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The function of long non-coding RNA MT1JP in the development and progression of gastric cancer

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ABSTRACT

Gastric cancer is one of the most common malignant tumors with the third mortality rate of cancer in world. A larger number of researches showed that genetic factors played an important role in the development of gastric cancer. More and more evidences have indicated that lncRNAs can regulate the gene expression through the transcription, post transcription and epigenetic levels including cancer invasion and metastasis, cell differentiation and apoptosis. However, the mechanisms of lncRNAs in the pathogenesis of gastric cancer remaining unclear.

LncRNA MT1JP overexpression vector was constructed and then transfected into gastric cancer cells. Further CCK-8 and transwell detection showed that lncRNA MT1JP exerted an evident inhibition role on proliferation, migration and invasion. By combining molecular cell biology experiments of the reporter gene, cell overexpression or interference and Western blot, we found that lncRNA MT1JP may regulate FBXW7 expression involved in the occurrence and development of gastric cancer. The results of this study not only explain the role of lncRNA MT1JP in the development of gastric cancer, but also provide important ideas and clues for the study of genetic regulation mechanism in gastric cancer.

1. Introduction

Gastric cancer is one of the most frequent malignant tumors in the world, especially in East Asian countries, and is also one of the leading causes of cancer death [1]. Due to the lack of effective diagnostic markers, most patients with gastric cancer missed the most appropriate time for diagnosis and treatment, which leading to advanced stage, metastasis of tumor and even terminal stage upon presentation [2].

The results of the entire human genome sequencing showed that only 1.5–2.0% of genes were protein coding gene (PCG). The remaining genes correspond to large noncoding protein regions, which include amounts of transcriptional regulatory elements and noncoding RNA (ncRNA) genes [3]. Usually, noncoding RNA cannot be translated into protein. According to the sizes of noncoding RNA, as well as the number of bases, noncoding RNAs are divided into long noncoding RNA (lncRNA) and small noncoding RNA (sncRNA) [4–6].

LncRNA is a kind of functional RNA which is longer than 200 nts, lacking in open reading frames and the ability to code for proteins [7]. Many recent studies have reported that lncRNA was closely related to human diseases, including cancers such as lung, breast, and gastric cancer [8]. Many lncRNA were expressed differentially in gastric cancer, and have been shown to be involved in both oncogenesis and tumor suppressor pathways of gastric cancer, such as cell proliferation and apoptosis [9].

However, lncRNA expression is often tissue-specific, and few lncRNAs with a consistent expression pattern in different types of cancer have been reported. Here, we investigated MT1JP, a lncRNA that appears to play a key regulatory role upstream of p53. By acting as a p53 regulator, MT1JP modulates a series of cancer hallmarks in which p53 participates, ranging from cell cycle, apoptosis, and proliferation to migration and invasion. More generally, its consistent expression pattern in numerous tumor specimens indicate that MT1JP may be a significant biomarker of diagnosis with a potential in cancer therapy.

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2. Materials and methods

2.1. Clinical specimens collection

This study included 50 pairs of primary gastric patients' cancer tissues and paired adjacent non-tumor tissue from patients who had undergone surgical resection between 2012 and 2016 at the Yantaishan Hospital, Shandong, China. All patients did not receive chemotherapy or radiotherapy before surgery. All collected tissue samples were immediately snap-frozen in liquid nitrogen and stored until required. The study was approved by the Ethics Committee of the Yantaishan Hospital, Shandong, China, and each patient participated after providing informed consent.

2.2. Cell culture

The gastric cancer cell lines (BGC-823, MGC-803 and SGC-7901) and the human gastric epithelial cell line (GES-1) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Science (Shanghai, China). All cell lines were maintained using standard and conditions (Dulbecco's Modified Eagle Medium supplemented with 10% FBS (Invitrogen, Carlsbad, CA, USA) and 100 U/mL penicillin, 100 mg/mL streptomycin (Invitrogen) in a 5% CO_2 cell culture incubator at 37 °C).

2.3. Quantitative real-time PCR

Total RNA was extracted from cells with TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) or from tissues samples with RecoverAll™ Total Nucleic Acid Isolation kit (Ambion, Foster City, CA, USA), according to the manufacturer's instructions. For qRT-PCR, RNA was reverse transcribed into cDNA using a Reverse Transcription Kit (Takara, Dalian, China). qRT-PCR analysis were performed using SYBR Premix Ex Taq (Takara, Dalian, China). The results were normalized to the expression of GAPDH. The primers for MT1JP were: forward, 5′-CTCCTGCAAGAAGAGCTGC-3′ and reverse, 5′-TGCAGCAAATGGCT CAGTA. The primers for GAPDH were: forward, 5′-AAGGTGAAGGTC GGAGTCAAC-3′ and reverse, GGGGTCATTGATGGCAACAATA-3′. All experiments were performed using the $2^{-\Delta\Delta Ct}$ method, and each experiment was performed in triplicate.

2.4. Cell transfection

siRNAs were designed by using webserver of Integrated DNA Technologies (http://www.idtdna.com). The cDNA of lncRNA *MT1JP* were synthesized by GENEWIZ and then cloned into pcDNA3.1.DNA with double enzyme digestion reactions. For transfection of siRNAs or plasmids, BGC-823, MGC-803 and SGC-7901 or GES-1 cells were cultured in 12-well or 10 cm plates overnight, and transfected with 20 μM siRNAs or plasmids using the lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

2.5. MTT assay

Cultured cells were grown in 6-, 24- and 96-well plates. Apoptosis was measured using flow cytometry. Cell viability was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) assay. MTT was performed at 24, 48, 72, 96, 120 and 144 h incubation. The absorbance at 492 nm was measured after incubation with 20 μL of MTT for 4 h. The curve of cell proliferation was then drawn and the proliferation efficiency was examined. The experiments were repeated three times independently.

2.6. Transwell cell migration and invasion assay

Migration and invasion potential of cells was measured using transwell chambers. For migration assay, 5×10^4 cells were seeded into the upper chamber of transwells (BD Bioscience). For invasion assay, 1×10^5 cells were added into the upper chamber precoated with matrigel (BD Bioscience). In both assays, cells were maintained in DMEM medium without serum in the upper chamber, and DMEM medium containing 10% FBS was added to the lower chamber as chemoattractant. After incubated for 24 h, the non-migration or non-invade cells that remained on the upper surface were removed with a cotton swab. Then the membranes were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.2% Triton X-100 at room temperature for 15 min and stained with 0.1% crystal violet for 5 min. Three random fields were counted per chamber using an inverted microscope (Olympus). The experiments were repeated three times independently.

2.7. Western blotting analysis

Extraction of Tissue Proteins Using General protein Kits (Beyotime, Haimen, China). All protein samples were adjusted to equal concentrations, followed by addition of bromophenol blue. Remove the bubbles under the board. Equal amounts of proteins were loaded on SDS/PAGE. Added 6 μL of protein marker at the same time. The protein samples were separated according to a predetermined voltage. Then the protein was transferred to nitro-cellulose membranes. Then the membrane was blocked with TBS containing 5% skim milk and 0.1% Tween-20 at room temperature, and incubated with primary antibodies against E-cadherin, N-cadherin, Fibronectin, Vimentin and GAPDH at a dilution of 1: 1000 for 24 h at room temperature, followed by incubation with secondary antibodies (anti-E-cadherin antibody, anti-N-cadherin antibody, anti-Fibronectin antibody, anti-Vimentin antibody and anti-GAPDH antibody) for 24 h at room temperature. Detection was performed using the LI − COR Odyssey Scanning Infrared Fluorescence Imaging System (LI − COR, Lincoln, NE, USA).

2.8. Statistical analysis

All statistical analyses were performed using SPSS version 17.0 software (IBM, Armonk, NY, USA). All data were presented as the mean ± SD and differences between groups were analyzed using Student's *t*-test or chi-square test analysis or Mann-Whitey analysis. Results were considered significant when P value < 0.05.

3. Results

3.1. MT1JP was down-regulated in gastric cancer

We first detected the lncRNA MT1JP expression in gastric cancer cell lines BGC-823, MGC-803 and SGC-7901 and the human gastric epithelial cell line GES-1. When compared with GES-1, lncRNA MT1JP expression was down-regulated in BGC-823, MGC-803 and SGC-7901 (Fig. 1A). We next examined the expression of lncRNA MT1JP in 50 paired gastric cancer tissues and adjacent non-tumor tissues by qRT-PCR. The results showed that lncRNA MT1JP was significant down expression in gastric cancer patients (Fig. 1B). Table 1 showed that lncRNA MT1JP expression was also correlated with cliniclpathologic characteristics including lymphatic metastasis, T stage and clinical stage. These results showed that lncRNA MT1JP might play a critical role in the progression of gastric cancer.

3.2. Up-regulated of MT1JP inhibited gastric cancer cell proliferation

We did lentivirus-mediated overexpression of lncRNA MT1JP in BGC-823 and MGC-803 cells and explored the effect of lncRNA MT1JP in BGC-823 and MGC-803 cell proliferation. Our data showed that the proliferation rate of gastric cells infected with lncRNA MT1JP overexpression was significantly decreased compared with negative control (Fig. 2).