

Original Article

Expression profile of miR-155 and miR-34a in peripheral blood and tissues of breast cancer patients

Xiaolang Tan¹, Lei Guo², Shayang Luo², Weiming Wang¹, Kejing Zhang², Feiyu Chen²

¹Department of Oncology, Changsha Central Hospital, Changsha 410004, PR China; ²Department of Breast Surgery, Xiangya Hospital, Central South University, Changsha 410008, PR China

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Abstract: Breast cancer severely affects women's health. The role of microRNA (miR) in the pathological process of tumors has been widely studied as one novel biological marker for diagnosis, typing and prognosis. Previous study has suggested the correlation between breast cancer and miR. This study thus investigated expression level of miR-155 and miR-34a in both blood and tissue samples from breast cancer patients, thus investigating their relationships with tumor occurrence and potential values in early diagnosis and prognosis prediction. A total of 40 breast cancer patients who received surgeries in our hospital were recruited as the experimental group, in parallel with 40 healthy controls. RT-PCR was used to test the expression of miR-155 and miR-34a level in blood samples, and tumor or adjacent tissues. Correlation analysis was performed regarding their expression level and pathological features of tumors. Blood samples of cancer patients had higher miR-155 and lower miR-34a compared to control group ($P < 0.05$). Breast cancer tissue had higher miR-155 and lower miR-34a than adjacent tissues ($P < 0.05$). The expression of miR-155 and miR-34a is correlated with TNM stage, lymph node metastasis and differentiation degree ($P < 0.05$). With advanced TNM stage, lymph node metastasis, and lower differentiation grade, miR-34a was up-regulated while miR-155 was down-regulated. MiR-155 expression was increased and miR-34a was down-regulated in breast cancer patients. Their expression level is related with TNM stage, lymph node metastasis and differentiation grade. Both miR-155 and miR-34a might participate in the occurrence, progression, infiltration and metastasis of breast cancer.

Keywords: Breast cancer, MiR-155, MiR-34a

Introduction

Breast cancer is one common malignant tumor in females, and can be caused by the activation of oncogenes and/or inactivation of tumor suppressor gene. Among various tumor suppressor genes, the most common one is p53, whose mutation and consequent over-expression may account for 25%~40% of all breast cancers [1]. MicroRNA (miR) is one endogenous non-coding single-stranded RNA composing of 17~25 base pairs, and is widely expressed in the body [2]. As one genetic modulating factor, miR participates in about 30% gene transcription in human, and plays a crucial role in cell differentiation, growth, apoptosis and metabolism [3]. As one research hotspot in epigenetics, serum miR-155 expression has been suggested to be closely related with tumor occurrence [4]. For example, in non-small cell lung cancer, p53 regulates miR-34a expression, which can be

down-regulated by p53 knock-down or dysfunction [5]. This study thus recruited breast cancer patients in our hospital, and tested serum levels of miR-155 and miR-34a by real-time quantitative PCR. The differential expression of miR-34a and miR-155 between tumor and adjacent tissues was expressed, in order to analyze their relationships with TNM stage, lymph node metastasis and other clinical/pathological features.

Materials and methods

General information

A total of 40 breast cancer patients (aging between 30 and 65 years old, average = 50.2 ± 3.6 years) who received surgery in Changsha Central Hospital from January 2014 to January 2015 were recruited as the experimental group, which consisted of 10 papilloma,

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Table 1. Primer sequence

Gene	Forward primer	Reverse primer
miR-155	5'-GCGAAAGCATTTGCCAAGAA-3'	5'-CATCACAGACCTGTTATTGC-3'
miR-34b	5'-AAATCACACCCTTCACAGCC-3'	5'-GTTGTAATGGTTCTCCTCCAGC-3'
β -actin	5'-AGCGGGAAATCGTGC GTGACA-3'	5'-GTGGACTTGGGAGAGGACTGG-3'

Table 2. Serum miR-155 and miR-34a levels

Group	miR-155	miR-34a
Experiment group	2.330 \pm 0.211*	0.122 \pm 0.105*
Control group	1.000	1.000

Note: *P<0.05 compared to control group.

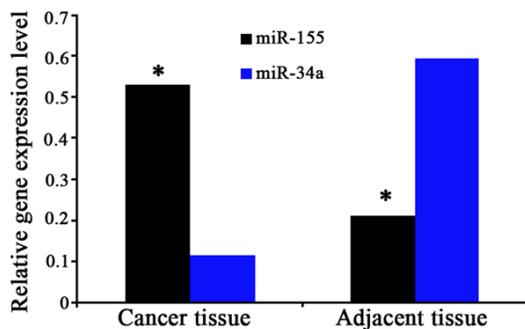


Figure 1. Expression of miR-155 and miR-34a in breast cancer and adjacent tissues. *P<0.05 compared to tumor adjacent tissues.

14 adenoma, 8 squamous carcinoma and 8 myeloma. Based on differentiation grade, there were 19 high, 10 moderate and 11 low differentiation cases. TNM staging revealed 14 stage I, 17 stage II and 9 stage III patients. Another cohort of 40 healthy females (aging between 25 and 65 years old, average = 49.7 \pm 3.2 years) were recruited as the control group. There was no significant difference regarding sex or age between two groups (P>0.05), which were thus comparable.

The study protocol was approved by the Research Ethics Committee of Changsha Central Hospital, and all patients gave their informed consent before study commencement.

Inclusive criteria: (1) Being diagnosed by pathological examinations; (2) No mesenchymal disease or immune dysfunction; (3) Not receiving radio-/chemo-/immune-/cyro-/laser-therapy before surgery.

Exclusive criteria: (1) Dysfunctions in major organs including heart, liver or kidney; (2) Complicated with other malignant tumors; (3)

Acute/chronic inflammation; (4) With mental or psychological conditions.

Reagents

RT-PCR test kit for miR-34b and miR-155 (Takara

Shuzo, Japan); PCR cycler model PTC-100TM (PE, US); Trizol reagent (Gibco BRL, US); RNase-free H₂O (Sangon, China); NanoDrop UV spectrometer (NanoDrop, US).

Primer design

Specific primers for RT-PCR were synthesized by Sangon (China) as shown in **Table 1**.

Expression of miR-155 and miR-34a in blood samples

Fasted blood samples were collected from both experimental and control groups. Blood samples were collected in EDTA-containing tube and were centrifuged at 1500 rpm of 20 min under 4°C. Serum was then saved and stored at -20°C.

Trizol reagent was employed to extract total RNA, which was then tested under electrophoresis for the integrity. Nano Drop was then employed to determine the concentration and purity of RNA. Total RNA (1 μ g) was used as the template for synthesizing cDNA by reverse transcription. The relative expression level of miR-155 and miR-34a in serum was then tested by quantitative PCR under the following conditions: 95°C pre-denature for 2 min, followed by 40 cycles each containing 94°C denature for 1 min, 60°C annealing for 1 min, and 72°C elongation for 1 min, and ended with 55°C~95°C gradient treatment (step: 0.5°C; duration: 30 sec). Curve analysis was then performed.

RT-PCR for the expression of miR-155 and miR-34a in breast cancer tissues

Tissue samples collected from surgery of breast cancer patients were frozen in liquid nitrogen and stored in -80°C.

Trizol reagent was used to extract mRNA from breast cancer tissues. Total concentration of RNA was determined by D260 nm/D280 nm. 200 ng total RNA was used to synthesize cDNA based on polyA tail. Using cDNA as the template, PCR amplification was performed using

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Table 3. Correlation between expression of miR-155 and miR-34a and clinical features of breast cancer patients

Item	N	miR-34a	P value	miR-155	P value
Age	40		0.257		0.187
>50	19	0.228±0.215		2.641±0.201	
≤50	21	0.245±0.121		2.628±0.103	
TNM stage	40		0.044		0.041
I	14	0.294±0.162		2.107±0.203	
II	17	0.261±0.141		2.363±0.227	
III	9	0.203±0.113		2.799±0.308	
Lymph node metastasis	40		0.021		0.011
Yes	21	0.201±0.132		2.898±0.314	
No	19	0.278±0.121		2.365±0.112	
Pathological type	40		0.134		0.212
Papilloma	10	0.241±0.126		2.673±0.121	
Adenoma	14	0.238±0.118		2.667±0.122	
Squamous carcinoma	8	0.241±0.113		2.673±0.133	
Myeloma	8	0.237±0.102		2.669±0.121	
Differentiation grade	40		0.042		0.046
High	19	0.287±0.512		2.201±0.103	
Moderate	10	0.238±0.317		2.459±0.142	
Low	11	0.201±0.101		2.689±0.172	

primers as shown in **Table 1**. In a 20 µL reverse-transcription system, 2 µL RNA plus 1 µL primer were added. 3 µL cDNA plus 1 µL primer and 0.2 µL Taq DNA polymerase were used for PCR amplification under the following conditions: 94°C pre-denature for 3 min, followed by 30 cycles each containing 94°C denature for 40 sec, 56°C annealing for 1 min, and 72°C elongation for 1 min, ended with 72°C elongation for 5 min. Quantitative PCR was employed using $2^{-\Delta\Delta Ct}$ method to determine the relative expression level of miR-34a and miR-155.

Statistical analysis

SPSS 17.0 software was employed to process all collected data, which were presented as mean ± standard deviation (SD). Measurement data were compared by student t-test while enumeration data were compared by chi-square test. LSD comparison was performed across multiple groups. A statistical significance was defined when $P < 0.05$.

Results

Serum miR-155 and miR-34a expression

The relative expression level of miR-155 and miR-34 against internal reference was tested in

serum from both groups. Results showed significantly elevated miR-155 (2.330 ± 0.211) and suppressed miR-23a (0.122 ± 0.105) compared to control group ($P < 0.05$, **Table 2**).

Expression of miR-155 and miR-34a in breast cancer and adjacent tissues

We further tested the mRNA level of miR-155 and miR-34a from both breast cancer and tumor adjacent tissues. Results showed significantly elevated miR-155 in breast cancer tissues (0.532 ± 0.124) higher than tumor adjacent tissues (0.212 ± 0.112) and depressed miR-34a levels ($0.117 \pm$

0.113 vs. 0.506 ± 0.142 , $P < 0.05$) as shown in **Figure 1**.

Correlation between miR expression and clinical/pathological features of patients

Correlation analysis was performed regarding the expression of miR-155 and miR-34a and clinical features of breast cancer was performed involving age, TNM stage, lymph node metastasis, pathological subtype, and differentiation grade. Results showed the correlation between miR-155 or miR-34a expression with TNM stage, lymph node metastasis and differentiation grade ($P < 0.05$) but not with age or pathological subtype ($P > 0.05$). With advanced TNM grade, occurrence of lymph node metastasis or lower differentiation grade, miR-34a expression was further lowered while miR-155 was up-regulated (**Table 3**).

Discussion

Breast cancer is the most common malignant tumor in females worldwide. A survey in 2010 revealed that about 28% of all newly diagnosed cancer patients in American women are breast cancer, whose incidence was significantly elevated [6, 7]. In China, the mortality of breast cancer is also increasing by years. The distal

metastasis is the major mortality reason of breast cancer [8]. MicroRNA (miR) is one endogenous non-coding short RNA molecule consisting of 20~25 nucleotides. Although having non open reading frame (ORF) itself, miR exerts crucial role in modulating multiple gene expression inside cells [9]. Recently increasing number of studies have shown the abnormal expression of miR in various cancers including breast cancer. MiR participates in multiple biological processes of cells and plays a critical role during tumor cell adhesion, migration, invasion and angiogenesis [10]. As the earliest discovered miR with tumor accelerating activity, miR-155 can bind onto 3'-non coding region at post-transcriptional level for forming RNA-induced silencing complex, which can inhibit the translation of target genes or degrade target mRNA, to mediate biological activities such as cell proliferation, differentiation and apoptosis [11]. Among ~1,000 miR that have been discovered, miR-155 was up-regulated in various malignant tumors, making it as one small "oncogene", which has been correlated with B cell lymphoma, gastric cancer, lung cancer and colorectal carcinoma [12]. MiR-34 is one highly-conserved miR, and locates on 1p36 including 22 nucleotides and 2 exons for separate gene transcription and expression. It can also facilitate cell apoptosis, arrest at G1 phase, accelerate cell aging, and inhibit tumor migration [13].

In this study, we selected breast cancer patients who received surgeries in our hospital as the experimental group, in addition to healthy individuals as the control group. Serum assay showed significantly higher miR-155 and lower miR-34a in cancer patients compared to control ones, suggesting significantly higher elevation of miR-155 and depression of miR-34a in breast cancer patients. A DNA microarray assay revealed abnormal expression of miR-155 in human tissues, indicating its potential role as an oncogene [14]. Other studies focusing on breast cancer cells with estrogen receptor expression indicated that p53 induced nuclear protein 1 might be the target of miR-155 [15]. MiR-34a can inhibit the progression of malignant tumors via regulating its downstream genes, and is abnormally expressed in various malignant tumors. A similar study in neuroblastoma cells also confirmed the correlation between miR-34a down-regulation and malignant proliferation of tumor cells [16], as consistent with our results.

This study further tested the mRNA and protein expression levels of miR-155 and miR-34a, and found significantly elevated miR-155 and suppressed miR-34a in breast cancer tissues compared to tumor adjacent tissues. Study has indicated the up-regulation of miR-155 in various human malignant tumors including pulmonary carcinoma, colorectal cancer, pancreatic cancer, nasopharyngeal cancer and cervical cancer. The expression of miR-155 was significantly higher in lung cancer tissues compared to normal tissues, indicating its potential role as oncogene to mediate the progression of tumors. Previous study has indicated significantly depression of miR-34a in breast cancer tissues compared to adjacent normal tissues. Furthermore, miR-34a has been significantly down-regulated in p53-mutated breast cancer cells compared to wild type p53 tumor cells or normal epithelial cells [17].

Further correlation analysis regarding the expression of miR-155 and miR-34a and pathological features of breast cancer patients revealed the correlation between miR-155, miR-34a and TNM stage, lymph node metastasis and differentiation grade of tumors. As advancement of TNM stage, occurrence of lymph node and lower differentiation grade, the expression of miR-155 was elevated while miR-34a was down-regulated. Previous study has indicated that miR-155 might work as one index predicting the prognosis of non-small cell lung cancer, as its up-regulation normally suggested unfavorable prognosis [18]. Basic research also found down-regulation of miR-34a in K562 cells, which also had elevated CCND1 and CDK6 expression, all of which may mediate cell cycle and facilitate cell proliferation [19]. As the down-regulation of miR-34a in prostate cancer, tumor cell proliferation and migration were all potentiated [20]. MiR-34a was also down-regulated in various solid malignant tumors and blood hematological tumors, as it can regulate cell proliferation, cell cycle and aging.

In summary, miR-155 was up-regulated in breast cancer cells, whose miR-34a expression level was suppressed, as contrast to adjacent tumors. The expression of miR was correlated with TNM stage, lymph node metastasis and differentiation grade. As advancement of TNM stage, occurrence of lymph node, lowering of differentiation grade, miR-155 expression was further enhanced while miR-34a was down-reg-

ulated. MiR-155 and miR-34a might participate in the occurrence, progression, infiltration and metastasis, and is one potential biomarker for breast cancer. The early assay of miR may benefit the diagnosis and prognostic prediction of breast cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lei Guo, Department of Breast Surgery, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha 410008, Hunan, PR China. Tel: +86-731-89753015; E-mail: guoleiyu@sina.com

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