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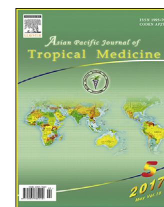

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MiR-200a and miR-200b target PTEN to regulate the endometrial cancer cell growth in vitro

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ABSTRACT

Objective: To study whether miR-200a and miR-200b target PTEN gene expression to regulate the endometrial cancer cell growth in vitro.

Methods: Endometrial cancer cells ECC-1 were cultured and transfected with the miR-200a and miR-200b mimics and inhibitors as well as the negative control mimics and inhibitors, and then the cell proliferation activity as well as the expression of PTEN and downstream genes in cells was determined; after transfection of miR-200a and miR-200b mimics as well as PTEN-3′UTR luciferase report gene plasmids, the fluorescence activity of luciferase reporter gene was determined.

Results: 12 h, 24 h and 48 h after transfection, the cell proliferation activity of miR-200a mimics group and miR-200b mimics group were significantly higher than those of NC mimics group while the cell proliferation activity of miR-200a inhibitor group and miR-200b inhibitor group were significantly lower than those of NC inhibitor group; 48 h after transfection, PTEN expression in cells and PTEN-3′UTR luciferase reporter gene fluorescence activity of miR-200a mimics group and miR-200b mimics group were significantly lower than those of NC mimics group while p-PI3K and p-Akt expression were significantly higher than those of NC mimics group; PTEN expression in cells and PTEN-3′UTR luciferase reporter gene fluorescence activity of miR-200a inhibitor group and miR-200b inhibitor group were significantly higher than those of NC inhibitor group while p-PI3K and p-Akt expression were significantly lower than those of NC inhibitor group.

Conclusion: miR-200a and miR-200b can promote the endometrial cancer cell growth in vitro by targeted inhibition of PTEN gene expression.

1. Introduction

Endometrial carcinoma is one of the common gynecological malignant tumors, invasive cancer cell growth is the most outstanding malignant biological behavior of endometrial carcinoma, but the mechanism of uncontrolled endometrial carcinoma cell growth has not been fully elucidated. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a newly discovered tumor suppressor gene in recent years, and it inhibits the cancer cell growth significantly [1,2]. In breast cancer [3], ovarian cancer [4], endometrial carcinoma [5] and other malignant tumor tissues, tumor suppressor gene PTEN is lowly expressed and will weaken the effect on inhibiting the growth of cells. The regulatory mechanism of PTEN gene expression is very complex, and microRNA (miR) can inhibit gene expression by acting on the gene mRNA 3′UTR. MiR-200a and miR-200b are two types of miR that have the characteristics of the proto-oncogene and are highly expressive in endometrial cancer [6]. However, it has not been reported about the effects of miR-200a and miR-200b on the proliferation of

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endometrial cancer cells and the expression of PTEN in cells. In the following studies, it was analyzed whether miR-200a and miR-200b targeted PTEN gene expression to regulate the endometrial cancer cell growth in vitro.

2. Materials and methods

2.1. Experimental materials

Endometrial cancer cells ECC-1 were bought in cell bank of Shanghai Gefan Biotechnology, fetal bovine serum, DEME medium and trypsin were purchased from Gibco company, miR-200a and miR-200b mimics and inhibitor as well as the negative control mimics and inhibitors were synthesized by Shanghai GenePharma Biotechnology Company, and the luciferase report gene pGL3-PTEN-3′UTR plasmids were synthesized by Shanghai Generay Biotech co., LTD; PTEN inhibitor peroxovanadate (PIC) was from Sigma Company; the MTS cell viability detection kits and the biluciferase report gene analysis system were purchased from Promega Company; the protein lysis buffer RIPA was purchased in Shanghai Beyotime Company, and PTEN, p-pi3k and p-akt monoclonal antibodies were purchased from Santa cruz Company.

2.2. Experimental methods

2.2.1. Cell culture and treatment methods

ECC-1 cells were recovered, cultured with DEME containing 10% fetal bovine serum, and then digested with 0.125% trypsin for subculture after the cell density reached 80–90%, and then the cells in logarithmic growth phase were collected for treatment. Transfection methods of miR mimics and inhibitors were as follows: the miR-200a and miR-200b mimics and inhibitors as well as NC mimics and inhibitors were mixed with transfection reagents and serum-free DMEM medium and then incubated for 20 min, the end concentration of mimics and inhibitor is 10 nmol/L, then the cell culture medium in culture plate was abandoned, and the DMEM medium containing miR mimics and inhibitors as well as transfection reagent was added in the culture well; PTEN inhibitor treatment method was as follows: PTEN inhibitor PIC was mixed with DMEM medium to make the PIC concentration reach 10 nmol/L, then the cell culture medium in culture plate was abandoned, the DMEM medium containing PIC was added in culture well, and the DMEM medium without PIC was used as control (Table 1).

Table 1

Regulating effect of miR-200a and miR-200 on endometrial cancer cell proliferation activity ($n = 5$).

Groups	12 h	24 h	48 h
NC mimics group	0.78 ± 0.09	0.94 ± 0.12	1.21 ± 0.18
miR-200a mimics group	1.02 ± 0.15*	1.33 ± 0.18*	1.64 ± 0.22*
miR-200b mimics group	1.07 ± 0.18*	1.36 ± 0.19*	1.67 ± 0.26*
NC inhibitor group	0.72 ± 0.08	1.05 ± 0.12	1.30 ± 0.17
miR-200a inhibitor group	0.52 ± 0.07#	0.75 ± 0.11#	0.82 ± 0.12#
miR-200b inhibitor group	0.49 ± 0.06#	0.77 ± 0.09#	0.85 ± 0.15#

*: Compared with NC mimics group, $P < 0.05$; #: compared with NC inhibitor group, $P < 0.05$.

2.2.2. Cell proliferation activity detection methods

The cells for cell viability detection were inoculated in 96-well culture plate, the fluid in MTS cell viability detection kit was added in the culture medium in clean bench 12 h, 24 h and 48 h after transfection, 20 μL/well, the cells continued to be incubated for 4 h in the incubator, the culture plate was taken out and fully shaken, and then the absorbance at 450 nm wavelength was determined in microplate reader and used as the value of cell proliferation activity (Table 2).

2.2.3. Protein expression detection methods

The cells for protein expression detection were inoculated in 12-well culture plate and transfected for 48 h, then the culture medium was abandoned, the cells were collected and added in RIPA lysis buffer to extract the total protein in the cells, BCA kits were used to determine the total protein concentration in the protein samples, 4% and 10% SDS-polyacrylamide gel were prepared, the standard of 50 ng total protein in each sample application well was followed to add the protein samples in the gel for vertical electrophoresis, the protein was separated and then transferred to nitrocellulose membrane, the antigen sites were closed in 5% skim milk at room temperature for 1 h, then with PTEN, p-PI3K, p-Akt and β-actin antibodies were used for incubation overnight, the second antibodies labeled by horseradish peroxidase were used the next day for 2 h of incubation, the protein bands were obtained after development and expose, the gray value was scanned, and β-actin was used as reference to calculate PTEN, p-PI3K and p-Akt protein expression (Table 3).

2.2.4. Biluciferase report gene detection methods

The cells for luciferase report gene detection were inoculated in 96-well culture plate, 100 ng firefly luciferase reporter gene pGL3-PTEN-3′UTR plasmid, 5 ng renilla luciferase reference plasmid and 10 nmol/L miRNA mimics or inhibitors were transfected into cells together with Lipofectamine2000 reagent, Promega company dual luciferase reporter gene analysis system and microplate reader were used 48 h after transfection to

Table 2

Regulating effect of miR-200a and miR-200 on PTEN-3′UTR luciferase reporter gene activity and PTEN expression in endometrial cancer cells ($n = 5$).

Groups	Fluorescence activity	PTEN	p-PI3K	p-Akt
NC mimics group	1.00 ± 0.16	1.00 ± 0.15	1.00 ± 0.13	1.00 ± 0.18
miR-200a mimics group	0.32 ± 0.06*	0.36 ± 0.06*	2.52 ± 0.37*	2.21 ± 0.35*
miR-200b mimics group	0.27 ± 0.08*	0.33 ± 0.05*	2.66 ± 0.41*	2.19 ± 0.38*
NC inhibitor group	1.00 ± 0.19	1.00 ± 0.14	1.00 ± 0.16	1.00 ± 0.17
miR-200a inhibitor group	2.31 ± 0.39#	1.98 ± 0.25#	0.37 ± 0.09#	0.27 ± 0.05#
miR-200b inhibitor group	2.89 ± 0.46#	2.07 ± 0.32#	0.40 ± 0.06#	0.35 ± 0.08#

*: $P < 0.05$ compared with NC mimics group; #: $P < 0.05$ compared with NC inhibitor group.

Table 3
Regulating effect of PTEN inhibitor on endometrial cancer cell proliferation activity and downstream gene expression (n = 5).

Groups	n	Cell proliferation activity			Target gene expression	
		12 h	24 h	48 h	p-PI3K	p-Akt
Control group	5	0.71 ± 0.09	0.98 ± 0.14	1.32 ± 0.18	1.00 ± 0.15	1.00 ± 0.17
PTEN inhibitor group	5	1.02 ± 0.15#	1.52 ± 0.19#	2.04 ± 0.32#	2.42 ± 0.39#	2.77 ± 0.48#

#: $P < 0.05$ compared with control group.

determine fluorescence value, and the renilla luciferase activity was used as reference to calculate the firefly luciferase activity.

2.3. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis between two groups was t test and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. Effect of miR-200a and miR-200 on endometrial cancer cell proliferation activity

12 h, 24 h and 48 h after miR-200a and miR-200b mimics and NC mimics treatment, analysis of endometrial cancer cell proliferation activity was as follows: (1) 12 h, 24 h and 48 h after intervention, the cell proliferation activity of three groups gradually increased; (2) the cell proliferation activity of miR-200a mimics group and miR-200b mimics group were significantly higher than those of NC mimics group. 12 h, 24 h and 48 h after miR-200a and miR-200b inhibitors and NC inhibitors treatment, analysis of endometrial cancer cell proliferation activity was as follows: (3) 12 h, 24 h and 48 h after intervention, the cell proliferation activity of three groups gradually increased; (4) the cell proliferation activity of miR-200a inhibitor group and miR-200b inhibitor group were significantly lower than those of NC inhibitor group.

3.2. Effect of miR-200a and miR-200 on PTEN-3′UTR luciferase reporter gene activity

48 h after miR-200a and miR-200b mimics and NC mimics treatment, analysis of the PTEN-3′UTR luciferase reporter gene activity in endometrial cancer cells was as follows: PTEN-3′UTR luciferase reporter gene fluorescence activity of miR-200a mimics group and miR-200b mimics group were significantly lower than that of NC mimics group. 48 h after miR-200a and miR-200b inhibitors and NC inhibitors treatment, analysis of the PTEN-3′UTR luciferase reporter gene activity in endometrial cancer cells was as follows: PTEN-3′UTR luciferase reporter gene fluorescence activity of miR-200a inhibitor group and miR-200b inhibitor group were significantly higher than that of NC inhibitor group.

3.3. Effect of miR-200a and miR-200 on PTEN expression in endometrial cancer cells

48 h after miR-200a and miR-200b mimics and NC mimics treatment, analysis of PTEN, p-PI3K and p-Akt expression in endometrial cancer cells was as follows: PTEN expression in cells of miR-200a mimics group and miR-200b mimics group were significantly lower than that of NC mimics group while p-PI3K and p-Akt expression were significantly higher than those of NC mimics group. 48 h after miR-200a and miR-200b inhibitors and NC inhibitors treatment, analysis of PTEN, p-PI3K and p-Akt expression in endometrial cancer cells was as follows: PTEN expression in cells of miR-200a inhibitor group and miR-200b inhibitor group were significantly higher than that of NC inhibitor group while p-PI3K and p-Akt expression were significantly lower than those of NC inhibitor group.

3.4. Effect of PTEN inhibitor on endometrial cancer cell proliferation activity and downstream gene expression

12 h, 24 h and 48 h after PTEN inhibitor treatment, analysis of endometrial cancer cell proliferation activity was as follows: (1) 12 h, 24 h and 48 h after intervention, the cell proliferation activity of both groups gradually increased; (2) the cell proliferation activity of PTEN inhibitor group were significantly higher than those of control group. 48 h after PTEN inhibitor treatment, analysis of p-PI3K and p-Akt expression in endometrial cancer cells was as follows: (3) p-PI3K and p-Akt expression in cells of PTEN inhibitor group were significantly higher than those of control group.

4. Discussion

MiR is a newly discovered type of non-coding small RNA with size of 18–25 bp, and it can influence the mRNA stability and protein translation process through targeted combination with target gene mRNA 3′UTR region. MiR is involved in the regulation of cell proliferation, invasion and other cellular biological behaviors in the body, miR-200a and miR-200b are the miR with proto-oncogene characteristics, they are significantly highly expressive in the lung cancer, gastric cancer, bile duct cancer, ovarian cancer and other malignant tumors [7–10] and they have promoting effect on the proliferation of esophageal cancer, liver cancer, nasopharyngeal carcinoma and other malignant tumor cells [11–13]. In the study, in order to define the miR-200a and miR-200b influence on endometrial carcinoma cell growth in vitro, the miR mimics and inhibitors were transfected into cells respectively and the cell proliferation activity was analyzed. The analysis after miR-200a and miR-200b mimics transfection showed that miR-200a and miR-200b mimics could significantly increase the endometrial cancer cell proliferation activity; analysis after miR-200a and miR-200b inhibitors transfection showed that miR-200a and miR-200b inhibitors could significantly reduce the endometrial cancer cell proliferation activity. This means that miR-200a and miR-200b have promoting effect on the endometrial cancer cell growth in vitro, the miR-200a and miR-200b mimics can promote the proliferation of endometrial cancer cells, and the miR-200a and miR-200b inhibitors can inhibit the proliferation of endometrial cancer cells.

The biological effects of miR-200a and miR-200b on promoting the development of malignant tumors have received more and more attention, and related bioinformatics research has confirmed that the expression of multiple tumor suppressor genes are under the targeted regulation of miR-200a and miR-200b. The tumor-suppressor gene PTEN is an important downstream target gene for miR-200a and miR-200b, and there is the binding site of miR-200a and miR-200b on the PTEN gene 3′UTR. Study of foreign scholars has shown that in colon cancer cells, miR-200a and miR-200b can be combined with PTEN mRNA 3′UTR in the form of complementary base pairing so as to inhibit PTEN mRNA translation and induce PTEN mRNA degradation [14]. In order to define whether the miR-200a and miR-200b could target and regulate the expression of PTEN gene in endometrial cancer cells, the miR-200a and miR-200b mimics and inhibitors as well as PTEN-3′UTR luciferase reporter gene plasmids were transfected into cells respectively, and the analysis of the luciferase reporter gene fluorescence activity showed that miR-200a and miR-200b mimics could significantly decrease the PTEN-3′UTR luciferase reporter gene fluorescence activity, and the miR-200a and miR-200b inhibitors could significantly increase the PTEN-3′UTR luciferase reporter gene fluorescence activity. This shows that miR-200a and miR-200b are able to target and combine the PTEN gene 3′UTR in the endometrial cancer cells.

The product encoded by tumor suppressor gene PTEN is the phosphatase specific to both protein phosphatase and lipid phosphatase substrates, and can cause dephosphorylation of a variety of kinases and implement negative regulation on PI3K/Akt, ERK, FAK/P130cas and multiple signal pathways [15,16]. PI3K/Akt are the important signaling pathways in mammals that regulate cell proliferation and invasion, PI3K phosphorylation will phosphorylate PIP2 into PIP3, and the phosphorylated PIP3 can activate Akt into p-Akt, and then promote cell proliferation and invasion through the role of p-Akt [17,18]. PTEN can inhibit the activity of PI3K/Akt pathway by dephosphorylation, and then inhibit cell proliferation and invasion [19,20]. It was found in the study that in endometrial cancer cells, PTEN inhibitors could significantly increase the cell proliferation activity and decrease cell the expression of p-PI3K and p-Akt. This indicates that PTEN has a definite tumor-suppressor gene activity in endometrial cancer cells. As mentioned earlier, miR-200a and miR-200b can target and combine PTEN mRNA 3′UTR in endometrial cancer cells, and in order to further clarify miR-200a and miR-200b effect on PTEN gene expression and downstream signaling pathway function, PTEN, p-PI3K and p-Akt expression were analyzed in the study, and the results showed that miR-200a and miR-200b mimics could inhibit the expression of PTEN, and increase the expression of p-PI3K and p-Akt; miR-200a and miR-200b inhibitors could increase the expression of PTEN, and inhibit the expression of p-PI3K and p-Akt. This shows that miR-200a and miR-200b are able to regulate the expression of PTEN genes and the function of the downstream signaling pathway PI3K/Akt in endometrial cancer cells.

To sum up, it is believed that miR-200a and miR-200b have promoting effect on endometrial cancer cell growth in vitro, and targeted inhibition of PTEN gene expression and activation of downstream PI3K/Akt signaling pathway are the specific molecular mechanism of miR-200a and miR-200b to promote cell growth.

Conflict of interest statement

We declare that we have no conflict of interest.

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