Down-Regulated microRNA-421 Was Associated with the Poor Prognosis of Breast Cancer Patients

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Abstract

Background: Breast cancer is one of the most common cancers among females with high morbidity and mortality. MicroRNAs (miRNAs) have been reported to play important roles in the development of cancers. However, the prognostic value of *microRNA-421* (*miR-421*) in breast cancer have not been extensively explored.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect the relative expression of *miR-421* in breast cancer tissue samples. Relationships between *miR-421* expression and clinicopathological factors were analyzed by chi-square test. The effects of several variables on survival status were tested by Kaplan-Meier curve and Cox proportional hazards regression analyses.

Results: *MiR-421* expression was significantly decreased in breast cancer tissues, compared with adjacent noncancerous tissues ($P<0.001$). Moreover, abnormal *miR-421* expression was closely correlated with lymph node metastasis ($P<0.001$), TNM stage ($P=0.021$), and differentiation ($P=0.044$) of breast cancer patients. Kaplan-Meier analysis revealed that patients with low *miR-421* expression had a shorter overall survival time than those with high *miR-421* expression ($P=0.001$). Furthermore, multivariate analysis demonstrated that *miR-421* ($P=0.014$, HR=2.000, 95%CI: 1.149-3.480) was an independent prognostic indicator in breast cancer patients, as well as lymph node metastasis ($P=0.016$, HR=1.987, 95%CI: 1.137-3.474).

Conclusion: The reduced expression of *miR-421* may predict the poor prognosis of breast cancer and *miR-421* may be involved in the progression of breast cancer.

Background

Breast cancer is the major cause of cancer-related mortality among females worldwide [1] and it is recognized to be a heterogeneous disease with different outcomes and responses to treatment [2]. Currently, numbers of potential risk factors in breast cancer have been identified, such as obesity, lack of physical exercise, alcohol consumption, hormone replacement therapy, using oral contraceptives, ever-breastfeeding, and genetic factors [3-5]. Despite many advances in tumor diagnostic and therapeutic strategies, the prevention and therapy of breast cancer remains a major public health concern [6]. Non-estrogen receptor (ER)-, non-progesterone receptor (PR)-, or non-human epidermal growth factor receptor 2 (Her2, triple negative)-expressing breast cancer patients are not sensitive response to hormonal therapy with Her2-targeted agents. This category of patients often display a inferior clinical outcome due to disease recurrence and distant metastasis [7-9]. Thus, it is urgently required to search for and identify powerful biomarkers to predict the clinical outcome and develop more promising treatment strategies to effectively control aggressive breast cancer.

microRNAs (miRNAs) are a series of short single-stranded RNA molecules and act as post-transcriptional regulators of gene expression by binding 3'-untranslated region (3'UTR) of target mRNA, resulting in an inhibition of translation or degradation of mRNA [10]. MiRNAs are reported to be involved in multiple

biological processes, including cell proliferation, migration, apoptosis, and differentiation [11]. Increasing evidences have proved that dysregulation of miRNA expression is observed in various human cancers, certainly including breast cancer [12]. Furthermore, miRNAs have recently been demonstrated to function as both oncogenes and suppresses of tumor progression [13]. However, the aberrant expression and prognostic performance of *microRNA-421* (*miR-421*) in breast cancer has not been investigated.

In the present study, we detected the relative expression of *miR-421* in breast cancer patients and analyzed the correlation between *miR-421* expression and clinicopathological characteristics of breast cancer. We also investigated the correlation of *miR-421* expression with the overall survival of breast cancer patients.

Methods And Materials

Study samples

The study was approved by the Ethic Committee of Southwest Hospital, Army Medical University and all patients had signed the written informed contents before sampling.

A total of 117 breast cancer tissues and paired adjacent noncancerous breast tissues were collected at the Department of Pathology, Southwest Hospital, Army Medical University. All the specimens were diagnosed by two pathologists separately to determine the pathological classification of breast cancer. None of patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. The clinical characteristics of patients were collected, including age, tumor size, lymph node metastasis, TNM stage, differentiation, ER status, PR status and Her-2 status. The fresh tissue samples were immediately frozen in liquid nitrogen after surgical removal and stored at -80°C for RNA extraction.

Then all patients were conducted a 5 year-follow-up for prognosis determination. Breast cancer patients who died from other adverse events were excluded.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from breast cancer tissues and matched noncancerous tissues with TRIZOL reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's recommendations. cDNA was synthesized from total RNA using the Taqman miRNA reverse transcription kit (Applied Biosystem, Foster City, CA). QRT-PCR was performed by using the Applied Biosystems 7500 Sequence Detection system. *U6* RNA was used as an endogenous reference for normalizing the expression levels of *miR-421*. The relative expression levels of *miR-421* compared to the normal controls were calculated using the method of $2^{-\Delta\Delta Ct}$. All assays were performed at least in triplicate.

Statistical analysis

All data were presented at mean $\pm$ SD (standard deviation) or n (%). All statistical analyses were performed using SPSS 18.0 (SPSS software, Chicago, Inc, IL, USA), and graphs were plotted by GraphPad

Prism 5.0 (GraphPad Software, Inc, CA, USA). The continuous variable of statistical differences between two groups were analyzed by Student's *t* test. The relationships between *miR-421* expression and clinicopathological characteristics were evaluated using chi-square test. Survival curves were plotted using Kaplan-Meier methods and compared with log-rank test. The Cox proportional regression model analysis were applied to evaluate the prognostic significance of *miR-421* in breast cancer patients. All tests were two-tailed, and when $P < 0.05$ was considered to be statistically significant.

Results

Down-regulation of *miR-421* expression in breast cancer

To investigate the role of *miR-421* in breast cancer, we detected the relative expression of *miR-421* level in 117 breast cancer tissues and matched noncancerous tissues using qRT-PCR. As shown in Figure 1, compared with paired noncancerous tissues, *miR-421* expression level was significantly down-regulated in breast cancer tissues ($P < 0.001$).

miR-421 expression was associated with clinicopathologic features of breast cancer patients

We also explored the association of *miR-421* expression with clinicopathological factors of breast cancer patients, 117 patients were divided into two groups based on the median value (0.56) of *miR-421* expression in breast cancer tissues: low *miR-421* expression group ($n=59$) and high *miR-421* expression group ($n=58$). As indicated in Table 1, abnormal *miR-421* expression was significantly correlated with lymph node metastasis ($P=0.000$), TNM stage ($P=0.021$), and differentiation ($P=0.044$) of breast cancer patients. Unfortunately, no significant difference was observed between *miR-421* expression and other clinicopathological factors, such as age, tumor size, ER status, PR status, or Her-2 status (all $P > 0.05$).

The prognostic value of *miR-421* expression in breast cancer

To investigate the prognostic significance of *miR-421* in breast cancer, Kaplan-Meier method was employed to analyze the relationship of *miR-421* with survival time of breast cancer patients. Results showed that patients with low *miR-421* expression had a worse overall survival than those with high *miR-421* expression ($P=0.001$, Figure 2).

Subsequently, we performed univariate and multivariate Cox regression analyses to define the prognosis role of *miR-421* in breast cancer with hazard ratio (HR) and 95%CI. In univariate analysis, *miR-421* expression ($P=0.002$) and TNM stage ($P=0.022$) were closely related to overall survival (Table 2). Multivariate analysis indicated *miR-421* expression ($P=0.014$, HR=2.000, 95%CI: 1.149-3.480) was an independent prognostic indicator for breast cancer, together lymph node metastasis ($P=0.016$, HR=1.987, 95%CI: 1.137-3.474) (Table 2).

Discussion

Breast cancer is the most prevalent malignancy and is also a leading cause of cancer-related deaths among females worldwide. Although there are tremendous advances made in developing multiple treatments, the clinical outcome of breast cancer patients is still unfavorable. It is difficult to coordinate effective treatment strategies for every patient, due to heterogeneity at phenotypic and molecular levels [2]. Therefore, it is of great significance to investigate the underlining molecular mechanisms and identify powerful biomarkers for prediction of prognosis in breast cancer.

Overwhelming evidences have strongly demonstrated that dysregulation of miRNAs is involved in the initiation and development of cancers, which explains the controls of multiple critical functions, such as cancer cell proliferation, migration, and resistance to therapeutic interventions [14]. In recent years, more and more studies indicate that miRNAs are proved to be served as biomarkers in a variety of cancers [15-18]. Fan *et al.* reported that *miR-196a* might play an important role in the progression of ovarian carcinoma, and could be acted as an independent prognostic biomarker for ovarian carcinoma [19]. Dong *et al.* revealed that the decreased expression of *miR-124* might be associated with tumor progression and poor prognosis in breast cancer patients [20]. Therefore, it is of great importance to understand the functions of miRNAs and provide new insights on the involved molecules in the development of cancers and novel markers for cancer prognosis, diagnosis and treatment.

MiR-421, located on X chromosome, was first implicated in gastric cancer oncogenesis [21]. It has been reported that *miR-421* is abnormally expressed in a variety of tumors and is a promising prognostic biomarker in cancers. For instance, Liu *et al.* found that *miR-421* was correlated with lymph node metastasis and prognosis of gastric carcinoma [22]. Jiang *et al.* also showed that *miR-421* might be involved in the early stage of stomach carcinogenesis and could be used as an efficient diagnostic biomarker [23]. In our present study, we focused on the abnormal *miR-421* expression in breast cancer tissues, indicating that *miR-421* play the important roles in the pathogenesis of breast cancer.

In the present study, we compared *miR-421* expression level between breast cancer tissues with matched noncancerous tissues. By qRT-PCR analysis, we found that *miR-421* expression was significantly decreased in breast cancer tissues compared with adjacent normal tissues. In addition, the relationship of *miR-421* expression with clinicopathological features was also explored. Results showed that down-regulated *miR-421* expression was connected to lymph node metastasis, TNM stage and differentiation of breast cancer patients. It indicated that abnormal *miR-421* expression might be involved in the progression of breast cancer patients. In a further survival analysis, patients with high *miR-421* expression had dramatically longer survival time than those with low *miR-421* expression, suggesting the correlation between *miR-421* expression and breast cancer clinical outcome. Multivariate analysis revealed that *miR-421* was an independent prognostic biomarker for breast cancer patients, as well as lymph node metastasis. Our findings were consistent with previous studies. For example, Pan *et al.* reported that *miR-421* was down-regulated in breast cancer tissues and metastatic cell lines, and was significantly associated with lymph node metastasis, recurrence/metastasis, or pTNM stage [24]. Wang *et al.* demonstrated that *miR-421* was down-regulated in lymph node metastasis group compared with non lymph node metastasis group of breast cancer [25].

Conclusion

In summary, *miR-421* expression is significantly down-regulated in breast cancer tissues, and may be associated with the progression of breast cancer. *miR-421* may be served as an independent prognostic biomarker of breast cancer. However, further studies should be conducted with larger sample size and longer follow-up time to confirm the clinical application of miR-421 in breast cancer.

List Of Abbreviations

MicroRNAs (miRNAs)

microRNA-421 (miR-421)

Quantitative real-time polymerase chain reaction (qRT-PCR)

Non-estrogen receptor (ER)

Non-progesterone receptor (PR)

3'-untranslated region (3'UTR)

hazard ratio (HR)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

K.Z. design of the work; T.M., Z.H. the acquisition, analysis, X.W., Y.P. interpretation of data; Y.C., Y.D. the creation of new software used in the work; Z.R., Z.W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1.

The relationships between *miR-421* expression and clinicopathological factors

Factors	Cases (n=117)	MiR-421 expression		χ^2	P-value
		Low (n=59)	High (n=58)		
Age (years)					
<50	36	19	17	0.115	0.735
≥50	81	40	41		
Tumor size (cm)					
<2	76	36	40	0.812	0.368
≥2	41	23	18		
Lymph node metastasis					
Absent	87	35	52	14.114	<0.001
Present	30	24	6		
TNM stage					
I-II	79	34	45	5.313	0.021
III-IV	38	25	13		
Differentiation					
Well and moderate	85	38	47	4.070	0.044
Poor	32	21	11		
ER status					
Positive	56	26	30	0.687	0.407
Negative	61	33	28		
PR status					
Positive	74	38	36	0.069	0.793
Negative	43	21	22		
Her2 status					
Negative	84	41	43	0.312	0.577
Positive	33	18	15		

ER: estrogen receptor; PR: progesterone receptor; Her-2: human epidermal growth factor receptor

Table 2.

The univariate and multivariate analyses for overall survival in breast cancer patients

Features	Univariate analysis		Multivariate analysis	
	HR (95%CI)	<i>P</i> -value	HR (95%CI)	<i>P</i> -value
Age	1.354 (0.787-2.327)	0.273	-	-
Tumor size	1.152 (0.674-1.968)	0.604	-	-
Lymph node metastasis	2.444 (1.429-4.181)	0.001	1.987 (1.137-3.474)	0.016
TNM stage	1.847 (1.095-3.117)	0.022	-	-
Differentiation	1.560 (0.901-2.701)	0.112	-	-
ER status	0.914 (0.546-1.530)	0.732	-	-
PR status	1.107 (0.651-1.882)	0.707	-	-
Her-2 status	1.217 (0.698-2.124)	0.488	-	-
<i>MiR-421</i> expression	2.351 (1.381-4.004)	0.002	2.000 (1.149-3.480)	0.014

Figures

Figure 1

The relative expression of miR-421 was detected using quantitative reverse transcription polymerase chain reaction in breast cancer tissues (qRT-PCR). Results showed that miR-421 expression was significantly reduced in breast cancer tissues compared with adjacent noncancerous tissues ($P < 0.001$).

Figure 2

The Kaplan-Meier analysis revealed that low miR-421 expression was associated with shorter overall survival of breast cancer patients (log-rank test, $P=0.001$).