

The Expression Level of Mir-210 and Mir-141 in Breast Cancer Patients

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Abstract

Background: Breast cancer is a clinically heterogeneous disease. Molecular classification of breast cancer has been proposed based on gene expression profiles of human tumors. Luminal, basal-like, normal-like, and erBB2+ subgroups were identified and were shown to have different prognoses. Breast cancer is the most common cancer among women worldwide, with 1.3 million women diagnosed each year and about 500,000 deaths per year from the disease. Recently molecular studies have been conducted on early diagnosis of breast cancer. In this study, expression levels of Mir-210 and Mir-141 were reported in breast cancer samples. **Methods:** The expression levels of Mir-210 and Mir-141 were checked by Real Time-PCR method in 35 breast cancer tissues and 35 adjacent normal tissues. **Results:** This study for Mir-210 reported that 42.8% of tumor samples have increased of expression levels comparison with normal samples and 45.8% of tumor samples showed an increase in expression level for Mir-141. Finally, was not observed significant difference between the levels of expression in these micro RNAs ($P > 0.05$).

Keywords - Breast Cancer, Mir-210, Mir-141, Real Time-PCR Method.

I. INTRODUCTION

Breast cancer comes from uncontrolled cell growth in breast tissue and is a heterogeneous disease. These cells usually form a tumor which is often felt as a mass. Breast cancer occurs almost entirely in women, accounting for 23% of the cancer in women but men can also get breast cancer (Nafissi, Saghafinia, Motamedi, & Akbari, 2012; Rouzier et al., 2005). GLOBOCAN statistics for 2012 announced that approximately 1.7 million women were diagnosed with breast cancer in that year, with 522,000 related deaths. Besides this, among the total cancer cases, breast cancer incidence represented about 11 % in 2008

while this number jumped to 12 % in 2012. According to American Cancer Society, one in eight women in the United States was developed breast cancer in her lifetime and approximately 232,340 new cases of invasive breast cancer and 39,620 breast cancer deaths were diagnosed for 2013 among US women (Siegel, Ma, Zou, & Jemal, 2014; Tao et al., 2015). The incidence of breast cancer in women around the world is projected to reach around 3.2 million new cases per year by 2050 (Hortobagyi et al., 2005). Breast cancer is divided into five subtypes that include Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2/ERBB2)-positive, basal-like, and normal-like breast

cancer(Reis-Filho & Pusztai, 2011).One of the categories of breast tumors is the presence or absence of estrogen (ER) and progesterone (PR) receptors so that patients with positive estrogen and progesterone receptors are more at risk than those with negative receptors(Anderson, Chu, Chatterjee, Brawley, & Brinton, 2001).Recent studies have focused on the depth of the molecular problems of this disease(Bidoki et al., 2018; Negari et al., 2018). MicroRNAs are emerging as important regulators of cancer-related processes that are known as post-transcription regulators (Lagos-Quintana, Rauhut, Lendeckel, & Tuschl, 2001; Lee & Ambros, 2001). MicroRNAs have been found implicated in a multitude of cellular processes including proliferation, differentiation, migration and apoptosisso that aberrant microRNA expression has been linked to diseases including cancer(Esquela-Kerscher & Slack, 2006; Meng et al., 2007).So examining different biomarkers like MicroRNAs can help in early diagnosis of cancers(Kota & Balasubramanian, 2010). In this paper, the expression levels of Mir210 and Mir141 were investigated in breast tumor samples. So far, numerous studies have been conducted on these microRNAs in breast cancer(Finlay-Schultz et al., 2015; Hong et al., 2012; Uhlmann et al., 2010).MiR-210 appears as an important regulator of the cellular response to hypoxia and has been called the “micromananger of the hypoxia pathway”(Huang, Le, & Giaccia, 2010). In malignant disease, hypoxia evolves because of insufficient vasculature supporting the growing number of cancer cells.

Regulation of miR-210 is reported to be dependent on the transcription factor HIF1(Crosby, Kulshreshtha, Ivan, & Glazer, 2009). Expression of Mir 210 is effective in the spreading and proliferation of the tumor and is recognized as a strong biomarker in breast cancer(Rothe et al., 2011).

Mir141 is a member of the mir-200 family which involved in the Tumor progression and especially in the EMT process, so the mir-200 family is particularly involved in immigration and invasion(Liep et al., 2016).Also, Mir141 directly targets PHLPP1 and PHLPP2 that reduces AKT activity and causes apoptosis and inhibits tumor growth(Mei et al., 2014).In addition, reducing the expression of Mir141 leads to an increase in PR protein levels(Finlay-Schultz et al., 2015).

II. MATERIAL AND METHODS

2.1 Human specimens

Human breast cancer specimens (n = 35) and adjacent non-tumor tissues were obtained from patients at Imam Khomeini Hospital, Tehran, Iran. with informed consent from each patient. Patient demographic and clinicopathologic characteristics are shown in Table 1.

Table 1. Clinicopathologic characteristics of thirty-five breast cancer patients.

Variable	Value (n = 35)
Gender	
Female	35 (100%)
Smoking history	
Never-smoker	28 (80%)
Smoker	7 (20%)
Stage	
I/II	19 (54.3%)
III	16 (45.7%)

2.2 RNA extraction and cDNA preparation

Total RNA was isolated from each tumor tissue and adjacent non-tumor tissue by using RiboEx (Gene All, Korea) according to the manufacturer’s specifications. The concentration of total RNA in the final eluate was

determined by spectrophotometry. The synthesis of cDNA (240 ng of total RNA per 20 µL reaction mixture) was performed using the Prime Script RT reagent kit (Perfect Real Time) RR037A (Takara, Japan) according to the

manufacturer's specifications. The obtained cDNAs were stored in -70°C until use.

2.3 Real-time quantitative PCR

Real-time PCR was performed using an StepOnePlus™ Real-Time PCR Systems (ABI Applied Bio-systems, Thermo Fisher Scientific, USA) in a 15-μl reaction containing 7.5-μl of Real Q Plus 2x Master Mix Green High ROX™ (Ampliqon, Denmark), 1-μl of cDNA, 5.5-μl of H₂O and 1-μl of mixed forward and reverse primers (6

Pmol/μl concentration). Real-time PCR amplifications were done as follows: for two selected miRNA, PCR amplification was set to an initial 95°C for 15 min and then for both miRNAs, a total of 40 cycles, 95°C for 15 seconds and 60°C for 1 min (step and hold). All samples were analyzed in duplicate. RNU44 was used as an internal control. Gene expression was calculated using the comparative threshold cycle ($2^{-\Delta\Delta CT}$) method. The primers used for real-time PCR are listed in Table 2.

Table 2. Primer sequences for selected miRNAs and RNU44.

Target Name	Sequences (5' → 3')	Length
miR-141	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCAC TGGATACGACCCATCT	50
miR-210	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCAC TGGATACGACTCAGCC	50
RNU44	GTCGTATCCAGTGCAGGGTCCGACCGGTATTTCGC ACTGGATACGACAGTCAG	52

2.4 Statistical Analysis

Statistical analysis was performed using the GraphPad Prism v7.03 (GraphPad Software Inc., USA) and T-test. For all tests, a P value <0.05 was considered statistically significant.

III. RESULTS

3.1 Results of the expression level of Mir 210 in breast cancer tissues using the Real Time-PCR method

The results of expression level of this microRNA in 35 breast tumor samples compared to 35 adjacent normal samples showed that out of 35 tumor samples, 15 samples (42.8%) showed an increase in expression level of Mir 210 compared to normal samples, in addition 20 samples (57.2%) indicated a decrease in expression of this

microRNA. Finally, was not shown significant difference between the expression levels of this Mir in tumor samples and adjacent normal samples ($P>0.05$) (Fig 1a and 2a show the consequence of the Mir 210).

3.2 Results of the expression level of Mir 141 in breast cancer tissues using the Real Time-PCR method

The changes in the expression level of Mir 141 in this study indicate that, this Mir has increased in 16 samples (45.8%) of 35 tumor samples compared with normal samples while 19 tumor samples (54.2%) report a decrease in expression level of this Mir. So that there was no significant difference between the expression levels of Mir 141 in tumor samples comparison with normal samples ($P>0.05$) (Fig 1b and 2b demonstrate the outcome of Mir 141).

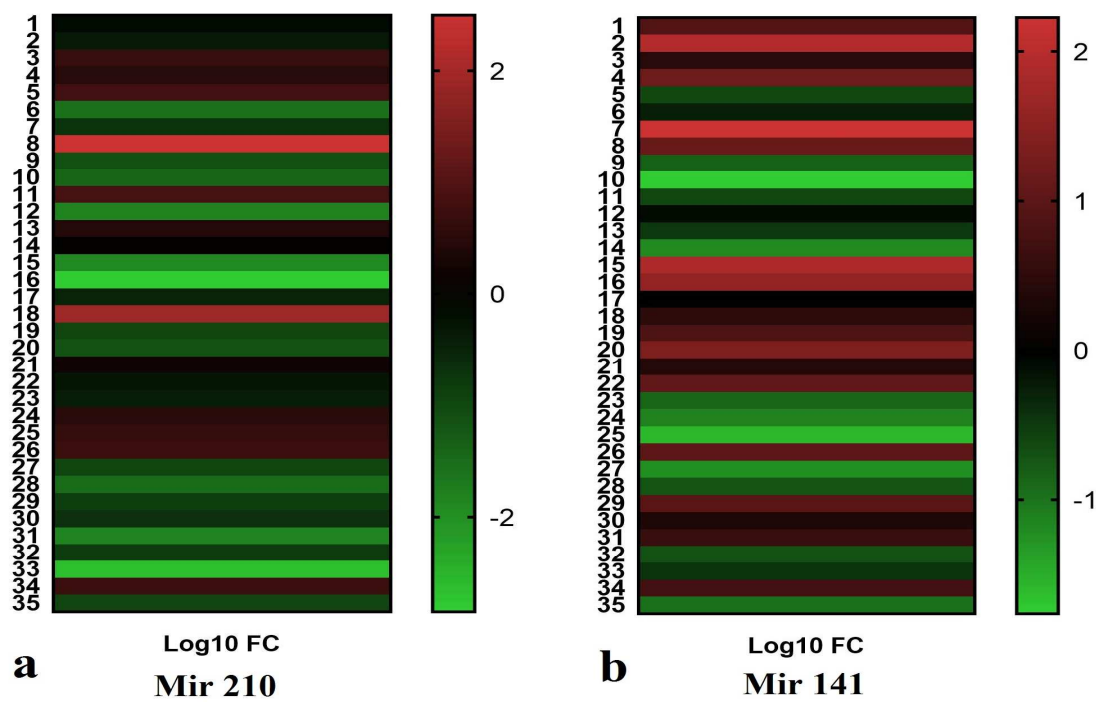


Fig.1 The graph (a) related to Mir 210 expression level and (b) related to Mir 141 expression level.

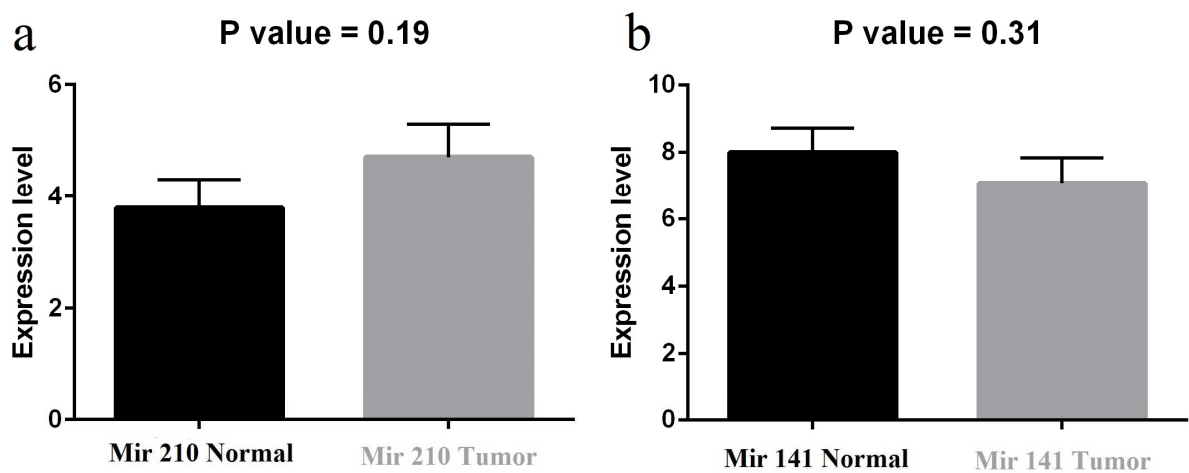


Fig.2. The graph (a) related to comparison of Mir 210 expression between tumor samples and adjacent normal samples and graph (b) related to comparison of Mir 141 expression between tumor samples and adjacent normal samples.

IV. DISCUSSION

Over the last few years, a great number of studies have reported aberrant patterns of miRNAs expressions in various

cancers including breast cancer. Therefore, understanding of the different mechanisms involved in the onset and progression of breast cancer can provide the basis for better

treatment. In this study, we have analyzed the expression level of mir210 and mir141 in thirty-five breast cancer patients by real-time quantitative PCR.

Earlier researches on these genes determine different results in various cancers. Puissegur et al. demonstrated that the expression rate of mir210 was significantly increased in lung cancer tissues rather than normal tissues (Puissegur et al. 2010). Also, miR-141 is frequently up regulated in colon cancer and is associated with cancer cell proliferation of CRC (Ding et al. 2017). In another study, Liu et al. showed MicroRNA-141 suppresses prostate cancer stem cells and metastasis by targeting a cohort of pro-metastasis genes (Liu et al. 2017). Considering the mentioned studies and the roles of this miRNA in normal breast tissues, we expect the over expression of mir210 occurs in these patients, but in this case, over expression of mir210 was observed only in 15 breast cancer patients (42.8%). Mir141 was also showed an increase in expression level in 16 breast cancer patients (45.8%).

In this study, we didn't see any significant difference in expression level between tumor and normal breast tissues for this miRNAs. However, the roles of the mir210 and mir141 in the evolution of breast cancer is complicated and requires more research and a larger population.

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