IncRNA MALAT1 regulates the expression level of miR-21 and interferes with the biological behavior of colon cancer cells

Bin Huang*, Xiongbo Guo*, Yan Li

1Department of General Surgery, Daping Hospital, Army Medical University, Chongqing 400042, P.R. China. 2Department of Pathology, the Affiliated Hexian Memorial Hospital Southern Medical University, Guangzhou 511400, P.R. China.

*These authors contributed equally to this study.

Summary

Purpose: This study aimed to verify whether the regulation of miR-21 expression by IncRNA MALAT1 interferes with the biological behavior and mechanism of colon cancer cells.

Methods: RT-qPCR was used to detect the expression of MALAT1 in colon cancer and paracancerous tissues and different colon cancer cell lines (HT-29, SW480, SW620, CaCo-2). The relationship between MALAT1 and clinicopathological parameters of colon cancer patients and the interaction of MALAT1 and miR-21 by dual luciferase reporter gene detection were detected. Transwell invasion assay detected the invasive ability of colon cancer cells after MALAT1 inhibition and scratch assay detected the migration ability of colon cancer cells after MALAT1 inhibition. Subcutaneous tumor formation was detected in nude mice to measure the inhibition of the tumor size and volume of MALAT1 colon cancer cells.

Results: Compared with paracancerous tissues, MALAT1 expression was significantly increased in colon cancer tissues. MALAT1 expression was the highest in HT-29 colon cancer cell line. MALAT1 was specifically bound to miR-21 3' UTR. Inhibition of MALAT1 could inhibit colon cancer cell invasion and migration ability, and tumor formation in nude mice showed that the tumor volume and weight of the tumor-bearing mice were reduced after inhibiting the expression of MALAT1.

Conclusion: In conclusion, IncRNA MALAT1 plays an important role in the development of colon cancer. MALAT1 can regulate miR-21 to regulate the migration and invasion of colon cancer cells.

Key words: colon cancer, invasion behavior, MALAT1, miR-21

Introduction

Colon cancer ranks third in mortality of patients with malignant tumors worldwide. There are approximately 1.2 million new cases each year [1]. Despite effective diagnostic methods and progress of cancer treatment the patient overall survival (OS) rate is still low [2]. The incidence of colon cancer is increasing, so investigation of progression and potential pathogenesis of colon cancer is warranted.

Recently, new evidence suggests that epithelial-mesenchymal transition (EMT) is a component of colorectal cancer and the molecules that are related to EMT may become new targets of clinical prognosis and treatment [3]. EMT is a process in which cell adhesion disappears and migration and invasion of cells strengthen [4]. The transdifferentiation from resting epithelial cells to mesenchymal cells is vital for embryogenesis, fibrosis, tissue
repair, wound healing and tumor progression [5]. In pathological conditions, the transition from epithelial to mesenchymal cells occurs in the initial stage of cancer. For example, colon cancer cells in the front invasive part tend to differentiate into mesenchymal cells with a poor differentiation and a high proliferation rate, resulting in the loss of cell polarity and the intensification of migration and invasion ability [6]. At present, many studies have confirmed a number of key transcription factors to facilitate the progression of EMT, such as TWIST, ZEB1, ZEB2, SNAIL1, and SNAIL2 [7,8]. In the transition from epithelial cells to mesenchymal cells, epithelial cells must break extracellular restriction made by cell adhesion molecules such as E-cadherin and p120 to strengthen their ability for migration and invasion [9]. At the same time, the expression of the mesenchymal marker Vimentin increases, facilitating the movement and adhesion of cells [10].

Long-chain non-coding RNAs (lncRNAs) are transcripts with a length of more than 200 nt and has no coding ability. Currently lncRNAs were shown to be involved in embryogenesis and have regulatory functions in the development of malignant tumors [11]. In addition, lncRNAs can also regulate the stability of proteins [12]. Recently, MALAT1, belonging to lncRNAs, has been identified as an oncogene in colon cancer [13]. Xia et al found that MALAT1 facilitated the proliferation of gastric cancer cells [14]. Recently, MALAT1 was shown to facilitate the development of osteosarcoma by activating dependent gene transcription [15,16]. It also facilitates the proliferation and migration of chondrosarcoma cells by activating mTOR signaling pathway [17]. Moreover, the up-regulation of MALAT1 expression is directly associated with poor prognosis of patients with non-small cell lung cancer [18]. However, whether MALAT1 plays a key role in colon cancer still needs to be confirmed.

In this study, it was found that the expression level of MALAT1 was up-regulated in colon cancer tissues compared to adjacent normal tissues. The results of this study revealed the new function of MALAT1 in colon cancer and can provide new ideas for the treatment of patients with colon cancer.

**Methods**

This study was approved by the research ethics committees.

**Collection of tissues and cell culture**

Colon cancer and adjacent normal tissue samples were obtained. The tumors and normal tissue samples were frozen in liquid nitrogen and stored at -80°C to extract RNA and proteins. The cells were cultured in RPMI 1640 medium in a humid atmosphere with 5% CO₂ at 37°C for 5-7 days and the confluence reached 70%.

**Quantitative real-time polymerase chain reaction**

TaqMan MicroRNA reverse transcription kits were used to carry out reverse transcriptions and synthesize cDNA sense strands. Then, qRT-PCR reaction was performed in a Real-Time PCR system. The reaction conditions: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 32 s, a total of 50 cycles. Then the solubility curve was detected. After this, the Ct value of each sample was automatically analyzed by a computer program. The relative expression level of miRNA was calculated by 2-ΔΔCt. The experiment was repeated for three times. MALAT1 primer sequences: 5'-GTGGCTAUCTAUGTTTTAGCATA'C-3' (forward) and 5'-CCTATGCTTAGGAGCAGTGCAAAGT-3' (backward). miR-21 primer sequences: 5'-TTGAGCTATCTAAGTAGGGCAAATCG-3' (forward) and 5'-GCTATTCTAAACCUAAGGGCAGGA-3' (backward).

**Dual luciferase assay**

MALAT1 fragments containing wild-type (WT) miR-21 binding site or mutant (MT) miR-21 binding site and downstream primers of luciferase genes of dual luciferase miRNA target expression vectors were synthesized chemically. The recombinant plasmids were named as pmirGLO-miR-21-WT and pmirGLO-miR-21-MUT, respectively. The cells were cultured in 12-well plates, then Lipofectamine 2000 and recombinant reporter plasmids were used to get and lyse the cells in 24 h. The activity of the luciferase was detected by a dual luciferase assay reporting system according to the manufacturer’s instructions. The experiments were repeated for three times.

**Wound healing assay**

The tip of a sterile pipette was used to scratch monolayer cells in a standard way to construct an area without cells. The RPMI 1640 medium was aspirated and replaced with a fresh complete medium. Then, the cells were incubated at 37°C for 24 h. After 24 h, the cell migration was recorded and photographed. The migration residual gap among the migratory cells was measured in five random intervals in each experimental condition, and the percentage between the migration residual gap and the original scratch width was used for comparison. The experiments were performed in triplicate.

**Transwell invasion assay**

The cell suspension with a concentration of 1×10⁵ cells/μL was placed in a serum-free RPMI-1640 medium with a volume of 200 μL, and transferred into the upper chamber. In order to create an environment with chemotactants in the lower chamber, RPMI-1640 medium with 20% of fetal bovine serum (FBS) was added. After incubation for 24 h, the cells on the surface of the upper chamber were removed, and the cells on the surface of the bottom chamber were fixed with 4% of polyoxymethylene and then were stained with 0.1% of crystal violet.
Finally the number of invading cells was counted. The experiment was repeated for three times.

The tumor xenograft model of nude mice

The cell suspension with a concentration of $1 \times 10^{6}$ cells/μL in si-MALAT1 group and NC group was injected into the armpits of nude mice aged between 4 to 6 weeks. The volume and mass of tumor xenografts of the nude mice were measured after 8 weeks. The size of the xenografts was measured according to the following formula: volume = $1/2 \times (\text{the shortest diameter}) \times (\text{the longest diameter})$.

Statistics

Statistical analyses were carried out using SPSS 16.0 software package (SPSS, Chicago, IL, USA). One-way ANOVA was used to evaluate the differences between cell migration and invasion tests. Student’s t-test was also used. P value <0.05 was considered as statistically significant.

Results

Expression of MALAT1 in colon cancer tissues and colon cancer cell lines

The results of RT-qPCR showed that, compared to adjacent normal tissues, the expression level of MALAT1 mRNA in colon cancer tissues increased significantly (1.52±0.18 vs 1.05±0.12, p<0.05) (Figure 1). Compared to other cell lines, the expression level of MALAT1 mRNA in colon cancer cells HT-29 was significantly higher (1.25±0.15 vs 1.21±0.13 vs 0.67±0.11 vs 0.72±0.13, p<0.05) (Figure 1). The differences were statistically significant.

Relationship between MALAT1 and clinicopathological parameters of patients with colon cancer

Colon cancer tissue samples and adjacent normal tissues of 50 patients were statistically ana-

**Table 1.** Relationship between expression of MALAT1 and clinicopathological features in tissues of patients with colon cancer

<table>
<thead>
<tr>
<th>Clinicopathological data</th>
<th>Number</th>
<th>High expression of MALAT1</th>
<th>Low expression of MALAT1</th>
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<td>24</td>
<td>18</td>
<td>6</td>
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**Figure 1.** Expression of MALAT1 in colon cancer tissues and cell lines (*p<0.05).
analyzed. There was no obvious difference among the expression level of MALAT1 in patients with colon cancer and different gender and age (p>0.05; Table 1). For the patients with colon cancer, the higher the stage of colon cancer, the higher the expression level of MALAT1. The expression level of MALAT1 was higher in tissues of the patients with lymph node metastasis (p<0.05; Table 1).

Dual luciferase assay to detect the correlation between MALAT1 and miR-21

To clarify the expression of miR-21 that was related to MALAT1 in breast cancer cells, a similar binding sequence of MALAT1 and miR-21 was shown, indicating that there is a mutual regulatory relationship between MALAT1 and miR-21(Figure 2A). The results of the dual luciferase assay showed that si-MALAT1 could inhibit the activity of the luciferase of miR-21 effectively and regulate its expression level (Figure 2B).

Effect of MALAT1 on wound healing assay of colon cancer cells

The results of the wound healing assay showed that the migration distance in si-MALAT1 group was significantly lower than in the control group (0.79±0.08 vs 0.45±0.05, p<0.05) (Figure 3). The results showed that migration ability of colon cancer cells weakened after MALAT1 was inhibited.

Effect of MALAT1 on the invasive behavior of colon cancer cells

The results of the Transwell invasion as-
say showed that the number of cells that passed matrigel was 51.52±5.24 in si-MALAT1 group, significantly less than in NC group (258.54±18.12) (p<0.05) (Figure 4). The results showed that the invasive ability of colon cancer cells weakened after MALAT1 was inhibited.

**Effect of MALAT1 on the growth of transplanted tumors in nude mice**

Compared to NC group, the average volume and weight of transplanted tumors reduced in si-MALAT1 group (p<0.05) (Figure 5), indicating that inhibiting the expression of MALAT1 of colon cancer cells in vivo is also inhibited.

**Discussion**

At present, it is imperative to develop new and effective treatments of colon cancer, but before this, the potential mechanisms of development and progression of colon cancer needs to be researched deeply. The expression of MALAT1 is up-regulated in lung cancer and MALAT1 acts as a carcinogenic non-coding RNA [19,20]. In this study, it was found that MALAT1 was a key factor in the proliferation and migration of colon cancer cells with results showing that the expression of MALAT1 was up-regulated in colon cancer tissues compared to adjacent normal tissues, the overexpression of MALAT1 facilitated the proliferation and migration of cells, while its down-regulation inhibited the proliferation and migration of cells.

Accumulating evidence shows that IncRNAs play an important role in tumorigenesis and cancer progression [21]. They act as tumor suppressors or oncogenes through a variety of mechanisms, including epigenetic regulation, transcriptional regulation, and post-translation regulation [22]. It is reported that IncRNAs are involved in the regulation of the proliferation, apoptosis, migration, and invasion of tumor cells [23]. The function of IncRNAs in tumors has been researched frequently. However, due to the specific expression pattern of IncRNAs in tissues, the relationship between the dysregulation of IncRNAs and the progression of colon cancer is largely unknown. It is necessary to investigate the function of IncRNAs deeply in researches of colon cancer.

In previous studies over the molecular mechanisms of tumors, the analysis of mRNA focused on finding effects of obvious changes of mRNA on tumors [24]. The mRNA microarray analysis can find effects of regulatory genes that are ascribed on the occurrence and development of tumors. In this study, firstly, the abnormality of expression levels of miR in colon cancer tissues and normal colon tissues was tested. Then, the effects of miR on the behavior of tumors and the interaction of upstream and downstream regulatory factors were tested. Studies have reported that miR-21 could...
be abnormally expressed in different cancers, including colon cancer, rectal cancer, hepatocellular carcinoma, etc. This result indicates that miR-21 may be a potential tumor regulatory factor [25]. The results of this study indicate that miR-21 can facilitate the migration and invasion of colon cancer cells effectively, revealing that it is likely to be involved in the occurrence and development of colon cancer. Also in this study the effects of miR-21 on colon cancer cells were investigated deeply. IncRNA is primarily used as an auxiliary to guide and regulate proteins and the activation of downstream molecules. The data in this study showed an innovative model for describing IncRNA and miR.

IncRNA regulates the biological behavior of colon cancer cells by regulating miR.

In conclusion, this study demonstrated that MALAT1 was highly expressed in colon cancer cells and facilitated the proliferation and migration of cells by regulating signaling pathways. At the same time, this study indirectly proved that MALAT1 could be used as a new biomarker in colon cancer and a potential therapeutic target for the treatment of this disease.

Conflict of interests

The authors declare no conflict of interests.

References

22. Wang D, Ding L, Wang L et al. LncRNA MALAT1 en-

