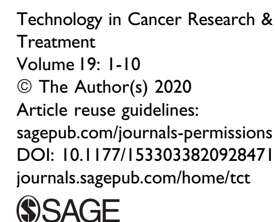

Technology in Cancer Research & Treatment
Volume 19: 1-10
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1533033820928471
journals.sagepub.com/home/tct

Circular RNA hsa_circ_0000376 Participates in Tumorigenesis of Breast Cancer by Targeting miR-1285-3p

Ziqi Peng, BM[1], Boyang Xu, BM[2], and Feng Jin, MD[1]

Abstract

This study was designed to identify novel circular RNAs and the related regulatory axis to provide research targets for the diagnosis and treatment of breast cancer. The circular RNA expression microarray "GSE101123" related to breast cancer was downloaded from the Gene Expression Omnibus database. The differentially expressed circular RNAs between tumor and normal samples were screened using Limma package. The targeted microRNAs of the differentially expressed circular RNAs and the targeted messenger RNAs of the microRNAs were predicted using miRanda and miRWalk, respectively, and a circular RNAs–microRNAs–messenger RNAs network was constructed. Then, functional enrichment analysis, protein–protein interaction network construction, and drug–gene interaction analysis were conducted for the messenger RNAs. A total of 11 differentially expressed circular RNAs were identified between the breast cancer and normal samples, of which 3 were upregulated, while 8 were downregulated. The circular RNA–microRNA–messenger RNA network contained 1 circular RNA (hsa_circ_0000376), 2 microRNAs (miR-1285-3p and miR-1286), and 353 messenger RNAs. The protein–protein interaction network contained 150 nodes and 240 interactions. The hub genes in the protein–protein interaction network were all targeted messenger RNAs of miR-1285-3p that were significantly enriched in the ubiquitin–proteasome system, apoptosis, cell cycle arrest–related pathways, and cancer-related pathways involving SMAD specific E3 ubiquitin protein ligase 1, β-transducin repeat containing E3 ubiquitin protein ligase, tumor protein P53 among others. Twenty-two drugs were predicted to target 4 messenger RNAs, including tumor protein P53. A novel circular RNA, hsa_circ_0000376, was identified in breast cancer that may act as a sponge targeting miR-1285-3p expression which through its target genes, *SMURF1, BTRC*, and *TP53*, may further regulate tumorigenesis.

Keywords

circular RNA, competing endogenous RNA, microRNA protein–protein interaction, differentially expressed circRNAs

Abbreviations

BP, biological process; BTRC, β-transducin repeat containing E3 ubiquitin protein ligase; ceRNA, competing endogenous RNA; circRNAs, circular RNAs; DAVID, Database for Annotation, Visualization, and Integrated Discovery; DGIdb, drug–gene interaction database; FC, fold changes; GEO, Gene Expression Omnibus; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNAs, microRNAs; mRNA, messenger RNA; PCA, principal component analysis; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PPI, protein–protein interactions; qPCR, quantitative polymerase chain reaction; TNBC, triple-negative breast cancer; SMURF1, SMAD specific E3 ubiquitin protein ligase 1

Received: August 28, 2019; Revised: January 10, 2020; Accepted: March 31, 2020.

Introduction

Circular RNA (circRNA) is a kind of endogenous RNA without a 5′ cap or 3′ poly (A) tail that forms a closed continuous loop structure through covalent bonds.[1] Recently, with the advances in high-throughput sequencing and bioinformatics, abundant circRNAs have been identified in the eukaryotic transcriptome that plays crucial roles in the regulation of gene expression at

[1] Department of Breast Surgery, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province, China
[2] Department of Gastroenterology, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province, China

Corresponding Author:
Feng Jin, Department of Breast Surgery, The First Affiliated Hospital of China Medical University, No. 155 Nanjing Road, Heping District, Shenyang, Liaoning Province 110001, China.
Email: pzq_6138@163.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

transcriptional and posttranscriptional levels through binding to microRNAs (miRNAs) or other RNA-associated proteins.[2] Circular RNAs are derived from exons or introns through a back splicing process and are characterized as being conserved and stable.[3] Circular RNAs are implicated in the development of various cancers through a competing endogenous RNA (ceRNA) mechanism in which the circRNAs serve as miRNA sponges.[2] For example, circ_0067934 acts as a sponge inhibiting miR-1324 expression which regulates its target gene, frizzled class receptor 5 (*FZD5*), to further regulate the progression of hepatocellular carcinoma.[4]

Breast cancer is the most common cancer in women and is considered as a great public health problem worldwide.[5] The incidence and mortality of breast cancer presents an increasing trend, and breast cancer–related mortality has increased by nearly 18% since 2008.[6] Recently, increasing number of studies have demonstrated that circRNAs play a crucial role in breast cancer. Circular RNAs can provide promising diagnostic value in breast cancer.[7] Liang *et al* reported overexpression of circABCB10 in breast cancer that accelerates tumor progression by targeting miR-1271.[8] In addition, circAGFG1 was found to be upregulated in triple-negative breast cancer (TNBC) and accelerates tumor progression through the circAGFG1–miR-195-5p–cyclin E1 regulatory axis, and the level of circAGFG1 was related to the tumor stage, grade, and unfavorable prognosis.[9] Nevertheless, there are several uncharacterized circRNAs that still need to be investigated. Therefore, we intended to identify novel circRNAs and the related regulatory axis to further provide research targets and theoretical basis for the diagnosis and treatment of breast cancer. In our study, we used the circRNA expression microarray data set "GSE101123" (contributed by Xu and Chen in July 2018). We first screened the differentially expressed circRNAs (DE-circRNAs) between the tumor and normal samples that were then used to predict the targeted interactions to further construct the DE-circRNAs–miRNAs–messenger RNA (mRNA) regulatory network. Finally, functional enrichment analysis, protein–protein interactions (PPI) network construction, and drug–gene interaction analysis were performed for the mRNA.

Materials and Methods

Microarray Data

The circRNA expression microarray data set "GSE101123" related to breast cancer was downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and contained 8 breast cancer tissue samples and 3 normal mammary gland tissue samples. The platform used was GPL19978 Agilent-069978 Arraystar Human CircRNA microarray V1.

Data Preprocessing and Identification of DE-circRNAs

The raw data were read using the Limma package[10] (Version 3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html), and the data were preprocessed using robust multi-array average method, including background correction, normalization, and expression calculation. The probes were matched to gene symbols based on the annotation files from the platform, in which, the probes were removed without the corresponding gene symbol, while the mean expression value was considered as the final expression value of this circRNA when multiple probes matched the gene symbol. The classical Bayes test in limma package was used to perform the DE analysis, and the DE-circRNAs were identified using the threshold of adjusted P value <0.05 and $|\log$ fold changes (FC)$| > 2$. Differentially expressed circRNAs were converted into universal name based on circBase, heatmaps, and volcano plots of the DE-circRNAs were constructed using the matrix data.

Differentially Expressed circRNA–miRNA–Target Regulatory Associations

The targeted miRNAs of DE-circRNAs were predicted using miRanda (Version: 3.3a, https://omictools.com/miranda-tool) with the parameter setting of score >170 and energy <-30. Then, the targeted genes of the miRNAs above were predicted using miRWalk2.0[11] (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) with the following parameter setting (species: human; other databases: miRWalk, miRanda, miRDB, miRMap, Pictar2, RNA22, and Targetscan; retrieval relation: AND). The target genes that emerged in more than 6 databases were selected. Finally, the DE-circRNA–miRNA–target regulatory network was constructed based on the DE-circRNA–miRNA regulatory pairs and miRNA–target regulatory pairs.

Functional Enrichment Analysis of Target Genes

The functional enrichment analysis of target genes was performed using Database for Annotation, Visualization, and Integrated Discovery (DAVID; Version 6.8, https://david-d.ncifcrf.gov/), including biological processes in Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways. The enrichment genes count was set as ≥ 2, and the P value <0.05 were selected to identify the significantly enriched results.

Protein–Protein Interaction Network of Target Genes

The interactions pairs between target genes were retrieved from the search tool for the retrieval of interacting genes (STRING) database (Version: 10.0, http://www.string-db.org/) with PPI score setting as 0.4 (median confidence). Then, based on the interactions pairs, the PPI network was visualized using Cytoscape (version 3.2.0, http://www.cytoscape.org/), and the topological properties of PPI network were analyzed using CytoNCA plugin[12] (Version 2.1.6, http://apps.cytoscape.org/apps/cytonca), with the parameter setting of "without weight." The hub genes were identified based on the degree score of the nodes in the network. In addition, the functional enrichment

analysis was performed for the top 10 hub genes using the method mentioned above.

Drug–Gene Interaction Analysis

The drug–gene interaction database (DGIdb) is an open public information platform of known and potential drug–gene interactions. Here, the drug–gene interactions were predicted for the top 10 hub genes mentioned above using DGIdb 2.0[13] (http://www.dgidb.org/) with default parameters, and the databases were limited to DrugBank and Food and Drug Administration. Then, based on the drug–gene interactions, the network was visualized using Cytoscape.

Tissue Samples and Quantitative Polymerase Chain Reaction

Ten luminal A breast cancer samples, 10 TNBC samples, and 10 nontumor breast samples were collected from The First Affiliated Hospital of China Medical University (April 2019 to October 2019). The nontumor breast samples were obtained from a minimum of 5 cm distance from the tumor. All samples were confirmed by histopathology and none of the patients had received neoadjuvant chemotherapy before the surgery. The samples were approved by the Ethics Committee of First Affiliated Hospital of Chinese University of Science.

Total RNA was extracted using TRIzol reagent. The TaKaRa PrimeScript RT reagent Kit with gDNA Eraser was used to reverse transcribe the RNA. The reactions were run in an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). U6 was chosen as the reference gene. The sequences of primers used for miR-1285-3p were Forward 5′-GCGTCTGGGCAACAAAGTG-3′, Reverse 5′-AGTGCAGGGTCCGAGGTATT-3′, and RT-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGTCT. The sequences of the primers used for miR-1286 were Forward 5′-CGCGTGCAGGACCAAGATG-3′, Reverse 5′-AGTGCAGGGTCCGAGGTATT-3′, and RT-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGCT. Quantitative polymerase chain reaction (qPCR) data were analyzed using relative quantification or ΔΔCt method based on mRNA copy number ratio (R) of target gene versus reference genes U6 in each tumor sample relative to another reference sample.

Results

Identification of DE-circRNAs

We first performed principal component analysis on the standardized matrix data, and the tumor sample (T04) that seriously deviated was removed in the following analysis (Figure 1A). A total of 11 DE-circRNAs were identified between tumor and normal samples, including 3 upregulated and 8 downregulated DE-circRNAs. As shown in the heatmaps and volcano plot of the DE-circRNAs in Figure 1B and C, the DE-circRNAs could clearly distinguish the samples, suggesting that it was significant for further analysis.

Differentially Expressed circRNA–miRNA–Target Regulatory Network

In all, 14 DE-circRNA–miRNA interaction pairs and 349 miRNA–target interaction pairs were obtained. A total of 351 DE-circRNA–miRNA–target regulatory associations were included based on the overlapped miRNAs, including 2 DE-circRNA–miRNA interaction pairs and 349 miRNA–target interaction pairs. The DE-circRNA–miRNA–target regulatory network was constructed based on the 351 DE-circRNA–miRNA–target regulatory associations (Figure 2), and there were 356 nodes in the network, including 1 DE-circRNA (hsa_circ_0000376), 2 miRNAs (miR-1285-3p and miR-1286), and 353 target genes.

Functional Enrichment Analysis of Target Genes

Functional enrichment analysis was performed to further explore the biological processes (BPs) and pathways of target genes involved using DAVID. A total of 30 GO_BPs and 9 KEGG pathways were significantly enriched for the target genes of miR-1285-3p, such as, "GO:0045944 ∼ positive regulation of transcription from RNA polymerase II promoter," "GO:0043161 ∼ proteasome-mediated ubiquitin-dependent protein catabolic process," and "hsa04144: Endocytosis." Similarly, 10 GO_BPs and 1 KEGG pathways were significantly enriched for the target genes of miR-1286, such as "GO:0006396 ∼ RNA processing," "GO:0007399 ∼ nervous system development," and "hsa04022: cGMP-PKG signaling pathway." The top 10 significantly enriched Gene ontology–biological processes (GO-BPs) and KEGG pathways are listed in Table 1.

Protein–Protein Interaction Network of Target Genes

The PPI network contained 150 nodes and 240 interactions (Figure 3). The top 10 hub nodes with high degrees were all target genes of miR-1285-3p (Table 2). Ubiquitin A-52 residue ribosomal protein fusion product 1 (UBA52) had the highest degree in the PPI network, followed by tumor protein P53 (TP53) and glycogen synthase kinase 3 beta (GSK3B).

Functional enrichment analysis was performed to further explore the functions of the top 10 hub genes. A total of 14 KEGG pathways and 37 GO-BPs were significantly enriched for the top 10 hub genes in PPI network, and the top 10 significantly enriched GO-BPs and KEGG pathways are listed in Table 3. Beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC), promyelocytic leukemia (PML), and SMAD specific E3 ubiquitin protein ligase 1 (SMURF1) were significantly enriched in "hsa04120: Ubiquitin mediated proteolysis," while BTRC, GSK3B, and TP53 were significantly enriched in "hsa04310: Wnt signaling pathway." In

Figure 1. The results of differential expression analysis. A, PCA before and after data standardization preprocessing; B, Heatmaps of the DE-circRNAs; C, Volcano plot of the DE-circRNAs. DE-circRNAs indicates differentially expressed circRNAs; PCA, principal component analysis.

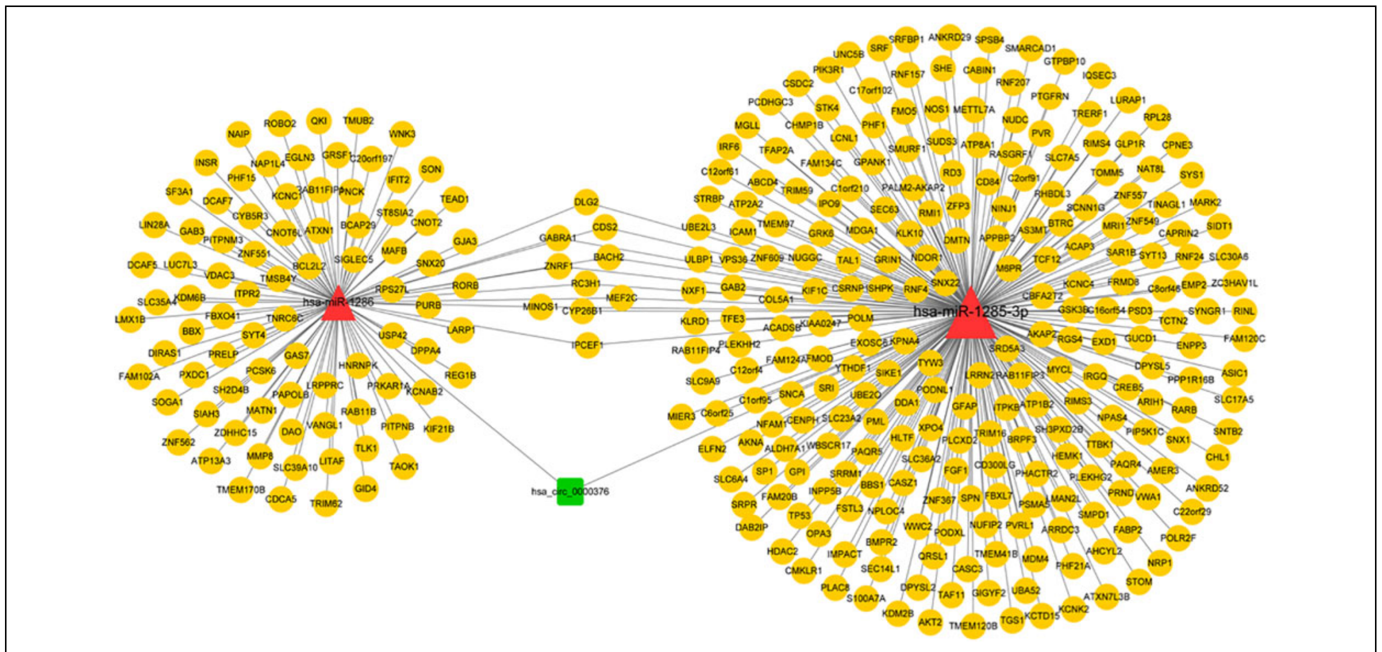

Figure 2. The circRNAs–miRNAs–mRNA regulatory network. Green squares represent circRNAs; red triangles represent miRNAs; yellow circles represent mRNAs. circRNA indicates circular RNA; miRNA, microRNA; mRNA, messenger RNA.

Table 1. The Results of Functional Enrichment Analysis for the Target Genes.

Category	Terms	Count	*P* value	Genes
Results of functional enrichment for the target genes of miR-1258				
GO_BP	GO:0045944—positive regulation of transcription from RNA polymerase II promoter	28	2.64E-04	MEF2C, AKNA, TFE3, BMPR2, PML, FSTL3, SRF, CBFA2T2, TAL1, RARB, FGF1, PIK3R1, DAB2IP, NOS1, GRIN1, TP53, NPAS4, HLTF, PLAC8, CAPRIN2, HDAC2, RNF4, SP1, GSK3B, CSRNP1, TFAP2A, TCF12, UBA52
GO_BP	GO:0043161—proteasome-mediated ubiquitin-dependent protein catabolic process	10	1.60E-03	GSK3B, PML, TP53, SMURF1, RNF4, PSMA5, BTRC, RNF24, ZNRF1, UBA52
GO_BP	GO:0030220—platelet formation	4	1.91E-03	MEF2C, TAL1, C6ORF25, SRF
GO_BP	GO:0043524—negative regulation of neuron apoptotic process	8	1.92E-03	MEF2C, GPI, NRP1, KDM2B, UNC5B, GRIN1, SNCA, CHL1
GO_BP	GO:0000209—protein polyubiquitination	9	3.24E-03	ARIH1, PSMA5, BTRC, RNF24, SMURF1, HLTF, ZNRF1, UBE2L3, UBA52
GO_BP	GO:0050900—leukocyte migration	7	5.78E-03	CD84, ICAM1, ATP1B2, PODXL, SLC7A5, PIK3R1, SPN
GO_BP	GO:0060079—excitatory postsynaptic potential	4	5.93E-03	MEF2C, GRIN1, SNCA, NPAS4
GO_BP	GO:0007616—long-term memory	4	5.93E-03	RASGRF1, GRIN1, NPAS4, SRF
GO_BP	GO:1901796—regulation of signal transduction by p53 class mediator	7	6.25E-03	TAF11, HDAC2, PML, TP53, MDM4, RMI1, UBA52
GO_BP	GO:0046621—negative regulation of organ growth	3	7.37E-03	SLC6A4, WWC2, STK4
KEGG_Pathway	hsa04144: Endocytosis	12	3.40E-04	RAB11FIP4, CHMP1B, RAB11FIP3, ACAP3, PML, PSD3, GRK6, SNX1, PIP5K1C, SMURF1, IQSEC3, VPS36
KEGG_Pathway	hsa05223: Non-small cell lung cancer	5	6.48E-03	TP53, RARB, STK4, PIK3R1, AKT2
KEGG_Pathway	hsa05220: Chronic myeloid leukemia	5	1.54E-02	HDAC2, GAB2, TP53, PIK3R1, AKT2
KEGG_Pathway	hsa04919: Thyroid hormone signaling pathway	6	1.86E-02	HDAC2, ATP1B2, GSK3B, TP53, PIK3R1, AKT2
KEGG_Pathway	hsa05169: Epstein-Barr virus infection	6	2.34E-02	ICAM1, HDAC2, TP53, PIK3R1, SPN, AKT2
KEGG_Pathway	hsa05215: Prostate cancer	5	2.98E-02	GSK3B, TP53, CREB5, PIK3R1, AKT2
KEGG_Pathway	hsa05213: Endometrial cancer	4	3.19E-02	GSK3B, TP53, PIK3R1, AKT2
KEGG_Pathway	hsa04070: Phosphatidylinositol signaling system	5	4.18E-02	CDS2, PIP5K1C, ITPKB, INPP5B, PIK3R1
KEGG_Pathway	hsa05210: Colorectal cancer	4	4.97E-02	GSK3B, TP53, PIK3R1, AKT2
Results of functional enrichment for the target genes of miR-1268				
GO_BP	GO:0006396—RNA processing	4	1.23E-02	ATXN1, HNRNPK, GRSF1, SF3A1
GO_BP	GO:0007399—nervous system development	6	1.39E-02	MEF2C, MAFB, NAIP, ST8SIA2, GAS7, DLG2
GO_BP	GO:0002634—regulation of germinal center formation	2	2.45E-02	MEF2C, RC3H1
GO_BP	GO:0045727∼positive regulation of translation	3	2.83E-02	RPS27L, LIN28A, LARP1
GO_BP	GO:0010606—positive regulation of cytoplasmic mRNA processing body assembly	2	2.93E-02	CNOT6 L, CNOT2
GO_BP	GO:0016567—protein ubiquitination	6	3.25E-02	DCAF5, DCAF7, BACH2, NAIP, TRIM62, RC3H1
GO_BP	GO:0031123—RNA 3′-end processing	2	3.41E-02	PAPOLB, LIN28A
GO_BP	GO:0000288—nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	2	3.89E-02	CNOT2, RC3H1
GO_BP	GO:0046007—negative regulation of activated T cell proliferation	2	4.36E-02	PRKAR1A, RC3H1
GO_BP	GO:2000008—regulation of protein localization to cell surface	2	4.36E-02	KCNAB2, RAB11B
KEGG_Pathway	hsa04022: cGMP-PKG signaling pathway	4	2.56E-02	MEF2C, VDAC3, INSR, ITPR2

Abbreviations: BP, biological process; BTRC, β-transducin repeat containing E3 ubiquitin protein ligase; GO, Gene ontology; GSK3, glycogen synthase kinase 3; KEGG, Kyoto Encyclopedia of Genes and Genomes; mRNA, messenger RNA; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PML, promyelocytic leukemia; SMURF1, SMAD specific E3 ubiquitin protein ligase 1; TP53, tumor protein P53.

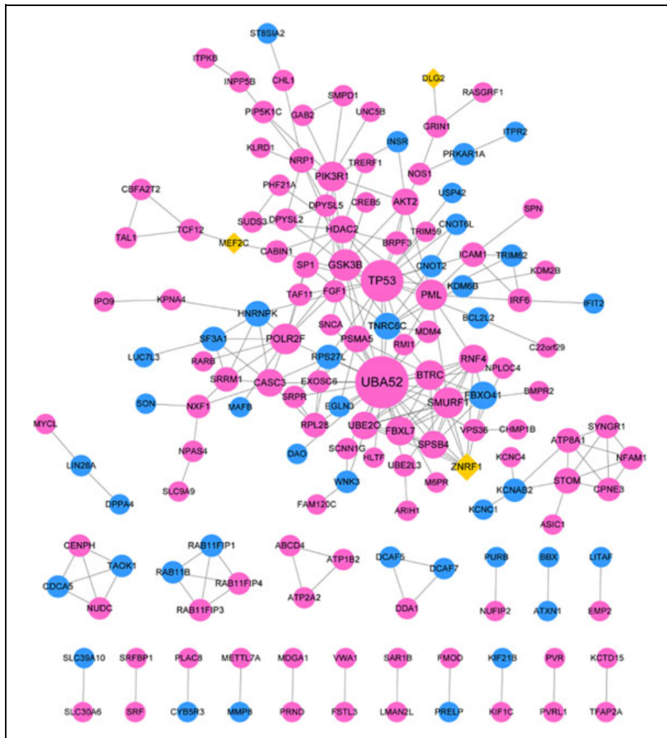

Figure 3. The PPI network of mRNAs. Blue circles represent the targeted mRNAs of miR-1286; purple circles represent the targeted mRNAs of miR-1285-3p; yellow rhombuses represent the overlapping targeted mRNAs of miR-1285-3p and miR-1286. mRNA indicates messenger RNA; PPI, protein–protein interaction.

Table 2. The Top 10 Nodes in the PPI Network Ranked by Degree.

Nodes	miRNA	Degree
UBA52	hsa-miR-1285-3p	32
TP53	hsa-miR-1285-3p	22
GSK3B	hsa-miR-1285-3p	12
PML	hsa-miR-1285-3p	11
SMURF1	hsa-miR-1285-3p	11
POLR2F	hsa-miR-1285-3p	11
BTRC	hsa-miR-1285-3p	10
PIK3R1	hsa-miR-1285-3p	10
RNF4	hsa-miR-1285-3p	9
FBXL7	hsa-miR-1285-3p	9

Abbreviations: BTRC, β-transducin repeat containing E3 ubiquitin protein ligase; GSK3B, glycogen synthase kinase 3 beta; miRNA, microRNA; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PML, promyelocytic leukemia; PPI, protein–protein interaction; SMURF1, SMAD specific E3 ubiquitin protein ligase 1; TP53, tumor protein P53.

addition, it was observed that GSK3B, TP53, and phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) were significantly enriched in various cancers or diseases. In addition, "GO:0043161 ∼ proteasome-mediated ubiquitin-dependent protein catabolic process" was the most significant enriched GO_BP, involving GSK3B, SMURF1, TP53, among others.

Drug–Gene Interaction Analysis

In addition, 22 drugs were predicted to be implicated with the 4 target genes of miR-1285-3p using DGIdb, including GSK3B, PIK3R1, TP53, and PML (Figure 4, Table 4). Isoprenaline and SF-1126 were predicted to be an agonist and inhibitor of PIK3B, respectively. Aspirin was predicted to regulate the acetylation of TP53, while alsterpaullone was an inhibitor of GSK3B.

Evaluation of Genes by qPCR

Quantitative polymerase chain reaction was used to detect the expression of miR-1285-3p and miR-1286 in cancer tissues and normal tissues. As shown in Figure 5, the expression level of miR-1285-3p in the tumor tissue was significantly lower than in the control ($P < .05$). The expression of miR-1286 in luminal A breast tissue and triple-negative tumor tissue was significantly lower than in the control ($P < .05$).

Discussion

In the current study, 11 DE-circRNAs were screened between breast cancer samples and normal samples, while only one circRNA was identified in the circRNA–miRNA–mRNA regulatory network, that was hsa_circ_0000376, a novel circRNA found to be downregulated in breast cancer. MiR-1285-3p and miR-1286 are hsa_circ_0000376 targets, and the targeted mRNAs of miR-1285-3p were the hub genes in PPI network that were significantly enriched in ubiquitin–proteasome system, apoptosis, and cell cycle arrest–related pathways and cancer-related pathways, involving *SMURF1, BTRC*, *TP53*, and other genes. In addition, the drugs including SF-1126 and aspirin were predicted to target PIK3B and TP53, and these drugs had been reported in the treatment of breast cancer in previous studies.[2] For example, Deng *et al* indicated that a combination of SF1126 and gefitinib triggered apoptosis in TNBC cells through the PI3K/AKT-mTOR pathway.[14]

MiR-1285 was first identified from a massive parallel sequencing of human embryonic stem cells.[15] It was reported that the expression of tumor suppressor p53 was repressed by miR-1285 ectopic expression, while its expression decreases upon depletion of miR-1285 in the cells of human neuroblastoma, hepatoblastoma, and breast cancer.[16] Gao *et al* indicated that downregulation of miR-1285 was observed in stage I lung squamous cell carcinoma and could be considered as a promising diagnostic biomarkers.[17] In addition, the proliferation, migration, and invasion of pancreatic cancer cells were significantly inhibited by miR-1285 through targeting Yes Associated Protein 1, showing a tumor suppressor role.[18] Further, a previous study revealed that miR-1285-3p expression was decreased in the plasma of hepatocellular carcinoma and could be used to predict the prognosis of patients receiving transarterial chemoembolization. Meanwhile, miR-1285-3p can also inhibit the expression of v-jun avian sarcona virus 17 oncogene homolog (JUN) oncogene in hepatocellular carcinoma cells,

Table 3. The Results of Functional Enrichment Analysis for the Top 10 Hub Genes in PPI Network.

Category	Terms	Count	*P* value	Genes
GO_BP	GO:0043161—proteasome-mediated ubiquitin-dependent protein catabolic process	7	2.36E-10	SMURF1, TP53, GSK3B, RNF4, BTRC, PML, UBA52
GO_BP	GO:0045944—positive regulation of transcription from RNA polymerase II promoter	6	6.97E-05	RNF4, GSK3B, PML, TP53, UBA52, PIK3R1
GO_BP	GO:0006977—DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	3	4.75E-04	PML, TP53, UBA52
GO_BP	GO:0016567—protein ubiquitination	4	7.40E-04	SMURF1, RNF4, BTRC, FBXL7
GO_BP	GO:0007179—transforming growth factor β receptor signaling pathway	3	1.04E-03	PML, SMURF1, UBA52
GO_BP	GO:0043066—negative regulation of apoptotic process	4	1.47E-03	GSK3B, TP53, UBA52, PIK3R1
GO_BP	GO:1901796—regulation of signal transduction by p53 class mediator	3	1.88E-03	PML, TP53, UBA52
GO_BP	GO:0000086—G2/M transition of mitotic cell cycle	3	2.29E-03	BTRC, FBXL7, UBA52
GO_BP	GO:0050852—T cell receptor signaling pathway	3	2.67E-03	BTRC, UBA52, PIK3R1
GO_BP	GO:0038095—Fc-epsilon receptor signaling pathway	3	3.83E-03	BTRC, UBA52, PIK3R1
KEGG_Pathway	hsa05213: Endometrial cancer	3	1.15E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa05210: Colorectal cancer	3	1.63E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa05215: Prostate cancer	3	3.26E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa05200: Pathways in cancer	4	5.45E-03	GSK3B, PML, TP53, PIK3R1
KEGG_Pathway	hsa04919: Thyroid hormone signaling pathway	3	5.51E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa04722: Neurotrophin signaling pathway	3	5.98E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa05162: Measles	3	7.31E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa05160: Hepatitis C	3	7.31E-03	GSK3B, TP53, PIK3R1
KEGG_Pathway	hsa04120: Ubiquitin-mediated proteolysis	3	7.74E-03	SMURF1, BTRC, PML
KEGG_Pathway	hsa04310: Wnt signaling pathway	3	7.85E-03	BTRC, GSK3B, TP53

Abbreviations: BP, biological process; BTRC, β-transducin repeat containing E3 ubiquitin protein ligase; GO, Gene ontology; GSK3B, glycogen synthase kinase 3 beta; KEGG, Kyoto Encyclopedia of Genes and Genomes; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PML, promyelocytic leukemia; PPI, protein–protein interaction; SMURF1, SMAD specific E3 ubiquitin protein ligase 1; TP53, tumor protein P53.

which suggests that it has a tumor suppressive effect.[19] However, few studies have reported the functions of miR-1285-3p in breast cancer. In our study, miR-1285-3p was the target of hsa_circ_0000376, and we speculated that hsa_circ_0000376 might be involved in the progression of breast cancer by targeting miR-1285-3p. In this study, we have detected the expression of miR-1285-3p and miR-1286 in cancer and normal tissues, and the result indicates that these 2 miRNAs showed lower expression level in cancer tissues than normal tissue.

Notably, the targeted mRNAs of miR-1285-3p were significantly enriched in ubiquitin–proteasome system, apoptosis, and cell cycle arrest–related pathways. Vriend and Reiter reported in a review that BRCA1, a susceptibility gene, served as an ubiquitin ligase, and suggested that ubiquitin–proteasome system was associated with the regulation of breast cancer susceptibility.[20] In addition, several studies have reported the role of ubiquitin–proteasome system in breast cancer.[2] In our study, SMURF1, BTRC, and TP53 were enriched in ubiquitin–proteasome system-related BPs and pathways. *SMURF1* encodes an E3 ubiquitin ligase that regulates SMAD ubiquitination and degradation.[21] Fukunaga *et al* revealed that *SMURF1* expression was improved by *SMURF2* knockdown that in turn increased cell migration and bone metastasis in breast cancer.[22] Yu *et al* indicated that the increased expression of SMURF1 by SND1 caused the ubiquitinated and degradation of RhoA which destroyed F-actin cytoskeletal structure and further accelerated metastasis in breast cancer.[23] Beta-transducin repeat containing E3 ubiquitin protein ligase, also called as β-TrCP1, is an E3-ubiquitin ligase receptor subunit that mediates protein stability and acts as a tumor inhibitor or oncogene.[24] It was reported that β-TrCP1 expression was downregulated in TNBC cells, and β-TrCP1 knockdown repressed cell proliferation, suggesting that it could be a potential target in the treatment of TNBC.[25] *TP53* encodes a tumor suppressor protein which plays a crucial role in monitoring following DNA damage, and it triggers damage repair or apoptosis.[26] Reportedly, *TP53* mutation was commonly found in the tumorigenesis of various cancers, including breast cancer, and *TP53* germline mutation is associated with highly increased risk of developing breast cancer.[27] *TP53* polymorphisms are related to the risk and type of breast cancer and could be a prognostic biomarker.[28] Hereby, we concluded that hsa_circ_0000376 could target the expression of miR-1285-3p to further regulate cell proliferation, metastasis, and tumorigenesis of breast cancer. The target genes of miR-1285-3p are *SMURF1, BTRC*, and *TP53*. A novel circRNA, hsa_circ_0000376, and its related ceRNA mechanism were identified in our study; however, experimental verification is needed to further confirm the role of this circRNA and its related regulatory mechanism in breast cancer. In addition, there are many target genes of miR-1285-3p. Further experiments are also needed to screen and identify the regulatory

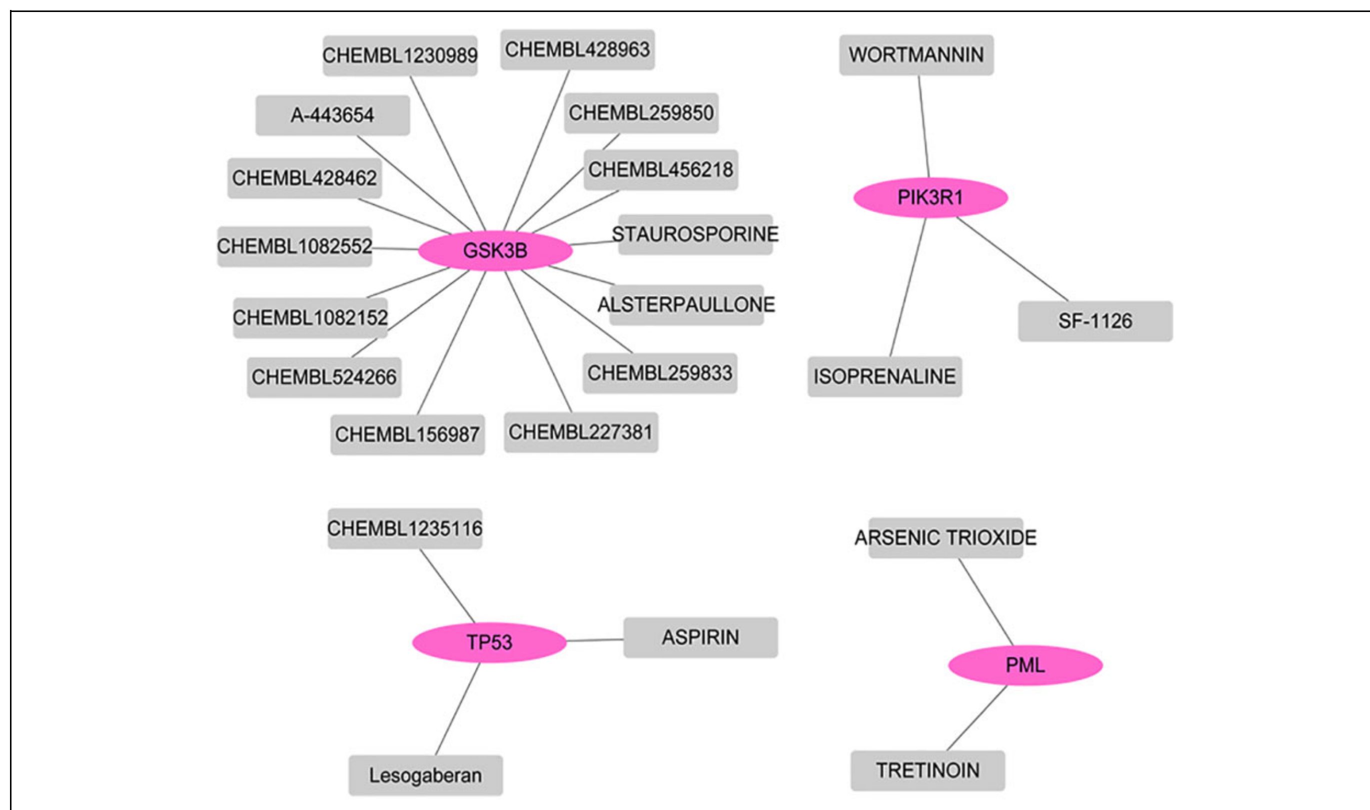

Figure 4. The results of drug–gene interaction analysis. Purple ellipses represent targeted mRNAs of miR-1285-3p; gray squares represent the predicted drugs. mRNA indicates messenger RNA.

Table 4. The Results of Drug–Gene Interaction Analysis.

Gene	Drug	Interaction types	Sources	Pmids
TP53	CHEMBL1235116	NA	DrugBank	10592235
TP53	ASPIRIN	acetylation	DrugBank	21475861
TP53	Lesogaberan	NA	DrugBank	NA
GSK3B	A-443654	NA	DrugBank	10592235
GSK3B	CHEMBL524266	inhibitor	GuideToPharmacologyInteractions|DrugBank	10592235
GSK3B	CHEMBL428963	NA	DrugBank	10592235
GSK3B	ALSTERPAULLONE	inhibitor	GuideToPharmacologyInteractions|DrugBank	10592235|17139284|17016423
GSK3B	CHEMBL156987	NA	DrugBank	10592235|17139284|17016423
GSK3B	CHEMBL227381	NA	DrugBank	10592235
GSK3B	CHEMBL428462	NA	DrugBank	10592235
GSK3B	CHEMBL259850	NA	DrugBank	10592235|17139284|17016423
GSK3B	CHEMBL456218	NA	DrugBank	10592235
GSK3B	CHEMBL1082152	NA	DrugBank	10592235|17139284|17016423
GSK3B	STAUROSPORINE	NA	DrugBank	17139284|17016423
GSK3B	CHEMBL1082552	inhibitor	GuideToPharmacologyInteractions|DrugBank	10592235|17139284|17016423
GSK3B	CHEMBL259833	NA	DrugBank	10592235
GSK3B	CHEMBL1230989	NA	DrugBank	17139284|17016423
PML	ARSENIC TRIOXIDE	NA	CGI|CIViC|FDA	11704842
PML	TRETINOIN	NA	PharmGKB|CGI|CIViC|FDA	21505136|8674046|21613260|23670176|11704842
PIK3R1	WORTMANNIN	NA	DrugBank	10592235
PIK3R1	SF-1126	inhibitor	MyCancerGenome|ChemblInteractions|DrugBank	NA
PIK3R1	ISOPRENALINE	agonist	DrugBank	15110780|15618457|15985706|15381832|15527548

Abbreviations: FDA, Food and Drug Administration; GSK3B, glycogen synthase kinase 3 beta; NA, not available; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PML, promyelocytic leukemia; TP53, tumor protein P53.

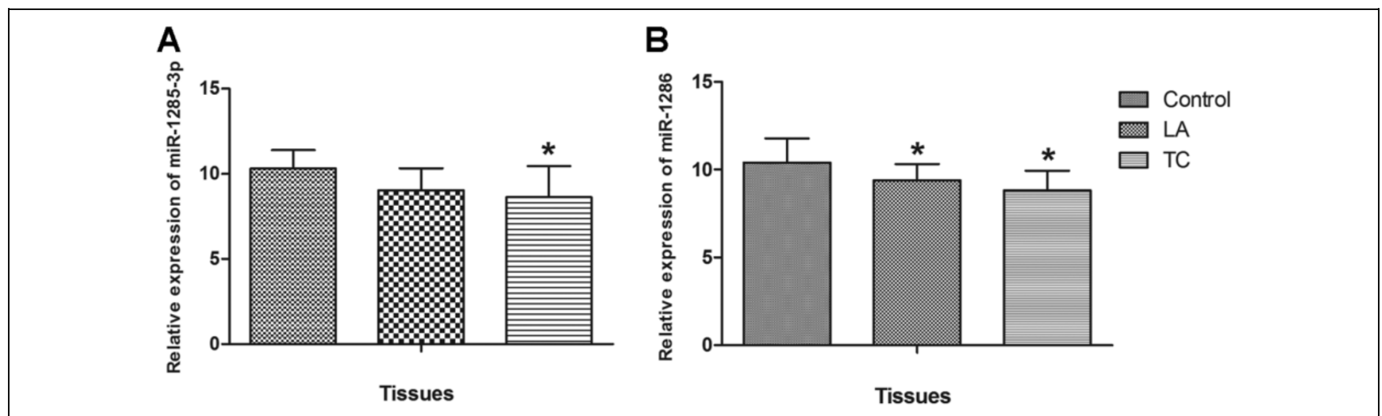

Figure 5. Relative expression level of miRNAs in tissues. A, Relative expression level of miR-1285-3p in tissues; B, Relative expression level of miR-1286 in tissues. Control represents the nontumor breast tissues; LA indicates luminal A breast tissues; TC, triple-negative breast cancer tissues. miRNA, microRNA.

mechanisms. In addition, the predicted drugs that were not reported needed to be tested experimentally.

In conclusion, we identified a novel circRNA, hsa_circ_0000376, and its related ceRNA mechanism in breast cancer. hsa_circ_0000376 might be a sponge, firstly targeted the expression of miR-1285-3p which mediates its target genes, *SMURF1*, *BTRC*, and *TP53*, to further regulate cell proliferation, metastasis, and tumorigenesis of breast cancer.

Authors' Note

Not applicable. Our study did not contain human or animal trials.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by National Natural Science Foundation of China (grant number 81773163).

ORCID iD

Feng Jin https://orcid.org/0000-0002-3072-8436

References

1. Chen LL, Yang L. Regulation of circRNA biogenesis. *RNA Biol*. 2015;12(4):381-388.
2. Xuan L, Qu L, Zhou H, et al. Circular RNA: a novel biomarker for progressive laryngeal cancer. *Am J Transl Res*. 2016;8(2): 932-939.
3. Li J, Yang J, Zhou P, et al. Circular RNAs in cancer: novel insights into origins, properties, functions and implications. *Am J Cancer Res*. 2015;5(2):472-480
4. Zhu Q, Lu G, Luo Z, et al. CircRNA circ_0067934 promotes tumor growth and metastasis in hepatocellular carcinoma through regulation of miR-1324/FZD5/Wnt/β-catenin axis. *Biochem Biophys Res Commun*. 2018;497(2):626-632.
5. Tao Z, Shi A, Lu C, Song T, Zhang Z, Zhao J. Breast cancer: epidemiology and etiology. *Cell Biochem Biophys*. 2015;72(2): 333-338.
6. 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):43-46.
7. Lü L, Sun J, Shi P, et al. Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. *Oncotarget*. 2017;8(27):44096-44107.
8. Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. *Am J Cancer Res*. 2017;7(7): 1566-1576.
9. Yang R, Xing L, Zheng X, Sun Y, Wang X, Chen J. The circRNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CCNE1 expression. *Mol Cancer*. 2019;18(1):4
10. Smyth GK. Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. Springer; 2011:397-420.
11. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods*. 2015;12(8):697.
12. Tang Y, Li M, Wang J, Pan Y, Wu F-X. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems*. 2015;127:67-72.
13. Wagner AH, Coffman AC, Ainscough BJ, et al. DGIdb 2.0: mining clinically relevant drug-gene interactions. *Nucleic Acids Res*. 2016;44(D1):D1036-D1044.
14. Deng M, Wang J, Chen Y, Zhang L, Liu D. Combination of SF1126 and gefitinib induces apoptosis of triple-negative breast cancer cells through the PI3K/AKT-mTOR pathway. *Anticancer Drugs*. 2015;26(4):422-427.
15. Morin RD, O'Connor MD, Griffith M, et al. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. *Genome Res*. 2008;18(4):610-621.
16. Tian S, Huang S, Wu S, Guo W, Li J, He X. MicroRNA-1285 inhibits the expression of p53 by directly targeting its 3' untranslated region. *Biochem Biophys Res Commun*. 2010;396(2):435-439.

17. Gao X, Wang Y, Zhao H, et al. Plasma miR-324-3p and miR-1285 as diagnostic and prognostic biomarkers for early stage lung squamous cell carcinoma. *Oncotarget*. 2016;7(37):59664-59675.
18. Huang H, Xiong G, Shen P, et al. MicroRNA-1285 inhibits malignant biological behaviors of human pancreatic cancer cells by negative regulation of YAP1. *Neoplasma*. 2017;64(3):358-366.
19. Liu J, Yan J, Zhou C, Ma Q, Jin Q, Yang Z. miR-1285-3p acts as a potential tumor suppressor miRNA via downregulating JUN expression in hepatocellular carcinoma. *Tumor Biol*. 2015; 36(1):219-225.
20. Vriend J, Reiter RJ. Breast cancer cells: modulation by melatonin and the ubiquitin-proteasome system—a review. *Mol Cell Endocrinol*. 2015;417:1-9.
21. Tao Y, Sun C, Zhang T, Song Y. SMURF1 promotes the proliferation, migration and invasion of gastric cancer cells. *Oncol Rep*. 2017;38(3):1806-1814.
22. Fukunaga E, Inoue Y, Komiya S, et al. Effects of grape seed-derived polyphenols on amyloid beta-protein self-assembly and cytotoxicity. *J Biol Chem*. 2008;283(47):35660-35687.
23. Yu L, Liu X, Cui K, et al. SND1 Acts downstream of TGFβ1 and upstream of smurf1 to promote breast cancer metastasis. *Cancer Res*. 2015;75(7):1275-1286.
24. Müerköster S, Arlt A, Sipos B, et al. Increased expression of the E3-ubiquitin ligase receptor subunit betaTRCP1 relates to constitutive nuclear factor-kappaB activation and chemoresistance in pancreatic carcinoma cells. *Cancer Res*. 2005;65(4):1316-1324.
25. Yi YW, Kang HJ, Bae EJ, Oh S, Seong Y-S, Bae I. β-TrCP1 degradation is a novel action mechanism of PI3K/mTOR inhibitors in triple-negative breast cancer cells. *Exp Mol Med*. 2015;47: 143.
26. Schon K, Tischkowitz M. Clinical implications of germline mutations in breast cancer: TP53. *Breast Cancer Res Treat*. 2018; 167(2):417-423.
27. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA*. 2006;295(12):1379-1388.
28. AL B-D. TP53 and breast cancer. *Hum Mutat*. 2003;21(3): 292-300.