

DUSP28 promotes cell proliferation, migration, and invasion by Akt/ β -catenin/Slug axis in breast cancer

Jiabo Zhang and Yu Guo✉

Department of Breast Surgery, Ningbo First Hospital, Ningbo city, Zhejiang Province, 31500, China

Background: Breast cancer (BCa) has been the most commonly diagnosed cancer worldwide and the leading cause of cancer-related death. Dual-specificity phosphatase 28 (DUSP28) is associated with various cancer progression, but its function and mechanism in breast cancer remain unclear. **Methods:** DUSP28 level was identified by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot assays. The proliferation, migration, and invasion of DUSP28 in MCF-7 and MDA-MB-231 cells were assessed by Cell Counting kit-8 (CCK-8), colony formation, and transwell assays. The xenograft tumor model was established to explore the effects of DUSP28 on tumor growth of nude mice. Immunohistochemistry (IHC) and western blot assays were performed to evaluate the expression of related signal molecules. **Results:** The expression of DUSP28 was up-regulated in BCa tissues and closely correlated with tumor size and distant lymphatic metastasis in The Cancer Genome Atlas (TCGA) dataset. Quantitative real-time PCR and western blot assays indicated that the expression of DUSP28 was up-regulated in BCa cells. DUSP28 was demonstrated to promote the proliferation, migration, and invasion of MCF-7 and MDA-MB-231 cells *in vitro*. Knockdown of DUSP28 inhibited tumor growth of xenograft tumor mice *in vivo* and reduced the levels of DUSP28 and Ki-67. Notably, further mechanism analysis indicated that DUSP28 promoted the activation of Akt/ β -catenin/Slug signaling. **Conclusion:** DUSP28 exerts its oncogene function *via* regulating Akt/ β -catenin/Slug signaling in BCa, indicating that DUSP28 may provide a promising therapeutic target for the treatment of BCa.

Keywords: DUSP28, breast cancer, proliferation, invasion, Akt/ β -catenin

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✉e-mail: guoyu308@163.com

Abbreviations: BCa, breast cancer; BCA, bicinchoninic protein acid; DUSPs, Dual-specificity phosphatases; EMT, epithelial-mesenchymal transition; ERK1/2, extracellular signal-regulated kinase 1/2; IHC, Immunohistochemistry; OD, optical density; RIPA, radio-immunoprecipitation assay; TCGA, The Cancer Genome Atlas

INTRODUCTION

Breast cancer (BCa) has surpassed lung cancer as the most commonly diagnosed cancer worldwide and the leading cause of cancer-related death, with an estimated 2.3 million women diagnosed with breast cancer occurred in 2020 (Sung *et al.*, 2021). The pathogenesis of BCa is very heterogeneous, with different subtypes presenting different biological behavior and progression (Zheng *et al.*, 2017; Feng *et al.*, 2018). The etiology of BCa includes age, genetics, diet, radiation, various envi-

ronmental chemicals, endocrine, and other factors (Peart, 2015). Despite great progress in research and clinical diagnosis and treatment, the prognosis of BCa still needs to be improved because long-term continuous chemoradiotherapy will produce certain toxic side effects and induce drug resistance, as well as local and distal metastasis in the treatment process, the mortality of BCa remain high (Tao *et al.*, 2015; Ullah, 2019). Understanding the mechanism of tumorigenesis is a necessary condition for the development and formulation of novel effective therapeutic measures, and it is necessary to explore new biomarkers to predict and track disease progression or treatment response.

Dual-specificity phosphatases (DUSPs) are protein phosphatases that regulate the activity of mitogen-activated protein kinases (MAPKs) (Dhillon *et al.*, 2007; Lang *et al.*, 2006). DUSPs are critical in regulating cancer cell growth and survival (Ramkissoon *et al.*, 2019; Xian *et al.*, 2018). These genes can be classified as “classical” or “non-classical” based on dephosphorylation or phosphorylation and involve specific residues in various cancer cells (Wang *et al.*, 2014; Patterson *et al.*, 2009; Prabhakar *et al.*, 2014; Rios *et al.*, 2014). A growing body of evidence suggests that dysregulation of DUSPs is a common phenomenon and plays a key role in human cancer, and their differential expression may be a potential biomarker for tumor prognosis (Gao *et al.*, 2021). Previously, blocking DUSP28 has been reported to inhibit chemotherapy resistance and migration in pancreatic cancer by inhibiting the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway (Lee *et al.*, 2015). Evidence suggests that DUSP28 promotes the progression of pancreatic cancer and hepatocellular carcinoma (Lee *et al.*, 2017; Lee *et al.*, 2016; Lee *et al.*, 2019; Wang *et al.*, 2014; Kim *et al.*, 2018). However, the role of DUSP28 in the development of breast cancer remains unclear.

In the present study, we analyzed the mRNA expression of DUSP28 in the BCa project extracted from The Cancer Genome Atlas (TCGA) samples through the Ualcan (<http://ualcan.path.uab.edu/analysis.html>) prediction dataset. DUSP28 was significantly overexpressed in BCa tissues and associated with tumor stage and lymph node metastasis. DUSP28 functioned as an oncogene to promote the growth of BCa cells both *in vitro* and *in vivo*. Moreover, DUSP28 promoted the migration, invasion, and epithelial-mesenchymal transition (EMT) of BCa cells *in vitro*. Further study revealed that the mechanism by which DUSP28 exerts its pro-cancer effect in BCa cells may be through the regulation of the Akt/ β -catenin/Slug axis. Our study provides novel directions and insights into the mechanism of DUSP28 in BCa, which may be a promising target for the treatment of BCa.

MATERIALS AND METHODS

Cell culture and transfection

Human breast cancer cell lines (MCF-7, MDA-MB-157, MDA-MB-453, and MDA-MB-231) and human normal mammary cell MCF10A were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle Medium or Roswell Park Memorial Institute 1640 medium (Gibco, USA), containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution at 37°C in a humidified 5% CO₂ atmosphere. The DUSP28 sequences inserted into the pc-DNA3.1 vector to overexpress DUSP28 (DUSP28), the control pc-DNA3.1 empty vector (Vector), the lentiviral vector containing short hairpin RNA against DUSP28 (sh-DUSP28#1 and sh-DUSP28#2) and negative control (sh-NC) were all designed and synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, Guangdong, China). Next, Lipofectamine 3000 (Invitrogen) was used to perform cell transfection based on the manufacturer's instructions.

Cell Counting Kit-8 (CCK-8) assay

The transfected cells (1×10^4 cells/well) were seeded into a 96-well plate incubated for 0, 24, 48, 72, and 96 h, respectively. Next, 10 μ L of CCK-8 solution (Beyotime, Shanghai, China) was added and incubated for 4 h. The optical density (OD) was measured with a microplate reader at 450 nm.

Colony formation assay

1×10^3 cells were placed in a 6-well plate and incubated for 2 weeks with fresh medium replaced every 2–3 days. Cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet for 15 min. Finally, colonies were counted and photographed.

Flow cytometry

48 hours after transfection, the cells were harvested, digested, and then fixed in 70% ethanol at 4°C for 12 h. Afterwards, the cells were washed in PBS, stained with propidium iodide (PI, Sigma-Aldrich) with Ribonuclease A (Takara Biotechnology, Dalian, China) at 37°C for 30 min. The cell cycle was detected using FACS Calibur (BD Biosciences, USA) and analyzed by FlowJo software.

Quantitative Real-time PCR

Total RNA was extracted using TRIzol Reagent (Invitrogen) and reverse transcribed using PrimeScript RT reagent Kit (Takara, Tokyo, Japan) according to the manufacturer's instructions. Quantitative PCR was conducted using SYBR Premix Ex Taq TM (Takara) Applied Biosystems 7500 Sequence Detection system (ABI, USA). The mRNA expression was calculated using a 2^{- $\Delta\Delta$ CT} method and GAPDH was used as an internal control to normalize the data. The primer sequences were as follows: DUSP28 (forward), 5'-CCTTCCAGATGGT-GAAGAGC-3' and (reverse), 5'-GGTGAATGTGGGT-GACACTG-3'; GAPDH (forward) 5'-ACACCCACTC-CTCCACCTTT-3' and (reverse) 5'-TTACTCCTTG-GAGGCCATGT-3'.

Western blot assay

Total proteins were extracted with a radio-immunoprecipitation assay (RIPA) lysis buffer (Solarbio, Beijing, China) and were quantified using the bicinchoninic protein acid (BCA) kit (Beyotime). The equal amount of protein was loaded and resolved with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat milk and incubated with primary antibodies overnight at 4°C. The primary antibodies were as followed: DUSP28 (# MA5-17248, 1:2000, Thermo Fisher Scientific, Inc., Waltham, MA, USA), E-cadherin (1:1000, Cat#ab133597, Abcam, Cambridge, MA, USA), N-cadherin (1:5000, Cat#ab76011, Abcam), Vimentin (1:500, Cat#ab8978, Abcam), Akt (1:5000, Cat#ab182729, Abcam), p-Akt (1:1000, Cat#ab38449, Abcam), GSK-3 β (1:5000, Cat#ab32391, Abcam), p-GSK-3 β (1:10000, Cat#ab75814, Abcam), β -catenin (1:15000, Cat#ab32572, Abcam), c-Myc (1:1000, Cat#ab32072, Abcam), Cyclin D1 (1:200, Cat#ab16663, Abcam), Slug (1:1000, Cat#ab27568, Abcam), GAPDH (1:2500, Cat#ab9485, Abcam). The membranes were incubated with the corresponding HRP-conjugated secondary antibodies (anti-rabbit, 1:1000, Cat#ab6721, Abcam; anti-mouse, 1:1000, Cat#ab6728, Abcam) at room temperature for 1 h. Afterwards, Bands were visualized and observed using an enhanced chemiluminescence reagent (ECL) kit (Beyotime). GAPDH was served as a loading control. The intensity of protein bands was quantified using Image J software.

Immunohistochemistry (IHC) assay

In brief, the paraffin-embedded tissues were cut into slides 4 μ m thick, dewaxed and dehydrated in gradient ethanol. The sections were then microwave boiled in 10 mmol/L citrate buffer to extract the antigen, incubated with 3% H₂O₂ and blocked with 5% bovine serum albumin (BSA) for 30 min. Afterwards, the sections were then incubated with primary antibodies against DUSP28 (# PA5-61523, 1:50, Thermo Fisher Scientific), Ki-67 (1:500, Cat#ab192742, Abcam) at 4°C overnight, followed by incubation with the peroxidase-conjugated antibody for 30 min at 37°C. Thereafter, the signal was visualized using 3,3'-diaminobenzidine (DAB, Proteintech, Wuhan, China) for 10 min and all the slides were counterstained with haematoxylin, dehydrated and coverslipped. Last, the images were photographed using a light microscope (Olympus, Japan).

Transwell assay

Cell migration and invasion were analyzed in a 24-well plate with transwell chambers (Millipore, Bedford, MA, USA) using an 8- μ m polycarbonate membrane (Corning, NY, USA) without or with Matrigel (Millipore) coating. Transfected cells (1×10^4 cells/well) were cultured with 100 μ L of serum-free medium in the upper chamber and 600 μ L of complete medium containing 10% FBS in the lower chamber. After 24 h, the cells in the lower chamber were fixed with 4% paraformaldehyde, washed in PBS twice, and stained with 0.5% crystal violet. The stained cells were observed under an inverted microscope (Olympus, Japan).

In vivo experiments

Animal studies were approved by the Institutional Animal Care and Use Committee of Ningbo First Hospital.

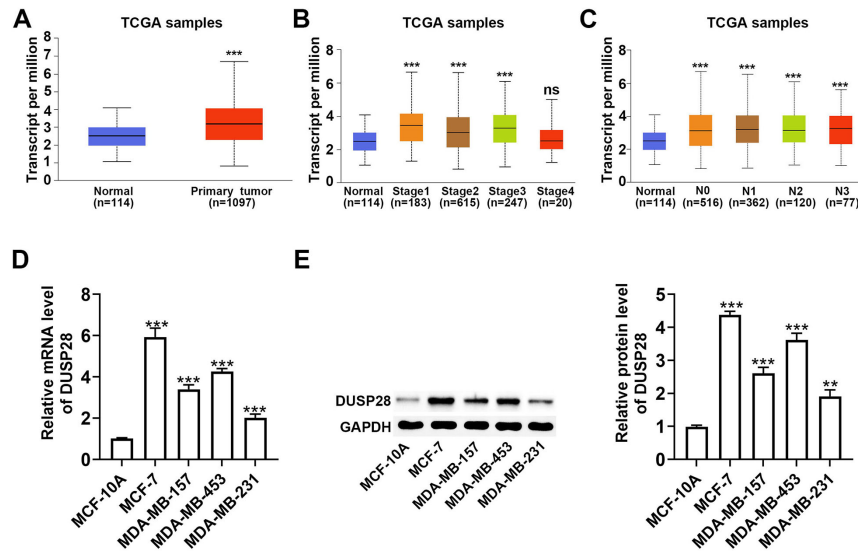


Figure 1. DUSP28 expression is up-regulated in BCa.

(A) UALCAN (<http://ualcan.path.uab.edu/analysis.html>) analysis of DUSP28 expression in The Cancer Genome Atlas (TCGA) database; (B) UALCAN analysis of DUSP28 expression with different clinical stages; (C) UALCAN analysis of DUSP28 expression with lymph node metastasis; (D) The mRNA expression of DUSP28 in breast cancer cell lines (MCF-7, MDA-MB-157, MDA-MB-453 and MDA-MB-231) and human normal mammary cell MCF10A was determined by qRT-PCR assay; (E) The protein level of DUSP28 in breast cancer cell lines (MCF-7, MDA-MB-157, MDA-MB-453 and MDA-MB-231) and human normal mammary cell MCF10A was determined by western blot assay. Data are shown as mean \pm S.D.; ** $P < 0.01$ and *** $P < 0.001$, ns represented as not significant.

A total of 10 nude mice (4-week-old) were randomly divided into two groups ($n=5$ per group). 1×10^6 MCF-7 cells stably transfected with sh-DUSP28, or sh-NC suspended in 200 μ L normal saline and subcutaneously injected into the right flank of mice. The tumor volume was measured every week and calculated using the formula: length \times width² \times 0.5. After 5 weeks of injection, the mice were anesthetized and ethically euthanized and the tumor tissues were excised for weighting and expression analysis.

RESULTS

DUSP28 expression is up-regulated in BCa

The expression of DUSP28 mRNA in BCa tissue samples was analyzed in the TCGA dataset of the UALCAN database website. It was found that the level of DUSP28 was significantly up-regulated in BCa tissues, which was related to tumor stage and lymph node metastasis (Fig. 1A–C). Subsequently, qRT-PCR and western blot assays showed that the expression level of DUSP28 was up-regulated in breast cancer cell lines (MCF-7, MDA-MB-157, MDA-MB-453 and MDA-MB-231) compared with human normal mammary cell MCF10A (Fig. 1D–E).

DUSP28 promotes the proliferation of BCa cells

Next, to identify the biological function of DUSP28 in BCa cells, we detected the knockdown or overexpression efficiency of DUSP28 in MCF-7 and MDA-MB-231 cells as shown in Fig. 2A–B. Due to the higher gene silencing efficiency of sh-DUSP28#1 in MCF-7 cells, we selected sh-DUSP28#1 (labeled as sh-DUSP28) for the subsequent assays. CCK-8 and colony formation assays demonstrated that knockdown of DUSP28 markedly hindered the proliferation of MCF-7 cells whereas overexpression of DUSP28 dramatically increased the proliferation of MDA-MB-231 cells (Fig. 2C–D). Flow cytometry results in-

dicated that compared with the sh-NC group, the proportion of cancer cells in the G0/G1 phase increased significantly, while that in the S phase decreased significantly in the sh-DUSP28 group (Fig. 2E). On the contrary, the proportion of G0/G1 cancer cells in the DUSP28 group was significantly lower than that in the Vector group, while the proportion of S cancer cells was significantly increased (Fig. 2E). These data suggested that DUSP28 promoted the proliferation of BCa cells *in vitro*.

Knockdown of DUSP28 inhibits BCa tumor growth *in vivo*

In vivo xenograft mice models were constructed to further evaluate the effects of DUSP28 on the tumorigenicity of MCF-7 cells. The representative image revealed that the tumor size was obviously inhibited in the sh-DUSP28 group (Fig. 3A). The growth curve of the tumor implied that knockdown of DUSP28 significantly slowed the BCa tumor growth (Fig. 3B). Consistently, the tumor weight of the sh-DUSP28 group was significantly reduced compared with the sh-NC group (Fig. 3C). Moreover, IHC staining displayed that knockdown of DUSP28 apparently inhibited the expression of DUSP28 and Ki-67 in xenograft tumors (Fig. 3D). Collectively, the results above suggested that knockdown of DUSP28 could inhibit BCa growth *in vivo*.

DUSP28 promotes the migration, invasion, and epithelial-stromal transformation (EMT) of BCa cells

The results of transwell assays indicated that knockdown of DUSP28 obviously inhibited cell migration and invasion in MCF-7 cells while overexpression of DUSP28 increased cell migration and invasion in MDA-MB-231 cells. Further western blot assay illustrated that knockdown of DUSP28 decreased the expression of E-cadherin and increased the expression of N-cadherin and vimentin in MCF-7 cells, whereas overexpression of DUSP28 exhibited an opposite result in MDA-MB-231

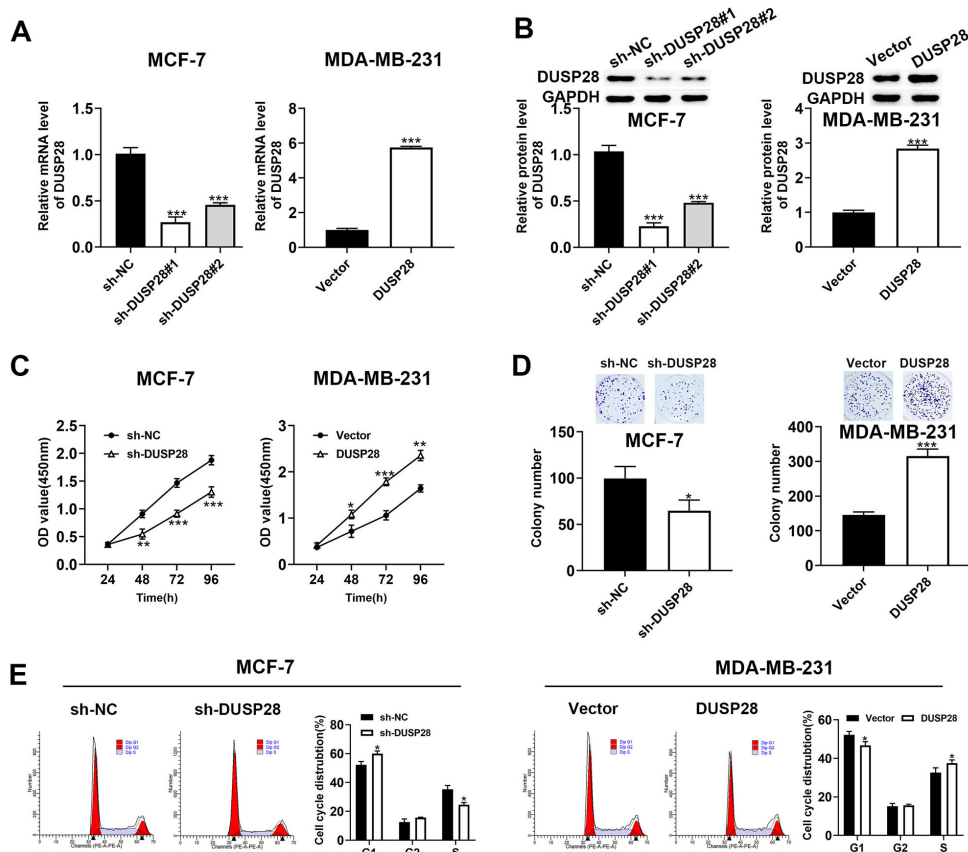


Figure 2. DUSP28 promotes the proliferation of BCa cells.

(A) The mRNA expression of DUSP28 in MCF-7 and MDA-MB-231 cells was measured by qRT-PCR assay; (B) The protein level of DUSP28 in MCF-7 and MDA-MB-231 cells was determined by western blot assay; (C) CCK-8 assay was used to determine the cell viabilities of MCF-7 and MDA-MB-231 cells; (D) Colony formation assay was used to detect the number of cell clones in MCF-7 and MDA-MB-231 cells; (E) Flow cytometry assay was used to detect the distribution of cell cycle in MCF-7 and MDA-MB-231 cells. Data are shown as mean \pm S.D.; * P <0.05, ** P <0.01 and *** P <0.001.

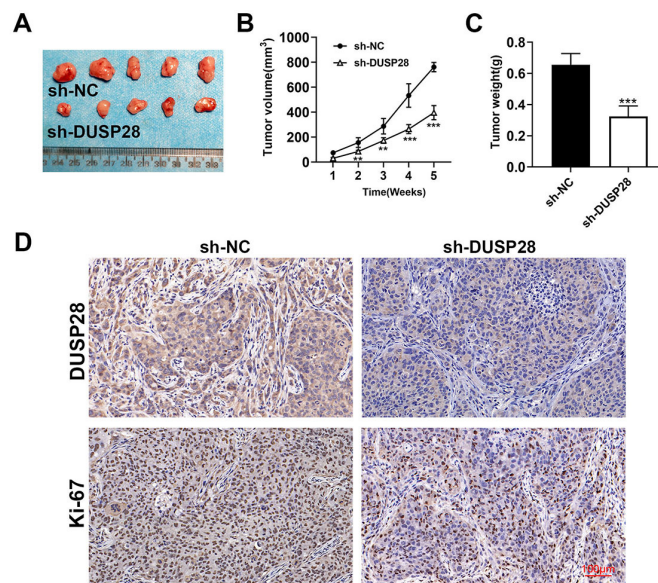


Figure 3. Knockdown of DUSP28 inhibits BCa tumor growth *in vivo*.

(A) Photographs of tumors excised from the mice injected with MCF-7 cells transfected with sh-NC or sh-DUSP28; (B) Tumor growth curves of mice injected with MCF-7 cells transfected with sh-NC or sh-DUSP28; (C) Tumor weight of mice injected with MCF-7 cells transfected with sh-NC or sh-DUSP28; (D) The levels of DUSP28 and Ki-67 in tumors from mice injected with MCF-7 cells were evaluated by IHC staining (Scale bar=100 μ m, magnification, 200 \times). Data are shown as mean \pm S.D.; ** P <0.01 and *** P <0.001.

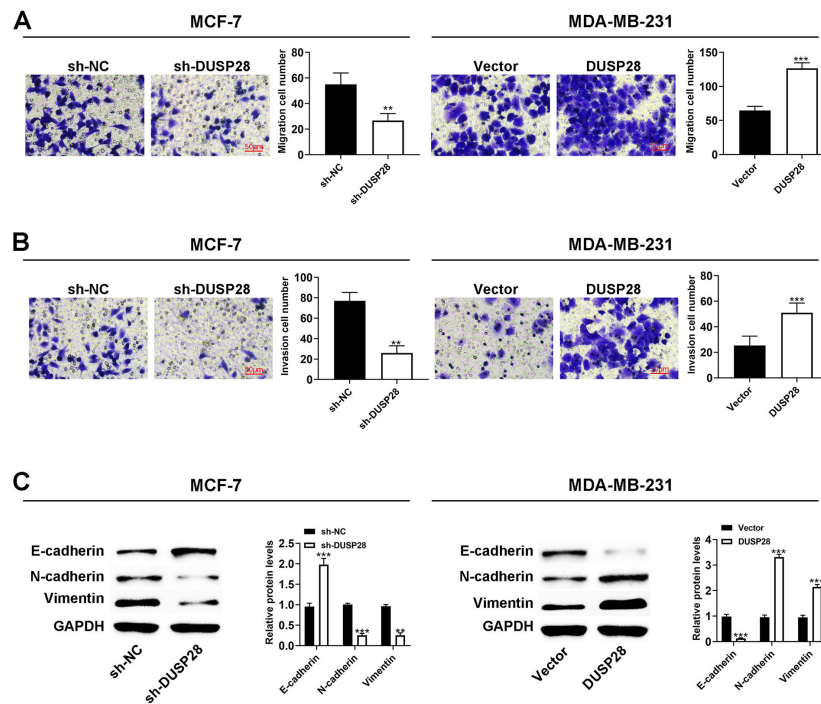


Figure 4 DUSP28 promotes the migration, invasion and epithelial-stromal transformation (EMT) of BCa cells

(A) Transwell assay was used to detect the migration capabilities of MCF-7 and MDA-MB-231 cells (Scale bar=50 μ m, magnification, 400 \times); (B) Transwell assay was used to detect the invasion capabilities of MCF-7 and MDA-MB-231 cells (Scale bar=50 μ m, magnification, 400 \times); (C) The levels of E-cadherin, N-cadherin and Vimentin in MCF-7 and MDA-MB-231 cells were measured by western blot assay. Data are shown as mean \pm S.D.; ** P <0.01 and *** P <0.001.

cells (Fig. 4C). These data suggested that DUSP28 promoted the migration, invasion and EMT of BCa cells.

DUSP28 plays an oncogenic role in BCa cells through Akt/ β -catenin/Slug axis

The Akt/ β -catenin signaling pathway is usually abnormally activated and plays an important regulatory role in the progression of breast cancer (Song *et al.*, 2018; Li *et al.*, 2017; Yu *et al.*, 2018). Western blotting was used to study the molecular mechanism of DUSP28 regulating the development of BCa. The results showed that knockdown of DUSP28 signifi-

cantly downregulated the levels of p-Akt, p-GSK-3 β , β -catenin, c-myc, cyclin D1 and Slug in MCF-7 cells, while overexpression of DUSP28 lead to the opposite results. To confirm whether Akt/ β -catenin engages in the occurrence of BCa mediated by DUSP28, the cells were cultured with 10mMol/L Akt-specific inhibitor MK2206. Treatment with MK2206 decreased the levels of p-Akt, p-GSK-3 β and β -catenin in MCF-7 cells and reversed the activation of the Akt/ β -catenin/Slug pathway triggered by DUSP28 (Fig. 5B). Furthermore, inhibition of Akt by MK2206 could counteract the effects of overexpression of DUSP28 on

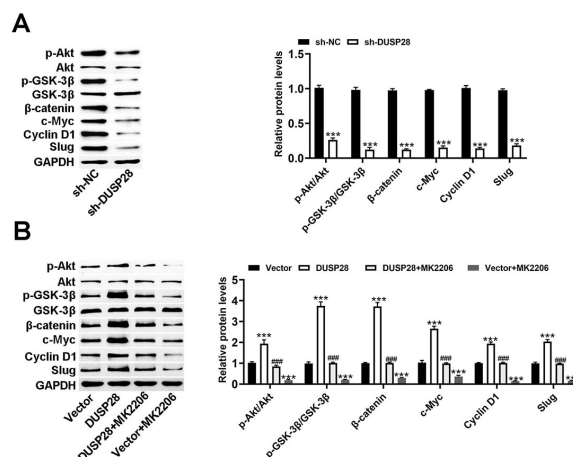


Figure 5. DUSP28 plays an oncogenic role in BCa cells through Akt/ β -catenin/Slug axis

(A) The levels of Akt, p-Akt, GSK-3 β , p-GSK-3 β , β -catenin, c-myc, cyclin D1 and Slug in MCF-7 cells were evaluated by western blot assay; (B) The levels of Akt, p-Akt, GSK-3 β , p-GSK-3 β , β -catenin, c-myc, cyclin D1 and Slug in MDA-MB-231 cells were evaluated by western blot assay. Data are shown as mean \pm S.D.; compared with sh-NC group or Vector group, *** P <0.001; compared with DUSP28 group, ### P <0.001

the proliferation (c-myc and cyclin D1), migration and invasion (Slug) related proteins of MDA-MB-231 cells (Fig. 5B). These results suggest that the Akt/ β -catenin/Slug pathway engages in the promotion of DUSP28 in BCa progression.

DISCUSSION

In the present study, we firstly provided evidence for the role of DUSP28 in regulating BCa proliferation, migration, invasion, and EMT. In addition, we demonstrated that Akt/ β -catenin/Slug axis is essential for DUSP28-mediated BCa progression. Therefore, DUSP28 functions as an oncogene in BC and may be a potential therapeutic target for BC.

DUSPs have many heterogeneous forms and functions, can dephosphorylate phosphorylated serine, threonine and tyrosine residues and up to 44 human DUSP genes have been identified (Gao *et al.*, 2021). DUSPs are divided into 6 subgroups, the phosphatase and tensin homolog protein phosphatases (PTENs), mitogen-activated protein kinase phosphatases (MKPs), cell division cycle 14 (CDC14) phosphatases (CDC14s), atypical DUSPs, phosphatases of the regenerating liver (PRLs) and slingshot protein phosphatases (SSHs) (Gao *et al.*, 2021). Among them, DUSP4 is considered a potential marker of chemotherapy resistance in triple-negative breast cancer (TNBC), and a low DUSP4 expression level can predict recurrence and mortality in TNBC patients (Baglia *et al.*, 2014). Inhibition of DUSP6 has been reported to increase the sensitivity of cancer cells to chemotherapeutic drugs (Wu *et al.*, 2018; James *et al.*, 2019). Studies have shown that DUSP28 promotes the proliferation and cycle progression of human liver cancer cells by regulating the P38 MAPK signaling pathway (Wang *et al.*, 2014). Evidence also reveals that DUSP28 can promote pancreatic cancer cell migration and drug resistance (Lee *et al.*, 2015; Lee *et al.*, 2017; Lee *et al.*, 2016). We found for the first time that the expression level of DUSP28 was significantly increased in BCa tissues and cells, and DUSP28 was associated with tumor stage and lymph node metastasis. In addition, DUSP28 not only significantly enhanced cell viability *in vitro*, but also significantly augmented tumorigenesis of BCa cells *in vivo*. It is considered a prerequisite for initial tumor cell migration and invasion, leading to metastasis and the recurrence of many cancers (Brabletz *et al.*, 2018). We identified that DUSP28 promoted BCa cell migration, invasion and EMT.

Many molecular mechanisms of cross-regulation between DUSPs and MAP kinases have been extensively studied (Wang *et al.*, 2014). Recent evidence suggests that recombinant DUSP28 therapy can significantly increase migration, invasion, and survival of metastatic pancreatic cancer cells by activating cAMP-response-element binding protein (CREB), Akt, and ERK1/2 signaling pathways (Lee *et al.*, 2019). However, other mechanisms have not been clearly studied. It is well known that the Wnt/ β -catenin signaling pathway that regulates a variety of biological processes, including tumorigenesis and metastasis (Yang *et al.*, 2016). Abnormalities in the Wnt/ β -catenin signaling pathway are common in many types of cancer, such as BCa. Overactivation of the Wnt/ β -catenin signaling pathway in BCa cells leads to the expression of many oncogenic target genes, such as C-myc and cyclin D1, inducing cell proliferation. β -catenin interacts with T cytokine 4 (TCF4) in Wnt signal transduction and regulates transcription of EMT-related target

genes including Slug (Hong *et al.*, 2009). When the Wnt signaling pathway is inactivated, cytoplasmic degradation complexes are formed and interact with β -catenin, which is phosphorylated and ubiquitinated, and subsequently degraded by intracellular proteasomes. The role of the degrading complex is largely dependent on the kinase activity of glycogen synthase kinase-3 β (GSK-3 β), which phosphorylates β -catenin ultimately resulting in β -catenin degradation (Tao *et al.*, 2020). In addition, GSK3 β is a phosphorylation substrate for Akt. Akt activation promotes phosphorylation of GSK3 β , thereby inhibiting β -catenin degradation and activating the Wnt/ β -catenin signaling pathway (Bray *et al.*, 2018). Our study suggested that DUSP28 significantly promoted Akt/ β -catenin signaling and increased the levels of downstream proliferation and EMT-related proteins.

In conclusion, DUSP28 promoted *in vitro* cell growth, cell migration and invasion and *in vivo* tumorigenesis of BCa cells. DUSP28 exerted its oncogene function by activating Akt/ β -catenin/Slug signaling, suggesting that DUSP28 may provide a promising therapeutic target for the treatment of BCa.

REFERENCES

- Baglia ML, Cai Q, Zheng Y, Wu J, Su Y, Ye F, Bao PP, Cai H, Zhao Z, Balko J, Zheng W, Lu W, Shu XO (2014) Dual specificity phosphatase 4 gene expression in association with triple-negative breast cancer outcome. *Breast Cancer Res Treatment* **148**: 211–220. <https://doi.org/10.1007/s10549-014-3127-z>
- Brabletz T, Kalluri R, Nieto MA, Weinberg RA (2018) EMT in cancer. *Nat Rev Cancer* **18**: 128–134. <https://doi.org/10.1038/nrc.2017.118>
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**: 394–424. <https://doi.org/10.3322/caac.21492>
- Dhillon AS, Hagan S, Rath O, Kolch W (2007) MAP kinase signaling pathways in cancer. *Oncogene* **26**: 3279–3290. <https://doi.org/10.1038/sj.onc.1210421>
- Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, Luo W, Liu B, Lei Y, Du S, Vuppapapati A, Luu HH, Haydon RC, He TC, Ren G (2018) Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis* **5**: 77–106. <https://doi.org/10.1016/j.gendis.2018.05.001>
- Gao PP, Qi XW, Sun N, Sun YY, Zhang Y, Tan XN, Ding J, Han F, Zhang Y (2021) The emerging roles of dual-specificity phosphatases and their specific characteristics in human cancer. *Biochim Biophys Acta (BBA) – Reviews on Cancer* **1876**: 188562. <https://doi.org/10.1016/j.bbcan.2021.188562>
- Hong CF, Chou YT, Lin YS, Wu CW (2009) MAD2B, a novel TCF4-binding protein, modulates TCF4-mediated epithelial-mesenchymal transdifferentiation. *J Biol Chem* **284**: 19613–19622. <https://doi.org/10.1074/jbc.M109.005017>
- James NE, Beffa L, Oliver MT, Borgstadt AD, Emerson JB, Chichester CO, Yano N, Freiman RN, DiSilvestro PA, Ribeiro JR (2019) Inhibition of DUSP6 sensitizes ovarian cancer cells to chemotherapeutic agents via regulation of ERK signaling response genes. *Oncotarget* **10**: 3315–3327. PMID: 31164954
- Lang R, M. Hammer, J. Mages (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. *J Immunol* **177**: 7497–504. <https://doi.org/10.4049/jimmunol.177.11.7497>
- Lee J, Hun Yun J, Lee J, Choi C, Hoon Kim J (2015) Blockade of dual-specificity phosphatase 28 decreases chemo-resistance and migration in human pancreatic cancer cells. *Sci Rep* **5**: 12296. <https://doi.org/10.1038/srep12296>
- Lee J, Lee J, Yun JH, Jeong DG, Kim JH (2016) DUSP28 links regulation of Mucin 5B and Mucin 16 to migration and survival of AsPC-1 human pancreatic cancer cells. *Tumour Biol* **37**: 12193–12202. <https://doi.org/10.1007/s13277-016-5079-x>
- Lee J, Lee J, Yun JH, Choi C, Cho S, Kim SJ, Kim JH (2017) Autocrine DUSP28 signaling mediates pancreatic cancer malignancy via regulation of PDGF-A. *Sci Rep* **7**: 12760. <https://doi.org/10.1038/s41598-017-13023-w>
- Lee J, Lee J, Kim J-H (2019) Scattered DUSP28 is a novel biomarker responsible for aggravating malignancy via the autocrine and paracrine signaling in metastatic pancreatic cancer. *Cancer Lett* **456**: 1–12. <https://doi.org/10.1016/j.canlet.2019.03.006>

- Li J, Yin J, Shen W, Gao R, Liu Y, Chen Y, Li X, Liu C, Xiang R, Luo N (2017) TLR4 promotes breast cancer metastasis via Akt/GSK3 β / β -catenin pathway upon LPS stimulation. *Anat Rec (Hoboken)* **300**: 1219–1229. <https://doi.org/10.1002/ar.23590>
- Patterson KI, Brummer T, O'Brien PM, Daly RJ (2009) Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem J* **418**: 475–489. <https://doi.org/10.1042/bj20082234>
- Peart, O. (2015) Breast intervention and breast cancer treatment options. *Radiologic Technol* **86**: 535M–558M
- Prabhakar S, Asuthkar S, Lee W, Chigurupati S, Zakharian E, Tsung AJ, Velpula KK (2014) Targeting DUSPs in glioblastomas – wielding a double-edged sword? *Cell Biol Int* **38**: 145–153. <https://doi.org/10.1002/cbin.10201>
- Ramkissoon A, Chaney KE, Milewski D, Williams KB, Williams RL, Choi K, Miller A, Kalin TV, Pressey JG, Szabo S, Azam M, Largaespada DA, Ratner N (2019) Targeted inhibition of the dual specificity phosphatases DUSP1 and DUSP6 suppress MP-NST growth via JNK. *Clin Cancer Res* **25**: 4117–4127. <https://doi.org/10.1158/1078-0432.ccr-18-3224>
- Ríos P, Nunes-Xavier CE, Tabernero L, Köhn M, Pulido R (2014) Dual-specificity phosphatases as molecular targets for inhibition in human disease. *Antioxid Redox Signal* **20**: 2251–2273. <https://doi.org/10.1089/ars.2013.5709>
- Song N, Zhong J, Hu Q, Gu T, Yang B, Zhang J, Yu J, Ma X, Chen Q, Qi J, Liu Y, Su W, Feng Z, Wang X, Wang H (2018) FGF18 enhances migration and the epithelial-mesenchymal transition in breast cancer by regulating Akt/GSK3 β /B-catenin signaling. *Cell Physiol Biochem* **49**: 1060–1073. <https://doi.org/10.1159/000493286>
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **71**: 209–249. <https://doi.org/10.3322/caac.21660>
- Tao C, Luo J, Tang J, Zhou D, Feng S, Qiu Z, Putti TC, Xiang T, Tao Q, Li L, Ren G (2020) The tumor suppressor Zinc finger protein 471 suppresses breast cancer growth and metastasis through inhibiting AKT and Wnt/ β -catenin signaling. *Clin Epigenetics* **12**: 173. Erratum in *Clin Epigenetics* 2022 J **14**: 17. <https://doi.org/10.1186/s13148-020-00959-6>
- Tao Z, Shi A, Lu C, Song T, Zhang Z, Zhao J (2015) Breast cancer: Epidemiology and etiology. *Cell Biochem Biophys* **72**: 333–338. <https://doi.org/10.1007/s12013-014-0459-6>
- Ullah MF (2019) Breast cancer: current perspectives on the disease status. *Breast Cancer Metastasis Drug Res* 51–64
- Wang D, Han S, Peng R, Jiao C, Wang X, Han Z, Li X (2014) DUSP28 contributes to human hepatocellular carcinoma via regulation of the p38 MAPK signaling. *Int J Oncol* **45**: 2596–604. <https://doi.org/10.3892/ijo.2014.2653>
- Wu QN, Liao YF, Lu YX, Wang Y, Lu JH, Zeng ZL, Huang QT, Sheng H, Yun JP, Xie D, Ju HQ, Xu RH (2018) Pharmacological inhibition of DUSP6 suppresses gastric cancer growth and metastasis and overcomes cisplatin resistance. *Cancer Lett* **412**: 243–255. <https://doi.org/10.1016/j.canlet.2017.10.007>
- Xian F, Hu X, Hu XS, Chen QL, Qin J, Bie J (2018) DUSP facilitates RPMI8226 myeloma cell aging and inhibited TLR4 expression. *Eur Rev Med Pharmacol Sci* **22**: 6030–6034. https://doi.org/10.26355/eur-rev_201809_15939
- Yang K, Wang X, Zhang H, Wang Z, Nan G, Li Y, Zhang F, Mohammed MK, Haydon RC, Luu HH, Bi Y, He TC (2016) The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies. *Lab Invest* **96**: 116–136. <https://doi.org/10.1038/labinvest.2015.144>
- Yu J, Wang X, Lu Q, Wang J, Li L, Liao X, Zhu W, Lv L, Zhi X, Yu J, Jin Y, Zou Q, Ou Z, Liu X, Zhou P (2018) Extracellular 5'-nucleotidase (CD73) promotes human breast cancer cells growth through AKT/GSK-3 β /B-catenin/cyclinD1 signaling pathway. *Int J Cancer* **142**: 959–967. <https://doi.org/10.1002/ijc.31112>
- Zheng T, Wang A, Hu D, Wang Y (2017) Molecular mechanisms of breast cancer metastasis by gene expression profile analysis. *Mol Med Rep* **16**: 4671–4677. <https://doi.org/10.3892/mmr.2017.7157>