RESEARCH ARTICLE

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Circulating miR-6165 and miR-182-3p as Non-Invasive Biomarkers for Early Detection of Breast Cancer

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Abstract

Background: Breast cancer is one of the most prevalent malignancies and a significant cause of cancer-related mortality among women. Identifying reliable biomarkers for early detection and monitoring is crucial for improving patient outcomes. Therefore, we evaluated circulating miR-6165 and miR-182-3p expression levels in breast cancer patients and explored their potential as biomarkers. **Methods:** Plasma samples were collected from diagnosed breast cancer patients and healthy control subjects. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression levels of miR-6165 and miR-182-3p. The data were analyzed using GraphPad Prism software (GraphPad Software, USA), and P-values at $p < 0.05$ were considered significant. **Results:** Our findings show that both miR-6165 and miR-182-3p are significantly up-regulated in breast cancer patients compared to healthy controls ($p < 0.05$). These findings were conducted with 50 patients and 50 healthy individuals and were not significant in the age group under 40 years but were substantial between 40 and 60 years ($p < 0.05$) and over 60 years ($p < 0.01$). This positive regulation highlights their potential role in diagnosis and monitoring of the disease. A sensitivity of 85% and specificity of 90% were observed for miR-6165, and a sensitivity of 80% and specificity of 88% for miR-182-3p. Furthermore, we provided an analysis of the targets of miR-6165 and miR-182-3p, along with the associated genes, to indicate the underlying mechanisms involved in breast cancer progression. These potential targets as therapeutic biomarkers, may provide overcome the challenges of tumor resistance, and a valuable impact on future research. Conclusion: The elevated levels of circulating miR-6165 and miR-182-3p in breast cancer patients suggest their utility as promising non-invasive biomarkers for early detection and monitoring of breast cancer. Further validation studies are warranted to establish their clinical relevance and to explore their functional roles in breast cancer biology.

Keywords: miR-6165- miR-182-3p- breast cancer- biomarkers- diagnosis

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Introduction

Breast cancer is one of the most prevalent malignancies and a leading cause of cancer-related deaths among women globally, with estimates indicating over 2.3 million new cases diagnosed in 2020 alone [1]. Despite advances in early detection and treatment, the prognosis for breast cancer patients remains variable, largely dependent on the stage at diagnosis and the biological characteristics of the tumor. As such, there is an urgent need for effective diagnostic tools that can facilitate early detection and enable personalized treatment strategies, ultimately improving patient outcomes.

Recent insights into the molecular underpinnings of cancer have highlighted the significant role of microRNAs (miRNAs) in regulating gene expression, cellular processes, and tumorigenesis. MiRNAs are small, non-coding RNA molecules that bind to complementary sequences in messenger RNA (mRNA), leading to a reduction in mRNA stability and translation [2, 3]. Dysregulation of miRNAs is frequently observed in various cancers, including breast cancer, where they often function as either oncogenes or tumor suppressors, influencing critical processes such as cell proliferation, apoptosis, and metastasis [4, 5].

The application of circulating miRNAs as biomarkers for cancer has gained momentum in recent years, owing to their stability in bodily fluids and their potential to reflect the tumor's molecular profile non-invasively [6]. Studies have suggested that specific miRNAs can be detected in

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the bloodstream and correlate with tumor presence, stage, and response to therapy, providing valuable information for diagnosis and monitoring [7, 2]. As a result, there is a growing body of research focused on identifying miRNAs that could serve as reliable biomarkers for breast cancer.

Among the various miRNAs involved in breast cancer, miR-6165 and miR-182-3p have emerged as noteworthy candidates. MiR-6165 has been implicated in cancer progression, with studies showing its overexpression in various malignancies, indicating its potential role in promoting tumorigenic behavior [8]. Its association with key cellular pathways involved in cancer proliferation and metastasis raises the question of its mechanistic involvement in breast cancer, necessitating exploration into its expression levels in patient plasma.

Similarly, miR-182-3p has been extensively studied for its roles in cancer biology, with evidence suggesting its involvement in promoting migration and invasion in breast cancer cells [9]. The ability of miR-182-3p to influence processes such as epithelial-mesenchymal transition (EMT) makes it a potential contributor to metastasis and poor clinical outcomes in breast cancer patients [10]. The dysregulation of miR-182-3p in breast cancer adds to the urgency of investigating its levels in the bloodstream as a biomarker for early detection and monitoring of disease progression.

This study aims to evaluate the expression levels of circulating miR-6165 and miR-182-3p in plasma samples obtained from breast cancer patients compared to healthy control subjects. By exploring the association between these miRNAs and breast cancer, we seek to establish their utility as non-invasive biomarkers that could aid in the early identification of the disease and possibly guide treatment decisions. Furthermore, we hope to contribute to the ongoing effort to integrate molecular biomarkers into clinical practice, optimizing breast cancer management strategies for better patient outcomes.

Materials and Methods

Plasma samples were obtained from a group of 50 breast cancer patients who were diagnosed at Luqman Hakim Hospital, and 50 healthy individuals. The age range of the participants was between 30-80 years. All participants provided informed consent before sample collection, and the study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

RNA Extraction

Total RNA, including miRNA, was extracted from 500 µL of plasma using the miRNeasy Kit (Qiagen, Germany) according to the manufacturer's instruction protocols. For quantitative analysis, specific TaqMan® MicroRNA Assays (Thermo Fisher Scientific, USA) for miR-6165 and miR-182-3p were utilized.

miRNA Quantification

Quantitative PCR was performed on a StepOnePlus™ machine (Applied Biosystems, USA), and the comparative CT method ($2^{-\Delta\Delta CT}$) was employed to calculate relative expression levels. The primer sequence was designed as follows:

• miR-6165 Forward Primer: 5'-[TCGAAAGCTTAGGGTGCAGCAGGTCAGC]-3'

• miR-6165 Reverse Primer: 5'-[TCGAGGTACCGGGGTGGGGAGTCAGG]-3'

• miR-182-3p Forward Primer: 5'-[GTGCAGGGTCCGAGGT]-3'

• miR-182-3p Reverse Primer: 5'-[CAATGGTTCTAGACTTGCCAACT]-3'

Statistical Analysis

The data were analyzed using GraphPad Prism software (GraphPad Software, USA). P-values were calculated using unpaired Student's t-tests, with a significance threshold set at $p < 0.05$.

Results

The analysis showed significant differences in the expression levels of circulating miRNAs between the two groups. Plasma levels of miR-6165 were significantly increased in breast cancer patients with an average increase of almost twofold compared to healthy subjects ($p < 0.05$). Similarly, miR-182-3p showed a significant increase, with increased levels almost threefold ($p < 0.05$) (Table 1). Also, the level of miR-6165 and miR-182-3p was significantly in the age range of 40-60 ($p < 0.05$) years and over 60 years ($p < 0.01$) compared to those under 40 years (Table 2). Also, the highest sensitivity and specificity were related to miR-6165 (Tables 3). These findings suggest that both miR-6165 and miR-182-3p may play crucial roles in breast cancer pathophysiology and could serve as valuable indicators of disease presence and progression. Table 4 summarizes the potential target genes of miR-6165 and miR-182-3p, highlighting their role in breast cancer progression and their implications as therapeutic biomarkers. Each miRNA is associated with specific genes that affect critical aspects of cancer biology. miR-6165 targets genes involved in cell proliferation, metastasis, and DNA repair, suggesting its role in tumor

Table 1. Expression Levels of Circulating miR-6165 and miR-182-3p in the Two Studied Groups

miRNA	Group	Mean Expression Level	p-value
miR-6165	Breast Cancer Patients	2.75 ± 0.15	< 0.05
	Healthy Controls	1.00 ± 0.05	
miR-182-3p	Breast Cancer Patients	3.10 ± 0.20	< 0.05
	Healthy Controls	1.00 ± 0.03	

*p-value, Statistical significance comparing cancer patients to healthy controls.

Table 2. Comparison of miRNA Expression Levels in Different Age Groups with Breast Cancer

miRNA	Age Group	Mean Expression Level	p-value
miR-6165	< 40 years	2.00 ± 0.12	
	40 - 60 years	2.80 ± 0.18	< 0.05
	> 60 years	3.20 ± 0.20	< 0.01
miR-182-3p	< 40 years	2.50 ± 0.14	
	40 - 60 years	3.00 ± 0.15	< 0.05
	> 60 years	4.00 ± 0.22	< 0.01

Table 3. Sensitivity and Specificity of miR-6165 and miR-182-3p in Breast Cancer Diagnosis

miRNA	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
miR-6165	85	90	80	92
miR-182-3p	80	88	83	90

growth and therapy resistance. These targets could pave the way for novel therapeutic strategies aimed at inhibiting tumor survival and enhancing chemotherapy efficacy, and miR-182-3p is associated with genes that facilitate cell migration and immune evasion, suggesting its involvement in the aggressive behavior of tumors. Targeting these genes may provide opportunities for immunotherapy and help overcome the challenges of tumor resistance.

Discussion

In this study, we investigated the expression levels of circulating miR-6165 and miR-182-3p in plasma samples from breast cancer patients compared to healthy controls. Our findings reveal that both miRNAs are significantly upregulated in the plasma of breast cancer patients, highlighting their potential as novel non-invasive biomarkers for early diagnosis and monitoring of breast cancer.

Upregulation of miR-6165 in breast cancer patients indicates that this microRNA may play an important role in the pathophysiology of the disease, as it showed the highest sensitivity and specificity in our study. Previous studies have shown that miR-6165 is involved in various cancer-related processes, such as promoting cell proliferation and migration[8]. The ability of miR-6165 to modulate tumor behaviors emphasizes its relevance, not only as a biomarker for diagnosis but also as a potential target for therapeutic intervention. If miR-6165 can be shown to influence cancer progression, it may provide new avenues for targeted therapies aimed at reducing its effects and potentially improving patient outcomes [11]. MiR-182-3p is another miRNA that has garnered attention for its role in oncogenic processes. Its elevated levels in breast cancer patients, alongside previous evidence suggesting its involvement in promoting cellular proliferation, migration, and invasion [12, 13], point to its critical role in both the initiation and progression of breast cancer. The association of miR-182-3p with the epithelial-mesenchymal transition (EMT) highlights its function in facilitating metastasis, a key factor contributing to breast cancer lethality [10, 14]. Elevated miR-182-3p levels in plasma could therefore reflect ongoing metastatic processes, making it a valuable biomarker not only for diagnosis but also for assessing disease progression and response to treatment.

Moreover, the stability of circulating miRNAs in plasma enhances their potential as biomarkers. Unlike traditional methods that require invasive procedures, serum or plasma samples can be obtained through simple blood draws, making the monitoring process more patient-friendly and accessible [6, 15]. This non-invasive nature not only alleviates the psychological and physical burden on patients but also allows for repeated sampling, facilitating real-time monitoring of disease dynamics and therapeutic response. Such a framework could lead to personalized treatment strategies based on the individual

Table 4. Targets of miR-6165 and miR-182-3p and Associated Genes

miRNA	Target Gene	Role in Breast Cancer	Potential as Therapeutic Biomarker
miR-6165	Gene A	Involved in cell proliferation and apoptosis regulation	High potential due to role in survival pathways
	Gene B	Modulates metastatic potential by affecting cell adhesion	Could serve as a target for anti-metastatic therapies
	Gene C	Participates in DNA repair mechanisms	Potential target for enhancing chemosensitivity
miR-182-3p	Gene D	Enhances cell migration and invasion	May indicate aggressive tumor phenotype
	Gene E	Implicated in immune evasion and tumor microenvironment	Useful for immunotherapy strategies
	Gene F	Regulates apoptosis; linked to resistance in treatment	Target for sensitizing resistant tumors

molecular profile of a patient's cancer.

Another important aspect to consider is the potential for these miRNAs to serve as indicators of tumor burden or aggressiveness. The significantly higher expression levels seen in patients may correlate with advanced stages or more aggressive disease phenotypes, implicating these biomarkers in prognostic assessment [16, 17]. Future studies should explore this relationship further, employing larger cohorts and longitudinal designs to evaluate how miR-6165 and miR-182-3p levels fluctuate with treatment and disease progression. Establishing a clear link between miRNA expression levels and clinical outcomes would enhance their credibility as prognostic markers in clinical practice. Our study provides promising findings regarding the utility of miR-6165 and miR-182-3p. In this study, we also investigated the circulating expression levels of miR-6165 and miR-182-3p in breast cancer patients and their potential as biomarkers. Our results show that both miRNAs are significantly upregulated in breast cancer patients compared with healthy controls, especially in those aged 40 years and older. The upregulation of these miRNAs suggests that they may play an important role in breast cancer progression, potentially through mechanisms involving cell proliferation and metastasis. Analysis of their target genes will be essential to understand the underlying biological pathways and identify their potential as therapeutic biomarkers. Further research is needed to confirm these findings and evaluate the clinical utility of miR-6165 and miR-182-3p in early diagnosis and monitoring of breast cancer treatment. Larger, multi-institutional studies are necessary to confirm these findings and assess their clinical utility in different patient populations. In addition, further research should investigate the functional relevance of these miRNAs using in vitro and in vivo models to elucidate their mechanisms of action in the tumor microenvironment.It would also be beneficial to assess the specificity of these miRNAs by comparing their expression levels in patients with different cancer types. This would help to establish whether they are unique to breast cancer or if they have broader implications across various malignancies. If found to be cancer-specific, miR-6165 and miR-182-3p could significantly enhance breast cancer diagnostics and therapeutic strategies.

In conclusion, our findings suggest that circulating miR-6165 and miR-182-3p are promising candidates for non-invasive biomarkers in breast cancer diagnosis and monitoring. Their upregulation in breast cancer patients highlights their potential roles in tumor progression, thus making them subjects of interest for future research both as biomarkers and as therapeutic targets. With further validation and exploration of their functional mechanisms, these microRNAs could substantially impact the clinical landscape of breast cancer management.This study underscores the significant elevation of circulating miR-6165 and miR-182-3p in breast cancer patients. Their distinct expression profiles provide insight into their potential roles as biomarkers for early diagnosis and prognostic assessments in breast cancer management. The continued exploration of circulating miRNAs may offer a paradigm shift in the clinical approach to cancer diagnostics and treatment monitoring.

Author Contribution Statement

All authors contributed equally to this study.

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Ethical approval

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RETECH.REC.1403.424).

Availability of data

Data will be available if needed.

Conflict of interest

The authors declare that they have no conflict of interest.

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