Biotechnology, Beijing, China). These cells were kept in flasks at 37°C with 5% CO₂.

Overexpression of lncRNA MEG3 in Gastric Cancer Cell Lines

We got overexpression of lncRNA MEG3 plasmid from GeneChem Company (Shanghai, China). The cells were seeded at 2×10⁵ cells/well in 2 ml of medium (RPMI 1640) in 6-well plates. Two hours before transfection, cells were washed twice with phosphate buffered saline (PBS) and placed in Opti-MEM I Reduced Serum Medium (Invitrogen, Carlsbad, CA, USA). 1 µg of plasmid and 2 µl of lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added into 250 µl opti-MEM respectively. Five minutes later, diluted plasmid and lipofectamine 2000 were mixed and incubated for 30 minutes. Then, the 500 ml of plasmid-lipid complex and another 500 µl Opti-MEM were added to the each well containing cells. After 12 h of incubation, 2 µl RPMI 1640 with 10% serum was added to each well.

CCK8 Assay

Target cells were seeded into 96-well plates with at the density of 2000 cells per well. Five replicates were set for each group. Each well contained 100 ml DMEM with 10% FBS and 10 ml Cell Counting Kit 8 (Dojindo Laboratories, Kumamoto, Japan). The plate was cultured for 2 hours at 37°C. The absorbance value of each well

**Figure 2.** lncRNA MEG3 was closely related with the proliferation and metastasis ability of gastric cancer cell lines. (A) Overexpression of lncRNA MEG3 in SGC7901 and BGC823. \( p<0.01; \) (B) CCK8 assay was applied to detect the proliferation ability of SGC7901 and BGC823 cells, after lncRNA MEG3 was overexpressed. \( p<0.05; \) (C) Wound Healing assay was used to detect the effect of lncRNA MEG3 on the metastasis of gastric cancer cells.
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was measured and collected at 450 nm. The data were collected for 3 days, and the whole experiment was repeated 3 times.

Wound Healing Assay

Target cells were seeded into 6-well plate. When cells grew to 90% confluent, cells monolayers were scraped with a sterile micropipette tip. The wounded monolayers were washed with phosphate buffer solution (PBS) to remove cell debris. The distance between the two edges of the wound was calculated at three different positions. And 24 hours later, the distance between the two edges should be measured again.

Western Blot Assay

Cells were washed 3 times with phosphate buffer saline (PBS) to remove the rest cell culture media. Total cell proteins were extracted using RIPA lysis buffer containing protein inhibitor PMAF (Beyotime Biotechnology, Beijing, China). The concentration of protein was detected by standard bovine serum albumin protein quantitation assay. 50 mg of proteins were added to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). These membranes were blocked in 5% non-fat milk for 1 hour at room temperature and then were treated as antibody protocol described overnight at 4°C. Moreover, respective secondary antibody was used to incubate these membranes according to protocol. The protein bands were quantified with ECL system (Thermo Fisher Scientific, Waltham, MA, USA) and were analyzed by GraphPad Prism software.

Figure 3. IncRNA MEG3 controlled the expression of p53 in gastric cancer cell lines. (A) qRT-PCR was used to detect the change of p53 at mRNA level, \( p<0.01 \); (B) The expression of p53 was detected at protein level.

Statistical Analysis

All of the experiments were performed at least three times and all the data were presented as means ± standard deviation (SD). The difference between two groups was analyzed by the Student’s t-test, and one-way ANOVA was used to analyzed data from more than two groups. We used “Brown-Forsythe test” to validate ANOVA. \( p\)-value<0.05 was considered statistically significant. All statistical analyses were performed using the GraphPad Prism 5.

Results

IncRNA MEG3 was Low Expressed in Gastric Cancer Tissue and Cell Lines

To explore the possible role of IncRNA MEG3 in gastric cancer, we first compared the expression of IncRNA MEG3 in gastric cancer tissue with that of the adjacent tissue by using qRT-PCR. We found that the expression of IncRNA MEG3 was lower in gastric cancer tissue, while the expression of IncRNA MEG3 was higher in normal gastric epithelia, when compared with gastric cancer tissue (Figure 1 A). Then, we detected the expression of IncRNA MEG3 in four gastric cancer cell lines (SGC7901, BGC823, MKN45, HGC27) and one normal gastric cell line.

IncRNA MEG3 was Closely Related to the Proliferation and Metastasis Ability of Gastric Cancer Cell Lines

To understand the role of IncRNA MEG3 in gastric cancer, we then overexpressed IncRNA MEG3 in SGC7901 and BGC823 cells (Figure 2A). We then used the CCK8 assay to detect the ef-
Influence of lncRNA MEG3 on the proliferation ability of gastric cancer cells. We found that overexpression of lncRNA MEG3 in gastric cancer cell line (SGC7901 and BGC823) could suppress the proliferation ability of gastric cancer cells (Figure 2B). What’s more, we also found that metastasis ability of gastric cancer cells was suppressed when lncRNA MEG3 was overexpressed (Figure 2C). Taken together, we found that lncRNA MEG3 could inhibit proliferation ability of gastric cancer cells, but the mechanism remained unclear.

**lncRNA MEG3 Controlled the Expression of p53 in Gastric Cancer Cell Lines**

Studies have found that lncRNA MEG3 acted as a tumor suppressor gene in various tumors via regulating p53 signaling pathway. We wondered whether lncRNA MEG3 could regulate the expression of p53 in gastric cancer. We found that expression of p53 was increased at both mRNA and protein level (Figure 3A and Figure 3B), when lncRNA MEG3 was overexpressed, suggesting that lncRNA MEG3 could regulate the expression of p53 in gastric cancer cells.

**lncRNA MEG3 Could be Used as a Potential Target for Predicting Prognosis of Gastric Cancer Patients**

We found that the expression of lncRNA MEG3 was low in gastric cancer tissue and cell lines, and overexpression of lncRNA MEG3 could suppress the proliferation and metastasis ability of gastric cancer cells. We then tried to detect the relation between the expression of lncRNA MEG3 and clinical pathological characteristics. Interestingly, we found that the expression of lncRNA MEG3 was negatively related to the tumor size of gastric cancer (Figure 4A), but no significance was found with metastasis of gastric cancer, which might be due to the number of clinical samples (Figure 4A). In addition, we found that lncRNA MEG3 was closely related with the survival time of patients (Figure 4B), suggesting that lncRNA MEG3 may be used as a potential biomarker for gastric cancer.

**Discussion**

Gastric cancer has been for a long time the leading cause of cancer related death in the world, especially in Asian countries. Radiotherapy and chemotherapy are the most common methods of treating gastric cancer, after surgery. Despite great advances have been made in the treatment of gastric cancer, the mortality remains high in patients with advanced gastric cancer, suggesting that resistance exists under present treatments. To improve the effectiveness of treatments in gastric cancer, new therapeutic strategies and targets should be further studied. Recently scholars have shown that lncRNAs could act as a critical regulator in the development of gastric cancer. Ma et
al reported that IncRNA XIST could promote cell growth and invasion by acting as a ceRNA to sponge miR-497, which controlled its downstream target MACC1. Shao et al. showed that IncRNA RMRP could sponge miR-206 to promote carcinogenesis of gastric cancer, and it could also be used as an effective biomarker for gastric cancer. These works showed that IncRNAs may be used as potential targets and biomarkers for gastric cancer. Notably, most IncRNAs in gastric cancer act as an oncogene, according to present studies. IncRNAs act as tumor suppressor gene in gastric cancer are rarely reported. IncRNA MEG3 is one of the tumor suppressor genes in various kinds of cancers, including gastric cancer. In our paper, we firstly detected the expression of IncRNA MEG3 in 31 patients with gastric cancer, and we found that the expression of IncRNA MEG3 was much lower in gastric cancer tissue, compared with the adjacent tissue. We then also found that the expression of IncRNA MEG3 was negatively related with the tumor size of the gastric cancer and positively related with the survival time of patients. But no significance in metastasis was observed according to the clinical pathological characteristics. Our data suggested that IncRNA MEG3 could be used as a potential biomarker to predict prognosis of gastric cancer patients. However, there are some differences between clinical results and cytology experiments in our work. Our results showed that overexpression of IncRNA MEG3 could inhibit proliferation and metastasis of gastric cancer. Wang et al. found that IncRNA MEG3 could suppress migration and invasion of thyroid carcinoma. Yin et al. found that MEG3 was remarkably correlated with deep tumor invasion and advanced tumor node metastasis. However, there was no significance found in the clinical results in our paper. It might be due to the small number of clinical samples. According to present investigation and our results, we assumed that MEG3 may play as a tumor suppressor gene in gastric cancer. Accumulating evidence showed that IncRNA MEG3 could inhibit the proliferation and metastasis of tumors through different pathways. Studies showed that MEG3 could act as a ceRNA which sponges different miRNAs, such as miR-21, miR-141, miR-181 and miR-29 to regulate the malignant activity in tumors. Recent works also revealed that IncRNA MEG3 could regulate different factors to achieve suppression role in tumors. Luo et al. showed that MEG3 could reduce Bcl-2 expression and enhance Bax and caspase 3 in prostate cancer. What’s more, MEG3 could also reduce the expression of Myc at both transcriptional and translational levels in lung cancer. Among these researches, we found that p53 signaling pathway was reported to interact with IncRNA MEG3. Studies showed that MEG3 could enhance stability and transcriptional activity of p53 to influence p53 target genes in suppressing the progression of tumors. However, whether MEG3 could regulate p53 in gastric cancer remained largely unclear. Then, we mainly focused on the relation between p53 and MEG3 in gastric cancer. We observed that overexpression of MEG3 promoted the expression of p53 in gastric cancer cell lines, suggesting that MEG3 may suppress the proliferation and metastasis of gastric cancer via regulating the expression of p53.

Conclusions

We showed that expression of IncRNA MEG3 was decreased in the gastric cancer tissue. The expression of IncRNA MEG3 was negatively related with the size of the gastric cancer, and positively related to the survival time of patients. We also found that MEG3 could inhibit both the proliferation and the metastasis of gastric cancer cells in vitro. Finally, we observed that MGE3 might regulate the expression of p53 in gastric cancer.

Conflict of interest

The authors declare no conflicts of interest.

References


