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THE miR-93-3p Has THE POTENTIAL TO DOWNREGULATE THE ACTIVITY OF NFATc4 AND NFAT5 GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF BREAST CANCER PATIENTS

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Breast cancer is currently recognized as the most prevalent malignant tumor affecting women. It exhibits significant biological complexity, partly due to the regulatory roles of microRNAs (miRNAs). Given the critical function of miRNAs in modulating gene activity, miRNA expression or function disruptions are frequently observed in cancer cells, including those in breast cancer. Also, NFATc4 and NFAT5 have been identified as targets of miR-93. This study aimed to evaluate the expression levels of miR-93-3p, along with NFATc4 and NFAT5 genes, in peripheral blood mononuclear cells (PBMCs) from healthy women and women diagnosed with breast cancer. In this case-control study, blood samples were collected from 15 healthy women and 15 women diagnosed with early-stage breast cancer. PBMCs were isolated from each sample, followed by RNA extraction and complementary DNA (cDNA) synthesis. Gene expression levels of the target genes were then quantified using real-time PCR. Statistical analyses were conducted using R-Studio (version 4.4.1). The results indicated a significant reduction in

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the expression of NFATc4 and NFAT5 genes in PBMC from breast cancer patients compared to healthy individuals. Conversely, miR-93-3p expression was elevated in breast cancer patients relative to healthy women. These differences in gene expression were statistically significant at the 0.05 level, as determined by the Student's T-test. In summary, this study reveals that miR-93-3p is upregulated, while NFATc4 and NFAT5 are downregulated in PBMCs of breast cancer patients. These expression changes underscore the potential of miR-93-3p and its targets as biomarkers for early breast cancer detection and may inform future therapeutic approaches. Further research with larger samples is recommended to validate these findings.

Keywords: Biomarkers, Breast Neoplasms; Gene expression, Human breast cancer, micro RNAs

INTRODUCTION

Cancer originates from the uncontrolled proliferation of body cells, remaining a leading cause of death and reduced life expectancy globally. The COVID-19 pandemic, particularly during mid-2020, significantly delayed cancer diagnoses and treatments (SIEGEL *et al.* 2023). In 2019, the World Health Organization (WHO) estimated that cancer was the leading cause of death before the age of 70 in 112 out of 183 countries and ranked as the second or third leading cause in an additional 23 countries (SUNG *et al.* 2021). Breast cancer is the most prevalent malignant tumor among women and exhibits significant biological complexity. It is characterized by heterogeneity and hormone dependence. Statistically, one in eight women and one in every 1,000 men will be diagnosed with invasive breast cancer at some point in their lifetime (DONEPUDI *et al.* 2014; VAN SEIJEN *et al.* 2019). Invasive breast cancer spreads to the adjacent breast tissue, with the most common types being invasive ductal carcinoma and invasive lobular carcinoma (DOLATKHAH *et al.* 2020). Prognostic factors identified for breast cancer are either directly or indirectly linked to the proliferation of cancer cells, highlighting the critical role of cell proliferation in the clinical behavior of aggressive breast cancer. Increased proliferation of breast cancer cells is associated with a poorer prognosis (HONG *et al.* 2022; KAMIŃSKA *et al.* 2015).

The nuclear factor of activated T cells (NFAT) gene plays a crucial role in regulating cancer initiation, progression, drug resistance, and the tumor microenvironment. Understanding the functions of NFAT is essential for elucidating the central mechanisms of NFAT signaling and developing effective cancer treatments (MANCINI and TOKER 2009). The NFAT was initially identified in nuclear extracts from activated T cells as a transcription factor that activates T cells by binding to the promoter of interleukin-2 (IL-2). Subsequent research revealed that NFAT transcription factors are expressed not only in immune cells but also in all cell types and tissues, including epithelial cells. The NFAT family comprises five members: NFAT1 (NFATc2/NFATp), NFAT2 (NFATc1/NFATc), NFAT3 (NFATc4), NFAT4 (NFATc3/NFATx), and NFAT5. NFAT proteins contain an amino-terminal transactivation domain (TAD) as well as a carboxy-terminal domain. NFAT1 through NFAT4 possess two additional conserved domains: the Rel homology region (RHR) and NFAT homology region (NHR). In contrast, NFAT5 lacks the NHR domain, rendering it unresponsive to activation by calcineurin and calcium. NFAT collaborates with other transcription factors within the nucleus, including activator protein 1 (AP1), GATA-binding protein 4, MEF, and FOXP3 family members, to initiate the transcription of target genes (SHOU *et al.* 2015). NFAT is a transcription factor in vertebrates, and its dysregulation can lead to the expression of various genes involved in cancer development.

However, NFAT isoforms exhibit distinct and varied functions in tumorigenesis. For example, activation of NFAT2 inhibits fibroblast cell differentiation, promoting cellular transformation and malignancy. In contrast, active NFAT1 promotes cell cycle arrest and cell death, inhibiting H-rasV12-induced cellular transformation. The findings indicate that NFAT1 functions as a tumor suppressor, while NFAT2 acts as an oncogene. Recent studies highlight the critical role of NFAT in regulating the migration of invasive cancer cells, particularly in breast cancer (MANCINI and TOKER 2009). Evidence suggests a potential link between NFAT5 and various types of cancer. In melanoma, NFAT5 is implicated in cell cycle regulation and promotes cancer development and metastasis. Conversely, in hepatocellular carcinoma, NFAT5 functions as a tumor suppressor gene. These findings suggest that NFAT5 plays a significant role in cancer biology (JIANG *et al.* 2019). NFAT5 is closely associated with inflammatory breast cancer (IBC), the rarest and most aggressive form of breast cancer. Studies indicate that NFAT5 positivity is higher in IBC patients compared to those with non-inflammatory breast cancer (nIBC). Activation of the NFAT signaling pathway may aid in identifying aggressive breast cancer subtypes, suggesting that NFAT5 could serve as a potential biomarker for the IBC phenotype in clinical practice. However, despite these associations, the precise role of NFAT5 in breast cancer remains unclear (REMO *et al.* 2015).

NFATc4 (also known as NFAT3) is implicated in cell growth, migration, tumor metastasis, and patient survival outcomes. It plays a critical role in regulating breast cancer cell proliferation, invasion, and migration. NFATc4 is overexpressed in breast cancer, with 65% positivity observed in cancerous tissue compared to 15% in non-cancerous tissue. Despite its significance, NFATc4 has received relatively less attention in research over recent decades compared to other NFAT proteins, presenting substantial potential for further investigation. However, NFATc4 has potential as a molecular therapeutic target in various human malignancies, including breast cancer, due to its roles in cell growth, migration, tumor metastasis, and patient survival.

MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression in most eukaryotes, primarily by binding to the 3' untranslated regions (3'UTRs) of messenger RNAs (mRNAs), resulting in mRNA degradation or inhibition of translation. Given their role in controlling gene expression, it is unsurprising that miRNA dysfunction or dysregulated expression is common in cancer cells. While most miRNAs downregulate their target genes, certain miRNAs act as oncogenes and are upregulated in cancer, influencing cancer characteristics such as cell proliferation, growth, apoptosis, invasion, metastasis, immune response, and metabolic alterations. Due to their high stability, miRNAs hold promise as non-invasive biomarkers for early cancer diagnosis, monitoring, and treatment response prediction, and even as potential therapeutic strategies for cancer patients. Given the importance of identifying biomarkers for predicting treatment response and the vital function of miRNAs in controlling basic biological processes, the role of these molecules in breast cancer treatment response should be highlighted (GARRIDO-CANO *et al.* 2022; HABIBI KHOEI *et al.* 2023)r. Circulating miRNAs hold potential as diagnostic, predictive, or prognostic biomarkers in various cancers, including breast cancer. Their stability, ease of measurement, and the ability to obtain information without invasive biopsy make circulating miRNAs particularly suitable for these applications (GARRIDO-CANO *et al.* 2022).

miR-93 plays an oncogenic or tumor suppressing role in the initiation and progression of breast cancer regarding to specific cell types and treatments. Overall, miR-93 suppresses cell proliferation, induces cell cycle arrest, and promotes apoptosis. Thus, miR-93 may serve as a potential therapeutic target for overcoming chemoresistance in breast cancer (BAO *et al.* 2020). miR-93 promotes breast cancer metastasis and enhances tumor cell survival and invasion. It influences interactions between tumor and endothelial cells and supports angiogenesis; by suppressing LATS2 expression, miR-93 further increases tumor angiogenesis and metastasis. Thus, inhibiting miR-93 may offer a practical approach to suppress tumor metastasis (FANG *et al.* 2012). In triple-negative breast cancer (TNBC) tissues, miR-93-3p and miR-105-5p are upregulated compared to non-TNBC tissues. Both miR-93-3p and miR-105-5p directly target SFRP1, thereby promoting TNBC cell stemness and resistance to cancer therapies via activation of Wnt/β-catenin signaling. Silencing these miRNAs has been shown to increase chemotherapy sensitivity (LI *et al.* 2017). NFATc4 and NFAT5 are known targets of miR-93, specifically its isoform miR-93-3p.

Few studies have examined the interactions between this miRNA and the genes encoding the transcription factors NFATc4 and NFAT5. Gaining new insights in this area could contribute to advancements in preventive, diagnostic, and therapeutic strategies, helping reduce treatment-related side effects, manage costs, and ultimately enhance patients' physical and mental well-being.

MATERIALS AND METHODS

Sample collection

The patient group consisted of 15 women with breast cancer, aged 30 to 55, who presented to the Breast Disease Research Center at Shiraz University of Medical Sciences following diagnostic testing. None of the patients had undergone chemotherapy, radiotherapy, or immunotherapy. Oncologists confirmed the presence of breast cancer and determined its stage. The control group was comprised of 15 healthy women aged 30 to 55, randomly selected with no history of acute or chronic disorders. All participants provided written informed consent prior to blood sampling. Exclusion criteria included advanced or metastatic cancer, significant clinical impairment, and use of psychiatric medications within the past 6 months. A 5 ml sample of fresh peripheral blood was collected from both healthy participants and breast cancer patients in anticoagulant tubes. All human-derived blood samples were collected from healthy individuals and patients with breast cancer at the Breast Disease Research Center, Shiraz University of Medical Sciences, who provided written informed consent in accordance with the guidelines under the approval of the Shiraz University of Medical Sciences and the Ethical Committee permissions. All experimental methods were carried out in accordance with approved guidelines stated in the declaration of Helsinki. All experimental protocols were also approved by an Ethics Committee of professionals at the Shahrekord University (Ethics committee approval code: IR.SKU.REC.1401.053) and all methods were carried out under the accordance of their guidelines.

Isolation of peripheral blood mononuclear cells (PBMCs), RNA extraction and cDNA synthesis

PBMCs were isolated using Ficoll-Lymphodex solution (Innotrain, Germany) according to the manufacturer's instructions and used for RNA extraction and further experiments.

Total RNA was extracted from PBMCs using RNX-plus solution (Sinaclon, Tehran, Iran) according to the manufacturer's instructions. The quality of the extracted RNA was assessed by agarose gel electrophoresis. Additionally, the purity and quantity of the RNA were determined by measuring the absorbance at 260 nm and 280 nm using a nanodrop spectrophotometer. Subsequently, complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Sinaclon, Tehran, Iran) with specific primers. The resulting cDNA was stored at -20 °C in accordance with the kit protocol.

Primer Optimization and Expression Profiling

Following RNA extraction and cDNA synthesis, the real-time PCR was employed to analyze the expression levels of mir 93-3p, NFATc4, NFAT5 and beta-actin (as an internal control) in the patients and concurrently in the control group by SYBR Green master mix kit (Ampliqon, Korea) and run on step one 96 Real-Time PCR System (Applied Biosystems, USA). The primer sequences are listed in Table 1. Following the reaction, raw data were extracted from the device in the form of cycle threshold (Ct) values. The expression levels of the genes were then compared using the 2-ΔΔCT method.

Table 1. Primer sequences used in the present study

Gene	Primer	Sequence
MiR-93-3p	F	GTGCAGGGTCCGAGGT
MiR-93-3p	R	CTTACTGCTGAGCTAGCACTTCC
NFAT5	F	5'-AACAACATGACACTGGCGGT-3'
NFAT5	R	5'-CTCGAAAAACCAATCTGGCACG-3'
NFATc4	F	5'-GAAGGGTGAGACGGACATCG-3'
NFATc4	R	5'-TTGGAGCCAGTCAGTACCAGT-3'
β -actin	F	AGACGCAGGATGGCATGGG
β -actin	R	GAGACCTTCAACACCCCAGCC
C mir-93-3p		GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACCGGGAAGT

Statistical analysis

Statistical analysis of the data obtained from real-time PCR was conducted using R-Studio version 4.3.3. After assessing the normality and variance of the data, the Student's t-test was employed to evaluate the relative changes in microRNA and target gene expression in PBMCs of both the control and patient groups. A significance level of $p < 0.05$ was considered statistically significant in this study. Additionally, the effect size was calculated using Cohen's d test using R-Studio 4.3.3.

RESULTS

Study Groups Data Profile

Table 2 shows the demographic and clinicopathological features of participants, including age and tumor grade which were gathered using a questionnaire from the pathology department.

Table 2. Characteristics of the study groups

<table>
<tr><td></td><td colspan="4">**controls**</td><td colspan="9">**patients**</td></tr>
<tr><td>**Age**</td><td>**(25-35)**</td><td>**(35-45)**</td><td>**(45-55)**</td><td>**(up to 55)**</td><td colspan="3">**(25-35)**</td><td colspan="2">**(35-45)**</td><td colspan="2">**(45-55)**</td><td colspan="2">**(up to 55)**</td></tr>
<tr><td></td><td>n=3</td><td>n=10</td><td>n=2</td><td>n=0</td><td colspan="3">n=1</td><td colspan="2">n=11</td><td colspan="2">n=3</td><td colspan="2">n=0</td></tr>
<tr><td>**Marriage**</td><td colspan="2">**Married**
n= 12</td><td colspan="2">**unmarried**
n= 3</td><td colspan="5">**Married**
n= 12</td><td colspan="4">**unmarried**
n= 3</td></tr>
<tr><td rowspan="2">**Pregnancy**</td><td colspan="4">(n=12)</td><td colspan="9">(n=12)</td></tr>
<tr><td colspan="4">mean (2.4±1.4)</td><td colspan="9">mean (2.4±1.4)</td></tr>
<tr><td rowspan="2">**Abortion**</td><td colspan="4">(n=0)</td><td colspan="9">(n=0)</td></tr>
<tr><td colspan="4">mean (0)</td><td colspan="9">mean (0)</td></tr>
<tr><td>**Breastfeeding (In Month)**</td><td colspan="4">(n=11)</td><td colspan="9">(n=11)</td></tr>
<tr><td></td><td colspan="4">mean (16.5±13.3)</td><td colspan="9">mean (16.5±13.3)</td></tr>
<tr><td>**Menopause**</td><td colspan="3">**Menopause**
n=0</td><td>**None – Menopause**
n=15</td><td colspan="5">**Menopause**
n=1</td><td colspan="4">**None – Menopause**
n=14</td></tr>
<tr><td>**Smoking**</td><td colspan="4">n=2</td><td colspan="9">n=0</td></tr>
<tr><td>**Alcohol consumption**</td><td colspan="4">**None**
n=0</td><td colspan="9">**None**
n=0</td></tr>
<tr><td>**Physical activity**</td><td colspan="2">**regular physical activity**
n=0</td><td colspan="2">**None - regular physical activity**
n=15</td><td colspan="5">**regular physical activity**
n=0</td><td colspan="4">**None - regular physical activity**
n=15</td></tr>
<tr><td>**Dietary pattern**</td><td colspan="2">**specific diet**
n=0</td><td colspan="2">**None - specific diet**
n=15</td><td colspan="5">**specific diet**
n=0</td><td colspan="4">**None - specific diet**
n=15</td></tr>
<tr><td rowspan="2">**BMI(Kg/m^2)**</td><td colspan="2">**Normal**</td><td>**overweight**</td><td>**Obesity**</td><td colspan="3">**Normal**</td><td colspan="4">**overweight**</td><td colspan="2">**Obesity**</td></tr>
<tr><td colspan="2">n=13</td><td>n=2</td><td>n=0</td><td colspan="3">n=12</td><td colspan="4">n=2</td><td colspan="2">n=1</td></tr>
<tr><td rowspan="2">**Invasive carcinoma Histology**</td><td colspan="4" rowspan="2"></td><td colspan="3">**Invasive ductal carcinoma (IDC)**</td><td colspan="4">**Invasive lobular carcinoma (ILC)**</td><td colspan="2">**In situ ductal carcinoma (DCIS)**</td></tr>
<tr><td colspan="3">n= 15</td><td colspan="4">n= 0</td><td colspan="2">n= 0</td></tr>
<tr><td>**Stage**</td><td colspan="4"></td><td>**IA**</td><td>**IB**</td><td>**IC**</td><td>**IIA**</td><td>**IIB**</td><td>**IIC**</td><td>**IIIA**</td><td>**B ^{III}**</td><td>**C ^{III}**</td></tr>
<tr><td></td><td colspan="4"></td><td>n=0</td><td>n=0</td><td>n=0</td><td>n=0</td><td>n=6</td><td>n= 4</td><td>n=5</td><td>n=0</td><td>n=0</td></tr>
<tr><td>**total**</td><td colspan="4">**n=15**</td><td colspan="9">**n=15**</td></tr>
</table>

RNA extraction

The extracted RNA was analyzed by loading it onto a 1.5% agarose gel. The presence of distinct bands corresponding to 28S and 18S ribosomal RNAs confirmed the quality of the extracted RNA.

Expression analysis of target genes for miR-93-3p

The expression levels of NFATc4 and NFAT5 target genes were significantly decreased in PBMCs of breast cancer patients compared to the control group. Real-time PCR analysis was conducted to evaluate changes in the expression of NFATc4, NFAT5, and β-actin housekeeping gene. The results demonstrated a significant reduction in NFATc4 and NFAT5 expression in the PBMCs of breast cancer patients ($p < 0.05$). The fold change in gene expression is presented in Table 3 and illustrated in Figures 1 and 2.

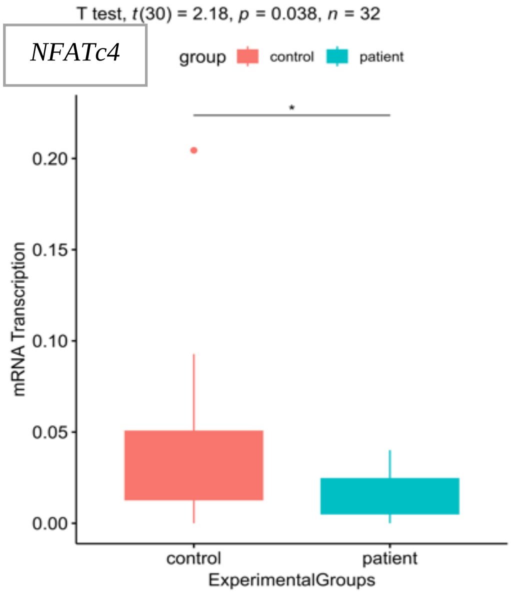

Fig 1. NFATc4 mRNA expression in PBMCs of healthy women and breast cancer patients

The expression of NFATc4 in PBMCs of breast cancer patients was significantly lower than that in healthy women. The effect size, measured using Cohen's d test, was determined to be medium, with a value of 0.77.

The expression of NFAT5 in PBMCs of breast cancer patients was significantly lower than that in healthy women. The effect size, calculated using Cohen's d test, was found to be medium with a value of 0.71.

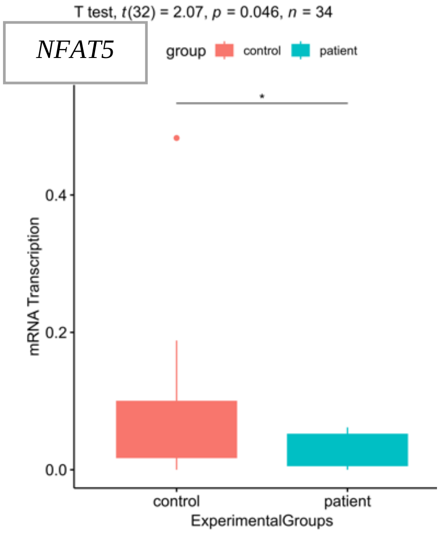

Fig 2. NFAT5 mRNA expression in PBMCs of healthy women and breast cancer patients

miR-93-3p expression in PBMCs of breast cancer patients

The expression of miR-93-3p was significantly elevated in PBMCs of breast cancer patients compared to the control group (P < 0.05). These changes are depicted in Table 3 and Figure 3.

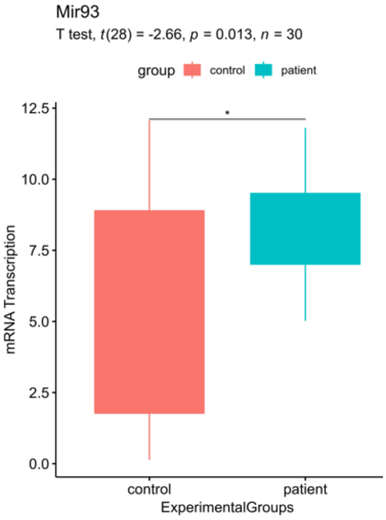

Fig 3. Expression of miR-93-3p in PBMCs of healthy women and breast cancer patients

*Table 3***.** *Changes in the expression of miR-93-3p, NFATc4, and NFAT5 genes in PBMCs of healthy women and breast cancer patients*

Gene names	Significance level	Fold change	Gene expression relative to control
Mir 93-3p	P<0.05	8.65	Up
NFATc4	P<0.05	0.32	down
NFAT5	P<0.05	0.36	down

The mRNA expression levels of miR-93-3p in peripheral blood mononuclear cells (PBMCs) were significantly higher in breast cancer patients compared to healthy women. The effect size, calculated using Cohen's d test, was found to be large with a value of 0.97.

DISCUSSIONS

The rising significance of cancer as a primary cause of mortality is evidenced by a notable reduction in deaths from stroke and coronary heart disease in many countries. Globally, the incidence and mortality rates of cancer are escalating swiftly, which can be attributed to the aging population, population growth, and shifts in the prevalence and distribution of key cancer risk factors—many of which are linked to socio-economic development. In the interplay between cancer cells and immune cells, the presence of cancer cells induces a range of phenotypic and functional alterations in immune cells. These compromised immune cells may either target and destroy cancer cells or inadvertently promote their proliferation and metastasis. Breast cancer is recognized as the most prevalent malignant neoplasm globally with a relatively high mortality rate. This condition not only poses considerable health risks but also imposes a substantial psychological burden on individuals diagnosed with it (SUZUKI *et al.* 2019; WANG *et al.* 2024). Over the past decade, the incidence of breast cancer has risen by 0.5% annually, while mortality rates have decreased by 1.3% per year, attributed to advancements in diagnostic techniques, enhanced early detection, and improvements in therapeutic options (KAUR *et al.* 2023). The gene expression profile in PBMCs exhibits distinct patterns in cancer patients, including those with renal cell carcinoma, pancreatic cancer, and lung cancer (SUZUKI *et al.* 2019). pbmcs isolated from breast cancer patients display characteristics that contribute to the aggressiveness of breast cancer cells, whereas pbmcs from healthy individuals do not possess these traits. the protein expression in pbmcs reflects the proteins expressed in the breast cancer tissue; however, the frequency of these proteins varies based on the cancer stage (MORADPOOR *et al.* 2020). Given that the initial diagnosis of breast cancer is effectively conducted through mammography and ultrasound examinations, identifying the gene expression profile of pbmcs could be crucial for understanding various aspects of the antitumor immune response in patients. This approach may also aid in developing appropriate treatment strategies, as it presents fewer challenges for patients compared to other diagnostic methods. nfat is implicated in regulating cell proliferation and apoptosis. observations from nfat-deficient mice indicate that this family of transcription factors likely plays a more extensive role in controlling cell growth and apoptosis (ROBBS *et al.* 2008). Recent key findings have highlighted the significant roles of NFATs in influencing phenotypes related to malignancy and tumor progression. Notably, NFAT isoforms are overexpressed in various human solid tumors and hematological malignancies, suggesting their involvement in cancer cell-independent functions such as invasive migration, differentiation, and survival of cells within the tumor and its microenvironment. Understanding the role of NFATs in tumor progression is believed to be crucial for developing effective therapies that target the NFAT signaling pathway in cancer progression and metastasis. Figure 4 shows some types of cancers associated with NFAT proteins. The results of the present study indicated a significant reduction in the expression of NFATc4 and NFAT5 genes in the PBMCs of women with breast cancer compared to healthy controls.

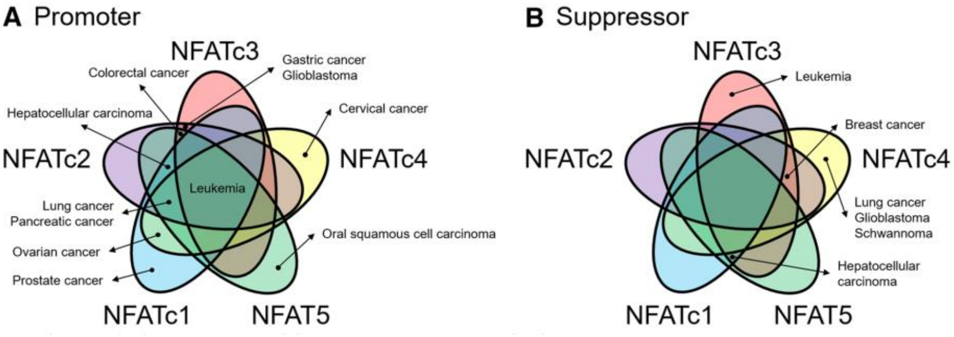

Fig 4. Venn diagrams showing common and distinct types of cancers associated with NFAT proteins. A) NFAT proteins act as promoters. B) NFAT proteins act as suppressors (ZHONG et al. 2022)

Furthermore, examining miR-93-3p levels in the PBMCs of breast cancer patients using real-time PCR revealed a substantial increase in expression within the patient group, whereas the control group exhibited lower levels of this microRNA. Consistent with our findings, NFATc4 has been reported to possess anti-metastatic functions in estrogen receptor α-positive (ERα+) breast cancer cells. NFATc4 collaborates with ERα to inhibit cancer cell migration by suppressing the expression of lipocalin 2, thereby reducing cancer cell invasion. Conversely, the downregulation of NFATc4 results in actin reorganization, which is linked to enhanced migration and invasion. Additionally, NFATc4 functions as a tumor suppressor (ZHONG *et al.* 2022). NFATc4 expression is detectable in breast cancer cells and a subset of breast cancer patients, where it exhibits strong co-expression with the androgen receptor (ER), present in 88% of ER-positive breast tumors. Survival analysis indicates that NFATc4 serves as an unfavorable prognostic factor in breast cancer. Collectively, these observations underscore the crucial role of NFATc4 in the development of breast cancer (ZHONG *et al.* 2022).

Extracellular vesicles (EVs) are bilayer-secreted vesicles that mediate cellular signal transduction and various pathological processes. EVs produced by NFATc4-expressing breast cancer cells have been shown to effectively inhibit cell invasion across different cancer types, including triple-negative breast cancer, invasive melanoma, glioblastoma, and pancreatic cancer. EVs derived from cells expressing NFATc4 decrease cell proliferation and induce apoptosis via macrophages in a 2D cell culture system. NFAT functions as a potent and multifunctional regulator of tumor progression and invasion in breast cancer, while NFAT5 is associated with promoting cell migration (NIKFARJAM *et al.* 2019). In breast cancer, a significant positive correlation exists between the expression of integrin α6β4 and both NFAT1 and NFAT5. The α6β4 integrin is released from cancer cell hemidesmosomes and binds to the actin cytoskeleton, which activates NFAT5 transcription. This activation facilitates cancer cell metastasis by engaging downstream targets, including COX-2. In the majority of NFAT5-positive breast cancer samples, an aberrant nuclear expression pattern was observed compared to control samples. This nuclear accumulation of NFAT5 occurs in a substantial proportion of breast cancer, independent of WNT/β-catenin signaling activation, suggesting that NFAT5 pathway activation may be significant in breast cancer pathogenesis. Additionally, serum levels of NFAT5 are significantly lower in breast cancer patients. The reduction in NFAT5 expression disrupts the Wnt pathway, resulting in increased β-catenin levels within the cell, which promotes tumor progression and migration (ZHONG *et al.* 2022). A study conducted by DASTGIR *et al.* (2024) demonstrated that the expression levels of NFATc1 and NFATc3 genes in the blood mononuclear cells of breast cancer patients were significantly reduced compared to those in healthy individuals (DASTGIR *et al.* 2024).

Conversely, the expression of miR-93-3p was elevated in breast cancer patients, indicating higher levels than in healthy women (DASTGIR *et al.* 2024). The upregulation of miR-93-3p is associated with poor survival outcomes in patients with TNBC. Research indicates that miR-93-3p enhances Wnt/β-catenin signaling by downregulating SFRP1, increasing chemoresistance and metastasis. Importantly, the combination of circulating miR-93-3p may be a powerful biomarker for TNBC, even in the early stages of the disease (LI *et al.* 2017).

CONCLUSIONS

The findings indicate that miR-93-3p has the potential to downregulate the activity of NFATc4 and NFAT5 genes. The overexpression of miR-93-3p and subsequent suppression of its target genes in PBMCs of breast cancer patients suggest that it could serve as a minimally invasive diagnostic marker for breast cancer.

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miR-93-3p IMA POTENCIJAL DA SMANJI AKTIVNOST GENA NFATc4 I NFAT5 U MONONUKLEARNIM ĆELIJAMA PERIFERNE KRVI (PBMC) KOD PACIJENATA OBOLELIH OD RAKA DOJKE

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Izvod

Rak dojke je trenutno prepoznat kao najčešći maligni tumor koji pogađa žene. Pokazuje značajnu biološku složenost, delimično zbog regulatornih uloga mikroRNK (miRNK). S obzirom na kritičnu funkciju miRNK u modulaciji aktivnosti gena, poremećaji ekspresije ili funkcije miRNK se često primećuju u ćelijama raka, uključujući i one kod raka dojke. Takođe, NFATc4 i NFAT5 su identifikovani kao mete miR-93. Cilj ove studije je bio da se procene nivoi ekspresije miR-93-3p, zajedno sa genima NFATc4 i NFAT5, u mononuklearnim ćelijama periferne krvi (PBMC) kod zdravih žena i žena sa dijagnozom raka dojke. U ovoj studiji slučaj-kontrola, uzorci krvi su prikupljeni od 15 zdravih žena i 15 žena sa dijagnozom raka dojke u ranom stadijumu. PBMC su izolovane iz svakog uzorka, nakon čega je usledila ekstrakcija RNK i sinteza komplementarne DNK (cDNA). Nivoi ekspresije gena ciljnih gena su zatim kvantifikovani korišćenjem PCR u realnom vremenu. Rezultati su ukazali na značajno smanjenje ekspresije gena NFATc4 i NFAT5 u PBMC kod pacijenata obolelih od raka dojke u poređenju sa zdravim osobama. Nasuprot tome, ekspresija miR-93-3p je bila povišena kod pacijenata obolelih od raka dojke u odnosu na zdrave žene. Ove razlike u ekspresiji gena bile su statistički značajne na nivou od 0,05, što je određeno Studentovim T-testom. Ukratko, ova studija otkriva da je miR-93-3p povećan, dok su NFATc4 i NFAT5 smanjeni u PBMC pacijenata obolelih od raka dojke. Ove promene ekspresije naglašavaju potencijal miR-93-3p i njegovih meta kao biomarkera za rano otkrivanje raka dojke i mogu informisati buduće terapijske pristupe. Preporučuju se dalja istraživanja sa većim uzorcima kako bi se potvrdili ovi nalazi.

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