DACT1, an antagonist to Wnt/β-catenin signaling, suppresses tumor cell growth and is frequently silenced in breast cancer

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Abstract

**Introduction:** Aberrant activation of Wnt/β-catenin signaling plays an important role in the pathogenesis of breast cancer. DACT1 (Dapper/Frodo) has been identified as involved in antagonizing Wnt/β-catenin signaling through interacting with Dishevelled (Dvl), a central mediator of Wnt signaling, whereas its role in breast tumorigenesis remains unclear.

**Methods:** We examined DACT1 expression in breast cancer cell lines and primary tumors with semiquantitative or quantitative RT-PCR and immunohistochemistry, and further evaluated the promoter methylation of DACT1 with methylation-specific PCR (MSP). We also explored the tumor-suppressive functions of DACT1 in vivo and in vitro, and its related mechanism in breast cancer.

**Results:** We identified DACT1 as a methylated target in our breast cancer epigenome study. Here, we further investigated DACT1 expression in multiple breast cell lines and primary tumors, and further studied its function and molecular mechanisms. We found that DACT1 expression was silenced in eight (88.9%) of nine breast cancer cell lines, and its protein levels were obviously reduced in breast tumors compared with paired surgical-margin tissues. Promoter CpG methylation of DACT1 was detected in five (55.6%) of nine breast cancer cell lines and 40 (29.9%) of 134 primary tumors, but not in surgical-margin tissues and normal breast tissues. Demethylation treatment of breast cancer cell lines restored DACT1 expression along with promoter demethylation, suggesting that an epigenetic mechanism mediates DACT1 silencing in breast cancer. Functional assays showed that ectopic expression of DACT1 could inhibit breast tumor cell proliferation in vivo and in vitro through inducing apoptosis, and further suppress tumor cell migration through antagonizing the Wnt/β-catenin signaling pathway.

**Conclusions:** Our study demonstrates that DACT1 could function as a tumor suppressor but was frequently downregulated in breast cancer.
crucial for the aberrant activation of WNT/β-catenin signaling in tumor pathogenesis [5,7]. DACT1, a homologue of Dapper, located at chromosomal region 14q23.1, was first identified as a Dishevelled (Dvl)-associated antagonist of Wnt/β-catenin and JNK signaling pathways [8,9], DACT1 is expressed during embryonic development in the adult brains of mice [10,11], but studies on its role in tumorigenesis are scanty. DACT1 has been shown to be reduced in several tumors, such as gastrointestinal stromal tumors [12] and non-small cell lung cancer (NSCLC) [13], but overexpressed in some other tumors [14,15]. Dysregulated DACT1 was associated with poor prognosis in tumor patients [13]. DACT1 was also reported to be a novel inhibitor of the WNT/β-catenin signaling in hepatocellular carcinoma (HCC) [16]. However, its expression and biologic functions in breast cancer pathogenesis are unknown.

We identified DACT1 as a methylated target in the MB231 breast cancer cell line as compared with normal tissue in our pilot cancer epigenome study. Here, we further examined the expression and promoter methylation of DACT1 in multiple breast cell lines and primary tumors, and evaluated its potential as a tumor biomarker for breast cancer. We further demonstrated the biologic functions of DACT1 in breast cancer cells in vitro and in vivo in the context of the Wnt/β-catenin signaling pathway.

Materials and methods

Cell lines, tumor samples, and normal tissues

Several breast cancer cell lines (BT549, MDA-MB-231, MDA-MB-468, MCF-7, T47D, SK-BR-3, YCC-B1, YCC-B3, and ZR-75-1) were studied. All cell lines were maintained at 37°C in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 U/ml of penicillin, and streptomycin.

Normal human breast tissue RNA samples were purchased commercially (Strategene, La Jolla, CA, USA; Millipore Chemicon, Billerica, MA, USA; BioChain Institute, Hayward, CA, USA). Primary breast tumor samples, paired surgical-margin tissues, and normal breast tissues were obtained from the First Affiliated Hospital of Chongqing Medical University, or elsewhere, as described previously [17-19]. All samples were evaluated and subject to histologic diagnosis by pathologists. Clinical information, including age, tumor grade, tumor size, follow-up data after initial diagnosis, and treatment, was obtained for the majority of tumor cases. Grading of tumors was achieved by staining with hematoxylin and eosin (H&E). All patients provided written consent for the study. The study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Approval notice: 2010/2012(23)).

Semiquantitative RT-PCR analysis

Total RNA was isolated from cell lines by using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA). Semiquantitative RT-PCR was performed as described previously [20]. GAPDH was amplified as a control. Primer sequences are listed in Table 1. RT-PCR was performed with 32 cycles for DACT1, and 23 cycles for GAPDH, by using Go-Taq (Promega, Madison, WI, USA).

5-Aza-2′-deoxycytidine and trichostatin A treatment

Cell lines were treated with 10 mM 5-aza-2′-deoxycytidine (Aza; Sigma-Aldrich, St Louis, MO, USA) for 3 days or further treated with 100 nM trichostatin A (TSA; Cayman Chemical Co., Ann Arbor, MI, USA) for 14 hours.

DNA bisulfite treatment and methylation-specific PCR

Genomic DNA was extracted from tumors, normal tissues, and cell pellets by using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Bisulfite modification of DNA and methylation-specific PCR (MSP) were performed as described previously [21,22]. Bisulfite-treated DNA was amplified by MSP with DACT1 methylation-specific primer set for DACT1 promoter (Table 1), by using AmpliTaq-Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA). Methylated and unmethylated MSP primer sets target the same CpG sites and do not amplify genomic DNA with no bisulfite treatment.

Quantitative reverse transcription polymerase chain reaction

Real-time PCR (rtPCR) was performed by using Maxima SYBR Green/ROX qPCR Master Mix (MBI Fermentas, St. Leon-Rot, Germany) (Table 1). Thermal-cycling reaction was performed in the 7500 Real-Time PCR System (Applied Biosystems). Melting-curve analysis and agarose gel electrophoresis of PCR products were further performed. Relative expression levels of DACT1 in breast tissues were standardized to β-actin levels.

Tissue microarray and immunohistochemistry

To evaluate DACT1 expression in breast tissues, tissue microarray (TMA) was used as described previously, containing 30 pairs primary tumors and corresponding tumor-margin tissues [18]. Immunohistochemistry was performed by using a two-step method. In brief, after deparaffinization, sections were hydrated and underwent sodium citrate antigen retrieval. Sections were then incubated with 3% hydrogen peroxide to block endogenous peroxidase activity. A rabbit polyclonal antibody against human DACT1 protein (Ab104.4; Abcam, Cambridge, UK) was used. Sections were incubated with primary
antibody (1:200 dilution) overnight at 4°C, detected by using polyperoxidase-anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA), and counterstained with hematoxylin. To eliminate nonspecific staining, a negative control was performed with PBS.

All immunohistochemical photographs were analyzed by using Image Pro Plus (IPP, version 6.0; Media Cybernetics, Silver Spring, MD, USA), as described previously [18]. The mean optical density (OD), as a quantitative measure of stain intensity, was analyzed to determine average protein expression.

**Immunofluorescence staining**

Cells transfected with pcDNA3.1-DACT1 or pcDNA3.1 plasmid were grown on glass coverslips. Transfected cells were washed with PBS, fixed with 4% paraformaldehyde in PBS for 15 minutes, permeabilized with 0.5% Triton X-100 for 30 minutes, and blocked with 3% bovine serum albumin in PBS at 37°C for 60 minutes. Cells were incubated with primary antibodies diluted in TBST at 4°C for 12 hours, washed twice with PBS, and then incubated with DyLight-conjugated anti-rabbit or anti-mouse antibody (CoWin Biotech Co., Ltd. (CWBIO), Beijing, China) for an additional 50 minutes. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (Roche, Palo Alto, CA, USA).

**Cell-proliferation assay**

MB231 cells were cultured in six-well plates at a density of 1 × 10⁴ cells/well and allowed to grow overnight. Cultures were then transfected with pcDNA3.1-DACT1 or pcDNA3.1 plasmid by using Lipofectamine 2000 (Invitrogen). At 24, 48, and 72 hours, cell proliferation was measured by using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) [23].

**Colony-formation assay**

Colonies from each well were stained with 0.1% crystal violet solution for 5 min. All experiments were performed 3 times.

**Caspase-3 colorimetric assay**

Cells were seeded in six-well plates and transfected with pcDNA3.1-DACT1 or pcDNA3.1 (4 μg each) plasmid by using Lipofectamine 2000 (Invitrogen). At 48 hours after transfection, cells were collected, replated, and selected for 2 weeks in the presence of G418 (0.4 mg/ml). Surviving colonies (≥50 cells/colony) were counted after staining with gentian violet. All experiments were performed 3 times.

**Western blot**

Transfected cells were lysed in M-PER Mammalian Protein Extraction Reagent (Pierce, Thermo Scientific, Cramlington, UK) containing a protease inhibitor cocktail (Sigma Aldrich). A total of 50 μg of protein lysate for each sample was separated by using sodium dodecyl-sulfate/polyacrylamide gel electrophoresis (SDS-PAGE). The lysates were then transferred to PVDF membranes for antibody incubation. After blocking with 5% nonfat milk and 0.1% Tween 20 in TBS, the membranes were incubated with DACT1 antibody (Abcam, Cambridge, UK), or antibodies to active β-catenin (Millipore, Billerica, MA, USA), cyclin D1, c-Myc, cleaved caspase 3, and cleaved PARP (Epitomics Inc., Burlingame, CA, USA).

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### Table 1 List of primers used in this study

| PCR                  | Primer   | Sequence (5’-3’)  | Product size (bp) | PCR cycles | Annealing temperature (°C) |
|----------------------|----------|------------------|-------------------|------------|--------------------------|
| MSP                  | DACT1m3  | CGGGATAGTAGTAGTCGGC | 118   | 41         | 60                       |
|                      | DACT1m4  | CGCTAAAACCTAGGCGG | 123   | 41         | 58                       |
|                      | DACT1u3  | GTGGGATAGTAGTAGGTTG | 123   | 41         | 58                       |
|                      | DACT1u4  | AAACACTAAAACACTAACAACA | 123   | 41         | 58                       |
| RT-PCR               | DACT1-F  | AGGAGAATGCTTGGAGGAG | 179   | 32         | 60                       |
|                      | DACT1-R  | TGGACTTGGCGGCTCTGTCG | 206   | 23         | 55                       |
|                      | GAPDH-F  | GGAAGTCACCGAGTTGTTG | 206   | 23         | 55                       |
|                      | GAPDH-R  | GTGAATGGGATTCATGGA | 206   | 23         | 55                       |
| Real-time PCR        | DACT1-F  | GAGGTGAGCAAGGACACACC | 158   | 40         | 60                       |
|                      | DACT1-R  | ACCGTGGAAAGGGGACAGA | 158   | 40         | 60                       |
|                      | β-actin-F| CCTGTTGATCCCAAGAACT | 314   | 40         | 60                       |
|                      | β-actin-R| GAAGGATTTTCCGGGACG | 314   | 40         | 60                       |

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Yin et al. Breast Cancer Research 2013, 15:R23
http://breast-cancer-research.com/content/15/2/R23
Page 3 of 12
The immunoblots were visualized by using an enhanced chemiluminescence detection system. GAPDH was used as a control.

**In vivo tumor model**

Stable DACT1-expressing MB231 cells or controls (1 × 10⁶ cells in 0.2 ml PBS) were subcutaneously injected into the right dorsal flank of female nude mice (6 weeks old, six mice per group). The weight of nude mice was measured every 7 days for 4 weeks. The xenograft tumor weight was assessed at the terminal time. The protocols for in vivo animal experiment were approved by the Committee on Ethical Use of Animals of the First Affiliated Hospital of Chongqing Medical University.

**Statistical analysis**

Statistical analyses were performed with SPSS version 16 (SPSS Inc., Chicago, IL, USA). Student t test was used to analyze the difference of DACT1 expression between breast cancer tissues and surgical margin tissues, χ² test and Fisher Exact test were used to assess the correlation between DACT1 methylation and clinicopathologic parameters. For all the tests, P < 0.05 was considered statistically significant.

**Results**

**DACT1 is frequently reduced in breast cancer**

We first examined DACT1 expression in a panel of human normal adult tissues and fetal tissues, as well as breast cancer cell lines, by using semiquantitative RT-PCR. Results showed that DACT1 was widely expressed in human normal tissues and fetal tissues, including normal breast tissues (Figure 1A, C), but was frequently silenced or downregulated in breast cancer cell lines studied (Figure 1C).

DACT1 expression in primary breast tumors was further examined at the RNA and protein levels. We found that DACT1 mRNA was obviously downregulated in breast cancer tissues, compared with normal breast tissues (P < 0.01), as measured with qPCR (Figure 2A). IHC was then performed to examine DACT1 expression in 30 cases of primary tumors and paired surgical-margin tissues (Figure 2B). An IPP system showed that the mean optical density of DACT1 protein expression was significantly decreased in primary breast tumors (0.222 ± 0.060), compared with that in surgical-margin tissues (0.287 ± 0.054) (**P < 0.001) (Figure 2C).

We further assessed DACT1 expression by using the Gene expression-based Outcome for Breast Cancer Online (GOBO) database [24]. Gene-Set Analysis (GSA)-Tumor showed that DACT1 is differently expressed among different subtypes of breast cancer, with relatively high expression in a normal-like subtype (P < 0.00001; Figure 2D). Furthermore, decreased expression of DACT1 was associated with ER− and higher histologic grade (P < 0.00001, Figure 2E). These results indicate that DACT1 expression is frequently downregulated in breast cancer and associated with clinicopathologic features.

**Promoter methylation of DACT1 contributes to its downregulation in breast cancer**

We next evaluated whether DACT1 repression was due to promoter methylation. A typical CpG island spanning the proximal promoter and exon 1 regions of the DACT1 gene was found (Figure 1B). MSP showed that DACT1 was methylated in five breast cancer cell lines (MB231, MB468, MCF7, T47D, and YCC-B1), although no methylation was found in another two cell lines (SK-BR-3 and ZR-75-1) with silenced DACT1 (Figure 1C). Pharmacologic demethylation was used to assess whether promoter methylation directly regulates DACT1 expression. Three cell lines (MB231, MCF7, and T47D) with methylated and silenced DACT1 were treated with Aza and/or histone deacetylase inhibitor TSA. After treatment, DACT1 expression was increased, accompanied by decreased methylated alleles of DACT1 (Figure 1D). The results indicate that promoter methylation is a major mechanism for DACT1 silencing in breast cancer cells.

**DACT1 is methylated in breast primary tumors**

We further investigated DACT1 methylation in primary tumors, surgical-margin tissues, and normal breast tissues. DACT1 methylation was detected in 40 (29.9%) of 134 breast cancer tissues, but not in surgical-margin tissues and normal breast tissues (Figure 3, Table 2), suggesting a tumor-specific methylation of DACT1 in breast cancer. We next analyzed the correlation between DACT1 methylation and clinicopathologic features of breast cancer patients, including age, tumor size, tumor grade, lymph node metastasis, status of estrogen receptor (ER), progesterone receptor (PR), and HER2. However, no significant correlation between DACT1 methylation and clinicopathologic features was found (Table 3).

**Ectopic expression of DACT1 inhibits breast cancer cell growth**

DACT1 repression by promoter methylation in breast cancer indicated that DACT1 is likely a tumor suppressor. Immunostaining showed that DACT1 is located mainly in the cytoplasm and membrane of DACT1-expressing MB231 cells (Figure 4A). Colony-formation assay and CCK-8 cell-proliferation assay were further performed to assess the effect of DACT1 on cell proliferation of breast cancer. About 40% to 60% reduction of colony numbers was observed in DACT1-transfected MB231 and MCF7 cells, compared with controls (*) (P < 0.05) (Figure 4B). Cell viability was significantly decreased at 24, 48, and 72 hours.
after transfection with DACT1 in MB231 cells (**\(P < 0.01\); *\(P < 0.05\) (Figure 4C).

To evaluate the molecular mechanism of DACT1 in the inhibition of cell proliferation, caspase-3 colorimetric assay was used. Results indicated that the concentration of active caspase-3 was increased in DACT1-expressing MB231 and MCF7 cells, compared with controls (**\(P < 0.01\) (Figure 4D), as further confirmed by upregulated cleaved caspase-3 and cleaved PARP (Figure 5C). Thus, DACT1 is a functional TSG, inhibiting tumor cell growth and inducing cell apoptosis of breast cancer.

**DACT1 decreases β-catenin activity and suppresses breast cancer cell migration**

We next investigated whether DACT1 could counteract Wnt/β-catenin signaling for its tumor-suppressive function. Expression and localization of active β-catenin were examined with immunostaining and Western blot. Reduced expression of active β-catenin and its downstream target gene c-MYC were detected in DACT1-expressing MB231 cells (Figure 5), suggesting that DACT1 antagonizes Wnt/β-catenin signaling by decreasing active β-catenin levels in breast cancer.

As the Wnt/β-catenin signaling pathway plays a key role in tumor metastasis, the effect of DACT1 on cell migration was further analyzed. Wound-healing assay showed that MB231 and MCF7 cells migrated into scraped areas within 42 and 48 hours, whereas DACT1 expression decreased their wound closure by about 55% after 42 hours and about 70% after 48 hours in these two cell lines (Figure 6), suggesting that DACT1 attenuates the wound-induced cell migration of breast cancer.

**DACT1 inhibits breast tumor growth in vivo**

Further to evaluate the tumor-suppressive functions of DACT1 in vivo, tumorigenicity of MB231 cells expressing
Figure 2 DACT1 was downregulated in primary breast tumors. (A) Analysis of DACT1 expression in normal breast tissues and primary breast tumors by using real-time PCR. (B) Representative immunohistochemical (IHC) staining for DACT1 in paired breast tumors and surgical-margin tissues. Magnification, 400×. (C) The mean optical density of DACT1 expression in each case (left panel). Quantitative analysis of DACT1 expression density is shown as values of mean ± SD (right panel). ***P < 0.001. (D, E) GSA-tumor analysis of DACT1 by using the 1,881-sample breast cancer data set. Box plot of DACT1 expression for tumor samples stratified according to Hu subtypes, ER status, and histologic grade.
Figure 3 Representative methylation-specific PCR (MSP) analysis of DACT1 methylation in primary breast tumors, surgical margin tissues, and normal tissues. M, methylated; U, unmethylated.

Table 2 Promoter methylation status of DACT1 in primary breast tumors

| Tissue                                | Samples (number) | DACT1 promoter Frequency of methylation |
|---------------------------------------|------------------|----------------------------------------|
| Breast cancer tissues                 | 134              | 40/94 (29.9%)                          |
| Breast cancer surgical-margin tissues | 11               | 0/11 (0%)                              |
| Normal breast tissues                 | 15               | 0/15 (0%)                              |

Table 3 DACT1 methylation and clinicopathologic features of breast tumors

| Clinicopathologic features | Number (N = 134) | DACT1 methylation status | P value |
|----------------------------|------------------|--------------------------|---------|
| Age (years)                |                  |                          |         |
| ≤40                        | 11               | 2/9                      | 0.229   |
| > 40                       | 86               | 30/56                    |         |
| Unknown                    | 37               | 8/29                     |         |
| Tumor grade                |                  |                          |         |
| I                          | 7                | 2/5                      | 0.425   |
| II                         | 56               | 21/35                    |         |
| III                        | 5                | 1/4                      |         |
| Unknown                    | 66               | 16/50                    |         |
| Tumor size                 |                  |                          |         |
| ≤2.0 cm                    | 44               | 14/30                    | 0.21    |
| ≥2.0 cm, ≤5.0 cm           | 48               | 18/30                    |         |
| > 5.0 cm                   | 4                | 0/4                      |         |
| unknown                    | 38               | 8/30                     |         |
| Lymph node metastasis      |                  |                          |         |
| Positive                   | 43               | 16/27                    | 0.38    |
| Negative                   | 41               | 12/29                    |         |
| Unknown                    | 50               | 12/38                    |         |
| Distant metastasis         |                  |                          |         |
| Positive                   | 1                | 1/0                      | 0.114   |
Table 3 DACT1 methylation and clinicopathologic features of breast tumors (Continued)

|              | Negative | 32 | 66 |
|--------------|----------|----|----|
| ER status    | Positive | 45 | 17 | 28 | 0.274 |
|              | Negative | 29 | 9  | 20 |
|              | Unknown  | 60 | 14 | 46 |
| PR status    | Positive | 34 | 11 | 23 | 0.296 |
|              | Negative | 40 | 15 | 25 |
|              | Unknown  | 60 | 14 | 46 |
| HER2 status  | Positive | 42 | 15 | 27 | 0.148 |
|              | Negative | 28 | 11 | 17 |
|              | Unknown  | 64 | 14 | 50 |

ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; PR, progesterone receptor.

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Figure 4 Tumor-suppressive functions of DACT1 in breast cancer cells. (A) Subcellular localization of DACT1 in MB231 cells was detected with immunofluorescence. (B) Representative colony-formation assay of DACT1 in MB231 and MCF7 (left panel). Quantitative analyses of colony numbers are shown as values of mean ± SD. *P < 0.05 (right panel, upper). DACT1 expression as measured with RT-PCR in vector- and DACT1- transfected MB231 and MCF7 cells (right panel, lower). (C) Cell Counting Kit-8 (CCK8) assay assessed the effect of DACT1 on cell proliferation in vector-, and DACT1- expressing MB231 cells. The values are shown as the mean ± SD. *P < 0.05; **P < 0.01. (D) The colorimetric assay for active caspase-3 was performed in vector-, and DACT1-expressing MB231 and MCF7 breast cancer cells at 24 and 48 hours.
DACT1 was evaluated in nude mice. Thirty days after injection, tumors were excised from tested mice for further analysis. The average volume of tumors induced by DACT1-expressing MB231 cells was significantly decreased, compared with control tumors (**P < 0.01; Figure 7A, B). Immunohistochemistry was further performed to analyze the expression of DACT1 and cell-proliferation marker Ki-67 in xenograft tumors. Numerous tumor cells with higher nuclear fragmentation were observed in H&E-stained sections from DACT1-expressing MB231 cells compared with controls, along with decreased proliferating cells (Figure 7C). These results indicate that DACT1 does act as a tumor suppressor in breast tumorigenesis.

**Discussion**

From this study, we report that DACT1 is widely expressed in normal breast tissues but frequently downregulated/silenced by promoter methylation in breast cancer. DACT1 is methylated in 29.9% of primary breast tumors, but not in surgical-margin tissues and normal breast tissues. DACT1 inhibits breast cancer cell proliferation by inducing apoptosis, and further suppresses tumor-cell migration through downregulating β-catenin activity, thus functioning as a tumor suppressor for breast cancer.

Epigenetic disruption of TSGs, including promoter methylation and histone modification, is a key mechanism regulating cancer gene expression [25,26]. DACT1 was frequently downregulated by promoter methylation in HCC, whereas another DACT family member, DACT3, was repressed by bivalent histone modifications in colon cancer [16,27]. We report that DACT1 was frequently methylated in breast cancer cell lines and primary tumors, which was correlated with its downregulation/silencing. No methylation was detected in some breast cell lines with...
silenced DACT1, suggesting that histone modifications or other mechanisms may be alternative mechanisms for DACT1 downregulation in some settings.

The Wnt/β-catenin signaling pathway plays an important role in tumor proliferation and migration [6]. Dact1 (Dapper), originally identified as an interacting protein for Dishevelled (Dvl), has been involved in distinct Wnt-dependent developmental processes of Xenopus, zebrafish, and mice [8,11,28-30]. Dact1 antagonizes Wnt signaling by binding with Dvl and promoting its degradation. Notably, this inhibitory activity is conserved from Xenopus to humans [8,9,31,32]. DACT1 has been identified disrupting the expression and localization of β-catenin, thus dysregulating Wnt/β-catenin signaling in NSCLC [13]. We found that DACT1, located mainly in the cytoplasm and membrane, reduced the expression of active β-catenin and its downstream target gene c-MYC in breast cancer cells, thus inhibiting cell proliferation of breast cancer by inducing apoptosis, as well as tumor cell migration.

DACT1 has been reported as a potential tumor marker associated with poor prognosis of NSCLC [13]. We observed tumor-specific methylation of DACT1 in breast cancer, indicating its potential as a tumor marker, although no obvious correlation between its methylation and clinicopathologic features was found, which must be further
confirmed by a large sample-sized study. Future study of circulating methylated DACT1 in serum or in combination with other methylated TSGs may be performed for the detection of breast cancer [5,33,34].

Conclusions
In summary, DACT1, as a Wnt/β-catenin signaling antagonist, is frequently downregulated/silenced in breast cancer, acting as a functional tumor suppressor in breast tumorigenesis, and may serve as a potential tumor marker for breast cancer.

Abbreviations
Aza: 5-aza-2-deoxycytidine; CGI: CpG island; DAPI: 4,6-diamidino-2-phenylindole; Dvl: Dishevelled; ER: estrogen receptor; H&E: hematoxylin and eosin; HCC: hepatocellular carcinoma; IHC: immunohistochemistry; IPP: Image Pro Plus; MSP: methylation-specific PCR; NSCLC: non-small cell lung cancer; OD: optical density; PR: progesterone receptor; qRT-PCR: quantitative reverse transcription polymerase chain reaction; SDS-PAGE: sodium dodecylsulfate/polyacrylamide gel electrophoresis; TMA: tissue microarray; TSA: trichostatin A; TSG: tumor-suppressor gene.

Authors’ contributions
XY, TX, WXS, XS, XL, JH, YY, and WP acquired data. XY, TX, and LL analyzed data and drafted the manuscript. MO and KK provided material. GR reviewed the manuscript. QT conceived of and supervised the study, analyzed data, and finalized the manuscript. All authors read and approved the manuscript for publication.

Competing interests
The authors declare that they have no competing interests.

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