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In Silico and Experimental Outcomes of the Expression of miR508-5p and miR-635 in Tumor Tissues of Patients with Breast Cancer

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Abstract

Background: Breast cancer (BC) is a malignant tumor that occurs in the epithelial tissue of the breast gland and has become the most common malignancy in women. Various studies have reported the effect of epigenetic changes, including DNA methylation and microRNAs, on breast carcinogenesis. microRNAs play an important role in the post-transcriptional regulation of genes and are important regulators of oncogenic pathways. Studying microRNAs in BC facilitates the development of targeted therapies and early detection of this cancer.

Objectives: This study aimed to evaluate the expression level of miR-508-5p and miR-635 in BC tumor tissues compared to healthy marginal tissues.

Methods: In silico analysis confirmed microarray datasets (GSE40525, GSE44124 and GSE45666) downloaded from the GEO database. The analysis was defined using the Affy packages in R software to screen remarkably dysregulated miRNAs attended by utilized to predict the potential biological processes and molecular pathways of miR-508-5p and miR-635. Experimental statistical significance of differences in miRNA relative expression results was analyzed by pair-wise fixed reallocation randomization test as a statistical model included in the REST (relative expression software tool).

Results: GEO microarray data set, similar to qPCR results, showed that miR-508-5p was downregulated in the sample group by a mean factor of 0.327 (S.E.M range is 0.031-2.000). Moreover, miR-635 was upregulated in the sample group by a mean factor of 2.361 (S.E.M range is 0.250-16.000).

Conclusion: miR-508-5p was downregulated, while miR-635 was upregulated in BC tissues. They may be proposed as diagnostic and therapeutic biomarkers for patients with BC.

Keywords: Breast cancer, Gene expression omnibus (GEO), In silico analysis, Microarray dataset, MicroRNA

1. Background

Breast cancer (BC) is the second most common cancer and a major women's health problem worldwide, affecting approximately one in eight women (1-3). It affects 2.1 million women annually and accounts for 14% of all cancer-related deaths and 23% of all cancer cases (4). Despite widespread advances in diagnosis and treatment (including radiotherapy, chemotherapy, hormone therapy, and targeted therapies), BC remains a major cause of death among women with cancer (5-7).

Various environmental, genetic and epigenetic factors influence the pathogenesis of BC. Various studies have reported the effect of epigenetic changes, including DNA methylation (8), histone acetylation modifications (9), long non-coding RNAs (lncRNA) (10) and miRNAs, on BC (8). These changes are transmissible and reversible, affecting gene expression without altering the gene sequence. Studies have shown that subtypes of BC are epigenetically different from healthy cells. Expanding the study of various changes in proteins, mRNAs, and miRNAs in BC allows the development of targeted therapies and early detection of this cancer (6, 11). The miRNAs are small non-coding RNAs (21-23 nucleotides) that play a key role in the post-transcriptional regulation of genes and are important regulators of oncogenic pathways. According to previous reports, miRNAs are involved in the regulation of approximately 60% of genes (12, 13). The influence of miRNAs on cancer pathogenesis has been well established. Some studies demonstrated the linkage between the pathological features of BC and the expression of miRNAs (14). Impaired expression of miRNAs is associated with various biological processes such as cell apoptosis (15), proliferation (16), metastasis (17), and invasiveness in BC (18, 19). Thus, miRNAs may be used as important biomarkers for diagnosis, prognosis and a promising treatment for BC (11, 19).

The miR-635 is involved as a tumor suppressor in inhibiting solid tumors (20).

2. Objectives

A previous study showed that miR-508-5p was downregulated in cancers, and overexpression of miR-508-5p reversed multidrug resistance (MDR) most efficiently (21). Currently, the highest number

of therapeutic targets and tumor biomarkers are related to coding genes, while non-coding sequences constitute 98% of the genome and produce a large number of regulatory non-coding RNAs (22). Therefore, miRNAs can be used as important non-invasive biomarkers in the early detection of cancers (23). The present study aimed to evaluate the expression level of miR-508-5p and miR-635 in BC tumor tissues compared to healthy marginal tissues.

3. Methods

3.1. In silico methods (Data gathering, process, and build networks)

Gene Expression Omnibus (GEO), a high-throughput genomic database, was investigated for miRNAs expression profiles in BC. The keywords were as follows: (("breast cancer") or Tumor) and [(healthy) or control or "non tumor") or adjacent)] and "Homo sapiens" Filters: miRNA, [Non-coding RNA profiling by array]. The criteria for selecting the qualified datasets consisted of datasets with miRNAs platforms that concomitantly comprised BC tissues and healthy (or adjacent non-tumor samples). Regarding the quality of raw data, three datasets, including GSE40525, GSE44124, and GSE45666, were chosen for the rest of the study. The GSE 40525 was used to study 64 patients with primary BC, comprising 56 matched tumor and adjacent peritumor BC tissues, five tumor tissues and three peritumor unmatched tissues. The miRNA microarray datasets GSE44124 which included 53 samples were analyzed. Fifty tumor samples and three pools of normal tissue (10 normal samples for each pool) were used. The GSE45666 included 101 BC and 15 adjacent breast normal tissue samples. All raw expression data files were subjected to background correction and quantile normalization using Robust Multi-Array Analysis (RMA) from Bioconductor package. The "limma" R package was used to identify differentially expressed items between tumor and normal samples. Among a multitude of miRNAs, miR-508-5p and miR-635 were selected since these miRNAs were confirmed in recent studies and dysregulated in several cancers.

3.2. Clinical Patients and Samples

3.2.1. Sampling

In this study, 100 cancerous and 100 non-cancerous marginal tissues were collected at BC patient from Noor Nejat Hospital in Tabriz, Iran, and stored at -80°C. The basic characteristics of the patients with BC included: 1) Tumor grade (Grade 3 = 5%, Grade 2 = 87.5%, Grade 1 = 7.5%), 2) Lymph node (Yes = 87.5%, No = 12.5%), 3) Family cancer history (Yes = 47.5%, No = 41.3%, Unknown = 11.2%), and 4) Abortion history (Yes = 36.2%). Institutional guidelines, including ethical approval and informed consent, were followed by the Ethics Committee of Tabriz Azad University, Tabriz, Iran (Ethics code: IR.IAU.TABRIZ.REC.1401.063), and was conducted following the Declaration of Helsinki.

3.3. Experimental Protocol

3.3.1. Tissue Processing, Total RNA isolation, comple-mentary DNA (cDNA) synthesis, and quantitative real-time PCR (QRT-PCR) analysis

For RNA extraction, we used liquid nitrogen for homogenizing tissue samples, extracted by Trizol reagent (Invitrogen, Massachusetts, USA), and for quality and quantity of extracted RNAs, we used a NanoDrop spectrometer (Thermo Scientific, USA). After extraction, obtained RNAs were eluted in 50 µL of RNase-free water (ROJE Technologies, Iran) and stored at -80°C (24).

In this study, cDNA of the miR-508-5p, miR-635, and RNU6 were synthesized using reverse transcriptase enzyme, dNTP (KIAGENE, FANAVAR, Iran), and their unique stem-loop-primers. For this purpose, three specific stem-loop primers were designed for miR-508-5p, miR-635, and also RNU6 (for normalization) were employed, and the conditions of PCR machines were 25 min at 10°C, 47 min at 60°C, and 5 min at 85°C. For conducting Real-time PCR reaction, SYBR Green master mix (SMOBIO, Taiwan) and miR-508-5p, miR-635, specific primers were used. These reactions were performed by PCR (Corbett RG6000 R010756, Australia) biomolecular system in two steps as follows: for miR-508-5p: 10 min at 95°C, 45 cycles of 15 s at 95°C and 60 s at 60°C. For miR-635: 10 min at 95°C, 40 cycles of 15 s at 95°C and 60 s at 58°C. Then, for RNU6 amplification: 10 min at 95°C, 40 cycles in 15 s at 95°C and 60 s at 56°C. The Oligonucleotide sequences in the present study are shown in Table 1.

3.4. Statistical Analysis

Most *in silico* statistical analyses were performed using the bioinformatic tools mentioned above. The threshold values for differentially expressed miRNAs (DEmiRs) were set to |logFC| > -0.05 and adjacent *P-value* < 0.05. Cut-off criteria between normal and tumor samples and volcano plots for the results of differential expression analysis were depicted using R software ggplot2 packages. Experimental statistical significance of differences in miRNA relative expression results was analyzed by pair-wise fixed reallocation randomization test as a statistical model using the Ct method ($2^{-\Delta\Delta Ct}$ method) in Excel software and REST (relative expression software tool). (The $2^{-\Delta\Delta Ct}$ formula was: $\triangle\triangle CT$ = (CT target − CT reference) normal − (CT target - CT reference) patient). Mann-Whitney U-test was used to compare demographic characteristics and miRNA. The results are shown as *P-value* < 0.05 and mean ± SEM using the GraphPad Prism software (version 8.4.3).

Table 1. Sequences of the primers for non-coding RNAs

	Accession numbers	Sequences
cDNA	miR-508-5p(STL)NIMAT0004778	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATGAGTGACGCCCTCTGGAGTA-3'
	miR-635(STL)NIMAT0003305	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGACATTGTTTCAGTGCCCAAGT-3'
	RNU6(STL)	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATAT3′
Real-time PCR	F(miR-5085p)	5'-GGCTCCAGAGGGCGTCA-3'
	Common(R)	5'-GTGCAGGGTCCGAGGT-3'
	F(miR-635)	5'-CTTGGGCACTGAAACAATGTCCG-3'
	Common(R)	5'-GTGCAGGGTCCGAGGT-3'
	RNU6 (F):	5'-GCTTCGGCAGCACATATACTAAAAT-3'

4. Results

4.1. MiR-508-5p was downregulated, while miR-635 was upregulated in breast cancer (BC) tissues

Based on miRTarBase, miRDB, and Target Scan Human 7.2, as miR-related databases, miR-508-5p and miR-635 were selected as potential miRNAs that might play a significant role in BC tissues. To determine the roles of miR-508-5p and miR-635 in human BC, we compared the relative expressions of both miRNAs in BC tissues and their normal adjacent tissues.

4.2. In silico analysis: identification of differentially expressed miRNAs

Considering |logFC| ≤ - 0.5 and $P < 0.05$ as the cut-off, we revealed that miR-508-5p was significantly downregulated in GSE44124 (logFC = -0.4), GSE4566 (logFC = -0.07), whereas miR-635 was significantly upregulated in only one array datasets, GSE40525 (logFC = 0.02), that they were in accordance with our experimental findings in Figure 1. In addition, differentially expressed miRNA results were identified in GSE44124 (7154 downregulated and 8585 upregulated), in GSE4566 (7148 downregulated and 8591 upregulated) and in GSE40525 (513 downregulated and 308 upregulated) (Figure 1). Furthermore, 634 and 339 predicted targets for hsa-miR-508-5p and hsa-miR-635 extracted from the miRDB database in Figure 2 and Tables 2-5.

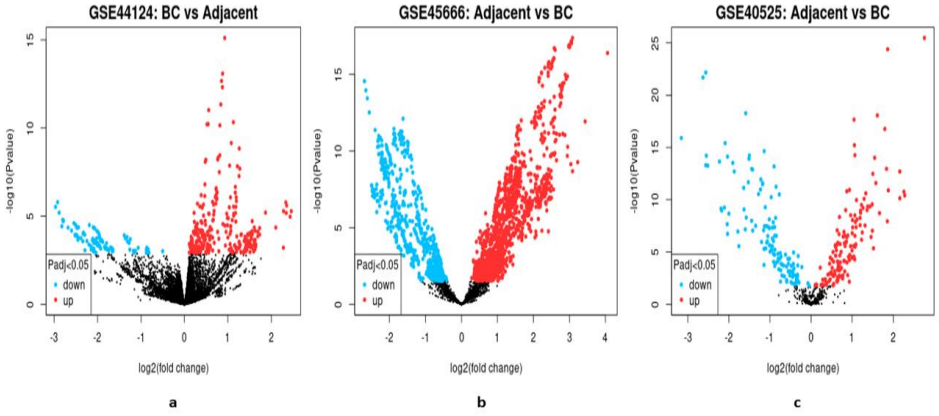

Figure 1. volcano plots of or differentially expressed genes in bc tissues unto normal tissues in gse gse44124, gse4566 (mir-508-5p), gse40525 (mir-635)

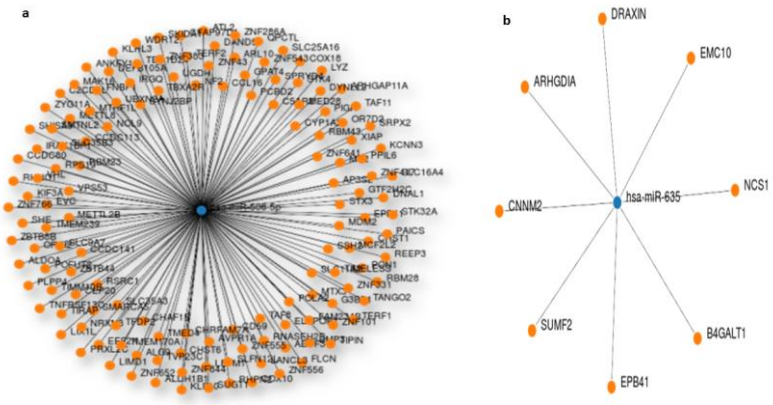

Figure 2. Illustrations of hsa-miR-508-5p and hsa-miR-635

Table 2. Enriched GO categories (Top 10 categories) mir-508-5p

GO ID	GO Name	C	O	Raw P-value	Adjusted P-value
GO:0032482	Rab protein signal transduction	48	6	0	0.1794
GO:0006886	Intracellular protein transport	581	18	0.0001	0.3135
GO:0046907	Intracellular transport	923	22	0.0003	1
GO:0071705	Nitrogen compound transport	980	22	0.0008	1
GO:0071702	Organic substance transport	1070	23	0.001	1
GO:0051649	Establishment of localization in cell	1019	22	0.0013	1
GO:0015031	Protein transport	907	20	0.0018	1
GO:0090070	Positive regulation of ribosome biogenesis	6	2	0.0019	1
GO:2000234	Positive regulation of rRNA processing	6	2	0.0019	1
GO:0070727	Cellular macromolecule localization	909	20	0.0019	1

Table 3. Enriched GO categories (Top 10 categories) mir-635

GO ID	GO Name	C	O	Raw P-value	Adjusted P-value
GO:0018193	Peptidyl-amino acid modification	483	13	0	0.346
GO:0035556	Intracellular signal transduction	1017	18	0.0001	0.58
GO:0051094	Positive regulation of developmental process	432	11	0.0002	0.58
GO:0006836	Neurotransmitter transport	80	5	0.0003	0.58
GO:0035864	Response to potassium ion	4	2	0.0003	0.58
GO:0035865	Cellular response to potassium ion	4	2	0.0003	0.58
GO:0033554	Cellular response to stress	810	15	0.0004	0.58
GO:0007268	Chemical synaptic transmission	191	7	0.0005	0.58
GO:0098916	Anterograde trans-synaptic signaling	191	7	0.0005	0.58
GO:0099537	Trans-synaptic signaling	192	7	0.0005	0.58

Table 4. Pathways that are enriched among target genes of miR-508-5p

Gene Set	Pathway	Size	Expect	Ratio	P-value
hsa04714	Thermogenesis	229	5.8553	2.0494	0.01449
hsa04211	Longevity regulating pathway	89	2.2757	2.6366	0.02617
hsa04152	AMPK signaling pathway	120	3.0683	2.6073	0.01169
hsa04115	p53 signaling pathway	72	1.841	3.2591	0.01004
hsa04070	Phosphatidylinositol signaling system	99	2.5313	3.1604	0.00375
hsa04068	FoxO signaling pathway	132	3.3751	2.9629	0.00201
hsa00900	Terpenoid backbone biosynthesis	22	0.56252	5.3331	0.01775
hsa00532	Glycosaminoglycan biosynthesis	20	0.51138	7.8219	0.00146
hsa00340	Histidine metabolism	23	0.58809	5.1013	0.02003
104300	Alzheimer disease	11	0.28126	7.1108	0.03075

Table 5. Pathways that are enriched among target genes of miR-635

Gene Set	Pathway	Size	Expect	Ratio	P-value
hsa04727	GABAergic synapse,	88	1.2336	4.8638	0.00143
613659	gastric cancer, intestinal cancer	8	0.11215	17.834	0.00517
hsa04724	Glutamatergic synapse	114	1.5981	3.7545	0.00523
hsa00072	Synthesis and degradation of ketone bodies	10	0.14018	14.267	0.00815
hsa04010	MAPK signaling pathway	295	4.1354	2.4182	0.00837
hsa05162	Measles	132	1.8504	3.2425	0.01049
hsa05168	Herpes simplex infection	185	2.5934	2.6992	0.01513
hsa04520	Adherents junction	72	1.0093	3.9631	0.0182
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	72	1.0093	3.9631	0.0182
hsa05212	Pancreatic cancer	75	1.0514	3.8046	0.02083

4.4. Experimental Analysis

4.4.1. MiR-508-5p and MiR-635

The findings of paired T-test indicated that relative expression of miR-508-5p was significantly down-regulated in the human BC tissues compared to the normal adjacent tissues (Table 6 and Figure 3). The miR-508-5p was downregulated in the sample group (in comparison to the control group) by a mean factor of 0.327 (SEM range is 0.031-2.000). The miR-635 was upregulated in the sample group (in comparison to the control group) by a mean factor of 2.361 (SEM range is 0.250-16.000). The results of paired T-test showed that relative expression of miR-635 was significantly upregulated in the human BC tissues compared to the normal adjacent tissues and Figure 3).

4.4.2. Receiver operating characteristic (ROC) curve analysis

The ROC curve was used to investigate the potential of miR-508-5p and miR-635 as diagnostic biomarkers in BC. According to Figure 4 and Table 7, the area under the curve was 0.9442 and 0.8779, respectively, which indicates the biomarker role of these miRNAs in the diagnosis of BC.

Table 6. Relative Expression Results of miR-508-5P

Gene	Type	Reaction Efficiency	Expression	Std. Error	95% C.I.	P(H1)	Result
miR-508-5p	TRG	1	0.327	0.031 - 2.000	0.008 - 16.000	0.000	Down
miR-635	TRG	1	2.361	0.250 - 16.000	0.031 - 128.000	0.000	Up
U6	REF	1	1				

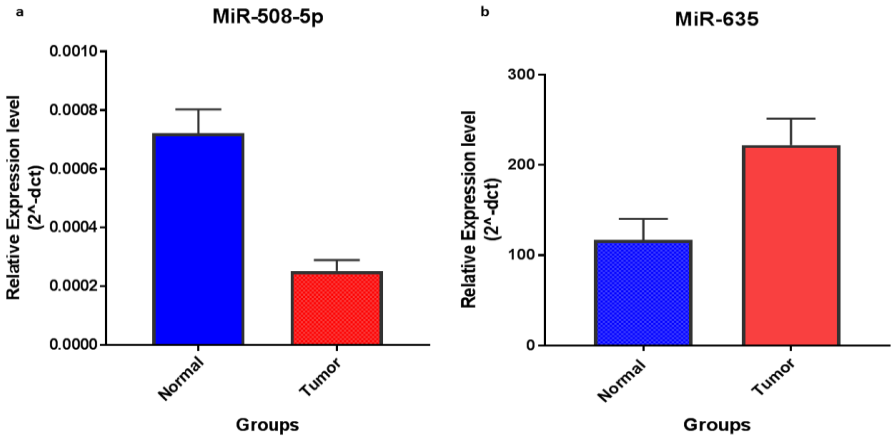

Figure 3. a) Expression of miR-508-5p in tumor tissues and normal adjacent tissues. b) Expression of miR-635 in tumor tissues and normal adjacent tissues

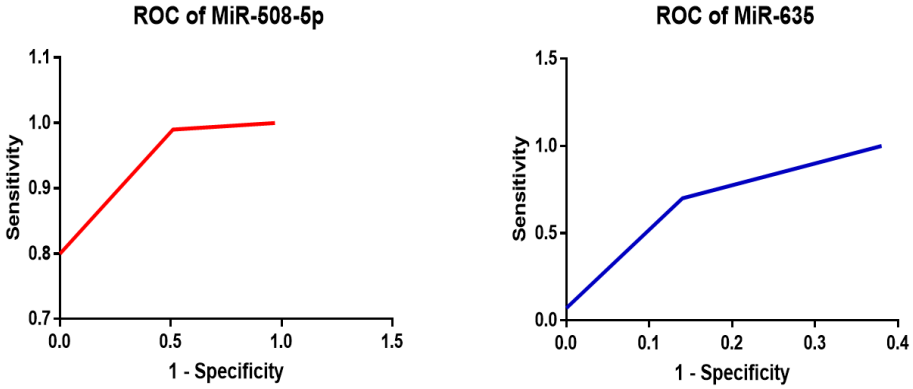

Figure 4. The receiver operating characteristic (ROC) curve for the miR-508-5p and miR-635

Table 7. Area under the receiver operating characteristic (ROC) curve analysis for the expression of miR-508-5p and miR-635

	miR-508-5P	miR-635
Area under the ROC curve	0.9442	0.8779
Std. Error	0.01528	0.02475
95% confidence interval	0.9142 to 0.9741	0.8294 to 0.9264
P-value	<0.0001	<0.0001

5. Discussion

According to studies performed on miRNAs, deregulation of miRNAs change the expression of tumor suppressors, oncogenes, and other genes (18). Given that the expression profile of miRNAs in BC cells differs from those in non-cancerous cells (25), they can be used as biomarkers in BC diagnosis and prognosis (24, 26). Changes in the miRNA expression profile induce metastasis, tissue invasion, apoptosis deregulation, and drug resistance in BC (6, 27, 28). Due to the high prevalence of BC (29), many studies have focused on identifying potential biomarkers that can effectively prevent complications through early detection. Bioinformatics studies have revealed the role of miR-635 and miR-508-5p in various intracellular processes. Therefore, in this study, we analyzed the expression levels of miR-635 and miR-508-5p in BC tissues compared to non-cancerous marginal tissues.

Previous studies showed that the expression level of miR-635 decreased in several tumors (30). Zhang et al. reported that miR-635 expression decreased in non-small cell lung cancer (NSCLC) and acted as a tumor suppressor. Moreover, miR-635 inhibits tumorigenesis in NSCLC by targeting Ying Yang 1 (20). Tian et al. reported that miR-635, as a tumor

suppressor, is involved in the inhibition of osteosarcoma tumor (31) and may also act as an essential mediator in various cancers (20). Zhu et al. demonstrated a negative relationship between miR-635 and PART1 expression in which the expression level of miR-635 decreased, while the expression level of PART1 increased in NSCLC. According to their results, miR-635, as a miRNA, is a target for PART1 gene. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway plays essential roles in regulating cytokine receptors (32). Binding of ligands such as cytokines to receptors at the cell surface via dimerization of receptors activates receptor-associated JAKs. Then JAKs activated by phosphorylation of the tyrosine residues. Subsequently, JAKs activate STATs by phosphorylation of the tyrosine subunit. Thus, activated STATs participate in regulating tumor progression (32). Therefore, JAK1-STAT3 messaging inhibitors have antitumor activity (33). Reports have shown that JAKs are involved in the pathogenesis of immune-related and inflammatory abnormalities and malignant tumors (34-36). JAKs have an important relationship with cytokine receptors, including IL-2, IL-4, IL-10, and IFNγ (37, 38) and have main inflammation and immunologic roles (32, 39).

They introduced JAK1 and JAK3 as key targets for miR-635 using the luciferase dual reporter assay (40). The effect of PART1/miR-635 on the JAK-STAT pathway depends on the genes (TIMP-1, JAK1, JAK3, STAT3, p-JAK1, P-JAK3, p-STAT3, Pim-1) (40). Yuan et al. demonstrated that miR-635 could inhibit the expression of these genes and inactivate JAK-STAT pathway (41). Also, the clinical significance of JAKs in BC has been reported abundantly. For example, JAK1 expression level is reversely related to lymph node status and tumor size of patients with BC (42). In addition, somatic mutations of JAKs (such as JAK1, JAK2, and JAK3) are commonly observed in BC and have potential features for clinical management (43-45). The TYK2 plays a prominent function in metastasis and growth of BC (46, 47). Various JAK inhibitors, including Lestaurtinib (multi-kinase target), CP-690,550 (JAK3 target), and AZ-01/AZ-60 (JAK2 target), are being developed to potentially treat hematological malignancies and autoimmune diseases (48-50).

In contrast to previous studies, our findings showed significant overexpression of miR-635 in BC tissues compared to normal adjacent tissues ($P<0.05$), in which BC tumor cells showed high expression of miR-635in bioinformatics studies of the GEO microarray dataset. The miR-635 is involved in different molecular signaling pathways and is affected by different molecules, clarifying the difference observed in the present study with other studies. Further studies on various molecular signaling pathways and evaluation of other molecules on miR-635 are needed to better understand this issue and find more accurate results. According to qPCR results and bioinformatics studies performed on miR-635, it can be mentioned that miR-635 may play a major role in the incidence and spread of BC. Furthermore, according to the ROC curve miR-635 can also be used as an important diagnostic and therapeutic biomarker in BC.

The miR-508-5p acts as a tumor inhibitor in various types of cancers. Liu et al. reported the miR-508-5p as an important prognostic biomarker in glioma, which could suppress migration and cell proliferation (51). One study demonstrated that miR-508-5p was an important suppressor of gastric cancer. Downregulation of miR-508-5p is associated with progression and malignancy in gastric cancer, and overexpression of miR-508-5p results in apoptosis, reduced invasion and migration of gastric tumor cells, and the cell cycle arrest in G0/G1 phase by targeting S-Phase Kinase Associated Protein 2 (SKP2) (52). The SKP2 is a significant factor for cell cycle transition from G phase to the S phase, which is involved in cell cycle progression at the G1/S and G2/M phases through the ubiquitin-dependent P27 pathway (53). Therefore, SKP2 can induce cell proliferation and tumorigenesis by degrading ubiquitin in various types of proteins (54). The SKP2 can act as an oncoprotein in the progression of various types of cancers (55). The level of miR-508-5p in metastatic gastric cancer reduce (56) and causes MDR in cancer chemotherapy. The high expression of miR-508-5p in gastric cancer leads to loss of or inhibition of MDR to chemotherapy drugs in gastric cancer and sensitization of cancer cells to chemotherapy drugs (57). The miR-27 is associated with drug resistance. The function of miR-27 is similar to miR-508-5p, leading to MDR in gastric cancer (58). The P53 protein is a tumor suppressor protein regulated by miRNAs (59). In MDR of gastric cancer cells, low expression of miR-27b increases the level of CCNG1, which suppresses P53, and low level of P53 directly suppresses miR-508-5p. Consequently, inappropriate expression of P53 directly affects the expression of miR-508-5p. Thus, the miR-27b/CCNG1/P53/miR-508-5p axis is involved in MDR of gastric cancer cells (58).

The miR-508-5p directly targets MESDC1 and increases the expression of miR-508-5p in HepG2 cells, which decreases the level of MESDC1 protein per se. The MESDC1 is an oncogenic factor involved in induction of cell migration, cancer cell survival, invasion and inhibition of apoptosis in bladder cancer (60). The LOC102724163 may affect miR-508-5p. Downstream target genes of miR-508-5p are identified using TargetscanHuman7.2 studies, among which MUC19 had the highest score. The expression of MUC19 in BC is very high and has a positive relationship with LOC102724163. The LOC102724163 increases the expression of MUC19 in BC by affecting miR-508-5p. The MUC19 is mainly expressed in mucosal cells of the salivary glands (61) and is

involved in the pathogenesis of Sjogren's syndrome (62). The MUC19 is significantly upregulated in BC (63) and increases proliferation, and cell invasion, whereas it decreases apoptosis (64).

In this study, corresponding to previous studies, the expression level of miR-508-5p was significantly reduced in tumor cells compared to healthy cells (P>0.05). In bioinformatics studies, GEO microarray data set, similar to qPCR results, showed a decrease in the expression of miR-508-5p in BC tissues. Furthermore, miR-508-5p plays a role in different molecular pathways and expression of miR-508-5p is affected by various molecules and proteins, which dysregulation ofmiR-508-5p may change cellular mechanisms, and subsequently, cause disease with different severities and MDR in cancer chemotherapy. Therefore, the study of miR-508-5p axis may help us obtain important information, which is important to propose effective treatments for various diseases. It can also be used as a major diagnostic and therapeutic biomarker in BC.

6. Conclusion

In general, it can be said that miR-635 may show different expression patterns in different types of cancer. Hence, miR-635 has tumor suppressor activity and oncogenic function in various cancers. In addition, some important molecular pathways changed by miR-635 make cells susceptible to tumorigenesis. Although the tumor suppressor activity of miR-508-5p has been identified in several common cancers, it can change the expression of various genes, which have an important role in cell cycle regulation and increases the incidence of cancers. According to our results and other studies, these miRNAs can be proposed as diagnostic and therapeutic biomarkers; however, further investigation is needed to shed light on different molecular pathways.

6.1. Limitation

The small number of tissue samples was one of the limitations of this study.

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Footnotes

Conflicts of Interest: The authors declare no competing interests.

Author Contribution: FZS conceived the study and drafted the first manuscript; CA, MRA, SG contributed to data collection, analysis and interpretation; MZ supervised the study and revised the manuscript. All authors read and approved the manuscript. FZS critically revised the manuscript for important intellectual contents.

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Ethical Statements: Institutional guidelines, including ethical approval and informed consent, were followed by the Ethics Committee of Tabriz Azad University, Tabriz, Iran (Ethics code: IR.IAU.TABRIZ.REC.1401.063) and was conducted according to the Declaration of Helsinki.

References

1. Zare M, Bastami M, Solali S, Alivand MR. Aberrant miRNA promoter methylation and EMT-involving miRNAs in breast cancer metastasis: diagnosis and therapeutic implications. *J Cell Physiol*. 2018;**233**(5):3729-44. doi: 10.1002/jcp.26116. [PubMed: 28771724].
2. Seif F, Vaseghi H, Ariana M, Ganji SM, Nazari M, Rad KK, et al. 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):141-7. doi: 10.4274/ejbh.galenos.2022.2021-10-4. [PubMed: 35445179].
3. Vahdanikia V, Maleki M, Fam RA, Abdi A. Assessment the effect of human umbilical cord wharton's jelly stem cells on the expression of homing genes; CXCR4 and VLA-4 in cell line of Breast Cancer. *Int J Hematol Oncol Stem Cell Res.* 2022; **16**(2):110-16. doi: 10.18502/ijhoscr.v16i2.9204. [PubMed: 36304731].
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca Cancer J Clin.* 2018;**68**(6):394-424. doi: 10.3322/caac.21492. [PubMed: 30207593].
5. Wong KK. DNMT1: A key drug target in triple-negative breast cancer. *Semin Cancer Biol.* 2021;**72**:198-213. doi: 10.1016/j.semcancer.2020.05.010. [PubMed: 32461152].
6. Safi A, Bastami M, Delghir S, Ilkhani K, Seif F, Alivand MR. miRNAs modulate the dichotomy of cisplatin resistance or sensitivity in breast cancer: an update of therapeutic implications. *Anticancer Agents Med Chem*. 2021;**21**(9):1069-81. doi: 10.2174/1871520620666200903145939. [PubMed: 32885760].
7. Mayahi S, Neshasteh-Riz A, Pornour M, Eynali S, Montazerabadi A. Investigation of combined photodynamic and radiotherapy effects of gallium phthalocyanine chloride on MCF-7 breast cancer cells. *J Biol Inorg Chem*. 2020;**25**(1):39-48. doi: 10.1007/s00775-019-01730-w. [PubMed: 31650249].
8. Idrissou M, Sanchez A, Penault-Llorca F, Bignon YJ, Bernard-Gallon D. Epi-drugs as triple-negative breast cancer treatment. *Epigenomics*. 2020;**12**(8):725-42. doi: 10.2217/epi-2019-0312. [PubMed: 32396394].
9. Guo P, Chen W, Li H, Li M, Li L. The histone acetylation modifications of breast cancer and their therapeutic implications. *Pathol Oncol Res*. 2018;**24**(4):807-13. doi: 10.1007/s12253-018-0433-5. [PubMed: 29948617].
10. Volovat SR, Volovat C, Hordila I, Hordila DA, Mirestean CC, Miron OT, et al. MiRNA and LncRNA as potential biomarkers in triple-negative breast cancer: a review. *Front Oncol.* 2020;**10**:526850. doi: 10.3389/fonc.2020.526850. [PubMed: 33330019].
11. 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**(2):589-603. doi: 10.1111/cas.14764. [PubMed: 33283362].
12. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;**12**(12):861-74. doi: 10.1038/nrg3074. [PubMed: 22094949].

13. Abdi A, Zafarpiran M, Farsani ZS. The computational analysis conducted on miRNA target sites in association with SNPs at 3'UTR of ADHD-implicated genes. *Cent Nerv Syst Agents Med Chem.* 2020;**20**(1):58-75. doi: 10.2174/1871524919666191014104843. [PubMed: 31660846].
14. Wang B, Li J, Sun M, Sun L, Zhang X. miRNA expression in breast cancer varies with lymph node metastasis and other clinicopathologic features. *IUBMB Life*. 2014;**66**(5):371-7. doi: 10.1002/iub.1273. [PubMed: 24846313].
15. Breunig C, Pahl J, Küblbeck M, Miller M, Antonelli D, Erdem N, et al. MicroRNA-519a-3p mediates apoptosis resistance in breast cancer cells and their escape from recognition by natural killer cells. *Cell Death Dis.* 2017;**8**(8):e2973. doi: 10.1038/cddis.2017.364. [PubMed: 28771222].
16. Xie F, Hosany S, Zhong S, Jiang Y, Zhang F, Lin L, et al. MicroRNA-193a inhibits breast cancer proliferation and metastasis by downregulating WT1. *PloS One.* 2017;**12**(10): e0185565. doi: 10.1371/journal.pone.0185565. [PubMed: 29016617].
17. Samaeekia R, Adorno-Cruz V, Bockhorn J, Chang YF, Huang S, Prat A, et al. miR-206 inhibits stemness and metastasis of breast cancer by targeting MKL1/IL11 pathway. *Clin Cancer Res*. 2017;**23**(4):1091-103. doi: 10.1158/1078-0432.CCR-16-0943. [PubMed: 27435395].
18. Fridrichova I, Zmetakova I. MicroRNAs contribute to breast cancer invasiveness. *Cells*. 2019;**8**(11):1361. doi: 10.3390/cells 8111361. [PubMed: 31683635].
19. Li C, Wang A, Chen Y, Liu Y, Zhang H, Zhou J. MicroRNA-299-5p inhibits cell metastasis in breast cancer by directly targeting serine/threonine kinase 39. *Oncol Rep.* 2020;**43**(4):1221-33. doi: 10.3892/or.2020.7486. [PubMed: 32020227].
20. Zhang Y, Sun Z, Zhang Y, Fu T, Liu C, Liu Y, et al. The microRNA-635 suppresses tumorigenesis in non-small cell lung cancer. *Biomed Pharmacother.* 2016;**84**:1274-81. doi: 10.1016/j. biopha.2016.10.040. [PubMed: 27810784].
21. Han P, Chang CP. Long non-coding RNA and chromatin remodeling. *RNA Biol.* 2015;**12**(10):1094-8. doi: 10.1080/ 15476286.2015.1063770. [PubMed: 26177256].
22. Bhatti GK, Khullar N, Sidhu IS, Navik US, Reddy AP, Reddy PH, et al. Emerging role of non-coding RNA in health and disease. *Metab Brain Dis.* 2021;**36**(6):1119-34. doi: 10.1007/s11011-021-00739-y. [PubMed: 33881724].
23. 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**(1):1-17. doi: 10.1186/s13578-021-00552-1. [PubMed: 33632341].
24. Ilkhani K, Delgir S, Safi A, Seif F, Samei A, Bastami M, et al. Clinical and in silico outcomes of the expression of miR-130a-5p and miR-615-3p in tumor compared with non-tumor adjacent tissues of patients with BC. *Anticancer Agents Med Chem*. 2021;**21**(7):927-35. doi: 10.2174/1871520620666200924105352. [PubMed: 32972352].
25. Gasparri ML, Casorelli A, Bardhi E, Besharat AR, Savone D, Ruscito I, et al. Beyond circulating microRNA biomarkers: Urinary microRNAs in ovarian and breast cancer. *Tumor biology*. 2017;**39**(5):1-13. doi: 10.1177/1010428317695525. [PubMed: 28459207].
26. Zografos E, Zagouri F, Kalapanida D, Zakopoulou R, Kyriazoglou A, Apostolidou K, et al. Prognostic role of micro-RNAs in breast cancer: A systematic review. *Oncotarget*. 2019;**10**(67):7156-78. doi: 10.18632/oncotarget.27327. [PubMed: 31903173].
27. Zhang L-F, Jiang S, Liu MF. MicroRNA regulation and analytical methods in cancer cell metabolism. *Cell Mol Life Sci.* 2017;**74**(16):2929-41. doi: 10.1007/s00018-017-2508-y. [PubMed: 28321489].
28. Zhan M-N, Yu XT, Tang J, Zhou CX, Wang CL, Yin QQ, et al. MicroRNA-494 inhibits breast cancer progression by directly targeting PAK1. *Cell Death Dis*. 2018;**8**(1):e2529. doi: 10.1038/cddis.2016.440. [PubMed: 28055013].
29. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;**71**(3):209-49. doi: 10.3322/caac.21660. [PubMed: 33538338].
30. Zhou M, Zhang P, Zhao Y, Liu R, Zhang Y. Overexpressed circRANBP17 acts as an oncogene to facilitate nasopharyngeal carcinoma via the miR-635/RUNX2 axis. *J Cancer.* 2021;**12**(14):4322-31. doi: 10.7150/jca.55794. [PubMed: 34093832].
31. Tian L, Guo Z, Wang H, Liu X. MicroRNA-635 inhibits the malignancy of osteosarcoma by inducing apoptosis. *Mol Med Rep*. 2017;**16**(4):4829-34. doi: 10.3892/mmr.2017.7127. [PubMed: 28765920].
32. 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**(1):1-13. doi: 10.1186/s12964-017-0177-y. [PubMed: 28637459].
33. Zhu F, Dai C, Fu Y, Loo JF, Xia D, Gao SP, et al. Physalin A exerts anti-tumor activity in non-small cell lung cancer cell lines by suppressing JAK/STAT3 signaling. *Oncotarget*. 2016; **7**(8):9462-76. doi: 10.18632/oncotarget.7051. [PubMed: 26843613].
34. Vainchenker W, Dusa A, Constantinescu SN. JAKs in pathology: role of Janus kinases in hematopoietic malignancies and immunodeficiencies. *Semin Cell Dev Biol.* doi: 10.1016/j.semcdb.2008.07.002. [PubMed: 18682296].
35. Cance WG, Liu ET. Protein kinases in human breast cancer. *Breast Cancer Res Treat.* 1995;**35**(1):105-14. doi: 10.1007/BF00694751. [PubMed: 7612897].
36. Seif F, Aazami H, Khoshmirsafa M, Kamali M, Mohsenzadegan M, Pornour M, et al. JAK inhibition as a new treatment strategy for patients with COVID-19. *Int Arch Allergy Immunol*. 2020;**181**(6):467-75. doi: 10.1159/000508247. [PubMed: 32392562].
37. Smith GA, Uchida K, Weiss A, Taunton J. Essential biphasic role for JAK3 catalytic activity in IL-2 receptor signaling. *Nat Chem Biol.* 2016;**12**(5):373-9. doi: 10.1038/nchembio.2056. [Pub Med: 27018889].
38. Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, et al. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell*. 1998;**93**(3):373-83. doi: 10.1016/s0092-8674(00)81166-6. [PubMed: 9590172].
39. Buchert M, Burns C, Ernst M. Targeting JAK kinase in solid tumors: emerging opportunities and challenges. *Oncogene*. 2016;**35**(8):939-51. doi: 10.1038/onc.2015.150. [PubMed: 25982279].
40. Zhu D, Yu Y, Wang W, Wu K, Liu D, Yang Y, et al. Long noncoding RNA PART1 promotes progression of non-small cell lung cancer cells via JAK-STAT signaling pathway. *Cancer Med.* 2019;**8**(13):6064-81. doi: 10.1002/cam4.2494. [PubMed: 31436388].
41. Yuan J, Ji H, Xiao F, Lin Z, Zhao X, Wang Z, et al. MicroRNA-340 inhibits the proliferation and invasion of hepatocellular carcinoma cells by targeting JAK1. *Biochem Biophys Res Commun*. 2017;**483**(1):578-84. doi: 10.1016/j.bbrc.2016. 12.102. [PubMed: 27998770].
42. Chen B, Lai J, Dai D, Chen R, Li X, Liao N. JAK1 as a prognostic marker and its correlation with immune infiltrates in breast cancer. *Aging*. 2019;**11**(23):11124-35. doi: 10.18632/aging. 102514. [PubMed: 31790361].
43. Balko JM, Schwarz LJ, Luo N, Estrada MV, Giltnane JM, Dávila-González D, et al. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. *Sci Transl Med*. 2016;**8**(334):334ra53. doi: 10.1126/scitranslmed.aad3001. [PubMed: 27075627].
44. Naeem MA, Shah TH, Zafar N, Khan M, Bhutto AA, Rabbani S. Detection of JAK2 gene mutation in Pakistani women with triple-negative breast cancer. *Breast J.* 2019;**26**(4):829-30. doi: 10.1111/tbj.13609. [PubMed: 31513718].
45. Jeong EG, Kim MS, Nam HK, Min CK, Lee S, Chung YJ, et al. Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. *Clin Cancer Res*. 2008;**14**(12):3716-21. doi: 10.1158/1078-0432.CCR-07-4839. [PubMed: 18559588].
46. Zhang Q, Sturgill JL, Kmieciak M, Szczepanek K, Derecka M, Koebel C, et al. The role of Tyk2 in regulation of breast cancer

growth. *J Interferon Cytokine Res*. 2011;**31**(9):671-7. doi: 10.1089/jir.2011.0023. [PubMed: 21864028].
47. Sang QX, Man YG, Sung YM, Khamis ZI, Zhang L, Lee MH, et al. Non-receptor tyrosine kinase 2 reaches its lowest expression levels in human breast cancer during regional nodal metastasis. *Clin Exp Metastasis*. 2012;**29**(2):143-53. doi: 10.1007/s10585-011-9437-1. [PubMed: 22116632].
48. Furumoto Y, Gadina M. The arrival of JAK inhibitors: advancing the treatment of immune and hematologic disorders. *BioDrugs*. 2013;**27**(5):431-8. doi: 10.1007/s40259-013-0040-7. [Pub Med: 23743669].
49. Harrington R, Al Nokhatha SA, Conway R. JAK inhibitors in rheumatoid arthritis: an evidence-based review on the emerging clinical data. *J Inflamm Res.* 2020;**13**:519-31. doi: 10.2147/JIR.S219586. [PubMed: 32982367].
50. Pesu M, Laurence A, Kishore N, Zwillich SH, Chan G, O'Shea JJ. Therapeutic targeting of Janus kinases. *Immunol Rev.* 2008;**223**(1):132-42. doi: 10.1111/j.1600-065X.2008.00644.x. [PubMed: 18613833].
51. Liu Y, Li B, Meng F, Qiu L. MiR-508-5p is a prognostic marker and inhibits cell proliferation and migration in glioma. *Eur Rev Med Pharmacol Sci*. 2017;**21**(1):76-81. [PubMed: 28121353].
52. Duan X, Bai J, Wei J, Li Z, Liu X, Xu G. MicroRNA-508-5p suppresses metastasis in human gastric cancer by targeting S-phase kinase-associated protein 2. *Mol Med Rep.* 2017;**16**(2):2163-71. doi: 10.3892/mmr.2017.6793. [PubMed: 28627698].
53. Lonjedo M, Poch E, Mocholí E, Hernández-Sánchez M, Ivorra C, Franke TF, et al. The Rho family member RhoE interacts with Skp2 and is degraded at the proteasome during cell cycle progression. *J Biol Chem*. 2013;**288**(43):30872-82. doi: 10.1074/jbc.M113.511105.
54. Zhang B, Ji LH, Liu W, Zhao G, Wu ZY. Skp2-RNAi suppresses proliferation and migration of gallbladder carcinoma cells by enhancing p27 expression. *World J Gastroenterol.* 2013;**19** (30):4917-24. doi: 10.3748/wjg.v19.i30.4917. [PubMed: 23946596].
55. Wang Z, Gao D, Fukushima H, Inuzuka H, Liu P, Wan L, et al. Skp2: a novel potential therapeutic target for prostate cancer. *Biochim Biophys Acta*. 2012;**1825**(1):11-7. doi: 10.1016/j.bbcan.2011.09.002. [PubMed: 21963805].
56. Wang Z, Wang J, Yang Y, Hao B, Wang R, Li Y, et al. Loss of has-miR-337-3p expression is associated with lymph node metastasis of human gastric cancer. *J Exp Clin Cancer Res*. 2013;**32**(1):1-9. doi: 10.1186/1756-9966-32-76. [PubMed: 24422944].
57. Shang Y, Zhang Z, Liu Z, Feng B, Ren G, Li K, et al. miR-508-5p regulates multidrug resistance of gastric cancer by targeting ABCB1 and ZNRD1. *Oncogene*. 2014;**33**(25):3267-76. doi: 10.1038/onc.2013.297. [PubMed: 23893241].
58. Shang Y, Feng B, Zhou L, Ren G, Zhang Z, Fan X, et al. The miR27b-CCNG1-P53-miR-508-5p axis regulates multidrug resistance of gastric cancer. *Oncotarget*. 2016;**7**(1):538-49. doi: 10.18632/oncotarget.6374. [PubMed: 26623719].
59. Hermeking H. MicroRNAs in the p53 network: micromanagement of tumour suppression. *Nat Rev Cancer.* 2012;**12**(9):613-26. doi: 10.1038/nrc3318. [PubMed: 22898542].
60. Wu S, Huang Y, Bao B, Wu L, Dong J, Liu X, et al. miR-508-5p acts as an anti-oncogene by targeting MESDC1 in hepatocellular carcinoma. *Neoplasma*. 2017;**64**(1):40-7. doi: 10.4149/neo_2017_105. [PubMed: 27881003].
61. Chen Y, Zhao YH, Kalaslavadi TB, Hamati E, Nehrke K, Le AD, et al. Genome-wide search and identification of a novel gel-forming mucin MUC19/Muc19 in glandular tissues. *Am J Respir Cell Mol Biol*. 2004;**30**(2):155-65. doi: 10.1165/rcmb.2003-0103OC. [PubMed: 12882755].
62. Yu D, Chen Y, Han J, Zhang H, Chen X, Zou W, et al. MUC19 expression in human ocular surface and lacrimal gland and its alteration in Sjögren syndrome patients. *Exp Eye Res.* 2008;**86**(2):403-11. doi: 10.1016/j.exer.2007.11.013. [Pub Med: 18184611].
63. Qiao K, Ning S, Wan L, Wu H, Wang Q, Zhang X, et al. Correction to: LINC00673 is activated by YY1 and promotes the proliferation of breast cancer cells via the miR-515-5p/MARK4/Hippo signaling pathway. *J Exp Clin Cancer Res.* 2020;**39**(1):1-5. doi: 10.1186/s13046-019-1421-7.
64. Liu Y, Zhang Q, Wu J, Zhang H, Li X, Zheng Z, et al. Long non-coding RNA A2M-AS1 promotes breast cancer progression by sponging microRNA-146b to upregulate MUC19. *Int J Gen Med.* 2020;**13**:1305-16. doi: 10.2147/IJGM.S278564. [PubMed: 33273850].