Original Article

Eur J Breast Health 2022; 18(2): 141-147
DOI: 10.4274/ejbh.galenos.2022.2021-10-4

Overexpression of miR-490-5p/miR-490-3p Potentially Induces IL-17-Producing T Cells in Patients With Breast Cancer

Farhad Seif[1,2], Hajar Vaseghi[3], Mehdi Ariana[4], Shahla Mohammad Ganji[5], Mohammad Nazari[3], Kowsar Kiani Rad[6], Majid Pornour[3]

[1]Department of Immunology and Allergy, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran
[2]Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran
[3]Department of Photo Healing and Regeneration, Medical Laser Research Center, Yara Institute, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran
[4]Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[6]Department of Genetic, Science Faculty, Shahrekord University of Medical Sciences, Shahrekord, Iran

ABSTRACT

Objective: Breast cancer (BC) is the most prevalent female cancer globally and this is also true in Iranian women. Alteration in circulating microRNAs affects the fate of immune cells, affecting immunological response to neoplasia.

Materials and Methods: We investigated the expression of *miR-490-5p* and *miR-490-3p* in peripheral blood mononuclear cells (PBMCs) and plasma of patients with BC. Moreover, the correlation of these microRNAs with the expression levels of *CD3d, interleukin 2 (IL-2), IL-2 receptor chain alpha (IL-2RA), forkhead box O1 (FOXO1)* and *nuclear factor of activated T cells 5 (NFAT5)* were investigated.

Results: Two groups, including 42 patients with BC, aged 22–75 years with stage I, II, III disease without administration of immunosuppressive chemotherapy regimens/radiotherapy and 40 healthy controls aged 27–70 years, participated. Overexpression and higher circulation levels of *miR-490-5p* and *miR-490-3p* were found in the patients with consequent down-regulation of all targets investigated in PBMCs. Furthermore, there was a significant negative correlation between the overexpression of these microRNAs and a reduction in levels of *CD3d*, *IL-2,* and *IL-2RA* in patients with BC.

Conclusion: These results suggest that down-regulation of the target genes by miR-490 may predispose and facilitate the production of Th17 lymphocytes and IL-17-producing Tregs. The variation in miR-490-5p/-3p and the investigated targets in the PBMCs of BC patients may be used as non-invasive diagnostic markers.

Keywords: miR-490; breast cancer; CD3d; FOXO1; IL-2; IL-2RA; NFAT5

Cite this article as: Seif F, Vaseghi H, Ariana M, Ganji SM, Nazari M, Rad KK, Pornour M. Overexpression of miR-490-5p/miR-490-3p Potentially Induces IL-17-Producing T Cells in Patients With Breast Cancer. Eur J Breast Health 2022; 18(2): 141-147

Key Points

- Overexpression and higher circulation of miR-490-5p and miR-490-3p were found in patients with stages I-III breast cancer.
- Furthermore, the expression of the targets of these microRNAs, including *FOXO1, CD3d, NFAT5, IL-2,* and *IL-2RA* were decreased in PBMCs of patients with breast cancer.
- These findings suggest a shift in lymphocyte population towards the production of Th17, Tregs, and IL-17-producing Tregs.

Introduction

Breast cancer (BC) is the most prevalent cancer amongst women worldwide, including in the Iranian population. Epigenetic factors play a crucial role in the initiation and progression of BC (1). One of these epigenetic factors is characterized by the variability in microRNAs in both tumoral tissues and circulation. These changes in microRNAs may act directly or indirectly to increase cancer cell proliferation or modification of the tumor microenvironment toward favorable tumor requirements, and drug resistance (2). MicroRNAs (miRNAs) are short (18–23 nucleotide), non-coding RNAs that regulate various complementary mRNAs post-transcriptionally. Secretory components, especially

Corresponding Author:
Majid Pornour; ma.pornour@gmail.com

Received: 04.11.2021
Accepted: 05.02.2022
Available Online Date: 01.04.2022

©Copyright 2022 by the the Turkish Federation of Breast Diseases Societies / European Journal of Breast Health published by Galenos Publishing House.

exosomes and microvesicles, have an important role in circulating and shuttling these regulatory factors throughout the body (3, 4).

Several studies have shown the effects of onco-microRNAs on the production of immune suppressor cells, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), M2 type macrophages, etc. (5-8). Tregs are a subpopulation of T-lymphocytes that have been shown to play a role in BC (9). Soheilifar et al. (6) showed that shuttling or concomitant overexpression of some of these onco-microRNAs, such as miR-182-5p and miR-182-3p, can target some proteins such as nuclear factor of activated T cell (NFAT) proteins, the T-cell receptor/complementarity determining region 3 (TCR/CD3) complex, and the interleukin 2/interleukin 2 receptor A (IL-2/IL-2RA) pathway to induce Tregs. The same study demonstrated that concomitant targeting FOXP3 inducer transcription factor (Forkhead box O1; FOXO1), NFATs that inhibited FOXP3 transcription factor, activation of interleukin-6 (IL-6) signaling, and inhibition of IL-2 signaling by miR-182-5p/-3p could induce an increase in the population of Tregs, including $FOXP3^+$ IL-17-producing Tregs and $FOXP3^+$ Tregs in BC patients (6).

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway has a pivotal impact on modulation of immune cells (10, 11). Activation of IL-2/IL-2RA induces STAT5 phosphorylation and activation (12). STAT5 is a transcription factor that induces FOXP3 expression, in addition to other inducer transcription factors, such as FOXO1 (13). In contrast, activation of NFAT proteins can activate T cells and induce their differentiation toward Th1 and Th2 subpopulations as well as suppress FOXP3 expression and Treg formation (14). Furthermore, initiation of IL-6 signaling causes STAT3 phosphorylation and inhibition of FOXP3 expression, as well (15). Activation of different members of the NFAT family, such as NFATc1 and NFATc2, recruit NFSATc4 that play a pivotal role in the induction of IL-2 by activation of T cells and activation of TCR/CD3 complex signal transduction (16-18). The expression or shuttling of miR-182/miR-183-96 cluster to immune cells in a BC microenvironment inhibits IL-2 and IL-2RA expression by targeting various TCR/CD3–associated signal transduction proteins (6). Moreover, miR-182-3p and miR-183 can negatively affect IL-2 production via targeting NFATc4 but also by directly targeting IL-2RA to prevent IL-2/IL-2RA signaling initiation (16).

However, NFAT5, the other member of NFAT family, induces IL-2 pathway and enhances the IL-17 inducer genes (19). The data derived from Gene Expression Omnibus (GEO) used to identify some microRNAs such as miR-490, etc. were upregulated in tumor tissues and over circulated in sera of patients with BC (6). Yang et al. (20) showed that miR-490-3p could directly target FOXO1. Also, Yang et al. (21) showed that targeting miR-490-5p inhibits the suppressive function of Tregs. However, some studies showed that miR-490-5p and miR-490-3p had potential in the production of Tregs and their polarization to IL-17-producing cells. Also, they potentially target *CD3d, IL-2, IL-2RA, FOXO1,* and *NFAT5* (22). Considering the role of miR-490 in IL-17 producing T cells formation and their potential in targeting *FOXO1, CD3d, IL-2, IL-2RA*, and *NFAT5*, after identification of miR-490-5p and miR-490-3p, we aimed to investigate their expression in peripheral blood mononuclear cells (PBMCs) and the plasma of the patients with BC. Finally, we evaluated the correlation of miR-490-5p and miR-490-3p with the expression of *CD3d, IL-2, IL-2RA, FOXO1*, and *NFAT5*.

Materials and Methods

Patient Selection

The participants were as follows. The patient group consisted of 42 patients with BC, aged 22–75 years who were patients in stages I, II, III of the disease without administration of immunosuppressive chemotherapy regimens/radiotherapy. The control group comprised 40 healthy individuals aged 27–70 years who were referred to Shohada Hospital. Written informed consent was obtained from the participants before the study. Demographic characteristics of all participants, including age, and marriage status, and pathologic data were gathered using a questionnaire from the pathology department. Exclusion criteria were advanced and metastatic cancer, neoadjuvant, a significant clinical disorder, psychiatric drug use for the past 5 months.

5 mL peripheral blood was collected from all participants in tubes containing EDTA and centrifuged at 150 g at 4 °C for 2 min. Then, we separated plasma, and then an equal volume of phosphate-buffered saline (PBS) was added to each blood sample and diluted gently. Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation was used to isolate the PBMCs and the buffy coat, that contained lymphocytes, was collected after centrifuging at 800 g at 4 °C for 15 min. This study was approved by the Ethics Committee of the Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (ethics code: IR.SBMU.RETECH.REC.1397.562).

RNA Extraction and qRT-PCR

Total RNA and circulating RNA were extracted from extracted PBMCs and 1 mL of plasma using the RiboEx LS reagent (Geneall, South Korea). Then, cDNA was synthesized for evaluation of the *CD3d, IL-2, IL-2RA, FOXO1*, and *NFAT5* using a first-strand cDNA synthesis kit (Thermo Fisher Scientific) followed by PCR according to the manufacturer's protocol. To assess microRNAs' levels (RNA-derived from plasma), specific hairpin loop primers were used to synthesize cDNA of the microRNAs of interest. The expression and variation of microRNAs (miR-490-5p and miR-490-3p) and their targets were evaluated by SYBR Green master mix kit (Genaxxon kit, Germany) on a MIC qPCR instrument (BioMolecular Systems, Australia). The specific primers are listed in Table 1. Eventually, qRT-PCR-derived data were analyzed by the $2^{-\Delta\Delta CT}$ and $2^{-\Delta CT}$ methods. Beta actin and GAPDH were utilized as housekeeping genes for comparison of the expression of target genes and RNU6 was used as the housekeeping gene for comparison of microRNAs.

Statistical Analysis

R-Studio 1.0.136 software was used to generate the correlation heatmap between miR-490-5p and miR-490-3p with their potential targets. In the current study, $p<0.05$ was considered statistically significant. Finally, multivariate analyses were performed to show the relationship between microRNAs and the expression level of their targets in the PBMCs of patients with BC. Comparison was made using the demographic and clinical characteristics of patients and controls. Statistical comparison was performed using SPSS, version 18 (IBM Inc., Armonk, NY, USA). Relative changes of microRNAs and their target genes in PBMCs of the patients with BC were assessed using student's t-test. Also, receiver operator characteristic (ROC) curve analysis was performed for miR-490-5p and -3p besides their targets in PBMCs samples using SPSS, version 18 (IBM Inc., Armonk, NY, USA).

Table 1. The primer sequences used in the current study

Stem loops for cDNA synthesis of microRNAs		
490-5p	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC ACCCACCT -3'	
490-3p	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC CAGCATGG -3'	
RNU6	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATAT-3'	
	Primers for qRT- PCR	
	Forward	**Reverse**
miR-490-5p	5'-GTGCAGGGTCCGAGGT-3'	5'-ATATCCATGGATCTCCAGGTGG-3'
miR-490-3p	5'-GTGCAGGGTCCGAGGT-3'	5'-TACAACCTGGAGGACTCCATG-3'
RNU6	5'-CGCTTCACGAATTTGCGTGTC-3'	5'-CGCTTCGGCAGCACATATACT-3'
GAPDH	5'-CCGAGCCACATCGCACAG-3'	5'-GGCAACAATATCCACTTTACCAG-3'
β-actin	5'-AGACGCAGGATGGCATGGG-3'	5'-GAGACCTTCAACACCCCAGCC-3'
CD3d	5'-AAGTGAGCCCCTTCAAGATACC-3'	5'-TCTGAGAGCAGTGTTCCCAC-3'
NFAT5	5'-AACAACATGACACTGGCGGT-3'	5'-CTCGAAAAACCAATCTGGCACG-3'
IL-2	5'-AAGGCCACAGAACTGAAACATC-3'	5'-ATTGCTGATTAAGTCCCTGGGT-3'
IL-2RA	5'- GATGCCAAAAAGAGGCTGACG-3'	5'-CCACATCAGCAGGTATGAATCCA-3'
FOXO1	5'- GAGGGTTAGTGAGCAGGTTACAC-3'	5'- TGCTGCCAAGTCTGACGAAAG-3'

Results

Pathologic Results

Pathology examinations showed that 23.8, 52.38, and 23.8 percent of the patients, respectively, were related to stages of I, II, and III. Almost 73.8% of patients expressed estrogen receptor (ER), and 64.2% were progesterone receptor (PR)-positive. Also, 21.42% of patients were human epidermal growth factor receptor 2 (HER-2)-positive. P53 mutation has been observed in 11.9 percent of these patients. Comparison between BC patients and control groups showed that patient groups had more abortions (nine abortions) even though the control group had more pregnancies (44 pregnancies) and longer time of breastfeeding (Table 2). However, there is not too much difference between other criteria between the two groups. Moreover, 36 of 40 people (90%) in the control group were married, and 36 of 42 people (86.7%) of patients were married. Of 42 patients, 35 were non-menopause (83.3%), while 87.5% of the control group were non- menopause (35 of 40 people). Moreover, there are no significant differences between these two groups in terms of menstruation (year). The pathological and clinical characteristics of patients with BC are summarized in Table 2.

Circulating miR-490-5p and miR-490-3p Were Increased in Plasma From Patients with BC

There was little published data on the expression of miR-490-3p in the plasma of the patients with BC. Thus miR-490-5p and miR-490-3p were evaluated separately. Expression analysis derived from real-time PCR showed that only miR-490-5p was significantly raised in the plasma of the patients with BC, whereas miR-490-3p in BC patients did not differ from healthy controls (Figure 1a). It was observed that miR-490-5p was increased 4.95-fold in patients with BC compared to controls ($p<0.01$).

MiR-490-5p and miR-490-3p Were Increased in PBMCs of Patients with BC

Both miR-490-5p and miR-490-3p were upregulated in PBMCs from patients with BC, by 15.6 times ($p<0.001$) and 13.14 times ($p<0.001$), respectively, compared to controls (Figure 1b).

Expression of Target Genes for miR-490-5p and miR-490-3p Were Decreased in PBMCs of Patients with BC

The expression levels of immune modulatory genes identified in the meta-analysis were compared between patients with BC and controls. This analysis showed significant down-regulation of the following genes: *FOXO1* 2.21 times ($p<0.01$), *CD3d* 3.9 times ($p<0.01$), *NFAT5* 4.8 times ($p<0.01$), *IL-2* 3.3 times ($p<0.05$), and *IL-2RA* 4.35 times ($p<0.01$) (Figure 1c). Correlation analysis was performed to investigate the relationship between expression changes in miR-490-5p and miR-490-3p and their respective target genes in patients and controls. There was a negative correlation between miR-490-5p and *CD3d* ($r = -0.658$, $p = 0.001$) and *IL-2RA* ($r = -0.670$, $p<0.001$), whereas there was a strong correlation between miR-490-5p and miR-490-3p in PBMCs of BC patients ($r = 0.823$, $p<0.001$). There was also a negative correlation between miR-490-3p and *CD3d* ($r = -0.698$, $p<0.001$), *IL-2* ($r = -0.462$, $p = 0.03$), and *IL-2RA* ($r = -0.725$, $p<0.001$). Moreover, there were a significant association between reduced expression of *CD3d* and *FOXO1* ($r = 0.41$, $p = 0.05$) and *CD3d* and *IL-2RA* ($r = 0.505$, $p = 0.014$). A significant relationship was found between *FOXO1* suppression and *NFAT5* ($r = 0.495$, $p = 0.016$). Finally, the decrease in *IL-2* expression was correlated with the decrease in *IL-2RA* ($r = 0.601$, $p = 0.002$) (Figure 2a).

ROC curve analysis was used to investigate the sensitivity and specificity of miR-490-5p, miR-490-3p, *FOXO1, CD3d, NFAT5, IL-2,* and *IL-2RA* expression levels in PBMCs of patients with BC compared to controls. The area under the curve (AUC) values for discrimination

Table 2. Pathological and clinical characteristics of patients with breast cancer

Age at diagnosis	(25–35)		(35–45)	(45–55)			(Up to 55)		
	(n = 9)		(n = 13)	(n =16)			(n = 2)		
Marriage	**Married**			**Unmarried**					
	n = 36			n = 4					
First menstruation (year)	Mean (n=40)								
	13±1.43								
Menopause	**Menopause**			**Non-menopause**					
	(n = 7)			(n = 33)					
Pregnancy	(n=36)								
	Mean (2.3±1.4)								
Abortion	(n = 9)								
	Mean (1.42±0.6)								
Breastfeeding (month)	(n = 36)								
	Mean (36.24±3.3)								
Axillary lymph nodes	N+			N-					
Invasive carcinoma histology	**Invasive lobular carcinoma (ILC)**			**Invasive ductal carcinoma (IDC)**					
	(n = 4)			(n = 36)					
Tumor grade	**Grade I**			**Grade II**			**Grade III**		
	(n = 10)			(n = 22)			(n = 10)		
Stage	**IA**	**IB**	**IC**	**IIA**	**IIB**	**IIC**	**IIIA**	**IIIB**	**IIIC**
	(n = 2)	(n = 3)	(n = 4)	(n = 13)	(n = 7)	(n = 2)	(n = 6)	(n = 4)	(n = 0)
Receptor status	ER+			PR+			HER-2+		
Positive	(n = 31)			(n = 27)			(n = 9)		
Negative	(n = 11)			(n = 15)			(n = 33)		
Total	(n = 40)								

ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2; n: number

of BC patients from healthy individuals were 0.840 ($p<0.001$) for miR-490-5p and 0.747 (p = 0.009) for miR-490-3p, respectively (Figure 2b). In the case of miR-490-5p and -3p, 73.5% and 69.7% of the positive outcomes would be correctly identified by diagnostic tests as positive. Also, 22.6% and 36.4% of the negative would be incorrectly specified by diagnosis test as positive for miR-490-5p and -3p, respectively. Moreover, 71.4, 68.8, 63.5, 62.5 and 61.5 percent of the positive outcomes would be correctly identified by diagnostic tests as positive for *IL-2, IL-2RA, CD3d, NFAT5* and *FOXO1*, respectively. Also, 33.3, 34.2, 28.6, 45 and 45.7 percent of the negative would be incorrectly specified by diagnosis test as positive for *IL-2, IL-2RA, CD3d, NFAT5,* and *FOXO1*, respectively. Similarly, the AUCs for BC patients compared to controls for expression levels of *CD3d*, *IL-2*, and *IL-2RA* were 0.680 (p = 0.014), 0.739 (p = 0.009), and 0.732 (p = 0.012), respectively. No significant difference was observed for *NFAT5* (p = 0.088) and *FOXO1* (p = 0.076) expression (Figure 2c). When the expression levels of miR490-5p and miR-490-3p and their target genes were examined in relation to clinical characteristics of the patients, no significant relationship was found.

Discussion and Conclusion

Previously, microarray-derived meta-analytical findings demonstrated an increased level of several microRNAs in both tumor tissue and plasma of patients with BC. Also, the immunosuppressive roles of some of these microRNAs have been described (6). Modulation of proteins involved in TCR/CD3 complex, IL-2/IL-2RA interactions and some transcription factors, such as NFATs, may direct T cells towards different Treg phenotypes (6). Furthermore, a concomitant decrease in FOXO1 level and reduction of NFATs has been associated with the production of IL-17-producing Treg (6). FOXO1, a transcription factor, induces *FOXP3* expression as the main step in directing T cells toward T regs, stimulated by STAT5 activation through some cytokine-related signaling pathways, such as the IL2/IL-2RA pathway (12, 13). In contrast, NFATs suppress the expression of *FOXP3* and induce Th1 and Th2 activation in normal conditions, together with *IL-2* expression, which stabilizes their functions (14, 15).

Several microRNAs have been reported to promote T-cell phenotype change from Th1 and Th2 toward Tregs or FOXP3^{+}IL-17-producing Tregs, including *miR-21, miR-182-5p, miR-182-3p, miR-183, miR-*

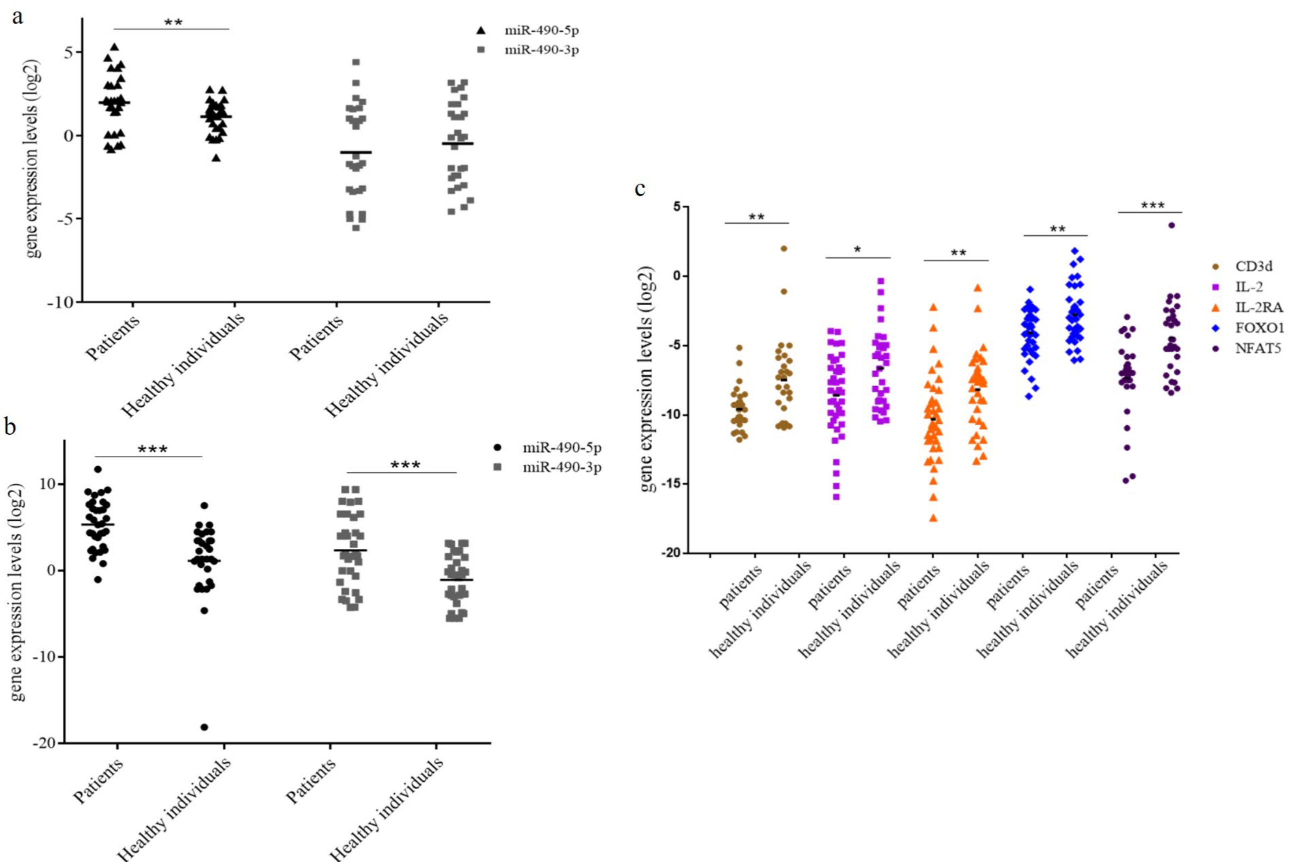

Figure 1. mir-490 cluster variation, circulation and possible effects in PBMC and plasma of BC patients. **a)** Variations of miR-490-5p and -3p in plasma of BC patients (derived from plasma data). **b)** Variations of miR-490-5p and miR-490-3p in PBMCs of patients with BC (derived from PBMC data), **c)** Variation of the miR-490-5p and -3p targets in PBMCs of patients with BC

BC: breast cancer; PBMCs: peripheral blood mononuclear cells

10a and Th17 cells are known to be involved in the progression of BC (5, 6, 23). Attenuation of TCR/CD3 signal transduction and reduction of NFATs, IL-2, and IL-2RA proteins may be effective in Treg production by activation of STAT5 (24). It has been shown that IL-2 expression is induced by NFATc1-4 and NFAT5 in different conditions (16-18, 25). However, Soheilifar et al. (6) showed that reduction of NFATs in patients with BC is associated with increased circulation of microRNAs, such as miR-182 and miR-183. Moreover, the negative effects of miR-490 on the expression of IL-2 have been confirmed by targeting NFAT5 (26).

The results of the current study showed a dramatic elevation of both miR-490-5p and miR-490-3p expression in PBMCs of BC patients, but only the plasma level of miR-490-5p was concomitantly increased while plasma levels of miR-490-3p were the same as in healthy controls. Therefore, increased expression of miR-490-5p in PBMCs and a concurrent high level in plasma means that these cells have both endogenous and exogenous sources of the microRNA whereas, because of the normal circulating levels of miR-490-3p, the only source potential pathogenic source for PBMCs is endogenous. Also, it was observed that the *CD3d* gene, coding for CD3d protein which is one of the CD3 complex proteins and a target for miR-490-3p, was significantly downregulated in PBMCs, and this reduction was associated with upregulation of miR-490-5p and miR-490-3p. So, both isoforms of miR-490-5p and miR-490-3p may be able to partially suppress the TCR/CD3 signal transduction cascade by downregulating *CD3d* expression, and may play a role in inhibition of T cell activation. In contrast, a significant reduction was observed in *IL-2RA* expression level. Moreover, this decrease in *IL-2RA* expression was associated with overexpression of both miR-490-5p and -3p isoforms. It is worth noting that a decrease in IL-2RA attenuates STAT5 phosphorylation and activation (12, 27). Such a decrease has been associated with increased levels of circulating onco-microRNAs, such as miR-182-3p, in sera of patients with BC (6). Furthermore, a significant reduction was found in *FOXO1* expression in the current study, which is targeted by miR-490-3p. This relationship, like other onco-microRNAs in BC, such as miR-182-3p, miR-183, has been confirmed in different studies (20, 28). However, simultaneous reduction of *FOXO1* and *NFAT* gene products may predispose to phenotype switch to IL-17-producing Tregs (6). Also, it has been shown that deficiency in the production of *FOXO1* has a key role in directing macrophages toward the M2-phenotype, which plays a critical role in the development of different kinds of cancers, especially BC (29).

It seems that miR-490 plays a role in decreased *IL-2* expression in PBMCs of patients with BC. Some studies have shown direct targeting by miR-490 of NFAT5, which induces IL-2 expression and production (5, 26). However, a significant reduction in *IL-2* expression level was

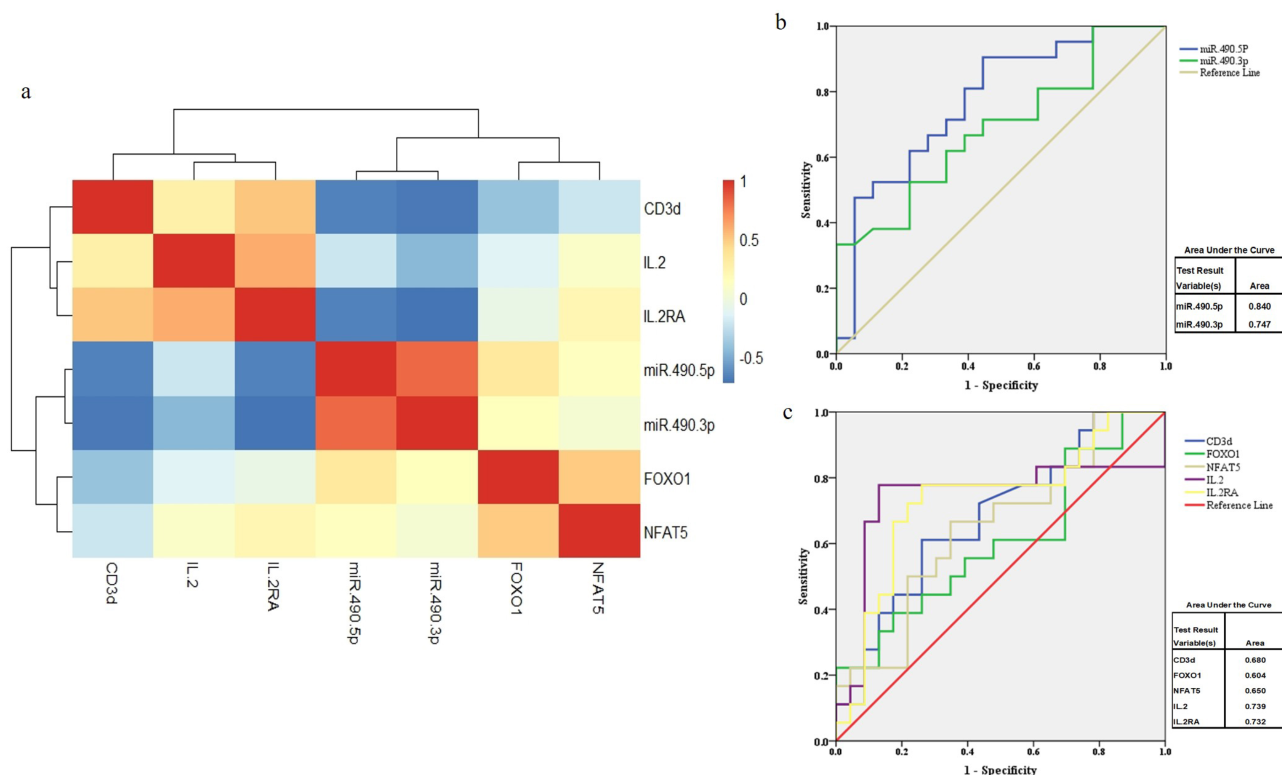

Figure 2. Hierarchical clustering analysis, Receiver operator characteristic (ROC) curve for mir-490 and area under the curves for target genes. **a)** Hierarchical clustering analysis showing the relationship between expression variability of miR-490-5p and miR-490-3p, *CD3d, NFAT5, IL-2, IL-2RA* and *FOXO1* in PBMCs of patients with BC, **b)** ROC curve was used for discrimination of BC patients. The areas under the curves are the gene expression level of *miR-490-5p*, *miR-490-3p* in PBMCs the patients with BC as compared with PBMCs of healthy individuals, **c)** The areas under the curves are the gene expression level of *CD3d*, *NFAT5*, *IL-2*, *IL-2RA*, and *FOXO1* in PBMCs of BC patients as compared with PBMCs of healthy individuals

BC: breast cancer; PBMCs: peripheral blood mononuclear cells

associated with miR-490-3p overexpression. Also, other studies have shown that *IL-2* was expressed irregularly in higher stages and in metastatic BC, and it is in line with our results in the case of IL-2 (30, 31). Thus, this study provides evidence that miR-490-5p and miR-490-3p may tip the balance between Treg and Th17 towards a Th17 T-cell phenotype through targeting *IL-2RA* as well as reduction of IL-2 by targeting *NFAT5*.

In conclusion, the results suggest potential for miR-490 to modulate the activity of *FOXO1, NFAT5, CD3d,* and *IL-2RA*. Over expression of mir-490-5p/-3p may facilitate the production of some phenotypes of T cells, which play a role in the progression of BC, including Th17, and IL-17-producing Tregs. A similar function has been suggested for other onco-microRNAs. Furthermore, the overexpression of both miR-490-5p and miR-490-3p and consequent suppression of their targets in PBMCs of BC patients may suggest a role as minimally invasive diagnostic markers in patients with BC.

Acknowledgements

We would like to thank Maryam Salari for her useful consultation regarding statistical analysis. This project was supported by grants 3008-10 and 11628 from the Academic Center for Education, Culture, and Research (ACECR) and the Cancer Research Center (CRC), respectively.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran with ethics number IR.SBMU.RETECH.REC.1397.562.

Informed Consent: Written informed consent was obtained from both groups prior to the initiation of the current study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A.; Concept: M.P.; Design: F.S., M.P.; Data Collection and/or Processing: H.V., S.M.G., M.N., K.K.R., M.P.; Analysis and/or Interpretation: H.V., S.M.G., M.N., M.P.; Literature Search: F.S., K.K.R., M.P.; Writing: F.S., M.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: Academic center for education, culture, and research (ACECR) and the Cancer Research Center (CRC), Grant/Award Number: 3008-10 and 11628, respectively.

References

1. Ariana M, Pornour M, Mehr SS, Vaseghi H, Ganji SM, Alivand MR, et al. Preventive effects of oxytocin and oxytocin receptor in breast cancer pathogenesis. Per Med 2018; 16: 25-34. (PMID: 30451597) [Crossref]

2. Torki Z, Ghavi D, Hashemi S, Rahmati Y, Rahmanpour D, Pornour M, et al. The related miRNAs involved in doxorubicin resistance or sensitivity of various cancers: an update. Cancer Chemother Pharmacol 2021; 88: 771-793. (PMID: 34510251) [Crossref]
3. Jafarzadeh N, Gholampour MA, Alivand MR, Kavousi S, Arzi L, Rad F, et al. CML derived exosomes promote tumor favorable functional performance in T cells. BMC cancer 2021; 21:1-1. (PMID: 34493241) [Crossref]
4. Heydarzadeh S, Ranjbar M, Karimi F, Seif F, Alivand MR. Overview of host miRNA properties and their association with epigenetics, long non-coding RNAs, and Xeno-infectious factors. Cell Biosci 2021; 11: 43. (PMID: 33632341) [Crossref]
5. Rodríguez-Galán A, Fernández-Messina L, Sánchez-Madrid F. Control of immunoregulatory molecules by miRNAs in T cell activation. Front Immunol 2018; 9: 2148. (PMID: 30319616) [Crossref]
6. Soheilifar MH, Vaseghi H, Seif F, Ariana M, Ghorbanifar S, Habibi N, et al. Concomitant overexpression of mir-182-5p and mir-182-3p raises the possibility of IL-17-producing Treg formation in breast cancer by targeting CD3d, ITK, FOXO1, and NFATs: A meta-analysis and experimental study. Cancer Sci 2021; 112: 589-603. (PMID: 33283362) [Crossref]
7. Hollen MK, Stortz JA, Darden D, Dirain ML, Nacionales DC, Hawkins RB, et al. Myeloid-derived suppressor cell function and epigenetic expression evolves over time after surgical sepsis. Crit Care 2019; 23: 355. (PMID: 31722736) [Crossref]
8. Julier Z, Park AJ, Briquez PS, Martino MM. Promoting tissue regeneration by modulating the immune system. Acta Biomater 2017; 53: 13-28. (PMID: 28119112) [Crossref]
9. Shevyrev D, Tereshchenko V. Treg heterogeneity, function, and homeostasis. Front Immunol 2020; 10: 3100. (PMID: 31993063) [Crossref]
10. Seif F, Khoshmirsafa M, Aazami H, Mohsenzadegan M, Sedighi G, Bahar M. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. Cell Commun Signal 2017; 15: 23. (PMID: 28637459) [Crossref]
11. Eslami N, Tavakol M, Mesdaghi M, Gharegozlou M, Casanova JL, Puel A, et al. A gain-of-function mutation of STAT1: a novel genetic factor contributing to chronic mucocutaneous candidiasis. Acta Microbiol Immunol Hung 2017; 64: 191-201. (PMID: 28597685) [Crossref]
12. Lin JX, Leonard WJ. The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene 2000; 19: 2566-2576. (PMID: 10851055) [Crossref]
13. Hedrick SM, Michelini RH, Doedens AL, Goldrath AW, Stone EL. FOXO transcription factors throughout T cell biology. Nat Rev Immunol 2012; 12: 649-661. (PMID: 22918467) [Crossref]
14. Lee W, Lee GR. Transcriptional regulation and development of regulatory T cells. Exp Mol Med 2018; 50: e456. (PMID: 29520112) [Crossref]
15. Maruyama T, Konkel JE, Zamarron BF, Chen W. The molecular mechanisms of Foxp3 gene regulation. Semin Immunol 2011; 23: 418-423. (PMID: 21752667) [Crossref]
16. Lee JU, Kim LK, Choi JM. Revisiting the concept of targeting NFAT to control T cell immunity and autoimmune diseases. Front Immunol 2018; 9: 2747. (PMID: 30538703) [Crossref]
17. Izsepi E, Himer L, Szilagyi O, Hajdu P, Panyi G, Laszlo G, et al. Membrane microdomain organization, calcium signal, and NFAT activation as an important axis in polarized Th cell function. Cytometry A 2013; 83: 185-196. (PMID: 23184643) [Crossref]
18. Rengarajan J, Tang B, Glimcher LH. NFATc2 and NFATc3 regulate T(H)2 differentiation and modulate TCR-responsiveness of naïve T(H) cells. Nat Immunol 2002; 3: 48-54. (PMID: 11740499) [Crossref]
19. Alberdi M, Iglesias M, Tejedor S, Merino R, López-Rodríguez C, Aramburu J. Context-dependent regulation of Th17-associated genes and IFNγ expression by the transcription factor NFAT5. Immunol Cell Biol 2017; 95: 56-67. (PMID: 27479742) [Crossref]
20. Yang X, Qu X, Meng X, Li M, Fan D, Fan T, et al. MiR-490-3p inhibits osteogenic differentiation in thoracic ligamentum flavum cells by targeting FOXO1. Int J Biol Sci 2018; 14: 1457-1465. (PMID: 30262997) [Crossref]
21. Yang L, Zhang C, Bai X, Xiao C, Dang E, Wang G. hsa_circ_0003738 Inhibits the Suppressive Function of Tregs by Targeting miR-562/IL-17A and miR-490-5p/IFN-γ Signaling Pathway. Mol Ther Nucleic Acids 2020; 21: 1111-1119. (PMID: 32871353) [Crossref]
22. Singh NP, Singh UP, Rouse M, Zhang J, Chatterjee S, Nagarkatti PS, et al. Dietary indoles suppress delayed-type hypersensitivity by inducing a switch from proinflammatory Th17 cells to anti-inflammatory regulatory T cells through regulation of microRNA. J Immunol 2016; 196: 1108-1122. (PMID: 26712945) [Crossref]
23. Kelada S, Sethupathy P, Okoye IS, Kistasis E, Czieso S, White SD, et al. miR-182 and miR-10a are key regulators of Treg specialisation and stability during Schistosome and Leishmania-associated inflammation. PLoS Pathog 2013; 9: e1003451. (PMID: 23825948) [Crossref]
24. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in T reg cell function. Nat Immunol 2016; 17: 1322-1333. (PMID: 27595233) [Crossref]
25. Aramburu J, López-Rodríguez C. Regulation of inflammatory functions of macrophages and T lymphocytes by NFAT5. Front Immunol 2019; 10: 535. (PMID: 30949179) [Crossref]
26. Griffiths R, Woods S, Cheng A, Wang P, Griffiths-Jones S, Ronshaugen M, et al. The transcription factor-microRnA Regulatory network during hESC-chondrogenesis. Sci Rep 2020; 10: 4744. (PMID: 32179818) [Crossref]
27. López-Rodríguez C, Aramburu J, Jin L, Rakeman AS, Michino M, Rao A. Bridging the NFAT and NF-kappaB families: NFAT5 dimerization regulates cytokine gene transcription in response to osmotic stress. Immunity 2001; 15: 47-58. (PMID: 11485737) [Crossref]
28. Kim KM, Park SJ, Jung SH, Kim EJ, Jogeswar G, Ajita J, et al. miR-182 is a negative regulator of osteoblast proliferation, differentiation, and skeletogenesis through targeting FoxO1. J Bone Miner Res 2012; 27: 1669-1679. (PMID: 2243139) [Crossref]
29. Yan K, Da TT, Bian ZH, He Y, Liu MC, Liu QZ, et al. Multi-omics analysis identifies FoxO1 as a regulator of macrophage function through metabolic reprogramming. Cell Death Dis 2020; 11: 800. (PMID: 32973162) [Crossref]
30. Kawaguchi K, Sakurai M, Yamamoto Y, Suzuki E, Tsuda M, Kataoka TR, et al. Alteration of specific cytokine expression patterns in patients with breast cancer. Sci Rep 2019; 9: 2924. (PMID: 30814616) [Crossref]
31. Dehqanzada ZA, Storrer CE, Hueman MT, Foley RJ, Harris KA, Jama YH, et al. Assessing serum cytokine profiles in breast cancer patients receiving a HER2/neu vaccine using Luminex technology. Oncol Rep 2007; 17: 687-694. (PMID: 17273752) [Crossref]