Int J Clin Exp Pathol 2016;9(11):11664-11669
www.ijcep.com /ISSN:1936-2625/IJCEP0039688

Original Article

Effect of miR-106b on the proliferation, invasion, and migration of breast cancer MCF-7 cells

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Received September 8, 2016; Accepted September 26, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Breast cancer is the number one common female malignancy. MiR-106b is found closely associated with the development of a variety of malignant tumors. This study analyzed the effect of miR-106b on proliferation, invasion and migration of breast cancer MCF-7 cells. miRNA-106b mimics, miRNA-106b inhibitor, mimic control were transfected into MCF-7 cells, untransfected cells were used as controls. miR-106b expression after transfection was detected by RT-PCR. MTT assay was used to measure proliferation of cells in each group after transfection. Transwell assay was used to measure the invasion and migration of cells in each group after transfection. Western blot was used to detect the expression levels of AKT and PTEN. miRNA-106b expression was significantly up-regulated after transfection of miRNA-106b mimic and the expression of miRNA-106b increased along with transfection time ($P<0.05$). miRNA-106b expression was significantly down-regulated after transfection of miRNA-106b inhibitor and the expression of miRNA-106b decreased along with transfection time ($P<0.05$). OD values and cell proliferation were significantly increased in miRNA-106b mimic group ($P<0.05$), but significantly decreased in miRNA-106b inhibitor group ($P<0.05$). Cells invasion and migration were significantly enhanced in miRNA-106b mimic group ($P<0.05$), while the invasion and migration were significantly suppressed in miRNA-106b inhibitor group invasion ($P<0.05$). Expression level of AKT was reduced and the expression level of PTEN was increased in miRNA-106b mimic group reduced, in contrast, the expression level of AKT was increased and the expression level of PTEN was decreased in miRNA-106b inhibitor group ($P<0.05$). miRNA-106b is expressed in breast cancer MCF-7 cells. miRNA-106b promotes proliferation, invasion, and migration of breast cancer MCF-7 cells via PTEN/AKT signaling pathway.

Keywords: miR-106b, MCF-7, proliferation, invasion, migration

Introduction

Breast cancer has the highest incidence in female malignant tumors in China. Statistics data showed that China's new patients with breast cancer increased by 3% to 4% each year in the past 20 years, suggesting that the incidence of breast cancer has an upward trend. It is known that malignant tumor has the capability of local invasion and distant metastasis. Metastasis of tumor cells accounted for more than 90% of the cause of death in cancer patients [1, 2]. MicroRNAs, which cause researchers' attention in recent years, are a class of cell-derived, small non-coding RNA molecules which can bind to 3' end non-coding region of target mRNA to regulate target genes at transcription and translation levels [3]. Recent research indicates that the expression changes of microRNAs are closely related to cancer occurrence, development, invasion and migration. microRNAs can promote overexpression of oncogenes and silence of tumor suppressor genes [4]. Studies showed that various miRNAs including miR-9, miR-205, and miR-106b had altered expression in breast cancer [5-7]. miR-106b is an oncogenic miRNA, which is highly expressed in colon cancer, head and neck squamous cell carcinoma, and esophageal cancer. miR-106b can significantly inhibit the metastasis of colon cancer, head and neck squamous cell carcinoma, and esophageal cancer [8]. In order to investigate the effect of miR-106b on the proliferation, invasion, and metastasis of MCF-7 cells, miR-106b was transfected into MCF-7 cells, MTT assay was used to measure the cell proliferation, Transwell assay was used measure cell invasion and migration, Western blot was used to measure the expression levels of AKT and PTEN.

Table 1. Primer sequences

Genes		Sequence (5'-3')
miRNA-106b	Forward	5'-AGCCGTCAAGAGCAATAACGAA-3'
	Reverse	5'-GTGCAGGGTCCGAGGT-3'
U6	Forward	5'-CTCGCTTCGGCAGCACA-3'
	Reverse	5'-AACGCTTCACGAATTTGCGT-3'

Material and methods

Experimental cell

Breast cancer MCF-7 cells were purchased from Shanghai Baili biotech.

Reagents and instruments

miRNA-106b mimics and miRNA-106b inhibitor were purchased from Gene Pharma. RPMI1640 medium, DMEM medium, penicillin/streptomycin, and fetal bovine serum were purchased from Gibco. LipofectamineTM 2000 was from Invitrogen. RT-PCR kit, AKT antibody, PTEN antibody, and goat anti-rabbit secondary antibody were purchased from Takara Shuzo. Trizol reagent was from Gibeo BRL. MTT was from sigma. Carbon dioxide incubator and inverted microscope were purchased from SANYO.

Methods

Conventional cell culture

MCF-7 breast cancer cells were cultured with RPMI1640 with 10% fetal bovine serum and incubated in a 37°C incubator with 5% CO_2 supply.

Cell transfection

MCF-7 cells were with 10% fetal bovine serum, 100 u/mL penicillin, 100 u/mL streptomycin. Cells were trypsinized and cultured in 96-well cell culture plates at a concentration of 104 cells/well. Lipofectamine 2000 was used to transfect miRNA-106b mimics, miRNA-106b inhibitor, and mimic control when the cells reach 60% of confluence for 48 hours.

RT-PCR detection of the expression of miRNA106b after transfection

Seventy-two hours after transfection of miRNA-106b mimics, miRNA-106b inhibitor, or mimic control, RNA was extracted with TRIzol reagent and quantified. 200 ng of total RNA was reverse transcripted into cDNA using synthetic miRNA polyA tail according to manufacturer's instruction. The cDNA was used as template for PCR amplification with the following primers (**Table 1**). PCR was performed with the following profile: an initial 5 min incubation at 95°C, 30 cycles of (30 s at 95°C, 30 s at 58°C, and 30 s at 72°C), and a final extension of 10 min at 72°C. U6 was used as a reference gene.

MTT assay of proliferation of MCF-7 cells after transfection

MCF-7 cells transfected with miRNA-106b mimics, miRNA-106b inhibitor, or mimic control were inoculated into 96-well cell culture plates at a density of 104/well and cultured at 37°C for 24, 48, or 72 h. After washing with PBS, 20 μL (5 mg/mL) of MTT solution was added to each well, mixed, and incubated for 4 h. 150 μL of dimethylsulfoxide was added to each well and shook for 10 min. Absorbance at 570 nm of each well was measured by a microplate reader to draw the growth curve.

Transwell assay of invasion and migration of MCF-7 cells after transfection

Invasion assay: Matrix was diluted with serum-free medium (1:8) and spread evenly to cover each chamber (60 μl/chamber). 200 μl of MCF-7 cells (106 cells/ml) was added to the upper chamber, and 1300 μl of complete medium was added to the lower well was added to each well. 24 h after incubation, cells were fixed with ethanol, stained, and observed under microscope.

Migration assay: 200 μl of MCF-7 cells (106 cells/ml) was added to the upper chamber, and 1300 μl of complete medium was added to the lower well was added to each well. 24 h after incubation, cells were fixed with ethanol, stained with crystal violet, and observed under microscope.

Western blot analysis of expression of AKT and PTEN in MCF-7 cells after transfection

MCF-7 cells were collected, lysed in RIPA buffer, centrifuged at maximum speed for 30 min. The supernatant was collected and stored at -80°C. Same amount of proteins were boiled, resolved by 10% SDS-PAGE gel electrophoresis, and transferred to PVDF membrane. After blocking for 1 h, membranes were incubated with primary antibodies (β-actin, ERK1/2, pERK1/2) for 2 h at room temperature. After 4 times of

Table 2. miRNA-106b expression in transfected MCF-7 cells

Time point	miRNA-27b mimic	miRNA-27b inhibitor	Mimic control	Control
miRNA-106b				
24 h	1.103±0.021a,b,c	0.294±0.022b,c	0.712±0.015	0.787±0.013
48 h	1.438±0.036a,b,c,d	0.067±0.011b,c,d	0.684±0.012	0.768±0.015
72 h	1.894±0.075a,b,c,d,e	0.022±0.003b,c,d,e	0.667±0.013	0.762±0.012

a: P<0.05, compared with miRNA-106b inhibitor group; b: P<0.05, compared with mimic control group; c: P<0.05, compared with control group; d: P<0.05, compared with that of 24 h; e: P<0.05, compared with that of 48 h.

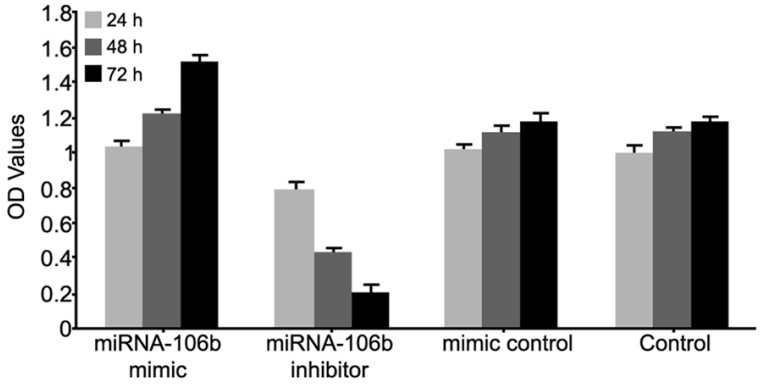

Figure 1. Proliferation capability of MCF-7 cells after transfection. a: P<0.05, compared with miRNA-106b inhibitor group; b: P<0.05, compared with mimic control group; c: P<0.05, compared with control group; d: P<0.05, compared with that of 24 h; e: P<0.05, compared with that of 48 h.

washing with TBST, membranes were incubated with secondary antibodies at room temperature for 1 h. Membranes were then washed, developed and fixed. Quantity One software was used for image analysis.

Statistical analysis

SPSS17.0 software was used to analyze the data. Data was expressed as mean ± standard deviation. Differences were analyzed using χ^2 test or ANOVA. P<0.05 was considered statistically different.

Results

miRNA-106b expression in transfected MCF-7 cells

RT-PCR results showed that miRNA-106b expression was significantly up-regulated after transfection of miRNA-106b mimic and the expression of miRNA-106b increased along with transfection time (P<0.05). miRNA-106b expression was significantly down-regulated after transfection of miRNA-106b inhibitor and the expression of miRNA-106b decreased along with transfection time (P<0.05) (**Table 2**).

MTT assay of proliferation of MCF-7 cells after transfection

MTT assay results showed that OD values and cell proliferation were significantly increased in miRNA-106b mimic group in a time-dependent manner (P<0.05). In contrast, OD values and cell proliferation were significantly decreased in miRNA-106b inhibitor group in a time-dependent manner (P<0.05) (**Figure 1**).

Transwell assay of invasion and migration of MCF-7 cells after transfection

Transwell assay results showed that cells invasion and migration were significantly enhanced in miRNA-106b mimic group (P<0.05), while the invasion and migration were significantly suppressed in miRNA-106b inhibitor group invasion (P<0.05) (**Figure 2**).

Expression levels of AKT and PTEN in MCF-7 cells after transfection

Western blot analysis results showed that compared with mimic control and blank control group, AKT expression was significantly suppressed while PTEN expression was significantly enhanced in MCF-7 cells transfected with miRNA-106b mimic. In contrast, the AKT expression was significantly enhanced while PTEN expression was significantly suppressed in MCF-7 cells transfected with miRNA-106b inhibitor (P<0.05) (**Table 3**; **Figure 3**).

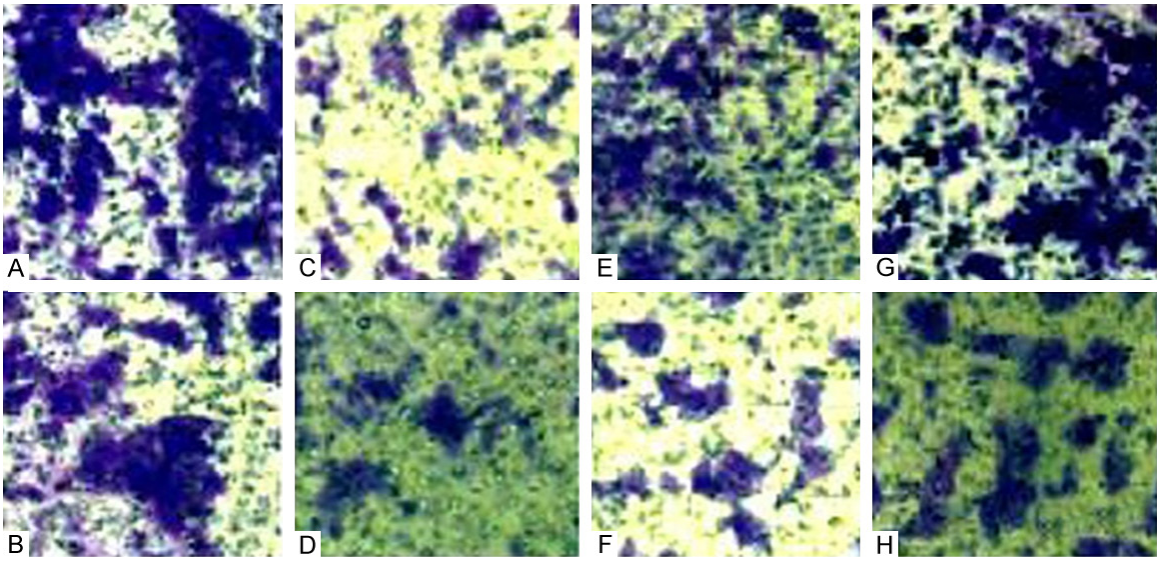

Figure 2. Transwell assay of invasion and migration of MCF-7 cells. A: miRNA-106b mimic, invasion; B: miRNA-106b mimic, migration; C: miRNA-106b inhibitor, invasion; D: miRNA-106b inhibitor, migration; E: Mimic control invasion; F: Mimic control, migration; G: Control, invasion; H: Control, migration.

Table 3. Expression levels of AKT and PTEN in MCF-7 cells after transfection

Group	AKT	PTEN
miRNA-106b mimic	0.24±0.28a,b,c	0.46±0.25a,b,c
miRNA-106b inhibitor	0.56±0.11b,c	0.23±0.12b,c
Mimic control	0.81±0.13	0.78±0.11
Control	0.87±0.66	0.81±0.53

a: P<0.05, compared with miRNA-106b inhibitor group; b: P<0.05, compared with mimic control group; c: P<0.05, compared with control group.

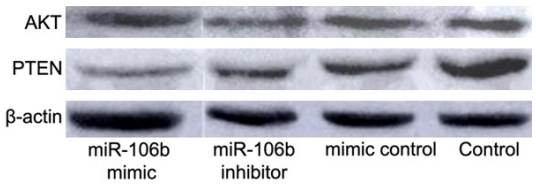

Figure 3. Expression levels of AKT and PTEN in MCF-7 cells after transfection.

Discussion

In recent years, the incidence of breast cancer in the female population of China shows an increasing trend. Breast cancer seriously threatens the health and quality of life of women. However, the mechanisms of breast cancer occurrence, development, invasion, and metastasis remain unclear. More and more studies point out that some miRNAs play an important role in the pathogenesis of cancer by playing a role of proto-oncogene or tumor suppressor. Among many of the studies on microRNA, the study of miR-106b attracts more interests [9]. MicroRNAs, as a newly discovered large class of short sequences, non-coding, regulatory, single-stranded small molecule RNA, play an important role in the development of malignant tumors [10]. Through binding of its 2-8 5'-end bases to 3'-UTR of target genes, microRNAs suppresses gene transcription, inhibits gene translation process, reduces the level of target proteins, regulates cell cycle, growth, differentiation, apoptosis and a variety of biological reactions [11-13]. Moreover, some miRNAs can also be involved in signal transduction pathways that promote or suppress tumor. Studies showed that various miRNAs involved in proliferation, invasion and metastasis of breast cancer via their target genes [14]. miR-106b, an important oncogenic miRNA which locates in chromosome 7q22.1, involves in a variety of cancers. It has been shown that overexpression of miR-106b promoted gastric cancer. miR-106b also plays a catalytic role in the local invasion and distant metastasis of by induction of TGF-α signaling [15]. These findings suggested that miR-106b is highly expressed in tumor cells and can promote tumor cell growth, proliferation, and differentiation of effect. This study was conducted

to investigate the effect of miRNA-106b on the proliferation, invasion and migration of breast cancer MCF-7 cells.

In the present study, miRNA-106b mimics, miRNA-106b inhibitor, or mimic control was transfected into the breast cancer MCF-7 cells. The expression of miRNA-106b was up-regulated in miRNA-106b mimic group and the expression of miRNA-106b was down-regulated in miRNA-106b inhibitor group in a time-dependent manner, suggesting that miRNA-106b is expressed in breast cancer MCF-7 cells. miR-106b is widely expressed in human lung, lymphatic system, spleen, testes, colorectal and other tissues. It has been shown that miR-106b is overexpressed in a variety of tumor cells. Clinical data also suggested that microRNA has a role in promoting development of breast cancer, and the level of microRNA might have some relevance to the prognosis of breast cancer patients [16].

The present study showed that OD values and cell proliferation were significantly increased in miRNA-106b mimic group, but significantly decreased in miRNA-106b inhibitor group. In addition, cells invasion and migration were significantly enhanced in miRNA-106b mimic group, while the invasion and migration were significantly suppressed in miRNA-106b inhibitor group invasion. These data suggested that miRNA-106b promotes proliferation, invasion and migration of breast cancer MCF-7 cells. It has been shown that miR-106b regulated the invasion and migration of tumor cells, affected normal cell cycle, and changed the microenvironment of tumor cells by regulating the expression of Bim, p21, PTEN, Smad7, and other proteins [17]. Studies showed that down-regulation of miR-106b inhibited invasion and metastasis of cervical cancer cells. Up-regulation of miR-106b promoted invasion and metastasis of cervical cancer cells [18].

Finally, we examined the expression of AKT and PTEN in MCF-7 cells after transfection. Expression level of AKT was reduced and the expression level of PTEN was increased in miRNA-106b mimic group reduced, in contrast, the expression level of AKT was increased and the expression level of PTEN was decreased in miRNA-106b inhibitor group. According to the prediction results of TargetScan, Pictar Mirnada and many other resources, PTEN might be the target gene of miR-106b. PTEN is a tumor suppressor gene which is down-regulated, deleted, or mutated in many human tumors. Down-regulation of PTEN resulted in the resistance of tumor cells to chemotherapy, enhancement of cell invasion and metastasis of tumor cells. On the contrary, up-regulation of PTEN suppressed invasion and metastasis of tumor cells to a certain extent [19]. AKT is downstream protein kinase which can promote malignant cell growth, differentiation, and survival to increase the invasion and metastasis [20]. The results of this study suggested that miR-106b might promote the proliferation, invasion, and migration of breast cancer MCF-7 cells by acting on the PTEN/AKT signaling pathway.

Conclusion

miRNA-106b is expressed in breast cancer MCF-7 cells. miR-106b might promote the proliferation, invasion, and migration of breast cancer MCF-7 cells by acting on the PTEN/AKT signaling pathway. The interaction and mutual regulation between miRNA-106b and PTEN/AKT signaling pathway need further exploration. Further study of miRNA-106b will provide new ideas and targets for a more rapid and accurate clinical diagnosis or prognosis of breast cancer.

Acknowledgements

This work was supported by Supported by Science and Technology Foundation of Guizhou Province (No. J[2009]2200).

Disclosure of conflict of interest

None.

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