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Downregulation of microRNA-214 and PTEN in tissue samples of patients with breast cancer

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ABSTRACT

Background: MicroRNAs (miRNAs) are a large group of small non-coding RNAs with critical functions in the regulation of important genes involved in cellular proliferation and apoptosis, at post-transcription level. Phosphatase and tensin homolog (PTEN) is a best example of miRNAs target genes. In the present study, we aimed to evaluate the expression levels of miR-214 and PTEN in tumor tissues and adjacent normal tissues in patients with breast cancer.

Methods: The expression levels of miR-214 and PTEN were measured in tumor tissue and compared to adjacent normal cells in 42 patients with breast cancer by qRT-PCR. Statistical analysis was processed using SPSS 21.0, GraphPad 7.00 software or the Student's *t*-test. All data from at least three separate experiments are presented.

Results: We found a significant downregulation of miR-214 expression levels in tumor tissues in comparison to normal tissues (0.85 ± 0.45 vs. 9.59 ± 1.71; $P < .001$). In addition, the expression levels of PTEN was also significantly lower in tumor tissues, as compared to adjacent normal tissues (0.75 ± 0.44 vs. 22.72 ± 6.76; $P < .001$). There was also a significant correlation between miR-214 and PTEN expression and clinicopathological factors including age, tumor size, and cancer grade ($P < .001$).

Conclusion: These findings suggest that miR-214 and PTEN have tumor-suppressor activity and thus, pharmaceutical interventions targeting miR-124 and PTEN may provide a promising therapeutic strategy for the treatment of breast cancer.

1. Introduction

Breast cancer, one of the most lethal types of human malignancies, is ranked as the second most common cancer, worldwide (Majidinia and Yousefi, 2017). In spite huge efforts in early diagnosis and designing novel most effective therapeutic strategies for breast cancer, potent proliferative ability of tumor cells, as well as development of drug resistance to conventional therapies result in the failure in the complete treatment of patients (Majidinia et al., 2017). Therefore, elucidation of exact molecular mechanisms involved in the initiation/progression of breast cancer is an urgent need. For this reason, the accumulating recent studies have been focused on the clearer understanding of genetic susceptibility to breast cancer. In addition to BRCA1/2 and p53, which are high-penetrance breast cancer predisposition genes in breast cancer, it is well-demonstrated that inhibition of the phosphatase and tensin homolog (PTEN) has also a critical function in the predisposition for this malignancy (Alimonti, 2010). PTEN is a dual protein/lipid phosphatase with major functions in

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phosphoinositide 3-kinase (PI3K)/Akt pathway, which converts Phosphatidyl-inositol 3,4,5 triphosphate (PIP3) to the PIP2 (Wong et al., 2010). Through this activity, PTEN negatively regulates PI3K/Akt signaling and contributes in the major biological processes such as metabolism, proliferation, and apoptosis (Wong et al., 2010). Down regulation of the PTEN gene results in a significant increase in the activity of PI3K/Akt transduction pathway, which ultimately not only leads to breast cancer tumorgenesis, but also confers resistance to targeted therapy (Papa and Pandolfi, 2019). Therefore, identifying and targeting factors and mechanisms that increase PTEN expression in cancer cells can be useful in the suppression of cancer cells growth and proliferation and can be considered as a new therapeutic strategy (Papa and Pandolfi, 2019).

MicroRNAs (miRNAs) are small non-coding RNA molecules with major function in the regulation of gene expression at post-transcriptional level, through direct targeting of mRNA molecules at 3′ untranslated region (UTR) (Aushev et al., 2018). Through wide range of targets of miRNAs, they are extensively contributed in the various biological events, as well as pathological process such as cancer (Søkilde et al., 2019). In this context it is suggested that miRNAs are involved in the regulation the expression levels of approximately 50% of all protein-coding genes (Søkilde et al., 2019). Regarding to contribution of decreased or increased expression levels of particular miRNAs in tumorigenesis, these RNAs may play either tumor suppressor or oncogenic roles in cancer. miR-214 is one of the miRNA with special importance in breast cancer, in which its expression levels were reported to be reduced (Sharma et al., 2015). More interestingly, it was demonstrated that downregulation of miR-214 was associated with a subset of tumors that are basal, estrogen receptor-negative, human epidermal growth factor-2-positive, of histological high grade and aggressive behavior (). In spite of the fact that overexpression of miR-214 significantly suppresses the proliferation of breast cancer cell, the exact mechanism underlying its anti-cancer effects is still remained unclear (Sharma et al., 2015). Importantly, PTEN is one of the well-known targets of miR-214, by which various pathological functions of miR-214 such as cell survival and drug resistance are mediated (Schwarzenbach et al., 2012b). Therefore, in the present study, the expression levels of miR-214 and PTEN were determined in tumor tissues and adjacent normal tissues, and their association with clinical pathologic data of patients was also compared. In this way, the possible role of this miRNA in the process of breast carcinogenesis, possibly through the regulation of PTEN expression could be determined.

2. Material and methods

2.1. Patients and tissue samples

Specimens were collected from 42 patients with breast cancer and matched adjacent non-tumoral breast tissues, which were diagnosed by pathological surgical resection at the Emam Reza Hospital, Tabriz University of Medical Sciences from January 2018 to February 2019 (IR.TBZMED.REC.1397.502). The histological grade of the cancer was defined according to the World Health Organization (WHO) grading system and patients with undergone chemotherapy or radiation therapy before surgery were excluded. In regarding of Declaration of Helsinki, All of the subjects provided written informed consent and all protocols and procedures were approved by the Ethics Board at the Tabriz University of Medical Sciences.

2.2. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

According to the manufacturer's instructions, total RNAs were extracted from breast cancer tissues, adjacent tissues by RNeasy plus mini kit (Qiagen, Dusseldorf, Germany). Complementary DNA (cDNA) was synthesized using Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Promega, Madison, WI). qRT-PCR was performed with SYBR Premix Ex Taq (TaKaRa Bio, Otsu, Japan) following standard protocol. PCR amplification was performed at 95 °C for 5 min prior to 42 cycles of 95 °C for 15 s, 59 °C for 30 s and 72 °C for 50 s, followed by a final incubation at 72 °C for 5 min. The fold changes were calculated via relative quantification ($2^{-\Delta CT}$).

2.3. Statistical analysis

All statistical analyses were expressed as mean ± standard deviation (SD). Statistical analysis was processed using the Statistical Product and Service Solutions (SPSS) Graduate Pack 21.0, GraphPad 7.00 software or the Student's *t*-test. Differences were considered statistically significant at $P < .001$. All data from at least three separate experiments are presented.

3. Results

In the present study, the expression levels of miR-214, as a tumor suppressor miRNA, was evaluated in tumor tissue and compared to adjacent normal cells in 42 patients with breast cancer by qRT-PCR. Our results showed a significant downregulation of miR-214 in breast cancer tissues compared with adjacent tissues (0.85 ± 0.45 vs. 9.59 ± 1.71; $P < .001$, Fig. 1). Additionally, the expression level of PTEN, which is a potential target of miR-214, was also evaluated in tumor and adjacent cells in breast cancer patients. Analysis by qRT-PCR demonstrated that the expression levels of PTEN was significantly decreased in tumor cells in comparison with normal tissue (0.75 ± 0.44 vs. 22.72 ± 6.76; $P < .001$, Fig. 2). In the next step, the correlation between miR-214 and PTEN expression and clinicopathological factors including age, tumor size, clinical stage, cancer grade and lymph node metastasis was assessed. We found that miR-214 and PTEN expression was associated with the age, tumor size and cancer grade (Table 1; $P < .001$). There was no correlation between the miR-214 or PTEN expression level clinical stage (Table 1). We also found a correlation between PTEN expression and lymph node metastasis. The correlation between miR-214 expression and lymph node metastasis was not significant. Further the correlation between miR-214 and PTEN was evaluated. We observed no significant correlation between the expression of miR-214 and the expression of PTEN in adjacent cells ($P > .05$).

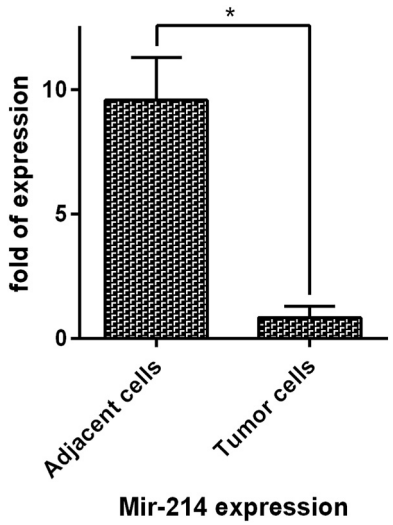

Fig. 1. Downregulation of miR-214 in tumor tissue of patients with breast cancer in comparison to adjacent normal cells. * means $P < .001$.

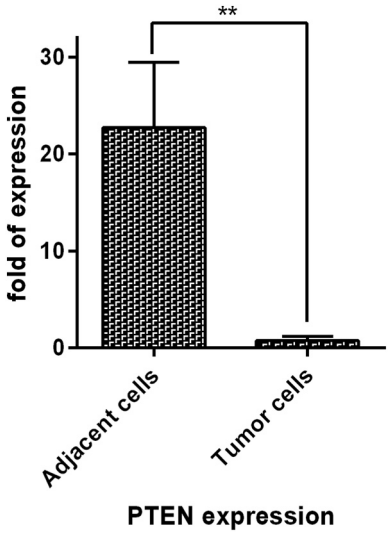

Fig. 2. Downregulation of PTEN in tumor tissue of patients with breast cancer in comparison to adjacent normal cells. * means P < .001.

4. Discussion

Downregulation of miR-214 is extensively reported in various human malignancies including hepatocellular carcinoma (Duan et al., 2012; Shih et al., 2012), human cervical cancer (Peng et al., 2012; Qiang et al., 2011; Yang et al., 2009), pancreatic cancer (Zhang et al., 2010), bladder cancer (Wang et al., 2015), and breast cancer (Liu et al., 2016; Wang et al., 2016). In spite its increasing significance in cancer pathogenesis, it's clear molecular biological functions is still not completely understood. As one of the important and well-studied miRNAs in breast cancer, in the present study we measured the expression levels of miR-214 in tumor tissues from breast cancer patients and evaluated its correlation with PTEN, as well as clinicopathological factors of patients. We showed the significant downregulation of miR-214 and its target PTEN in tumor tissues as compared with adjacent normal cell. In addition, there was a significant correlation between miR-214 or PTEN expression and the age, tumor size and cancer grade.

MiRNAs, as novel regulators of gene expression, have been demonstrated to play critical functions in various aspects of cancer, including cell proliferation, apoptosis, angiogenesis, metastasis, and development of drug resistance by targeting various molecules within a complicated regulatory network (Bach et al., 2017; Shafiei-Irannejad et al., 2018; Jafri et al., 2017). Among these miRNAs, miR-214 gains much more attention in recent years because of its dual function in development of different types of cancer (Penna et al., 2011; Shih et al., 2012). In other words, miR-214 may have tumor suppressing functions, which is reported in breast cancer and hepatoma (Shih et al., 2012), or may also have oncogenic functions and promote carcinogenesis as it proved in melanoma (Penna et al., 2011). However, in breast cancer finding are controversial. For example, Wang et al. (Wang et al., 2016). Showed that miR-214 acts as an oncogenic miRNA in breast cancer and promotes tumor cell growth by targeting the PTEN-PI3K/Akt pathway (Wang et al., 2016). They reported upregulated values of miR-214 in tumor tissues form breast cancer patients in comparison with adjacent normal tissues (Wang et al., 2016). In contrast, Schwarzenbach et al. (2012a). Reported that miR-214 plays tumor suppressor function in breast cancer, because of downregulation in serum samples of patients with breast cancer. More importantly, the authors showed that miR-214 could discriminate malignant from benign tumors and healthy controls (Schwarzenbach et al., 2012a). Similar results were found in a study by Liu et al.(Liu et al., 2016). In which it was revealed that the miR-214 was significantly downregulated in breast cancer tissues compared with adjacent tissues. Overexpression of this miRNA in breast cancer cell lines resulted in the decrease in cell proliferation and migration, induction in apoptosis, and disruption in cell cycle (Liu et al., 2016). In accordance with the finding of Liu and Schwarzenbach, our study showed decreased expression levels of miR-214 in tumor tissues as compared with adjacent normal tissues in patients with breast cancer. Therefore, we showed tumor suppressor function of miR-214 in breast cancer, downregulation of whom resulted in the progression of breast cancer.

miRNAs affect the various aspects of cancer cells through targeting particular mRNAs and altering their expression levels. PTEN, a negative regulator of PI3K/Akt signaling pathway, is reported to be targeted by miR-214, which is demonstrated in immortalized ovarian surface epithelial cell lines (Yang et al., 2008), T cells (Jindra et al., 2010), and monocytes (Yousefi et al., 2015). By targeting PTEN, miR-214 was shown to increase cell proliferation and delay apoptosis. In breast cancer, the loss of function of PTEN was reported to be associated with reduced survival of these patients (Razis et al., 2011). Therefor we planned to evaluate the expression levels of PTEN in breast cancer tissue samples and adjacent normal cells. We found significant downregulation of PTEN in tumor tissues as compared with adjacent normal tissues. In addition, there was also a positive correlation between the expression levels of miR-214 and PTEN in tumor and normal tissue; however, it was not statistically significant.

In conclusion, the most important finding of this study was the distinguishing value of tissue levels of miR-214 and PTEN in malignant and normal breast tissues and their correlation with the age, tumor size and cancer grade. Our results showed a significant down regulation of miR-214 and levels of PTEN in breast cancer tissues compared with adjacent tissues. Further, the correlation between miR-214 and PTEN was evaluated; we observed no significant correlation between the expression of miR-214 and the expression of PTEN in adjacent cells. Therefore, pharmaceutical interventions targeting miR-214 and PTEN may provide a promising therapeutic strategy for the treatment of breast cancer.

Table 1
Correlation of Mir-214 and PTEN expression with clinicopathological characteristics of breast cancer patients.

Parameters	Number of Patients	Relative Mir-214 expression	P value	Relative PTEN expression	p value
Age < 50 years	14	1.25 ± 0.27	< 0.001	0.47 ± 0.18	< 0.001
Age > 50 years	28	0.45 ± 0.16		1.27 ± 0.27	
Tumor size < 2 cm	26	0.92 ± 0.45	< 0.001	0.96 ± 0.41	< 0.001
Tumor size > 2 cm	16	0.35 ± 0.37		0.33 ± 0.09	
Stage I	22	0.78 ± 0.37	=0.3	0.76 ± 0.38	=0.8
Stage II and III	20	0.65 ± 0.51		0.74 ± 0.53	
Grade I	23	1.01 ± 0.41	< 0.001	1.01 ± 0.41	< 0.001
Grade II and III	19	0.44 ± 0.24		0.44 ± 0.24	
Lymph node metastasis	24	0.47 ± 0.19	=0.9	1.25 ± 0.27	< 0.001
No lymph node metastasis	16	0.46 ± 0.25		0.42 ± 0.19	

Credit author statement

B.Y and M.M: Conceptualization; M.Y, A.K: qRT-PCR; N.T and V.S: Analysis; S.S, N.T and A.S: Investigation; N.F and H.S: Methodology; B.Y: Supervision; M.B: Validation; S.S and S.R: Writing draft; M.M and B.Y: review & editing.

Declaration of Competing Interest

None.

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