

Expression of miRNA-206 and miRNA-145 in breast cancer and correlation with prognosis

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Abstract. Correlation between microRNA (miRNA)-206 and miRNA-145 expression and prognosis in breast cancer was investigated. Breast cancer specimens and paracancerous tissues of 372 breast cancer patients who underwent surgical resection in the First Affiliated Hospital of Shantou University Medical College from September 2010 to September 2014 were included. qRT-PCR was used to detect the expression of miR-206 and miR-145 in breast cancer and paracancerous tissues, and patients were divided into high and low expression groups according to the median expression level to plot survival curve. Expression levels of miR-145 and miR-206 in breast cancer tissues were 2.24 ± 1.23 and 0.76 ± 0.24 , respectively. Expression level of miR-145 was significantly lower, while expression level of miR-206 was significantly higher in tumor tissues than in paracancerous tissues ($p < 0.05$). The 3-year survival rates of miR-145 low expression group and miR-206 high expression group were also lower than that of miR-145 high expression group and miR-206 low expression group, respectively ($p < 0.05$). Expression of miR-206 is upregulated and expression of miR-145 is downregulated in breast cancer, which may have an impact on the prognosis of patients. miR-206 and miR-145 may serve as important indicators to predict prognosis of patients with breast cancer in the future.

Introduction

Breast cancer is one of the most common malignant tumors in women, accounting for 8-12% of all malignancies (1). Coates *et al* (2) showed that in 2015 breast cancer affected approximately 1.4 million new cases, and the incidence is rising. Breast cancer frequently occurs in developed countries in Europe and North America, and incidence is

highest in the United States (3). Turner *et al* (4) predict that the incidence of breast cancer will exceed 50% within the next 50 years and will become the second most common malignant tumor after gastric cancer. In addition, breast cancer at early stage usually shows no obvious symptoms, and can be easily ignored, leading to the high mortality rate. Hindié and Groheux (5) showed that the 5-year survival rate of breast cancer patients was only 62.4%. Because of its high incidence and mortality, breast cancer has long been a hot clinical research topic. MicroRNAs (miRNAs) have been proven to participate in the development of many types of tumors (6-8). Among them, miRNA-206 and miRNA-145 were proved by Sun *et al* (9) to be associated with female ovarian cancer. Therefore, we speculate that miRNA-206 and miRNA-145 may also show unique expression in breast cancer. Our study investigated the application values of miRNA-206 and miRNA-145 as prognostic or therapeutic indicators for breast cancer.

Patients and methods

Patient data. Breast cancer specimens and paracancerous tissues (within 5 cm around the tumor) of 372 breast cancer patients who underwent surgical resection in the First Affiliated Hospital of Shantou University Medical College (Shantou, China) from September 2010 to September 2014 were included. Patients were aged 30-75 years with an average age of 45.32 ± 7.21 years (Table I). Pathological classification and staging were based on the 2007 International Breast Cancer Typing Guidelines (10).

Inclusion and exclusion criteria. Inclusion criteria were: Patients confirmed with breast cancer by pathological biopsy in the First Affiliated Hospital of Shantou University Medical College. Cancerous tissue was placed in liquid nitrogen and stored at -80°C immediately after surgical resection. Before the operation, patients were not treated with radiotherapy or chemotherapy and patients with complete medical record. Exclusion criteria were: Combined with other cardiovascular and cerebrovascular diseases, respiratory tract and gastrointestinal disease patients, pregnant women, long-term bedridden patients, patients with physical disabilities, surgery-intolerant patients, patients transferred to other hospitals during treatment and patients who received

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Table I. Basic information of the patients.

Cases (n=372)	No.	%
Age (years)		
<50	152	40.9
≥50	220	59.1
Body weight (kg)		
<60	164	44.1
≥60	208	55.9
Residence		
City	254	68.3
Countryside	118	31.7
Ethnicity		
Han nationality	364	97.8
Minority	8	2.2
Types		
Non-invasive cancer	97	26.1
Early invasive cancer	134	36.0
Special type invasive cancer	51	13.7
Non-special type invasive cancer	90	24.2
Pathological staging		
I	46	12.4
II	96	25.8
III	136	36.6
IV	94	25.3
T staging		
Tis	35	9.4
T1	94	25.3
T2	127	34.1
T3	62	16.7
T4	54	14.5
N staging		
N1	86	23.1
N2	174	46.8
N3	112	30.1
Distant metastasis		
Yes	243	65.3
No	129	34.7

unauthorized treatment. The study was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. Signed informed consents were obtained from the patients or guardians.

Main instruments and reagents. LightCycler real-time PCR instrument (Roche Diagnostics, Basel, Switzerland), total RNA extraction TRIzol kit (Invitrogen; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA), M-MLV reverse transcriptase kit was from Vazyme Biotech Co., Ltd., (Nanjing, China). miR-206, miR-145 and real-time PCR kit from Biomiga China (Shanghai, China). Primers of miR-206, miR-145 and U6 (endogenous control) used in PCR reaction were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table II).

Table II. Primers of miR-206, miR-145 and U6.

Primer sequences	
miR-206	F 5'-ATCCAGTGCCTGTCGTG-3'
	R 5'-TGCTTGGAATGTAAGGAAG-3'
miR-145	F 5'-ACACTCCAGCTGGGCAGGTCAAAA
	GGGTCC-3'
	R 5'-GGTGTCGTGGAGTCG-3'
U6	F 5'-GCTTCGGCAGCACATATACTAAAAT-3'
	R 5'-CGCTTCACGAATTTGCGTGTCA-3'

F, forward; R, reverse.

Detection methods. Breast cancer tissue (80 mg) was ground in liquid nitrogen. TRIzol reagent was added and mixed, and the mixture was kept at room temperature for 30 min. Total RNA was extracted in strict accordance with the manufacturer's instructions. The extracted RNA was tested by ultraviolet spectrophotometer (Bio Rad, Hercules, CA, USA) and electrophoresis to determine the concentration and purity. Total RNA was then reverse-transcribed according to the instructions of reverse transcription kit, and cDNA samples were stored at -20°C. PCR reaction system was prepared according to the manufacturer's instructions (10.5 μl), and DEPC water was added to make a 20 μl volume. PCR reaction conditions: 94°C for 10 min, followed by 40 cycles of 94°C for 45 sec, 60°C for 45 sec and 72°C for 45 sec. Data were analyzed using the software provided by the manufacturer by $2^{-\Delta\Delta C_q}$ method (11). U6 was used as endogenous control. The average of three replicates was used as the final result.

Statistical analysis. SPSS 22.0 statistical software was used for data analysis. Measurement data are expressed as mean x standard deviation (SD), comparisons between two groups was performed by t-test. Enumeration data were expressed as rate. Survival curves were plotted using Kaplan-Meier method and compared by log-rank test. $P<0.05$ indicated that the difference was statistically significant.

Results

miR-145 expression. Expression level of miR-145 in breast cancer tissues was 2.24 ± 1.23 , and in paracancerous tissues was 6.24 ± 1.51 . Expression level of miR-145 in breast cancer tissues was significantly lower than that in paracancerous tissues ($t=39.61$, $p<0.001$) (Fig. 1).

miR-206 expression. Expression level of miR-206 in breast cancer tissues was 0.76 ± 0.24 , and in paracancerous tissues was 0.12 ± 0.08 . Expression level of miR-206 in breast cancer tissues was significantly higher than that in paracancerous tissues ($t=48.79$, $p<0.001$) (Fig. 2).

Prognosis of patients. According to the median value of miR-145 and miR-206 expression, patients were divided into miR-145 high expression group (≥ 2.24 , 219 cases), miR-145 low expression group (<2.24 , 153 cases), miR-206 high expression

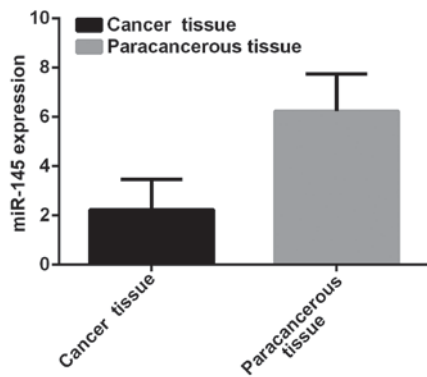


Figure 1. miR-145 expression in breast cancer and paracancerous tissues. Expression level of miR-145 in breast cancer tissues was 2.24 ± 1.23 and that in paracancerous tissues was 6.24 ± 1.51 .

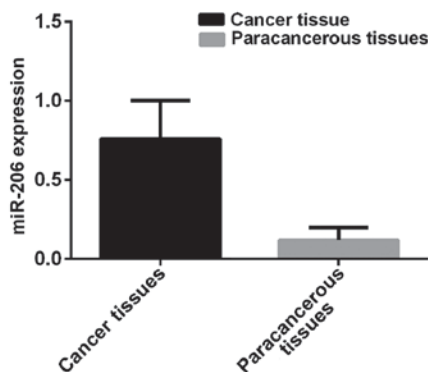


Figure 2. miR-206 expression in breast cancer and paracancerous tissues. Expression level of miR-206 in breast cancer tissues was 0.76 ± 0.24 and that in paracancerous tissues was 0.12 ± 0.08 .

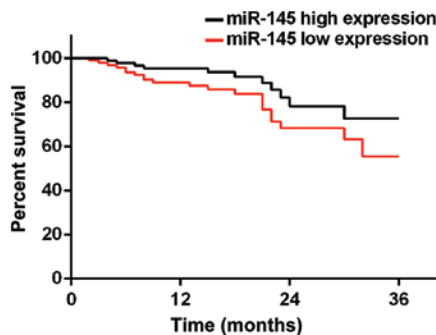


Figure 3. Three-year survival curve of patients with high and low expression level of miR-145. Survival rates at 1, 2 and 3 years in miR-145 low expression group were 83.1, 71.2 and 59.8%, respectively. Survival rates at 1, 2 and 3 years in miR-145 high expression group were 89.5, 79.1 and 70.6%, respectively.

group (≥ 0.76 , 194 cases) and miR-206 low expression group (< 0.76 , 178 cases). Patients were followed up for 3 years by telephone, review and mail. Follow-up was performed until December 2017 or death of the patients. A total of 354 patients finished the follow-up, and follow-up success rate was 95.2%. Survival rates at 1, 2 and 3 years in miR-145 low expression group were 83.1, 71.2 and 59.8%, respectively, while survival rates at 1, 2 and 3 years in miR-145 high expression group were 89.5, 79.1 and 70.6%, which were significantly better than

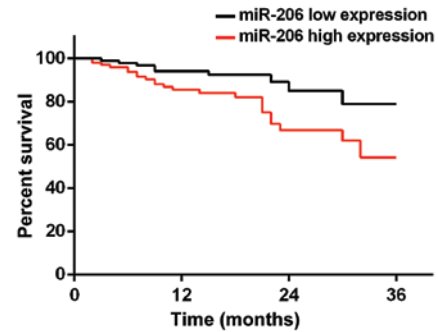


Figure 4. Three-year survival curve of patients with high and low expression level of miR-206. Survival rates at 1, 2 and 3 years in miR-206 high expression group were 77.3, 67.5 and 55.7%, respectively. Survival rates at 1, 2 and 3 years in miR-206 low expression group were 89.9, 81.5 and 75.8%, respectively.

those in miR-145 low expression group ($p=0.028$). Survival rates at 1, 2 and 3 years in miR-206 high expression group were 77.3, 67.5 and 55.7%, respectively, while survival rates at 1, 2 and 3 years in miR-206 low expression group were 89.9, 81.5 and 75.8%, respectively, which were significantly higher than those in miR-206 high expression group ($p=0.034$) (Figs. 3 and 4).

Discussion

Breast cancer is a malignant tumor that seriously affects life and health of females. Incidence and mortality of breast cancer rank in the top area among all malignancies (12). Breast cancer at early stage shows no obvious symptoms and most patients are diagnosed at advanced stages, and thus missing the best treatment time, leading to poor prognosis. At present, pathogenesis of breast cancer is still unclear. Poortmans *et al* (13) believed that the occurrence of breast cancer is mainly caused by genetic factors, while Tutt *et al* (14) showed that the occurrence of breast cancer is closely related to cancer stem cells. miRNAs as a group of endogenous non-protein-coding RNA (15,16) have been proved to be directly involved in tumorigenesis and development. Most miRNAs are highly conserved, cell-specific, and have a strong ability to regulate cell proliferation and apoptosis (17). Among them, miR-206 plays a key role in the regulation of cell proliferation, apoptosis, invasion and migration. miR-206 may play a role as a tumor suppressor gene and may also have oncogenic functions and has been proven to promote muscle differentiation by downregulating the P180 subunit of DNA polymerase and muscle transcription factors (18). miR-145 is expressed in many eukaryotic organisms and plays a role in regulating gene expression and has multiple targets that are associated with oncogenes (19). With the deepening of research, miR-206 and miR-145 have been proven to be closely correlated with breast cancer. Therefore, in this study, expression levels of miR-206 and miR-145 in breast cancer and paracancerous tissues were measured, and the correlation with prognosis were analyzed with an expectation of providing references for diagnosis and treatment of breast cancer.

The results of this study indicate that miR-206 is upregulated in breast cancer tissues and miR-145 is downregulated

in breast cancer tissues. This is in agreement with the finding by Kim *et al* (20) and Oksuz *et al* (21) on the role of miR-206 and miR-145 in ovarian and uterine cancer, suggesting that miR-206 and miR-145 may be involved in the occurrence and development of breast cancer. Expression of miR-206 and miR-145 may be related to the severity of breast cancer, the degree of differentiation, lymph node metastasis and the depth of invasion. miR-206 and miR-145 can be used as tumor markers in the early diagnosis of breast cancer because they can downregulate mRNA expression of ER α and its coregulatory proteins, inhibit the proliferation of breast cancer cells and participate in the development of breast cancer. However, the mechanism of miR-206 and miR-145 in breast cancer remains unclear.

miR-206 and miR-145 may also inhibit the development of breast cancer. Because miR-206 is upregulated in breast cancer and miR-145 is downregulated, and high level of miR-206 expression and level of miR-145 expression was correlated with poor prognosis, suggesting that miR-206 and miR-145 can be used as a prognostic indicator for patients with breast cancer.

There are still some shortcomings in this study. For example, the sample size was small, and it is not ruled out that there may be differences in expression levels of miR-206 and miR-145 among different age groups. We will conduct a longer follow-up survey of patients in this study to confirm our conclusions.

In conclusion, miR-206 is upregulated and miR-145 is downregulated in breast cancer tissues, which may affect the prognosis of patients. miR-206 and miR-145 may be used as important prognostic indicators for patients with breast cancer in the future.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YQ wrote the manuscript and was responsible for collecting the tissues. XH contributed to the extraction of RNA. XQ performed PCR. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College (Shantou, China). Signed informed consents were obtained from the patients or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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