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Assessment of Non-Coding RNAs (miR-506 and circRNA 000284) and their Target Gene SNAIL-2 in Breast Tumors: Implications for Prognosis and a Possible Circulating Biomarker

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ABSTRACT

Background: Breast cancer is the most common malignancy among women, and early diagnosis and targeted therapy have garnered significant attention. Non-coding RNAs have emerged as potential diagnostic, prognostic, and treatment biomarkers for breast cancer. This study aimed to evaluate the expression of non-coding RNAs, specifically miR-506 and circular RNA 000284, and their target gene SNAIL-2 in breast cancer patients compared to normal controls. The study also focused on clinicopathological characteristics, and plasma was monitored for expression of circ0000284 to identify a possible accessible cancer-related marker.
Methods: Using the SYBR-Green Real-time PCR technique, circ0000284, miR-506, and SNAIL2 expression were analyzed in total RNA extracted from 80 breast tumors compared with normal control. Also, the expression of circ0000284 was monitored in the plasma of breast cancer patients. The association of circ0000284, miR-506, and SNAIL2 expression with clinicopathological characteristics were studied.
Results: The results showed overexpression, down-regulation, and up-regulation of circRNA 000284, miR-506, and SNAIL-2, respectively. These expression changes were associated with advanced stages of the disease and lymph nodal involvement, which are signs of a poor prognosis (<0. 0001). Additionally, a positive direct correlation was observed between circRNA000284 expression in tumors and plasma (<0. 0001). Moreover, it was discovered that circ-0000284 sponged miR-506, causing up-regulation of SNAIL-2 as its mRNA target.
Conclusion: The upregulation of SNAIL-2 as an epithelial-mesenchymal-transition (EMT) factor leads to poor prognosis in breast cancer and is epigenetically regulated by miR-506 and circRNA 000284. Therefore, the overexpression of circRNA000284 in plasma could be considered an indicator of lymph nodal involvement and advanced stages of cancer, and nominated as a poor prognostic biomarker for future considerations.

Keywords: breast cancer, circRNA000284, miR-506, noncoding RNAs, prognostic biomarker

INTRODUCTION:

Breast cancer is a prevalent malignancy among women worldwide [1], with higher estimated prevalence and mortality rates in Asia. In Iran, ductal and lobular invasive carcinoma account for 89.5% and 5.4% of cases, respectively [2, 3]. Early diagnosis and screening strategies can greatly impact patient survival and cancer management. Therefore, investigating biological elements that influence the initiation and development of breast cancer is vital for identifying related cancer biomarkers.

Epigenetics, initially coined by Conard Waddington during the early 1940s, pertains to heritable modifications in gene expression caused by non-permanent changes in DNA sequence [4]. The pathogenesis of numerous diseases, including cancer, is influenced by epigenetic changes. These changes involve DNA methylation, modifications to histones, and the involvement of epigenetic regulators like non-coding RNAs (ncRNA) [5]. Circular RNAs (circRNAs) were initially discovered in RNA viruses in 1976 and have since been identified in other organisms, including humans, mice, rats, and fungi [6, 7]. Initially considered as byproducts of RNA splicing errors, circRNAs are now recognized to have various functions in cells [8, 9].

Circular RNAs (circRNAs) are generated through a distinct splicing mechanism known as back splicing, where an upstream 3' splice site is connected to a downstream 5' splice site. These RNAs are categorized into three primary types: exonic circular RNAs (ecircRNAs), circular intronic RNAs (ciRNAs), and exon-intron circular RNAs (EIciRNAs) [10]. Unlike linear RNAs, circRNAs do not possess 3' to 5' polarity, which grants them a unique characteristic. Their closed structure confers high stability within the cytoplasm, safeguarding them from degradation by RNA exonucleases [11]. CircRNAs possess notable characteristics such as abundant presence, stability, and conservation of sequences across species. These attributes enable them to fulfill diverse functions, including the regulation of transcription and alternative splicing, interaction with RNA-binding proteins (RBPs), acting as sponges for miRNAs, modulation of translation, and involvement in epigenetic modifications [8, 12]. Additionally, circRNAs exhibit tissue-specific expression and varied expression patterns in pathological conditions, which have sparked interest in their potential roles in human diseases and cancer [13, 14]. MicroRNAs (miRNAs), on the other hand, are non-coding RNAs that are approximately 18-25 nucleotides long and regulate gene expression post-transcriptionally. These mature miRNAs bind to the 3' UTR region of target mRNA via the seed region, leading to mRNA degradation or inhibition of translation. MiRNAs play crucial roles in various biological processes, including cell division, proliferation, and apoptosis [18].

Recently, it has been discovered that miRNAs can interact with other non-coding RNAs, such as circRNAs. Circular RNAs bind to miRNAs via a special region called the miRNA response element (MRE), and they can indirectly regulate the expression of miRNAs [19].

Circular RNA0000284, also known as circHIPK3, is derived from exon 2 of the HIPK3 gene situated on chromosome 11p.13 and has a length of 1099 bp [20, 21]. Although the association of circRNA000284 with various cancers, such as prostate, thyroid, colorectal, and cervical, has been reported, its significance in breast cancer remains unknown. In a 2018 study on cervical cancer, miR-506 was identified as the miRNA associated with circRNA-000284. Moreover, the direct targeting of SNAIL-2 by miR-506 was discovered, and circRNA-000284 was found to positively regulate SNAIL-2 expression. The study concluded that circRNA-000284 enhances cellular proliferation and invasion in cervical cancer and could potentially represent a potential therapeutic target for individuals diagnosed with cervical cancer. Hence, silencing circRNA-000284 has the potential to be a prospective avenue for the development of a novel treatment strategy [21]. Based on this evidence, our investigation examined the expression of Circular RNA0000284,

miR-506, and SNAIL-2 in breast cancer to identify possible breast cancer-related markers.

Materials and methods

Study Population

To explore the association between the expression of circRNA00284, miR-506, and SNAIL-2 gene in ductal carcinoma breast cancer tumor tissue and its relationship with histopathological and hormone receptors characteristics, this study was carried out in 80 breast cancer tumor and plasma samples, 80 normal adjacent and 20 normal breast samples retrieved from mammoplasty in non-affected individuals for cosmetic purposes as the control groups. The clinical and demographic features of patients are demonstrated in Table 1. All patients received no radiotherapy or chemotherapy before surgery. The Ethics Committee approved the protocol of the National Institute of Genetic Engineering and Biotechnology based on the Helsinki Declaration.

Sampling

During the patient's surgery, the surgeon obtained fresh tissue samples, which were subsequently transferred

Table 1. The features of individuals with breast cancer and the control group

	Patient N (%)	Control N (%)
number	80	20
Age (years)		
Mean	45.9±11.6	48.5± 16.4
Range	27-84	25-82
Stage at diagnosis		
Stage I	6 (7.5)	
Stage II	34 (42.5%)	
Stage III	28 (35%)	
Stage IV	12 (15%)	
Lymph node status		
N0	36 (45%)	
N+	44(55%)	
Distance metastasis		
yes	10 [2 bone, 8 lung] (12.5%)	
No	70 (87.5%)	
Hormone receptor status (IHC)		
ER positive	48 (60%)	
ER negative	32 (40%)	
PR positive	42 (52.5%)	
PR negative	38 (47.5%)	
HER-2 status (IHC)		
+++	24 (30%)	
Negative	48 (60%)	
Triple-negative breast cancer	8 (10%)	

to a sterile cryotube in a liquid nitrogen storage tank. Pathological reports were then conducted to confirm the tumor and normal samples. Within three hours of collection, the samples were transported to the laboratory and stored at a temperature of -70°C. Before surgery, patients did not receive any radiotherapy or chemotherapy. Additionally, Complete blood samples were gathered using aseptic tubes containing EDTA. To separate the plasma from the blood, a two-step centrifugation process was employed. First, the blood was centrifuged at 3000 rpm at 4°C for 10 minutes, and then the plasma was further centrifuged at 12000 rpm at 4°C for 10 minutes to remove any remaining cellular debris.

RNA Extraction and Complementary DNA Synthesis

To isolate total RNAs from the samples (both breast tissue and plasma), a Sigma-Aldrich total RNA purification kit (Germany) was used according to the manifested protocol with some modifications. The quality and quantity of the extracted RNAs were confirmed via electrophoresis on a 0.8% agarose gel and the nanodrop 2000 instrument was utilized to determine the relative absorbance ratio at 260/280 (Thermo, Wilmington, USA), respectively. For the synthesis of complementary DNA for SNAIL-2 and circular RNA000284, 1 µg of extracted total RNA was used along with a cDNA synthesis kit (YEKTA TAJHIZ AZMA, Iran). Additionally, cDNA for miR-506 was synthesized using the protocol outlined in the BONmiR kit (Bonyakhteh company, Iran).

Real-time reverse-transcription polymerase chain reaction

All PCRs were conducted using the Rotor-Gene 6000 real-time system (QIAGEN). YTA SYBR Green qPCR Master Mix 2X was used to assess circRNA000284 and SNAIL-2 expression. A total of 500 ng/µL of complementary DNA was prepared for each sample combined with 0.4 µM of both the forward and reverse primers (Table 2) along with 5 µL of SYBR Green qPCR MasterMix2X (YTA, Iran) to achieve a final reaction volume of 10 µL. Additionally, BON-miR QPCR was used to assess miR-506 expression. For each sample, 500 ng/µL of total complementary DNA was combined with 0.2 µM of each forward and reverse primer and 6.5 µL of miRNA QPCR Master Mix (BON miR, Iran). The miRNA and SNORD-47 primers were ordered from Bon yakteh Co. for design and construction, and the sequence of the designed primers belongs to the patent of the company. For circRNA000284 and SNAIL-2 expression analysis, the thermal cycling protocol included an initial denaturation step of 5 minutes at 95°C, followed by 40 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. For miR-506 expression analysis, the thermal cycling protocol involved an initial denaturation step of 2 minutes at 95°C, followed by 40 cycles at 95°C for 5 seconds, 60°C for 15 seconds, and 72°C for 15 seconds. Duplicate tests were performed for each data point, and negative controls were incorporated into every reaction. The relative expression was quantified in the presence of a suitable internal control, beta-actin (for both SNAIL-2 and circRNA000284) and SNORD-47 (for miR-506). The efficiency for each primer set of experimental interests and reference gene was calculated using LinRegPCR software (about 100%). For comparative relative quantification, the $2^{-\Delta\Delta CT}$ method was employed, and the resulting data were presented as the fold change in gene

Table 2. sequences of oligonucleotides employed as primers for real-time polymerase chain reaction (PCR)

Primer name	Primer Sequence (5′-3′)
SNAIL-2	Forward:CGAACTGGACACACATACAGTG Reverse:GAGAGGCCATTGGGTAGCTG
CircRNA000284	Forward:GGTATGTTGGTGGATCCTGTTCGGCA Reverse:CGTGGTGGGTAGACCAAGACTTGTGA
Beta actin	Forward: GAGACCTTCAACACCCCAGC Reverse: AGACGCAGGATGGCATGG

The primers for SNAIL-2 and Beta actin were designed using Primer Blast and all primers were ordered for synthesis from Bionneer Company (South Korea).

expression. The expression levels were normalized to an endogenous reference gene (beta-actin and SNORD-47) and compared to the controls. RNA expression that exhibited a two-fold or greater change was considered upregulated, expression between 0.5 and 2-fold was considered normal, and expression that was 0.5-fold or less was considered downregulated.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism software (version 9.0) to compute the results. The Kolmogorov-Smirnov test was utilized to assess the normal distribution of data within each group. Parametric and non-parametric tests were employed for comparison purposes. A significance level of $P < 0.05$ was considered statistically significant. The numerical data are presented as mean ± standard deviation.

Results:

Assessment of circRNA000284 Expression in Breast Cancer Patients

In this investigation, the expression of circRNA000284 was evaluated among individuals diagnosed with breast cancer. The results showed that 63% of the patients had a normal level of circRNA000284 expression compared to control groups, while 37% of the patients showed upregulation of circRNA000284. None of the patients showed downregulation of circRNA000284. As depicted in Figure 1, the mean expression level of circRNA000284 in breast tumors and plasma was significantly higher than normal adjacent and normal control groups ($P < 0.0001$). The mean expression of circRNA000284 in tumor tissue and plasma was 2.18 ± 1.48-fold and 1.77±1.33 fold higher compared to the normal control groups. However, no statistically significant differences in circRNA000284 expression were observed between the normal adjacent and normal control groups and also between plasma and tumor groups.

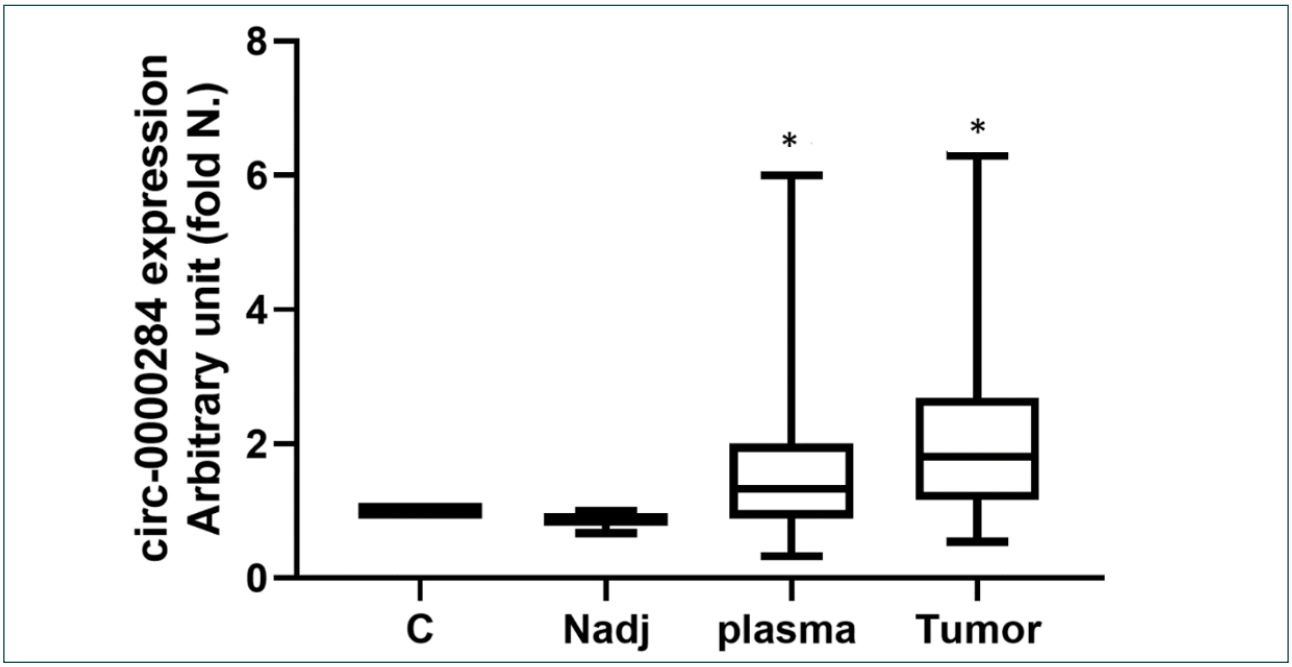

Figure 1. The mean expression of CircRNA-0000284 in breast cancer tissues (plasma and tumor) compared to the normal adjacent and control groups. Results are expressed as fold number increase versus control assumed as 1 ± standard deviation. The circRNA0000284 values were previously normalized to beta actin RNA.
* : P < .0001
C:Control
Nadj: Normal adjacen
T:Tumor

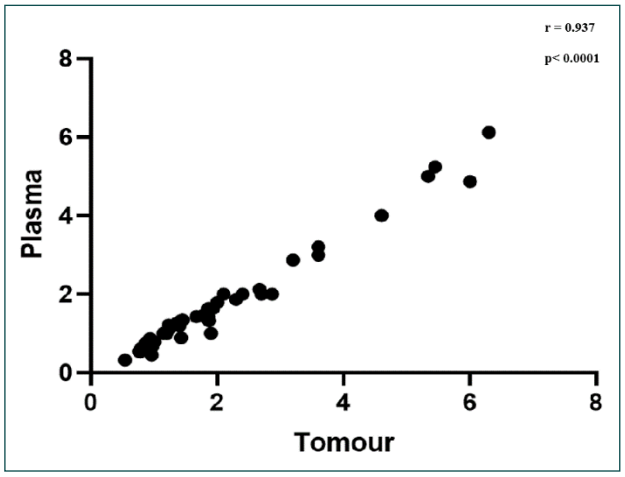

Figure 2. The correlation of circular RNA 000284 expression between tumors and plasma in breast cancer patients. "The Spearman test was used for this analysis.

Figure 2 illustrates a positive correlation between circRNA000284 expression in breast tumor tissue samples and plasma samples from breast cancer patients.

Association of circRNA000284 Expression with Clinicopathological Characteristics in Breast Cancer Patients

Table 3 displays the relative average expression of breast tumors circRNA000284 in various clinicopathological conditions. The findings reveal that patients with lymph node involvement and advanced stages of breast cancer exhibited higher expression of circRNA000284 ($P < 0.0001$). However, there were no notable differences in the expression of circRNA000284 regarding the hormone receptor and HER-2 status of the patients.

Assessing the Relative Expression of miR-506 in Breast Tumors and its Association with Clinicopathological Characteristic

In the study group, 60% of patients exhibited normal expression of miR-506 compared to the control group. However, the downregulation of miR-506 was observed in 40% of patients. Figure 3 illustrates that the average expression of miR-506 in breast tumors was 0.56 ± 0.24 fold lower than that of the control group, which confirms downregulation ($P < 0.0001$).

Table 4 presents the relative mean expression of miR-506 in various clinicopathological conditions. The results demonstrate a significant downregulation of miR-506 in cases of lymph node involvement and advanced stages

Table 3. The average expression of circRNA000284 in breast cancer patients based on different clinicopathological conditions.

Various factors	Mean of circRNA000284 exp. ± SD	P value	Statistical test	Significantly different (P < 0.05)
ER+	2.30 ±1.45	P= 0.34	Mann-Whitney test	No
ER-	2.01±1.5			
PR+	2.33±1.56	P= 0.6	Mann-Whitney test	No
PR-	2.02±1.36			
HER2+	2.44± 1.52	P= 0.5	Mann-Whitney test	No
HER2-	2.11±1.36			
TN	2.54±2.07	P=0.92	Mann-Whitney test	No
Non-TN	2.36±1.5			
LN+	3.03±1.51	<0.0001	Mann-Whitney test	Yes
LN-	1.14±0.35			
Stage I	0.7 ±0.11	<0. 0001	Kruskal-Wallis test	Yes
Stage II	1.24±0.31			
Stage III	2.35±0.56			
Stage IV	5.21±0.89			

PR: progesterone receptor, ER: the estrogen receptor, HER2: human epidermal growth factor receptor, LN: Lymph node, TN: triple-negative (ER-, PR-, HER2-)

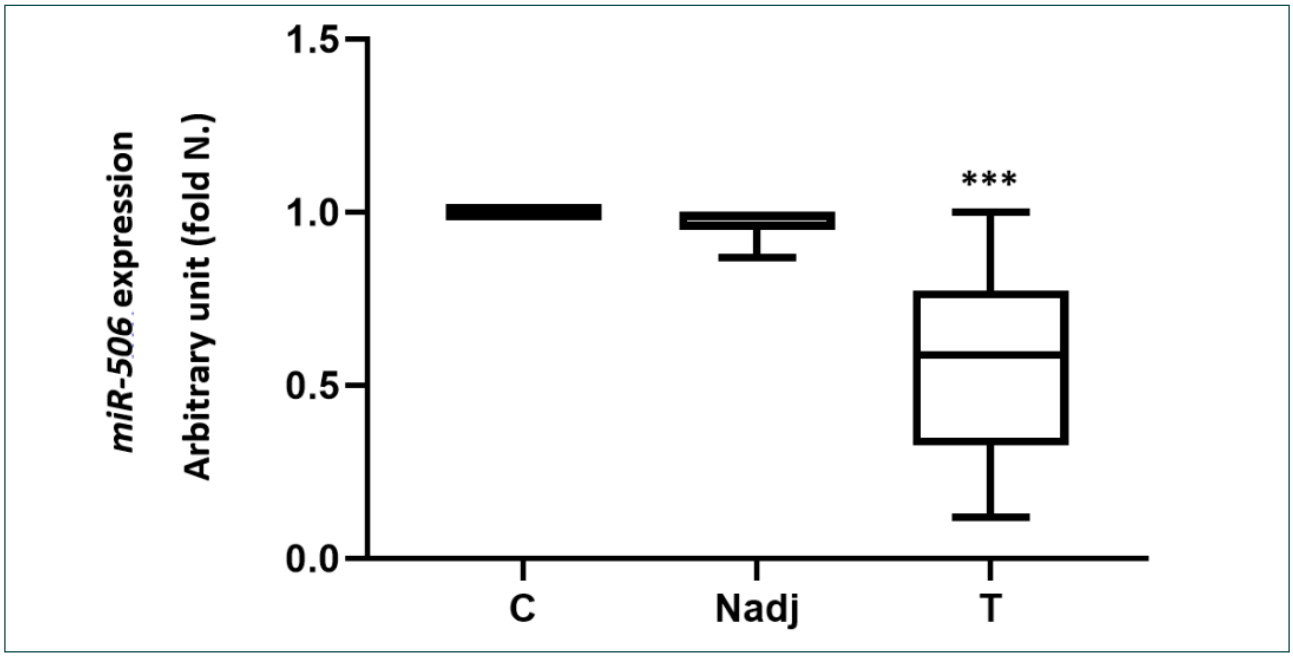

Figure 3. The expression of miR-506 in breast cancer tumors compared to the normal adjacent and control groups. The results are reported as the fold increase compared to the control, which is assumed to have a value of 1. Before analysis, the miR-506 values were normalized to SNORD-47 RNA.
***P < .0001

Table 4. The average expression of miR-506 in breast cancer patients based on different clinicopathological conditions.

Various factors	Mean of miR-506 exp. ± SD	P value	Statistical test	Significantly different (P < 0.05)
ER+	0.50 ±0.24	P= 0.25	t test	No
ER-	0.60±0.23			
PR+	0.51±0.22	P= 0.36	t test	No
PR-	0.58±0.26			
HER2+	0.60± 0.23	P= 0.3	t test	No
HER2-	0.51±0.24			
TN	0.50±0.28	P=0.48	t test	No
Non-TN	0.55±0.24			
LN+	0.39±0.17	<0.0001	t test	Yes
LN-	0.77±0.14			
Stage I	0.91 ±0.03	<0. 0001	ANOVA test	Yes
Stage II	0.73±0.13			
Stage III	0.42±0.13			
Stage IV	0.22±0.08			

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor, LN: Lymph node, TN: triple-negative (ER-, PR-, HER2-)

of breast cancer ($P < 0.0001$). However, there were no notable differences in miR-506 expression regarding the hormone receptors and HER-2 status of the patients.

Evaluation of SNAIL-2 Expression in Patients with Breast Cancer and its Correlation with Clinicopathological Characteristics

In 55% of the patients, SNAIL-2 expression was within the normal range compared to the control group, while upregulation of SNAIL-2 was observed in 45% of cases. As shown in Figure 4, the average expression of SNAIL-2 in breast tumors was 2.48 ± 1.72 fold higher than that of the normal control group. As depicted in Table 5, a statistically significant increase in SNAIL-2 expression was observed in breast cancer cases with lymph node involvement and advanced stages ($P < 0.0001$). However, no significant differences were found in SNAIL-2 expression based on hormone receptor status and HER-2 status of the patients.

The Correlation between circRNA000284 and miR-506/SNAIL2 Expressions in Breast Cancer

The correlation between circRNA000284 and the expressions of miR-506 and SNAIL2 was evaluated using Pearson's correlation coefficient and is presented in Figure 5 a and b, respectively. Pearson's correlation coefficient of -0.8163 indicates an inverse correlation between miR-506 and circular RNA 000284 expression, suggesting that an increase in circular RNA 000284 expression is accompanied by a decrease in miR-506 expression (Figure 5a).

In contrast, Pearson's correlation coefficient of 0.9523 between circRNA000284 and SNAIL2 expression suggests a direct association, indicating that an increase in circular RNA000284 expression is accompanied by an upregulation of SNAIL-2 (Figure 5b).

Discussion:

For several years, circular RNAs were regarded as molecular anomalies [18]. However, with the emergence of next-generation sequencing, numerous circRNAs were recognized as highly stable and abundantly expressed in

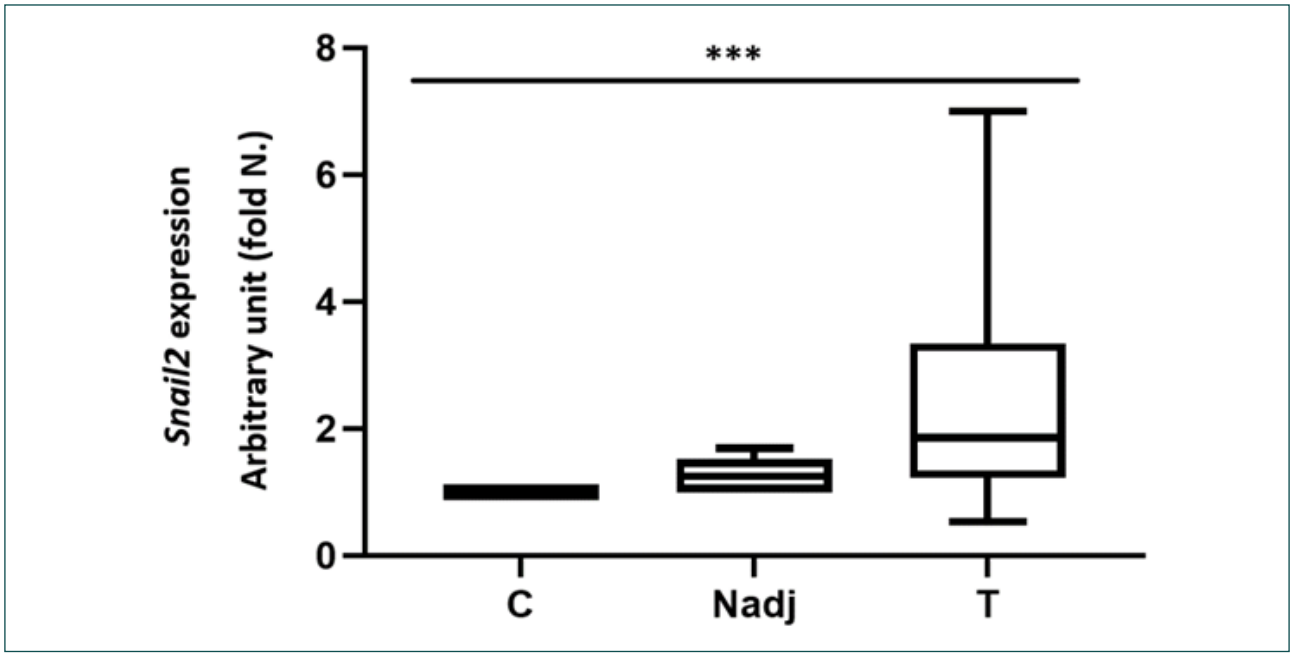

Figure 4. The expression of SNAIL-2 gene in breast cancer tissues compared to the normal adjacent and control groups. Results are expressed as fold number increase versus control assumed as 1. The SNAIL-2 gene values were previously normalized to beta actin RNA.
***$P < 0.0001$

Table 5. The average expression of SNAIL-2 in breast cancer patients based on different clinicopathological conditions.

Various factors	Mean of SNAIL-2 exp. ± SD	P value	Statistical test	Significantly different (P < 0.05)
ER^+	2.78 ±1.81	P= 0.29	Mann-Whitney test	No
ER^-	2.18±1.63			
PR^+	2.84±1.92	P= 0.26	Mann-Whitney test	No
PR-	2.13±1.43			
HER2+	2.7± 1.6	P= 0.69	Mann-Whitney test	No
HER2-	2.44±1.69			
TN	2.57±2.04	P=0.56	Mann-Whitney test	No
Non-TN	2.7±1.75			
LN+	3.51±1.72	<0.0001	Mann-Whitney test	Yes
LN-	1.21±0.45			
Stage I	0.71 ±0.20	<0. 0001	Kruskal-Wallis test	Yes
Stage II	1.32±0.39			
Stage III	2.78±0,79			
Stage IV	5.95±0.77			

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor, LN: Lymph node, TN: triple-negative (ER-, PR-, HER2-)

various disease conditions. This has largely reshaped the conventional perspective on circRNAs. The objective of the current study was to evaluate the expression of circRNA-000284 and its reported downstream targets in breast cancer, building upon the results of a previous study that provided the first evidence of its potential prognostic role in cervical cancer [21]. Breast cancer is a prevalent and diverse form of cancer affecting women globally [22]. Accordingly, identifying novel biomarkers with diagnostic, treatment and prognostic value is of utmost importance.

The circular RNAs (circRNAs) represent a newly discovered group of long non-coding RNA molecules that exhibit a wide range of biological functions and pathological implications. Compared to other non-coding RNAs such as microRNAs (miRNAs) and other long non-coding RNAs (lncRNAs), circRNAs exhibit highly conserved sequences and a high degree of stability in mammalian cells, making them ideal biomarkers for the diagnosis and prognosis of cancers [21, 22]. Depending on the circumstances, circular RNAs may act as oncogenes, tumor suppressor genes, or both.

One of the prominent roles of circRNAs is their ability to function as "miRNA sponges," influencing the post-transcriptional translation of target mRNAs by binding to miRNA response elements (MRE). Through computational analyses, MREs have been identified within the miRNA target sites of circRNA molecules, enabling them to serve as miRNA sponges and indirectly modulate the expression of miRNA targets.

A study conducted in 2018 provided compelling evidence through in vitro experiments that circRNA-000284 acts as an oncogene, promoting the proliferation and invasion of cervical cancer cells, which aligns with its expression in clinical samples. The study further validated the interaction between circRNA-000284 and miR-506, revealing that the SNAIL-2 gene is a direct target of miR-506 and is regulated by circRNA-000284. Moreover, functional assays involving the gain and loss of circRNA-000284 function indicated its regulatory role in cell proliferation and invasion through miR-506 sequestration, ultimately targeting SNAIL-2 in cervical cancer. Based on these findings, the authors concluded that circRNA-000284 holds promise as a novel diagnostic biomarker and prognostic indicator in cervical cancer [21].

CircRNA000284, also known as circHIPK3, is derived from exon 2 of the HIPK3 gene and exhibits a high degree of conservation and stability across various tissues [23]. Numerous studies have demonstrated the association

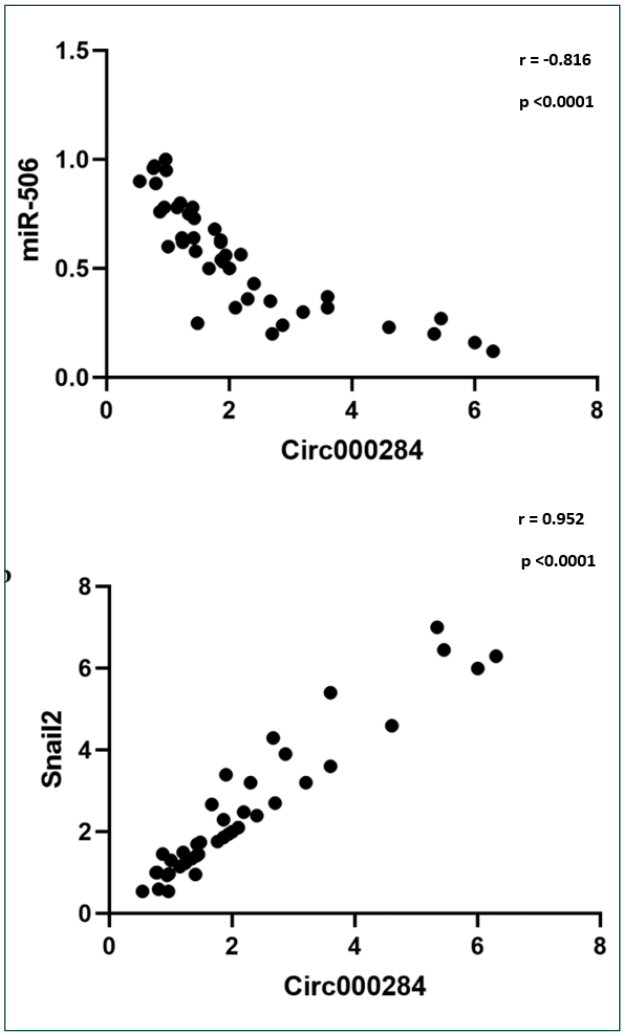

Figure 5. Evaluation of the relationship between circular RNA 000284 and miR-506 expression in breast cancer tissue (a) the association between circular RNA 000284 and SNAIL-2 expression in breast cancer tissue (b).

between the dysregulation of circRNA000284 and various cancers. For instance, the upregulation of circRNA000284 in colorectal cancer promotes cell proliferation, invasion, and metastasis by sponging miR-7 [24]. Another study on bladder carcinoma demonstrated that downregulation of circRNA000284 causes malignancy, and miR-558, a target of circRNA000284, is released and binds to the promoter of the HPSE gene, leading to proliferation and angiogenesis of bladder carcinoma [25]. In 2019, a study on prostate cancer showed that upregulation of circRNA000284 leads to proliferation and metastasis by sponging miR-338-3p, resulting in the release of the target mRNA of miR 338-3p, ADAM 17 [26]. In 2018, another study found that circRNA000284 promotes cell proliferation and invasion in cervical cancer via the circRNA000284/miR-506/SNAIL-2 network [21].

Based on previous studies, the present research was designed to clarify the association between circRNA000284 expression and breast cancer. The data revealed that circRNA000284 expression was upregulated in approximately 37% of breast cancer patients, and this increase in expression was associated with lymph node involvement and advanced stages of cancer. Conversely, miR-506 was downregulated in about 40% of patients, and miR-506 expression was significantly negatively correlated with lymph node involvement and cancer development. In the case of SNAIL-2 expression, specific data from the present study indicated upregulation of SNAIL-2 in about 45% of patients, indicating lymph node involvement and higher stages of the disease. A negative correlation was observed between circRNA000284 and miR-506 expression, while a positive correlation was observed between circRNA000284 and SNAIL-2 expression, respectively, confirming the circRNA000284/miR-506/SNAIL-2 network introduced earlier in cervical cancer. The circRNA000284 molecule is believed to function as a competing endogenous RNA (ceRNA), which means it can sponge or sequester miRNAs and control their availability for binding to target mRNA. In breast cancer, the miR-506 miRNA has been found to be dysregulated and acts as a tumor suppressor by targeting various genes involved in cancer progression. One such gene is SNAIL-2, also known as SNAI2 or SLUG, which is a transcription factor that plays a crucial role in the process of epithelial-mesenchymal transition (EMT). EMT is a biological process that occurs during cancer metastasis, where epithelial cells acquire mesenchymal-like characteristics, enabling them to invade nearby tissues and spread to distant sites. SNAIL-2 is upregulated in breast cancer and is associated with tumor aggressiveness and metastasis.

The circRNA000284/miR-506/SNAIL-2 network represents a regulatory pathway in breast cancer.

In this pathway, circRNA000284 acts as a ceRNA, competing with other mRNAs for binding to miR-506. miR-506, in turn, targets SNAIL-2 mRNA, suppressing its expression. By regulating SNAIL-2, miR-506 may influence the EMT process and potentially inhibit cancer cell invasion and metastasis.

The Cancer Genome Atlas (TCGA) project has provided comprehensive genomic data for various cancer types, revealing associations between gene expression changes and clinicopathological features like tumor stage, histological subtype, and patient survival. For instance, TCGA studies have identified specific gene expression patterns linked to different subtypes of breast cancer [27], lung cancer [28], and glioblastoma [29], offering insights into molecular diversity and potential therapeutic targets. Studies investigating neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease, have revealed gene expression changes associated with disease progression and specific pathological features. For instance, research has identified the upregulation of genes involved in neuroinflammation and downregulation of genes associated with synaptic function in Alzheimer's disease [30, 31]. Studies on autoimmune disorders like rheumatoid arthritis and systemic lupus erythematosus have identified gene expression changes linked to disease activity and specific clinical manifestations. These studies have revealed increased expression of genes involved in immune response and inflammation, along with decreased expression of genes related to tissue homeostasis [32]. In infectious diseases like HIV and viral hepatitis, gene expression alterations have been observed in relation to disease progression, treatment response, and clinical outcomes. For example, specific gene expression changes have been associated with the transition from HIV infection to AIDS and response to antiretroviral therapy [33, 34].These examples represent a fraction of the extensive research exploring the connection between gene expression changes and clinicopathological conditions. A range of approaches, including transcriptomic analysis, gene set enrichment analysis, network analysis, validation studies, and integration of multi-omics data, can be employed to investigate these associations. Additionally, functional experiments involving gene knockdown or overexpression studies in relevant models can help uncover the roles of differentially expressed genes in the development and progression of clinicopathological conditions.

The present data revealed that circRNA000248 expression in both tumor and plasma of the patients was equivalent, implying that due to the less invasiveness and more availability of plasma markers than breast tissues on one hand, and the relative stability of circRNAs compared with other non-coding RNAs in blood on the other hand, circRNA000248 expression could be nominated as a possible poor prognosis indicator associated with metastasis and advanced cancer stages. Future consideration and follow-up studies are recommended to confirm this claim.

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