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Expression of Bioinformatically Candidate miRNAs including, miR-576-5p, miR-501-3p and miR-3143, Targeting PI3K Pathway in Triple-Negative Breast Cancer

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Abstract

Background: Triple-negative breast cancer (TNBC) is an invasive and lethal form of breast cancer. PI3K pathway, which often activated in TNBC patients, can be a target of miRNAs. The purpose of this study was bioinformatic prediction of miRNAs targeting the key genes of this pathway and evaluation of the expression of them and their targets in TNBC. **Materials and Methods:** We predicted miRNAs targeting PIK3CA and AKT1 genes using bioinformatics tools. Extraction of total RNA, synthesis of cDNA and quantitative real-time polymerase chain reaction were performed from 18 TNBC samples and normal adjacent tissues and cell lines. **Results:** Our results demonstrated that miR-576-5p, miR-501-3p and miR-3143 were predicted to target PIK3CA, AKT1 and both of these mRNAs, respectively and were down-regulated while their target mRNAs were up-regulated in clinical samples and cell lines. The analysis of the receiver operating characteristic curve was done for the evaluation of the diagnostic value of predicted miRNAs in TNBC patients. **Conclusion:** The findings of our study demonstrated the reverse correlation between miRNAs and their target genes and therefore the possibility of these miRNAs to be proposed as new candidates for TNBC targeted therapies. [GMJ.2019;8:e1646] DOI: [10.31661/gmj.v8i0.1646](https://doi.org/10.31661/gmj.v8i0.1646)

Keywords: Triple Negative Breast Cancer; MicroRNA; PIK3CA; AKT1; Bioinformatics

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Introduction

Breast cancer is one of the most prevalent neoplasms in women [1]. About 7.1 million women worldwide are diagnosed with this type of cancer and approximately 500,000 of them die from it annually [2]. Triple-negative breast cancer (TNBC) is a highly invasive and lethal form of breast cancer that does not represent any of estrogen receptors, progesterone receptors and human epidermal receptors 2 (HER2) [3, 4]. Concerning the fact that traditional breast cancer treatments are not so effective, modern and accessible therapeutic methods are required for treating this type of breast cancer. One of these developing methods is targeted therapies [5, 6]. miRNAs are small non-protein coding RNAs with an approximate length of 20-25 nucleotides. Binding of these molecules to 3'-untranslated regions (3'-UTR) of their target genes results in mRNA cleavage or translation repression [7-9]. In the past few years, different reports indicated a large number of miRNAs were involved in the pathophysiology of breast cancer. In general, the expression of tumor suppressor miRNAs can be significantly down-regulated whilst oncogenic miRNAs are up-regulated in breast cancer. Furthermore, different subtypes of this malignancy including TNBC show a specific expression profile of miRNAs [6, 10, 11]. Phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling, which is also called PI3K signaling pathway, promotes cell proliferation, survival, metabolism, and metastases and its aberrant activation is considered as a center for growth, survival and dynamics of cancer [6, 12-14]. PI3K from class IA of PI3Ks is the major role-player in this pathway. This enzyme phosphorylates phosphatidylinositol 4 and 5-bisphosphate (PIP₂) to phosphatidylinositol 3, 4 and 5-triphosphate (PIP₃). PIP₃ activates AKT, a serine/threonine kinase, which affects the growth, survival and cell cycle of cancerous tissue [15-17]. Several studies have shown dysregulation of this pathway in triple-negative and other subtypes of breast cancer that leads to proliferation and cellular invasion [14]. Bioinformatics are up-to-date and low-cost methods that absorbed a lot of attention today. These software tools and programs can predict miRNAs targeting a specific

target gene in a short time based on biology and computer sciences [18]. To achieve more valuable results, several programs can be simultaneously utilized. One advantage of using these methods is to limit target sites for experimental verification [19-21]. Hence, the first purpose of the present study was to recruit bioinformatics for the prediction of miRNAs targeting PIK3CA and AKT1 mRNAs. Then, the expression of predicted miRNAs and their targets was investigated in breast cancer clinical samples and cell lines. The results of this study could be the starting point for further investigation of the diagnosis and treatment of TNBC.

Material and Methods

miRNAs Prediction

To predict miRNAs which target PI3K and AKT mRNAs, bioinformatic software applications including TargetScan [22], MicroCosm [23], DIANA Tool [24], miRanda [25], microPIR [26], miRTarBase [27], miRDB [28], miRWalk [29], miRNAMap [30], miR-Gator [31], miRSearch [32] were used.

Primer Design for Investigating the Expression of Genes and miRNAs

mRNA sequences of target genes were received from GenBank, NCBI (www.ncbi.nlm.nih.gov). Then by Using Allele ID 7.0 and Oligo7 software, the best pair of forward and reverse primer was designed. Due to the small size of miRNAs, their expression analysis by conventional primer pairs is not possible. Therefore, the Stem-loop structures were used for cDNA synthesis based on the previously published studies [33, 34].

Cell Culture

MDA-MB-231 and MCF-10A, as TNBC and normal breast cell lines respectively, were received from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran, Iran). MDA-MB-231 is an aggressive triple-negative breast ductal carcinoma cell line with estrogen, progesterone, and human epidermal growth factor receptors deficiencies. The normal breast cell line, MCF-10A, was used as control. MDA-MB-231 was cultured in Dulbecco's Modified Eagle Medium (DMEM)

including 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and MCF-10A was cultured in DMEM containing 10% horse serum and 1% penicillin-streptomycin, at 37°C incubator with 5% CO₂ and 98% humidity.

Tumor and Normal Breast Clinical Samples

Eighteen clinical samples of triple-negative invasive ductal carcinoma and 18 adjacent normal tissues were received from the tumor bank of Imam Khomeini Hospital (Tehran, Iran). Samples were stored in RNA Later (Qiagen, Germany) in a -70°C freezer. The patients were not undergone previous treatments, including surgery, chemotherapy or radiotherapy. Written informed consent was taken from all participants. This study was under the supervision of the ethics committee of Semnan University of Medical Sciences (ethics code: IR.SEMUMS.REC.1394.120). The characteristics of breast cancer samples

Table 1. Investigated Parameters of Breast Cancer Samples (N=18)

Variable	Abundance	Percentage
Age (year)		
≤35	3	17
35-50	7	39
>51	8	44
Grade		
I	2	11
II	2	11
III	14	78
Lymphatic invasion		
+	9	50
-	9	50
Vascular invasion		
+	10	56
-	8	45
Perineural invasion		
+	6	33
-	12	67

are presented in Table-1.

Extraction of Total RNA and Synthesis of cDNA

The extraction of total RNA was performed by Gene All Hybrid-RTM purification kit (Korea) according to the protocol presented by manufacturer. Extracted RNA's concentration was evaluated applying bio photometer (Eppendorf, Germany) and the quality of RNAs was investigated by gel electrophoresis. Then, synthesis of cDNA for genes and miRNAs was performed using Random hexamer and RT Stem-loop primers, respectively, and RevertAid® RT enzyme (Thermo scientific TM, USA) [33]. SNORD 47 and HPRT1 house-keeping genes were used in order to normalize the expression of miRNAs and target genes, respectively.

Quantitative Real-Time Polymerase Chain Reaction (PCR)

Analysis of gene expression was performed in triplicates by Real-time PCR assay and the final volume was 13 µL, containing 4.5 µL dH₂O, 1 µL cDNA (diluted at a ratio of 1/3), 0.5 µL of each reverse and forward primers (10 µM) and 6.5 µL RealQ Plus 2X Master Mix Green, High ROX™ (Amplicon, Denmark). Real-time PCR cycling profile for target and house-keeping genes was as follows: enzyme activation (95°C for 15 min), denaturation (95°C for 15 sec), annealing and extension (60°C for 1 min) for 40 cycles. At the end of amplification, analysis of melting temperature (T_m) was performed by raising the temperature from 60°C to 95°C by 0.2°C per sec increment. Analysis of predicted miRNAs by real-time PCR was done in triplicates with a total volume of 13 µL, containing 4.5 µL dH₂O, 1 µL cDNA (diluted at a ratio of 1/3), 0.2 µL TaqMan® Probe (Macrogen, Korea), 0.4 µL for each reverse and forward primer (10 µM) and 6.5 µL RealQ Plus 2X Master Mix for Probe, High ROX™ (Amplicon, Denmark). Profile of real-time PCR reaction for target miRNAs and housekeeping was as follows: enzyme activation (95°C for 15 min), denaturation (95°C for 15 sec), annealing and extension (60°C for 1 min) for 40 cycles. The results were reported as Ct (Threshold Cycles). Finally, the increase or decrease in the

level of the expressions was reported as fold changes.

Receiver Operating Characteristic (ROC) Curve Analysis

For assessing the diagnostic value of predicted miRNAs, the area under curve (AUC) was calculated by ROC curve analysis. To achieve the best cut off point, the Youden index ($[\text{specificity} + \text{sensitivity}] - 1$) was applied.

Statistical Analysis

SPSS version 18 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism were used for statistical analysis and graph preparation, respectively. REST® 2009 was applied to analyze the results of Real-time PCR. This program applies $\Delta\Delta\text{CT}$ method for analysis. This software compares the expression level in groups of treated and control samples using iterations. Furthermore, the Mann-Whitney non-parametric test was used for comparing differences between tumor samples and normal surrounding tissues. Statistically, the P-value less than 0.05 was considered significant.

Results

Predicting miRNAs Targeting PIK3CA and AKT1

miRNAs were predicted applying bioinformatic software mentioned before. We arranged the results of TargetScan from the

highest probable seed match 8mer to the lowest probable interactions. Then, miRNAs were investigated in other software and eventually miRNAs with the highest achieved-score were chosen. The hsa-miR-576-5p was selected as the miRNA targeting PIK3CA mRNA, hsa-miR-501-3p as the miRNA targeting AKT1, and hsa-miR-3143 as the miRNA targeting both of these mRNAs (Table-2). The bioinformatics analysis also revealed that the selected miRNAs had highest score and at least one binding site in the 3'-UTR of their target mRNAs (Table-3).

MiRNAs and Genes Expressions in MDA-MB-231

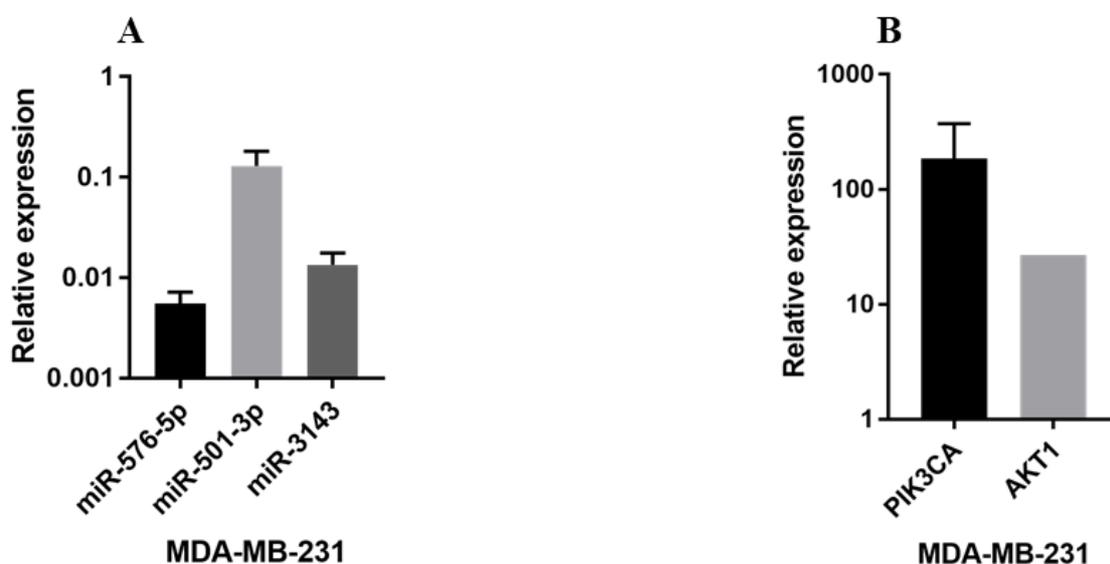
Analysis of real-time PCR assay demonstrated the expression of miR-576-5p, miR-501-3p, and miR-3143 in the MDA-MB-231 cell line was decreased by 18.18, 9.4 and 7.4 folds, respectively in comparison with MCF-10A cells (Figure-1A). Our findings also indicated the expression of both PIK3CA and AKT1 genes were significantly increased by 185 and 26 folds, respectively in the MDA-MB-231 cell line compared to normal cells (Figure-1B). According to statistical analysis, the relative expression of all three miRNAs was statistically significant ($P < 0.05$). Furthermore, the increase in the expression of target genes and the decline in the expression of miRNAs were significant between MDA-MB-231 and MCF-10A cell lines ($P < 0.05$).

Table 2. Prediction of miRNAs Predicting PIK3CA and AKT1

Gene	miRNA	TargetScna	MicroCosm	DIANA Tool-microT-CDS	DIANA Tools-TarBase	miRanda	microPIR	miRTarBase	miRDB	miRWalk	miRNAMap	miRGator	miRSearch	SUM
PIK3CA	hsa-miR-576-5p	1	1	1	0	1	1	0	0	1	0	1	0	7
AKT1	hsa-miR-501-3p	1	1	0	0	1	1	0	0	1	0	0	0	5
PIK3CA	Has-miR-3143	1	0	1	0	1	0	0	0	1	0	0	0	4
AKT1	Has-miR-3143	1	0	0	0	1	0	0	0	0	0	0	0	2

Table 3. miRNAs Binding Site on 3'-UTR of Target mRNAs

Predicted miRNA	Position of AKT1 3' UTR pair to miRNA	Site type
miR-501-3p	Position <u>90-96</u> : 5'...GUUUUUAUUUCUCGGGUGCAUU ... 3'	7mer-m8
miR-3143	Position <u>276-283</u> : 5'... UCCGAUUCACGUAGGGAAAUGUUAA... 3'	8mer
Predicted miRNA	Position of PIK3CA 3' UTR pair to miRNA	Site type
	Position <u>109-115</u> : 5'...CAAUCCAUGAACAGCAUUAGAAU ... 3'	7mer-m8
	Position <u>2380-2386</u> : 5'...AACCAAUUAUUUCUAUUAGAAG ... 3'	7mer-m8
	Position <u>3015-3022</u> : 5'...UUAUAUUAAAAGCCCAUUAGAAA ... 3'	8mer
	Position <u>5460-5466</u> : 5'... UGGUAUAACUGCAUUCAUUAGAAG... 3'	7mer-m8
	Position <u>443-449</u> : 5'... AGUAAACUGGAGUUUAUGUUAAA... 3'	7mer-A1
miR-3143	Position <u>909-915</u> : 5'...GAAAAUAGAAGUAUUAUUGUUAAU... 3'	7mer-m8
	Position <u>90-96</u> : 5'...UGCUUGAUUUUAGGUAUGUUAAU... 3'	7mer-A1

**Figure 1.** Expression of miRNAs and genes in cell lines; A: Down-regulation of miR-576-5p, miR-501-3p and miR-3143 in MDA-MB-231 cell line compared to MCF-10A; B: Up-regulation of PIK3CA, AKT1 in MDA-MB-231 cell line compared to MCF-10A.

miRNAs and Genes Expressions in Clinical Specimens of Triple-Negative Invasive Ductal Carcinoma

Analysis of the miRNAs expression in 18 tumor samples compared with the tumor's marginal tissue demonstrated that, interestingly, the expression of miR-576-5p, miR-501-3p, and miR-3143 decreased with an average of 16.9, 5.4, and 22 folds, respectively (Figure-2). The results also demonstrated that the expression of the PIK3CA gene in %83 of samples had an average increase of 3.1 folds (Figure-3A). The results also showed the expression of AKT1 in %83 of samples has an average increase of 82.5 folds and in one of the samples, it had been increased by even 6315 folds (Figures-3B). Furthermore, the relative expression of all genes and miRNAs was significant ($P < 0.05$) in tumor samples compared to the normal ones.

Diagnostic Value of miR-576-5p, miR-501-3p, and miR-3143 Expressions in TNBC

ROC curve analysis of miR-576-5p revealed that the AUC value was 0.872 (95% confidence interval [CI]=0.764- 0.981), and the best cut off point was 0.191 with the Youden index of 0.650, sensitivity of 80%, and spec-

ificity of 85%. For miR-501-3p, AUC value was 0.770 (95% CI=0.623-0.917), and the cutoff point of 0.178 with the Youden index of 0.450 was selected as the best. The sensitivity of 85% and specificity of 60% were obtained for this point. Eventually, the best-calculated cut off point for miR-3143 was 0.159 with Youden index of 0.650, sensitivity of 80%, and specificity of 85%. The AUC value of this miRNA was 0.894 (95% CI=0.785-1, Figure-4).

Discussion

miRNAs are a large family of regulatory RNAs that cause post-transcriptional gene silencing. Changes in miRNAs expression profiles are one of the characteristics of different kinds of cancers including TNBC. TNBC, an aggressive subtype of breast cancer with poor prognosis, contains 15-20% of all cases of breast cancer. This type of breast cancer lacks specific markers for therapies, hence the main method in its treatment is chemotherapy. miRNAs-based targeted therapy is one of the developing new strategies [16, 35-39]. Concerning different pathways that are dysregulated in TNBC, PI3K/AKT/mTOR pathway

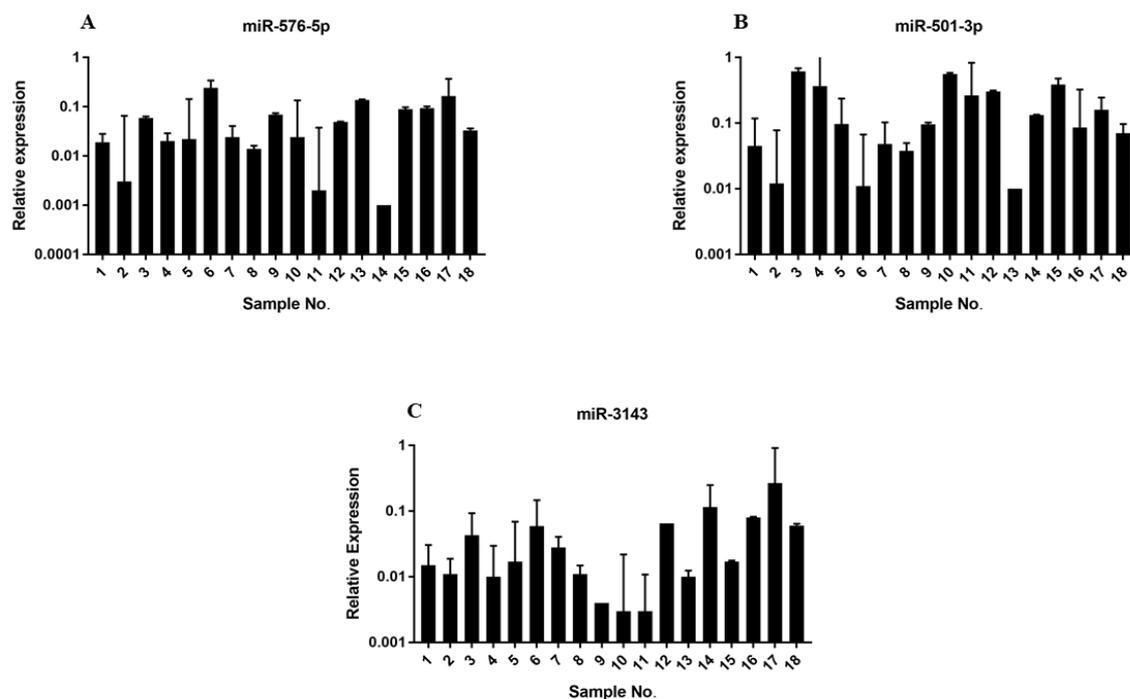


Figure 2. Down-regulation of miRNAs in tumor samples compared to normal samples; A: miR-576-5p, B: miR-501-3p, C: miR-3143.

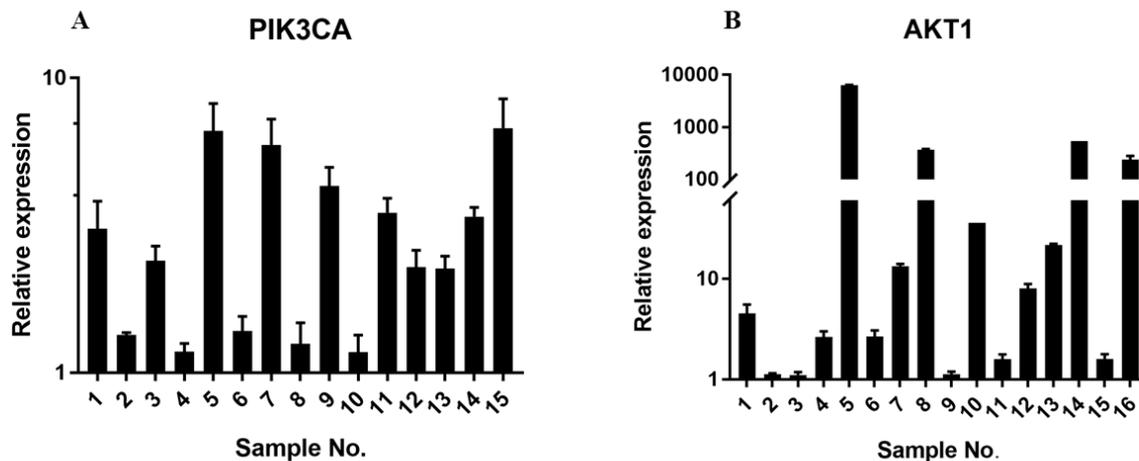


Figure 3. Relative expression of PIK3CA and AKT1 in TNBC clinical samples compared to normal adjacent tissues. A: Up-regulation of PIK3CA. B: Up-regulation of AKT1

was selected in this study due to its relation to the proliferation and survival of the patients. PI3K is one of the critical components of this pathway. PIK3CA gene that encodes catalytic α subunit of PI3K and also AKT1 gene, which is the downstream mediator of PI3K, were chosen as the target genes in the study [6, 12, 16, 38, 40, 41]. PIK3CA (catalytic subunit alpha of PI3K) is an oncogene located on chromosome 3q26.32 and is composed of 23 exons. AKT1 gene (AKT serine/threonine kinase 1) is an oncogene on chromosome 14q32.33 and is composed of 16 exons. Previous researches were inconsistent with our study and have shown up-regulation and the role of these two genes in cancers such as breast cancer. Cossu-Rocca *et al.* found that in 23.7% of TNBC cases, PIK3CA gene has been mutated. Therefore, PI3K pathway dysregulation and mutations in PIK3CA are commonly reported in TNBC [42]. The results of a study performed by Aleskandarany *et al.* showed that PIK3CA is an oncogenic biomarker, and its overexpression is significantly associated with the higher tumor grade, tumor invasion, axillary lymph node metastasis and vascular invasion in breast cancer patients. Therefore, it can consider a biomarker of breast cancer progression associated with poor prognosis. Also, in their study, 82.9 % of no special type case and 88.2% of medullary carcinoma showed overexpression of PIK3CA [43]. The results of the study performed by Ahmad *et al.* showed that AKT1 in breast cancer cell lines

is overexpressed regardless of their estrogen receptor status [44]. Sun *et al.* found that the activity of AKT1 gene in breast, prostate, and ovarian cancers was increased, particularly in advanced tumors, and it has an important role in tumor development and progression [45]. Zhang *et al.* showed that miR-409-3p directly targets and inhibits AKT1 expression that leads to suppression of proliferation and invasion in breast cancer [46]. In this study, bioinformatics software and programs as mentioned in material and method part were used to predict miRNAs targeting PIK3CA and AKT1 genes. Finally, miR-576-5p and miR-501-3p, with the highest scores, were predicted as miRNAs targeting PIK3CA and AKT1 mRNAs, respectively. Moreover, miR-3143 was also predicted targeting both genes. To the best of our knowledge, there wasn't any previous report about the role of these miRNAs in TNBC, except our previous in the parallel study that its results were in line with the current study and demonstrated down-regulation of these three miRNA in the MCF-7 cell line [47]. A study conducted on lung tumors by Mairinger *et al.* suggests that miR576-5p is significantly related to cell survival [48]. In the study of Dong *et al.*, the miRNAs expression pattern in the peripheral blood of glioblastoma patients and normal cases were obtained by microarray. The results showed that the expression of miR-576-5p increased on average 3.05 folds in glioblastoma patients compared to healthy individuals [49] while in

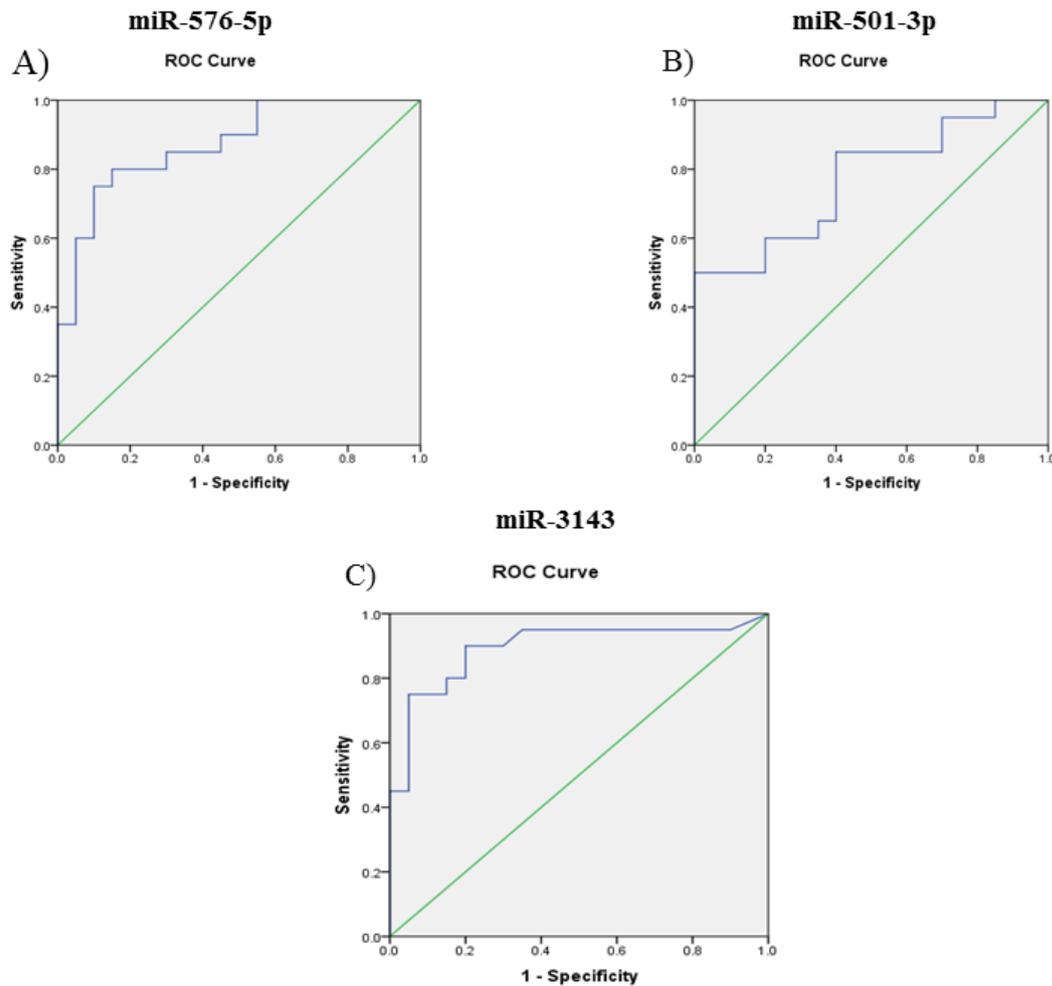


Figure 4. ROC curves of miRNAs including miR-576-5p (A) miR-501-3p (B) miR-3143 (C)

the study of Diaz-Prado *et al.* on patients with osteoarthritis, the expression of miR-576-5p in osteoarthritis chondrocyte pellets have been fallen 4.74 folds [50]. Soronen *et al.* have shown a significant increase in miR-576-5p expression in non-alcoholic fatty liver disease [51]. According to Haldrup *et al.* study, expression of miR-501-3p in serum and tumor samples of patients with prostate cancer has been increased [52]. In the case of miR-3143, the literature review also showed that there was only one article in PubMed referring to this miRNA. This study by Yilmaz *et al.* examined the expression of miR-3143 and some other miRNAs in the peripheral blood of Parkinson-suffering patients. By using real-time PCR method, they found that median expression value of miR-3143 was 0.4466 [53]. Our results showed that miR-576-5p, miR-501-3p, and miR-3143 were down-regulated in MDA-

MB-231 and TNBC clinical specimens. ROC curve analysis showed that the declined expression of these miRNAs has a predictive value, i.e. AUC value of miR-3143 was nearly 0.9, which indicates the excellent discrimination between TNBC and normal samples. In addition, based on our findings, PIK3CA and AKT1 genes were up-regulated in TNBC samples and cell lines.

Conclusion

The findings are in line with our hypotheses and indicate the possible negative correlation between the candidate miRNAs and their target genes. Given that PIK3CA and AKT1 genes promote proliferation and survival of tumor cells, their down-regulation by miRNAs results in a decline in the proliferation of cancerous cells. Therefore, miR-576-5p, miR-

501-3p, and miR-3143 can be considered as novel candidates for miRNAs-based therapies in TNBC. Among these miRNAs, miR-3143, which targets both PIK3CA and AKT1 mRNAs and has the best discriminating value, can be the first suggestion. However, to practically confirm the effect of predicted miRNAs on their target genes further validation methods such as luciferase assay are needed.

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Conflict of Interest

Not declared.

References

- Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. *Asian Pac J Cancer Prev*. 2016;17(S3):43-6.
- Cao Z-G, Li J-J, Yao L, Huang Y-N, Liu Y-R, Hu X et al. High expression of microRNA-454 is associated with poor prognosis in triple-negative breast cancer. *Oncotarget*. 2016;7(40):64900-909.
- Briggs KJ, Koivunen P, Cao S, Backus KM, Olenchok BA, Patel H et al. Paracrine Induction of HIF by Glutamate in Breast Cancer: EglN1 Senses Cysteine. *Cell*. 2016;166(1):126-39.
- Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. *Cell*. 2015;163(1):174-86.
- Mohammadi-Yeganeh S, Mansouri A, Paryan M. Targeting Of miR9/NOTCH1 Interaction Reduces Metastatic Behavior in Triple-negative Breast Cancer. *Chem Biol Drug Des*. 2015;86(5):1185-91.
- Zhang K, Zhang Y, Liu C, Xiong Y, Zhang J. MicroRNAs in the diagnosis and prognosis of breast cancer and their therapeutic potential (review). *Int J Oncol*. 2014;45(3):950-8.
- Kurozumi S, Yamaguchi Y, Kurosumi M, Ohira M, Matsumoto H, Horiguchi J. Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. *J Hum Genet*. 2017;62(1):15-24.
- Mulrane L, McGee SF, Gallagher WM, O'Connor DP. miRNA dysregulation in breast cancer. *Cancer Res*. 2013;73(22):6554-62.
- Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer*. 2015;15(6):321-33.
- Wang W, Luo YP. MicroRNAs in breast cancer: oncogene and tumor suppressors with clinical potential. *J Zhejiang Univ Sci B*. 2015;16(1):18-31.
- Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Kerin MJ. MicroRNAs as Novel Biomarkers for Breast Cancer. *J Oncol*. 2009;2009:950201, 7 pages.
- Misso G, Di Martino MT, De Rosa G, Farooqi AA, Lombardi A, Campani V et al. Mir-34: a new weapon against cancer? *Mol Ther Nucleic Acids*. 2014;3:e194.
- Singh R, Mo YY. Role of microRNAs in breast cancer. *Cancer Biol Ther*. 2013;14(3):201-12.
- Cidado J, Park BH. Targeting the PI3K/Akt/mTOR pathway for breast cancer therapy. *J Mammary Gland Biol Neoplasia*. 2012;17(3-4):205-16.
- Markman B, Dienstmann R, Taberero J. Targeting the PI3K/Akt/mTOR pathway--beyond rapalogs. *Oncotarget*. 2010;1(7):530-43.
- Paplomata E, O'Regan R. The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. *Ther Adv Med Oncol*. 2014;6(4):154-66.
- Huemer F, Bartsch R, Gnant M. The PI3K/AKT/MTOR Signaling Pathway: The Role of PI3K and AKT Inhibitors in Breast Cancer. *Curr Breast Cancer Rep*. 2014;6(2):59-70.
- Karami F, Mohammadi-Yeganeh S, Abedi N, Koochaki A, Kia V, Paryan M.

- Bioinformatics Prediction and In Vitro Analysis Revealed That miR-17 Targets Cyclin D1 mRNA in Triple Negative Breast Cancer Cells. *Chem Biol Drug Des.* 2016;87(3):317-20.
19. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature.* 2007;449(7163):682-8.
 20. Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol.* 2010;11(8):R90.
 21. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L, Congdon CB. Common features of microRNA target prediction tools. *Frontiers in genetics.* 2014;5:23.
 22. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife.* 2015;4:e05005.
 23. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res.* 2008;36(suppl 1):D154-D8.
 24. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Res.* 2013;41(W1):W169-W73.
 25. Betel D, Wilson M, Gabow A, Marks DS, Sander C. The microRNA.org resource: targets and expression. *Nucleic Acids Res.* 2008;36(suppl 1):D149-D53.
 26. Piriyaopongsa J, Bootchai C, Ngamphiw C, Tongshima S. microPIR: an integrated database of microRNA target sites within human promoter sequences. *PloS one.* 2012;7(3):e33888.
 27. Chou C-H, Chang N-W, Shrestha S, Hsu S-D, Lin Y-L, Lee W-H et al. miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database. *Nucleic Acids Res.* 2016;44(D1):D239-D47.
 28. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res.* 2014;gku1104.
 29. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods.* 2015;12(8):697.
 30. Hsu S-D, Chu C-H, Tsou A-P, Chen S-J, Chen H-C, Hsu PW-C et al. miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. *Nucleic Acids Res.* 2008;36(suppl 1):D165-D9.
 31. Cho S, Jang I, Jun Y, Yoon S, Ko M, Kwon Y et al. MiRGator v3.0: a microRNA portal for deep sequencing, expression profiling and mRNA targeting. *Nucleic Acids Res.* 2012;41(D1):D252-D7.
 32. Garcia DM, Baek D, Shin C, Bell GW, Grimson A, Bartel DP. Weak seed-pairing stability and high target-site abundance decrease the proficiency of Isy-6 and other microRNAs. *Nat Struct Mol Biol.* 2011;18(10):1139-46.
 33. Mohammadi-Yeganeh S, Paryan M, Samiee SM, Soleimani M, Arefian E, Azadmanesh K et al. Development of a robust, low cost stem-loop real-time quantification PCR technique for miRNA expression analysis. *Mol Biol Rep.* 2013;40(5):3665-74.
 34. Mohammadi Yeganeh S, Vasei M, Tavakoli R, Kia V, Paryan M. The effect of miR-340 over-expression on cell-cycle-related genes in triple-negative breast cancer cells. *Eur J Cancer Care (Engl).* 2016; 6: e12496.
 35. Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD et al. Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. *Cell.* 2014;156(5):893-906.
 36. Cimino D, De Pittà C, Orso F, Zampini M, Casara S, Penna E et al. miR148b is a major coordinator of breast cancer progression in a relapse-associated microRNA signature by targeting ITGA5, ROCK1, PIK3CA, NRAS, and CSF1. *The FASEB Journal.* 2013;27(3):1223-35.
 37. D'Ippolito E, Iorio MV. MicroRNAs and triple negative breast cancer. *Int J Mol Sci.* 2013;14(11):22202-20.
 38. Massihnia D, Galvano A, Fanale D, Perez A, Castiglia M, Incorvaia L et al. Triple negative breast cancer: shedding light onto the role of pi3k/akt/mtor pathway. *Oncotarget.* 2016; 7(37): 60712–60722.
 39. Adams JR, Schachter NF, Liu JC, Zacksenhaus E, Egan SE. Elevated PI3K signaling drives multiple breast cancer subtypes. *Oncotarget.* 2011;2:435-47.
 40. de Lint K, Poell JB, Soueidan H, Jastrzebski K, Rodriguez JV, Lieftink C et al. Sensitizing Triple-Negative Breast Cancer to PI3K Inhibition by Cotargeting IGF1R. *Mol Cancer Ther.* 2016;15(7):1545-56.
 41. Rudolph M, Anzeneder T, Schulz A, Beckmann G, Byrne AT, Jeffers M et al.

- AKT1 E17K mutation profiling in breast cancer: prevalence, concurrent oncogenic alterations, and blood-based detection. *BMC cancer*. 2016;16(1):622.
42. Cossu-Rocca P, Orrù S, Muroli MR, Sanges F, Sotgiu G, Ena S et al. Analysis of PIK3CA mutations and activation pathways in triple negative breast cancer. *PLoS One*. 2015;10(11):e0141763.
 43. Aleskandarany MA, Rakha EA, Ahmed MA, Powe DG, Paish EC, Macmillan RD et al. PIK3CA expression in invasive breast cancer: a biomarker of poor prognosis. *Breast Cancer Res Treat*. 2010;122(1):45-53.
 44. Ahmad S, Singh N, Glazer RI. Role of AKT1 in 17 β -estradiol-and insulin-like growth factor I (IGF-I)-dependent proliferation and prevention of apoptosis in MCF-7 breast carcinoma cells. *Biochem Pharmacol*. 1999;58(3):425-30.
 45. Sun M, Wang G, Paciga JE, Feldman RI, Yuan Z-Q, Ma X-L et al. AKT1/PKB α kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. *Am J Pathol*. 2001;159(2):431-7.
 46. Zhang G, Liu Z, Xu H, Yang Q. miR-409-3p suppresses breast cancer cell growth and invasion by targeting Akt1. *Biochem Biophys Res Commun*. 2016;469(2):189-95.
 47. Razaviyan J, Hadavi R, Mohammadi-Yeganeh S. Expression Analysis of miRNAs Targeting PIK3CA and AKT1 Genes of PI3K Signaling Pathway in Breast Cancer Cells. *GMJ*. 2017;6(4):338-45.
 48. Mairinger FD, Ting S, Werner R, Walter RFH, Hager T, Vollbrecht C et al. Different micro-RNA expression profiles distinguish subtypes of neuroendocrine tumors of the lung: results of a profiling study. *Mod Pathol*. 2014;27(12):1632-40.
 49. Dong L, Li Y, Han C, Wang X, She L, Zhang H. miRNA microarray reveals specific expression in the peripheral blood of glioblastoma patients. *Int J Oncol*. 2014;45(2):746-56.
 50. Díaz-Prado S, Cicione C, Muiños-López E, Hermida-Gómez T, Oreiro N, Fernández-López C et al. Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculoskelet Disord*. 2012;13(1):144.
 51. Soronen J, Yki-Järvinen H, Zhou Y, Sädevirta S, Sarin AP, Leivonen M et al. Novel hepatic microRNAs upregulated in human nonalcoholic fatty liver disease. *Physiol Rep*. 2016;4(1):e12661.
 52. Haldrup C, Kosaka N, Ochiya T, Borre M, Høyer S, Orntoft TF et al. Profiling of circulating microRNAs for prostate cancer biomarker discovery. *Drug Deliv Transl Res*. 2014;4(1):19-30.
 53. Yılmaz ŞG, Geyik S, Neyal AM, Soko ND, Bozkurt H, Dandara C. Hypothesis: Do miRNAs Targeting the Leucine-Rich Repeat Kinase 2 Gene (LRRK2) Influence Parkinson's Disease Susceptibility? *Omic*. 2016;20(4):224-8.