Expression and correlation of miR-124 and miR-126 in breast cancer

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Received November 16, 2018; Accepted March 14, 2019

DOI: 10.3892/ol.2019.10184

Abstract. The expression of micro ribonucleic acid (miR)-124 and miR-126 in different stages of breast cancer (BC) and their correlation were investigated to analyze the role of miR-124 and miR-126 in the occurrence and development of BC. BC tissues of 83 BC patients treated in First Teaching Hospital of Tianjin University of Traditional Chinese Medicine from March 2016 to January 2018 were selected as the research group, while the corresponding para-carcinoma normal tissues were selected as the control group. The relative expression levels of miR-124 and miR-126 in both groups were detected via reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and the miR-124 and miR-126 expression asociated with clinicopathological parameters of patients and the correlation between miR-124 and miR-126 was analyzed. The relative expression levels of miR-124 and miR-126 in the research group were significantly lower than those in the control group (P<0.001). miR-124 and miR-126 in the research group were associated with clinicopathological parameters (clinical stage, degree of pathological differentiation and lymph node metastasis) of patients (P<0.001). According to Pearson's correlation analysis, there was a positive correlation between the relative expression levels of miR-124 and miR-126 in cancer tissues (r=0.497, P<0.001). The expression levels of miR-124 and miR-126 were downregulated in BC tissues, which were associated with clinicopathological parameters (clinical stage, degree of pathological differentiation and lymph node metastasis). There was a positive correlation between the expression levels of miR-124 and miR-126 in BC tissues. Thus, miR-124 and miR-126 may be involved in the occurrence, development, invasion and metastasis of BC, and both can be used as targeted biological indexes for treatment of BC.

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Key words: breast cancer, miR-124, miR-126, clinical stage, degree of differentiation, lymph node metastasis

Introduction

As one of the most common malignant tumors in women, breast cancer (BC) (1) frequently occurs in mammary epithelial tissues and the incidence rate accounts for 8-11% of all systemic malignant tumors, greatly affecting the physical and mental health of females, and even threatening their life in severe cases. According to statistics (2), the incidence rate of BC has shown an increasing trend year by year worldwide. Surgical resection and radiotherapy dominate among the treatment methods of BC (3). However, most patients are at the late stage of BC when diagnosed, and BC is very likely to metastasize even if cancer tissues are resected. Statistical data (4) have shown that the postoperative recurrence rate of patients with advanced BC is up to 60%, and the survival rate of patients also significantly declines after recurrence and metastasis of BC, with a 5-year survival rate of less than 15%. The pathogenesis of BC (5) remains unclear. Current studies (6) have shown that micro ribonucleic acid (miRNA) is associated with a variety of cancers and malignant tumors, and various miRNAs can serve as specific diagnostic markers and new therapeutic targets for different tumors.

miRNA (7) is an endogenous non-coding RNA with a regulatory function, which is involved in cell proliferation, differentiation and apoptosis, and expressed in a variety of tissues. Based on its role in different tumors, miRNA is divided into oncogene (8) and tumor suppressor gene (9). Studies have confirmed (10) that miR-124 expression is low in ovarian cancer tissues, BC tissues and colorectal cancer tissues. Some studies (11) on the expression of miR-126 in BC have also demonstrated that miR-126 expression is low in human BC cells, and has an inhibitory effect on invasion of BC cells. However, there are few studies on simultaneous action of miR-124 and miR-126 on BC. In this study, the expression of miR-124 and miR-126 in tissues of BC patients was investigated, and the association of miR-124 and miR-126 expression with clinicopathological parameters of patients as well as the correlation between miR-124 and miR-126 were analyzed.

Patients and methods

Collection of specimens. BC tissues of 83 female patients, aged 25-60 years with an average age of 50.78±7.04 years,

preserved in the Department of Breast Surgery in First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China) from March 2016 to January 2018 were selected as the research group, while the corresponding para-carcinoma normal tissues of BC patients were selected as the control group. Inclusion criteria: i) patients treated in First Teaching Hospital of Tianjin University of Traditional Chinese Medicine and diagnosed with BC via joint examination of tissue specimens by the Department of Breast Surgery as well as the Pathology Department, and ii) patients receiving no chemoradiotherapy and other treatments before operation. Exclusion criteria: i) patients whose cancer tissues were obtained, but corresponding para-carcinoma normal tissues were not obtained, or ii) patients whose specimens were unqualified with RNA degradation due to improper preservation, or whose specimens were not embedded into paraffin. Patients were informed of the collection of specimens in advance, and signed the informed consent. The study was approved by the Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine.

Main reagents and instruments. Total RNA extraction reagent TRIzol and reverse transcription kit were both purchased from Nanjing Cobioer Biotechnology Co., Ltd. (Nanjing, China), miR-124 polymerase chain reaction (PCR) kit and miR-126 PCR kit were purchased from Thermo Fisher Scientific China Co., Ltd. (Shanghai, China), while the fluorescence quantitative PCR instrument was purchased from Xi'an Tianlong Science & Technology Co., Ltd. (Xi'an, China). The primers of miR-124 and miR-126 as well as the internal reference U6 are shown in Table I.

Experimental procedure. BC tissues or corresponding para-carcinoma normal tissues stored in liquid nitrogen were taken, placed in a pre-cooled homogenizer, and added with TRIzol reagent at a ratio of 100 mg/ml for homogenization. The total RNA was extracted from tissues according to the manufacturer's instructions of the TRIzol reagent, dissolved in 20 μl diethylpyrocarbonate (DEPC)-treated water, sub-packaged and cryopreserved at -80˚C. Then, the total RNA was reverse transcribed to synthesize complementary deoxyribonucleic acid (cDNA) using the reverse transcription kit, followed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) amplification with cDNA as a template. Finally, the expression levels of miR-124 and miR-126 in the research and control groups were detected via RT-qPCR according to the instructions of the miR-124 and miR-126 SYBR-Green PCR kits (Takara, Beijing, China), respectively. The reaction conditions were as follows: 95˚C for 1 min, 95˚C for 15 sec and 60˚C for 20 sec for a total of 39 cycles. A total of 3 repeated wells were set for each specimen, and the experiment was performed 3 times. U6 was used as an internal reference for both miR-124 and miR-126. After reaction, the amplification curve and dissociation curve of RT-qPCR were confirmed, and the relative level of target gene was calculated based on result parameters. The target gene was quantified using the $2^{-\Delta Cq}$ method (12).

Statistical analysis. Statistical Product and Service Solutions (SPSS) 17.0 [Asia Analytics (formerly SPSS, Beijing, China)] software was used for the statistical analysis of research results. Measurement data were expressed as mean ± standard deviation. t-test was used for the comparison of means between the two groups, and the correlation between the two groups was analyzed using Pearson's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of miR-124 and miR-126 in the research and control groups. The expression level of miR-124 in the research group (1.36±0.14) was significantly lower than that in the control group (2.43±0.16) (t=45.850, P<0.001), and the difference was statistically significant. The expression level of miR-126 in the research group (0.24±0.11) was also significantly lower than that in the control group (1.07±0.28) (t=25.140, P<0.001), and the difference was statistically significant (Fig. 1).

Expression of miR-124 and miR-126 in different clinicopathological features of BC patients in the research group. The experimental results revealed that miR-124 in the research group was not related to age and tumor size, but it was related to clinical stage, degree of pathological differentiation and lymph node metastasis (P<0.001; Table II). In the research group, the expression level of miR-124 in tumor-node-metastasis (TNM) stage III-IV (0.70±0.21) was obviously lower than that in stage I-II (2.02±0.53) (t=13.600, P<0.001), and there was a statistically significant difference. In the research group, the expression level of miR-124 with high pathological differentiation (2.36±0.27) was significantly higher than that with moderate-low differentiation (0.36±0.01) (t=38.360, P<0.001), and there was a statistically significant difference. Moreover, in the research group, the expression level of miR-124 with lymph node metastasis (0.40±0.32) was obviously lower than that without lymph node metastasis (2.32±0.16) (t=32.610, P<0.001), displaying a statistically significant difference.

The experimental results manifested that miR-126 in the research group was not related to age or tumor size, but it was related to clinical stage, degree of pathological differentiation and lymph node metastasis (P<0.001; Table III). In the research group, the expression level of miR-126 with high pathological differentiation (0.30±0.07) was significantly higher than that with moderate-low differentiation (0.18±0.14) (t=5.222, P<0.05). In the research group, the expression level of miR-126 in TNM stage III-IV (0.15±0.08) was obviously lower than that in stage I-II (0.33±0.12) (t=7.570, P<0.001), and there was a statistically significant difference. Moreover, in the research group, the expression level of miR-126 with lymph node metastasis (0.19±0.13) was obviously lower than that without lymph node metastasis (0.29±0.10) (t=3.802, P<0.001), displaying a statistically significant difference.

Correlation between miR-124 and miR-126 expression levels in cancer tissues in the research group. According to Pearson's correlation analysis, there was a positive correlation between the relative expression levels of miR-124 and miR-126 in the research group (r=0.497, P<0.001; Fig. 2).

Discussion

BC is a female cancer with high morbidity, recurrence and mortality rate worldwide. According to related statistical

Table I. Primers of miR-124, miR-126 and internal reference U6.

Groups	Forward primers	Reverse primers
miR-124	5'-GCTAAGGCACGCGGTG-3'	5'-GTGCAGGGTCCGAGGT-3'
miR-126	5'-CATTATTACAGGGCAGCGGTCGC-3'	5'-CATTATTACGCGGCAGGTGCCGT-3'
U6	5'-CTCGCTTCGGCAGCACATATACT-3'	5'-ACGCTTCACGAATTT-GCGTGTC-3'

Table II. Expression of miR-124 in different clinicopathological features of BC patients in the research group (mean ± standard deviation).

Factor	n=83	miR-124	t	P-value
Age (years)			0.188	0.850
≤50	40	1.37±0.55		
>50	43	1.35±0.41		
Tumor size (cm)			0.430	0.669
≤2	31	1.38±0.54		
>2	52	1.34±0.31		
TNM stage			13.600	<0.001
I-II	50	2.02±0.53		
III-IV	33	0.70±0.21		
Degree of pathological differentiation			38.360	<0.001
Moderate-low	27	0.36±0.01		
High	56	2.36±0.27		
Lymph node metastasis			32.610	<0.001
No	35	2.32±0.16		
Yes	48	0.40±0.32		

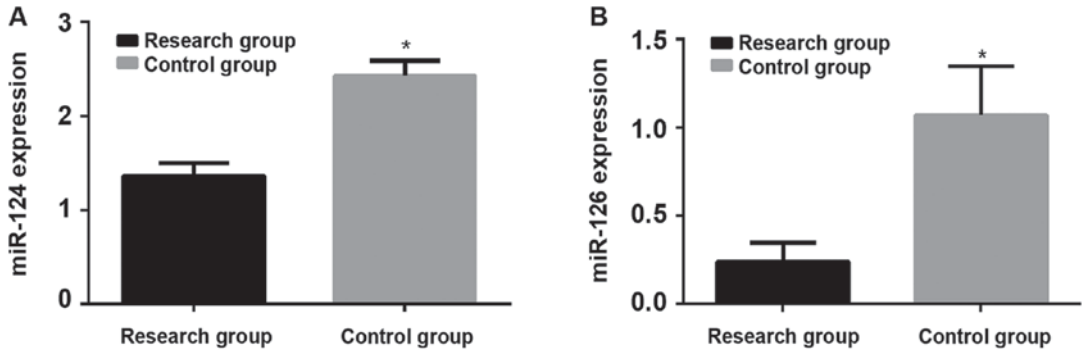

Figure 1. Expression of miR-124 and miR-126 in the research and control groups. (A) Results of RT-qPCR reveal that the expression level of miR-124 in the research group is significantly lower than that in the control group (t=45.850, *P<0.001), showing a statistically significant difference. (B) Results of RT-qPCR reveal that the expression level of miR-126 in the research group is significantly lower than that in the control group (t=25.140, *P<0.001), displaying a statistically significant difference. miR, micro ribonucleic acid; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

data (13), there are more than 1.3 million people suffering from BC every year, but the specific pathogenesis of BC remains unclear. Currently, clinical studies (14) have confirmed that the pathogenesis of BC is related to a variety of factors, such as the endocrine regulation in patients, familial inheritance, benign breast lesions, malignant transformation of BC and geographical environment (15). In the biological study on cancer-related genes in recent years, researchers have focused on protein genes (16), such as S-phase kinase-associated protein 2 (SKP2) (p45). A kind of small non-coding RNA, miRNA, directly related to tumorigenesis has been found recently, which, as an important regulatory factor for tumors, is involved in the occurrence, development, invasion and metastasis of tumor. The expression of miRNA varies from tumor to tumor. Thus, the expression of oncogene in cancer tissues or cells is upregulated, while the expression of tumor suppressor gene in cancer tissues or cells is downregulated (7). miR-126 was first discovered in human BC tissues, and its

Table III. Expression of miR-126 in different clinicopathological features of BC patients in the research group (mean ± standard deviation).

Factors	n=83	miR-126	t	P-value
Age (years)			0.988	0.326
≤50	40	0.26±0.11		
>50	43	0.23±0.16		
Tumor size (cm)			0.802	0.425
≤2	31	0.25±0.09		
>2	52	0.23±0.12		
TNM stage			7.570	<0.001
I-II	50	0.33±0.12		
III-IV	33	0.15±0.08		
Degree of pathological differentiation			5.222	<0.001
Moderate-low	27	0.18±0.14		
High	56	0.30±0.07		
Lymph node metastasis			3.802	<0.001
No	35	0.29±0.10		
Yes	48	0.19±0.13		

expression is low in most tumor tissues (17). miR-124 is also involved in the occurrence and development of a variety of tumors (18).

In this study, the relative expression levels of miR-124 and miR-126 in BC tissues (research group) and para-carcinoma tissues (control group) were detected via RT-qPCR. According to experimental results, the relative expression levels of miR-124 and miR-126 in cancer tissues in the research group were significantly lower than those in the control group (P<0.001), which are basically consistent with research results of Dong *et al* (19). They also detected the expression of miR-124 in cancer tissues and para-carcinoma normal tissues in BC patients using RT-qPCR, and it was found that the expression level of miR-124 in para-carcinoma normal tissues was obviously higher than that in cancer tissues (P<0.001), showing a statistically significant difference. It has been proven that the expression of miR-126 is downregulated in BC tissues (11). The association of miR-124 and miR-126 expression with clinicopathological parameters of patients (age, tumor size, TNM stage, degree of pathological differentiation and lymph node metastasis) in the research group was analyzed, and results revealed that miR-124 and miR-126 had no association with age and tumor size in BC patients, but was associated with TNM stage, degree of pathological differentiation and lymph node metastasis (P<0.01). Finally, the correlation between relative expression levels of miR-124 and miR-126 in BC tissues was analyzed, and a positive correlation in BC was found. The above experimental results are basically consistent with research results of Danesh *et al* (20). Danesh *et al* (20) detected the expression level of miR-124 in BC tissues through immunohistochemical method, and reached the conclusion that the expression level of miR-124 in BC tissues is significantly lower than that in para-carcinoma normal tissues, and miR-124 was associated with clinical stage, lymph node metastasis and differentiation degree of BC, verifying the results in this study.

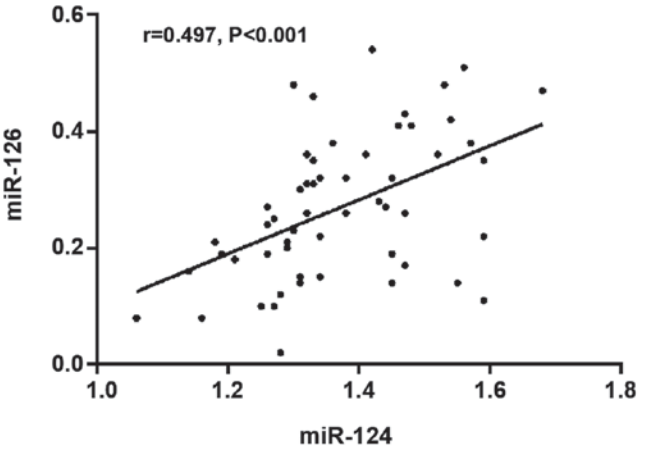

Figure 2. Correlation between miR-124 and miR-126 in cancer tissues in the research group. According to the results of the Pearson's correlation analysis, there is a positive correlation between the relative expression levels of miR-124 and miR-126 in cancer tissues in the research group (r=0.497, P<0.001). miR, micro ribonucleic acid.

In the study of Baldassari *et al* (21), miR-126 was taken as a target gene of BC to investigate its role in BC, and results demonstrated that miR-126 expression was low in BC cells, and increasing the expression level of miR-126 in BC cells, combined with anticancer drugs, can inhibit the development of BC cells. The above research results indicate that miR-126 is a tumor suppressor gene for BC, and it is speculated that miR-126 is related to the occurrence, development, invasion and signal transduction pathway (22) of BC.

In this study, the sample size was small, and there were different regional, familial and racial influences, thus causing certain limitations. However, patients in the experimental group will be followed up regularly according to the data, and results will be detected and analyzed, to further verify the results of this study.

In conclusion, the expression levels of miR-124 and miR-126 were significantly higher in TNM stage I-II than that in stage III-IV, significantly higher with high pathological differentiation than that in moderate-low differentiation, and also obviously higher without lymph node metastasis than that with lymph node metastasis. Moreover, there was a positive correlation between relative expression levels of miR-124 and miR-126 in BC. The above results suggest that miR-124 and miR-126 play an important role in the occurrence and development of BC. Therefore, it is speculated that both miR-124 and miR-126 may serve as clinical monitoring targets for BC, thus, providing new ideas for the specific pathogenesis of BC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and material

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

Authors' contributions

FL wrote the manuscript, performed the experiment and analyzed the data. The author read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China) and informed consents were signed by the patients or guardians.

Patient consent for publication

Not applicable.

Competing interests.

The authors declare that they have no competing interests

References

1. Lantz PM and Booth KM: The social construction of the breast cancer epidemic. Soc Sci Med 46: 907-918, 1998.
2. Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, Colditz GA and Manson JE; Nurses' Health Study: Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care 26: 1752-1758, 2003.
3. Houssami N, Turner RM and Morrow M: Meta-analysis of pre-operative magnetic resonance imaging (MRI) and surgical treatment for breast cancer. Breast Cancer Res Treat 165: 273-283, 2017.
4. Pogoda K, Niwińska A, Murawska M and Pieńkowski T: Analysis of pattern, time and risk factors influencing recurrence in triple-negative breast cancer patients. Med Oncol 30: 388, 2013.
5. Nichols HB, Trentham-Dietz A, Egan KM, Titus-Ernstoff L, Holmes MD, Bersch AJ, Holick CN, Hampton JM, Stampfer MJ, Willett WC, *et al*: Body mass index before and after breast cancer diagnosis: associations with all-cause, breast cancer, and cardiovascular disease mortality. Cancer Epidemiol Biomarkers Prev 18: 1403-1409, 2009.
6. Slattery ML, Herrick JS, Mullany LE, Samowitz WS, Sevens JR, Sakoda L and Wolff RK: The co-regulatory networks of tumor suppressor genes, oncogenes, and miRNAs in colorectal cancer. Genes Chromosomes Cancer 56: 769-787, 2017.
7. Schueller F, Roy S, Vucur M, Trautwein C, Luedde T and Roderburg C: The Role of miRNAs in the pathophysiology of liver diseases and toxicity. Int J Mol Sci 19: 2261, 2018.
8. Wu SG, Huang YJ, Bao B, Wu LM, Dong J, Liu XH, Li ZH, Wang XY, Wang L, Chen BJ, *et al*: miR-508-5p acts as an anti-oncogene by targeting MESDC1 in hepatocellular carcinoma. Neoplasma 64: 40-47, 2017.
9. Gao W, Li W, Xiao T, Liu XS and Kaelin WG Jr: Inactivation of the PBRM1 tumor suppressor gene amplifies the HIF-response in VHL-/-clear cell renal carcinoma. Proc Natl Acad Sci USA 114: 1027-1032, 2017.
10. Zhang H, Wang Q, Zhao Q and Di W: MiR-124 inhibits the migration and invasion of ovarian cancer cells by targeting SphK1. J Ovarian Res 6: 84, 2013.
11. Zhang Y, Yang P, Sun T, Li D, Xu X, Rui Y, Li C, Chong M, Ibrahim T, Mercatali L, *et al*: miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. Nat Cell Biol 15: 284-294, 2013.
12. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402 408, 2001.
13. Mariotto AB, Etzioni R, Hurlbert M, Penberthy L and Mayer M: Estimation of the number of women living with metastatic breast cancer in the United States. Cancer Epidemiol Biomarkers Prev 26: 809-815, 2017.
14. Alakwaa FM, Chaudhary K and Garmire LX: Deep learning accurately predicts estrogen receptor status in breast cancer metabolomics data. J Proteome Res 17: 337-347, 2018.
15. Notaridou M, Quaye L, Dafou D, Jones C, Song H, Høgdall E, Kjaer SK, Christensen L, Høgdall C, Blaakaer J, *et al*; Australian Ovarian Cancer Study Group/Australian Cancer Study (Ovarian Cancer); Ovarian Cancer Association Consortium: Common alleles in candidate susceptibility genes associated with risk and development of epithelial ovarian cancer. Int J Cancer 128: 2063-2074, 2011.
16. Ye D, Li Z and Wei C: Genistein inhibits the S-phase kinase-associated protein 2 expression in breast cancer cells. Exp Ther Med 15: 1069-1075, 2018.
17. Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, Freund M, Lichtner P, Hartmann L, Schaal H, *et al*: Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet 42: 410-414, 2010.
18. Li S, Mei Z, Hu HB and Zhang X: The lncRNA MALAT1 contributes to non-small cell lung cancer development via modulating miR-124/STAT3 axis. J Cell Physiol 233: 6679-6688, 2018.
19. Dong LL, Chen LM, Wang WM and Zhang LM: Decreased expression of microRNA-124 is an independent unfavorable prognostic factor for patients with breast cancer. Diagn Pathol 10: 45, 2015.
20. Danesh H, Hashemi M, Bizhani F, Hashemi SM and Bahari G: Association study of miR-100, miR-124-1, miR-218-2, miR-301b, miR-605, and miR-4293 polymorphisms and the risk of breast cancer in a sample of Iranian population. Gene 647: 73-78, 2018.
21. Baldassari F, Zerbinati C, Galasso M, Corrà F, Minotti L, Agnoletto C, Previati M, Croce CM and Volinia S: Screen for MicroRNA and drug interactions in breast cancer cell lines points to miR-126 as a modulator of CDK4/6 and PIK3CA inhibitors. Front Genet 9: 174, 2018.
22. Wu ZJ, Li Y, Wu YZ, Wang Y, Nian WQ, Wang LL, Li LC, Luo HL and Wang DL: Long non-coding RNA CCAT2 promotes the breast cancer growth and metastasis by regulating TGF-β signaling pathway. Eur Rev Med Pharmacol Sci 21: 706-714, 2017.