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Original Research

Circular RNA PVT1 Promotes Breast Cancer Progression via miR-30b-5p/AEG-1 Axis

Yingdong Li[1], Wenfang Li[1,*], Yongbiao Ai[1], Tianxiang Zhang[1]

[1]Department of General Surgery, The Taihe Hospital Affiliated to Hubei University of Medicine, 442000 Shiyan, Hubei, China
*Correspondence: wenfangli79@163.com (Wenfang Li)

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Abstract

Background: Circular RNA PVT1 (circPVT1) is involved in malignant tumorigenesis. However, the function of circPVT1 in breast cancer (BC) has not been investigated. The purpose of this research is to explore the mechanisms of circPVT1 in breast cancer from different perspectives. **Methods**: In this study, the expressions of circPVT1 and microRNA-30b-5p (miR-30b-5p) were analyzed by quantitative real-time polymerase chain reaction (q-PCR). The Kaplan-Meier was adopted to compare disease free survival (DFS) and overall survival (OS). CircPVT1 was depleted with special si-RNA, Cell Counting Kit-8 (CCK-8) to evaluate the cell viability and transwell assays to examine invasion ability. Astrocyte elevated gene 1 (AEG-1) protein level was measured by western blot. The competitive endogenous functions among circPVT1, miR-30b-5p and AEG-1 were verified by bioinformatics analysis and luciferase assay. **Results**: In this study, it was found that the circPVT1 was significantly elevated in BC tissues and cell lines compared to the control group. The Kaplan-Meier results indicated that higher circPVT1 expression had a worse prognosis than the low circPVT1 expression group in DFS ($\chi^2 = 7.174$, $p = 0.007$) and OS ($\chi^2 = 3.946$, $p = 0.047$). CircPVT1 regulated the BC cell viability, migration, and invasion ability. Besides, miR-30b-5p was found as a molecular target of circPVT1, and AEG-1 was identified as a target of miR-30b-5p. The suppression of circPVT1 promoted the expression of miR-30b-5p and inhibited AEG-1 expression. Moreover, simultaneous inhibition of miR-30b-5p expression in the circPVT1 knockout group could reverse the inhibition of AEG-1. **Conclusions**: Our results indicate circPVT1 regulates AEG-1 expression by binding to miR-30b-5p in breast cancer cells. CircPVT1 promoted AEG-1 expression by inhibiting miR-30b-5p in order to enhance the BC cell viability, migration, and invasion. Our results reveal a new molecular therapy target for breast cancer.

Keywords: circular RNA PVT1; breast cancer; miRNA-30b-5p; AEG-1

1. Background

Circular RNAs (circRNAs) includes exon sequences and splicing at classical splicing sites [1]. The biological functions of circRNAs are an important topic of clinical research in recent years, and function in a vital role in malignant tumor progression, acting as an oncogene or tumor suppressor involved in the malignant progression of a variety of cancers [2]. CircRNAs participate in regulating physiological and pathological processes by competitively binding to microRNAs (miRNAs or miRs) and reversing miRNA-mediated inhibition of downstream mRNA protein translation [3,4].

Circular RNA PVT1 (circPVT1) is located on chromosome 8q24 and plays a carcinogenic role in cancers which include lung, neck, and oral cancers [5–7]. Several studies have reported that circPVT1 might function in cancer progression by regulating c-Myc protein [8,9]. It has been demonstrated that circPVT1 regulates cellular radiosensitivity in breast cancer (BC) [10]. However, the detailed regulatory mechanism of circPVT1 in BC has not been fully revealed. This purpose of this study is to investigate the expression of circPVT1 in breast cancer and its biological roles, and to elucidate its potential biological mechanisms.

2. Materials and Methods

2.1 Patients and Tissue Samples

The Ethical Committee of Taihe Hospital, Hubei University of Medicine approved the present study (NO.2024KS03). Cancer and para-cancer tissue specimens of patients undergoing breast cancer surgery in Taihe Hospital, Hubei University of Medicine from July 2017 to August 2019 were collected. Permission for use of breast tissues in this research was signed by all the participating patients. A total of 142 female patients, aged between 35–55 years old, with an average age of 43.26 ± 4.31 years old, were included. All the patients were followed up successfully and the follow-up data were recorded and analyzed.

Inclusion criteria: (1) pathological diagnosis of invasive breast cancer; (2) complete clinical medical records; (3) no radiotherapy, chemotherapy or endocrine therapy administered prior to surgery. Exclusion criteria: (1) patients with other primary malignant tumors; (2) defecits in clinical data and incomplete treatment. All patients signed informed consent forms. All sample tissues were stored at –80 °C.

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2.2 Cell Culture

The MDA-MB-231 and MCF-7 cell lines were obtained from Academia Sinica Cell Bank (Shanghai, China), MCF-10A, MDA-MB-468, T47D and BT20 were bought from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and were identified with the short tandem repeat (STR) method. The cell lines used have been tested for Mycoplasma. Cells were cultured under the condition of 37 °C with 5% carbon dioxide. Dulbecco Modified Eagle Medium (DMEM) (11965092, Thermo Fisher Scientific, Waltham, MA, USA) was added with 10% fetal bovine serum (FBS, Life Technologies, Carlsbad, CA, USA) as the culture medium, with antibiotics being streptomycin (100 µg/mL) (Beyotime, Shanghai, China) and penicillin (100 U/mL) (Beyotime, Shanghai, China).

2.3 Cell Transfection

The shRNA that specifically targets for circPVT1 (si-circPVT1) (20 nM) and a negative control that targets nothing (si-NC) were chemical synthesized by Shengong Biotechnology (Shanghan, China). The sequences of siRNA were si-circPVT1#1: 5′-GAGCTTCGTTCAAGTATTT-3′, si-circPVT1#2: 5′-GAAATGTCCTCTCGCCTGC-3′ and si-NC: 5′-UUCUCCGAACGUGUCACGUTT-3′. Lipofectamine 2000 (Invitrogen, Shanghai, China) was added for the transfer of MDA-MB-231 and MCF-7. After 48 h transfection, the silenced cells were harvested and applied for the following experiments.

2.4 Cell Counting Kit-8 (CCK-8) Assay

The breast cancer cell lines MCF-7 and MDA-MB-231 were cultured without serum in 6-well plates. They were divided into si-circPVT1#1, si-circPVT1#2, and siRNA NC with a final concentration of 100 nM. After transfection for 24 h, 1000 cells were inoculated in 96-well plates with a volume of 200 µL per well. The light absorption values of cells were detected at culture time points 1, 2, 3 and 4 days. According to the description of CCK-8 proliferation detection reagent, each well had added 10 µL proliferation detection reagent, incubated for 2 h, and the optical density (OD) value of each well was measured at 450 nm wavelength. The growth curves of different groups of breast cancer cells were drawn.

2.5 RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (q-PCR)

The TRIzol reagent (Invitrogen, Shanghai, China) was adopted to extract tissue or cell RNA. The NanoDrop ND-1000 (Thermo Scientific, Waltham, MA, USA) was utilized to measure the RNA concentration and quality. The total RNA was reverse transcribed (Takara, Tokyo, Japan). Quantitative real-time PCR was completed with SYBR Green PCR Master Mix reagents (Takara, Tokyo, Japan). The primer sequences were as follows: circPVT1 forward, 5′-CTTCCTGGTGAAGCATCTGAT-3′ and reverse, 5′-TTCAGCCTCCACTTAAAGTACC-3′; and GAPDH forward, 5′-CATGAGAAGTATGACAACAGCCT-3′ and reverse, 5′-AGTCCTTCCACGATACCAAAGT-3′. The expression level of circPVT1 mRNA was relatively normalized to the GAPDH. The $2^{-\Delta\Delta Ct}$ method was utilized to compare the quantification difference.

2.6 Wound-Healing Assay

A wound-healing assay was adopted to observe cell migration. The cells were seeded with a concentration of 5×10^4 cells per well in six-well plates and were cultured for 24 hours in serum-free medium. Cells were individually scratched by a sterile 10 µL micropipette tip. Photos were taken at 0 and 24 hours after the scratching with the microscope (Leica, Malvern, PA, USA). Wound closure was measured and compared.

2.7 Transwell Invasion Assay

The 8 µm pore transwell (BD Biosciences, San Jose, CA, USA) was utilized to observe cell invasion. Briefly, 1×10^5 cells in 200 µL serum-free culture medium were placed in the upper transwell chamber with Matrigel (Corning, New York, NY, USA), and 10% FBS medium was added to the transwell bottom chamber. Incubation time was 48 hours. The non-invading cells were moved away by a cotton swab from the upper chamber. The invaded cells on the transwell membrane were fixed with 4% paraformaldehyde (NO.P1110, Maichen Gene Technology, Beijing, China) and then dyed with 1% crystal violet (Sigma-Aldrich Co., St Louis, MO, USA). The invaded cells were randomly calculated in five different fields with a microscope (Leica, Malvern, PA, USA).

2.8 Western Blot

Sixty micrograms of total protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis and electrophoretically transferred onto PVDF (poly vinylidene fluoride) membranes. Five percent milk was adopted to block the membrane. The membrane was incubated with astrocyte elevated gene 1 (AEG-1) or GAPDH (1:1000) antibodies overnight at 4 °C. AEG-1 (1:1000) was purchased from Abcam (No. ab229128, Cambridge, MA, USA) and GAPDH was from Cell Signaling Technology, Inc. (No. #5174, Beverley, MA, USA). The proteins were observed by enhanced chemiluminescence (Millipore, Beijing, China) following the manufacturer's procedure. Image J 1.53 (https://imagej.net/ij/) to compare western blot (WB) gray value. AEG-1 gray value is normalized to GAPDH.

2.9 Luciferase Assay

The luciferase assay kit was purchased from Vigorous Biotech (Beijing, China) and the TOP/FOP Flash lu-

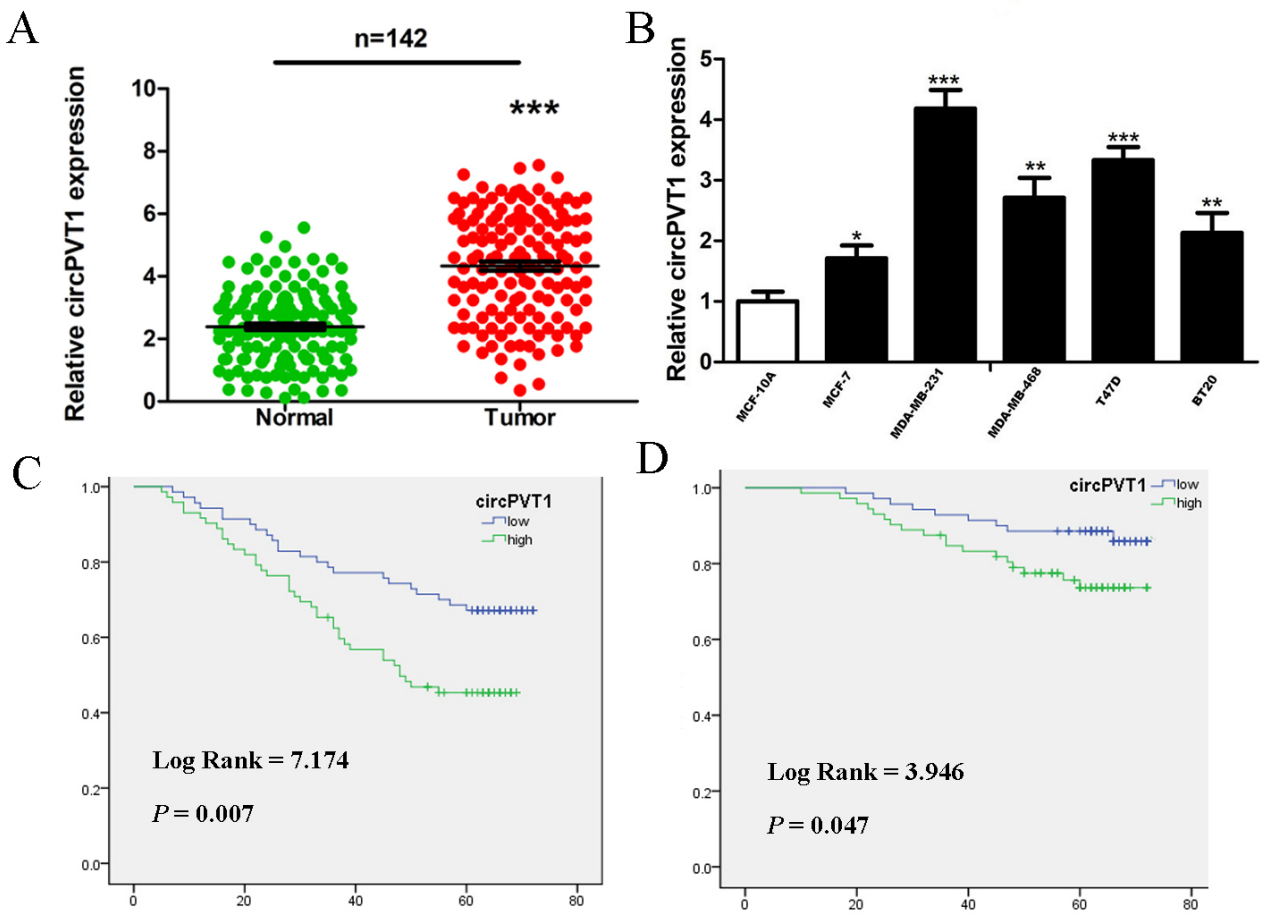


Fig. 1. Increasing expression of circPVT1 in breast cancer tissues and cells. (A) CircPVT1 expression was elevated in breast cancer tissues compared to the corresponding paracancer tissues, n = 142. GAPDH was adopted as the internal control. Data come from triplicate experiments. (B) q-PCR to detect circPVT1 RNA levels in different breast cancer cell lines. n = 3. MCF-10A is a normal mammary cell. (C) The Kaplan-Meier results indicated that higher circPVT1 expression correlated with poorer disease free survival. (D) The Kaplan-Meier results indicated that higher circPVT1 expression correlated with poorer overall survival. circPVT1, circular RNA PVT1; q-PCR, quantitative real-time polymerase chain reaction. *$p < 0.05$ *vs.* the control; **$p < 0.01$ *vs.* the control; ***$p < 0.001$ *vs.* the control.

ciferase reporter plasmid was bought from Biovector NTCC Ltd (Beijing, China). Luciferase assays were completed following by the manufacturer's protocol. The miRNA-30b-5p binding site sequence of circPVT1 or AEG-1's 3′untranslated region (3′-UTR) was cloned into pGL3 vector (wild type), respectively. The 3′-UTR wild type (WT) and mutatin (MUT) luciferase reporter plasmids containing circPVT1 or AEG-1 were synthesized and transfected into MDA-MB-231 or MCF-7 cells cultured in 24-well plates. Simultaneously transfected 100 ng wild type or mutate type plasmid, 100 nm invalid nucleotide or miRNA-30b-5p in the MDA-MB-231 or MCF-7 cells were placed in 24-well plates. The luciferase assay was done after 24 hours transfection. Firefly luciferase was utilized as a reference, and Renilla luciferase was utilized as the internal control.

2.10 Tumor Formation in Vivo

The 6-week-old female Balb/c mice were obtained from Charles River (Beijing, China). MDA-MB-231 cells transfected with si-circPVT1 or si-NC and were separately injected into the dorsum of the mice (n = 7 per group), 5 000 000 cells/200 µL phosphate buffered saline (PBS) were inoculated. Tumor sizes were carefully measured weekly and the formula L × S/2 (L: tumor's long diameter; S: tumor's short diameter) was applied to calculate tumor volume. At 25 days post-injection, all mice were euthanasia executed by decapitation and tumor tissues were removed. Quantitative real-time polymerase chain reaction (q-PCR) was used to examine the relative circPVT1 expression in tumor tissues. Animal studies had obtained the agreement of the Animal Care and Use Committee of Taihe Hospital (NO.2024KS03).

Table 1. Association between circPVT1 expression in the breast cancer patients and clinicopathological characteristics.

Characteristics	circPVT1			χ^2	*p* value
	n	Low (n = 70)	High (n = 72)		
Age (years)				0.031	0.861
≤50	74	37 (50.0%)	37 (50.0%)		
>50	68	33 (48.5%)	35 (51.5%)		
Menopausal status				0.099	0.753
Premenopausal	81	39 (48.1%)	42 (51.9%)		
Postmenopausal	61	31 (50.8%)	30 (49.2%)		
LN metastasis				4.108	0.043
Negative	75	43 (57.3%)	32 (42.7%)		
Positive	67	27 (40.3%)	40 (59.7%)		
Tumor size				0.236	0.627
≤2 cm	62	32 (51.6%)	30 (48.4%)		
>2 cm	80	38 (47.5%)	42 (52.5%)		
ER status				0.099	0.753
Negative	61	31 (50.8%)	30 (49.2%)		
Positive	81	39 (48.1%)	42 (51.9%)		
PR status				0.001	0.973
Negative	57	28 (49.1%)	29 (50.9%)		
Positive	85	42 (49.4%)	43 (50.6%)		
HER2				0.770	0.380
Negative	107	55 (51.4%)	52 (48.6%)		
Positive	35	15 (42.9%)	20 (57.1%)		
Ki67 status				0.127	0.721
Negative	79	40 (50.6%)	39 (49.4%)		
Positive	63	30 (47.6%)	33 (52.4%)		
Differentiation				4.823	0.028
Well	54	34 (60.7%)	22 (39.3%)		
Poor	88	36 (41.9%)	50 (58.1%)		
TNM				0.671	0.413
I, II	101	52 (51.5%)	49 (48.5%)		
III	41	18 (43.9%)	23 (56.1%)		

circPVT1, circular RNA PVT1; ER, estrogen receptor; PR, progesterone receptor; LN, lymph node; HER2, human epidermal growth receptor 2; TNM, T: tumor, N: lymph node, M: metastasis.

2.11 Immunohistochemical Staining Methods

Tumor tissue samples were collected, embedded in paraffin with 10% formaldehyde solution. Antigen retrieval was enhanced by microwaving in sodium citrate solution antigen repair liquid antigen of 98 °C for 15 minutes to repair, rabbit anti human AEG-1 (Abcam, No. ab229128, Cambridge, MA, USA) or PCNA monoclonal antibody (Abcam, No. ab92552, Cambridge, MA, USA) (1:100) 4 °C for the night, horseradish peroxidase labelled goat anti-rabbit second antibody (Shenggong Bioengineering Company, NO. E670020, Shanghai, China) incubation for 1 hour, colour reaction with 3,3-diaminobenzidine (DAB) (NO.DAB-0031, MaiXin Bioengineering Company, Fuzhou, Fujian, China), and then all the sections were lightly counterstained with hematoxylin and examined by light microscopy. The results were finally evaluated by the staining intensity and staining area score. The staining intensity was negative to strong (0–3), negative (0 point), weak positive (1 point), moderate positive (2 points), and strong positive (3 points). The staining positive area score was 0 (<10%); 1 (10–25%); 2 points (26–50%); 3 (51–75%); 4 (>76%). Staining area score × staining intensity score was the final score, with a score ≥4 to judge positive and a score <4 to judge negative.

2.12 Statistical Analysis

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the data. The measurement data were analyzed with *t* test, and the Kaplan–Meier method was used to evaluate disease free survival (DFS) and overall survival (OS). The Chi-square Test was adopted to compare the difference between circPVT1 expression in breast cancer patients and clinicopathological characteristics. Values of $p < 0.05$ were defined as being statistically significant.

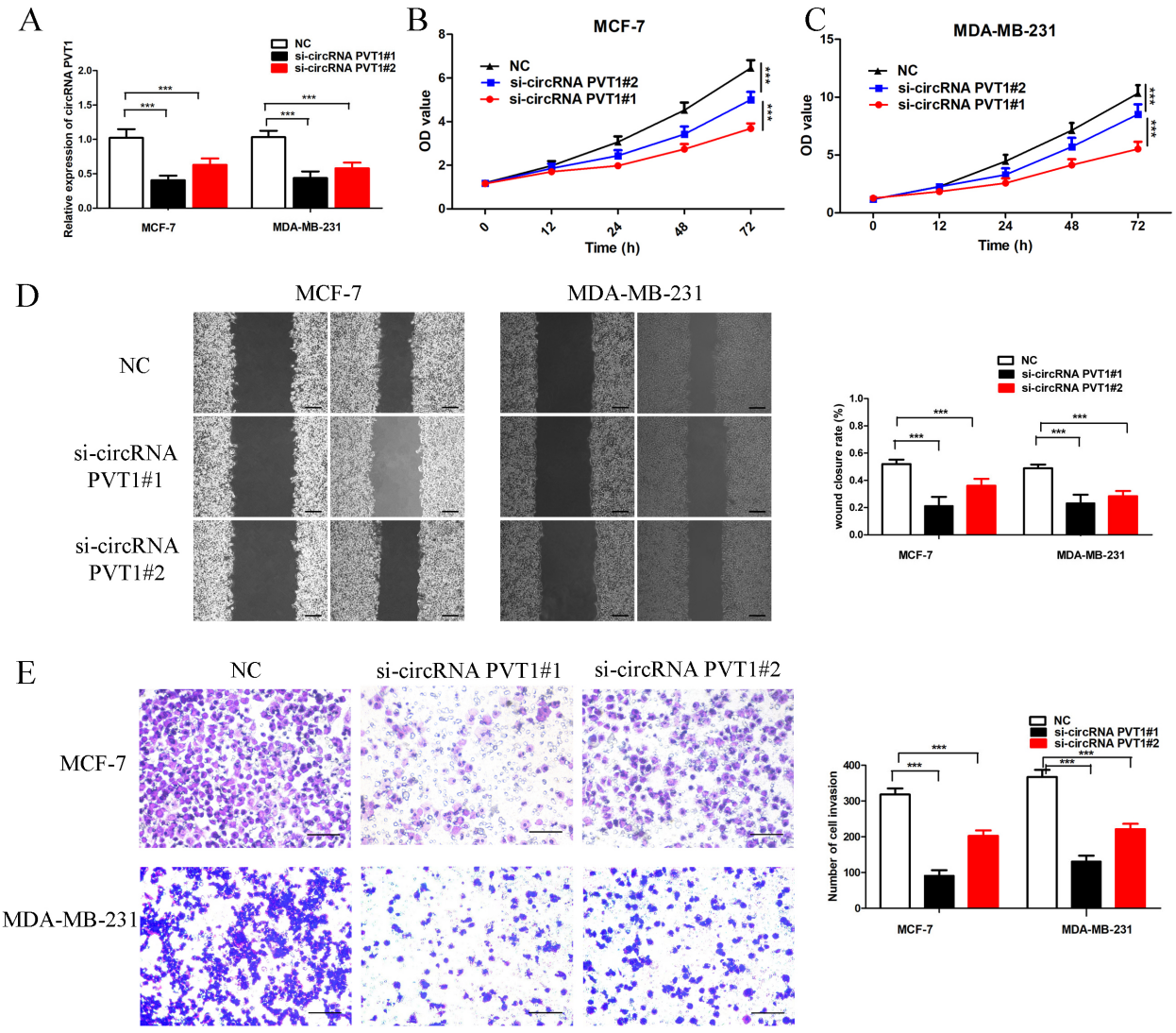


Fig. 2. Knockout of circRNA PVT1 in breast cancer cells. (A) Knockout of circRNA PVT1 expression compared to negative control (NC) group. (B,C) Cell proliferation assay results suggested that knockout of circRNA PVT1 inhibited the breast cancer cell proliferation. (D) Scratch assay suggested that knockout of circRNA PVT1 inhibited the migration of MCF-7 and MDA-MB-231 breast cancer cells, scale bar = 200 µm. (E) Cell invasion assay suggested that knockout of circRNA PVT1 effectively inhibited the breast cancer cell invasion, scale bar = 100 µm. ***$p < 0.001$ *vs.* the control. n = 3.

3. Results

3.1 CircPVT1 Expression in Breast Cancer and Corresponding Paracancer Tissues

CircPVT1 was expressed in 142 cases of breast cancer and corresponding paracancer tissues. The results indicated that the relative expression level of circPVT1 in breast cancer tissues was 4.21 $\pm$ 0.17, while that in corresponding paracancer tissues was 2.57 $\pm$ 0.12. Statistical analysis using matched data showed a significant difference (t = 8.613, $p < 0.001$), indicating that circPVT1 expression was increased in cancer tissues (Fig. 1A). q-PCR was used to detect the circPVT1 expression in breast cancer cells. Compared with normal breast cancer epithelial cells, the expression of circPVT1 in breast cancer cell line was increased (Fig. 1B). The difference was statistically significant ($p < 0.05$), and the highest expression level was found in MDA-MB-231 cells ($p < 0.01$). MCF-7 is a widely used cell line in breast cancer research. The Kaplan-Meier results indicated that high circPVT1 expression correlated with a worse prognosis as compared to those with low circPVT1 expression in DFS (χ^2 = 7.174, p = 0.007) and OS (χ^2 = 3.946, p = 0.047) (Fig. 1C,D).

3.2 Relationship between circPVT1 Expression and Breast Cancer Pathological Characteristics

The mean expression level of circPVT1 in breast cancer tissues was 4.21. High expression of circPVT1 was related to axillary lymph node metastasis (χ^2 = 4.108, p =

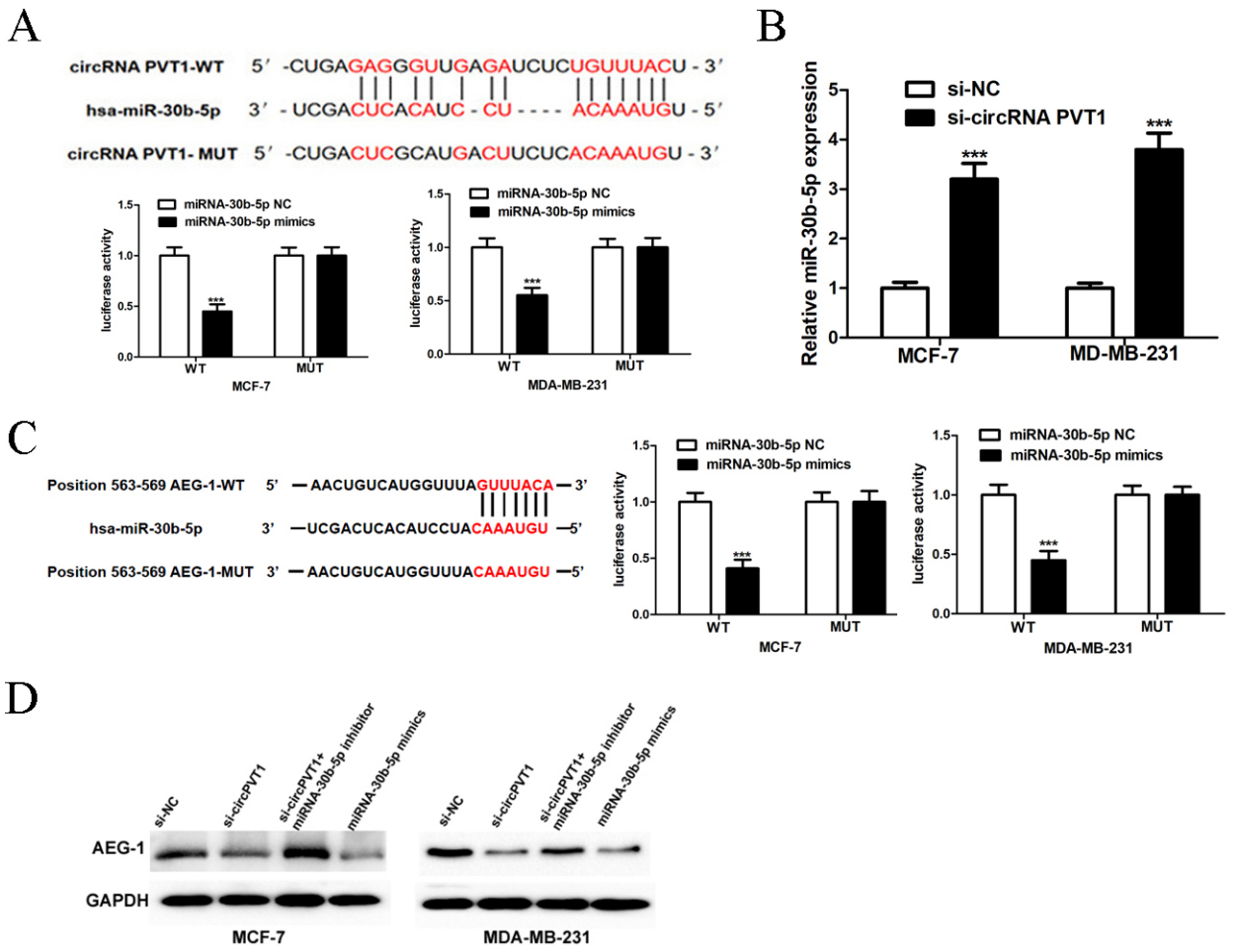


Fig. 3. CircRNA PVT1 regulates miR-30b-5p/AEG-1 pathway. (A) The binding places between miR-30b-5p and circRNA PVT1. Luciferase assay results indicated that miR-30b-5p mimics could target circRNA PVT1 3′UTR-WT (3′-untranslated region-wild type). (B) CircRNA PVT1 knockout by the small interference RNA could up-regulate miR-30b-5p expression. (C) The binding sites between miR-30b-5p and AEG-1. Luciferase assay results indicated that miR-30b-5p could target AEG-1 3′UTR-WT. (D) Small si-RNA knockout circRNA PVT1 in MCF-7 and MDA-MB-231 cells or miR-30b-5p mimics could downregulate AEG-1 protein expression. MiR-30b-5p inhibitor reversed si-circRNA PVT1 function. AEG-1, astrocyte elevated gene 1; MUT, mutatin. ***$p < 0.001$; n = 3.

0.043) and differentiation degree ($\chi^2 = 4.823$, $p = 0.028$). However, there was no correlation with patient age, Ki67 index, HER2 (human epidermal growth receptor 2), ER (estrogen receptor), PR (progesterone receptor), tumor diameter or tumor stage ($p > 0.05$) (Table 1).

3.3 Knockout of circPVT1 Inhibited Breast Cancer Cell Proliferation, Migration and Invasion

In order to further study the role of circPVT1 in breast cancer, cell lines with circPVT1 knockout were established in breast cancer cells. The cell lines with circPVT1 knockout were successfully established by q-PCR (Fig. 2A). Further cell count assay showed that the elimination of circPVT1 significantly inhibited the proliferation ability of MCF-7 and MDA-MB-231 cells (Fig. 2B,C) ($p < 0.05$).

In order to further study the functions of circPVT1 on breast cancer cell biology, the cell scratch test was applied to study the migration ability. The elimination of circPVT1 significantly inhibit the breast cancer cell migration ability (Fig. 2D). The transwell invasion assay further indicated that the invasion ability of knockout circPVT1 in MCF-7 and MDA-MB-231 cells was significantly suppressed compared to the control group (Fig. 2E).

3.4 CircPVT1 Promotes the Expression of AEG-1 by Sponging with miR-30b-5p

Since circular RNA can act as a miRNA sponge, by searching the circinteractome database, we found that the circPVT1 sequence contained the binding site of microRNA-30b-5p (miR-30b-5p) (Fig. 3A), and further confirmed the relationship between circPVT1 and miR-

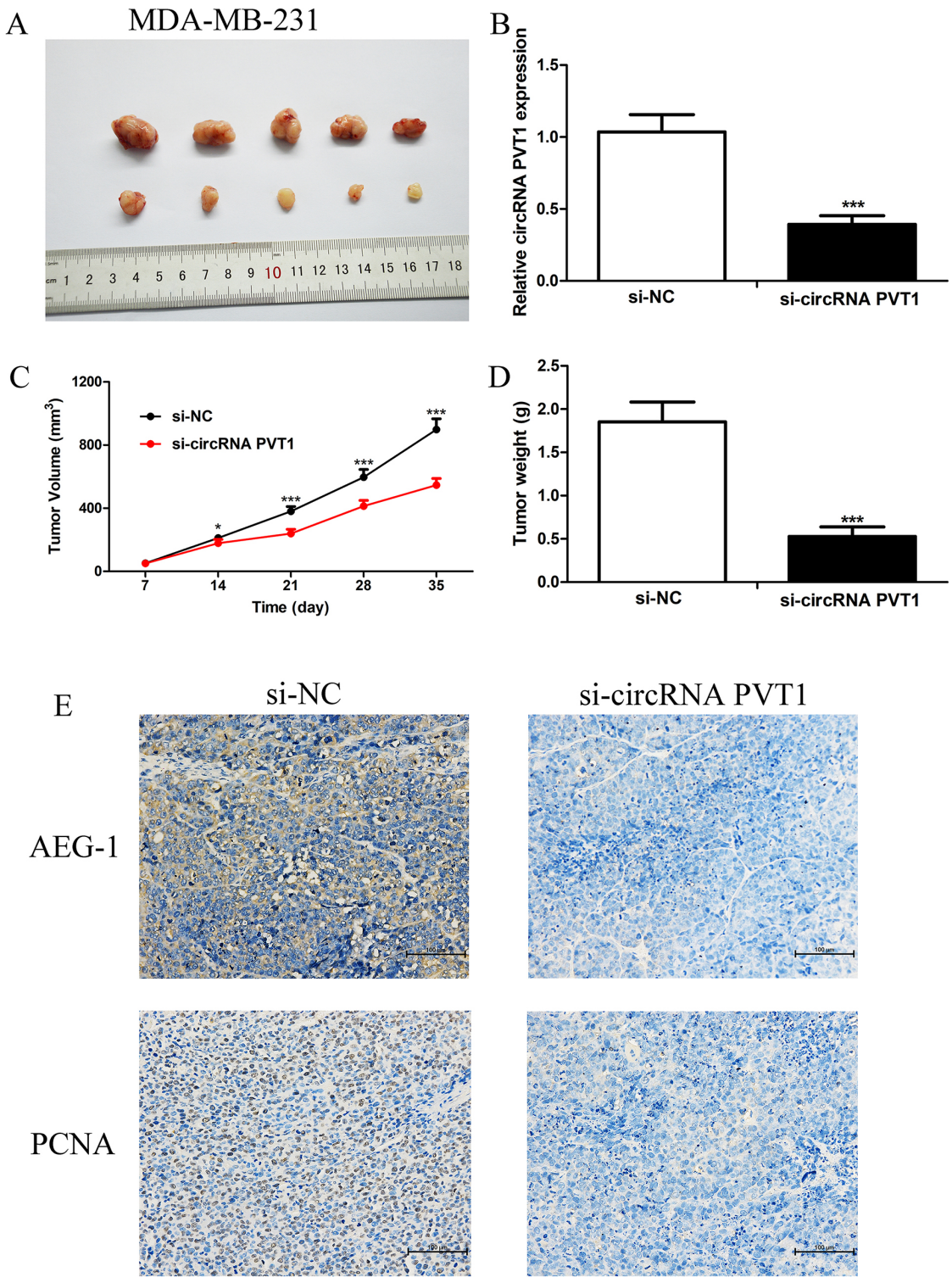


Fig. 4. CircPVT1 knockdown inhibited tumor growth *in vivo*. (A) The MDA-MB-231 cells were transfected with si-circPVT1 or si-NC respectively, and injected into the dorsum of Balb/c mice. Tumor tissues were collected after 5 weeks. (B) q-PCR was used to detect the relative circPVT1 expression in tumor tissues. (C) Tumor sizes were carefully measured weekly and the formula L × S/2 was adopted to calculate tumor volume. (D) The difference of tumor weight between the si-circPVT1 and si-NC groups. *$p < 0.05$ *vs.* the control; ***$p < 0.001$ *vs.* the control. n=5, there are five mice in si-circPVT1 and si-NC respectively. (E) CircPVT1 knockdown inhibit tumor AEG-1and proliferating cell nuclear antigen (PCNA) expression in nude mice. Immunohistochemical method was used to detect AEG-1and proliferating cell nuclear antigen (PCNA) expression in nude mice (representative images). Scale bar = 100 μm.

30b-5p. The luciferase results indicated that overexpression of miR-30b-5p could inhibit luciferase activity in circPVT1 3′UTR wild type group. When the circPVT1 3′UTR binding site was mutated, the overexpression of miR-30b-5p could not effectively inhibit luciferase activity (Fig. 3A). To confirm the reliability of our results, q-PCR was applied to detect the expression level of miR-30b-5p in MCF-7 and MDA-MB-231 cells, miR-30b-5p expression was significantly increased after circPVT1 knockout (Fig. 3B, $p < 0.05$). Therefore, it could be inferred that circPVT1 might be involved in breast cancer progression through sponging miR-30b-5p.

By searching the target scan database, we found the presence of miR-30b-5p binding site 3′UTR in AEG-1. Moreover, the biding between AEG-1 and miR-30b-5p was confirmed by luciferase reporter assay and the results revealed that overexpression of miR-30b-5p could inhibit luciferase activity in AEG-1 3′-UTR wild type group. The inhibitory effect disappeared in the mutation group of 3′-UTR binding site of AEG-1 (Fig. 3C). In order to confirm the reliability of our results, we used Western blotting to detect the overexpression of miR-30b-5p in MCF-7 and MDA-MB-231 or the elimination of circPVT1 with si-circPVT1#1, both of which inhibited the expression of AEG-1. However, simultaneous inhibition of miR-30b-5p expression in the circPVT1 knockout group could reverse the inhibition of AEG-1 expression (Fig. 3D).

3.5 Suppression of circPVT1 Inhibited Tumor Formation in Vivo

The expression level of MDA-MB-231 in mice was transfected with si-NC or si-circPVT1#1, and the results demonstrated that si-circPVT1 effectively inhibited the tumor growth (Fig. 4A) and the expression of circPVT1 (Fig. 4B), reduced the tumor volume and weight (Fig. 4C,D), and inhibited the expression levels of AEG-1 and proliferating cell nuclear antigen (PCNA) in nude mice (Fig. 4E).

4. Discussion

CircPVT1 (circBase ID: hsa_circ_0001821) is located on chromosome 8q24, and the amplification of circPVT1 has been frequently observed in various cancers [8]. CircPVT1 regulates epithelial-mesenchymal transition and tumor stemness in tumor progression, promoting tumor proliferation, invasion and metastasis [9,10]. Zheng *et al*. [11] found increased circPVT1 expression in thyroid cancer and it promoted the progression of thyroid cancer. Typically, circPVT1 functions as a miRNA "sponge" and increases special miRNA-targeted mRNA expression, thereby stimulating cancer progression [12]. To elucidate the roles of circPVT1 in breast cancer, we investigated the functions of knockout circPVT1 in breast cancer cells. Our results revealed that high circPVT1 expression was positively correlated with axillary lymph node metastasis and differentiation degree in breast cancer. CircPVT1 can promote breast cancer cell proliferation, migration, and invasion. Further results revealed that circPVT1 inhibits AEG-1 degradation by binding to miRNA-30b-5p. The oncogene AEG-1 demonstrated that it could regulate the expression of genes associated with breast cancer malignant progression [13]. Our data indicates that circPVT1 is a key regulator of AEG-1 in breast cancer and might be a novel target for treatment.

CircRNAs usually localize in the cytoplasm and perform their biological roles via sponging miRNAs. The circRNA-miRNA regulatory networks are involved in the genesis and development of tumors [14]. Our results indicated that circPVT1 was up-regulated in breast carcinoma cells, suggesting that circPVT1 might be involved in the progression of breast cancer through regulation of specific miRNAs. Further dual luciferase reporter experiments indicated that miR-30b-5p was the target of circPVT1. MiR-30b-5p participates in malignant progression in a variety of cancers including pancreatic cancer [15], esophageal carcinoma [16], and papillary thyroid cancer [17]. In our study, miR-30b-5p inhibitors partially reversed circPVT1 depletion-mediated inhibition in breast cancer cells. Therefore, the circPVT1/miR-30b-5p regulatory axis might have important roles in breast cancer.

AEG-1 has approximately a 64 kda molecular weight, also known as MTDH (metadherin), and the coding gene is located in human chromosome 8q22 [18]. AEG-1 was first reported to be related to lung metastasis of breast cancer in a mouse tumor model in 2004 [19]. High MTDH expression in breast cancer is closely associated to high Ki67 expression and poor prognosis, and is related to invasion, metastasis and EMT of breast cancer [20]. Through activation of NF-κB (nuclear factor kappaB), Wnt/β-catenin, and STAT3 (transcription 3) signal pathway, AEG-1 played an important roles in drug resistance, stemness and self-renewal [21,22]. MTDH-SND1 (staphylococcal nuclease domain-containing 1) interaction intimately correlates with expansion of tumor-initiating cells in mammary tumors [23]. This study demonstrated that silencing circPVT1 inhibits breast cancer cell biology through sponging miR-30b-5p to inhibit AEG-1. These findings revealed a key role of circPVT1 in breast cancer progression by regulating the miR-30b-5p/AEG-1 axis.

5. Conclusions

In our study, the circPVT1 expression is higher in breast cancer tissues compared to corresponding paracancer tissues and higher circPVT1 expression correlates with worse prognosis in breast cancer patients. Silencing circPVT1 could inhibit breast cancer cell malignant biology through sponging miR-30b-5p to promote AEG-1 expression. These findings reveal the key role of the circPVT1/miR-30b-5p/AEG-1 axis in breast cancer malignant biology progression, providing a vital therapeutic target for treatement of breast cancer patients.

Abbreviations

circPVT1, circular RNA PVT1; q-PCR, quantitative real-time polymerase chain reaction; si-NC, negative control siRNA; DMSO, dimethyl sulfoxide; EMT, epithelial/mesenchymal transition.

Availability of Data and Materials

All the data supporting our findings could be found in the "Results" section of the paper. Please contact authors for data request.

Author Contributions

YL and WL designed this study. YL and TZ performed the research. YA and TZ collected the patients medical materials and checked the pathological diagnosis. YA analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Taihe Hospital affiliated to Hubei University of Medicine (approval number: NO.2024KS03).

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Conflict of Interest

The authors declare no conflict of interest.

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