MiRNA-139 regulates oral cancer Tca8113 cells apoptosis through Akt signaling pathway

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Abstract: Oral cancer threatens people’s life and health seriously. Traditional treatment (surgery, radiotherapy, chemotherapy and traditional Chinese medicine treatment) is lack of pertinence that affects curative effect and prognosis. Therefore, it is necessary to explore the specific targets for oral cancer treatment. MiRNA-139 was transfected into oral cancer Tca8113 cells. Methyl thiazolyl tetrazolium (MTT) was applied to test cell proliferation. Flow cytometry was used to detect oral cancer Tca8113 cells apoptosis. miR-139 significantly inhibits oral cancer Tca8113 cells proliferation and induces cell apoptosis. SH-5 obviously weakened the cell apoptosis caused by miR-139. miR-139 could induce Tca8113 cell apoptosis through Akt signaling pathway. It may develop a more effective method for oral cancer treatment by this target.

Keywords: miR-139, Akt signaling pathway, Tca8113, apoptosis

Introduction

Oral cancer seriously threatens people’s life and health [1]. Various malignant tumors occurred in the oral cavity are collectively referred to oral cancer. It is thought that oral cancer is one of the most common malignant tumors in head and neck [2]. Almost all of the oral cancers are squamous cell carcinoma, which means mutation happened in the oral mucosa [3]. Clinically, the oral cancer could be classified as maxillary sinus carcinoma, lip cancer, salivary gland carcinoma, gingival cancer, jaw bone cancer, tongue cancer, oropharyngeal cancer, palate carcinoma, oral floor carcinoma, and carcinoma of skin mucous membrane [4].

Early stage oral cancer does not appear the neck lymphatic metastasis and invasion. Radiotherapy and surgery both have certain curative effect for the early detected oral cancer, but often together with side effects [5]. While the effect of radiotherapy and surgery is limited for local advanced patients with strong systemic side effects [6]. In short, traditional treatment (surgery, radiotherapy, chemotherapy and traditional Chinese medicine therapy) is lack of pertinence that affects curative effect and prognosis. It is necessary to explore the specific targets for oral cancer treatment.

MicroRNAs is a type of small non-coding RNA with 18-26 nucleotide in length that has an important regulation role [7]. Further studies suggested that multiple miRNAs are closely related to the occurrence and development of many kinds of cancers, and these miRNAs play roles in tumor development and metastasis [8-10]. There are numerous miRNAs, mainly including miRNA-139, miR-143, miR-145, miR-125b, miR-15a and miR-16-1 [11-13]. In vitro and in vivo studies suggested that the miRNA related to cancer can be divided into promoting cancer miRNAs and inhibiting cancer miRNAs [14-18]. It has been confirmed that miRNA-139 can activate Akt signaling pathway and regulate cell proliferation [19-21]. This study aims to investigate the role of miRNA-139 in regulating oral cancer cell proliferation and Akt signaling pathway activation.

MiRNA-139 is one of the newly discovered miRNAs [22], and there is still lack of research about the relationship between miRNA-139 and
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![Graph](image)

**Figure 1.** MiR-139 significantly inhibited oral cancer Tca8113 cells proliferation.

It is only found that [22] clinically miRNA-139 is associated with liver cancer patients' occurrence risk and prognosis. However, it is still unclear whether miRNA-139 participates in oral cancer occurrence, development, and prognosis. Our study tried to investigate its role in oral cancer cell line.

Oral cancer cell line Tca8113 is widely used for basic research [1-4, 22-25]. At present, it is still lack of investigation about the role and its mechanism of miRNA-139 in oral cancer cells. Some studies [14-18] implied that miRNA-139 may associate with tumor growth, but still lack of experimental evidence. The purpose of this study is to explore the effect of miRNA-139 on oral cancer Tca8113 cells proliferation and apoptosis. This study will provide information about miRNA-139 regulating role in oral cancer cells.

**Materials and methods**

**Main reagents**

DMEM medium (Gibco); Akt activity kit (Gibco); Trypsin (DIFCO); Ethylenediamine tetraacetic acid (EDTA, domestic); poly-L-lysine (Sigma); Hanks balanced salt solution (Sigma); PBS solution; DMSO (Sigma); penicillin and streptomycin (domestic); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma); miRNA-139 was supplied by Genepharma (Shanghai, China); the other reagents were from Sigma.

**Cell culture and transfection**

Oral cancer cell line Tca8113 was purchased from ATCC cell bank in the United States. The cells were unfreezed in 37°C water bath and centrifuged at 800 rpm for 8 min. After removing the supernatant, the cells were resuspended in DMEM and maintained in the incubator with 37°C and 5% CO₂ [1-4].

Cell transfection was in accordance with the previous reports [1-4]. The cells were seeded one day before transfection. Transfection started when the cell density reached 95%. After transfection for 24 h, the medium was changed and the cells continue culture for 36 h.

**MTT assay**

MTT assay was applied to evaluate cell survival [2]. 100 μl medium was added to each hole in the 24-well plate, and 10-25 μl MTT dissolved in the PBS (0.1 M, pH 7.2) was added into the holes. After cultured for 4 h, remove the MTT solution and add DMSO. The absorbance at 570 nm was read on microplate reader. The experiments were performed in five duplicate wells.

**Flow cytometry for ΔΨm depolarization detection**

1 × 10⁵ cells in each tube were added with 100 nM TMRE and incubated at 37°C for 20 min under dark shaking. After inserting 250 μl PBS, the cells were tested by flow cytometry.

**Flow cytometry for cell PS expose detection**

1 × 10⁵ Tca8113 cells in each tube were added with 250 μl 1 x annexin V buffer and 5 μl FITC labeled annexin V and incubated at room temperature for 30 min under dark shaking. Then the cells were tested by flow cytometry.

**Caspase-3 activity detection**

1 × 10⁵ Tca8113 cells were tested for caspase-3 activity according to the manufacture's specification.

**Akt activity detection**

1 × 10⁵ Tca8113 cells were tested for Akt activity according to the manufacture's specification.

**Statistical analysis**

Numerical data were presented as means and standard deviation. Levene’s test was applied
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Results

MiR-139 inhibits Tca8113 cells proliferation

To study the effect of miRNA-139 on Tca8113 cells, miRNA-139 was transfected to Tca8113 cells. MTT assay was applied to test miRNA-139 effect on Tca8113 cell proliferation.

As shown in Figure 1, compared with control, miR-139 significantly inhibited oral cancer Tca8113 cells proliferation ($P<0.05$). It indicated that miRNA-139 can obviously impede Tca8113 growth.

MiRNA-139 induce Tca8113 cell apoptosis

To study the effect of miRNA-139 on Tca8113 cell apoptosis, miRNA-139 was transfected to Tca8113 cells. Flow cytometry was applied to test miRNA-139 effect on Tca8113 cell apoptosis.

As shown in Figures 2-4, compared with control, miRNA-139 markedly decreased mitochondrial membrane potential, induced phosphatidylinerse eversion, and activated caspase-3 ($P<0.05$). It suggested that miRNA-139 may induce Tca8113 cell apoptosis.
MiRNA-139 can induce Akt activation in multiple cells. To further investigate the mechanism of miRNA-139 inducing Tca8113 cell apoptosis, we used Akt signaling pathway inhibitor SH-5 to pretreat cells. After transfected with miRNA-139, the cell apoptosis was tested by flow cytometry.

As shown in Figure 5, miRNA-139 induced Akt activation in Tca8113 cells, which indicated that miRNA-139 can activate Akt signaling pathway.

To study the role of Akt signaling pathway in miRNA-139 induced oral cancer apoptosis, we used SH-5 to pretreat Tca8113 cells. After transfected with miRNA-139, caspase-3 activation was tested by flow cytometry.

SH-5 significantly inhibited Tca8113 cell apoptosis induced by miRNA-139 (Figure 6). It suggested that miRNA-139 induced Akt signaling pathway dependent Tca8113 cell apoptosis.

Discussion

Oral cancer seriously threatens people's life and health [1]. However, there is still lack of report...
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Our work studied the influence of miRNA-139 on the oral cancer Tca8113 cells. In our study, miRNA-139 significantly inhibited Tca8113 cells proliferation and induced cell apoptosis. To study the effect of miRNA-139 on Tca8113 cells, we tried different doses (1 μM, 5 μM, 10 μM, and 20 μM) and different time (12, 18, 24, 30, and 36 hours) of transfection on Tca8113 cell growth and apoptosis. We observed that miRNA-139 decreased mitochondrial membrane potential, induced phosphatidylserine eversion, and activated caspase-3 in dose dependent in oral cancer Tca8113 cells. Transfection for 36 hours showed the most obvious effect on cell apoptosis, while longer than 36 hours treatment did not exhibit further apoptosis. It implied that miRNA-139 might be degraded by intracellular nuclease. During 18-36 h transfection, cell apoptosis degree increased following the longer duration of transfection, which is consistent with the previous study [3, 12, 16]. Limited to the expenditure and condition, we only performed one time for dose and time dependent experiment, which result in no statistical analysis. Thus, we showed the most significant result here, as 20 μM miRNA-139 transfected for 36 h.

The difference of our results from previous studies was the time and dose of transfected miRNA [3, 12, 16], this may be caused by the difference of tumor sensitivity to apoptosis. Another reason might be the difference of experimental conditions. Our results suggested that different cell state (change or not change fresh medium before transfection) may also affect cell sensitivity to miRNA-139. Changing fresh medium before transfection let Tca8113 cells has strong sensitivity to miRNA-139, while not changing fresh medium before transfection made cells sensitivity to miRNA-139 decreased. It suggested that cells in rich nutrition are sensitive to extrinsic stimuli.

There are two classic signaling pathways on cell apoptosis: the external death receptor mediated signaling pathway and internal mitochondrial mediated cell apoptosis pathway. We also tested the signaling pathway during miRNA-139 induced Tca8113 cells apoptosis. We did not detect caspase-8 activation, which suggested that the miRNA-139 induced Tca8113 apoptosis is not through receptor mediated death. We analyzed the caspase 3 activity and found that miRNA-139 induced caspase-3 activation. Since caspase-3 activation is a strong evidence for apoptosis, we confirmed that miRNA-139 can induce oral cancer Tca8113 cells apoptosis. In addition, phosphatidylserine eversion appears in the late stage of apoptosis, we speculated that miRNA-139 is a kind of strong apoptosis inducer.
Akt signaling pathway plays an important role in cell apoptosis, we also tested Akt activation in the process of miRNA-139 induced Tca8113 cells apoptosis. The results showed that Akt signaling pathway inhibitor SH-5 significantly impeded Tca8113 cells apoptosis induced by miRNA-139. This is in agreement with previous results [1-4], as miRNA-139 can promote Akt activity and inhibit cell viability. A variety of miRNAs are closely related to many kinds of cancer occurrence and development. MiR-143, miR-145, miR-125b, miR-15a and miR-16-1 [11-13] were found can inhibit tumor cell apoptosis [14-18], which is not consistent with our result. It might be caused by the different cell lines and miRNAs, and this may also explain the difference of tumor sensitivity to drugs. At the same time, our results also suggested that the miRNA-139 may be a potential target for cancer treatment.

Our study also has some drawbacks, as the exact mechanism of miRNA-139 influence on Akt activity still needs further research. Moreover, different types and different stages of oral cancers should be collected to detect Akt activity and cell apoptosis state. It would provide meaningful information for miRNA-139 potential clinical value.

In conclusion, miRNA-139 induced Akt signaling pathway dependent cell apoptosis in oral cancer Tca8113 cells.

Disclosure of conflict of interest

None.

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References

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