Original Article
MicroRNA-101 is a novel biomarker for diagnosis and prognosis in breast cancer

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Abstract: Objective: The purpose of this study was to evaluate the diagnostic and prognostic significance of miR-101 in breast cancer. Methods: The relative level of miR-101 in plasma of breast cancer patients and healthy volunteers were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square analysis was used to analyze the relationship between miR-101 level and clinical characteristics and the diagnostic value of the gene was evaluated by receiver operating characteristic (ROC) analysis. Besides, Kaplan-Meier method was used for evaluating overall survival of the patients according to miR-101 level and the prognostic value was analyzed by Cox regression analysis. Results: Decreased level of miR-101 was detected in patients plasma, compared with healthy controls. The level was significantly associated with tumor size, histological grade, TNM stage, lymph node metastasis and recurrence ($P < 0.05$ for all). The ROC curve indicated that miR-101 could act as a diagnostic marker with the sensitivity of 91.7% and the specificity of 80.8%, and the AUC and the cut-off value were 0.940 and 0.965, separately. The results of Kaplan-Meier suggested that patients with high level of miR-101 had longer survival time than those with low level ($P = 0.002$). Besides, miR-101 could serve as an independent biomarker for breast cancer prognosis (HR = 1.808, 95% CI = 1.193-2.740, $P = 0.005$). Conclusion: MiR-101 may be a novel marker for diagnosis and prognosis in breast cancer, which might improve the clinical outcomes of patients with breast cancer.

Keywords: MicroRNA-101, breast cancer, diagnosis, prognosis

Introduction

Breast cancer is one of the common malignancies and the leading cause of cancer death among the females worldwide [1]. The poor prognosis is due to late diagnosis and rapid metastasis [2]. If patients were diagnosed at early stage without metastasis, the clinical outcomes will be significantly improved. However, there are still no validated biomarkers for detection of the disease in early stages with effective power in diagnosis and therapeutic approaches [3].

MicroRNAs (miRNAs) are highly conservative RNA molecular with length of ~22 nt [4]. They involve in various biological processes by regulating their targets at a post- transcriptional level [5]. It has been widely accepted that miRNAs play key roles in various biological processes, including development, metabolism, cell proliferation, differentiation and apoptosis [6].

MiRNAs has been confirmed to play very important role in many diseases especially in cancers. MiRNAs may function as tumor suppressors or oncogenes, and play critical roles in carcinogenesis [7, 8]. Growing evidence has also indicated the possible use of miRNA expression profiles to distinguish between normal and neoplastic tissues, leading to the identification of new diagnostic and/or prognostic markers [9].

MiR-101 belongs to a family of miRNAs involving in cell proliferation, invasion and angiogenesis [10]. Evidences have demonstrated that abnormal expression levels of miR-101 are associated with various types of cancer, such as colorectal cancer, gastric cancer, epithelial ovarian cancer, liver cancer and so on [11-14]. The functional roles of miR-101 in breast cancer had been proved in the previous studies. Wang L et al., had reported that over-expression of miR-101 in cultured breast cancer cells

Table 1. Sequences of primers used in this study

Primers		Sequences
miR-101	Forward	5'-CCCTGGCTCAGTTATCAC-3'
	Reverse	5'-ATGGACAGCATCAGCACT-3'
U6	Forward	5'-CGCTTCGGCAGCACATATAC-3'
	Reverse	5'-TTCACGAATTTGCGTGTCAT-3'

MCF-7 and MDA-MB-231 could inhibit proliferation and induce apoptosis [15]. The function of miR-101 in breast cancer tissues was proved in the study of Wang R et al. [16], who had demonstrated that down-regulation of miR-101 is linked to the increase of cellular proliferation and invasiveness. And the serum miR-101 level was studied by Eichelser et al. They found cancer-specific increase in serum miR-101 in breast cancer patients compared with patient with benign breast disease and healthy women [17]. However, the clinical significance of miR-101 in breast cancer had been rarely reported.

In this study, we aimed to evaluate the diagnostic and prognostic significance of miR-101 in breast cancer.

Materials and methods

Ethics statement

Approval for this study was obtained from the Ethics Committee of Guizhou Provincial People's Hospital. The participants or their legal guardians provided written informed consents for research purposes.

Patients and plasma specimens

This study was conducted in Guizhou Provincial People's Hospital and 108 female patients confirmed with breast cancer in the hospital and 78 healthy volunteers were enrolled in this study. 5 mL serum specimen was collected from each participator before they didn't have any food in the morning. The collected serum specimen was kept in a tube with EDTA at 4°C, and then centrifuged at 2,500 rpm for 5 min, to get plasma. The plasma specimens were stored at -80°C until use. All the patients were enrolled in a follow-up investigation and the clinical information of the cases were collected for evaluating the prognostic significance of miR-101 in breast cancer. A 5-years' follow-up was conducted and the information was updated via a telephone or questionnaire. The death of the participants was ascertained by a report from the family and verified by the review of public records.

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

Total RNA were extracted from collected plasma specimens using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's introduction. DNase I (Sigma, St. Lousi, MO, USA) was used to hydrolyze residual DNA in RNA samples. The concentration and quality of the extracted RNA were detected by UV absorbance (A260/A280) and 1% agarose gel electrophoresis, respectively. Prime Scrip RT reagent kit (Takara Biotechnology Co., Ltd) was used for synthesizing cDNA and qRT-PCR was performed with Hairpin-it TM miRNAs qPCR Quantitation Kit (Genepharma, Shanghai, China). Data analysis was performed with $2^{-\Delta\Delta Ct}$ method. U6 was used as internal inference. The primers sequences, designed by Primer 5.0, were listed in **Table 1**.

Statistic analysis

SPSS 18.0 (SPSS Inc, IL, USA) was performed for statistic analysis and Sigma Plot 12.5 (Systat Software Inc., CA, USA) was used for drawing. The significant expression of the gene was analyzed by Student's t test. Relationship between miR-101 level and clinical characteristics was evaluated by Chi-square analysis and the diagnostic value of miR-101 was evaluated by receiver operating characteristic (ROC) analysis. Kaplan-Meier method was used for analyzing overall survival of the patients according to miR-101 levels and the prognostic value was evaluated by Cox regression analysis. $P < 0.05$ was considered to be statistically significant.

Results

Level of miR-101 was decreased in breast cancer

The relative level of plasma miR-101 in patients was 0.608 ± 0.270, while the level in the healthy controls was 1.224 ± 0.290. There was a significant difference between the groups ($P < 0.001$, **Figure 1**).
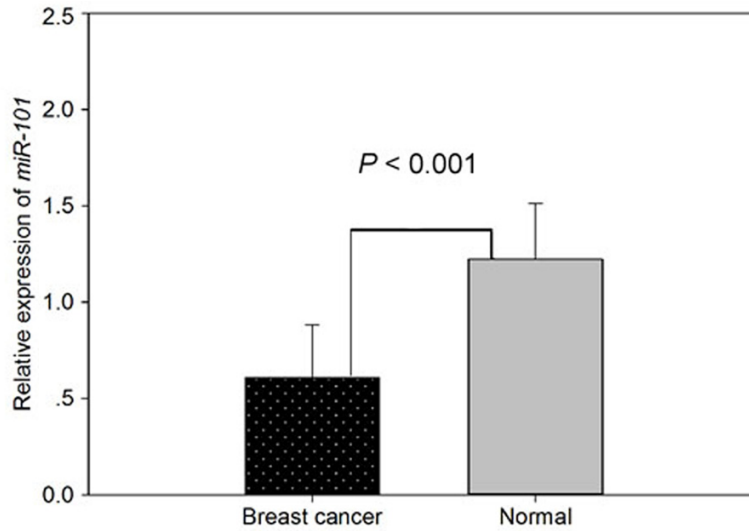
Figure 1. Relative level of plasma miR-101 in patients with breast cancer and healthy controls (U6 as internal control).

Table 2. Relationship between miR-101 level and clinical characteristics

| Characteristic | Total number (n) | miR-101 level | | X^2 | P |
		High (n)	Low (n)		
Age				0.156	0.693
≥ 50	66	34	32		
< 50	42	20	22		
Tumor location				0.037	0.847
Unilateral	55	27	28		
Bilaterl	53	27	26		
Tumor size				10.707	0.001
≥ 3 cm	53	18	35		
< 3 cm	55	36	19		
Histological stage				8.336	0.004
G1+G2	53	34	19		
G3	55	20	35		
TNM stage				10.707	0.001
T1+T2	55	36	19		
T3+T4	53	18	35		
Lymph node metastasis				6.313	0.012
Yes	49	18	31		
No	59	36	23		
Recurrence				6.261	0.012
Yes	55	21	34		
No	53	33	20		
Pathological classification				8.174	0.043
Invasive ductal	40	24	16		
Invasive lobular	36	11	25		
Mixed invasive	17	10	7		
Others	15	9	6		

Clinical characteristics of patients and their association with miR-101 level

The average age of the collected patients was 54.1 years old and that in the healthy control group was 56.3. The clinical characteristics of the patients with breast cancer were listed in **Table 2**. In order to analyze the association between miR-101 level and clinical features, the patients were divided into high and low relative level group according to their average level.

The level of miR-101 was significantly associated with tumor size (P = 0.001), histological grade (P = 0.004), TNM stage (P = 0.001), lymph node metastasis (P = 0.012) and recurrence (P = 0.012). However, there were no significant difference between miR-101 level and age or tumor location (P > 0.05 for all, **Table 2**).

Diagnostic value of miR-101 in breast cancer

ROC curve was used for analyzing the diagnostic significance of miR-101 in breast cancer and the results were shown in **Figure 2**. Form the figure, we found that miR-101 was a potential biomarker for differentiating breast cancer patients with ROC curve area of 0.940. The cut-off value was 0.965. The sensitivity of miR-101 for breast cancer diagnosis was 91.7%, while the specificity was 80.8%.

Level of miR-101 in breast cancer determines prognosis

The results of Kaplan-Meier method suggested that the overall survival of patients with low miR-101 level were
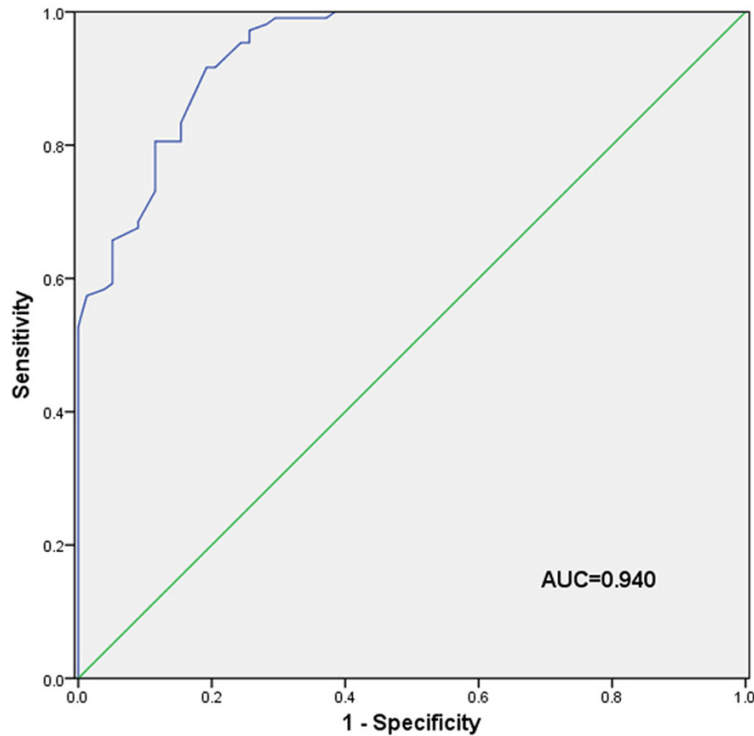
Figure 2. ROC curve of miR-101 in the diagnosis of breast cancer.
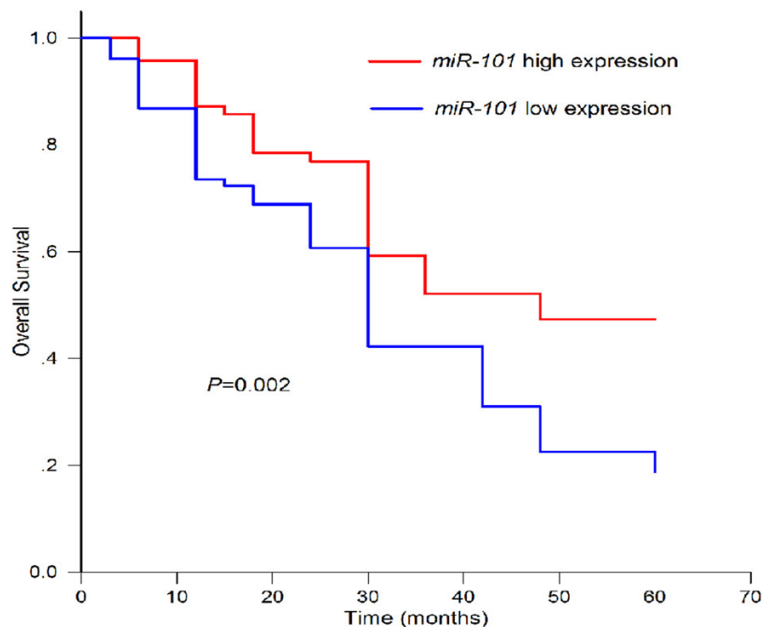
Figure 3. The overall survival of patients with breast cancer according to miR-101 level.

analysis were used to analyze the prognostic factors in breast cancer and the results were listed in **Table 3**. The data indicated that miR-101 was associated with prognosis (P = 0.003) and it could act as an independently prognostic biomarker for breast cancer (HR = 1.808, 95% CI = 1.193-2.740, P = 0.005).

Discussion

MiRNAs are small single-stranded RNA molecules composed of 18-23 nts, they act as oncogenes or tumor suppressor genes playing important roles in the processes of tumor formation, infiltration and metastasis [18]. Recently, a number of reports have indicated that the levels of miRNA in serum can serve as diagnostic and prognostic markers for malignancies [19, 20]. For example, serum miR-638 levels are associated with non-small cell lung cancer prognosis [21]. MiR-210 acts as a potential noninvasive biomarker for the diagnosis and prognosis of glioma [22]. MiR-141 level is associated with diagnosis and prognosis of patients with bladder cancer [23]. The serum miRNA associated with breast cancer progression may be helpful for improving the early detection and clinical outcomes of patients. Our results indicated that serum miR-101 is a molecular marker for the diagnostic and prognostic evaluation of breast cancer.

In this study, we detected the plasma miR-101 level by qRT-PCR. The result suggested that the level of miR-101 in patients plasma was lower than that in the healthy controls. Wang R et al., had detected the expression

significantly shorter than those with high miR-101 level (Log rank test, P = 0.002, **Figure 3**). Univariate and multivariate Cox regression

Table 3. Cox regression analysis

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
MiR-101 low vs high	1.857	1.231-2.801	0.003	1.808	1.193-2.740	0.005
Age ≥ 50 vs < 50	1.075	0.726-1.593	0.717	1.128	0.751-1.696	0.561
Tumor size ≥ 3 cm vs < 3 cm	1.350	0.901-2.023	0.145	1.379	0.587-2.133	0.149
Tumor location unilateral vs bilateral	1.034	0.697-1.533	0.869	0.887	0.891-1.342	0.571
Histological stage G3 vs G1+G2	1.175	0.784-1.759	0.435	1.148	0.762-1.729	0.509
TNM stage T3+T4 vs T1+T2	1.136	0.761-1.697	0.532	1.216	0.798-1.853	0.363
Lymph node metastasis yes vs no	1.253	0.838-1.875	0.272	1.201	0.792-1.823	0.389
Recurrence yes vs no	1.192	0.803-1.767	0.384	1.223	0.814-1.838	0.333
Pathological classification Invasive ductal vs Invasive lobular vs Mixed invasive vs others	1.211	0.645-1.824	0.405	1.173	0.725-1.903	0.389

level of miR-101 in breast cancer tissues and the similar results were obtained [16]. Interesting, in the study of Eichelser et al., increased miR-101 level was found in serum of breast cancer patients compared with that in patient with benign breast disease and healthy women, but decreased level was found in lymph node-negative breast cancer, suggesting a dual role for miR-101 in breast cancer [17]. So further work is needed to study the serum miR-101 level in breast cancer. The miR-101 level was significantly associated with tumor size, histological grade, TNM stage, lymph node metastasis and recurrence. These data indicated that miR-101 was correlated with breast cancer progression.

The diagnostic significance of miR-101 was proved in this study and the results demonstrated that miR-101 could serve as a diagnostic biomarker for breast cancer. As non-invasive markers, miRNAs were highly stable, resistant to degradation and easily detected [24]. Lin et al., reported that use of down-regulation of miR-101 and up-regulation of Cox-2 as markers may play a role in early diagnosis of cervical cancer in Uygur women [25]. In this study, ROC curve was used to estimate the diagnostic value of miR-101 for breast cancer patients. The area under curve (AUC) value of miR-101 was 0.940, with the sensitivity of 91.7% and the specificity of 80.8% at the cut-off of 0.965. The result indicated that miR-101 could act as an independent diagnostic biomarker for breast cancer.

In the previous studies, the prognostic significance of miR-101 had been proved in cancers. For example, Zhang et al., indicated that miR-101 may be a potential prognostic marker and therapeutic target for HCC [26]. Luo et al., claimed that miR-101 may play important roles as biomarkers for prognosis and therapeutic targets in NSCLC [27]. Zhang et al., had reported that down-regulated level of miR-101 was found in bladder transitional cell carcinoma and the level was associated with tumor progression, which might be a prognostic marker for the cancer [28]. But the prognostic value of miR-101 in breast cancer is still unclear. So in our study, we analyzed the prognostic value of miR-101 in breast cancer, we found that decreased miR-101 level predicted unfavorable prognosis using Kaplan-Meier curves. The Cox regression analysis demonstrated that miR-101 was an independent prognostic factor for breast cancer patients.

In conclusion, the aberrant serum miR-101 level is detected in the patients with breast cancer and the level is significantly associated with tumor progression. MiR-101 may be a potential biomarker for diagnosis and prognosis in breast cancer, which might improve the early detection and clinical outcomes of the patients.

Disclosure of conflict of interest

None.

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