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

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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 was also studied. Our data showed that plasma miR-4270 is significantly up regulated 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 non tumoral 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 et 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 °C 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

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

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 polyA 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.

![Figure 1](image1.png)

**Fig. 1.** Relative mean expression of miR-4270 in plasma 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 more than 0.05 is not significant.
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.

References


