

Correlation Analysis between lncRNA HOXA-AS2 of Circulating Tumor Cells and Radiotherapy Sensitivity in Colon Cancer

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Abstract: The aim of this study is to analyze the association between lncRNA HOXA-AS2 in circulating tumor cells (CTC) and the radiotherapy sensitivity of colon cancer. The researchers detected the expression of HOXA-AS2 in cancer tissues and peripheral blood CTC of colon cancer patients with radiotherapy sensitivity and radiotherapy tolerance by RT-PCR method. Subsequently, radiotherapy-tolerant colon cancer cells were cultured in vitro, and HOXA-AS2 was silenced by cell transfection, followed by radiation therapy. The apoptosis of cells after radiotherapy was observed by MTT assay. The results showed that compared with radiotherapy-tolerant patients, HOXA-AS2 was significantly down-expressed in cancer tissues and CTC of radiotherapy-sensitive patients, and the difference was statistically significant ($P < 0.05$). The radiotherapy sensitivity of colon cancer cells with silenced HOXA-AS2 was significantly improved, and the difference was also statistically significant ($P < 0.05$). This indicates that the high expression of HOXA-AS2 in CTC is negatively associated with the radiotherapy sensitivity of colon cancer cells.

Keywords: Circulating tumor cells, lncRNA HOXA-AS2, colon cancer, radiotherapy sensitivity

1. Introduction

Colorectal cancer (CRC) is a common malignancy, with its incidence on a global rise. Statistically, approximately 1.36 million individuals are diagnosed with CRC annually, accounting for about 10% of all cancer cases worldwide^[1]. Radiotherapy plays a pivotal role in the treatment of CRC, as it can reduce tumor size, alleviate pain and other symptoms, and lower the risk of recurrence. However, due to the heterogeneity of cancer cells, including variations in gene expression, metabolism, and growth rates, some cells develop tolerance to radiotherapy, thereby impeding its therapeutic efficacy^[2]. Recent advancements in understanding the mechanisms of CRC resistance, particularly studies on circulating tumor cells (CTC), have identified several biomarkers related to the radiotherapy sensitivity of colorectal cancer^[3].

CTCs, originating from primary tumors or metastatic sites, can enter and circulate within the bloodstream^[4]. Containing the complete genomic information of the primary lesion, CTCs hold significant value for disease detection^[5]. Earlier studies, through transcriptomic sequencing, have identified significant differences in the expression of long non-coding RNAs (lncRNAs) between radiotherapy-sensitive and radiotherapy-tolerant CRC patients, with a notable upregulation of HOXA-AS2 in the latter. Research has shown that HOXA-AS2 is aberrantly expressed in colorectal cancer and is closely associated with biological behaviors such as tumor cell proliferation, invasion, and metastasis^[6-8]. However, the correlation between HOXA-AS2 and radiotherapy sensitivity in CRC has not been studied.

This study initially examines the expression of HOXA-AS2 in cancer tissues and peripheral blood CTCs of radiotherapy-sensitive and radiotherapy-tolerant colon cancer patients via RT-PCR. Subsequently, through in vitro cellular models, this research aims to elucidate the impact of HOXA-AS2 on the radiotherapy sensitivity of colon cancer cells, thereby clarifying the association between HOXA-AS2 expression in CTCs and the radiotherapy sensitivity of colon cancer cells.

2. Materials and Methods

2.1 Material Acquisition and Storage

This study procured ten samples of radiotherapy-sensitive and ten samples of radiotherapy-tolerant colorectal cancer tissues from the Sixth People's Hospital of Nanning. These samples were preserved in an ultra-low temperature freezer at -80°C following ethical approval. The ethical approval number is LL2022010306.

2.2 Methods

2.2.1 Circulating Tumor Cell (CTC) Extraction

Blood samples were collected from both radiotherapy-sensitive and radiotherapy-tolerant colorectal cancer patients, with each sample containing approximately 10 mL of blood. Immunomagnetic bead technology was employed to enrich CTCs. Firstly, leukocytes were removed using specific biomarkers CD45, CD16, and CD66b. Subsequently, colorectal cancer cell biomarker A33 was used to isolate CTCs. The entire processing was completed within three hours of sample collection.

2.2.2 Isolation and Culture of Colorectal Cancer Cells

Under aseptic conditions, freshly obtained colorectal cancer tissues were dissected into small pieces of approximately 1mm^3 . These tissue pieces were then washed several times in pre-cooled, sterile PBS solution containing double antibiotics to remove blood vessels, fat, and necrotic tissues. Next, the tissues were digested with 0.25% trypsin for 30 minutes and then with 2g/mL of Type IV collagenase for four hours at 37°C . After digestion, the supernatant was discarded through centrifugation, and the precipitate was resuspended in modified Eagle's medium, adjusting the cell concentration to 5×10^6 cells/L. These cell suspensions were seeded in 100mm culture dishes at 10mL/dish. After seeding, cells were incubated at 37°C with 5% CO_2 . The medium was replaced after 24 hours and subsequently every three days. Cells were sub-cultured at a 1:2 ratio upon reaching over 80% confluence, using third-generation cells for subsequent experiments.

2.2.3 RT-PCR Detection of CTC and Cancer Cells

RNA extraction was completed using a rapid extraction kit from Beijing Solabao Technology Co., Ltd., and cDNA was synthesized using reverse transcription kits from Thermo Fisher Scientific. Quantitative detection was performed using a fluorescent quantitative PCR kit. β -actin served as an internal control, and relative expression of HOXA-AS2 was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method. Primers for HOXA-AS2 were: upstream 5'-TTCAGCCACTCCAGACACAG-3', downstream 5'-CAAGCGTTGTGGGTAGGTTT-3'.

2.2.4 Cell Transfection to Silence HOXA-AS2 Expression

Third-generation radiotherapy-tolerant colorectal cancer cells were seeded in 6-well plates, approximately 2×10^4 cells per well. These cells were divided into non-silenced and silenced groups. HOXA-AS2 silencing virus was designed and synthesized, and transfection was performed according to the Lipofectamine 3000 kit instructions. Twenty-four hours post-transfection, interference efficiency was confirmed by PCR, followed by subsequent experiments.

2.2.5 Radiation Exposure

Cells were irradiated using a Precision X RAD 320 machine with a dose of 10Gy. The machine parameters were set to 320kVp and 12.5mA.

2.2.6 MTT Assay

Cells were divided into three groups: radiotherapy-sensitive, non-silenced, and silenced. They were seeded in 96-well plates and irradiated. After the treatment duration, the culture medium was removed, and a solution containing 5 g/L MTT was added, incubated at 37°C for 4 hours. The supernatant was carefully removed, and 200 μL of dimethyl sulfoxide was added to each well, agitated for 10 minutes until complete dissolution. Absorbance at 490 nm was measured using an enzyme-linked immunosorbent assay reader.

2.3 Statistical Methods

Data were processed using Graphpad 5.0 statistical software. Quantitative data were expressed as mean \pm standard deviation ($x \pm s$), and intergroup comparisons were made using the F-test, with $P < 0.05$ indicating statistical significance.

3. Results

3.1 Expression of HOXA-AS2 in CTCs and Cancer Cells

As depicted in Figure 1A, PCR analysis confirmed a significantly higher expression of HOXA-AS2 in radiotherapy-tolerant CTCs ($P < 0.05$). Further illustrated in Figure 1B, a significant difference in the expression of HOXA-AS2 was observed between radiotherapy-sensitive and radiotherapy-tolerant colorectal cancer cells, with a notably higher expression in the latter ($P < 0.05$).

3.2 Detection of HOXA-AS2 Post Gene Silencing

As shown in Figure 1C, 24 hours post-transfection, RT-PCR assessment of HOXA-AS2 in radiotherapy-tolerant colorectal cancer cells indicated a significant reduction in expression in the silenced group compared to the non-silenced cells ($P < 0.05$).

3.3 Cell Death Assessment

Figure 1D demonstrates that, 24 hours post-radiotherapy, the MTT assay revealed the number of dead cells in the gene-silenced group was comparable to that in the radiotherapy-sensitive group. In contrast, the non-silenced group exhibited a significantly lower number of dead cells compared to the other two groups ($P > 0.05$). This suggests that the silencing of HOXA-AS2 enhances the radiotherapy sensitivity of colorectal cancer cells.

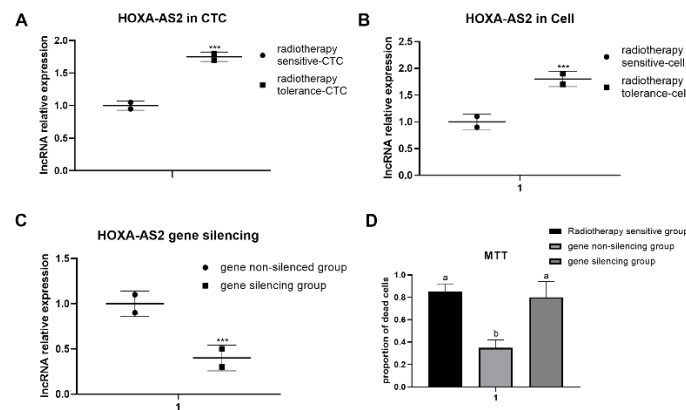


Figure 1: RT-PCR Analysis and MTT Assay.

4. Discussion

This study delves into the relationship between the long non-coding RNA (lncRNA) HOXA-AS2 in circulating tumor cells (CTCs) and the radiotherapy sensitivity of colorectal cancer. Previous research has substantiated the significant role of HOXA-AS2 in cancer [9-14], with studies like that of Biyun Lu et al. revealing its high expression correlating with poor prognosis and clinical pathological features in cancer patients [15]. In this study, researchers employed RT-PCR to precisely measure the expression levels of HOXA-AS2 in CTCs and cancer cells of patients with radiotherapy-sensitive and radiotherapy-tolerant colorectal cancer, uncovering its notably lower expression in the cancer tissues and CTCs of radiotherapy-sensitive patients. This discovery provides preliminary evidence suggesting a potential association between HOXA-AS2 and the radiotherapy response in colorectal cancer cells.

To further test this hypothesis, radiotherapy-tolerant colorectal cancer cells were cultured in vitro, and the HOXA-AS2 gene was silenced using cell transfection techniques. Subsequent radiation therapy

experiments demonstrated a marked increase in radiotherapy sensitivity in cells with silenced HOXA-AS2, further endorsing our hypothesis that high expression of HOXA-AS2 might be linked to radiotherapy resistance in colorectal cancer cells.

The MTT assay was employed to observe cell apoptosis post-radiotherapy, revealing a significant increase in the apoptosis rate of cells with silenced HOXA-AS2. This indicates that HOXA-AS2 is not only related to the radiotherapy sensitivity of colorectal cancer cells but might also affect the efficacy of radiotherapy through the regulation of apoptotic pathways.

In summary, the findings of this study suggest that high expression of HOXA-AS2 in CTCs is inversely correlated with the radiotherapy sensitivity of colorectal cancer cells. This provides new insights into therapeutic strategies, suggesting that modulating the expression of HOXA-AS2 could enhance the radiotherapy sensitivity of colorectal cancer cells, thereby improving patient outcomes. However, further research is needed to understand the mechanisms behind HOXA-AS2's role in radiotherapy resistance in colorectal cancer and its potential clinical applications.

5. Conclusions

The high expression of HOXA-AS2 in CTCs of patients with colon cancer is negatively correlated with the sensitivity of colon cancer cells to radiotherapy.

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