Up-Regulation of *Mir-105-5p* Promotes The Development of Breast Cancer By Targeting *AKT1* / *GRB2* Genes In Patients With Breast Cancer

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Abstract

Objective: *miR-105-5p* either acts as an oncomiR or tumor suppressor that has been shown to have various expression levels in a wide range of diseases and targets RAC-alpha serine/threonine-protein kinase (*AKT1*) and Growth Factor Receptor-bound protein 2 (*GRB2*) genes. *GRB2* is a signaling protein that takes part in various signaling pathways. Due to the fact that miRNAs by acting on target genes, and regulates the expression of them, so the aim of this study is to evaluate the expressions of *miR-105-5p*, *AKT1*, and *GRB2* genes and the correlation between them in breast cancer (BC) tissue compared to normal-adjacent tissues (NATs) in women with breast cancer.

Materials and methods: We utilized bioinformatics analysis to searching for target genes of *miR-105-5p*. Then, 35 BC and NATs tissues were taken. In this experimental study, quantitative Real-Time PCR was performed to evaluate *miR-105-5p*, *AKT1* and *GRB2* genes expression, and the correlation between them was evaluated. The relation between expression and features of clinicopathological was explored.

Results: The *miR-105-5p* expression was increased significantly in BC tissues in comparison to NATs (P 0.05). The expression levels of *AKT1* and *GRB2* genes were decreased significantly in BC tissue in comparison to NATs (P 0.05). An inverse correlation was observed between *miR-105-5p*, *AKT1* and *GRB2* expression which was not significant (P>0.05). Also, a direct correlation was observed between *GRB2* and *AKT1* gene expression (P 0.05).

Conclusion: Our findings revealed that increased expression levels of *miR-105-5p* in patients with breast cancer caused a decrease in the expression level of its target genes, *AKT1* and *GRB2*, and subsequently lead to tumor progression and invasion of the tumor. *miR-105-5p* may serve as a promising target for BC therapy.

Introduction

Breast cancer (BC) is widely assumed to be the main cause of cancer death in women around the world. Every year, 2,088,849 new cases are diagnosed with BC with over 626,679 deaths estimated worldwide [1]. It is a multifactorial disorder associated with the effects of multiple genes in combination with environmental factors [2,3,4]. BC is classified into two forms of sporadic BC, which approximately attributes to 95 percent of BCs, and familiar BC which accounts for the rest of the BCs and it is caused by mutations in genes like Breast cancer susceptibility gene 1 (*BRCA1*) and Breast cancer susceptibility gene 2 (*BRCA2*) [5]. There are five major molecular subtypes of BC. They are grouped based on the genes expressed: Luminal A BC is hormone-receptor positive (progesterone-receptor positive and/or estrogen-receptor), HER2 negative, and the protein Ki-67 is in low levels. **Luminal B** BC is hormone-receptor positive (progesterone-receptor positive and/or estrogen-receptor), and either HER2 positive or HER2 negative with high levels of Ki-67. **Triple-negative/basal-like** BC is HER2 negative and hormone-receptor negative (progesterone-receptor and estrogen-receptor negative). **HER2-enriched** BC is HER2 positive and hormone-receptor negative (progesterone-receptor and estrogen-receptor negative). **Normal-like** BC is similar to

luminal A disease: HER2 negative and hormone-receptor positive (progesterone-receptor and/or estrogen-receptor positive), and the protein Ki-67 is in low levels [6].

In the past decade, with the usage of genome-wide studies on DNA sequence, gene expression, and so forth, many aberrant genes and miRNAs have been identified which might contribute to breast tumor [7, 8]. *miR-105-5P* targets many genes in various cancers and can act either as tumor suppressor or oncomiR [9]. Some studies showed that miR-1 to be associated with several diseases such as cancer (BC, human gliomas, colorectal cancer, gastric cancer, prostate cancer and hepatocellular carcinoma) and heart diseases [9]. miR-105 functions as an oncogene in several of these cancers such as colorectal cancer and BC, and high expression of this miR causes metastasis and invasion. But in cancers such as prostate cancer, hepatocellular carcinoma, human gliomas, and lung cancer, miR-105 functions as a tumor suppressor. miR-105 can alter the expression levels of its target gene, so it has an impact on cancer progression [9]. *miR-105-5P* is secreted by cancer cells and in BC, it works as oncomiR via targeting ZO1 protein, which acts as a tight junction adaptor protein, and therefore, it contributes to metastasis. *miR-105-5P* targets when this micro RNA is suppressed in BCs it can lead to a decrease in the metastasis rate [10, 11].

Other genes such as *AKT1* which are involved in different cell signaling pathways and any dysregulation of their expression may lead to creation of cancer cells [12].

AKT1 is serine-threonine protein kinase which regulates numerous processes such as proliferation, cell survival, growth, and so on [13, 14]. *AKT1* is a mediator of various cellular pathways including *PI3K / PTEN / AKT / mTORC1*, therefore, it has distinct roles in the development of BC [15, 16]. It has been assumed that overexpression and activation of *AKT1* might lead to BC progression by contributing to being resistant to anti-proliferative signals [17, 18].

Growth Factor Receptor-bound protein 2 (*GRB2*) gene is an adapter protein that plays an important link between cell surface growth factor receptors and the Ras signaling pathway and it may have a vital role in cell survival, proliferation, differentiation and angiogenesis [19]. It has been reported that *GRB2* is involved in development and progression of various malignancies such as BC, lung cancer, gastric cancer, colorectal cancer and so forth [20,21,22]. The aim of the current study is to evaluate the expression of miR-105-5p, *AKT1*, and *GRB2* genes and the correlation between them in BC tissues from Iranian patients with BC and adjacent normal tissue (NATs) from same patients.

Material And Methods

Subjects

In this experimental study, fresh breast cancer (BC) tissues and paired normal adjacent tissues (NATs) (>5 cm from tumor) from 35 women with BC were collected from Imam Khomeini Hospital, Tehran, Iran. Tissues were carried to the laboratory via liquid nitrogen tank. The BC patients had the average age of 50 years when diagnosed (range 30-55 years). Patients did not undergo any radiation therapy or

chemotherapy. The diagnosis, histological grade and the clinical stage of each case were independently confirmed by a pathologist. Patients had no other diseases and the ones that did, were excluded from the study. The study was approved by the Ethics Committee and all of the contributors provided written informed consent.

RNA isolation and cDNA synthesis

Total RNA was extracted from the fresh BC tissues using XS spin kit (Macherey-Nagel, Germany, LOT No: 1301/005), based on the manufacturer's instruction. After applying DNase-I for removing DNA contamination, complementary DNA strand (cDNA) was synthesized using the PrimeScript™ RT Reagent Kit (Takara, Japan, Cat No: RR037A) according to the manufacturer's protocol. For evaluation of *miR-105-5p* expression, stem loop cDNA was synthesized. The RNA samples were evaluated on Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) for both quantity and also quality

Target genes prediction of miR-105-5p

miRNA targets were predicted and determined using the algorithms of TargetScan 7.1 [23], miRBase [24], miRanda [25], miRDIP 4.1 [26], miRWALK 3.0 [27] and miRDB 5.0 [28].

Real-time quantitative PCR (qPCR)

Quantitative Real-time PCR was carried out using ABI 7900HT instrument (ABI Inc.), WizPure™ qPCR Master (SYBR) (Korea, Cat No: w1711). U6 snRNA was used for miR-105-5p normalization. Specific primers were brought as follow: U6 forward: 5'-GCTTCGGCAGCACATATAC-3', and U6 reverse: 5'-ATTCCGTTTCTGGGAGGG-3', GRB2 forward: 5'-ATTCCTGCGGGACATAGAACA-3', GRB2 reverse: 5'-AGTTCCAACCAAAGTGAGAGGG-3', AKT1 forward: 5'-TCTATGGCGCTGAGATTGTG-3', AKT1 reverse: 5'-CTTAATGTGCCCGTCCTTGT-3', miR-105-5p forward: 5'-TCAAATGCTCAGACTCCTGTGGTGTCTCGT-3' and miR-105-5p reverse: 5'-CCAGTGCAGGGTCCGAGGTA-3. The qPCR reaction for AKT1 and GRB2 genes and miR-105-5p were performed with the following reaction: 20 µL volume reaction containing 1 µl of template cDNA (12.5 ng/µL), 0.5 µl of each 10 pmol/ul primer (Sinaclone, Iran), 10 µl of SYBR Green PCR master mix and 8 µl dH₂O and followed by initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 secs, annealing at 60°C for 30 secs. GAPDH gene was used for AKT1 and GRB2 genes for normalization. GAPDH forward (F): 5'-ATTTGGTCGTATTGGGCG-3' and GAPDH reverse (R): 5'-GTAATCAGCGCCAGCATC-3'. The relative gene expression level was assessed by using $2^{-\Delta\Delta Ct}$ quantitative method (29), each sample was analyzed in duplicate.

Statistical analysis

All experimental information was expressed as mean $\pm$ SEM. Statistical comparisons between the BC tissues and NATs were carried out using paired t-test and one-way analysis of variance was carried out to compare the differences among more than two groups followed by Tukey's test. Differences were considered statistically significant at $P \leq 0.05$. The correlation between *AKT1*, and *GRB2* genes and *miR-*

105-5p in BC patients was assessed by Pearson's correlation. In this study, GraphPad Prism was used to carry out all statistical analyses (GraphPad Software, La Jolla California USA).

Results

The clinicopathological characteristics of patients are presented in Table 1.

Table 1. Clinicopathologic characteristics of patients with BC

Characteristics	Number of patients	Number analyzed (%)
<i>Age at disease onset (year)</i>		
	19	53.9%
	16	46.1%
<i>Clinical Stage</i>		
stage I	2	5.6%
stage II	24	67.2%
stage III	9	27.2%
<i>Histological grade</i>		
Grade- I	5	14%
	24	67.2%
Grade- II	6	16.8%
Grade- III		

miR-105-5p is upregulated in Breast cancer

Quantitative real-time PCR was used to evaluate the expression levels of *miR-105-5p* in breast cancer (BC) and paired normal adjacent tissues (NATs). We observed that there was a significant up-regulated in BC samples relative to that in NATs as the control group (Fig. 1) (p=0.0194). This finding indicates that *miR-105-5p* may be a regulator of molecular mechanisms in BC progression. The relationship between

miR-105-5p expression and clinicopathological characteristics of BC patients including histological grade, clinical stages and patients' age were examined. An increase in the expression level of *miR-105-5p* was observed in stage I and grade I compared with other grade (II – III) and stage (II-III). BC patients were divided according to age into the younger and older groups (women aged ≤ 50 and >50 years, respectively). In older women (aged >50 years) *miR-105-5p* expression was increased compared with younger women (aged ≤ 50 years) but no difference was found in the rate of *miR-105-5p* mRNA expression in according to age. In our study, statistical analysis showed that the *miR-105-5p* expression was not significantly related to clinicopathological features including histological grade, clinical stages and patients' age ($p > 0.05$) (Table 2).

Table 2: Correlation between *miR-105-5p* expression and Clinicopathological Characteristics in Breast Cancer patients

Characteristics	SE of difference	P value
Age at disease onset (year)		
<50 vs ≥ 50	0.2952	0.5777
Histological grade		
Grade I vs. Grade II	0.4478	0.8908
Grade I vs. Grade III	0.5515	0.5352
Grade II vs. Grade III	0.4157	0.6231
Breast cancer staging		
Stage I vs. Stage II	0.7149	0.0004
Stage I vs. Stage III	0.7593	<0.0001
Stage II vs. Stage III	0.3796	0.2733

Prediction of target genes of *miR-105-5p*

Various target prediction programs (TargetScan, miRBase, miRanda, mirDIP, miRWALK, and miRDB) were used to explore the potential mechanism and analyze the AKT1 and GRB2 target genes of *miR-105-5p* (ACCESSION: MIMAT0000102). Based on the predicted genes, score class of *AKT1* gene is top up 5% as high-throughput *miR-105-5p* targeting and Score class of *GRB2* gene is top up 1% as very high-throughput *miR-105-5p* targeting.

***AKT1* is downregulated in Breast cancer**

We first investigated the expression of *AKT1* in BC tissues and NATs by quantitative RT-PCR. We observed the expression levels of *AKT1* was decreased in BC tissues compared to that in NATs significantly (Fig. 2) ($p= 0.0141$). Next, we compared the expression levels of *AKT1* with the clinicopathological features including histological grade, clinical stages and patients' age. The lowest expression of *AKT1* was observed in Stage III and grade I. But, the *AKT1* gene expression was not significantly related to clinicopathological features including histological grade, and clinical stages.

To better understand the relationship between *AKT1* expression and patients' age, we considered the expression of this gene in younger and older groups (≤ 50 year and > 50 year). No significant difference was observed between aged ≤ 50 year and aged > 50 -year groups ($P=0.680$). However, there was a trend toward decreasing *AKT1* expression in aged > 50 -year groups (Table 3).

Table 3: Correlation between AKT1 expression and Clinicopathological Characteristics in Breast Cancer Patients

Characteristics	SE of difference	P value
Age at disease onset (year)		
<50 vs ≥ 50	0.6882	0.6808
Histological grade		
Grade I vs. Grade II	0.6956	0.5266
Grade I vs. Grade III	0.8569	0.9801
Grade II vs. Grade III	0.6459	0.6311
Breast cancer staging		
Stage I vs. Stage II	1.621	0.3079
Stage I vs. Stage III	1.722	0.1682
Stage II vs. Stage III	0.8608	0.6434

AKT1* is a target of *miR-105-5p

Bioinformatics prediction revealed potential binding sites of *miR-105-5p* on the 3'-untranslated region (3'-UTR) of *AKT1* (Fig. 3a). Spearman's rank order correlation was used to determine the relation between

miR-105-5p expression and its target gene. We observed a negative correlation between *miR-105-5p* and *AKT1* in BC tissues (slope=-0.008862, P=0.9602) but not significantly (Fig. 3b). In the other hand, the expression of *miR-105-5p* was up-regulated, whereas *AKT1* was downregulated in the tumor tissues. These findings collectively indicate that *AKT1* is a target of *miR-105-5p* in BC.

GRB2 is downregulated in Breast cancer

The expression level *GRB2* was quantified by quantitative real-time PCR. We observed that there was a significant decrease in *GRB2* expression in BC tissue samples in comparison to NATs ($p= 0.0473$) (Fig .4). We next explored the relationship between *GRB2* and clinicopathological characteristics of BC patients. The lowest expression of *GRB2* was observed in Stage III and grade I. But the significant difference was not found when the gene expression was compared between different histological grades (I to III) and different clinical stages (I to III). Also, the expression of *GRB2* in patients aged > 50-year groups was lower than aged< 50 year, but no correlation was observed between *GRB2* expression and age (< 50 year and > 50-year groups) (P= 0.585) (Table 4).

Table 4: Correlation between GRB2 expression and Clinicopathological Characteristics in Breast Cancer Patients

Characteristics	SE of difference	P value
Age at disease onset (year)		
<50 vs ≥50	4.104	0.5853
Histological grade		
Grade I vs. Grade II	7.184	0.9198
Grade I vs. Grade III	8.849	0.3992
Grade II vs. Grade III	6.670	0.3946
Breast cancer staging		
Stage I vs. Stage II	10.11	0.5194
Stage I vs. Stage III	10.74	0.2622
Stage II vs. Stage III	5.368	0.5123

GRB2 is a target of miR-105-5p

Bioinformatics prediction indicated that *GRB2* is the potential target gene of *miR-105-5p*. The 3'-UTR of *GRB2* mRNA has a specific sequence which is complementary to the seed region of *miR-105-5p* (Fig. 5a)

We analyzed 35 BC tissues and NATs to explore the relationship between *miR-105-5p* and the target *GRB2* gene. The Spearman's correlation demonstrated that *miR-105-5p* expression had negative correlation with the *GRB2* expression (slope = -0.02614, $p=0.4563$) but not significantly shown in (Fig. 5b). Showed *miR-105-5p* was up-regulated in BC tissues while *GRB2* was down-regulated in BC tissues.

Moreover, we explored the relationship between two target genes, *AKT1* gene and *GRB2* gene. We observed a significant positive correlation between *AKT1* gene and *GRB2* gene (slope = 3.582, $p<0.0001$) in BC tissues. This finding indicates that *AKT1* gene and *GRB2* gene can be a regulator of signaling pathway in BC progression.

Discussion

Our study indicated that *miR-105-5p* expression was up-regulated in BC tissues in comparison to NATs. that it was statistically significant. In some studies, it was observed that *miR-105-5p* acts as tumor suppressor. Honeywell et al. and Liu et al. found out that *miR-105-5p* inhibits prostate tumor growth and human glioma cell progression respectively and its reduced expression can lead to these types of cancers [10, 30]. The same result was found with non-small cell lung cancer [31]. It was also revealed that in some studies, *miR-105-5p* acts as onco-miR. *miR-105-5p* expression level was increased in gastric cancer cells and colorectal cancer cells [9, 32]. Our results indicated *miR-105-5p* acts as onco-miR in BC tissue because the expression level of *miR-105-5p* is increased in BC tissue compared with NATs, and it is revealed that *miR-105-5p* may be relevant with cell proliferation. miRNAs have been known to have a role in regulating most biological processes and therefore, they can be used in order to gain an insight into the complex processes, including cell proliferation in cancer. Our bioinformatics studies showed that *miR-105-5p* targets *AKT1* pathway and *GRB2* gene. *AKT* signaling pathway has been a major signaling node within the cells [33]. *AKT1* pathway dysregulation could possibly lead to disruption of the balance between survival and death and it can also have a major impact on cancer development and therapy. Our results demonstrated that *AKT1* expression level significantly decreased in BC tissue compared with NATs even the expression of this gene is more in the advanced stages of BC tissues. Our results agreement with previous studies. Heng et al. found out that *AKT1* expression level was significantly lowered in breast tumor in comparison to matched adjacent normal tissues [34]. We also observed *miR-105-5p* had negative correlation with expression of *AKT1* in BC tissues. This result can be justified by Shen et al. 's finding in which they demonstrated that *miR-105-5p* can inhibit PI3K/*AKT* pathway in hepatocellular cancer [12].

GRB2 is an essential signaling mediator in various oncogenic signaling pathways and it has role-playing in several human malignancies [35]. The *GRB2* has a wide association with a number of irregularities. So, it is an important target for the design of remedial anticancer strategies [36]. Lin-Yan et al. found that

overexpression of *GRB2* had correlation lymph node metastasis [37]. Yu et al. demonstrated that *GRB2* is over expressed in gastric cancer [22]. Watanabe et al. found that *GRB2* expression was significantly increased in human bladder cancer cell lines [38]. Lim et al. found out that *GRB2* downregulation led to AKT inactivation and consequently it caused BC [38]. Our results revealed that *GRB2* expression level significantly decreased in BC tissue compared with NATs. Its expression level lowered more in the advanced stages of BC. We demonstrated that *miR-105-5p* negatively modulated *GRB2* by combining with the 3'-UTR of *GRB2* gene.

Furthermore, another important aspect of this study showed that strong positively correlated between the expression levels of *AKT1* and *GRB2* genes. So, that downregulation of *GRB2* genes suppressed the activation of *AKT1* pathway. Lim et al. found that downregulation of *GRB2* can lead to *AKT1* inactivation and subsequently can cause lowered expression of *AKT1* [39]. These findings support the pivotal role of AKT pathway in modulating the BC development. Based on our results, we speculate that *miR-105-5p* may contribute to the development of BC via downregulation of *AKT1* and *GRB2* genes.

miR-105 affects the genes which are involved in intracellular signaling pathways and this leads to the downregulation of *GRB2* and *GRB2* downregulates *AKT1* through p13k signaling pathway. Consequently, these alterations lead to more invasion and metastasis in BC.

Conclusion

Our findings demonstrated *miR-105-5p* was up-regulated in human BC. *miR-105-5p* promotes cell proliferation in BC by targeting *AKT1* and *GRB2* genes. Up-regulation of *miR-105-5p* could promote BC growth by decreasing the activity of AKT1 signaling pathways and GRB2.

Our Insilco analyses showed that *AKT1* and *GRB2* are the direct targets of *miR-105-5p* and basically micro RNAs effect on the cell through regulating their target genes. Increased expression of *miR-105-5p* downregulate the expression of *GRB2* and *AKT1* and therefore these changes cause tumor progression and tumor invasion. These two genes are important genes in intracellular signaling pathways and reduced expression of them can dysregulate the cell cycle and cause tumor formation. Therefore, identifying a specific biomarker, and gaining an understanding into the underlying mechanism might provide feasible therapeutic approaches to overcome cancer. By targeting *miR-105-5p*, it might be considered as a possible therapeutic strategy for BC treatment. However, the detailed mechanism of *miR-105-5p* in BC progression needs further investigations.

Declarations

Conflict interest

The authors declare no conflict of interests in this study.

Authors' contributions

F.F. Participated in study design. F.R. and F.A. collected the samples. F.F, F.R. and F.A. participated in data collection and evaluation, drafting. F.F and F.R. participated in RT-qPCR analysis and statistical analysis. F.R. prepared lab working, and doing molecular experiments. F.F. and F.R. Contributed extensively in the interpretation of the data and the conclusion. All authors performed editing and approving the final version of this paper for submission, also participated in the finalization of the manuscript, and approved the final draft.

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Ethical issues

Approval for the study was obtained from the Ethics Committee of Islamic Azad Tehran Medical Sciences University-Pharmacy and Pharmaceutical Branches Faculty. Ethical code is IR.IAU.PS.REC.1398.069.

References

- 1- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jmal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries . **CA. 2018;68(6):394–424.**
- 2- Pharoah PD, Dunning AM, Ponder BA, Ponder BA, Easton DF. Association Studies for Finding Cancer-Susceptibility Genetic Variants. *NAT.* 2004;4(11):850–860.
- 3- Blackburn GL, Wang KA. Dietary Fat Reduction and Breast Cancer Outcome: Results from the Women's Intervention Nutrition Study (WINS). *Am J CLIN NUTR.* 2007;86(3):878-881.
- 4- Kaiser J. Cholesterol Forges Link between Obesity and Breast Cancer. *AAAS.* 2013; 342(6162):1028
- 5- Garber JE, Offit K. Hereditary Cancer Predisposition Syndromes. *AM. J Clin. Oncol.* 2005;23(2):276–292.
- 6- Al-thoubaity FKH. Molecular classification of breast cancer: A retrospective cohort study. *AMS.* 2019;49:44-48
- 7- Gray J, Druker B. The Breast Cancer Landscape. *Nature.* 2012; 486(7403): 328–329.
- 8- Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The Landscape of Cancer Genes and Mutational Processes in Breast Cancer. *Nature.* 2012;486(7403):400–404.
- 9- Li J, Zhang Z, Chen F, Hu T, Peng W, Gu Q, et al. The Diverse Oncogenic and Tumor Suppressor Roles of MicroRNA-105 in Cancer. *FRONT ONCOL.* 2019.

- 10- Honeywell DR , Cabrita MA, Zhao H, Dimitroulakos J, Addison CL. MiR-105 Inhibits Prostate Tumour Growth by Suppressing CDK6 Levels. *PloS One*. 2013;8(8):70515.
- 11- Zhou W, Fong MY, Min Y, Somlo G, Liu L, Palomares MR, et al. Cancer-Secreted MiR-105 Destroys Vascular Endothelial Barriers to Promote Metastasis. *Cancer Cell*. 2014;25(4):501–515.
- 12- Shen G, Rong X, Zhao J, Yang X, Li H, Jiang H, et al. MicroRNA-105 Suppresses Cell Proliferation and Inhibits PI3K/AKT Signaling in Human Hepatocellular Carcinoma. *Carcinogenesis*. 2014;35(12):2748–2755.
- 13- Manning BD, Cantley LC. AKT/PKB Signaling: Navigating Downstream. *Cell*. 2007;129(7):1261–1274.
- 14- Yoeli-Lerner M, Toker A. Akt/PKB Signaling in Cancer: A Function in Cell Motility and Invasion. *Cell Cycle*. 2006;5(6):603–605.
- 15- Davis NM , Sokolosky M, Stadelman K, Abrams SL, Libra M, Candido S, et al. Deregulation of the EGFR/PI3K/PTEN/Akt/MTORC1 Pathway in Breast Cancer. Possibilities for Therapeutic Intervention. *Oncotarget*. 2014;5(13):4603–4650.
- 16- 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;6:59–70.
- 17- Chin RY, Toker A. The Actin-Bundling Protein Palladin Is an Akt1-Specific Substrate That Regulates Breast Cancer Cell Migration. *Molecular Cell*. 2010;38(3):333–344.
- 18- Liang J, Zubovitz J, Petrocelli T, Kotchetkov R, Connor MK, Han K, et al. PKB/Akt Phosphorylates P27 Impairs Nuclear Import of P27 and Opposes p-27 Mediates G1 Arrest. *Nature Medicine*. 2002;8:1153–1160.
- 19- Ward AC, Smith L, De Koning JP, van Aesch Y, Touw IPT. Multiple Signals Mediate Proliferation, Differentiation, and Survival from the Granulocyte Colony-Stimulating Factor Receptor in Myeloid 32D Cells. *J B C*. 1999;274(21):14956–14962.
- 20- Pendergast AM, Quilliam LA, Cripe LD, Bassing CH, Dai Z, Li N, et al. BCR-ABL-Induced Oncogenesis Is Mediated by Direct Interaction with the SH2 Domain of the GRB-2 Adaptor Protein. *Cell*. 1993;75(1):175–185.
- 21- Timsah Z, Berrou J, Suraokar M, Behrens C, Song J, Lee JJ, et al. Expression Pattern of FGFR2, Grb2 and Plcy1 Acts as a Novel Prognostic Marker of Recurrence-Free Survival in Lung Adenocarcinoma. *Am. J Cancer Res*. 2015;5(10):3135.
- 22- Yu GZ, Chen Y, Wang JJ. Overexpression of Grb2/HER2 Signaling in Chinese Gastric Cancer: Their Relationship with Clinicopathological Parameters and Prognostic Significance. *J. Cancer Res. Clin. Onco*.

2009;135:1331–1339.

23- Riffo-Campos AL, Riquelme I, Brebi-Mieville P. Tools for Sequence-Based MiRNA Target Prediction: What to Choose? *I J M S*. 2016;17(12):1987.

24- Kozomara A, Birgaoanu M, Griffiths-Jones S. MiRBase: From MicroRNA Sequences to Function. *Nucleic Acids Res*. 2019;47(D1):D155–D162.

25- Zhao B, Xue B. Significant Improvement of MiRNA Target Prediction Accuracy in Large Datasets Using Meta-Strategy Based on Comprehensive Voting and Artificial Neural Networks. *BMC Genomics*. 2019;20(1):158.

26- Tokar T, Pastrello Ch, Rossos AEM, Abovsky M, Hauschild AC, Tsay M, et al. MirDIP 4.1—Integrative Database of Human MicroRNA Target Predictions. *Nucleic Acids Res*. 2018;46(D1):360–370.

27- Sticht C, Torre CDL, Parveen A, Gretz N. MiRWalk: An Online Resource for Prediction of MicroRNA Binding Sites. *PloS One*. 2018;13(10):0206239.

28- Chen Y, Wang X. MiRDB: An Online Database for Prediction of Functional MicroRNA Targets. *Nucleic Acids Res*. 2020;48(D1):127–131. _

29- Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method. *Methods*. 2001;25(4):402–408.

30- Liu X, Wang H, Zhu Z, Ye Y, Mao H, Zhang S. Micro RNA-105 Targets SOX 9 and Inhibits Human Glioma Cell Progression. *FEBS Letters*. 2016;590(23):4329–4342.

31- Lu G, Fu D, Jia C, Chai L, Han Y, Liu J, et al. Reduced MiR-105-1 Levels Are Associated with Poor Survival of Patients with Non-small Cell Lung Cancer. *Onco. Lett*. 2017;14(6):7842–7848.

32- Liu D, Hu X, Zhou H, Shi G, Wu J. Identification of Aberrantly Expressed MiRNAs in Gastric Cancer. *GASTROENT RES PRACT*. 2014. 2014;2014:1–9.

33- Liu P, Cheng H, Roberts T M, Zhao JJ. Targeting the Phosphoinositide 3-Kinase Pathway in Cancer. *Nat. Rev. Drug Discov*. 2009;8:627–644.

34. Heng J, Guo X, Wu W, Wang Y, Li G, Chen M, et al. Integrated Analysis of Promoter Mutation, Methylation and Expression of AKT1 Gene in Chinese Breast Cancer Patients. *PloS One*. 2017;12(3):1–14.

35- Giubellino A, Burke TR, Bottaro DP. Grb2 Signaling in Cell Motility and Cancer. *Expert Opin. Ther*. 2008;12(8):1021–1033.

36- Ijaz M, Shahabz M, Jiang W, Fathy AH, Nesa EU, Wang D, et al. Oncogenic Role of Grb2 in Breast Cancer and Grb2 Antagonists as Therapeutic Drugs. *Canc Therapy & Oncol Int J*. 2017;3(4):1–7.

37- Li LY, Li EM, Wu ZY, Cao HH, Shen JH, Xu XE, et al. Overexpression of GRB2 Is Correlated with Lymph Node Metastasis and Poor Prognosis in Esophageal Squamous Cell Carcinoma. *Int. J. Clin. Exp. Pathol.* 2014;7(6):3132–3140.

38- Watanabe T, Shinohara N, Moriya K, Sazawa A, Kobayashi Y, Ogiso Y, et al. Significance of the Grb2 and Son of Sevenless (Sos) Proteins in Human Bladder Cancer Cell Lines. *IUBMB Life.* 2000;49(4):317–320.

39- Lim SJ, Lopez-Berestein G, Hung MC, Lupu R, Tari AM. “Grb2 Downregulation Leads to Akt Inactivation in Heregulin-Stimulated and ErbB2-Overexpressing Breast Cancer Cells. *Oncogene.* 2000;19(54):6271–6276.

Figures

Figure 1

miR-105-5p is up-regulated in BC tissues and NATs. Relative *miR-105-5p* expression levels in BC tissues and NATs were determined by qRT-PCR. The expression of *miR-105-5p* is up-regulated in BC tissues compared with NATs (* $p < 0.05$). *U6snRNA* was used as an internal control. Abbreviation: BC: breast cancer, NATs: paired normal adjacent tissues.

Figure 2

AKT1 mRNA expression is downregulated in BC tissues and NATs. Relative *AKT1* expression levels in BC tissues and NATs were determined by qRT-PCR. The expression of *AKT1* is downregulated in BC tissues compared with NATs (* $p < 0.05$). *GAPDH* was used as an internal control. Abbreviation: BC: breast cancer, NATs: paired normal adjacent tissues.

Figure 3

AKT1 is a direct target of miR-105-5p in breast cancer. a) *miR-105-5p* and its putative binding sequence sites in the *AKT1* 3'-UTR. b) *miR-105-5p* expression has negative correlation with *AKT1* expression in BC tissues, (slope=-0.02466, P=0.7569).

Figure 4

GRB2 mRNA expression is downregulated in BC tissues and NATs. Relative *GRB2* expression levels in BC tissues and NATs were determined by qRT-PCR. The expression of *miR-105-5p* is significantly downregulated in BC tissues compared with NATs (* p 0.05). *GAPDH* was used as an internal control. Abbreviation: BC: breast cancer, NATs: paired normal adjacent tissues.

Figure 5

GRB2 is a direct target of miR-105-5p in breast cancer. a) miR-105-5p and its putative binding sequence sites in the *GRB2* 3'-UTR. *miR-105-5p* expression had negative correlation with the expression of *GRB2* in BC tissues, (slope = -0.02098, p=0.1664)