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ORIGINAL ARTICLE

Correlation between miR-19a inhibition and radiosensitivity in SiHa cervical cancer cells

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Summary

Purpose: *MicroRNAs (miRs) have been implicated in many aspects of tumor cell development and survival, including sensitivity to radiotherapy. In particular, miR-19a regulates the proliferation of cervical cancer cells, but its role in radiosensitivity is not known. Here, we describe the consequence of silencing miR-19a using antisense oligonucleotides (ASO) on the radiosensitivity of human cervical cancer SiHa cells.*

Methods: *Following transfection with miR-19a-ASO or control-ASO, SiHa cells were exposed to X-rays to determine their proliferation.*

Results: *Silencing of miR-19a significantly improved the sensitivity of SiHa cells to radiotherapy by reducing proliferation, increasing apoptosis, upregulating BAX, and downregulating Bcl-2.*

Conclusion: *Overall, inhibiting miR-19a significantly improves the sensitivity of SiHa cells to radiotherapy, which could lead to new methods for the treatment of cervical cancer.*

Key words: *cervical cancer, miR-19a, radiosensitivity, SiHa cells*

Introduction

Currently, cervical cancer is one of the most common gynecologic malignant tumors and the second major cause of cancer-related death in females. Cervical cancer accounts for 9% of new cancers in female patients and 8% of the total number of cancer death [1]. Radiotherapy is an important method to treat cervical cancer with good activity on the majority of patients, but a small percentage see no benefits from radiotherapy, most likely due to the low sensitivity of tumor cells to radiation [2].

miRs are single-stranded, noncoding RNAs about 22 nucleotides in length that interact with the 3'UTR region of the target gene mRNA to regulate translation [3]. In recent years, the role of miRs as potential targets for tumor treatment has become a hot spot. Research has proved that miRs can be targeted to treat tumors and some miRs can alter the sensitivity of tumor cells to radiotherapy and chemotherapy [4]. The latest relevant study has found that miR-19 expression is high in breast cancer, lymphoma, and other tumors, and miR-19 has an important significance for tumor occurrence and development [5,6]. MiR-19 can regulate the proliferation and metastasis of cervical cancer cells, but there is no research on the influence of inhibiting miR-19 on the radiosensitivity of cervical cancer [7].

This study used miR-19a antisense oligonucleotide (ASO) to inhibit the expression of miR-19 in SiHa cells and examined the influence of miR-19a on radiotherapy sensitivity of SiHa cells to provide an experimental foundation for improving the radiosensitivity of cervical cancer.

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Methods

SiHa cell culture

SiHa cells (Union Institute of Cellular Biology, Beijing, China) were cultured in the incubator with 5% CO2 at 37°C using RPM1640 medium (containing 10% FCS) (Gibco, New York, USA). The medium was replaced after 48 hrs, and SiHa cells were digested and sub-cultured after cell confluence reached 80%.

SiHa cell transfection

The sequence of the ASO fragments (2'- oxygen methylation modification) were: miR-19a-ASO 5'- UCAGUUUUGUAUGGAUUUGCACA-3'; negative control (NC) 5'-UAUCUCCGAACGUGUCACGU-3' (Nanjing GenScript Biology, Nanjing, China). The method of transfection was as follows: the cells were cultured using 6-well culture plate, and 2 μg ASO per well was transfected in accordance with the instructions of the Lipofectamine 2000 kit. The transfection experiment was divided into the normal group, negative control group (NC) group and miR-19a-ASO group.

Real-time quantitative PCR

24 and 48 hrs after ASO transfection, total RNA was extracted from the cells by the TRIzol method. 10g RNA was taken from the total RNA in each group, followed by reverse transcription in accordance with the instructions in the TaqMan MicroRNA Assays kit, and then miR-19a primer was added. PCR quantitative determination (Ambion, Texas, USA) of miR-19a expression was performed using two-step method, and U6 RNA was selected as the internal reference. PCR conditions were as follows: 95°C for 10 min, 95°C for 15 sec, 60°C for 60 sec, 40 cycles.

Sensitivity of SiHa to radiotherapy after transfection

SiHa cells were transfected with ASO, digested with trypsin after 24 hrs, and the cell suspension was prepared using DMEM medium. 104 cells per well were transferred to 96-well culture plates. After cell adherence, plates were radiated using 8 Gy adsorbed dose (6 MV X-ray, 100 cm source-plate distance, dose rate 300 cGy per min), and 20 μl MTT solution (concentration 5 μg/μl) was added at 0, 12 and 24 hrs after irradiation (Sigma, New York, USA). The supernatant was removed after incubation in the dark for 4 hrs and 150 μl DMSO was added (Sigma, New York, USA). The solution was mixed in the oscillator for 10 min, the optical density value (OD value) at 490 nm was measured, and the cell viability value was calculated.

The cell suspension prepared above was inoculated into 6-well plates at 105 cells/ well. The cells were processed according to the above method, and the supernatant was removed after 24 hrs. The cells were washed using the pre-cooled PBS for 3 times. DAPI solution (1 μg/mL) was added to each well, and cells were incubated in the incubator at 37°C for 5 min, washed using the pre-cooled PBS, and examined by fluorescence microscope.

Western blot

SiHa cells were treated with different ASOs for 24 hrs and cell lysis solution was added. The total protein was extracted according to the specification of protein extraction kit. The protein concentration was measured using BCA, followed by electrophoresis on 12% gel. Then the membrane was transferred and sealed with 5% BSA. Bcl-2, Bax, and GAPDH antibodies (Wuhan Sanying Biological Technology, Wuhan, China) were added and incubated at 4°C overnight. After washing in TBST, the membrane was incubated with horseradish peroxidase-labeled secondary antibody (Wuhan Sanying Biological Technology, Wuhan, China) for 2 hrs. The membrane was developed using ECL developer in the dark , and scanned using GAPDH as the internal reference for result analysis.

Statistics

The research data was presented as mean ± standard deviation. One-way ANOVA was used for comparison between groups and a p value ≤0.05 meant that the difference was statistically significant. For all analyses SPSS 17.0 software was used.

Results

Expression of miR-19a in SiHa cells

To examine the role of miR-9a in the radiosensitivity of cancer cells, ASO was used to knockdown the miR-9a expression. We first determined the transfection effectiveness of the miR-9a-ASO by quantifying miR-9a expression in SiHa cells. After SiHa cells were transfected with miR-9a-ASO or control ASO, it was found that miR-9a-ASO induced a significant reduction in the expression of miR-9a in SiHa at 24 and 48 hrs (Figure 1). As expected, the control group had no effect on the expression of miR-9a (Figure 1).

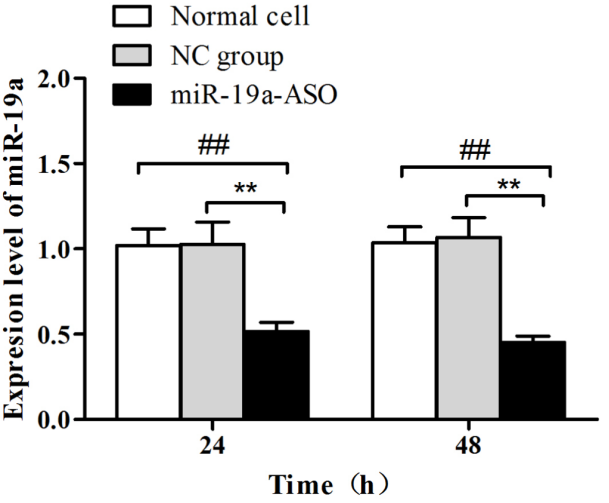

Figure 1. Expression of miR-19a in cervical cancer SiHa cells after ASO transfection. Normal cell: SiHa cells not transfected; miR-19a-ASO: SiHa cells transfected with miR-19a-ASO; NC: negative control group: SiHa cells transfected with NC-ASO. After SiHa cells were transfected with miR-9a-ASO or control ASO, it was found that miR-9a-ASO induced a significant reduction in the expression of miR-9a in SiHa cells at 24 and 48 hrs. ##p<0.05, **p<0.05.

Effect of miR-19a-ASO on SiHa cells radiosensitivity

Next, we examined the effect of miR-19a knockdown on cell proliferation using the MTT assay (Table 1). The proliferation of SiHa cells decreased over time in the NC-ASO-treated cells, showing a 25% reduction in cell number by 24 hrs. However, SiHa cells transfected with miR-19a-ASO demonstrated a significant reduction in cell number at 12 and 24 hrs compared to the NC-ASO cells, with a total decrease in cell proliferation of 49% (Table 1). These results showed that inhibition of miR-19a enhanced the sensitivity of cervical cancer SiHa cells to radiotherapy.

DAPI results showed that 24 hrs after SiHa cells were transfected with ASOs and exposed to X-ray, the nuclei of cells in the NC group showed good integrity, whereas the nuclei of the miR-19a-ASO group were condensed or broken with intense blue fluorescence (Figure 2). Thus, inhibiting miR-19 promoted the apoptosis of irradiated SiHa cells.

Table 1. Cell viability after ASO-treated SiHa cells were exposed to X-ray

Time (h)	*Survival rate (% non-irradiated cells)*	
	NC- ASO	*miR-19a-ASO*
0	99.35 ± 4.03	99.26 ± 3.33
12	85.72 ± 3.69	73.85 ± 3.01**
24	74.20 ± 4.81	51.04 ± 2.97**

**p<0.01

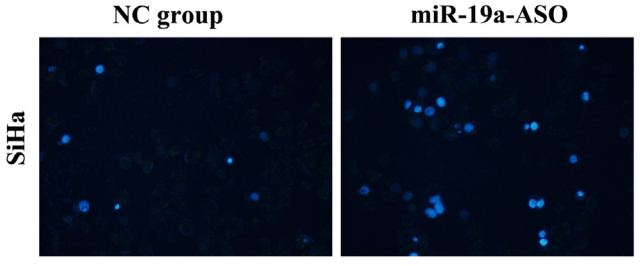

Figure 2. Expression of miR-19a in cervical cancer SiHa cells using real-time quantitative PCR. NC group: negative control group; miR-19a-ASO: miR-19a-ASO transfection group. 24 hrs after SiHa cells were transfected with ASO and exposed to x-rays, the nuclei of cells in the NC group showed good integrity, whereas the nuclei of the miR-19a-ASO group were condensed or broken with intense blue fluorescence.

Effect of miR-19a-ASO on BAX and Bcl-2 expression

Next, we examined the expression of cell death regulators BAX and Bcl-2 in transfected SiHa cells. MiR-19a silencing induced upregulation of the cell death factor BAX and downregulation of the cell death inhibitor Bcl-2 (Figure 3). Hence, inhibiting miR-19a had direct effects on the cell death regulatory machinery.

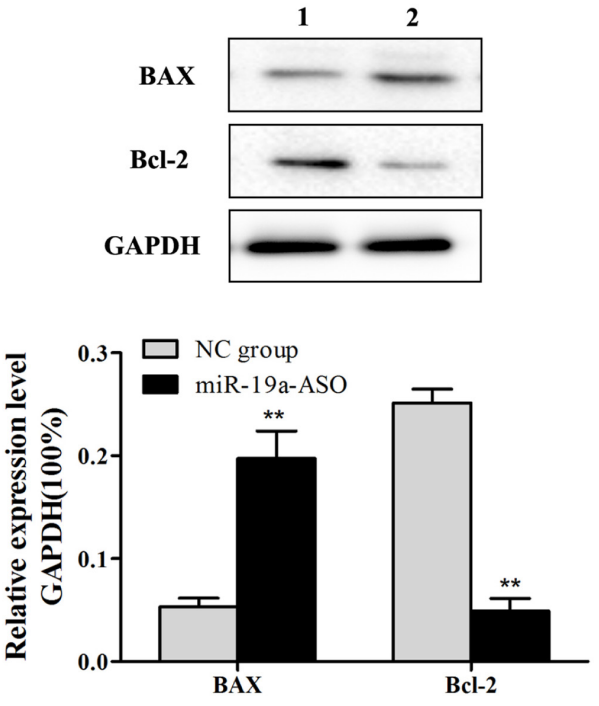

Figure 3. BAX and Bcl-2 protein expression in SiHa cells after inhibiting miR-19a. NC group: negative control group; miR-19a-ASO: miR-19a-ASO transfection group. 1: Negative control (NC) group; 2: NC group: negative control group; miR-19a-ASO: miR-19a-ASO transfection group: miR-19a silencing induced upregulation of the cell death factor BAX and downregulation of the cell death inhibitor Bcl-2. **p<0.05.

Discussion

Cervical cancer is one of the most prevalent malignant tumors in females. The morbidity of cervical cancer has increased year by year among women in China, seriously threatening the life of women. Abnormal expression of miRs in malignant cells often leads to abnormal expression of other genes, which are closely related to resistance to chemotherapy and sensitivity to radiotherapy. Therefore, research on miRs has a profound significance for the prediction of resistance to chemotherapy and sensitivity to radiotherapy of tumor cells [8-10].

MiRs have a variety of functions, such as regulating the cell DNA damage, regulating cell apoptosis, adjusting cell cycle, and changing the microenvironment of tumor cells [11]. Experiments have proved that miRs expression and function have a close relationship with malignant tumors [12-14] and the application of miRs has good potential in tumor diagnosis. Among other critical functions, studies on the expression of miRs changes under ionizing radiation have shown that miRs are involved in determining the radiosensitivity of tumor cells [15,16].

Clinical research on cervical cancer has found abnormal expression of miR-21, miR-218, miR-9, miR-200a, and miR-203 in tumor cells, and such expression changes are closely related to the increase in radiosensitivity of cervical cancer cells [17]. Chinese authors have found that miR-218 can improve the sensitivity of cervical cancer to radiotherapy, significantly increasing the ratio of cervical cancer cell apoptosis [18].

The miR-17-92 gene cluster can be processed into miR-19a. Specific ASO can inhibit the expression of miR-19 in esophageal cancer cells and induce esophageal cancer cell apoptosis [19]. The same gene cluster can edit a variety of miRs, among which miR-19 is one of the key carcinogenic miRs [20]. Here, we described that inhibiting miR-19a using miR-19a-ASO enhances the sensitivity of SiHa cells to radiotherapy, increases the expression of pro-apoptotic protein BAX, and decreases the expression of anti-apoptotic protein Bcl-2. Thus, miR-19 regulates the sensitivity of SiHa cells to radiotherapy, providing a new method to improve the radiosensitivity of cervical cancer.

Conflict of interests

The authors declare no conflict of interests.

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