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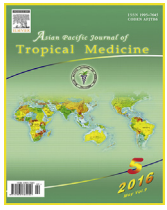

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Exploration of the regulatory effect of miR-21 on breast cancer cell line proliferation and invasion as well as the downstream target genes

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ABSTRACT

Objective: To study the regulatory effects of miR-21 on breast cancer cell line proliferation and invasion as well as the downstream target genes.
Methods: Breast cancer cell lines MCF-7 were cultured and transfected with miR-21 mimics and the corresponding negative control mimics (NC mimics), and then MTS kits were used to detect cell viability. Transwell experiment was used to detect cell invasion ability, and fluorescence quantitative PCR was used to detect the expression of proliferation and invasion-related genes in cells.
Results: 24 h after transfection of miR-21 mimics and NC mimics, cell OD value and the number of invasive cells of miR-21 group were significantly higher than those of NC group, and mRNA contents of PDCD-4, FasL, PTEN, RhoB, Maspin, TIMP3 and RECK in cells were significantly lower than those of NC group.
Conclusion: miR-21 can promote the proliferation and invasion of breast cancer cell lines, and its downstream target genes include PDCD-4, FasL, PTEN, RhoB, Maspin, TIMP3 and RECK.

1. Introduction

Breast cancer is malignant tumor with highest morbidity in women and also the main cause of cancer death in women. In recent years, both morbidity and mortality of breast cancer are showing a rising trend. Breast cancer is derived from the mammary epithelial cells, the prognosis of the disease is related to the abnormal expression of cell proliferation and invasion-related genes, and the stronger the cell proliferation and invasion ability, the worse the prognosis of tumor. At present, the upstream molecular mechanism regulating the expression of breast cancer cell proliferation and invasion-related genes is not yet clear [1,2]. Micro RNA (miRNA) is the single-stranded small RNA that has received more and more attention in recent years, and it can induce mRNA degradation or inhibit mRNA translation at posttranscriptional level, thus achieving the regulation of the expression of a variety of target genes. In a variety of miRNAs, miR-21 is confirmed to have antitumor effect and is associated with the pathogenesis of solid malignant tumors such as breast cancer, stomach cancer and colon cancer [3–5]. This study aims to clarify the relationship between miR-21 and the occurrence and progression of breast cancer and to identify its regulating effects on the biological behavior of breast cancer cells, breast cancer cell lines were used as research tools to specifically analyze the regulating effects of miR-21 on breast cancer cell line proliferation and invasion as well as the expression of downstream target genes.

2. Materials and methods

2.1. Experimental materials

Human breast cancer cell lines MCF-7 were purchased from the cell bank of Chinese Academy of Sciences in Shanghai, DMEM, fetal bovine serum and trypsin for cell culture were purchased from Gibco company. MiR-21 mimics and the corresponding negative control mimics (NC mimics) were synthesized by Shanghai GenePharma company; MTS cell viability detection kits were purchased from Promega company. Transfection reagents Lipofectamine 2000 and RNA extraction kits Trizol were purchased from Invitrogen company. Primers for

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fluorescence quantitative PCR were synthesized by Shanghai Sangon company, and PCR kits were purchased from Beijing Tiangen company.

2.2. Cell culture and transfection

MCF-7 cells were recovered, cultured with DMEM containing 5% fetal bovine serum until cell density reached 70%–80%, and then digested and amplified with 0.125% trypsin, amplified cells were divided into two, one was inoculated in culture bottle, continued to be cultured and used for subsequent digestion and amplification, and the other was inoculated in culture plate, continued to be cultured and used for subsequent transfection. For cell transfection, miR-21 mimics or NC mimics were mixed with Lipofectamine 2000 according to the proportion of 1:1, let stand at room temperature for 20 min, then added to the culture wells of cell plate, continued to be cultured for 24 h and used for subsequent detection.

2.3. Cell viability detection

For cell viability detection, cells were inoculated in 96-well cell culture plate and transfected for 24 h, then 20 μL MTS detection solution was added to the culture system for 4 h of continuous incubation, culture plate was placed in microplate reader and the absorbance (OD value) at 490 nm wave length was detected.

2.4. Cell invasion detection

For cell invasion detection, cells were inoculated in Transwell chambers and transfected for 24 h, then cell membrane at the bottom of the chambers was taken out, stained with DAPI dye and were observed under fluorescence microscope, and the number of cells within 3 high-power fields was counted.

2.5. mRNA contents detection

For detection of mRNA contents in cells, cells were inoculated in 12-well cell culture plate and transfected for 24 h, culture medium was discarded, cells were washed with PBS twice. Then Trizol lysate was added, total RNA was extracted, 1 μg RNA was collected for reverse transcription, obtained cDNA was amplified according to the following procedure: 95 °C and 10 s, specific annealing temperature and 20 s, 72 °C and 30 s, repeating for 40 cycles, and after amplification curves and take-off cycle number Ct were obtained, mRNA contents were calculated according to $2^{-\Delta\Delta Ct}$.

2.6. Statistical methods

Data were analyzed with SPSS20.0 software, and comparison between two groups was conducted by t test and differences were considered to be statistically significant at a level of $P < 0.05$.

3. Results

3.1. Cell viability

24 h after transfection of miR-21 mimics and NC mimics, cell OD value of miR-21 group was 0.980 ± 0.101 which was significantly higher than 0.561 ± 0.077 of the NC group showing significant differences between two groups ($P < 0.05$).

3.2. Cell invasion

24 h after transfection of miR-21 mimics and NC mimics, the number of invasive cells of miR-21 group was (131.00 ± 19.54) which was significant higher than (71.25 ± 8.34) of NC group, and difference between two groups was statistically significant ($P < 0.05$).

3.3. Proliferation-related gene expression

24 h after transfection of miR-21 mimics and NC mimics, mRNA contents of PDCD-4, FasL, PTEN and RhoB in cells of miR-21 group were significantly lower than those of NC group (Table 1).

3.4. Invasion-related gene expression

24 h after transfection of miR-21 mimics and NC mimics, mRNA contents of Maspin, TIMP3 and RECK in cells of miR-21 group were significantly lower than those of NC group (Table 2).

Table 1
Effect of transfection of miR-21 mimics on proliferation-related gene expression.

	PDCD-4/β-actin	FasL/β-actin	PTEN/β-actin	RhoB/β-actin
miR-21 group	0.384 ± 0.074	0.504 ± 0.102	0.306 ± 0.043	0.559 ± 0.059
NC group	1.000 ± 0.081	1.000 ± 0.168	1.000 ± 0.138	1.000 ± 0.114
t	11.216	5.042	9.626	6.844
P	<0.05	<0.05	<0.05	<0.05

Table 2
Effect of transfection of miR-21 mimics on invasion-related gene expression.

	Maspin/β-actin	TIMP3/β-actin	RECK/β-actin
miR-21 group	0.663 ± 0.105	0.461 ± 0.065	0.334 ± 0.079
NC group	1.000 ± 0.110	1.000 ± 0.098	1.000 ± 0.129
t	4.426	9.146	8.817
P	<0.05	<0.05	<0.05

4. Discussion

MicroRNAs is a type of non-coding small RNA, and the length of mature miRNA is about 18–24 bp. Primary transcript Pri-miRNA becomes mature miRNA by the hydrolysis of Drosha, Dicer and other enzymes, then is assembled into RNA-induced silencing complex (RISC) and combined with mRNA 3′ non-translated region in the form of complementary base pairing to induce mRNA degradation or inhibit mRNA translation, thus achieving the posttranscriptional regulation of gene expression [6,7]. The pathogenesis of breast cancer is associated with the abnormal expression of multiple genes, but upstream molecules regulating gene expression are unclear. MicroRNA is involved in the regulation of more than 50% of genes in the body by means of posttranscriptional regulation, and miR-21 is confirmed to be related to the occurrence, metastasis and poor prognosis of tumor. A number of studies have shown that miR-21 expression in breast cancer tissue is significantly higher than that in normal breast tissue suggesting that miR-21 has the function of oncogene in the development process of cancer [8–10].

In the occurrence and development process of breast cancer, the strong proliferation ability and invasive ability of cancer cells are the key links causing local tumor growth and distant metastasis. In this study, the *in vitro* cultured breast cancer cell lines were chosen as research tools, transfection of miR-21 mimics was adopted to simulate high miR-21 expression state in breast cancer tissue, and then cell proliferation and invasion ability were evaluated. Analysis of the detection results of cell vitality by MTS showed that OD value of the cells of miR-21 group was significantly higher than that of NC group; analysis of the detection results of cell invasion by transwell showed that the number of invasive cells of miR-21 group was significantly higher than that of NC group suggesting that miR-21 played a promoting role in breast cancer cell proliferation and invasion, and high expression of miR-21 in breast cancer tissue could increase the cell vitality and promote invasive growth of cells.

The main way for miRNA to play its role is to be combined with mRNA 3′ non-translated region, and its effect is to cause mRNA degradation or inhibit mRNA transcription. In the occurrence and development process of breast cancer, a variety of molecules are involved in regulating cell proliferation and invasion, and there are miR-21 binding sites in mRNA 3′ non-translated region of a variety of proliferation and invasion-related molecules. PDCD-4, FasL, PTEN and RhoB gene-encoded molecules are closely related to the regulation of breast cancer cell proliferation, and Maspin, TIMP3 and RECK gene-encoded molecules are closely related to the regulation of breast cancer cell invasion. Studies have reported that miR-21 can target and regulate PDCD-4, FasL, PTEN, RhoB, Maspin, TIMP3, RECK and other proliferation and invasion-related genes in bladder cancer, stomach cancer, esophageal cancer and other malignant tumor cells [11–15]. However, it has not been reported whether miR-21 regulates the above expression of proliferation and invasion-related genes in breast cancer cells.

PDCD-4, FasL, PTEN and RhoB are tumor suppressor genes, and their expression significantly reduces in breast cancer, lung cancer and other malignant tumors. Fatty acid synthase ligand (FasL) belongs to the tumor necrosis factor receptor super family, is cell apoptosis signal receptor, and can be combined with Fas to induce cell apoptosis and activate cellular immunity to kill target cells [16]; programmed cell death receptor 4 (PDCD4) is a kind of tumor suppressor gene, and the protein expressed by it is located in the nucleus and can inhibit cell proliferation and promote cell apoptosis through Sp-transcription factors; PTEN-encoded protein contains 4 cyclic structures of amino acid residue insertion sequence, which can induce cell cycle arrest and apoptosis through PI3K/Akt pathway [17]. After transfection of miR-21 mimics, mRNA contents of proliferation-related molecules PDCD-4, FasL, PTEN and RhoB in breast cancer cell lines decreased significantly. This meant that miR-21 could target and regulate proliferation-related molecules PDCD-4, FasL, PTEN and RhoB in breast cancer cells.

Maspin, TIMP3 and RECK are genes with invasion-inhibiting effect, and they show a trend of low expression in breast cancer and lung cancer, which will cause enhanced cancer cell invasion ability. Maspin belongs to the serpin super family, and is a kind of secretory protein that can block the combination of uPA with receptor uPAR to inhibit cell invasion [18]. RECK-encoded protein contains three serine hydrolase inhibitors regions, has inhibitory effect on the secretion and activity of several kinds of MMPs, and can prevent MMP2 and MMP9 degradation of extracellular matrix [19]; tissue inhibitor of metalloproteinase TIMPs can directly combine with MMPs and suppress their function, blocking the degradation of extracellular matrix [20]. After transfection of miR-21 mimics, mRNA contents of invasion-related molecules Maspin, TIMP3 and RECK in breast cancer cell lines decreased significantly. This meant that miR-21 could target and regulate invasion-related molecules Maspin, TIMP3 and RECK in breast cancer cells.

To sum up, miR-21 can promote the proliferation and invasion of breast cancer cell lines, and its downstream target genes include PDCD-4, FasL, PTEN, RhoB, Maspin, TIMP3 and RECK.

Conflict of interest statement

We declare that we have no conflict of interest.

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