EXPLORATION ON THE MECHANISM OF HSA-MIR-877-3P IN THE REGULATION OF THE CELL BIOLOGICAL BEHAVIOR OF BREAST CANCER CELLS BY PARTICIPATING IN HER-2 SIGNALING PATHWAY

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ABSTRACT

Objective: *To analyse the mechanism of hsa-miR-877-3p in the Regulation of the Cell Biological Behaviour of Breast Cancer Cells by Participating in HER-2 Signalling Pathway.*

Methods: *The human breast cancer cell line MCF-7 with a low HER-2 expression was cultured in vitro, with the human breast cancer cell line HER-2/MCF-7 being constructed by overexpressing HER-2 transfected MCF-7 cells. Expression of hsa-miR-877-3p in MCF-7 cells and HER-2/MCF-7 cells was detected using real-time quantitative PCR; HER-2/MCF-7 cells transfected with an overexpression of hsa-miR-877-3p were used as the transfection group, and HER-2/MCF-7 cells transfected with empty plasmids were the control group. The cell biological behaviours of the two groups were detected through a scratch test, MTT, and Transwell experiments.*

Results: *The expression of hsa-miR-877-3p in HER-2/MCF-7 cells were significantly lower than that in MCF-7 cells, and the difference was statistically significant (P<0.05); the results of the scratch test showed that the scratch healing rate after the HER-2/MCF-7 cells was transfected with hsa-miR-877-3p was significantly slower than that of the control group, and the difference was statistically significant (P<0.05). The results of the MTT assay showed that the proliferation after HER-2/MCF-7 cells were transfected with hsa-miR-877-3p was significantly lower than that of the control group (P<0.05), and the difference was statistically significant (P<0.05); The results of the MTT assay showed that the proliferation of the HER-2/MCF-7 cells which were transfected with hsa-miR-877-3p was significantly lower than that of the control group (P<0.05), and the difference was statistically significant (P<0.05).*

Conclusion: *The hsa-miR-877-3p expression is obviously down-regulated in human breast cancer HER-2/MCF-7 cells, and this can significantly inhibit the proliferation, invasion, and migration of HER-2 positive breast cancer cells. Consequently, this can be a new target for the treatment of breast cancer.*

Keywords: *hsa-miR-877-3p, HER-2 signalling pathway, breast cancer, biological behaviour.*

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Introduction

Breast cancer is a malignant tumour that occurs in the epithelial tissues of the breast gland, with an incidence rate of 7% to 10% in the systemic malignant tumours. This can occur in women before and after menopause. In contrast, the male incidence rate is only 1% to 2%, and as such, has become one of the malignant tumours that severely affect women's physical and mental health[(1)]. Breast cancer patients often present breast tumours, nipple discharge, skin-like changes on the skin and abnormalities of the nipple-areola, with its pathogenesis is not fully understood. It is believed to be closely related to heredity, early menarche, and an undiagnosis of benign breast disease[(2)]. Indeed, breast cancer has not been found to have a clear molecular target at this point. Although clinical surgery, radiotherapy, and chemotherapy are effective, long-term efficacy is unsatisfactory. Therefore, it is of great significance to explore improved treatment options of breast cancer genetics and molecular biology. miRNAs are a

class of endogenous short-chain RNAs that are approximately 22 nt in length, and this can regulate the expression of genes after transcription through specific bases of the targeted miRNAs, inhibiting their translation[3]. Studies have confirmed that the abnormal expression of miRNA can activate related genes to participate in the occurrence and development of multiple tumours[4]. Human epidermal growth factor receptor-2 (HER-2) is one of the important biomarkers for tumours, and this is highly expressed in highly malignant tumours such as gastric cancer and breast cancer[5]. In recent years, studies have also shown that HER-2 overexpression and miRNA dysregulation are closely related to the occurrence and development of breast cancer[6]. Thus, this study is aimed at analysing the mechanism of hsa-miR-877-3p in the regulation of the cell biological behaviour of breast cancer cells by its participation in the HER-2 signalling pathway.

Materials and methods

Experimental reagents and instruments

Human breast cancer cell line MCF-7 with a low expression of HER-2 was purchased from the University of Science and Technology of China; RPMI1640 medium, fetal bovine serum was purchased from Gibco, USA; Trizol was purchased from Invitrogen; protein absorbance detection kit was purchased from BIO-RAD in the United States; mouse anti-human GAPDH antibody was purchased from Signa, USA; agarose was purchased from Shanghai Shenggong Bioengineering Co., Ltd.; RNase inhibitor was purchased from Epicentre.

The CO_2 incubator was purchased from Thermo Fisher Scientific Inc.; the inverted microscope was purchased from the Japanese Olympus company; the ultraviolet spectrophotometer was purchased from the US company, Backman; the PCR instrument was purchased from the US PE2000 company; the -20 °C refrigerator was purchased in Haier, China. Company; gel imaging system was purchased from Shanghai Tianneng Technology Co., Ltd.; and the imaging acquisition system was purchased from the Nikon Corporation of Japan.

Cell culture

MCE-7 cells were cultured in RPMI-1640 medium that contained 10% fetal bovine serum and was cultured in 37 °C and 5% CO_2 incubator. In addition, 0.5 mmol/ml G418 was added into DMEM high glucose medium for HER-2/MCF-7 cells culture, which was then placed in a 37 ° C and 5% CO_2 incubator, and cells that were grown in the log phase were subjected to trypsin digestion and passaged for experiments. The human breast cancer cell line HER-2/MCF-7 was constructed by overexpressing the HER-2 transfected MCF-7 cells; HER-2/MCF-7 cells transfected with overexpressed hsa-miR-877-3p were used as transfection group, and the HER-2/MCF-7 cells were transfected with empty plasmids as a control group.

Experimental methods

• *The expression of hsa-miR-877-3p in MCF-7 cells and HER-2/MCF-7 cells were detected by real-time quantitative PCR:*

2μl of 10×RT buffer, 2μl of MMLV reverse transcriptase, 0.5μl of RT-specific primer, 1μg of Total RNA, 2μld of NTP, and 0.3μl of RNase inhibitor were added into 20μl of the total reaction system in turn, which was then carefully and gently pipetted. After centrifugation, reverse transcription reaction was performed in a PCR amplifier, and the reaction product was stored in a refrigerator at -20 °C for future use; The ROX, cDNA, and U6/miRNAs primers were diluted 40-fold with sterile water, and then subjected to real-time quantitative PCR reaction, and the miRNAs had hsa-miR-877-3p as the purpose.

• *The cell healing rate of the transfection group and control group was detected by a scratch test:*

After transfection of hsa-miR-877-3p, it was covered with 90% under a cytoscopic microscope and starved for 12 hours in a serum-free DMEM medium. The scratches on the central axis of the 6-well plate were observed with a 20 μL pipette. Three fields of view under the microscope were randomly selected for photographing and recorded as 0h. The culture was carried out in a 37°C and 5% CO_2 incubator, and photographs were taken 24 h and 48 h after the scratch test, respectively. Scratch healing rate = (0h scratch width - 24h or 48h scratch width) / 0h scratch width×100%.

• *Cell proliferation in transfected and control groups was detected by the MTT method:*

DMEM medium was used as the zero-adjusting well, while the negative control group and the hsa-miR-877-3p simulation experiment group were set. After transfecting HER-2/MCF-7 cells with 6-well plates for 48 hours, trypsinization, centrifugation, resuspension, and counting were performed. 1 well, which contained 1×103 cells per well, was inoculat-

ed into 96 wells and added into 200 μl of medium. 200 μl of DMEM medium containing 10% of FBS was added to the withering holes, and 96 well plates were placed in a 37°C and 5% CO_2 incubator, while 96-well plates were taken at four time-points of 24, 48, 72, and 96 h, respectively. 20 μl of MTT solution (5 mg/ml) was added into 96 wells and then cultured in a 37°C and 5% CO_2 incubator for 4 hours. The liquid of each well of the 96-well plate was aspirated, and the culture was then terminated. 150 μl of dimethyl sulfoxide liquid was then added into each well, which was then shaken vigorously for 15 min to sufficiently dissolve the crystals. The absorbance value was measured at a wavelength of 490 nm using a microplate reader.

• *Cell invasion and migration in transfected and control groups were detected by transwell assay:*

HER-2/MCF-7 cells transfected with hsa-miR-877-3p were cultured in 6-well plates for 48 h and starved for 12 h in serum-free DMEM medium. Indeed, while transwell invasion experiments required glue laying, migration experiments did not require glue. After dissolving the matrigel, the DMEM culture was diluted and mixed at a ratio of 1:7, before being carefully uniformed; 60 μl of Matrigel was added into a pre-cooled tip, and the 24-well plate was immediately shaken. It is also to be noted that the aforementioned steps needed to be completed on ice. After sealing the 24-well plate, it was placed in a refrigerator overnight at 4°C, and take it out in a 37°C incubator 4 h before the experiment. The transfected HER-2/MCF-7 cells were then washed with PBS, and the fetal bovine serum was added to stop the digestion reaction after the trypsinization completed. The cell suspension in the 6-well plate was added to a centrifuge tube, and the cells were resuspended by adding PBS. After centrifugation, the supernatant was discarded, and a 24-well plate that contained a chamber was placed in a 37°C and a 5% CO_2 incubator for cultivation. In the 24-well plate, a new small hole was selected and washed with PBS. The cells on the chamber and the matrigel were also wiped off using the cotton swab, before being observed under an inverted microscope, photographed, and counted.

Statistical methods

In this study, the t-test was used to compare the data of the experimental group and the control group. At $P<0.05$, it was considered statistically significant. The data of this study were analysed by the SPSS21.0 software package.

Results

Expression of hsa-miR-877-3p in MCF-7 cells and HER-2/MCF-7 cells

The results of real-time quantitative PCR showed that the expression of hsa-miR-877-3p in HER-2/MCF-7 cells was significantly lower than that in MCF-7 cells, and the difference was statistically significant ($P<0.05$), as seen in picture 1.

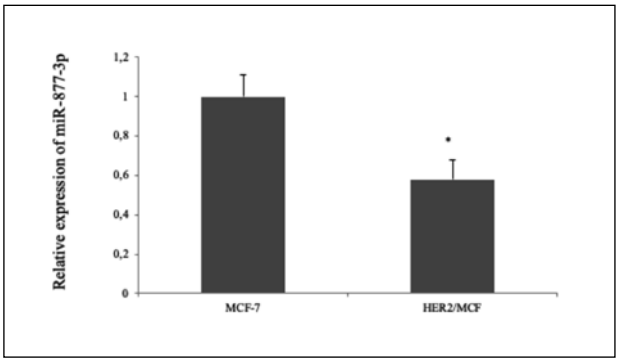

Figure 1: Expression of hsa-miR-877-3p in MCF-7 cells and HER-2/MCF-7 cells.
*Note: Compared with MCF-7 cell group *P<0.05.*

Results of scratch test after the transfection of hsa-miR-877-3p in HER-2/MCF-7 cells

The results of the scratch test showed that the scratch healing rate after HER-2/MCF-7 cells that were transfected with hsa-miR-877-3p was significantly slower than that of the control group, and the difference was statistically significant ($P<0.05$). This is shown in figure 2.

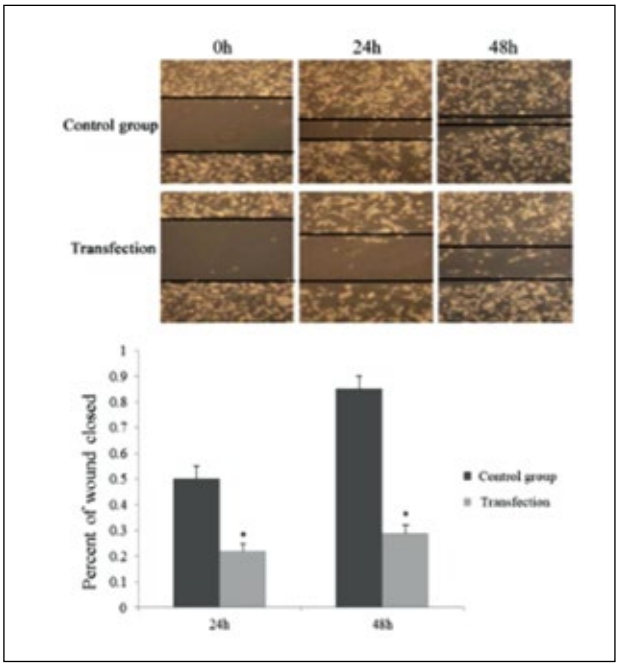

Figure 2: Results of the scratch test after HER-2/MCF-7 cells were transfected with hsa-miR-877-3p.

Results of proliferation after HER-2/MCF-7 cells transfected with hsa-miR-877-3p

The results of the MTT assay showed that the proliferation of HER-2/MCF-7 cells transfected with hsa-miR-877-3p was significantly lower than that of the control group, and the difference was statistically significant (P<0.05). This is shown in figure 3.

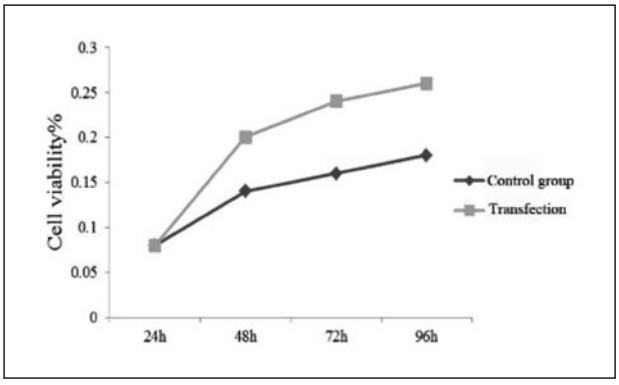

Figure 3: Results of proliferation after HER-2/MCF-7 cells were transfected with hsa-miR-877-3p.

Invasion and migration ability after HER-2/MCF-7 cells transfected with hsa-miR-877-3p

The results of the Transwell invasion and migration experiments showed that the number of cells permeated by HER-2/MCF-7 cells transfected with hsa-miR-877-3p was significantly lower than that of the control group, and the difference was statistically significant (P<0.05), as shown in Figure 4.

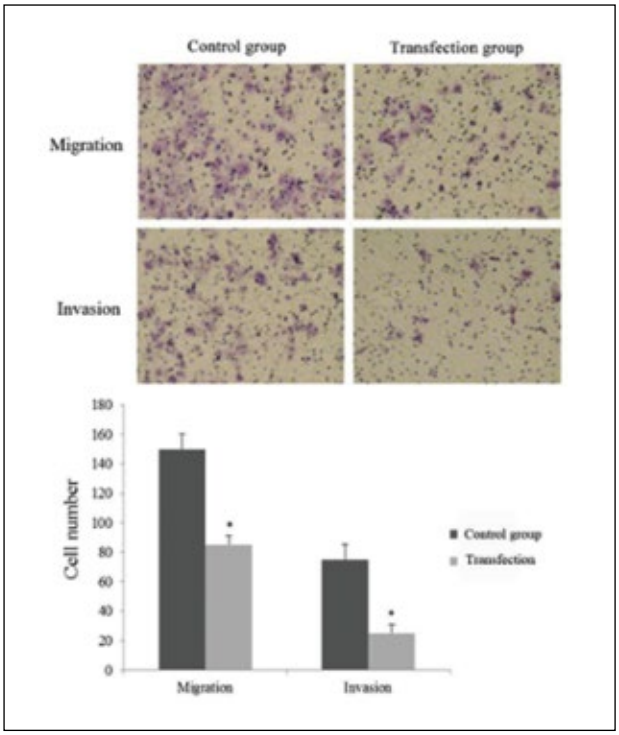

Figure 4: Invasion and migration ability after HER-2/MCF-7 cells transfected with hsa-miR-877-3p.

Discussion

Breast cancer is a common malignant tumour in women. The number of incidence cases accounts for an increasing percentage in the total number of people who are diagnosed with cancer globally. The number of new cases annually exceeds that of women who suffer from lung cancer and colorectal cancer, and the mortality rate is second only to lung cancer. The pathogenesis of the disease is still unclear, and women who are considered to have high-risk factors for breast cancer are more likely to develop breast cancer[7-8].

Currently, the treatment of breast cancer often utilises surgery combined with radiotherapy and chemotherapy in the clinic, but 30% to 40% of breast cancer patients have the risk of developing metastatic breast cancer after chemotherapy.

In recent years, due primarily to the advancement of medical molecular biology technology, many clinical studies have found that there are many factors in the occurrence and development of breast cancer can affect the biological behaviour of tumour cells, so early detection and diagnosis would be the key to improve the efficacy of clinical treatment[9].

The HER-2 is located on the q21 region of chromosome 17 and is highly expressed in placenta, embryonic epithelial tissues, and many tumour cells. It is a proto-oncogene that is closely related to the occurrence and development of breast cancer[10]. Relevant data has shown that HER-2 is highly expressed in 20%~30% of breast cancer patients, and its high expression often indicates that the tumour is highly malignant and prone to metastasis, with the prognosis being poor[11].

When the gene is amplified, HER-2 can cause subtypes of malignant tumours, and patients can express high expression of HER-2 protein tumours. As such, the clinical targeted therapy of HER-2 gene can significantly reduce patients' death rates. The expression of some miRNAs in tumours is reduced during tumour development, which further enables tumours to specifically recognize downstream target genes.

Studies have found that overexpression of miRNAs upstream of HER-2 gene can increase HER-2 enrichment and have sought to further analyse its downstream target genes, as it can provide more ideas for clinical drug targeted therapy. In tumours, including prostate cancer and glioma, some miRNAs are highly expressed or under-ex-

pressed to regulate target genes, and directly participate in the proliferation, invasion and metastasis of tumour cells. Some scholars have analysed the expression of miR-24 in the blood of patients that suffer from acute leukemia and found that its level is significantly higher than the normal population. Indeed, an increase of its level has been found to be inversely proportionate to the survival time of patients, and the prognosis is closely related[12]. In breast cancer, miRNAs play different roles in the HER-2 signalling pathway.

In oncogene miRNAs, miR-194 can directly regulate the downstream target gene talin2, while the HER-2 inhibitor Herceptin can inhibit talin2 activity. Therefore, inhibition of miR-194 expression can induce the occurrence of breast cancer[13]. Furthermore, miR-221 can inhibit apoptosis and promote HER-2 positive breast cancer metastasis. The expression of tumour suppressor PTEN can thus inhibit miR-221-induced malignant cell phenotype, suggesting that it can promote Herceptin resistance and the metastasis of HER-2 positive breast cancer, while multiple miRNAs can jointly regulate the HER-2 gene, which can also be regulated by the HER-2 gene in turn[14-15].

Related data has shown that[16], miRNA and HER-2 related genes are 214, and this study has screened hsa-miR-877-3p for observing breast cancer cell proliferation, invasion, migration and other biology behaviours that involve the participation of the HER-2 signalling pathway.

The real-time quantitative PCR assay showed that the expression of hsa-miR-877-3p in MCF-7 transfected HER-2 cells was significantly lower than that in MCF-7 cells ($P<0.05$). The results also showed that the scratch healing rate after HER-2/MCF-7 cells transfected with hsa-miR-877-3p was significantly slower than that of the control group ($P<0.05$). The results of the MTT assay showed that the proliferation after HER-2/MCF-7 cells transfected with hsa-miR-877-3p was significantly lower than that of the control group ($P<0.05$); and the results of MTT assay showed that the proliferation after HER-2/MCF-7 cells transfected with hsa-miR-877-3p was significantly lower than that of the control group ($P<0.05$), suggesting that hsa-miR-877-3p has a significant inhibitory effect on the proliferation, invasion, and migration of breast cancer cells.

In conclusion, hsa-miR-877-3p expression is significantly down-regulated in human breast cancer HER-2/MCF-7 cells, and this can significantly inhibit the proliferation, invasion, and migration of HER-2 positive breast cancer cells. This would also mean that it can be a new target for the treatment of breast cancer in clinics.

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