MicroRNA-1297 contributes to tumor growth of human breast cancer by targeting PTEN/PI3K/AKT signaling

CHAO LIU^{1*}, ZHIKUI LIU^{2*}, XIAO LI³, XIAOJIANG TANG³, JIANJUN HE³ and SHAOYING LU¹

Departments of ¹Vascular Surgery, ²Hepatobiliary Surgery, and ³Breast Surgery,
The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P.R. China

Received February 17, 2017; Accepted July 24, 2017

DOI: 10.3892/or.2017.5884

Abstract. Increasing evidence confirms that aberrant miRNA expression contributes to breast cancer (BC) development and progression. However, the roles of different miRNAs in BC remain to be explored. In the present study, we demonstrated that miR-1297 expression was increased in BC tissues and cell lines. Our clinical analysis revealed that the upregulated miR-1297 expression was significantly correlated with poor prognostic features including advanced TNM stage and larger tumor size. Moreover, we found that miR-1297 was a novel independent prognostic marker for predicting 5-year survival of BC patients. The ectopic overexpression of miR-1297 promoted cell proliferation, cell cycle progression and inhibited apoptosis while miR-1297 knockdown reversed the effect. In addition, miR-1297 modulated PTEN by directly binding to its 3'-UTR, resulting in activation of AKT signaling. In clinical samples of BC, miR-1297 inversely correlated with PTEN, which was down-regulated in BC. Alternation of PTEN expression or AKT inhibitor at least partially abolished the biological effects of miR-1297 on BC cells. In conclusion, our results indicated that miR-1297 functioned as an oncogene in regulating the proliferation, cell cycle and apoptosis of BC via targeting PTEN/PI3K/AKT signaling, and may represent a novel potential therapeutic target and prognostic marker for BC.

Introduction

Breast cancer (BC) is one of the most common malignancies and causes 15% of cancer-related deaths among females worldwide (1,2). Due to recent advances in clinical treatment and substantial experimental effort, the mortality rate of breast cancer obviously decreased in the past decades. However, the incidence of BC is increasing and the long-term survival of BC patient remains poor because of its heterogeneity and cancer recurrence and metastatic relapse (3). However, the discriminant prognostic predictors and detailed mechanism underlying the progression of breast cancer remains poorly elucidated (4). Therefore, it is urgent to illustrate the molecular mechanisms of BC and identify new biomarkers to develop novel therapeutic targets and improve the prognosis of BC patients.

Recently, emerging evidence has demonstrated that microRNAs (miRNAs), a group of endogenous evolutionarily conserved non-coding small RNAs, are identified as therapeutic biomarkers with diagnostic and prognostic potential (5). Dysregulation of miRNAs play either oncogenic or tumor suppressor roles in cancer initiation, growth and progression by interacting with complementary sequences within the 3'-untranslated region (UTR) of target mRNA to induce mRNA degradation or suppress translation (6-8). Increasing studies confirm that aberrant miRNAs play critical roles in diverse biological progresses in breast cancer, including cell proliferation, apoptosis, drug-resistance, metastasis and stem cell renewal and have been recognized as promising prognostic biomarkers in BC diagnosis and treatment (9,10).

miR-1297, a novel cancer-related microRNA, has been found to play a vital role in the pathogenesis of human cancers (11-15). miR-1297 promotes apoptosis and inhibits the proliferation and invasion of hepatocellular carcinoma cells by targeting HMGA2 or EZH2 (16,17). MicroRNA-1297 inhibits prostate cancer cell proliferation and invasion by targeting the AEG-1/Wnt signaling pathway (18). Moreover, miR-1297 regulates the growth, migration and invasion of colorectal cancer cells by targeting cyclo-oxygenase-2 (19). However, miR-1297 mediates PTEN expression and contributes to cell progression in laryngeal squamous cell carcinoma (20). In addition, miR-1297 regulates growth of testicular germ cell tumor through PTEN/PI3K/AKT pathway (21,22). Therefore, the functional roles of miR-1297 in human cancers are cancer-

Correspondence to: Professor Shaoying Lu, Department of Vascular Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, 277 Yanta West Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: lusy_xjtu@126.com

Professor Jianjun He, Department of Breast Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, 277 Yanta West Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: chinahjj@163.com

*Contributed equally

Key words: microRNA-1297, breast cancer, PTEN, proliferation, apoptosis

type specific. Nevertheless, the functional importance of miR-1297 and the molecular mechanisms in breast cancer are still unclear.

In the present study, we investigated the expression and biological role of miR-1297 in breast cancer progression. Our results showed that miR-1297 was significantly upregulated in breast cancer tissues and cells. Its ectopic expression was associated with poor clinicopathological features and poor survival of BC patients. Gain- and loss-of-function experiment revealed that miR-1297 promoted breast cancer cell proliferation and cell cycle progression and apoptosis resistance *in vitro*. Furthermore, miR-1297 knockdown inhibited the tumor growth of BC *in vivo*. Notably, phosphatase and tensin homolog (PTEN) was identified as direct targets of miR-1297, resulting in activation of AKT signaling in cell growth.

Materials and methods

Clinical specimens. BC tissues (116) and matched adjacent non-tumor tissues were obtained from our hospital during January 2004 to December 2011. Pathological diagnosis was performed according to the World Health Organization (WHO) criteria. None of the patients received chemotherapy or radiotherapy before surgery. All patients had written informed consent and the present study was approved by the Ethics Committee of Xi'an Jiaotong University.

The human BC cell lines T47-D, MCF-7, MDA-MD-231, MDA-MB-453, BT-549 and the normal mammary epithelial cell line MCF-10A were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China) and were cultured in complete Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen), 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) in a humidified atmosphere at 37°C with 5% CO₂.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Total RNA from BC tissues and cells was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was reverse-transcribed from 2 µg total RNA using a Reverse Transcription kit (Takara Bio, Tokyo, Japan). cDNA was then amplified with a SYBR® Premix Ex Taq™ II (Perfect Real-Time) kit (Takara). The gene expression levels were calculated using the $\Delta\Delta C_t$ method with U6 or GAPDH as an internal control. Hsa-miR-1297 primer was synthesized by Sangon Biotech, Co., Ltd., (Shanghai, China), snRNA U6 qPCR Primer (HmiRQP9001), FAK (HQP015535) and GAPDH (HQP006940) were purchased from GeneCopoeia (Guangzhou, China).

Cell transfection. miRNA vectors, including miR-1297 expression vector, the control vector for miR-1297, miR-1297 inhibitor and the negative control were synthesized by Shanghai GenePharma, Co., Ltd. (Shanghai, China). The PTEN overexpression plasmid and specific siRNA against PTEN and a scramble siRNA were synthesized by Sangon Biotech. Cells were transfected with the above vectors using Lipofectamine 2000 reagent (Invitrogen-Life Technologies) in accordance with the manufacturer's protocol.

Western blot analysis. The whole proteins were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors (Roche) and the concentrations were quantified with BCA protein assay kit (Tiangen Biotech, Co., Ltd., Beijing, China), and an equal amount of 40 µg protein was separated by 10% SDS-PAGE gel and then transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk in TBST for 2 h at room temperature and incubated overnight with specific primary antibodies (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) at 4°C. Then the membranes were washed three times by TBST and incubated with HRP-conjugated secondary antibody for 2 h at room temperature (ZSGB-Bio, Beijing, China). Detection was performed by enhanced chemiluminescence kit (Amersham, Little Chalfont, UK). GAPDH was used as protein loading control. The antibodies against PTEN, cyclin D1, p27, AKT and p-AKT were purchased from Cell Signaling Technology.

Cell proliferation, cell cycle and apoptosis detection. For the proliferation assay, cells were seeded in 24-well plate and grown on coverslips (Thermo Fisher Scientific, Pittsburgh, PA, USA) were incubated with BrdU for 1.5 h and then stained with anti-BrdU antibody (Sigma-Aldrich) according to the manufacturer's instruction. The images were taken under a laser scanning microscope (Axioskop 2 plus; Carl Zeiss GmbH, Jena, Germany). Flow cytometry was performed using the fluorescence-activated cell sorting (FACS) Calibur and CellQuest software (both from Becton-Dickinson, San Jose, CA, USA). For cell cycle assay, the cells were seeded in 6-well plates at 2×10^5 /well. Forty-eight hours after the transfection, the cells were fixed in 70% ethanol at 4°C for 24 h and stained with 50 µg/ml propidium iodide (PI; Nanjing KeyGen Biotech, Co., Ltd., Nanjing, China). An Annexin V-Fluos staining kit (Roche) was used to analyze apoptosis levels.

Luciferase reporter assay. The 3'-UTR sequence of PTEN predicted to interact with miR-1297, together with a corresponding mutated sequence within the predicted target sites, were synthesized and inserted into the pmiR-GLO Dual-luciferase miRNA target expression vector (Promega, Madison, WI, USA) called wt-PTEN 3'-UTR and mt-PTEN 3'-UTR. Subsequently, MCF-7 cells that were plated into 24-well plate and were transfected with miR-1297 inhibitor or negative control. Cells were co-transfected with the wild-type or mutant 3'-UTR of PTEN vector using the Lipofectamine 2000 reagent (Invitrogen). After 48 h, cells were harvested and measured according to the manufacturer's instructions (Dual-luciferase assay system; Promega). pRL-TK expressing *Renilla* luciferase was cotransfected as an internal control to correct the differences in both transfection and harvest efficiencies.

In vivo experiments. Four-to-six-week-old female BALB/c nude mice (Centre of Laboratory Animals, The Medical College of Xi'an Jiaotong University, Xi'an, China) were used to establish the nude mouse xenograft model. MCF-7 (5×10^6) cells that were transfected with miR-1297 or miR-control vectors were mixed in 150 µl of Matrigel and were inoculated subcutaneously into the flank of nude mice. The tumor volume for each mouse was determined by measuring two of its dimensions and then calculated as tumor volume = length

Figure 1. miR-1297 is highly expressed in BC tissues and cell lines. (A) Relative miR-1297 expression levels in BC tissues and matched adjacent non-tumor tissues were determined by qRT-PCR (n=116). (B) The expression of miR-1297 in five BC cell lines was significantly increased compared to that in the MCF-10A cells. U6 snRNA was used as internal control. *P<0.05, **P<0.01.

x width x width/2. After 3 weeks, the mice were sacrificed by cervical dislocation under anesthesia with ether and the xenograft tumor tissue was explanted for examination. Animal protocols were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University.

Statistical analysis. Data are presented as the mean $\pm$ SD and performed at least three independent replicates. SPSS software, 16.0 (SPSS, Inc., Chicago, IL, USA) and Graphpad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for a two-tailed Student's t-test, Pearson's correlation analysis, Kaplan-Meier method and the log-rank test to evaluate the statistical significance. Differences were defined as P<0.05.

Results

miR-1297 is significantly increased in breast cancer specimens and cells. To explore the potential role of miR-1297 in breast cancer, we first performed qRT-PCR to determine the expression in 116 pairs of breast cancer tissues and corresponding tumor-adjacent tissues. The data revealed that the mean level of miR-1297 in BC tissues was significantly higher than that in the tumor-adjacent tissues (P<0.01; Fig. 1A). Furthermore, we assessed miR-1297 expression in cell lines. All BC cell lines (T-47D, MCF-7, MDA-MB-231, MDA-MB-453 and BT-549) exhibited high expression as compared to the normal mammary epithelial cell line MCF10A (P<0.05; Fig. 1B). These results indicated that miR-1297 may be involved in the development of breast cancer.

Clinical significance of increased miR-1297 in BC tissues. We set the median level of miR-1297 as a cut-off value to distinguish

Table I. Correlation between miR-1297 expression and clinicopathological characteristics in breast cancer (n=116).

Clinical parameters	Cases (n)	Expression level		P-value
		miR-1297 ^{high} (n=64)	miR-1297 ^{low} (n=52)	
Age (years)				0.897
<50	61	34	27	
$\geq$ 50	55	30	25	
Tumor size (cm)				0.005 ^a
<2	78	36	42	
$\geq$ 2	38	28	10	
Tumor location				0.964
Left	89	49	40	
Right	27	15	12	
Differentiation				0.599
Moderate/high	70	40	30	
Poor	46	24	22	
T stage				0.806
I/II	81	43	38	
III/IV	35	21	14	
TNM stage				0.013 ^a
I/II	93	46	47	
III/IV	23	18	5	
ER status				0.485
Negative	64	37	27	
Positive	52	27	25	
Her2 status				0.361
Negative	58	33	25	
Positive	58	31	27	
PR status				0.947
Negative	42	23	19	
Positive	74	41	33	

^aP<0.05 statistically significant.

different subgroups to investigate the relationship between the miR-1297 expression and the clinical characteristics and prognosis of breast cancer patients. The high miR-1297 expression was obviously correlated with tumor node metastasis (TNM) stage (III+IV; P=0.013) and large tumor size ($>$ 2 cm, P=0.005) (Table I). Moreover, Kaplan-Meier survival curves suggest that high miR-1297 expression was markedly associated with shorter overall survival (OS, P=0.0035; Fig. 2A) and progression-free survival (PFS, P=0.0069; Fig. 2B) in BC patients. In addition, miR-1297 was an independent factor for predicting both 5-year OS and PFS in BC patients (P=0.002, P=0.009, respectively; Table II). These data reinforced miR-1297 as a potential biomarker for the prognosis outcome of breast cancer patients.

miR-1297 promotes cell proliferation, cell cycle progression and inhibits apoptosis of breast cancer cells. To investigate

Figure 2. The prognostic value of miR-1297 for BC patients assessed by the Kaplan-Meier analysis. BC patients with high expression of miR-1297 had worse (A) overall survival (OS) and (B) progression-free survival (PFS). ** $P < 0.01$.

Figure 3. miR-1297 promotes BC cell proliferation, cell cycle progression and inhibits cell apoptosis. (A) MCF-7 cells and MDA-MB-453 cells transfected with corresponding miRNA vectors were subjected to qRT-PCR for miR-1297. Overexpression of miR-1297 promoted cell proliferation (B), cell cycle progression (C) and inhibited apoptosis (D) in MCF-7 cells, while downregulation of miR-1297 inhibited cell proliferation (B), cell cycle progression (C) and promoted apoptosis (D) in MDA-MB-453 cells. $n = 6$ independent experiments. * $P < 0.05$, ** $P < 0.01$.

the biological role of miR-1297 in the progression of BC, we transduced BC cell lines with miR-1297 expression vector or anti-miR-1297 vector which contained different endogenous miR-1297 levels. As measured by qRT-PCR, we confirmed that miR-1297 effectively upregulated miR-1297 in MCF-7 ($P < 0.05$; Fig. 3A) or downregulated miR-1297 in MDA-MB-453 cells ($P < 0.05$; Fig. 3A). As determined by BrdU incorporation

assays and flow cytometric analysis, miR-1297 overexpression significantly promoted cell proliferation ($P < 0.05$; Fig. 3B) and cell cycle progression ($P < 0.05$; Fig. 3C) of MCF-7 cells, otherwise the percentage of apoptotic cells was obviously decreased ($P < 0.05$; Fig. 3D), whereas miR-1297 knockdown obviously inhibited cell proliferation ($P < 0.05$; Fig. 3B) and cell cycle progression ($P < 0.05$; Fig. 3C) of MDA-MB-453 cells, but the

Table II. Multivariate Cox regression analysis of 5-year OS and PFS of 96 GC patients.

Variables	Overall survival			Progression-free survival		
	HR	95% CI	P-value	HR	95% CI	P-value
miR-1297	4.845	1.862-12.054	0.002 ^a	3.945	1.364-10.135	0.009 ^a
Tumor size	3.324	1.372-7.689	0.014 ^a	1.223	1.029-5.258	0.023 ^a
TNM stage	3.194	1.426-7.846	0.007 ^a	1.748	1.212-4.513	0.018 ^a

HR, hazard ratio; CI, confidence interval; ^aP<0.05 statistically significant.

Figure 4. PTEN was identified as a direct target of miR-1297 in BC. (A) miR-1297 and its putative binding sequence in the 3'-UTR of PTEN. The mutant binding site was generated in the complementary site for the seed region of miR-1297. (B) miR-1297 significantly suppressed the luciferase activity that carried wild-type (WT) but not mutant (MUT) 3'-UTR of PTEN. (C) qRT-PCR analysis of PTEN mRNA expression in MCF-7 cells with miR-1297 or miR-control vector transfection and MDA-MB-453 cells with anti-miR-1297 or anti-miR-NC vector transfection. (D) Overexpression of miR-1297 reduced the expression of PTEN protein in MCF-7 cells and knockdown of miR-1297 increased the level of PTEN protein in MDA-MB-453 cells. (E) The expression of PTEN mRNA in miR-1297 high-expressing tumors was significantly lower than that in miR-1297 low-expressing tumors. (F) The expression of PTEN protein in miR-1297 high-expressing tumors was significantly lower than that in miR-1297 low-expressing tumors. n=six repeats with similar results, *P<0.05.

percentage of apoptotic cells was markedly increased (P<0.05; Fig. 3D). In conclusion, these results revealed that miR-1297 could regulate cell proliferation, cell cycle and apoptosis of BC cells.

miR-1297 directly targets PTEN in BC cells. To analyze the mechanism of miR-1297 on the regulation in BC cells, we used miRNA prediction bioinformatic algorithms (TargetScan) to identify the target genes of miR-1297 and

Figure 5. Alterations of PTEN partially abolish miR-1297-mediated BC cell proliferation, cell cycle progression and apoptosis. (A) miR-1297-overexpressing MCF-7 cells transfected with EV or PTEN expression plasmid and miR-1297-suppressed MDA-MB-453 cells that were transfected with control siRNA or PTEN siRNA were subjected to western blot analysis for PTEN. Three repeats with similar results. The correlations between miR-1297 effects and PTEN knockdown or overexpression are shown in the (B) cell proliferation, (C) cell cycle progression and (D) apoptosis. PTEN knockdown abrogated the effects of miR-1297 knockdown on MDA-MB-453 cells. PTEN overexpression induced effects that were opposite to those stimulated by miR-1297. Three independent experiments. * $P < 0.05$.

found that PTEN 3'-UTR had putative miR-1297 target sites (Fig. 4A). To validate that PTEN was a direct target of miR-1297, our luciferase reporter assay confirmed that the ectopic overexpression of miR-1297 significantly decreased luciferase activity of wild-type (wt) PTEN 3'-UTR, compared with mutant-type (mt) PTEN 3'-UTR ($P < 0.05$; Fig. 4B). In contrast, miR-1297 knockdown increased the luciferase activity of wt PTEN 3'-UTR ($P < 0.05$; Fig. 4B) but had no effect on mt PTEN 3'-UTR. Furthermore, overexpression of miR-1297 obviously inhibited PTEN mRNA and protein levels in MCF-7 cells, while the downregulation of miR-1297 markedly increased PTEN mRNA and protein expression in MDA-MB-453 cells ($P < 0.05$; Fig. 4C and D). In addition, we confirmed the relationship between miR-1297 expression and PTEN in BC tissues. Our data showed that the mRNA and protein of PTEN in the miR-1297 high-expressing cancer tissues were significantly lower than those in the miR-1297 low-expressing cancer tissues ($P < 0.05$, respectively; Fig. 4E and F). Taken together, the results demonstrated that miR-1297 directly binds to PTEN 3'-UTR and regulates its expression in BC cells.

Functional importance of PTEN in miR-1297-mediated biological effect. To further explore the biological function of PTEN in miR-1297-mediated effect on BC cells, we restored PTEN expression in MCF-7-miR-1297 cells by transfecting PTEN expression plasmid ($P < 0.05$; Fig. 5A). Functionally, PTEN overexpression inhibited cell proliferation, cell cycle progression and promoted cell apoptosis ($P < 0.05$; Fig. 5B-D). In contrast, PTEN knockdown by a specific siRNA in miR-1297-suppressed MDA-MB-453 cells ($P < 0.05$; Fig. 5A)

significantly increased cell proliferation, cell cycle progression and suppressed cell apoptosis ($P < 0.05$; Fig. 5B-D). Above results suggest that PTEN is a downstream mediator in the function of miR-1297 in BC.

PTEN/PI3K/AKT signaling mediated by miR-1297 is involved in the biological function of breast cancer cells. Previous studies confirmed that PTEN, as a tumor suppressor gene, could negatively regulate the activation of PI3K/AKT signaling and play a critical role in the development and progression of BC (23). As shown in Fig. 6A, overexpression of miR-1297 significantly increased, while miR-1297 knockdown decreased the AKT phosphorylation in BC cells ($P < 0.05$; Fig. 6A). but the total AKT protein had no change ($P < 0.05$; Fig. 6A). Moreover, the downstream effectors of PI3K/AKT, cyclin D1 and p27, were also significantly changed by the miR-1297 expression ($P < 0.05$; Fig. 6A). These results revealed that miR-1297 promoted the PI3K/AKT pathway in BC cells. To investigate whether AKT phosphorylation mediated miR-1297-induced promotion of cell proliferation, cell cycle progression and apoptosis inhibition in BC cells, we treated miR-1297-overexpressing MCF-7 cells with the inhibitor of AKT phosphorylation MK2206. We found that MK2206 at least partially inhibited the miR-1297-induced promotion of cell proliferation, cell cycle progression and apoptosis inhibition in BC cells ($P < 0.05$; Fig. 6B-E). Conversely, the insulin-like growth factor 1 (IGF-1), which is an activator of PI3K/AKT pathway, rescued the effects of miR-1297 knockdown on cell proliferation, cell cycle progression and apoptosis inhibition ($P < 0.05$; Fig. 6B-E) in miR-1297-suppressed MDA-MB-453 cells. In conclusion, our results indicate that PI3K/AKT signaling plays an essential

Figure 6. PI3K/AKT signaling is essential for the biological function of miR-1297 in BC. (A) Western blot analysis of p-Akt (Ser473), total Akt, cyclin D1 and p27 protein levels in MCF-7-miR-1297 or MDA-MB-453-anti-miR-1297 cells. Quantification of cell proliferation (B), cell cycle progression (C) and apoptosis (D) of MDA-MB-453-anti-miR-1297 treated with 100 ng/ml IGF-1 for 24 h or MCF-7 cells stably expressing miR-1297 treated with 1 μ M MK2206 for 24 h. (E) Western blot analysis of indicated proteins in MDA-MB-453 cells stably expressing anti-miR-1297 treated for 24 h with 100 ng/ml IGF-1 or MCF-7 cells stably expressing miR-1297 inhibitor treated with 1 μ M MK2206 for 24 h. * P <0.05.

role in miR-1297-mediated BC cell proliferation, cell cycle progression and apoptosis inhibition.

miR-1297 promotes the growth of BC cells in vivo. To validate miR-1297 as an oncogene in BC cells, we conducted subcutaneous tumor formation assay by miR-1297 overexpressing MCF-7 cells. The tumor growth curve indicated that miR-1297 overexpression significantly promoted the tumor growth of BC cells *in vivo* (P <0.001; Fig. 7A and B). Moreover, the tumor weight of ectopic expression of miR-1297 group was larger than that in the control group (P <0.05; Fig. 7C). Notably, miR-1297 overexpression in subcutaneous models (P <0.05, Fig. 7D), promoted the activation of PI3K/Akt signaling (P <0.05; Fig. 7E). In conclusion, these data indicated that miR-1297 promotes the growth of BC cells by regulating the PI3K/AKT pathway.

Discussion

Increasing evidence has confirmed that aberrant miRNA expression plays a crucial role in carcinogenesis and progression of breast cancer (24). miRNAs have been identified as novel prognostic biomarkers and effective therapeutic targets of BC (25). Therefore, searching for new cancer-related miRNAs and elucidation of their molecular mechanisms in the regulating biological function of cancers are urgent. In previous studies, miR-1297 was shown to promote apoptosis and inhibit the proliferation, migration and invasion of hepatocellular carcinoma cells by directly targeting HMGA2 (16). Liang *et al* (18) demonstrated that microRNA-1297 inhibits prostate cancer cell proliferation and invasion by targeting the AEG-1/Wnt signaling pathway. However, Li *et al* (20) showed that miR-1297 mediates PTEN expression and contributes to

Figure 7. miR-1297 promotes BC growth by activating Akt signaling *in vivo*. (A) Representative pictures of BC xenografts from both MCF-7-miR-1297 (upper panel) and MCF-7-miR-control cells (lower panel). Tumor growth curve (B) and weight (C) revealed that miR-1297 overexpression significantly promoted tumor growth *in vivo*. (D) Expression of miR-1297 in MCF-7-miR-1297 treated tumors from the subcutaneous model. (E) Expression of p-Akt, Akt, cyclin D1, p27 were assessed in the subcutaneous model by western blotting. * $P < 0.05$, ** $P < 0.01$.

cell proliferation, migration and tumor genesis in laryngeal squamous cell carcinoma. miR-1297 induces cell proliferation by targeting PTEN in testicular germ cell tumor cells (22). Hence, the functional significance of miR-1297 in cancer initiation and progression was cancer-type specific.

In the present study, we report that the mean level of miR-1297 was significantly upregulated in 116 BC tissues compared to matched adjacent non-tumor tissues. Moreover, the phenomenon was also found in BC cells. Elevated miR-1297 expression was obviously correlated with malignant clinicopathological features of BC patients, including advanced TNM stage and larger tumor size. In addition, we found that higher miR-1297 group had a worse 5-year OS and PFS for BC patients. Multivariate Cox regression analysis suggest that miR-1297 was an independent prognostic biomarker for predicting clinical outcome of BC patients. Taken together, these data indicated that miR-1297 is vital for prognosis of BC patients. Functionally, gain- and loss-function assays revealed that miR-1297 promoted cell proliferation, cell cycle progression and inhibited cell apoptosis, at least partially by targeting PTEN mediated PI3K/AKT signaling pathway *in vitro* and *in vivo*. In addition, miR-1297 was inversely associated with PTEN expression, which was decreased in BC tissues. Moreover, miR-1297 could negatively modulate PTEN accumulation in BC cells. In conclusion, these results demonstrated that miR-1297 functions as an oncogene in the cell proliferation, cell cycle progression and apoptosis inhibition of BC by directly inhibiting PTEN/PI3K/AKT pathway.

Accumulating studies demonstrated that the activation of PI3K/AKT signaling pathway was involved in carcinogenesis, development and progression of breast cancer and regulate the malignant biological function of BC, including cell proliferation, migration, invasion and apoptosis (26,27). PTEN, which is a negative modulator of PI3K/AKT pathway, was decreased in BC tissues because of gene mutation or deletion (28). PTEN/

PI3K/AKT protein expression is related to clinicopathological features and prognosis in BC with axillary lymph node metastases (29). Additionally, we found that miR-1297 promoted cell cycle regulator cyclin D1 and inhibited p27 through the PTEN/PI3K/AKT pathway. These results suggest the exact role of miR-1297 in breast cancer.

In conclusion, we demonstrated that miR-1297 was upregulated in BC tissues and cell lines, and its elevated expression was correlated with malignant clinicopathological features. Furthermore, we confirmed that miR-1297 promoted cell proliferation, cell cycle progression and apoptosis inhibition by directly targeting PTEN mediated PI3K/AKT signaling pathway. These results suggest that miR-1297 is a potential oncogene biomarker in BC. In summary, the deregulation of miR-1297 may play an important role in tumor growth and may be a novel prognostic factor and potential therapeutic target for BC.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 65: 87-108, 2015.
2. Perou CM, Sørli T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, *et al*: Molecular portraits of human breast tumours. *Nature* 406: 747-752, 2000.
3. Sørli T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, *et al*: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98: 10869-10874, 2001.
4. Taneja P, Maglic D, Kai F, Zhu S, Kendig RD, Fry EA and Inoue K: Classical and novel prognostic markers for breast cancer and their clinical significance. *Clin Med Insights Oncol* 4: 15-34, 2010.
5. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
6. Winter J, Jung S, Keller S, Gregory RI and Diederichs S: Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 11: 228-234, 2009.

7. van Rooij E: The art of microRNA research. *Circ Res* 108: 219-234, 2011.
8. Rosa A and Brivanlou AH: MicroRNAs in early vertebrate development. *Cell Cycle* 8: 3513-3520, 2009.
9. Ren Y, Chen Y, Liang X, Lu Y, Pan W and Yang M: MiRNA-638 promotes autophagy and malignant phenotypes of cancer cells via directly suppressing DACT3. *Cancer Lett* 390: 126-136, 2017.
10. Zhan MN, Yu XT, Tang J, Zhou CX, Wang CL, Yin QQ, Gong XF, He M, He JR, Chen GQ, *et al*: MicroRNA-494 inhibits breast cancer progression by directly targeting PAK1. *Cell Death Dis* 8: e2529, 2017.
11. Wang C, Li Q, Liu F, Chen X, Nesa EU, Guan S, Liu B, Han L, Tan B, Wang D, *et al*: Serum miR-1297: a promising diagnostic biomarker in esophageal squamous cell carcinoma. *Biomarkers* 21: 517-522, 2016.
12. Ju HQ, Lu YX, Chen DL, Tian T, Mo HY, Wei XL, Liao JW, Wang F, Zeng ZL, Pelicano H, *et al*: Redox regulation of stem-like cells through the CD44v-xCT axis in colorectal cancer: Mechanisms and therapeutic implications. *Theranostics* 6: 1160-1175, 2016.
13. Zhang C, Chi YL, Wang PY, Wang YQ, Zhang YX, Deng J, Lv CJ and Xie SY: miR-511 and miR-1297 inhibit human lung adenocarcinoma cell proliferation by targeting oncogene TRIB2. *PLoS One* 7: e46090, 2012.
14. Wang J, Xu X, Mo S, Tian Y, Wu J, Zhang J and Zhao J: Involvement of microRNA-1297, a new regulator of HMGA1, in the regulation of glioma cell growth in vivo and in vitro. *Am J Transl Res* 8: 2149-2158, 2016.
15. Wu XJ, Pu XM, Zhao ZF, Zhao YN, Kang XJ, Wu WD, Zou YM, Wu CY, Qu YY, Zhang DZ, *et al*: The expression profiles of microRNAs in Kaposi's sarcoma. *Tumour Biol* 36: 437-446, 2015.
16. Liu Y, Liang H and Jiang X: MiR-1297 promotes apoptosis and inhibits the proliferation and invasion of hepatocellular carcinoma cells by targeting HMGA2. *Int J Mol Med* 36: 1345-1352, 2015.
17. Liu F, He Y, Shu R and Wang S: MicroRNA-1297 regulates hepatocellular carcinoma cell proliferation and apoptosis by targeting EZH2. *Int J Clin Exp Pathol* 8: 4972-4980, 2015.
18. Liang X, Li H, Fu D, Chong T, Wang Z and Li Z: MicroRNA-1297 inhibits prostate cancer cell proliferation and invasion by targeting the AEG-1/Wnt signaling pathway. *Biochem Biophys Res Commun* 480: 208-214, 2016.
19. Chen P, Wang BL, Pan BS and Guo W: MiR-1297 regulates the growth, migration and invasion of colorectal cancer cells by targeting cyclo-oxygenase-2. *Asian Pac J Cancer Prev* 15: 9185-9190, 2014.
20. Li X, Wang HL, Peng X, Zhou HF and Wang X: miR-1297 mediates PTEN expression and contributes to cell progression in LSCC. *Biochem Biophys Res Commun* 427: 254-260, 2012.
21. Yang NQ, Luo XJ, Zhang J, Wang GM and Guo JM: Crosstalk between Meg3 and miR-1297 regulates growth of testicular germ cell tumor through PTEN/PI3K/AKT pathway. *Am J Transl Res* 8: 1091-1099, 2016.
22. Yang NQ, Zhang J, Tang QY, Guo JM and Wang GM: miRNA-1297 induces cell proliferation by targeting phosphatase and tensin homolog in testicular germ cell tumor cells. *Asian Pac J Cancer Prev* 15: 6243-6246, 2014.
23. Yang Z, Liu Y, Shi C, Zhang Y, Lv R, Zhang R, Wang Q and Wang Y: Suppression of PTEN/AKT signaling decreases the expression of TUBB3 and TOP2A with subsequent inhibition of cell growth and induction of apoptosis in human breast cancer MCF-7 cells via ATP and caspase-3 signaling pathways. *Oncol Rep* 37: 1011-1019, 2017.
24. Li Z, Meng Q, Pan A, Wu X, Cui J, Wang Y and Li L: MicroRNA-455-3p promotes invasion and migration in triple negative breast cancer by targeting tumor suppressor EI24. *Oncotarget* 8: 19455-19466, 2016.
25. Xue Y, Xu W, Zhao W, Wang W, Zhang D and Wu P: miR-381 inhibited breast cancer cells proliferation, epithelial-to-mesenchymal transition and metastasis by targeting CXCR4. *Biomed Pharmacother* 86: 426-433, 2017.
26. Atmaca H, Özkan AN and Zora M: Novel ferrocenyl pyrazoles inhibit breast cancer cell viability via induction of apoptosis and inhibition of PI3K/Akt and ERK1/2 signaling. *Chem Biol Interact* 263: 28-35, 2017.
27. Hohensee I, Chuang HN, Grottke A, Werner S, Schulte A, Horn S, Lamszus K, Bartkowiak K, Witzel I, Westphal M, *et al*: PTEN mediates the cross talk between breast and glial cells in brain metastases leading to rapid disease progression. *Oncotarget* 8: 6155-6168, 2017.
28. Rangel R, Lee SC, Hon-Kim Ban K, Guzman-Rojas L, Mann MB, Newberg JY, Kodama T, McNoe LA, Selvanesan L, Ward JM, *et al*: Transposon mutagenesis identifies genes that cooperate with mutant Pten in breast cancer progression. *Proc Natl Acad Sci USA* 113: E7749-E7758, 2016.
29. Wang LL, Hao S, Zhang S, Guo LJ, Hu CY, Zhang G, Gao B, Zhao JJ, Jiang Y, Tian WG, *et al*: PTEN/PI3K/AKT protein expression is related to clinicopathologic features and prognosis in breast cancer with axillary lymph node metastases. *Hum Pathol* 61: 49-57, 2016.