Effects of long non-coding RNA URHC on proliferation, apoptosis and invasion of colorectal cancer cells

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Abstract. – OBJECTIVE: To investigate the effect of long non-coding RNA URHC on the proliferation, apoptosis and invasion of colorectal cancer cells.

PATIENTS AND METHODS: The expression of lncRNA-URHC in tissues and cells was tested by Real-time quantitative PCR. The expression of lncRNA-URHC was down-regulated by RNA interference (siRNA). The Real-time quantitative polymerase chain reaction (PCR) method was used to detect the interference efficiency. Cell counting kit-8 (CCK-8), flow cytometry, and transwell were used to detect the effect of lncRNA-URHC on the proliferation, apoptosis and invasion of colorectal cancer cells. The effect of lncRNA-URHC on epithelial-mesenchymal transition (EMT)-related markers was detected by Western blot.

RESULTS: LncRNA-URHC expression was significantly increased in colorectal cancer cells compared with normal cells, and the expression of lncRNA-URHC in colorectal cancer cells was higher than that in the normal cell. After down-regulated the expression of lncRNA-URHC, the proliferation and invasion of colorectal cancer cells were decreased, while cells apoptosis was promoted. Down-regulation of lncRNA-URHC could enhance the expression of E-cadherin and reduce the expression of N-cadherin, vimentin and snail.

CONCLUSIONS: Down-regulation of lncRNA-URHC can inhibit the progression of colorectal cancer.

Key Words: Colorectal cancer, Long non-coding RNA-URHC, Cell proliferation, Cell invasion, EMT.

Introduction

Colorectal cancer is regarded as one type of the most common malignancies. Although the traditional treatment methods, such as surgical resection, radiotherapy chemotherapy, and targeted therapy technology are improving, its 5-year survival rate is still low. Therefore, it is urgent to investigate the mechanisms of colorectal cancer cell proliferation and metastasis process, to find new molecular targets, and to improve the clinical treatment effects.

LncRNAs are noncoding RNAs greater than 200 nt in length and participate in the development of various diseases. In recent years, the researchers found that lncRNA in malignant tumors in the abnormal expression, and tumor cell proliferation, invasion, metastasis and other biological processes, are closely related. LncRNA H19 was able to serve as a competing endogenous gene to regulate EMT-related genes, so that break the regulatory association of miRNA and EMT. In the patients with colorectal cancer, upregulation of lncRNA-ATB was involved in metastasis and tumor size, and might be a potential marker of poor prognosis. High expression of lncRNA-BANCR was contributed into the patients with gastric cancer clinical characters. In gastric cancer, abnormal expression of Linc00152 is associated with cell apoptosis, cycle arrest, cell migration, invasion.

However, the current expression and function of lncRNA-URHC in colorectal cancer is not clear. This article will focus on lncRNA-URHC in cancer tissues and cells in the expression of the initial exploration of its colorectal cancer cell proliferation, apoptosis and invasion process, for clinical diagnosis and treatment to provide a strong theoretical basis.
Patients and Methods

Patients
All specimens of cancer tissue and its adjacent matching tissue were collected from our hospital in patients with colorectal cancer after surgery. Then, specimens were quickly placed into the liquid nitrogen, and immediately saved in -80°C ultra-low temperature refrigerator. This study was approved by the Ethics Committee of the First People’s Hospital of Wujiang District Suzhou. Signed written informed consents were obtained from all participants before the study.

Materials and Reagents
Colorectal cancer cells and normal cells were purchased from Shanghai Institute of Biochemistry and Cell Biology, Institute of Life Sciences (Shanghai, China). Roswell Park Memorial Institute-1640 (RPMI-1640) medium and fetal bovine serum (FBS) were purchased from Gibco Corporation (Rockville, MD, USA), RNA extraction reagent TRIzol and reverse transcription kit were purchased from TaKaRa Corporation (Dalian, China), lncRNA-URHC interferon RNA (siRNA lncRNA-URHC) and negative control si-Negative Control (si-NC) were obtained by Shanghai Gemma Co., Ltd. (Shanghai, China). Cell transfection reagent Lipofectamine 2000 and cell counting kit-8 (CCK-8) reagent were purchased from Invitrogen (Carlsbad, CA, USA), matrigel matrix and transwell chamber were purchased from BD (Franklin Lakes, NJ, USA), dimethyl sulfoxide, crystalline violet dye and phosphate-buffered saline (PBS) were purchased from HyClone Company (South Logan, UT, USA).

Methods

Real-time Quantitative PCR
The expression of lncRNA-URHC and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in tissues and cells were detected by Taqman probe method. The expression of lncRNA-URHC and GAPDH in tissues and cells were detected by TRIZol method. lncRNA-URHC upstream primer: 5'-TGTATGTGAGAGAAAGGAAG-3', downstream primer: 5'-CACTAGAGGTCTGCAAAATAGTA-3'. GAPDH upstream primer: 5'-ACCTAGAGACTGTTGATTG-3'; downstream primer: 5'-TTCAGACGGCAGTACCTG-3'.

Cell Transfection Experiments
Cells were cultured in medium (RPMI-1640) containing 10% FBS and placed in a 5% CO₂ at 37°C incubator. When the cells were in logarithmic growth phase, they were seeded in a six-well plate at a density of about 40% with 5 μL of Lipofectamine 2000 and 200 pmol siRNA lncRNA-URHC mixture. At the same time, 5 μL of Lipofectamine 2000 and si-NC were added into cells in the control group.

Cell Proliferation in CCK-8 Experiments
The transfected cells were seeded in plates (96-well) and 100 μL of 10% fetal bovine serum (FBS) medium were added to each well to ensure that the number of cells per well was about 2,000. After incubation for 24 h, 48 h and 72 h, 10 μL of CCK-8 reagent were added to each well. After incubation for 2 h, OD values were measured at 450 nm. Each group was repeated three times.

Flow Cytometry Assay
After transfection for 24 h, cells apoptosis was detected using annexin V labeling. An annexin V-APC labeled Apoptosis Detection Kit (Abcam, Cambridge, MA, USA) was purchased; next, we performed flow cytometry assay according to the protocol.

Cell Invasion Transwell Experiment
The Matrigel gel was diluted with 10% FBS RPMI-1640 medium at a ratio of 1:5 and 50 μL of diluent were spread evenly in the transwell chamber. Cells in logarithmic growth phase were digested and resuspended in 10% FBS RPMI-1640 medium and added to the transwell chamber. The number of cells in each cell was about 2 × 10⁴ cells. 700 μL of medium containing 10% FBS were cultured in a cell incubator for 24 h. The medium was discarded, washed three times with PBS, fixed with anhydrous methanol for 20 min, stained with crystal violet dye for 30 min, and placed under a microscope randomly selected five view camera count.

Statistical Analysis
SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. Comparison between groups was done using One-way ANOVA test followed by least significant difference (LDS). p < 0.05 as the difference was statistically significant.
Results

LncRNA-URHC Expression in Cancer Samples and Cells Was Significantly Increased

The expression of lncRNA-URHC in colorectal cancer tissues was higher than that in adjacent tissues ($p < 0.05$, Figure 1A). Combined with clinical information, we found that the expression of lncRNA-URHC was not related to the gender and age of the patient, but was closely related to tumor size, metastasis and staging (Table I). At the same time, we detected the expression of lncRNA-URHC in colorectal cancer cells by qRT-PCR. The results suggested that the expression of lncRNA-URHC in colorectal cancer cells was higher than that in human normal cells ($p < 0.05$, Figure 1B). These results suggest that lncRNA-URHC plays an essential role in cells proliferation and invasion of colorectal cancer.

siRNA Can Inhibit the Expression of LncRNA-URHC in Colorectal Cancer Cells

The expression of lncRNA-URHC was detected by siRNA-lncRNA-URHC, and the interference efficiency was verified by qRT-PCR. Compared with si-NC, the expression of lncRNA-URHC in the cells transfected with siRNA lncRNA-URHC was significantly decreased ($p < 0.05$, Figure 2).

Table I. LncRNA-URHC expression and clinical characteristics of patients with colorectal cancer.

<table>
<thead>
<tr>
<th>Factor</th>
<th>NO.</th>
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<th>Low</th>
<th>$p$</th>
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<td>21</td>
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<tr>
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</table>
The results showed that siRNA lncRNA-URHC could inhibit the expression of lncRNA-URHC in colorectal cancer cells.

**Down-regulation of LncRNA-URHC Expression Can Suppress the Proliferation and Promote Apoptosis**

SiRNA lncRNA-URHC and si-NC were transfected into the cells, and the proliferation ability of the cells was detected by CCK-8 proliferation assay. The results showed that the proliferation ability of the cells was not significantly different after transfection for 24 h, and the proliferation ability of the cells was significantly different after transfection at 48 h and 72 h ($p < 0.05$). The proliferation capacity decreased significantly in cells with downregulation of lncRNA-URHC (Figure 3A). Moreover, cells apoptosis was evaluated by flow cytometry assay. Above results showed that down-regulation of lncRNA-URHC expression can promote apoptosis of colorectal cancer cells (Figure 3B).

**Down-regulation of LncRNA-URHC Expression Inhibits the Invasion of Colorectal Cancer Cells**

Subsequently, in order to detect the effect of lncRNA-URHC on the invasion ability of colorectal cancer cells, transwell invasion assay presented that the number of invasive colorectal cancer cells transfected with siRNA lncRNA-URHC was significantly lower than that of transfected si-NC control group. The results showed that down-regulation of lncRNA-URHC expression could inhibit the invasion of colorectal cancer cells ($p < 0.05$) (Figure 4A and B).

**LncRNA-URHC Regulates the Expression of EMT-related Markers**

To further explore whether lncRNA-URHC had an effect on the EMT process, we examined the expression levels of E-cadherin, N-cadherin, vimentin, snail protein by Western blot. The results...
showed that down-regulation of lncRNA-URHC could enhance the expression of E-cadherin and reduce the expression of N-cadherin, vimentin and snail (Figure 5A and B). These results suggest that abnormal expression of lncRNA-URHC regulates the expression level of EMT-related markers.

**Discussion**

The incidence of colorectal cancer is seriously endangering people's life and health\(^\text{10}\). From the point of view of molecular level, it is very important to explore the occurrence and development of colorectal cancer and find out the early diagnosis and prognosis, the prognosis evaluation index and the radiotherapy target to improve the clinical diagnosis and treatment of colorectal cancer. In recent years, more and more reports confirmed that abnormal expression of lncRNAs play an important regulatory role in the development of colorectal cancer. For instance, decreased expression of lncRNA BANCR promotes cancer cells proliferation through modulating the expression of p21 gene\(^\text{11}\). MALAT1 expression was increased in colorectal cancer tissues, and might be acted as
a poor prognosis in the patients with stage II/III. Xue et al. reported that two lncRNAs (HOTAIR and lncRNA-422) were involved in the progression of human colorectal cancer via genome-wide analysis. The genetic variants of HOTAIR were participated in the risk of patients with colorectal cancer, and rs7958904 might be a predictive marker for diagnosis. LncRNA MALAT1 could enhance tumor cells growth and invasion by bound to SFPQ and releasing PTBP2 from SFPQ/PTBP2 complex.

We focused on the role of lncRNA-URHC in the proliferation, apoptosis and invasion of colorectal cancer cells. Firstly, clinical data analysis revealed that lncRNA-URHC expression in cancer tissues was higher than the adjacent normal tissues. LncRNA-URHC was also related to colorectal cancer development process, such as tumor size, metastasis and staging. All these findings indicated that lncRNA-URHC was a cancer-promoting gene. Subsequently, the results of in vitro cell experiments indicated the expression of lncRNA-URHC in colorectal cancer cells was higher than that in normal cell. The transfection specific siRNA lncRNA-URHC could reduce the expression of lncRNA-URHC in colorectal cancer cells. We further confirmed that down-regulation of lncRNA-URHC could inhibit cell proliferation and invasion by CCK-8 and transwell experiments. Through flow cytometry assay, we also discovered that down-regulation of lncRNA-URHC could promote cells apoptosis.

EMT acts as an important factor in the process of tumor metastasis. EMT-related markers include E-cadherin, N-cadherin, vimentin, snail and so on. The protein expression of genes involved in EMT process predicts the prognosis and metastasis in ovarian cancer patients. Subsequently, we detected the EMT-related markers by Western blot, and found that down-regulation of lncRNA-URHC could enhance the level of E-cadherin and reduce the levels of N-cadherin, vimentin and snail.

Conclusions

We showed that down-regulation of lncRNA-URHC can inhibit the proliferation and promote apoptosis of colorectal cancer cells. Moreover, down-regulation of lncRNA-URHC can inhibit cells invasion and EMT process. Our work provides a potential targeted therapy for the clinical treatment of colorectal cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


