

## Original Research

## The tumor suppressor DACT3 sensitizes triple-negative breast cancer to apatinib by inhibiting the Wnt/β-catenin pathway

Jing Wu <sup>a</sup>, Rui Tian <sup>b</sup>, Mei Liu <sup>a</sup>, Yijing Liu <sup>a</sup>, Bianfei Shao <sup>b,1,\*</sup>, Xiaohua Zeng <sup>a,1,\*\*</sup>

<sup>a</sup> Department of Breast Center, Chongqing Key Laboratory for Intelligent Oncology in Breast Cancer (iCQBC), Chongqing University Cancer Hospital, Chongqing 400030, China

<sup>b</sup> Chongqing Key Laboratory of Translational Research for Cancer Metastasis and Individualized Treatment, Chongqing University Cancer Hospital, Chongqing 400030, China

## ARTICLE INFO

## ABSTRACT

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Apatinib, a small-molecule inhibitor of vascular endothelial growth factor receptor 2 (VEGFR2), shows efficient antitumor activity in heavily pretreated metastatic triple-negative breast cancer (TNBC). However, not all patients respond to apatinib, indicating that it is necessary to identify response biomarkers for more precise treatment and investigate the underlying mechanisms of apatinib resistance to develop new treatment strategies for TNBC. In this study, we identified the disheveled binding antagonist of beta-catenin 3 (DACT3) as a biomarker for apatinib sensitivity, as its expression level is significantly higher in apatinib-sensitive patients and positively correlates with longer survival. Furthermore, we explored that the exogenous expression of DACT3 could downregulate the IC<sub>50</sub> of apatinib (Vector vs DACT3: 16.04 μM vs 8.81 μM in MDA-MB231 cells, 19.65 μM vs 9.42 μM in YCCB1 cells) by inhibiting the Wnt/β-catenin signaling, a pro-malignancy pathway that leads to apatinib resistance through crosstalk with the VEGF/VEGFR2 pathway. In summary, our results indicate that DACT3 is a potential biomarker for predicting the response to apatinib and a new therapeutic target for improving TNBC sensitivity to apatinib.

## Introduction

Breast cancer (BC) has a five-year overall survival (OS) rate as high as 90 %, but metastatic BC (mBC) still remains an incurable disease ([1,2]). Because of the negative estrogen receptor (ER), progesterone receptor (PR) and nonamplified human epidermal growth factor receptor 2 (HER2) expression, chemotherapy is still the main way to treat triple-negative breast cancer (TNBC) that has strong heterogeneity and dismal prognostic outcome ([3,4]). Apatinib can selectively bind to vascular endothelial growth receptor 2 (VEGFR2) and achieve anti-cancer efficacy by inhibiting tumor neovascularization ([5,6]). For heavily pretreated metastatic TNBC, apatinib combined with traditional chemotherapy drugs has good antitumor activity and controllable toxicity [7–10]. However, not all patients are sensitive to apatinib, therefore, identifying the mechanism of apatinib resistance will be

beneficial to accurately find the suitable population and the method to overcome resistance.

The Wnt/β-catenin pathway makes a vital impact on proliferation, migration and invasion in multiple malignancies, including breast cancer [11–14]. Without Wnt signaling, β-catenin can bind to Axin, GSK3-β and APC to form a complex and is phosphorylated for degradation. While with Wnt signaling, the binding of Wnt to low density lipoprotein receptor-associated protein (LRP) co-receptor and frizzled receptor enhances Dishevelled (Dvl) dependent LRP phosphorylation. Then phosphorylated LRP contributes to recruiting Axin in the complex, which causes β-catenin dephosphorylation and nuclear translocation. In the nucleus, β-catenin can bind to LEF/TCF transcription factors, therefore activating downstream target genes [14–16]. Notably, previous studies have shown that apatinib suppresses tumor development through inhibiting β-catenin signaling besides its antiangiogenic effect ([17,18]).

\* Corresponding author at: Chongqing Key Laboratory of Translational Research for Cancer Metastasis and Individualized Treatment, Chongqing University Cancer Hospital, Chongqing 400030, China.

\*\* Co-corresponding author at: Department of Breast Center, Chongqing Key Laboratory for Intelligent Oncology in Breast Cancer (iCQBC), Chongqing University Cancer Hospital, 181, Hanyu Road, Shapingba District, Chongqing 400030, China.

E-mail addresses: [shaobianfei@126.com](mailto:shaobianfei@126.com) (B. Shao), [zxiaohuacqu@cqu.edu.cn](mailto:zxiaohuacqu@cqu.edu.cn) (X. Zeng).

<sup>1</sup> These authors contributed equally to this work.

DACT (Dapper antagonist of  $\beta$ -catenin) family, called Dapper/Frodo as well, is a direct binding partner of Dvl and has negative effect on intercellular  $\beta$ -catenin signaling pathway ([19,20]). There are three members belonging to DACT family, namely, DACT1, DACT2 and DACT3. Our previous studies have shown that DACT1 and DACT2, as the Wnt/ $\beta$ -catenin pathway inhibitors, could significantly inhibit biological processes in breast cancer cells and were silenced by promoter CpG methylation ([21,22]). Unlike the above two genes, DACT3 has been reported to be silencing mainly by bivalent histone modification and its role in breast cancer is unclear [23]. Moreover, no studies have concentrated on the association between DACT3 and apatinib resistance.

In this work, we explored the biological activity and molecular efficacy of DACT3 in TNBC cells. In addition, we confirmed that DACT3 can affect the antitumor efficacy of apatinib. The identification of the mechanism may be conducive to developing innovative therapeutic strategies.

## Materials and methods

### Cells and tissues

Human BC cells are MDA-MB231 and YCCB1 belonging to TNBC. MDA-MB231 cells were acquired in American Type Culture Collection (ATCC), whereas YCCB1 cells were provided by our collaborators. These cells were cultivated within RPMI-1640 (Gibco BRL, Karlsruhe, Germany) that contained 10 % fetal bovine serum (FBS; Invitrogen, Carlsbad, CA), and 100 U/ml penicillin and streptomycin (GibcoBRL, Karlsruhe, Germany). Additionally, human BC and matched non-carcinoma tissue samples were gathered from Chongqing University Cancer Hospital. The Ethics Committee of Chongqing University Cancer Hospital approved human sample collection (Approval No CZLS2019112-B). All patients had received informed consent.

### Polymerase chain reaction (PCR) assay

RNA was extracted from cell lines without enzymes by TRIzol (Invitrogen) reagent. A RT system (Promega, Madison, WI) was performed for reversing RNA samples to cDNA. Thereafter, the reaction mixture (10  $\mu$ L), consisting of Go-Taq DNA polymerase (Promega) and cDNA (2  $\mu$ L), was used for Semi-quantitative PCR (RT-PCR). SYBR Green (Thermo Fisher) was applied to Quantitative real-time PCR (qRT-PCR) detection following manufacturer's instructions (LightCycler96 real-time PCR system). Supplementary Table 1 listed primers for PCR.

### Immunohistochemistry (IHC)

Serial sections (5- $\mu$ m) were cut from paraffin blocks of breast cancer patients who received apatinib treatment. Immunohistochemical staining was performed on 14 tumor specimens from apatinib-resistant and apatinib-sensitive patients (5 pairs of breast cancer tissue and 2 pairs of lymphatic metastatic cancer tissue). Antibodies utilized in immunohistochemistry included rabbit anti-DACT3 (#bs-11837R; 1:200 dilution, Bioss). Images were explored with the Image Pro Plus (IPP) program. The mean integrated optical density (IOD) was explored to identify average protein expression.

### Construction of DACT3-expressed cell lines

Recombinant lentivirus (LV) encoding DACT3 sequences and LV control vector were constructed and produced by Sino Biological company. Lipofectamine 3000 reagent was used to transfer the viral plasmid into 293T cells. Viral supernatant was collected to infect cells and polybrene was added at a ratio of 2 $\mu$ L/mL to improve infection efficiency. Cell lines with instantaneous infection could be acquired by collecting cells 48 h after infection, and the stable cell lines with GFP fluorescence

signal could be obtained by flow sorter after continuous culture. QRT-PCR and western blot were carried out for DACT3 expression identification prior to additional assays.

### Cell proliferation assay

MDA-MB231 and YCCB1 cells (3000/well) were cultured within 96-well plates. If anti-tumor drugs are added in the experiment, about 8000–10,000 cells need to be planted in each well because of their obvious inhibitory effect on cells. Cell proliferation was assessed with Cell Counting Kit-8 (CCK-8, Beyotime) at 0, 24, 48, and 72 h or 0, 12, 24, and 36 h. The microplate reader was employed for measuring absorbance optical density (OD) value at 450 nm.

### Transwell assay

Cell migration capacity was assessed by conducting Transwell assay. Matrigel (BD Biosciences, San Jose, CA) was added into transwell chamber (8  $\mu$ m, Corning, Tewsbury, USA) for assessing cell invasion. 150  $\mu$ l of serum-free medium containing appropriate cells was added above the chamber, and 750  $\mu$ l medium including 10 %FBS was introduced below the chamber. Following 48 or 72 h of incubation, cells were subjected to 30 min of 4 % paraformaldehyde fixation and additional 20 min of crystal violet staining to observe the penetration of cells out of the chamber.

### Wound healing assay

According to the growth rate of the cells, the appropriate concentration of transfected cells was cultured in the six-well plate, and it was appropriate for the cells to fill the six-well plate the next day. A sterile pipette gun tip was used to carefully damage the cell layer, cells were later rinsed twice using PBS, prior to reculture within a freshly prepared serum free PRMI-1640 medium. Following 12, 24 and 48 h of incubation, imaging was performed using a microscope to compare the rate of wound healing between the groups.

### Colony formation assay

After transfection, we uniformly spread MDA-MB231 and YCCB1 cells on 6-well plates (800–1000 cells each well) and cultured them in 2 ml medium for a 10–14-day duration. After fixation and staining of surviving colonies, colonies were photographed with the microscope, whereas colony number was counted with Image J program.

### Flow cytometry

Cell cycle and apoptosis were analyzed with flow cytometry. To analyze cell cycle, cells after transfection were gathered and immersed in 100 % cold ethanol overnight for fixation, followed by incubation at 37°C for 30 min using RNase A (100  $\mu$ L) and then additional 30 min of staining using 5  $\mu$ L propidium iodide (PI) under room temperature in dark. For apoptosis analysis, transfected cells underwent 5 min of staining using 5  $\mu$ L PE-Annexin V under ambient temperature under dark conditions, then PI was added and filtered for machine detection. Both the cell cycle data and apoptotic cell percentage were explored using Novocyt flow cytometer.

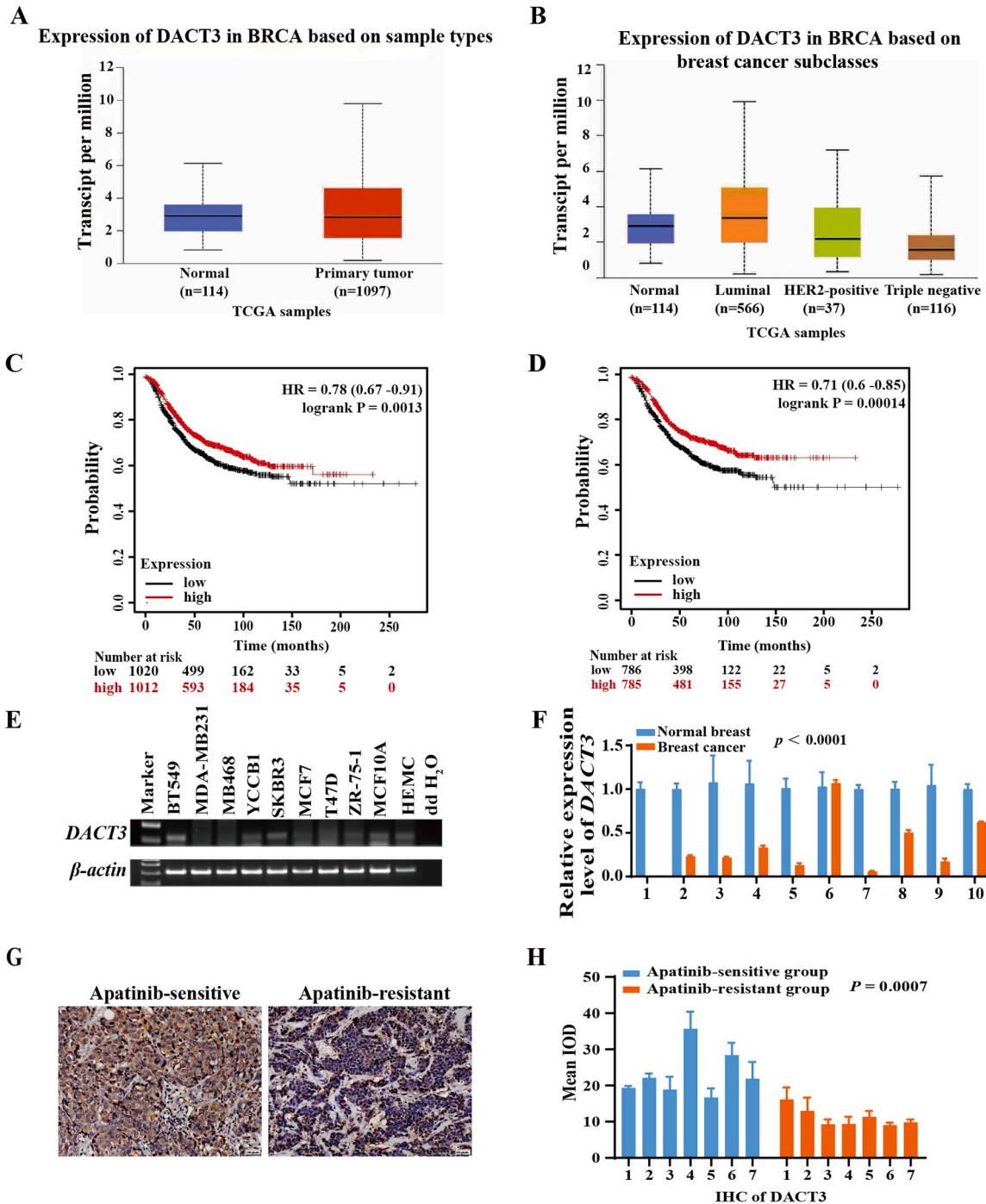
### Immunofluorescence staining

Appropriate number of cells transfected with DACT3 and control vector were planted on glass coverslips and incubated overnight. After transfection, cells underwent 30 min of fixation with 4 % paraformaldehyde, followed by 10 min of permeabilization using 0.5 % Triton X-100, and additional 60 min of blocking using Immunol Staining Blocking Buffer (Beyotime) for 60 min. Following overnight incubation

using primary antibodies total- $\beta$ -catenin (#8480; 1:200), non-phospho (active)  $\beta$ -catenin (Ser45) (#19,807; 1:200), phospho-VEGFR2 (Tyr951) (#4991; 1:200, Cell Signaling Technology), and VEGFR2 (#bs-10412R; 1:200, Bioss Antibodies) under 4 °C, DyLight-conjugated anti-rabbit antibody (CoWin Biotech Co., Ltd.) was added for further cell treatment at 37°C for 60 min. And then nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (Roche, Palo Alto, CA, USA).

#### Western blot

After protein separation through sodium dodecyl-sulfate-polyacrylamide gel electrophoresis, proteins were subsequently transferred onto polyvinylidene fluoride membranes for incubation with primary antibodies, including DACT3(#AF5767; 1:1000, R&D Systems); total  $\beta$ -catenin (#8480; 1:1000), active  $\beta$ -catenin (#19,807; 1:1000), c-Myc (#18,583; 1:1000), Cyclin D1 (#55,506; 1:1000, Cell Signaling Technology); and  $\beta$ -actin (sc-8432; 1:2000, Santa Cruz). The next day,



**Fig. 1.** Expression of DACT3 was lower in BrCa tissues and associated with clinical prognosis and apatinib sensitivity. (A, B) Online analysis of DACT3-expression in BC tissues compared with non-carcinoma counterparts. (C, D) Correlation between the expression of DACT3 and prognostic outcome in BC subclasses. (E) RT-PCR results of DACT3 in BC and normal breast cells. (F) QRT-PCR results of DACT3 in BC tissues relative to non-carcinoma tissues. (G) IHC staining of DACT3 in apatinib-sensitive (left) and apatinib-resistant tumor tissues (right). (H) Average IOD of DACT3 protein level of apatinib-sensitive and apatinib-resistant group. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001).

membranes were washed prior to incubation using Goat Anti-Rabbit, Goat Anti-Mouse, or Rabbit Anti-Goat IgG secondary antibodies. Proteins were visualized with the enhanced chemiluminescence detection system.

#### Tumor xenograft nude mouse model

Female nude mice purchased from Gempharmatech (China) were utilized to establish the xenograft model. The experimental protocols gained approval from Experimental Animal Management Committee of Chongqing University Cancer Hospital (Approval No CQCH-LAE-A0000202005). MDA-MB231 cells ( $2 \times 10^6$ ) with stable infection of LV-DACT3 and LV-control were exposed to subcutaneous inoculation into the right side of their backs ( $n = 5$ ). When tumors developed, tumor length and width were monitored with a caliper. Tumor volume was identified by  $0.5 \times \text{length} \times \text{width} \times \text{width}$ .

#### Statistical analysis

GraphPad Prism 8.0 and SPSS 25.0 (IBM, Almonck, USA) were employed for statistical analysis. Every experiment was carried out thrice. Data were indicated by mean  $\pm$  standard deviation. Between-group data were analyzed by T test, whereas among-group data were analyzed by analysis of variance. Statistical analysis of categorical variable data was performed by Fisher exact probability or Chi-square test. The difference was significant when  $p < 0.05$ .

#### Results

##### *DACT3 expression was reduced in breast cancer*

Online analysis using the TCGA database of UALCAN (<https://ualcan.path.uab.edu/index.html>) has showed that DACT3 is decreased within BC tissues compared with non-carcinoma counterparts (Fig. 1A). Among different molecular types, DACT3 expression decreased most significantly in TNBC (Fig. 1B). The relation of DACT3 expression with BC prognostic outcome was analyzed by referring to the K-M plotter database (<http://kmplot.com/>). As a result, the greater DACT3 expression, the better prognostic outcome in BC patients (Fig. 1C), especially for HER2-negative breast cancer patients (Fig. 1D). RT-PCR was applied to examine DACT3 levels within BC cells and non-carcinoma breast cells. Results showed that DACT3 generally showed silencing or down-regulation within BC cells (Fig. 1E). QRT-PCR also revealed that DACT3 mRNA expression evidently decreased within BC samples relative to non-carcinoma samples (Fig. 1F).

##### *DACT3 expression was lower in apatinib-resistant group*

Immunofluorescence staining (IHC) of apatinib-treated breast cancer tissues gained from our hospital indicated that DACT3 was located mainly in the cytoplasm (Fig. 1G). As demonstrated by the IPP program, the average IOD of DACT3 protein level of apatinib-resistant group remarkably declined compared with apatinib-sensitive group (Fig. 1H). The results demonstrated that increased expression of DACT3 may predict better clinical efficacy.

##### *Construction of MDA- MB231 and YCCB1 cells overexpressing DACT3*

We constructed MDA-MB231 and YCCB1 cells with stable DACT3 expression mainly through viral infection and flow separation, and verified them by qRT-PCR and Western blot. Transient cell line expression is shown in Fig. 2A and Fig. 2B, whereas stable cell line expression is presented in Fig. 2C. Results demonstrated that these two cell lines infected with DACT3 was significantly up-regulated relative to the control group, which proved that stable strain with DACT3 over-expression was successfully constructed.

##### *DACT3 inhibited proliferation ability of TNBC cells and increased apatinib sensitivity*

With the purpose of determining how DACT3 affected TNBC cell growth, CCK-8 assay using DACT3 and control vector transfected with MDA-MB231 and YCCB1 were performed. After transfection with DACT3, cell viability notably decreased at 24, 48, and 72 h (Fig. 2D). Following treatment with 10  $\mu\text{mol/L}$  apatinib, it was observed that DACT 3 and apatinib synergically inhibited TNBC proliferation capacity (Fig. 2E). Moreover, IC<sub>50</sub> value of apatinib decreased after over-expression of DACT3 by CCK-8 assay, indicating that DACT3 expression enhanced the sensitivity of MDA-MB231 and YCCB1 cells to apatinib (Fig. 2F).

Clonal formation assay was another effective method to measure cell proliferation ability besides CCK8, and our results indicated that the number of colonies was obviously reduced at 10–14 days compared with control cells (Fig. 2G). Both CCK8 and clonal formation assays suggested that DACT3 could suppressed TNBC cell proliferation.

##### *DACT3 inhibited TNBC cell migration and invasion ability*

After transfection with DACT3, MDA-MB231 and YCCB1 cells had decreased migration and invasion capacities, as evidenced by Transwell assay (Fig. 2H, 2I), suggesting that DACT3 attenuated cell migration and inhibited the invasiveness of TNBC cells. In addition, wound healing tests were carried out to reveal how DACT3 affected cell migration, as a result, DACT3-overexpressed cells showed a significantly slower spreading rate along wound edge relative to control group (Fig. 2J), indicating that DACT3 inhibited cell migration ability.

##### *DACT3 induced cell apoptosis and arrest of cell cycle at G0-G1 phase*

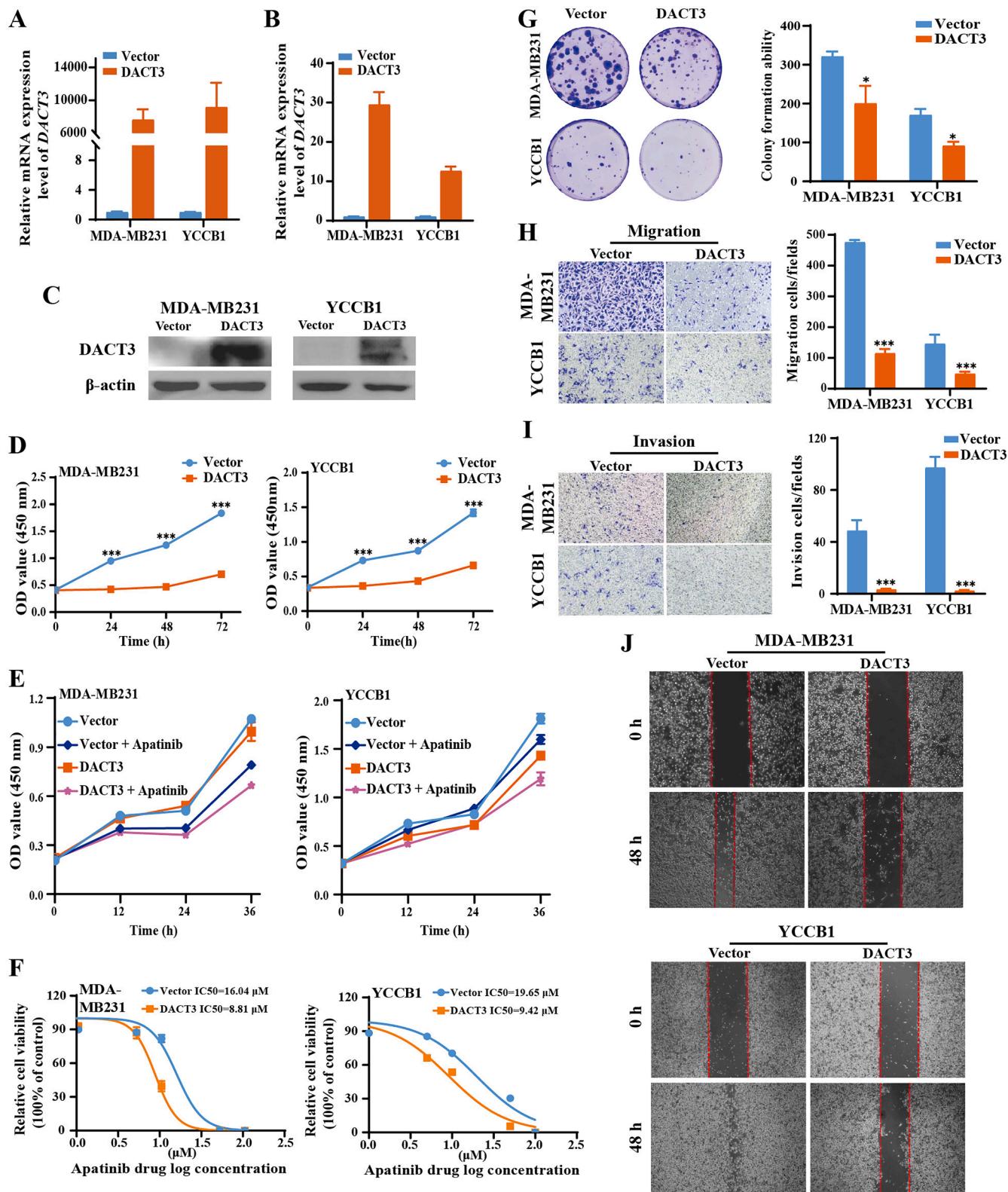
This study explored how DACT3 affected TNBC cell apoptosis via flow cytometry. As presented in Fig. 3A, MDA-MB231 and YCCB1 cells ectopically expressing DACT3 displayed increased apoptosis in comparison with control group, demonstrating that DACT3 could stimulate cancer cell apoptosis and hinder the growth of TNBC cells. In addition, we also investigated the effect of DACT3 on cell cycle progression through flow cytometry. Cell cycle tests showed that DACT3 enhanced the number of MDA-MB231 and YCCB1 cells at G0-G1 phase (Fig. 3B). Furthermore, we evaluated the effect of apatinib (10  $\mu\text{M}$ , 48 h) on cell cycle and found that apatinib could also block cells at G0-G1 phase compared with wild type (WT) breast cancer cell line (Fig. 3C), which suggested that DACT3 and apatinib might exert a synergistic effect on the G0-G1 cell cycle.

##### *DACT3 inhibited BC cell proliferation in vivo*

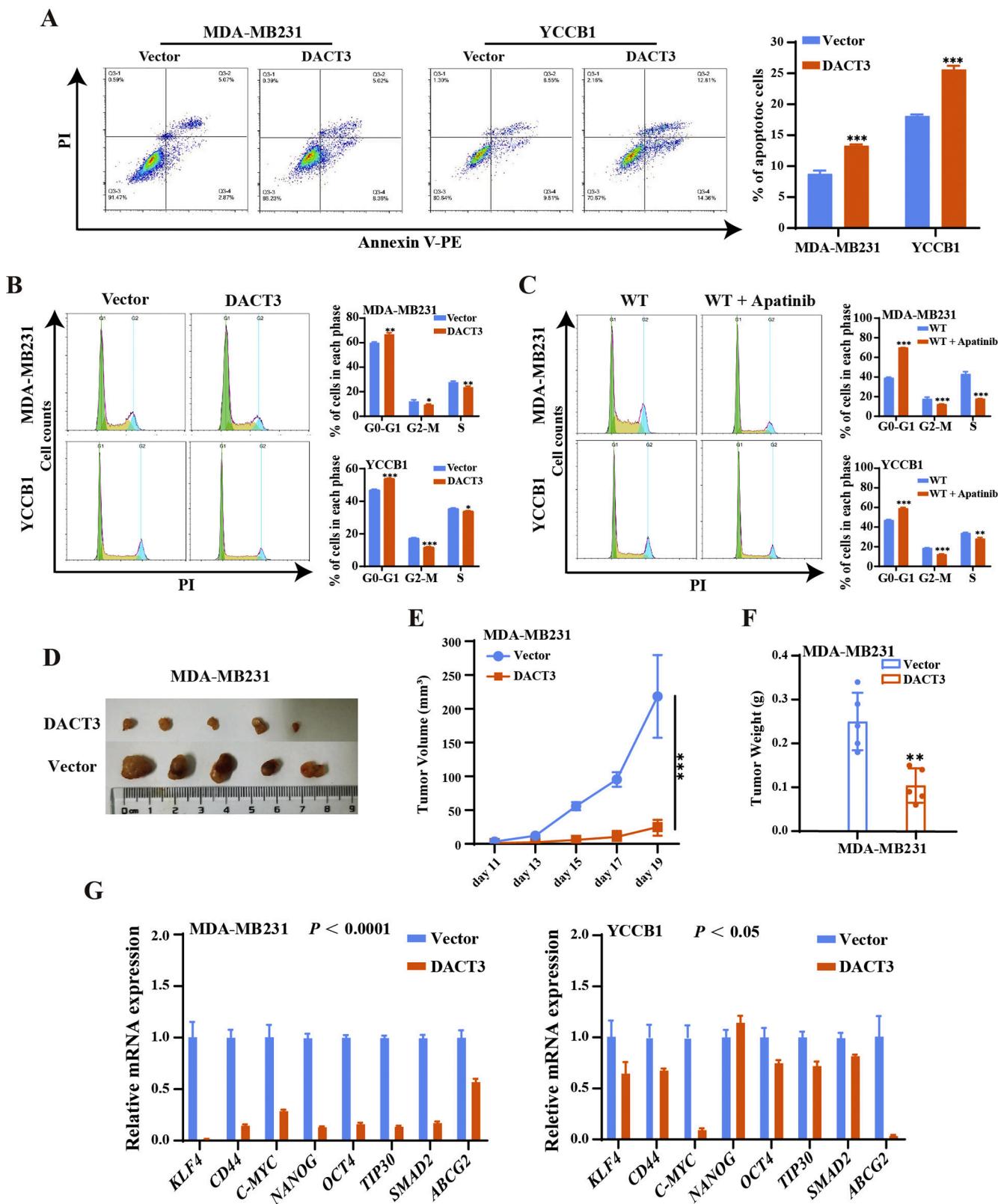
Subcutaneous tumor formation in nude mice was further used to test whether DACT3 can also play a role in tumor inhibition in vivo. We injected constructed MDA- MB231 cells with stable expression of DACT3 and control vector plasmid into female nude mice. Compared with mice injected with vector-transfected cells, the tumor volume of mice injected with DACT3-transfected cells was significantly reduced (Fig. 3D). The growth curves of the tumors in DACT3 group evidently decreased relative to control vector group (Fig. 3E). The mean xenograft weight of the DACT3 group decreased (Fig. 3F). The results indicated that ectopic DACT3 expression can also suppress TNBC cell proliferation in vivo.

##### *DACT3 suppressed the stemness of TNBC cells*

The stemness of cancer cells was closely related to tumor cell proliferation and invasion, besides, cells exhibiting stronger stemness are more likely to develop drug resistance. Therefore, we used qRT-PCR assay to detect stem-related indicators for evaluating how DACT3 affected the malignant phenotype and drug sensitivity of tumor cells.



**Fig. 2.** Overexpression of DACT3 inhibited the malignant phenotype of breast cancer in vitro. (A, B, C) The expression of DACT3 in MDA-MB231 and YCCB1 cells was detected by qRT-PCR and western blot. (D) The cell viability of DACT3-overexpressing cell lines was measured at 24, 48 and 72 h. (E) The interaction between DACT3 and apatinib was detected by CCK8 assay. (F) The IC50 of apatinib in DACT3-overexpressing group and control group. (G) Colony-forming capacity of Dact3-transfected MDA-MB231 and YCCB1 cells. (H, I) Transwell assay demonstrated the migration and invasion capacity of MDA-MB231 and YCCB1 transfected with DACT3. (J) The motility of transfected-DACT3 MDA-MB231 and YCCB1 cells was detected by wound healing assay. (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

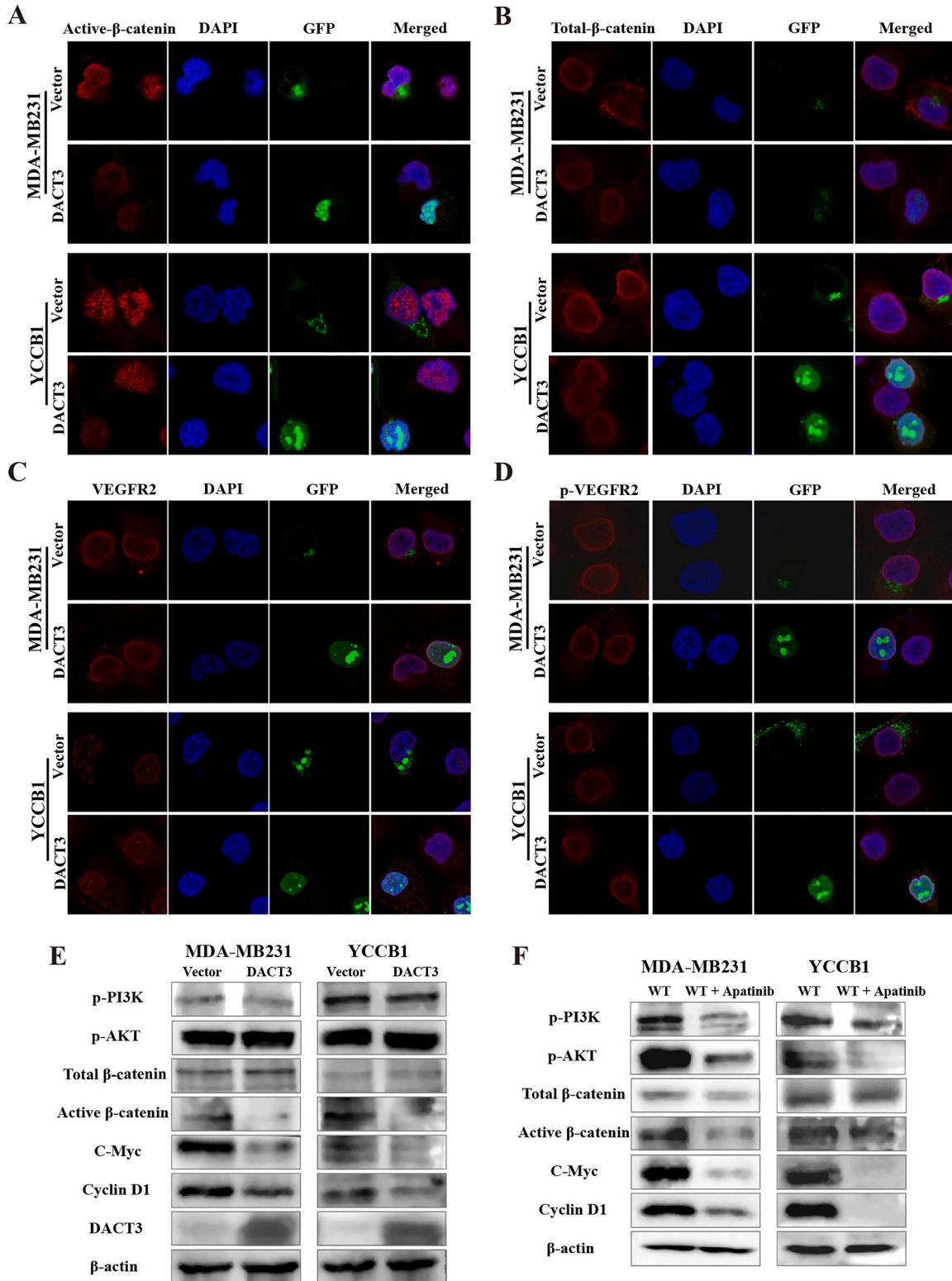


**Fig. 3.** DACT3 induced cell apoptosis and arrest of cell cycle at G0-G1 phase. DACT3 inhibited BC cell proliferation in vivo and suppressed the stemness of TNBC cells. (A) Apoptosis was detected by flow cytometry. (B, C) The cell cycle was measured by flow cytometry. (D) Tumor images transfected with DACT3 and vector-transfected MDA-MB231 cells by subcutaneous injection. (E) Tumor growth curve of DACT3 and vector-transfected MDA-MB231 cells in mice. (F) Weight histograms of two groups of tumors formed by MDA-MB231 cells transfected with DACT3 and vector. (G) The relationship between DACT3 and tumor stem-related markers was analyzed by qRT-PCR. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001).

Consequently, after DACT3 overexpression, several stemness-related genes had markedly reduced expression (Fig. 3G), supporting a role for DACT3 restraining the stemness of TNBC cells, which may be one of the mechanisms by which DACT3 inhibits cell proliferation and invasion as well as reversed drug resistance.

#### *DACT3 and apatinib both inhibited β-catenin signalling pathway*

DACT3 is previously suggested to regulate Wnt/β-catenin pathway ([19,20,24]). Since the main anti-tumor mechanism of apatinib is to block angiogenesis by acting on the VEGFR2 signaling pathway, immunofluorescence staining was carried out to examine the association



**Fig. 4.** DACT3 and apatinib both inhibited β-catenin signalling pathway. (A, B, C, D) Immunofluorescence results of interaction between DACT3 and active catenin, total catenin, VEGFR2, p-VEGFR2. (E, F) Expression of Wnt/β-catenin-related proteins in cells transfected with DACT3 and treated with Apatinib.

between DACT3 and  $\beta$ -catenin, as well as between DACT3 and VEGFR2. The results confirmed decreased concentration of active  $\beta$ -catenin in DACT3-transfected cells compared to controls (Fig. 4A). Meanwhile, the result showed that DACT3 did not interact with total  $\beta$ -catenin (Fig. 4B), VEGFR2 (Fig. 4C), and p-VEGFR2 (Fig. 4D).

For verifying how DACT3 and apatinib affected Wnt/ $\beta$ -catenin pathway, related protein levels in this pathway were measured through Western blot. According to our findings, DACT3 and apatinib both down-regulated active  $\beta$ -catenin and certain downstream targets including cyclin D1 and c-Myc within MDA-MB231 and YCCB1 cells (Fig. 4E, 4F). In addition, apatinib did not affect the expression of DACT3 (Figure S1). Our results also suggested that apatinib could affect  $\beta$ -catenin through PI3K/Akt pathway, consistent with previous literature [17]. However, our results show that DACT3 has no significant effect on PI3K/Akt (Fig. 4E, 4F). Furthermore, the western blot experimental results showed that DACT3 combined with Apatinib can improve AXIN1 and inhibit phospho-P65 and phospho-P44/42 MAPK (Figure S2), indicating that the sensitizing effect of DACT3 operates through multiple pathways such like Wnt/ $\beta$ -Catenin, NF- $\kappa$ B and MAPK simultaneously, and the underlying mechanisms still require further investigation (Fig. 5, Figure S2).

## Discussion

Increasing studies have confirmed that DACT3 inhibits tumor development and invasion [24–26]. TCGA database-based analysis reflected the reduced DACT3 level within BC tissues especially in TNBC tissues. According to our results, DACT3 expression markedly decreased within BC tissues, compared with non-carcinoma counterparts. Furthermore, DACT3 inhibited malignant phenotype of TNBC. Next, we confirmed that apatinib-treated breast cancer patients with high DACT3 expression displayed better response when compared with patients showing low DACT3 expression. On the whole, DACT3 is the candidate target for tumor inhibition, which may act as a potential marker to reflect the sensitivity of apatinib.

DACT3 belongs to DACT family, which similarly interacts with Dvl2, blocking the phosphorylation of LRP. Dephosphorylated LRP fails to recruit Axin, resulting in  $\beta$ -catenin degradation, which then inhibits TCF/LEF-mediated transcription, together indicating that DACT3 suppresses Wnt/ $\beta$ -catenin pathway ([14,27]). In this work, ectopic DACT3 expression in TNBC significantly down-regulated active  $\beta$ -catenin and

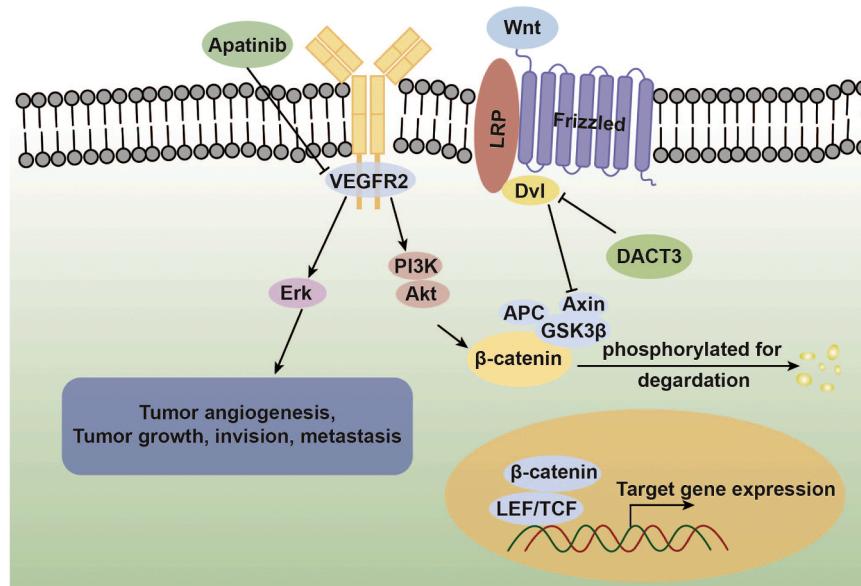
downstream targets including cyclin D1 and c-Myc. Wnt/ $\beta$ -catenin pathway has been suggested to be related to drug resistance of TNBC. Therapeutics plus Wnt/ $\beta$ -catenin pathway inhibitor can become the candidate way for treating TNBC [28–30]. Therefore, DACT3 may be proposed to be the key target for BC treatment breast cancer therapies which focus on monitoring Wnt/ $\beta$ -catenin pathway.

VEGF and VEGFR have been identified to be associated with cancer-related pathological angiogenesis. Apatinib targeting VEGFR2 is tested as a strategy for breast cancer treatment by preventing angiogenesis [31–33]. However, antiangiogenic therapies targeting a single pathway are prone to the development of drug-resistance and even promote tumor growth ([34,35]). At present, the mechanism of resistance to antiangiogenic drugs needs to be further comprehended, but some related explorations and reasonable explanations have emerged. Studies have shown that the resistance may be related to vascular remodeling, aberrant epigenetic modifications, and CD11b+Gr1+ bone marrow cell mediated infiltration of tumor tissue and other reasons [36–38]. Therefore, exploring the mechanisms of resistance to antiangiogenic drugs such as apatinib is beneficial for the development of new drugs with synergistic anti-tumor effects for clinical practice. Our study demonstrated that the expression of DACT3 shows a positive relationship to the efficacy of apatinib in tissues of apatinib-treated patients. In addition, our experiments also indicated the synergistic effect of apatinib and DACT3 in vitro. DACT3 negatively regulates Wnt/ $\beta$ -catenin pathway, and may overcome apatinib resistance.

To conclude, the tumor suppressor DACT3 of TNBC cells functions as the Wnt/ $\beta$ -catenin pathway antagonist. Furthermore, DACT3 is the candidate tumor marker for BC treatment with apatinib. Developing drugs targeting DACT3 may be beneficial to enhance the efficacy of apatinib, and more studies are warranted.

## CRediT authorship contribution statement

**Jing Wu:** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Rui Tian:** Project administration, Methodology, Data curation. **Mei Liu:** Project administration, Data curation. **Yijing Liu:** Project administration, Methodology, Investigation. **Bianfei Shao:** Writing – review & editing. **Xiaohua Zeng:** Validation, Supervision.



**Fig. 5.** Diagram of mechanism of DACT3 synergistically inhibiting Wnt/ $\beta$ -Catenin pathway.

## Declaration of competing interest

The authors declare no conflict of interest.

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## Ethical approval

This research was approved by the ethics committee of Chongqing University Cancer Hospital abode by the Declaration of Helsinki.

## Informed Consent

Written informed consent was obtained from all the patient.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.tranon.2025.102509](https://doi.org/10.1016/j.tranon.2025.102509).

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