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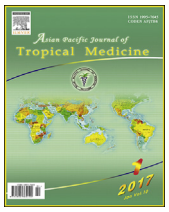

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miR-21 targets and inhibits tumor suppressor gene PTEN to promote prostate cancer cell proliferation and invasion: An experimental study

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ABSTRACT

Objective: To study whether miR-21 targets and inhibits tumor suppressor gene PTEN can promote prostate cancer cell proliferation and invasion.

Methods: Prostate cancer cell lines PC-3 were cultured and divided into negative control group (NC group), miR-21 group, pcDNA3.1 group, miR-21+pcDNA3.1 group and miR-21+PTEN group that were transfected with different miR and plasmid, respectively. After 12 h and 24 h of transfection, the cell viability and invasive cell number were determined; after 24 h of transfection, *Bcl*-2, Survivin, MMP2, MMP9, PTEN, PI3K, and AKT expression in cells were determined.

Results: After 12 h and 24 h of transfection, OD value and invasive cell number of miR-21 group were significantly higher than those of NC group; after 24 h of transfection, *Bcl*-2, Survivin, MMP2, MMP9, PI3K and AKT expression levels were significantly higher than those of NC group while PTEN expression level was significantly lower than that of NC group; after 12 h and 24 h of transfection, OD value and invasive cell number of miR-21+pcDNA3.1 group were significantly higher than those of pcDNA3.1 group, and the OD value and invasive cell number of miR-21+PTEN group were significantly lower than those of miR-21+pcDNA3.1 group; after 24 h of transfection, *Bcl*-2, Survivin, MMP2 and MMP9 content of miR-21+pcDNA3.1 group were significantly higher than those of pcDNA3.1 group, and *Bcl*-2, Survivin, MMP2 and MMP9 content of miR-21+PTEN group were significantly lower than those of miR-21+pcDNA3.1 group.

Conclusions: miR-21 can target and inhibit tumor suppressor gene PTEN expression to promote prostate cancer cell proliferation and invasion.

1. Introduction

Prostate cancer is a common malignant tumor of male urinary system, its incidence is rising year by year, and the common clinical treatments include surgical resection, radiotherapy and chemotherapy as well as castration [1–3]. Cell proliferation and invasion are the most prominent biological features of prostate cancer, but the regulatory mechanism of prostate cancer cell proliferation and invasion is not very clear, and the corresponding clinical targeted drugs are also short. MicroRNA (miR) is a class of small non-coding RNAs discovered in recent years, which adjusts the expression of a variety of genes to influence the biological behaviors of cells. miR-21 is a miRNA widely studied in malignant tumors, which shows high expression in cervical cancer, colorectal cancer, breast cancer and other malignant tumors, has the characteristics of proto-oncogene and can promote the cancer cell proliferation and invasion [4–6]. Domestic cytological study by Xiao Li [7] shows that miR-21 can target and inhibit the expression of tumor suppressor gene PTEN so as to weaken pro-apoptotic effects mediated by PTEN. However, it is not clear whether miR-21 can control the prostate cancer proliferation and invasion by PTEN. The following study aimed to confirm whether miR-21 targeted and inhibited PTEN to promote prostate cancer cell proliferation and invasion.

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2. Materials and methods

2.1. Research materials

Prostate cancer cell lines PC-3 were bought from the cell bank of Chinese Academy of Sciences, the RPMI1640 medium and fetal bovine serum for cell culture as well as the trypsin for cell digestion were purchased from Gibco Company, miR-21 analogs were synthesized by Shanghai Genepharma Company, pcDNA3.1(+) plasmid containing PTEN coding sequence were built by Shanghai Jerry Company, and transfection reagents Lipofectamine™2000 were purchased from Invitrogen Company. Cell viability detection kits were bought from Promega Company, and enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang Biotechnology Company.

2.2. Experimental methods

2.2.1. Cell culture methods

PC-3 cell lines were recovered and then cultured with RPMI1640 culture medium containing 10% fetal bovine serum, the cell culture medium was replaced every 2 d, the status of cell growth was observed, the cells were digested with 0.25% trypsin after the cell density reached 80–90%, then centrifuged, collected and re-suspended, some cells were inoculated in the culture bottle and continued to be digested, digested and sub-cultured, and some cells were inoculated in the cell culture plate for treatment.

2.2.2. Cell grouping and treatment methods

According to the different treatment conditions, the cells were divided into negative control group (NC group), miR-21 group, pcDNA3.1 group, miR-21+pcDNA3.1 group and miR-21+PTEN group, and Lipofectamine™2000 instructions were followed for miRNA sequence and plasmid transfection. NC group were only transfected with negative control miRNA sequences, miR-21 group were transfected with miR-21 sequences, pcDNA3.1 group were transfected with pcDNA3.1 blank plasmids, miR-21+pcDNA3.1 group were transfected with pcDNA3.1 blank plasmids and miR-21 sequences, and miR-21+PTEN group were transfected with pcDNA3.1 plasmid containing PTEN coding sequences as well as miR-21 sequences.

2.2.3. Cell viability detection methods

The cells for cell viability detection were inoculated in 96-well plate, 20 μL cell viability detection fluid was added in each wells after miRNA sequence and plasmid transfection for 12 h and 24 h, cells continued to be incubated for four hours in the incubator, shaken and mixed, then the cell plate was placed on microplate reader to measure the absorbance value (OD value) at 450 nm wavelength, and the OD value was used to reflect the cell viability.

2.2.4. Cell invasion detection methods

The cells for cell invasion detection were inoculated in Transwell Chambers, the Transwell Chambers were taken out after miRNA sequence and plasmid transfection for 12 h and 24 h, the filter membrane at the bottom of chambers was taken down and stained with crystal violet, and then the number of cells within 3 random fields was observed under the microscope.

2.2.5. Protein content detection methods

The cells for protein content detection were inoculated in 6-well plate and transfected with miRNA sequences and plasmids for 24 h, the culture medium was abandoned and the cells were kept and washed with PBS buffer for 2–3 times, then 120 μL protein lysis buffer was added in the culture wells, the cells were fully broken and then centrifuged, the residue was abandoned and the supernatant was kept, enzyme-linked immunosorbent assay kits were used to determine the *Bcl*-2, Survivin, MMP2, MMP9, PTEN, PI3K and AKT content in the supernatant.

2.3. Statistical analysis

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

3. Results

3.1. Regulatory effect of miR-21 analog transfection on cell proliferation activity and invasion ability

Analysis of cell viability OD value and invasive cell number between NC group and miR-21 group was as follows: after 12 h and 24 h of transfection, OD value and invasive cell number of miR-21 group were significantly higher than those of NC group. Differences in OD value and invasive cell number were statistically significant between NC group and miR-21 group.

3.2. Regulatory effect of miR-21 analog transfection on cell proliferation- and invasion-related gene expression

Analysis of the proliferation-related genes *Bcl*-2 and Survivin as well as the invasion-related genes MMP2 and MMP9 expression between NC group and miR-21 group was as follows: after 24 h of transfection, *Bcl*-2, Survivin, MMP2 and MMP9 content of miR-21 were significantly higher than those of NC group. Differences in *Bcl*-2, Survivin, MMP2 and MMP9 content were statistically significant between NC group and miR-21 group.

3.3. Regulatory effect of miR-21 analog transfection on PTEN and its downstream signaling molecule expression

Analysis of PTEN and its downstream signaling molecules PI3K and AKT expression between NC group and miR-21 group was as follows: after 24 h of transfection, PTEN content of miR-21 group was significantly lower than that of NC group while PI3K and AKT content were significantly higher than those of NC group. Differences in PTEN, PI3K and AKT content were statistically significant between NC group and miR-21 group.

3.4. Regulatory effect of miR-21 analog and PTEN-overexpression plasmid transfection on cell proliferation and invasion

Analysis of cell viability OD value and invasive cell number after miR-21 analog and PTEN-overexpression plasmid transfection was shown in Table 1: after 12 h and 24 h of transfection, OD value and invasive cell number of miR-21+pcDNA3.1

Table 1

Cell proliferation activity, invasion ability, proliferation- and invasion-related gene expression, PTEN and its downstream signaling molecule expression of NC group and miR-21 group.

Groups	Repeated batches	Cell viability OD value		Invasive cell number		Proliferation genes (ng/mL)		Invasion genes (pg/mL)		PTEN (ng/mL)	PI3K (pg/mL)	AKT (pg/mL)
		12 h	24 h	12 h	24 h	*Bcl*-2	Survivin	MMP9	MMP2			
NC group	5	0.67 ± 0.09	0.78 ± 0.08	14.42 ± 1.85	18.35 ± 2.15	6.48 ± 0.93	4.76 ± 0.55	113.42 ± 15.58	92.31 ± 10.34	10.32 ± 1.45	77.65 ± 9.34	113.42 ± 16.58
miR-21 group	5	0.94 ± 0.11	1.25 ± 0.14	20.39 ± 2.77	31.26 ± 4.48	9.42 ± 1.14	8.97 ± 0.93	189.34 ± 22.38	157.54 ± 16.78	4.62 ± 0.65	125.42 ± 14.58	187.87 ± 22.38
T		7.182	6.684	7.615	7.932	7.029	9.652	8.327	8.758	12.482	8.947	9.571

Table 2

Cell proliferation activity, invasion ability, proliferation- and invasion-related gene expression after miR-21 analog and PTEN-overexpression plasmid transfection.

Groups	Repeated batches	Cell viability OD value		Invasive cell number		Proliferation genes (ng/mL)		Invasion genes (pg/mL)	
		12 h	24 h	12 h	24 h	*Bcl*-2	Survivin	MMP2	MMP9
pcDNA3.1 group	5	0.62 ± 0.08	0.73 ± 0.09	12.67 ± 1.68	16.78 ± 2.24	6.03 ± 0.67	4.25 ± 0.58	88.94 ± 11.38	105.56 ± 12.58
miR-21+pcDNA3.1 group	5	1.17 ± 0.22*	1.39 ± 0.23*	18.95 ± 2.48*	24.15 ± 3.41*	9.77 ± 1.07*	9.14 ± 1.18*	167.31 ± 19.34*	197.33 ± 25.62*
miR-21+PTEN group		0.88 ± 0.10△	0.94 ± 0.12△	14.52 ± 1.67△	19.87 ± 2.33△	7.86 ± 0.80△	5.89 ± 0.74△	113.24 ± 13.48△	135.42 ± 16.70△
F		7.658	8.491	6.537	7.757	6.938	9.282	9.681	8.658

*Compared with pcDNA3.1 group, $P < 0.05$.

△Compared with miR-21+pcDNA3.1 group, $P < 0.05$.

group were significantly higher than those of pcDNA3.1 group, and the OD value and invasive cell number of miR-21+PTEN group were significantly lower than those of miR-21+pcDNA3.1 group. Analysis of proliferation- and invasion-related gene expression in cells was shown in Table 2: after 24 h of transfection, *Bcl*-2, Survivin, MMP2 and MMP9 content of miR-21+pcDNA3.1 group were significantly higher than those of pcDNA3.1 group, and *Bcl*-2, Survivin, MMP2 and MMP9 content of miR-21+PTEN group were significantly lower than those of miR-21+pcDNA3.1 group.

4. Discussion

MicroRNA (miR) is a class of non-coding small RNAs with extensive biological effects, and a number of basic researches have confirmed that miR-21 has characteristics of proto-oncogene and plays a significant role in promoting cancer cell growth, proliferation and invasion [8,9]. Prostate cancer is a common malignant tumor of the male reproductive system, the incidence increases year by year, but the mechanism is still not clear [10]. Cell study of domestic Xiao Li [7] shows that miR-21 shows the trend of high expression in prostate cancer tissues and miR-21 can target and inhibit the expression of tumor suppressor gene PTEN in prostate cancer cells; the study of Yang B shows that the highly expressed miR-21 has certain value for the prognosis of prostate cancer [11]. The above study results indicate that miR-21 has shown the characteristics of proto-oncogene in the occurrence and development process of prostate cancer, and has a promoting effect on the occurrence and development of tumor. So it was speculated that miR-21 played an important role in regulating prostate cancer proliferation, invasion and other processes. To test this theory, miR-21 analog was transfected into prostate cancer cells, the cell viability, invasive cell number and related gene expression were analyzed in the study, and the results showed that miR-21 analog transfection can increase the prostate cancer cell viability, increase the invasive cell number and up-regulate the expression of anti-apoptotic genes *Bcl-2* and Survivin as well as pro-proliferation genes MMP2 and MMP9. This means that miR-21 plays a significant role in promoting prostate cancer proliferation and invasion.

In recent years, the specific ways for miRNA to exert biological effects have received more and more attention, there are rich miRNA binding sites in the miRNA-regulated target gene mRNA 3′ non-coding region (3′UTR), miRNA can be combined with the corresponding target gene mRNA 3′UTR and then induce the corresponding mRNA degradation or inhibit the corresponding mRNA transcription, which is finally characterized by the decrease of corresponding target gene expression [12–15]. In the study, miR-21 was highly expressed in prostate cancer tissue, the genes under the targeted regulation of miR-21 should show the trend of low expression, and miR-21 analog should be able to inhibit the expression of corresponding target genes in prostate cancer cells. However, it was observed after miR-21 analog transfection that the expression of anti-apoptotic genes *Bcl*-2 and Survivin as well as pro-proliferation genes MMP2 and MMP9 significantly increased. Thus it indicates that miR-21 does not directly target the *Bcl*-2 and Survivin as well as MMP2 and MMP9, and its possible way of action is to target and adjust tumor suppressor gene and decrease tumor suppressor gene expression so as to enhance the malignant biological behaviors of cancer cells and promote the expression of malignant molecules.

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is now known as a relatively clear tumor suppressor gene, and the encoded protein has both lipid phosphatase and protein phosphatase activity, and can make the PI3K 3-phosphorylation sites in cells dephosphorylate and inhibit downstream signaling molecule Akt phosphorylation. When the expression of PTEN gene in cells is reduced or deleted, the dephosphorylation effect on PI3K/Akt signaling pathway is weakened, the function is correspondingly enhanced and it promotes cell proliferation and invasion [16,17]. Studies have shown that the expression of PTEN decreases significantly in prostate tissue and is closely related to the TNM staging of the tumor [18,19], and in the experiments in vitro of breast cancer, colorectal cancer and other malignant tumor cells, the researchers have found that miR-21 has targeted inhibition effect on the expression of PTEN [19,20]. In the study, the PTEN/PI3K/Akt expression in prostate cancer cells was analyzed after miR-21 analog transfection, and the results showed that PTEN content of miR-21 group was significantly lower than that of NC group while PI3K and AKT content were significantly higher than those of NC group. This means that miR-21 can target and inhibit the expression of tumor suppressor gene PTEN and enhance the function of the downstream signaling pathways PI3K/Akt in prostate cancer cells.

After confirming the regulatory effect of miR-21 on prostate cancer proliferation and invasion as well as PTEN expression, and in order to further clarify whether miR-21 directly regulated the expression of PTEN to influence the proliferation and invasion of prostate cancer cells, PTEN-overexpression plasmid and miR-21 analog were both transfected into prostate cancer cells, and the cell viability, the invasive cell number and the related gene expression were analyzed. The analysis results showed that miR-21 and blank plasmid transfection could significantly increase the cell viability, the invasive cell number as well as the *Bcl*-2, Survivin, MMP2 and MMP9 expression, and transfection of both miR-21 and PTEN-overexpression plasmid could reverse the miR-21 effects on cell viability and cell invasion, which was characterized that the OD value, invasive cell number as well as *Bcl*-2, Survivin, MMP2 and MMP9 expression of miR-21+PTEN group were significantly lower than those of miR-21+pcDNA3.1 group. It illustrates that the promoting effect of miR-21 on prostate cancer cell proliferation and invasion is realized by targeting inhibition of the expression of PTEN.

In conclusion, miR-21 can target and inhibit tumor suppressor gene PTEN expression to promote prostate cancer cell proliferation and invasion.

Conflict of interest statement

We declare that we have no conflict of interest.

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