University of Mazandaran

Journal of Genetic Resources
J Genet Resour2018;4(2):85-89
http://sc.journals.umz.ac.ir
doi: 10.22080/jgr.2018.14802.1111

Iranian Biology Society

Study of the Expression of *miR-4270* in Plasma of Patients with Breast Invasive Ductal Carcinoma

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Received: 11 March 2018 Accepted: 27 May 2018

Abstract

Detection of tumor-specific microRNAs (miRs) in the blood of cancer patients may provide a unique and valuable biomarker for diagnosis and prognosis. The aim of this study was to investigate whether plasma levels of microRNA-4270 could serve as a potential marker for breast invasive ductal carcinoma. A total of 40 breast cancer patients and 28 controls were recruited in this study. Total RNA was extracted from plasma samples and *miR-4270* expression was evaluated by real-time polymerase chain reaction (PCR). The correlation between *miR-4270* expression and clinico-pathological characteristics were also studied. Our data showed that plasma *miR-4270* is significantly upregulated in patients compared to control group (P-value=0.00). In addition, data analysis illustrated a correlation between low plasma levels of *miR-4270* and larger tumor size, lymph node metastasis and higher stages of malignancy (P-value > 0.05). The area under the curve of the ROC revealed that *miR-4270* expression was not able to distinguish between tumor plasmas and nontumoral specimens. Current work shows preliminary data on the expression profile of *miR-4270* in plasma of breast cancer patients. However, further studies are required to fully elucidate the role of circulating *mir-4270* in breast ductal carcinoma.

Keywords: Circulating microRNA; *miR-4270*; Breast cancer; Real-Time PCR

Introduction

MicroRNAs (miRNAs) are a principle class of short non-coding RNAs that play a main role in gene regulation (MacFarlane *et al*., 2010). miRNAs act through their completely or partially complementary binding with target mRNAs that results in their disruption and hence, translation inhibition (Murphy *et al*., 2010; Ling *et al*. 2013). The mature miRNA consists of 20-22 nucleotides (nt) processed from pre-miRNA of approximately 70 nt in length (Tafrihi *et al.,* 2019). They have regulatory roles in fundamental biological processes, including cell differentiation, proliferation, and apoptosis. Therefore, any changes in the miRNAs' expression and/or mutation could affect cellular behavior (Croce 2009; Fan *et al*., 2013).

Recent reports showed that cellular RNAs could be released in circulating bio fluids. The tumor and healthy cells contribute to the dissemination of nucleic acids like microRNAs in plasma and/or serum (Schwarzenbach *et al*., 2009). Therefore, blood miRNAs could reflect any physiological condition and malignancy in tissues. (Anker *et al*., 1999; Wang *et al*., 2010). Breast cancer is a heterogeneous disease with a high prevalence rate among women throughout the world (Kruk, 2014; Hamam *et al*., 2016). Differential expression of miRNAs illustrates the biomarker potential of these small non-coding RNAs for diagnosis and prognosis of breast malignancies (Assi e*t al*., 2013). Numerous

circulating miRNAs, like *miR-21*, *195*, *200b*, *145*, *155* and *200c* have been identified as potential breast cancer biomarkers (Tuna *et al;* Zhang *et al*., 2012). *MiR-4270* is a novel non-coding RNA that is located in chromosome 3p25.1, and its higher expression is associated with breast cancer progression. It has been reported that *miR-4270* expression level is increased in the plasma of a patient with breast cancer (Hamam *et al*., 2016). The aim of this study was to quantitatively evaluate the expression pattern of *miR-4270* in the plasma of patients with invasive ductal carcinoma (IDC), which is the most common type of aggressive breast cancer in the northwest of Iran. In addition, the association between the deregulation of *miR-4270* and clinico-pathological outcomes in patients has been evaluated.

Materials and Methods

Patients and blood collection

Forty blood samples were collected from women with invasive ductal breast cancer who were referred to Noor-Nejat Tabriz hospital from 2015 to 2017. The breast cancer patients were aged between 27 and 80 years (mean age of 51.8 years) and had undergone breast surgery. All blood samplings were performed in the pre-operative stage. Twenty-eight blood specimens were also taken from healthy women volunteers who had no history of cancer in their first-degree relatives. The research ethics committee of Noor-Nejat hospital approved the study in accordance with the institutional protocol and informed consent was obtained from all patients. The plasma was isolated from blood specimens and maintained at -80 ºC temperature. The clinical and pathological information of the patients is shown in Table 1.

Table 1. *miR-4270* expression and clinico-pathological characteristics of patients with breast cancer

Parameters	No. of patients	Statistic	p-value
Age (year)			
≤50	26		
>50	14		
Tumor size (cm)		NS	0.327
≤2	19		
>2	21		
Lymph node metastasis		NS	0.626
Negative	18		
Positive	22		
Tumor grade		NS	0.355
1(well differentiate)	17		
2 (intermediate/moderate grade)	23		

NS, not statistically significant (P-value ≥0.05)

RNA extraction and cDNA synthesis

Total RNA was extracted from plasma of all patients and healthy controls using RiboEX (Gene All, South Korea), following the manufacturer's instruction. DNase I treatment was employed to elute the probable genomic DNA contamination. Before synthesizing cDNA, polyA tail was added by poly A polymerase to the 5' end of miR-4270. *miR-4270* was reverse transcribed to cDNA using specific microRNA synthesis kit (Pars genome Co, Tehran, Iran).

Quantitative real-time polymerase chain reaction

Quantitative real-time PCR was performed in a total volume of 10µl using SYBR green master mix kit (Takara Co., Japan) by Illumina ECO instrument (Illumina Co., France). The housekeeping 5s rRNA was also used as endogenous control. The reaction included 1µl of diluted RT product, 5µl SYBR Green Master Mix, 0.3µl primer and 3.7µl RNase-free water in each well. All tests were performed in triplicate.

The identity of *microRNA-4270* was confirmed by sequencing.

Statistical analysis

All experiments were performed in triplicate. Data obtained from real-time PCR were analyzed using ANOVA and t-test and the significant level was set at $P < 0.05$. Receiver operating characteristic (ROC) curve was also plotted to evaluate the biomarker potential of *miR-4270*.

Results

MiR-4270 is significantly released in plasma of breast cancer patients

The plasma level of *miR-4270* was quantified in plasma of 40 breast cancer patients and 28 healthy controls. Real-time PCR data demonstrated that miR-4270 could be detected in blood. Furthermore, as Fig. 1 A illustrates, the expression level of this non-coding RNA is significantly higher than normal plasma specimens (p-value = 0.00).

To further evaluate the potential role of miR-4270 in breast invasive ductal carcinoma, the association between *miR-4270* expression and clinico-pathological features were studied. As Fig. 1 (B-D) shows, *miR-4270* expression is decreased in patients with grade 2 (P = 0.271), lymph node invasion (P = 0.268) and larger tumor size (P-value = 0.355). However, these differences were not significant (P-value > 0.05).

ROC curve analysis

ROC curve analysis was employed to evaluate the potential biomarker value of *miR-4270*. As Fig. 2 shows, an area of AUC 0.5 was calculated for *miR-4270* that does not validate the biomarker potential of *miR-4270*.

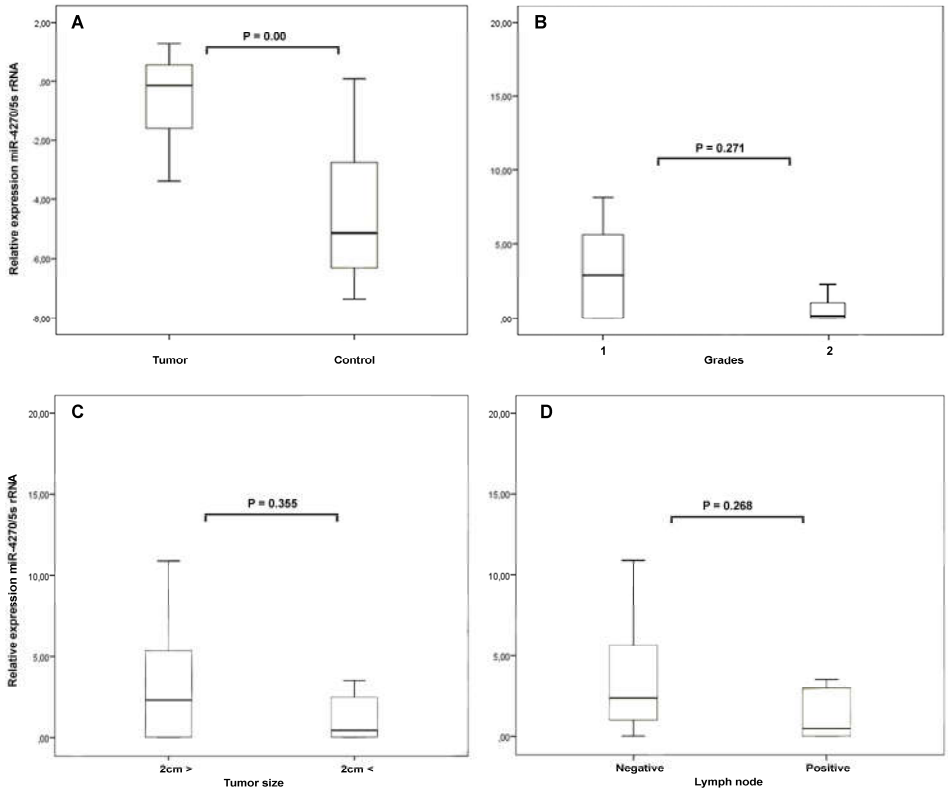

Fig. 1. Relative mean expression of *miR-4270* in plasma of A: tumor and control specimens, B: tumor grades 1 & 2, C: tumor size and D: samples with low and high incidence of lymph node metastasis. P-value > 0.05 is not significant.

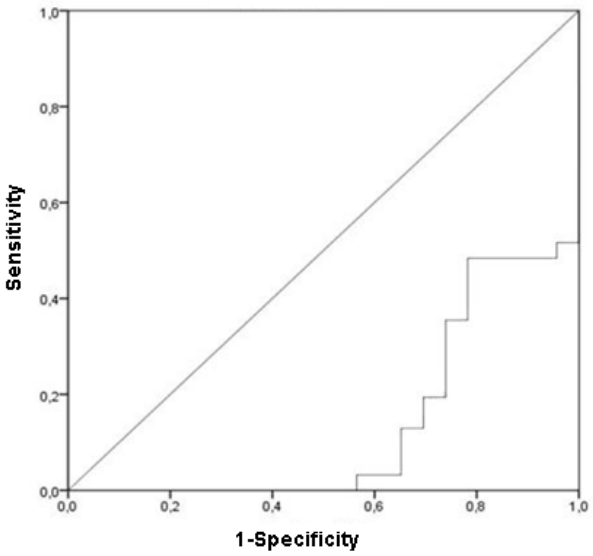

Fig. 2. Receiver operating characteristic (ROC) curve for miR-4270 yielded an AUC value of 0.5.

Discussion

Breast cancer is the leading cause of cancer-associated mortality among women worldwide. Despite recent advances in breast cancer diagnosis and novel therapeutic approaches, early diagnosis and prognosis remain poor (Ferlay *et al*., 2010; Hamam *et al*., 2016). Therefore, there is a need for newly non-invasive detection approaches to overcome the current drawbacks. miRNAs are small non-coding regulatory RNAs involved in different pathophysiological processes that illustrate great potential as a diagnostic and prognostic marker for breast cancer (Moore, *et al*., 2012; Mar-Aguilar *et al*., 2014). Furthermore, microRNAs have remarkable stability in blood as the RNase-rich environment. Lawrie *et al*. in 2008 and Roth *et al*. in 2010 showed that miRNAs could be detected in the blood of patients with B-cell lymphoma and breast cancer, respectively. Because the blood samples could be collected at different times during the course of the disease and circulating miRNAs could be quantified by real-time PCR, these non-coding molecules have recently been considered as non-invasive indicators of cancer.

Here, our investigation showed significantly higher plasma level of *miR-4270* in breast cancer patients compared to controls (P = 0.00). This finding is in accordance with microarray data reported recently by Hamam *et al.* demonstrating the upregulation of miR-4270 in plasma of breast cancer patients. The microarray analysis by Tokuhisa *et al*. also showed that miR-4270 is upregulated in gastric carcinoma.

Furthermore, our results illustrated that although *miR-4270* expression level is decreased in tumors with larger size, lymph node metastasis and grade 2, this down-regulation was not statistically significant. Accordingly, a recent work on breast cancer showed that *miR-4270* expression is significantly down-regulated in higher stages (Hamam *et al.,* 2016). However, they did not study the correlation between *miR-4270* expression, tumor size, and lymph node metastasis.

The number of studies reporting that blood miRNAs could serve as non-invasive biomarkers for breast cancer is increasing. Based on these reports, we found that *miR-4270* expression is associated with breast cancer initiation. Nevertheless, this is a preliminary report demonstrating the release of miR-4270 and its expression profile in plasma of breast cancer patients. Further investigations should be performed in a large cohort of specimens to evaluate the biomarker potential of plasma and/or serum *miR-4270* for diagnosis and/or prognosis of breast cancer. In future works, we will collect more samples with different pathological features

to evaluate in detail, the potential role of miR-4270 in breast cancer.

Acknowledgments

This work has been done at University of Tabriz and supported by the center for international scientific studies & collaboration (CISSC), Tehran-Iran.

Author Disclosure Statement

The authors disclose that there is no conflict of interest for this manuscript.

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