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*Original Article*

# Reduced PDZRN4 promotes breast cancer progression and predicts poor prognosis

Yu-Long Lu[1], Xin Yang[1], Ya-Kui Liu[2]

[1]*Department of General Surgery, Xiangya Second Hospital, Central South University, Changsha, Hunan, PR China;* [2]*Department of Thoracic Surgery, Jingmen First People's Hosptial, Jingmen, Hubei, PR China*

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**Abstract:** Breast cancer (BC) is one of the most lethal types of cancer throughout the world due its proliferation and invasion. PDZ domain containing ring finger 4 (PDZRN4) belongs to the LNX family, which has E3 ubiquitin ligase activity and is involved in the progression of cancer. However, the role of PDZRN4 in the progression of BC remains unknown. In the present study, the public database Oncomine was used to detect PDZRN4 expression for primary screening. BC tissues and matched normal tissues were collected for detection of expression and cohort analysis. BC cells were used for invasion and proliferation function tests *in vitro* and *in vivo*. The results revealed that both the mRNA and protein levels of PDZRN4 in the BC tissues were markedly reduced compared to normal tissues. The expression of PDZRN4 was significantly correlated with LNM, histological grade and perineural invasion. Prognostic analysis confirmed that PDZRN4 expression is an independent risk factor for overall survival and disease-free survival of patients with BC. The functional experiments demonstrated that knockdown of PDZRN4 significantly improved the invasive and proliferative abilities of BT-20 cells *in vitro* and *in vivo*. Furthermore, overexpression of PDZRN4 markedly inhibited the malignant aspects of MDA-MB-231 cells. Therefore, reduced PDZRN4 correlates with the progression of BC and may act as a novel prognostic marker for patients with BC.

**Keywords:** PDZRN4, breast cancer, proliferation, metastasis, prognosis

## Introduction

Breast cancer (BC) is one of most common types of cancer in females and in cancer mortalities globally [1]. There are various medical treatments for BC and they have considerable effects and the outcome of BC with a 5-year survival rate is around 70% in developing countries while 90% in some developed countries. BC patients are still subject to eventual cancer-caused death [2]. Invasion and metastasis are the main causes of poor prognosis for patients with BC [3]; however, the mechanism of the invasion-metastasis cascades remains unclear. Thus, it is important and urgent to investigate the metastatic factors that modulate BC progression.

The E3 ubiquitin ligase family has been reported to serve important roles in the regulation of cell migration, which is crucial for tumor cells to infiltrate into normal tissues and migrate to distant places [4, 5]. E3 ubiquitin ligase-related genes have been found to be abnormal at different levels of genetic modification in numerous types of cancer [6]. Furthermore, E3 ubiquitin ligase proteins have been identified to be involved in the modulation of numerous types of cancer [7, 8]. E3 ubiquitin ligases serve an important role in cancer processes and may act as potential tumor-related targets or prognostic biomarkers.

PDZ domain containing ring finger 4 (PDZRN4), a member of the LNX (ligand of numb protein-X) family of RING-type ubiquitin E3 ligases [9], is located on human chromosome 12. Previous studies have demonstrated that the LNX family is involved in numerous types of cancer [10, 11], and one study indicated that PDZRN4 is involved in the progression of liver cancer [12]. However, the role of PDZRN4 in other types of cancer, particularly in BC, has not yet been reported to the best of our knowledge. We hypothesized that PDZRN4 may participate in the invasion and metastasis of BC.

In the present study, the expression of PDZRN4 in BC tissues was significantly lower than that in the matched normal tissues. Furthermore, low PDZRN4 expression was closely associated with aggressive clinicopathologic characteristics and poor prognosis of patients with BC. Functional experiments indicated that reduced PDZRN4 facilitated the progression of BC *in vitro* and *in vivo*. Thus, this study identifies a potential targeted treatment for BC and suggests that PDZRN4 acts as a prognostic factor for patients with BC.

## Materials and methods

### *Patients and samples*

The human tissues and cohort study were conducted according to the protocol set by Jingmen No.1 People Hospital (Jingmen, China). BC tissues and matched normal tissues from 81 BC patients and other 24 fresh BC tissues were collected at the Department of Pathology, Jinmen First Hospital between April 2010 and June 2012. Informed consents were obtained from all individual participants. The tumor tissue samples collected were identified by two pathologists.

### *Cell culture and transfection*

The human breast cell line MCF10A and the BC cell lines MDA-MB-231, MDA-MB-453, MDA-MB-468, BT-20 and MCF7 were gifted from the Department of General Surgery, Zhongnan Hospital, Wuhan University (Wuhan, China) and all cell lines were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37°C in 5% $CO_2$. ShRNA was designed (sh-PDZRN4) and used to infect the BT-20 cells. The PDZRN4 shRNA sequence primers were as follows: sh1: 5'-GACACTTTGGGATTCAATATT-3'; and sh2: 5'-GGTCGACCAAATCAGAATAAT-3'. A plasmid was also designed (en-PDZRN4) and used to transfect the MDA-MB-231 cells. All siRNAs and plasmids were purchased from Fubio Biosciences Co., Ltd. (Shanghai, China).

### *Immunohistochemistry*

Immunohistochemistry was done in 81 BC tissues to detect the expression of PDZRN4 protein (1:100; Proteintech, USA) in the BC tissues. The PDZRN4 expression levels were scored by the percentage of stained breast cells [13] as follows: 0 (0-10%), 1 (11-25%), 2 (26-50%) and 3 (51-100%) [14]. According to the PDZRN4 expression level, the patients with BC were divided into two groups: 0-25%, low expression group; and 26-100%, high expression group.

### *Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)*

*m*RNA was extracted from 24 fresh BC samples and BC cells using a TRIzol kit (Leagene Biotech. Co. Ltd., Beijing, China) and transformed into cDNA using a reverse transcription kit (Roche Applied Science, Penzberg, Germany). A PCR kit (Roche Applied Science) was used to perform the RT-qPCR. The primers of PDZRN4 were as follows: Forward, 5'-ATGAAGAGGCAGTGGAAGCT-3'; and reverse, 5'-CCAGAGCCATGATGTGTTCG-3'. The primers of GAPDH (Forward, 5'-CAATGACCCCTTCATTGACC-3'; reverse, 5'-GACAAGCTTCCCGTTCTCAG-3') was used as the internal control. The expression levels of PDZRN4 and GAPDH were analyzed using the comparative $2^{-\Delta\Delta Ct}$ method [15]. Each assay was repeated three times.

### *Western blot analysis*

Radioimmunoprecipitation assay buffer (Yushen Biotech. Co. Ltd., Shanghai, China) was used to decompose the cultured cells. After detecting the concentrations of different proteins, these proteins were transferred to a polyvinylidenedifluoride (PVDF) membrane (EMD Millipore, Billerica, MA, USA). The membranes were incubated with PDZRN4 antibody (1:1,000; Protein TechGroup, Inc., Chicago, IL, USA) at 4°C overnight and then with the secondary antibody (1:5,000; ProteinTech Group, Inc.). The membranes were exposed on X-ray films by using an ECL kit (Ebioscience, Shanghai, China). β-actin was detected as the control. Each assay was repeated three times.

### *Invasion experiment*

BC cells ($1\times10^5$) were plated in the upper small chamber of a Transwell plate and supplied with serum-free DMEM. The Transwell membrane was precoated with Matrigel (QcbioScience and Technologies Co., Ltd., Shanghai, China). Normal DMEM with 10% FBS was added to the lower chamber. After 48 h, cells on the lower

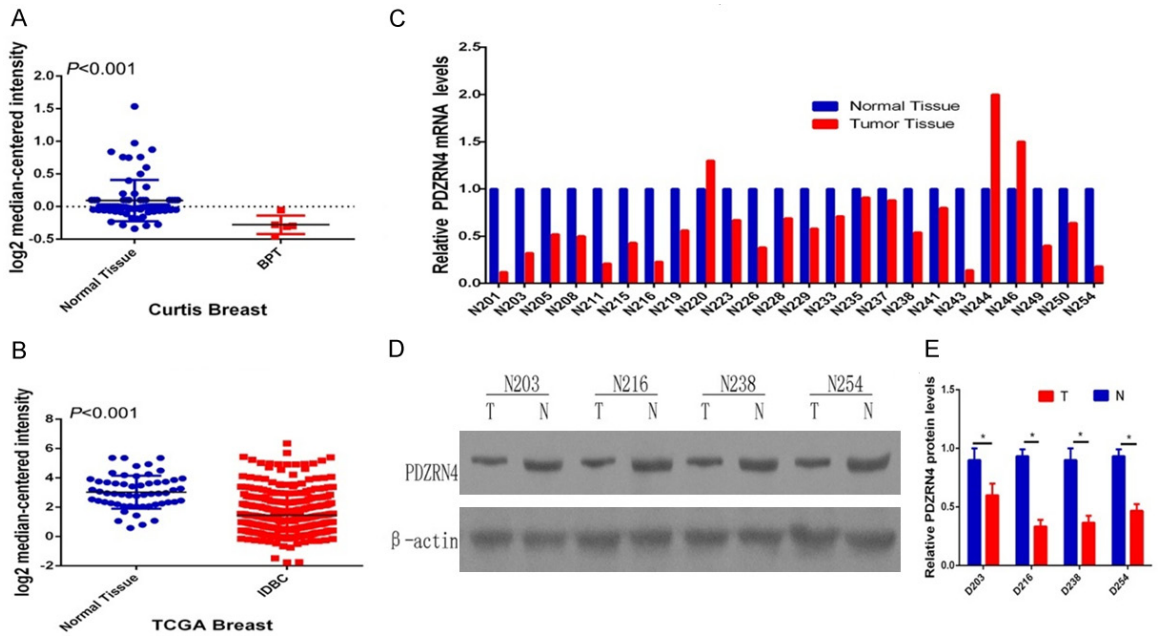


**Figure 1.** PDZRN4 is significantly decreased in BC tissues. A, B. PDZRN4 mRNA levels in public database (Oncomine) were significantly lower in BC tissues compared to normal tissues (P<0.001). C. 24 pairs of fresh BC tissues and their matched normal tissues were analyzed to detect the mRNA expression of PDZRN4 by qRT-PCR. D, E. Four BC tissues and matched normal tissues were collected to detect the PDZRN4 protein expression by western blot. β-actin as internal reference was used. The normal breast tissue is marked N and tumor tissue is marked T.

surface of the membrane were fixed with 20% methanol and stained with 0.1% crystal violet solution. Each assay was repeated three times.

*Colony formation assay*

A total of 500 BC cells were plated in six-well plates and cultured for 2 weeks in DMEM with 10% FBS. After 2 weeks, the plates were washed gently and dyed with crystal violet. Each assay was repeated three times.

*Subcutaneous (SC) tumor formation experiment*

BALB/c-immunodeficient mice were purchased from the Slk Jing Da Laboratory Animal Co., Ltd (Hunan) and the experiments were performed in a SPF-level sterile laboratory at the Animal Testing Center of Central South University (Changsha, China). The transfected and control cells that were in a good state were digested with 0.25% trypsin, and the concentration was adjusted to $1\times10^6$ cells/ml. A 200-μl cell suspension was injected into the right forelimb of the BALB/C-immunodeficient mice. From the second day after injection, the growth rate of the SC tumors in each cell-derived group was compared by measuring the short diameter and long diameter of the SC tumors every day. After 4 weeks, the SC tumors were removed and photographs were captured. The longest diameter and the shortest diameter of the SC tumors were measured using a Vernier caliper. The formula for SC tumor volume was as follows: Volume ($cm^3$) = (long diameter × short diameter$^2$)/2.

*Pulmonary metastasis experiment*

The cell concentration was adjusted to $5\times10^5$ cells/ml by using a cell counting instrument. Each nude mouse was injected with 200 μl cells into the tail vein every 2 days for two weeks. After 4 weeks, the mice were sacrificed by cervical dislocation. The lungs of mice were removed and soaked in 10% formalin solution. The lungs were embedded with paraffin and then cut into slices with a thickness of ~5 μm. H&E staining was used to identify any metastatic lesions in the lungs.

*Statistical analysis*

SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6 (GraphPad

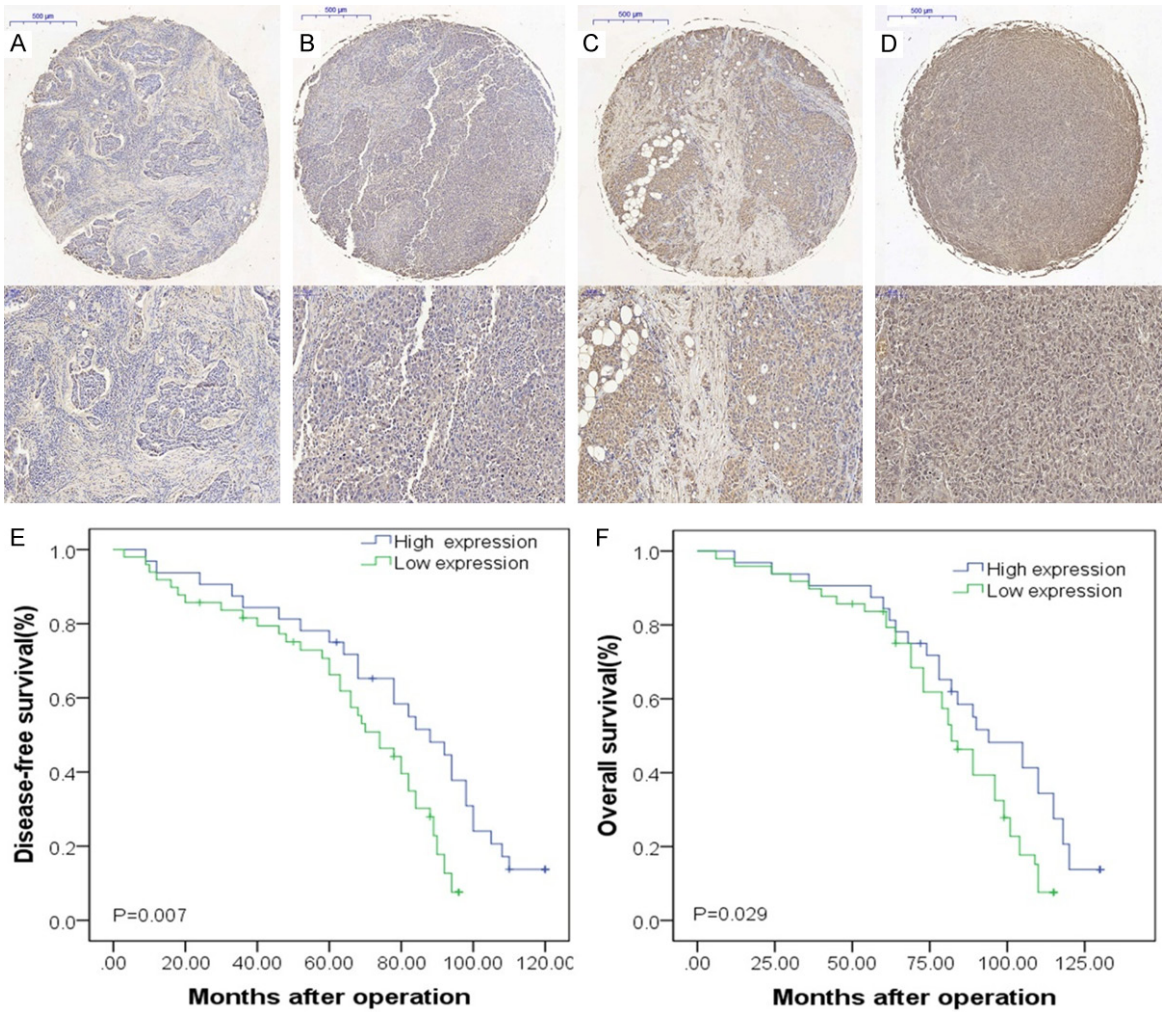


**Figure 2.** Decreased PDZRN4 indicates poor prognosis. Different PDZRN4 expression levels are shown as (A) negative, (B) 10-24%, (C) 25-49%, (D) over 50%. (E, F) According to the PDZRN4 expression, 81 cases of BC patients were analyzed for Disease-free survival and Overall survival by Kaplan-Meier and log-rank test.

Software, Inc, Armonk, CA, USA) was used for data analysis. Quantitative Data were expressed as means ± SD from at least three independent experiments. One way ANOVA test (multiple comparisons) was applied to check the differences in multiple comparisons of different cell groups in **Figure 3**. Student's t-test was applied to check the differences between paired tissue groups in **Figure 1** and PDZRN4-treated cell groups and their control cell groups in in vitro and in vivo experiments. Pearson's Chi-square test was used to analyze the association of the clinicopathologic characteristics of BC and PDZRN4 expression in **Table 1**, and Fisher's exact test was used when the number of intra-groups was five or less. The survival rates were determined using Kaplan-Meier analysis. Independent factors for tumor progression were determined using Cox regression analysis in **Tables 2** and **3**. $P<0.05$ was considered significant.

## Results

### *PDZRN4 expression is significantly reduced in BC tissues*

Prior to detecting the expression of PDZRN4 in the BC tissues, the levels of PDZRN4 in BC in publicly available datasets (https://www.oncomine.org) were analyzed. The data revealed that the levels of PDZRN4 mRNA were significantly reduced in BC tissues compared to normal tissues (**Figure 1A** and **1B**). RT-qPCR was then performed in the BC tissues and matched normal tissues of 24 patients with BC and the

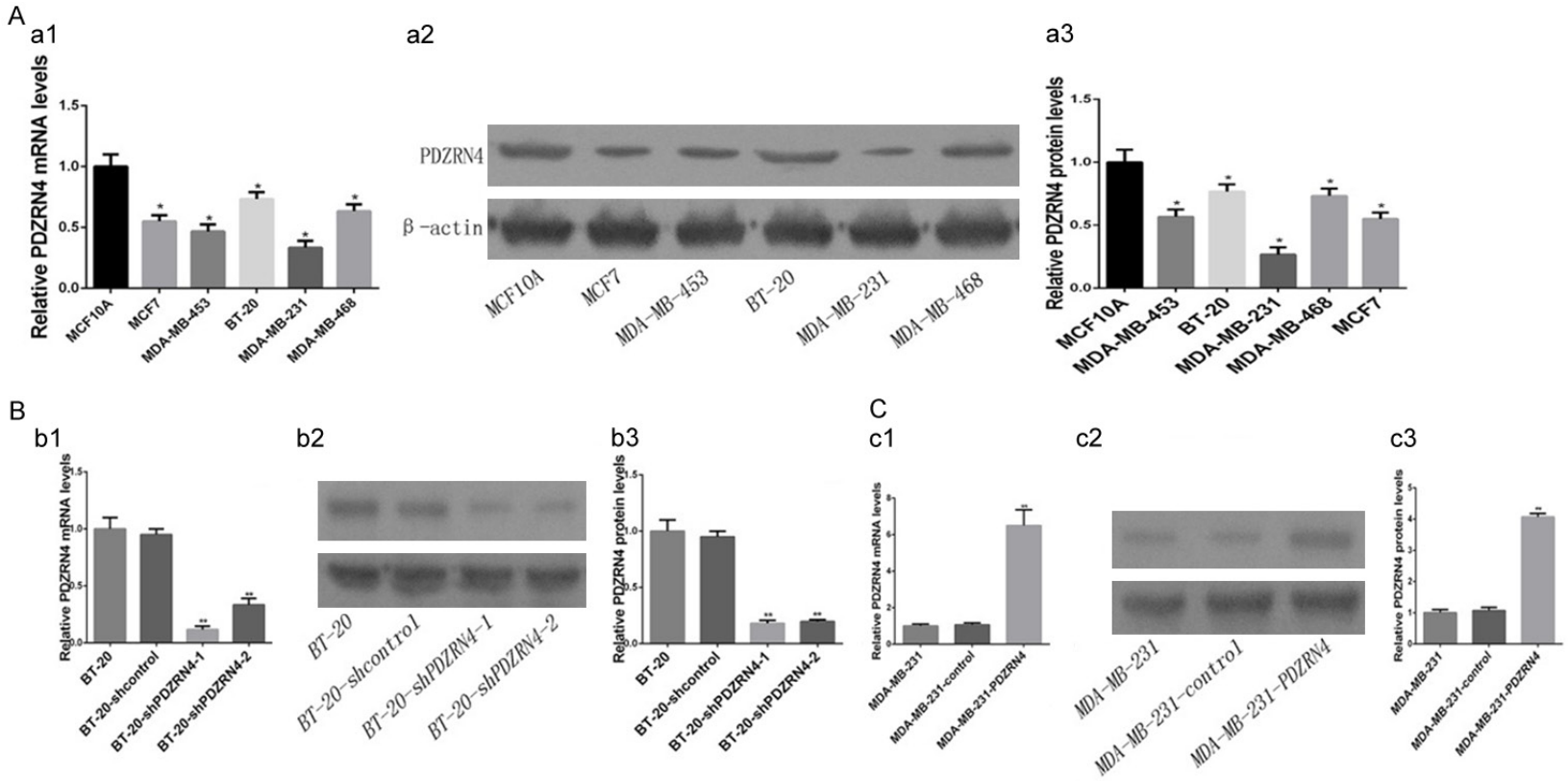


**Figure 3.** PDZRN4 is significantly reduced in BC cell lines. Aa1. PDZRN4 mRNA expression in human BC cell lines MDA-MB-231, MDA-MB-453, MDA-MB-468, BT-20, MCF7 and Human breast cell line MCF10A were tested by qRT-PCR. Aa2, Aa3. The protein expressions of PDZRN4 were detected by western blot. β-actin as an internal reference was used. B, C. qRT-PCR and western blot were performed to analyze the PDZRN4 mRNA and protein levels after transfection.

**Table 1.** Correlations between PDZRN4 and clinicopathologic variables of BC

| Variable | Number | PDZRN4 | | *P* value |
|---|---|---|---|---|
| | | Low | High | |
| Tumor size | | | | |
| <2 | 33 | 21 | 12 | 0.652 |
| ≥2 | 48 | 28 | 20 | |
| LNM | | | | |
| Negative | 37 | 19 | 18 | 0.044 |
| Positive | 44 | 30 | 14 | |
| Histological grade | | | | |
| 1 or 2 | 30 | 11 | 19 | 0.001 |
| 3 | 51 | 38 | 13 | |
| Perineural invasion | | | | |
| Negative | 30 | 6 | 24 | <0.001 |
| Positive | 51 | 43 | 8 | |

results demonstrated that the mRNA expression levels of PDZRN4 were significantly lower in the BC tissues than those in the matched normal tissues (**Figure 1C**). As the PDZRN4 mRNA levels in the BC tissues were significantly reduced (P<0.05), whether the expression of mRNA was coordinated with the expression of protein in BC tissues was investigated. Four pairs of BC tissues and matched normal tissues were selected, in which the BC tissues had relatively low PDZRN4 levels according to the RT-qPCR results, in order to detect the protein expression of PDZRN4 using western blot analysis and verify the consistency. As indicated in **Figure 1D** and **1E**, the protein expression of PDZRN4 was significantly downregulated in the BC tissues compared to the normal tissues. The results demonstrated that PDZRN4 was significantly downregulated in the BC tissues and reduced PDZRN4 may serve a role in promoting the malignant progression of BC.

*Low PDZRN4 expression correlates with malignant clinicopathologic features and poor prognosis of patients with BC*

The relationship between PDZRN4 expression and the clinicopathologic features of BC was analyzed. According to the PDZRN4 expression levels, the patients with BC (n=81) were divided into two groups: Scored 0 or 1, low PDZRN4 expression (n=49) (**Figure 2A** and **2B**); and scored 2+, high PDZRN4 expression (n=32) (**Figure 2C** and **2D**). The PDZRN4 expression was significantly correlated with LNM (*P*= 0.044), histological grade (*P*=0.001), and perineural invasion (*P*<0.001) (**Table 1**). As these clinicopathologic characteristics are representative of the malignant factors of cancer, the significance of PDZRN4 in the prognosis of patients with BC was evaluated. The results revealed that patients with BC in the high PDZRN4 group had better rates of overall survival (OS) and disease-free survival (DFS) than those in the low PDZRN4 group (DFS, P=0.007, **Figure 2E**; OS, P=0.029, **Figure 2F**). In addition, Cox regression analysis demonstrated that LNM, histologic grade, perineural invasion, and PDZRN4 expression were statistically significant for the OS and DFS in the univariate survival analysis (**Tables 2** and **3**). Furthermore, multivariate survival analysis demonstrated that PDZRN4 expression and perineural invasion were independent risk factors for OS and DFS in patients with BC (**Tables 2** and **3**). Therefore, low PDZRN4 expression is associated with malignant progression and predicts poor prognosis of patients with BC.

*PDZRN4 suppresses BC cell invasion and proliferation in vitro*

In order to identify the biological function of PDZRN4 in BC, the expression of PDZRN4 in human BC cell lines (MDA-MB-231, MDA-MB-453, MDA-MB-468, BT-20 and MCF7) and the human breast cell line MCF10A was analyzed. As shown in **Figure 3Aa1-Aa3** the levels of PDZRN4 mRNA and protein in the normal breast cell line (MCF10A) were higher than those in most of the BC cell lines (P<0.05). Among the BC cell lines, the BT-20 cells exhibited the highest expression levels of PDZRN4, while the MDA-MB-231 cells displayed the lowest levels. Therefore, the MDA-MB-231 and BT-20 cells were the most appropriate cell lines for the PDZRN4 functional tests. A specific shRNA was designed in order to interfere with PDZRN4 expression in the BT-20 cells and a plasmid was designed to overexpress PDZRN4 in the MDA-MB-231 cells for gain-and-loss-of-function assays. Compared to the control cells, the transfected BT-20 cells exhibited markedly reduced PDZRN4 mRNA levels (**Figure 3Bb1**), while the transfected MDA-MB-231 cells displayed upregulated PDZRN4 mRNA levels (**Figure 3Cc1**). The protein levels of PDZRN4 were tested using western blot analysis and the results were in accordance with the mRNA levels (**Figure 3Bb2**, **3Bb3** and **3Cc2**, **3Cc3**, P<0.001). Furthermore, a Transwell assay was used to investigate the role of PDZRN4 in the

**Table 2.** Univariable and multivariable analysis of disease-free survival (DFS) and PDZRN4 by Cox proportional hazards regression model

| Variable | Number | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Tumor size: <2 vs. ≥2 | 33 vs. 48 | 1.210 (0.735-1.993) | 0.453 | 1.104 (0.516-2.362) | 0.799 |
| Histological grade: 1 or 2 vs. 3 | 37 vs. 44 | 0.611 (0.374-0.998) | 0.049 | 0.735 (0.267-1.473) | 0.386 |
| LNM: positive vs. negative | 51 vs. 30 | 1.913 (1.159-3.156) | 0.011 | 1.660 (0.856-3.218) | 0.134 |
| Perineural invasion: positive vs. negative | 51 vs. 30 | 2.773 (1.678-4.583) | <0.001 | 2.146 (1.108-4.158) | 0.024 |
| PDZRN4: low vs. high | 49 vs. 32 | 1.840 (1.126-3.007) | 0.015 | 1.830 (1.032-3.242) | 0.039 |

**Table 3.** Univariable and multivariable analysis of overall survival (OS) and PDZRN4 by Cox proportional hazards regression model

| Variable | Number | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Tumor size: <2 vs. ≥2 | 33 vs. 48 | 0.671 (0.407-1.105) | 0.117 | 0.834 (0.391-1.779) | 0.639 |
| Histological grade: 1 or 2 vs. 3 | 37 vs. 44 | 0.533 (0.326-0.874) | 0.013 | 1.537 (0.758-3.117) | 0.234 |
| LNM: positive vs. negative | 51 vs. 30 | 1.743 (1.064-2.855) | 0.027 | 1.044 (0.546-1.995) | 0.897 |
| Perineural invasion: positive vs. negative | 51 vs. 30 | 2.343 (1.425-3.852) | 0.001 | 2.191 (1.110-4.324) | 0.024 |
| PDZRN4: low vs. high | 49 vs. 32 | 1.663 (1.013-2.731) | 0.044 | 2.292 (1.146-4.584) | 0.019 |

invasive ability of the BC cell lines. As presented in **Figure 4A**, knockdown of PDZRN4 significantly increased the invasive capacity of the BT-20 cells and overexpression of PAZRN4 in the MDA-MB-231 cells produced the opposite effects compared to the respective control groups (P<0.001). Furthermore, a colony formation experiment was used to test whether PDZRN4 expression affects the proliferative ability of BC cells. The data revealed that reduced PDZRN4 in the BT-20 cells resulted in a significant increase in the number of colonies (P<0.001; **Figure 4B**). Upregulation of PDZRN4 reduced the ability of the MDA-MB-231 cells to form colonies. Thus, these results demonstrate that PDZRN4 suppresses cell invasion and proliferation in BC.

*Reduced PDZRN4 facilitates BC growth and metastasis in vivo*

To further investigate the effect of PDZRN4 on BC progression *in vivo*, a SC xenograft tumor model was established. The results revealed that low PDZRN4 expression cell-derived tumors (MDA-MB-231 and BT-20-shPDZRN4) at the SC implantation sites grew more rapidly and to a much larger size than the high PDZRN4 expression cell-derived tumors (BT-20 and MDA-MB-231-PDZRN4) (P<0.001; **Figure 5A**). A lung metastasis model was also established in order to verify the role of PDZRN4 in BC metastasis and H&E staining was used to examine the metastatic foci in the lungs. The results demonstrated that the number of lung metastases in the BT-20-shPDZRN4 group was significantly higher than that in the BT-20-sh control group, and the number of lung metastases in the MDA-MB-231-PDZRN4 group was significantly less than that in the MDA-MB-231-control group (P<0.05; **Figure 5B**). These results suggest that reduced PDZRN4 promotes tumor growth and metastasis of BC *in vivo*. Taken together, reduced PDZRN4 facilitates BC progression and predicts poor prognosis.

## Discussion

The main reason for cancer-associated mortality is the invasion and metastasis of cancer cells [16], including those associated with BC. In the present study, PDZRN4 expression was reduced in the BC tissues and was associated with poor prognosis of patients with BC. Furthermore, it was also demonstrated that reduced PDZRN4 promoted invasion and proliferation in the *in vitro* experiments. Thus, PDZRN4 acts as a potential targeted treatment of BC and a valuable prognostic indicator for patients with BC.

The LNX (PDZRN) family possess a number of advantages due to their structures, with a RING domain involved in E3 ubiquitin ligase activity and PDZ domains involved in different cell sig-

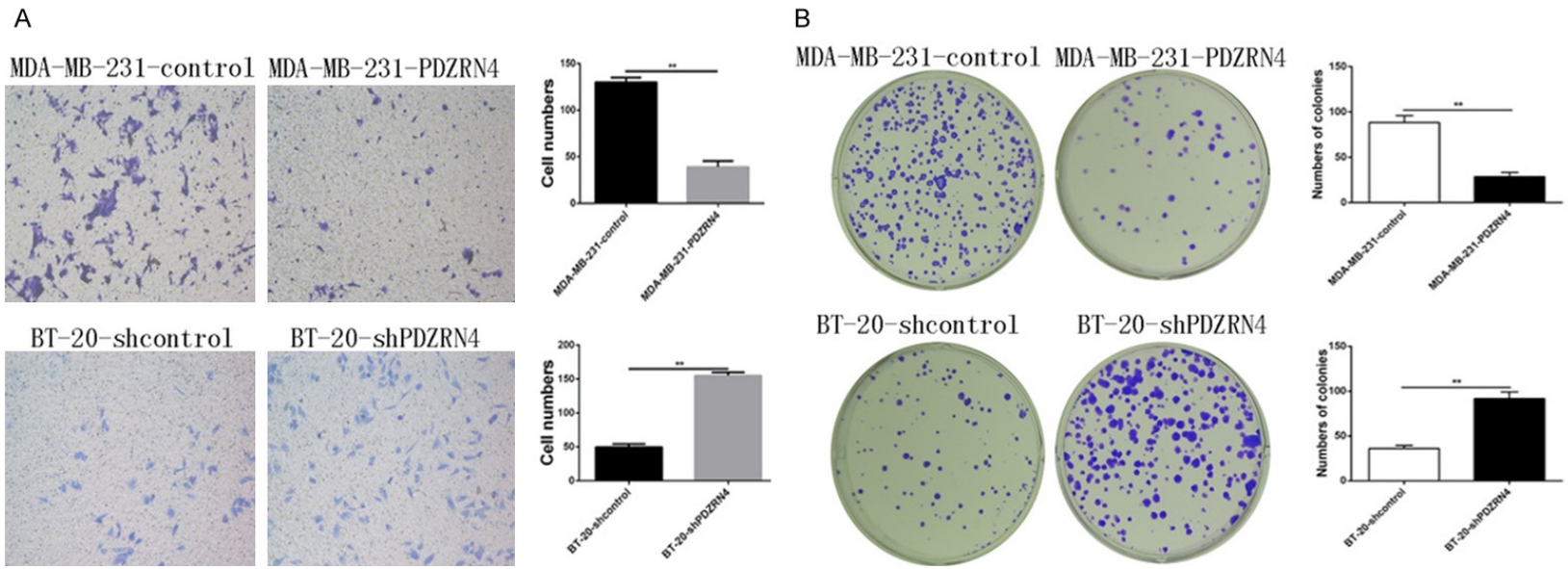


**Figure 4.** Reduced PDZRN4 promotes proliferation and metastasis of BC *in vitro*. Transwell assay (A) was performed to test the ability of cell invasion. (B) Colony formation assays were implemented to test the proliferative ability. Data are means ± SD.

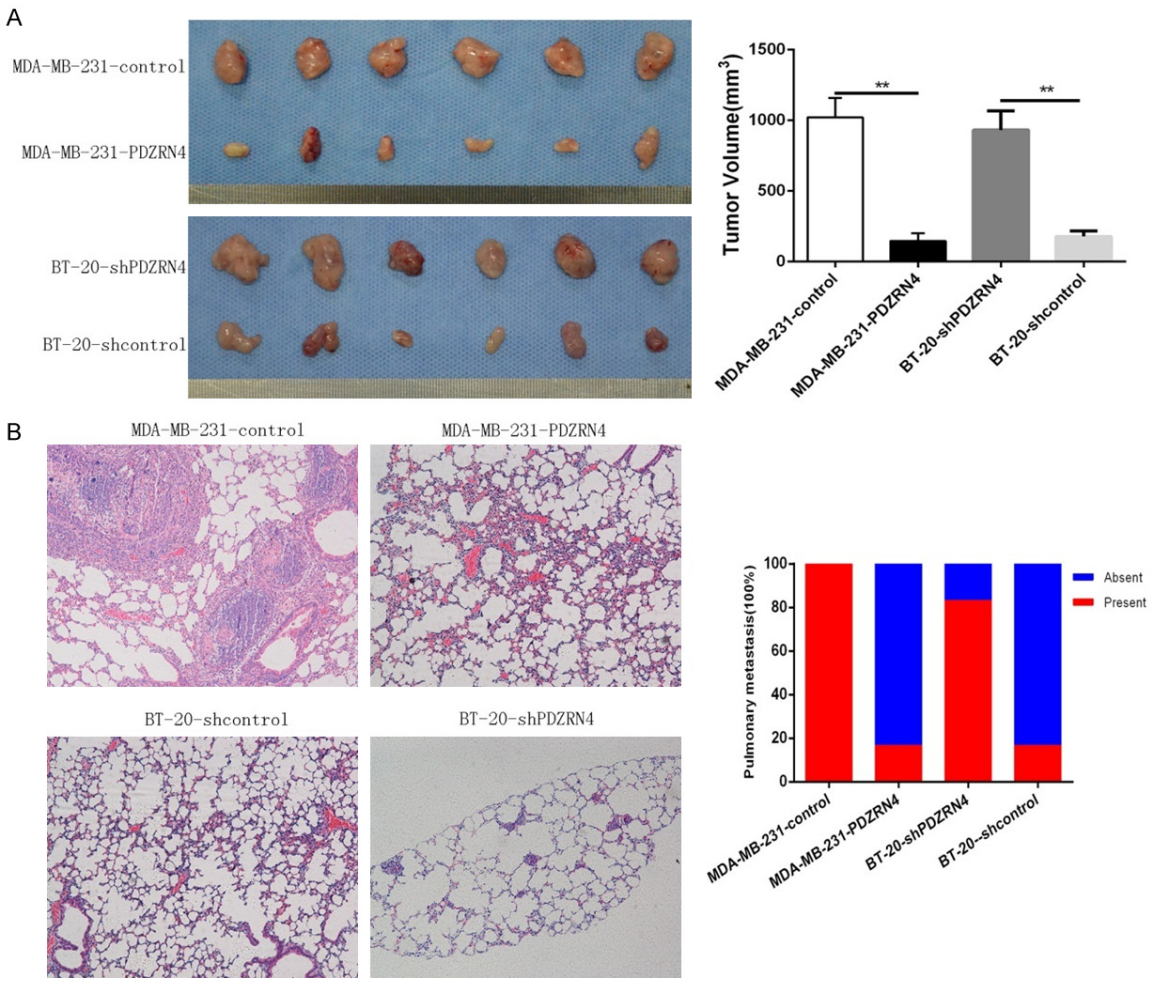


**Figure 5.** Reduced PDZRN4 facilitates proliferation and metastasis of BC *in vivo*. A. Tumors derived from MDA-MB-231-PDZRN4 and BT-20-shPDZRN4 with their control groups are shown. B. Different groups of lung metastasis are represented; metastatic lesions of lungs were analyzed.

naling assemblies [10, 11]. Therefore, the biological functions of the LNX family attract interest, particularly for cancer research. A previous study demonstrated that LNX1 was lowly expressed in glioma tissues compared with the expression levels in normal adult brains [17]. In addition, LNX1 was demonstrated to be reduced in colonospheres or side population cells of colorectal carcinoma cells [18]. Furthermore, the expression of LNX2, another member of the LNX family, was consistently amplified in colorectal cancer [19]. A previous study demonstrated that the expression of PDZRN4 is downregulated in liver cancer [12]. Based on these results, we hypothesized that the LNX family was likely to be distinctly expressed in different types of cancer. Based on a public database (https://www.oncomine.org), PDZRN4 mRNA was identified as markedly reduced in BC tissues compared to normal tissues, which suggested that there is a strong relationship between PDZRN4 and BC progression. We hypothesized that this relationship would present as altered expression levels in BC tissues. Therefore, BC tissues and matched normal tissues were investigated to clarify that there was differential expression at the mRNA level. In order to test whether mRNA and protein modification causes a change in protein expression, the accordance of mRNA and protein expression in the tissues was tested and the results demonstrated that the mRNA expression was coordinated with the protein expression in these BC tissues and the expression levels of PDZRN4 were significantly downregulated in the BC tissues compared to the normal tissues. Based on these results, PDZRN4 is significantly downregulated in BC tis-

sues and reduced PDZRN4 may serve a role in promoting the malignant progression of BC.

Clinically, BC is highly invasive and metastatic [20]. In clinical practice, receptor tyrosine-protein kinase erbB-2, estrogen receptor and progesterone receptor are accepted as biomarkers and biotargets of BC [21], and there are no other well-accepted biomarkers for targeting BC as a potential treatment. As PDZRN4 exhibited differential expression in BC tissues and, to the best of our knowledge, there were no studies that certify the significance of PDZRN4 in the clinicopathologic features of cancer, the relationship between PDZRN4 and the clinicopathologic features of BC was analyzed in the present study. The expression of PDZRN4 was significantly associated with the AJCC stage, LNM, histological grade, and perineural invasion, which indicates that PDZRN4 affects the malignant aspects of BC and the prognosis of patients with BC. Kaplan-Meier analysis identified that the BC patients with high PDZRN4 expression had better rates of OS and DFS compared to the group of BC patients with low PDZRN4 expression, which indicates that PDZRN4 expression in BC tissues may act as a candidate for predicting the survival of patients with BC. These results demonstrate that low PDZRN4 expression is associated with malignant progression and poor prognosis of patients with BC.

In patients with malignant tumors, the features that affect prognosis most are invasion/metastasis and proliferation [22]. In a previous study, PDZRN4 negatively affected the proliferation of liver cancer and overexpression of PDZRN4 was demonstrated to restrain the colony formation of HCC cells [12]. As PDZRN4 was involved in the OS and BCR-free survival of patients with BC, we hypothesized that PDZRN4 may be involved in the regulation of the malignant aspects of BC progression, particularly invasion/metastasis and proliferation. In order to investigate this, two BC cell lines that have opposite PDZRN4 expression levels were used to test the biological functions *in vitro*. The results revealed that downregulation of PDZRN4 significantly improved the migration and invasion abilities of BC cells in a transwell assay, and upregulation of PDZRN4 exhibited a negative effect on the migration and invasion of BC cells. Furthermore, knockdown of PDZRN4 expression in the BC cells enhanced the proliferation ability *in vitro*, as tested using a colony formation assay. Reduced PDZRN4 was demonstrated to promote the invasion and metastasis of BC cells not only *in vitro* but also *in vivo*. The tumor tissues of groups with low PDZRN4 expression were larger compared to the high-PDZRN4 expression groups. Furthermore, more metastatic lesions were formed in the low expression groups than in the high expression groups. Therefore, reduced PDZRN4 may serve a role in promoting the modulation of BC growth and progression.

In order to determine the intrinsic mechanism of PDZRN4 in BC progression, the BioGRID (https://thebiogrid.org/) and STRING databases (https://string-db.org/) were used and PDZRN4 was revealed to interact with Kidins220. A number of studies have indicated that Kidins220 serves an important role in tumor progression by affecting the abilities of proliferation, invasion and migration [23, 24]. Kidins220 not only promotes cell proliferation in tumors by activation of the ERK pathway [25, 26], but is also involved in the regulation of cytoskeletal remodeling. Cytoskeletal remodeling is necessary for tumor cell migration and invasion in epithelial-mesenchymal transition (EMT), which is important for tumor cell movement [27]. Kidins220 is highly expressed in numerous types of tumor and high expression of Kidins220 promotes tumor progression [25, 28, 29]. PDZRN4 has ubiquitination modification ability and Kidins220 has ubiquitination modification sites, as identified in the BioGRID database. We hypothesized that PDZRN4 may maintain cell signal stability via degradation of Kidins220 by ubiquitination, and a reduction in PDZRN4 relatively increases the expression of Kidins220, resulting in the progression of cancer. This hypothesis is only deduced from the literature and a public database, so a follow-up study is required to verify this hypothesis.

In summary, the present study demonstrates that PDZRN4 is an independent prognostic indicator for patients with BC. Further, the functional experiments confirmed the role of PDZRN4 in the growth and metastasis of BC cells. Therefore, PDZRN4 may act as a tumor suppressor in BC. The results of the present study indicate that PDZRN4 is a prognostic indicator in BC and a potential targeted treatment.

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## Disclosure of conflict of interest

None.

## Abbreviations

BC, breast cancer; PDZRN4, PDZ Domain Containing Ring Finger 4; mRNA, messenger RNA; LNM, Lymph Node Metastasis; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; IDBC, Invasive Ductal Breast Carcinoma; BPT, Breast Phyllodes Tumor.

**Address correspondence to:** Dr. Ya-Kui Liu, Department of Thoracic Surgery, Jingmen First People's Hosptial, Jingmen, Hubei, PR China. E-mail: hubeilyk@163.com

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