MiR-324-5P Functions as a Novel Serum Biomarker and Modulates Breast Cancer Malignant Activity

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Abstract

Introduction: The best management practices for patients with breast cancer (BC) involve early detection and deterioration prevention. Therefore, it is important to study the occurrence of BC and its development mechanism and explore more sensitive diagnostic and prognostic indicators.

Objective: Our study explored the significance and the serum miR-324-5p application in the diagnosis and prognosis of BC and evaluated possible molecular mechanisms.

Methods: MiR-324-5p expressions in the serum and tissue of patients with BC were observed by real-time fluorescence quantitative polymerase chain reaction (PCR). Next, the miR-324-5p diagnostic ability in BC was evaluated by the receiver operating characteristic curve. Kaplan-Meier analyzed the relationship between expression of miR-324-5P and prognosis. The Cox regression model evaluated the serum miR-324-5P prognostic value in predicting disease-free survival (DFS) and overall survival (OS). In functional studies, cell counting Kit-8 assay and Cloning experiment were employed to assess BC cells' proliferation level. Transwell assays and wound healing assays were performed to determine the effect of miR-324-5p on BC cell invasion and migration. In addition, the flow cytometry test was conducted to determine the effect of miR-324-5p on the apoptotic rate of BC cells. Finally, bioinformatics tools were employed to detect the potential target genes of miR-324-5p in BC.

Results: miR-324-5p expression is up-regulated in BC cells, which is related to tumor stage ($p=0.0291$) and age ($p=0.0278$). In addition, a BC diagnosis based on serum miR-324-5p levels had an 66% sensitivity, 78% specificity, and the area under the curve was approximately 0.7581. DFS ($p=0.027$) and OS ($p<0.017$) in the low miR-324-5P expression group were higher than those in the high expression group. The tumor node metastasis staging (Ki-67 and miR-324-5P) were independent prognostic factors for the survival of patients with BC. In addition, miR-324-5p had a positive effect on BC cell invasion and proliferation, thereby preventing apoptosis. Finally, bioinformatics synthesis showed that the miR-324-5p target genes in BC are cysteine-rich hydrophobic domain 1 (CHIC1) and kruppel-like factor 7 (KLF7).

Conclusion: MiR-324-5p may perform its oncogenic functions by interacting with the CHIC1 and KLF7 genes, and its existence in serum could serve as a diagnostic and prognostic biomarker of BC.

Introduction

Breast cancer (BC), is one of the most common malignancies in women and a leading cause of death in the 21st century. It is expected to become a primary obstacle to extended life expectancy [1]. However, there is a lack of accurate and early diagnostic and screening methods because of limited progress in BC molecular mechanisms determination. Thus, many patients with BC missed the opportunity for radical resection [2]. Therefore, studies should focus on BC molecular mechanisms to determine new biomarkers capable of identifying and predicting genes as potential treatment targets.

Micro-ribonucleic acid (miRNA) is a non-coding RNA composed of 21–25 nucleotides, widely distributed in the mammalian genome [3]. Thus far, the cancer cells regulation by miRNAs has been identified in many processes, such as cell proliferation, apoptosis [4], angiogenesis [5], radiosensitivity [6], and migration [7]. The miRNAs abnormal expression in malignant tumors would impact the carcinogenic expression or tumor suppressor genes during tumorigenesis and metastasis [8]. Research has revealed that miR-937-5p overexpression can regulate the cell proliferation and cell cycle via the Wnt signaling pathways [9]. Noyans et al. found that the miR-770-5p down-regulation could explain the epithelial-mesenchymal transition in BC [10]. Overall, these studies revealed the miRNAs important role in BC. Hence, abnormal miRNA expression may be a good biological marker for BC diagnosis and prognosis; besides, the involved miRNAs may also serve as potential treatment targets.

MiR-324-5p has been shown to either inhibit or promote human cancer. For example, it is inhibitory for gallbladder [11] and colorectal cancer [12] but can show carcinogenic effects in thyroid papillary [13], pancreatic [14], and gastric cancer [15]. However, whether the expression levels of miR-324-5p can serve as a predictor for BC development remains to be revealed. Therefore, this study evaluated the miR-324-5p relative expression levels in the serum and tissues of patients with BC and studied the correlation between the miR-324-5p expression levels and patients with BC clinicopathological features. In addition, the miR-324-5p feasibility as a marker for BC diagnosis and prognosis was evaluated. Finally, we studied miR-324-5p for its functional effects on BC and analyzed its potential target genes.

Methods And Materials

2.1.1 Collection of clinical samples

Serum samples include 100 serum samples of BC patients and 50 healthy control sera from Shaoxing People's Hospital.. Among them, the patient was newly diagnosed as a female patient with BC, aged 27 to 73 years old. Blood was collected from all patients before they received any form of treatment. Collect whole blood into anticoagulant blood collection tubes. Following that, the tubes were gently inverted 4 to 5 times to mix the anticoagulant with the blood. Quickly transfer to a separate cryopreservation tube or centrifuge tube, store in -80°C refrigerator.

The tissue samples included 41 pairs of BC and adjacent tissues obtained from female patients, all of which were newly diagnosed BC in Shaoxing People's Hospital, aged 35 to 72 years. The tissue specimens were quick-frozen in liquid nitrogen within 30 minutes of in vitro and then transferred to the -80°C refrigerator for storage. All experimenters signed a written informed consent form.

2.1.2 Cell culture

Human BC cell lines T-47D, MCF-7, Hs578T and non-tumorigenic epithelial cell line MCF-10A are provided by the Cell Resource Center of Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. Among them, MCF-10A cells use DMEM/F12 medium (Gibco), and additionally add 5% horse

serum (HyClone), insulin, epidermal growth factor, and hydrocortisone. Other cell lines use DMEM medium with 10% fetal bovine serum (Hyclone). All cells were cultured at 37°C and 5% CO₂.

2.1.3 Transfection

3 × 10⁵ MCF-7 cells were inoculated in one well of the six-well plate. These were then transfected at a final concentration of 50 nM miR-324-5p mimics or with the appropriate miRNA mimic control using lipofectamine 6000 transfection reagent (Beyotime Biotechnology). The Hs578T cells were seeded at 2×10⁵ cells per well into 6-well plates and were transfected with miR-324-5p inhibitors at a final concentration of 100 nM or with the appropriate miRNA inhibitor control using Lipofectamine 6000 transfection. All subsequent functional assays were performed 48-72h post-transfection. Guangzhou RiboBio provides transfection reagents.

2.1.4 RNA isolation and quantitative real-time PCR (RT-qPCR)

We chose to use Trizol (Haoxin) to collect total RNA from cultured cells and tissues. Serum miRNAs was harvested from each 200µl serum sample by using a Serum/plasma miRNA extraction and separation kit (TianGen). The total RNA then underwent reverse transcription using Prime Script™ RT reagent Kit with gDNA Eraser. For microRNAs detection, stem-loop RT-PCR was carried out by using the cDNA product as templates by SYBR® Premix Ex Taq™ (TaKaRa) in the Light Cycler 480 System (Roche Diagnostics). For internal reference, primers of U6 and U6. miR-324-5p were obtained from RiboBio.

2.1.5 Cell Counting Kit-8 tests

48 hours after transfection, MCF-7 cells and Hs578T cells were seeded in several 96-well culture dishes at 1000 and 3000 cells/well, respectively. Cell Counting Kit-8 (CCK-8, MedChemExpress) tests the cell growth rate at different times.

2.1.6 Cloning experiment

48 hours after transfection, MCF-7 cells and Hs578T cells were inoculated in several 6-well culture dishes with 1000 cells/well, respectively. After 10 days of culture, the cells were stained with crystal violet and counted.

2.1.7 Wound healing assay

When the cell density after transfection reaches 90-95%, Scratch with 200 µl grab head and wash the residual cells on the scratch with sterile PBS. Then, add fresh medium with 1% FBS into the 6-well plates, and microscopic images were taken over the plates at 0-24 h. The wound healing rates were calculated and compared to the width at 0 h.

2.1.8 Transwell assay

For migration assays, MCF-7 cells and Hs578T cells (5×10^4 cells/well) were cultured in the upper compartment of the transwell chamber (BD Biosciences) in serum-free medium. The lower chamber contains 600 μ l medium (containing 10% fetal bovine serum). The transwell chambers were coated with 50 μ l Matrigel, and cell invasion was evaluated at a dilution of 1:5 in serum-free DMEM. After transfection, place the transfected cells in the upper part of the chamber. Stain the cells for 48 hours. Finally, five random fields under the fluorescence microscope (Olympus Corporation) were selected for each group and had their cell numbers counted.

2.1.9 Cell apoptosis assay

Annexin V-FITC/PI Apoptosis Detection Kit (KeyGen Biotech) was used to detect cell apoptosis. After 24 hours of transfection, 1×10^5 cells were made into 100 μ l solution with culture medium and transferred to a centrifuge tube. Flow cytometry detection was performed for Annexin V-FITC and PI using a LSRFortessa-Flow Cytometer (Becton Dickinson).

2.1.10 Bioinformatics analysis

The expression of miR-324-5p in BC is searched for online database The Cancer Genome Atlas. The miR-324-5p target gene is combined with Targetscan, miRDB and miRtarbase databases. Inclusion criteria requires at least three databases. The expression of miR-324-5p and the correlation between such expression and BC were analyzed via StarBase and GEPIA.

2.1.11 Statistical analyses

Prism 9.0 software (GraphPad Software) was used to analyze the data. The relative quantification of miR-324-5p was calculated by the $2^{-\Delta\Delta Ct}$ method. SPSS was used for the COX regression analysis. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Results

3.1.1 MiR-324-5p expression in BC tissue

We first analyzed miR-324-5p expression in 1,103 BC patients included in The Cancer Genome Atlas (TCGA) database (Fig. 1A) to confirm whether the miR324-5P expression in BC was disordered. By comparing the results obtained from 41 BC breast lesion samples and 41 adjacent normal samples, we confirmed that miR-324-5p levels increased in BC (Fig. 1B). The miR-324-5P expression was relatively higher in patients with positive lymph node metastasis and advanced breast cancer (Fig. 1C–D). Overall, the data showed that miR-324-5p might promote BC progression.

3.1.2 Expression and diagnostic value of serum miR-324-5p in BC.

The miR-324-5p relative expression in BC serum ($n = 104$) and normal serum ($n = 50$) was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), which revealed that BC

serum contained higher miR-324-5p levels than the normal serum (Fig. 2A). After evaluating the potential of serum miR-324-5p levels as a diagnostic biomarker for BC, further evaluation results showed that the receiver operating characteristic (AUC) value for the miR-324-5p based diagnosis of BC was 0.7581 (95% confidence interval (CI: 0.6828- 0.8335), the sensitivity was 66%, and the specificity was 78%. (Figure 2B).

3.1.3 Correlation between clinicopathological features and miR-324-5p expression in patients with BC

We analyzed whether miR-324-5p in serum had significant differential expressions due to different clinicopathological features. Patients were categorized into a high expression (miR-324-5p expression in serum > 4.36) and a low expression (miR-324-5p expression in serum < 4.36) groups. The results showed that the high miR-324-5p expression was not related to age, tumor size and lymph node metastasis, human epithelial growth factor receptor 2, estrogen receptor, progesterone receptor or type, Ki-67 but is correlated with the specific TNM stage ($p=0.0291$) and age ($p=0.0278$) (Table 1).

3.1.4 Serum miR-324-5p prognostic value for BC

In survival analysis, patients with low miR-324-5p had a significantly better 5-year DFS ($p=0.027$; Figure 3A) and OS ($p=0.017$; Figure 3B) than the high miR-324-5p group. However, miR-324-5p (HR: 2.256; 95% CI: 1.06-4.802; $p=0.035$), TNM stage (HR: 3.622; 95% CI: 1.682-7.798; $p=0.001$), and Ki-67 (HR: 2.818; 95% CI: 1.164-6.826; $p=0.022$) were significantly associated with DFS. miR-324-5p (HR: 2.722; 95% CI: 1.061-6.983; $p=0.037$), TNM stage (HR: 3.791; 95% CI: 1.632-8.804; $p=0.002$), and Ki-67 (HR: 3.59; 95% CI: 1.136-11.345; $p=0.029$) which are independent prognostic factors for OS in the multivariate analysis (Table 2 and Table 3).

3.1.5 MiR-324-5p promoted the proliferation and inhibited apoptosis of BC cells

We further analyzed miR-324-5p expression in BC cells (MCF-7, Hs578T, and T-47D) and found a significant increase in miR-324-5p expression compared to human breast epithelial cells (MCF-10A) (Fig. 4A). The MCF-7 and Hs578T cell lines were transfected with agomiR-324-5p/antagomiR-324-5p to reveal whether miR-324-5p dysregulation would influence BC cell function and to induce miR-324-5p overexpression or inhibition. We transfected negative control miRNA (Fig. 4B). The CCK-8 assay and Cloning experiment result (Fig. 4C-D) showed that miR-324-5p overexpression enhanced the MCF-7 cells proliferation, while the growth potential of Hs578T cells with inhibited miR-324-5p expression was weakened. As expected, the Hs578T cells apoptosis level increased with miR-324-5p inhibition, while the MCF-7 cells apoptosis decreased with miR-324-5p overexpression (Fig. 4E-F). These results indicate that miR-324-5p may promote proliferation and inhibit BC cells apoptosis.

3.1.6 MiR-324-5p promoted BC cell migration and invasion

The wound healing results showed that the miR-324-5p up-regulation could stimulate wound closure after 24 h of scratch injury in MCF-7 cells. However, in Hs578T cells the inhibitory expression of miR-324-5p had a contrasting effect (Fig. 5A-B). In addition, attenuating the miR-324-5p expression can inhibit the

migration and invasion of HS578T cells, while a miR-324-5p mimic can enhance MCF-7 cells, as shown in Fig. 5C-D. These results indicate that miR-324-5p has a positive effect on BC cells migration and invasion.

3.1.7 MiR-324-5p target genes exploration

Next, our research on miR-324-5p explored the possible molecular regulation mechanisms. We used online bioinformatics databases to find potential miR-324-5p target genes. Ten genes appeared in the miDBR, TargetScan, and miRTarBase databases (Fig. 6A). These genes included CDP-diacylglycerol synthase 1 (CDS1), CUE domain-containing 2 (CUEDC2), cysteine-rich hydrophobic domain 1 (CHIC1), kruppel like factor 7 (KLF7), the mediator of cell motility 1 (MEMO1), ras-related nuclear protein (RAN), sublingual immunotherapy (SLIT), neurotrophic tropomyosin-receptor kinase (NTRK)-like family member 4 (SLITRK4), specific protein 1 (SP1), YOD1 deubiquitinase (YOD1), transmembrane and coiled-coil domain family 1 (TMCC1). There is an inverse correlation between target genes and miRNA expression. In the starBase database (Fig. 6B–K), we observed that CDS1, CHIC1, CUEDC2, KLF7, SP1, and SLITRK4 genes in BC were negatively correlated with miR-324-5p. We used the Kaplan-Meier plotter to evaluate miR-324-5p in relation to the six genes and their prognostic role in BC. In BC, a high expression of miR-324-5p indicates an adverse prognosis, but a high expression of CHIC1, KLF7, and CUEDC2 is a better prognostic indicator (Fig. 7A–E). Subsequently, the expression of these three genes in BC was analyzed with the Gene expression profiling interactive analysis (GEPIA) database, and the results showed that CHIC1 and KLF7 are valuable (Fig. 7F–H). However, CUEDC2 revealed no significant differences in its expression between BC and normal breast tissues. Based on these findings, we propose a potential miR-324-5p-mRNA network pathway named miR-324-5p-CHIC1/KLF7 to explore BC pathogenesis and progression further.

Discussion

BC is the most prevalent tumor that threatens the health of women worldwide [1]. Although there are increasingly new strategies to prevent BC, its incidence and recurrence rates are increasing, illustrating the critical need for early detection and treatment [2]. MiRNAs are widely expressed in eukaryotes, and they participate in tissues and organs development [16], involving the regulation of multiple functions, such as cell proliferation, apoptosis, invasive migration, metabolism, neuronal patterns, immune regulation, and cellular stress responses [17]. Thus, miRNAs have been identified as one of the important factors in human cancer [18]. Some studies report that many miRNAs are unbalanced in BC and either promote or inhibit BC occurrence and metastasis [19-21]. Research has shown that the expression of various miRNAs can be detected in 12 kinds of body fluids, including serum, urine, and saliva, suggesting that miRNA could be detected in a liquid biopsy [22]. Mitchell et al. [23] initially described serum miRNAs, and subsequent in-depth studies suggested that serum miRNA can be used as a biomarker for various malignant tumors, including BC. For example, the diagnostic specificity of miR-195, which is up-regulated in the patients with BC serum, is approximately 90% [24]. In addition, up-regulated serum miR-103 possesses 84% sensitivity and 70% specificity, demonstrating its diagnostic

potential [25]. D. LI found serum exosomal miR-148a as a novel prognostic biomarker for breast cancer [26]. These findings make serum miRNA a promising potential BC biomarker.

Studies have shown that miR-324-5p affects tumor occurrence and development by regulating target genes in various human cancers. MiR-324-5p promotes the progression of thyroid papillary cancer via the PTPRD/CEBPD [13]. In addition, miR-324-5p reduced the tumor protein expression, phosphatase, and tensin homolog and may improve gastric cancer's invasion and proliferation ability [14]. Furthermore, Wan et al. [15] discovered that miR-324-5p promoted pancreatic cancer progression due to the tumor suppressor gene expression and kruppel-like factor 3.

In this study, we discovered that the miR-324-5p expression in BC tissues increased significantly compared to the normal breast tissues for the first time. Furthermore, the miR-324-5p levels in the tissues of patients with BC and at an advanced stage of TNM or positive lymph node metastasis were higher than those in early-stage patients or negative lymph node metastasis. In addition, we noted that the expression trend of serum miR-324-5p levels was consistent with that in the tissues of patients with BC, suggesting a possibility of miR-324-5p as a BC biomarker. The role of serum miR-324-5P in the diagnosis and prognosis evaluation of breast cancer was explored. The receiver operating characteristic (AUC) of BC diagnosis based on miR-324-5p was 0.7208 and the sensitivity was 83%. The expression of miR-324-5p was related to TNM stage, progesterone receptor, tumor size and lymph node metastasis. In addition, patients with low expression of miR-324-5P significantly increased DFS and OS in 5 years. COX regression showed that miR-324-5p was an independent prognostic factor of OS in multivariate analysis of breast cancer. MiR-324-5P is a sensitive indicator, and its prognostic value is greater than the diagnostic value. The number of serum samples in this study was limited. As a result, the outcomes of the study need to be validated with a larger sample size.

In addition, the mechanism study of miR-324-5P on BC shows that it can promote proliferation, BC migration and inhibit apoptosis. MiRNAs inhibit the expression of multiple target genes. In this study, 10 genes from the target gene database were selected as candidates to validate miR-324-5p potential target genes and test the miR-324-5p predictive ability in BC. Furthermore, miR-324-5p was inversely correlated with CDS1, CHIC1, CUEDC2, KLF7, SLITRK4, and SP1, CHIC1, CUEDC2, and KLF7 were overexpressed and associated with better prognosis. In addition, we observed that CHIC1 and KLF7 from the GEPIA database were significantly lower in BC tissues than normal breast tissue. Currently, CHIC1-related diseases include ovarian epithelial tumors and Allan-Herndon-Dudley syndrome. [27]. KLF7 is known as the ubiquitous kruppel-like factor and is a key factor in the development of many cancers and an independent predictor of poor prognosis in several cancers [28]. In vitro, functional experiments have shown that KLF7 can affect tumor proliferation and migration [29–31]. Therefore, miR-324-5p may target CHIC1 and KLF7 to facilitate the progression of BC, resulting in a decreased survival rate of BC patients.

Conclusion

In conclusion, the serum miR-324-5p expression may be used as a biomarker for the BC diagnosis and prognosis. MiR-324-5p may promote the occurrence and development of BC cells by inhibiting the CHC1 and KLF7.

Declarations

Ethics approval and consent to participate

Written informed consent was signed by all breast cancer patients and volunteers. This study was approved by the Ethics Committee of Shaoxing People's Hospital and conducted according to the guidelines of Helsinki Declaration.

Consent for publication

Not applicable

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

JZ and AL have made great contributions to the concept and design. JZ and YZ are responsible for collecting specimens and analyzing clinical data. SL, HW and LG contributed to the writing, review and/or revision of manuscripts. And xdlz producer manuscript. All authors have read and approved the final manuscript.

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Tables

Table III Univariate and multivariate analysis of the prognostic variables influencing overall survival in breast cancer patients.				
Variables	Univariate analysis		Multivariate analysis	
	HR95%CI	p-value	HR95%CI	p-value
Age≤54 vs ≥55	0.9540.428-2.123	0.907	NA	NA
Tumor size(≤2 vs ≥2)	2.391(0.991-5.767)	0.052	NA	NA
Lymph node metastasisyes vs no	2.242(0.996-5.049)	0.051	NA	NA
TNM stageI-II vs III-IV	4.387(1.911-10.068)	<0.0001	3.791(1.632-8.804)	0.002
ER status(negative vs positive)	0.6870.284-1.659	0.404	NA	NA
PR status(negative vs positive)	0.3920.174-0.883	0.024	0.684(0.282-1.655)	0.399
HER2 status(negative vs positive)	0.6550.244-1.756	0.4	NA	NA
TypeLUM vs NLUM	1.996(0.984-4.048)	0.055	NA	NA
Ki-67(≤30 vs ≥30)	3.993(1.364-11.686)	0.012	3.59(1.136-11.345)	0.029
miR-324-5P expression(low vs high)	3.224(1.279-8.128)	0.013	2.722(1.061-6.983)	0.037
HR:hazard ratio;CI:confidence interval; TNM:tumor-node-metastasis classification system; ER:Estrogen receptor;PR:Progesterone receptor;HER2:Epidermal growth factor receptor 2;NA:not analyzed.				

Figures

Figure 1

Table 1 Clinicopathological variables and the serum expression of miR-324-5p in BC

Factors	Total(n=100)	miR-324-5p expression		<i>p</i> -value
		High(n=50)	Low(n=50)	(chi-square test)
Age				
≤ 54 years	49	30	19	0.0278
> 54 years	51	20	31	
Tumor size				
≤ 2cm	47	23	24	0.8412
>2cm	53	27	26	
Lymph node metastasis				
Yes	41	23	18	0.3093
No	59	27	32	
TNM				
I/II	84	38	46	0.0291
III	16	12	4	
ER status				
Negative	24	12	12	>0.9999
Positive	76	38	38	
PR status				
Negative	39	23	16	0.1512
Positive	61	27	34	
HER2				
Negative	15	10	5	0.1614
Positive	85	40	45	
Type				
LUM	76	37	39	0.6396
NLUM	24	13	11	
Ki-67				
<30%	42	20	22	0.6853

HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; LUM, luminal;NLUM,NO-luminal

Table II Univariate and multivariate analysis of the prognostic variables influencing disease-free survival in breast cancer patients.				
	Univariate analysis		Multivariate analysis	
Variables	HR95%CI	p-value	HR95%CI	p-value
Age≤54 vs ≥55	1.0460.522-2.094	0.9	NA	NA
Tumor size(≤2 vs ≥2)	1.9030.917-3.949	0.084	NA	NA
Lymph node metastasisyes vs no	1.3870.692-2.777	0.356	NA	NA
TNM stageI-II vs III-IV	3.5011.652-7.421	0.001	3.6221.682-7.798	0.001
ER status(negative vs positive)	0.5850.227-1.236	0.16	NA	NA
PR status(negative vs positive)	0.4530.226-0.909	0.026	0.6260.3-1.308	0.213
HER2 status(negative vs positive)	0.6880.283-1.672	0.409	NA	NA
TypeLUM vs NLUM	1.9120.993-3.682	0.053	NA	NA
Ki-67(≤30 vs ≥30)	2.9941.294-6.926	0.01	2.8181.164-6.826	0.022
miR-324-5P expression(low vs high)	2.4731.17-5.227	0.018	2.2561.06-4.802	0.035
HR:hazard ratio;CI:confidence interval; TNM:tumor-node-metastasis classification system; ER:Estrogen receptor;PR:Progesterone receptor;HER2:Epidermal growth factor receptor 2;NA: not analyzed.				

The dysregulated performance of miR-324-5p in BC tissue.

(A) In the TCGA database, the expression results of miR-324-5p in BC tissues. (B) Analysis of miR-324-5p expression difference between BC and paracancerous tissues in 41 pairs. (C) The expression difference of miR-324-5p between negative and positive lymph node metastasis (D) The expression difference of miR-324-5p in early and late stages.

Figure 2

Expression and diagnostic value of serum miR-324-5p in BC

(A) The abundance of miR-324-5p in BC serum and normal serum, measured by RT-qPCR. (B) The ROC analysis of miR-324-5p for the diagnosis of BC.

Figure 3

Relationship between miR-324-5P and survival.

(A) The disease-free survival in the miR-324-5P low expression group was higher than that in the miR-324-5P high expression group. (B) The overall survival in the miR-324-5P low expression group was higher than that in the miR-324-5P high expression group.

Figure 4

Study on the effect of miR-324-5p on proliferation and apoptosis of BC cells. (A) The expression of miR-324-5p in breast epithelial cells (MCF-10A) and tumor cell lines (MCF-7, T-47D, Hs578T). (B) Validation of the transfection efficacy of the overexpression/inhibition of miR-324-5p. (C–D) The Cell Counting Kit-8 detects the proliferation level. (E–F) The result of detecting apoptotic rates using flow cytometry.

Figure 5

The effect of miR-324-5p in promoting cancer migration and invasion [A–B] The effect of miR-324-5p on the wound healing ability of Hs578T and MCF-7 cells for 24 h, determined by a scarification test. [C–D] The effect of miR-324-5p on the invasive and migratory abilities of Hs578T and MCF-7 cells for 24 h, observed by transwell assays.

Figure 6

Identification of miR-324-5p targeted genes in BC. (A) Venn diagram of the intersection of three target gene prediction databases. (B–K) Correlation data between target genes and miR-324-5p in the starBase database.

Figure 7

Further authentication of the targeting relationship between target genes and miR-324-5p. A–E The prognostic analysis of miR-324-5p in relation to CDS1, CHIC1, CUEDC2, KLF7, SP1, and SLITRK4 shown by the Kaplan–Meier plotter. F–H The expression of target genes (CUEDC2, KLF7, and CHIC1) in BC, obtained from the GEPIA database.