Talin2 regulates breast cancer cell migration and invasion by apoptosis

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Abstract. Talin is a key component molecule of the extracellular matrix-integrin-cytoskeleton. It serves an important role in the activation of integrin, which, in turn, is known to mediate physiological and pathological processes, including cell adhesion, growth, tumorigenesis, and metastasis. In vertebrates, there are two Talin genes, Talin1 and Talin2. Talin1 is known to regulate focal adhesion dynamics, cell migration and cell invasion; however, the precise role of Talin2 in cancer remains unclear. In the present study, the functional role of Talin2 was examined in the MDA-MB-231 breast cancer cell line. Talin2 knockdown significantly inhibited growth, migratory capacity and invasiveness of MDA-MB-231 cells, and promoted apoptosis. The expression levels of Talin2 in breast cancer cells and in the peritumoral normal breast tissues were also determined by immunohistochemistry. Talin2 was identified to be overexpressed in breast cancer tissues compared with that in the peritumoral breast tissues. In addition, the knockdown of Talin2 by specific RNA interference markedly inhibited cell growth, and caused the downregulation of the apoptotic markers, cleaved Caspase-3 and phosphorylation of poly ADP-ribose polymerase. These findings demonstrate that Talin2 expression is upregulated in human breast cancer and that downregulation of Talin2 may serve as a useful therapeutic target in patients with breast cancer.

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Introduction

Cancer is one of the leading causes of mortality and a major public health issue worldwide. In 2015, there were 17.5 million cancer cases globally and 8.7 million cases resulting in mortality. Between 2005 and 2015, cancer cases increased by 33% (1). By 2030, the annual incidence of new cancer cases and cancer-associated mortalities is projected to increase to, 26.4 million and 17.0 million, respectively (2). The incidence and mortality of malignant tumor has exhibited a sustained growth trend in China (3). The primary cause of mortality in patients with cancer is tumor cell invasion and metastasis (4).

Talin was discovered ~3 decades ago as a highly abundant cytosolic protein important for cytoskeleton organization and cell-extracellular matrix (ECM) adhesion (5). Extensive genetic and cell biological studies have established that Talin is essential for the regulation of a variety of integrin-mediated cell adhesion dependent processes, including cell-shape changes, growth, differentiation and migration (6-8). In vertebrates, there are two Talin genes, Talin1 and Talin2 (9,10), which encode similar proteins (74% amino acid sequence identity) (11,12). Talin1 is expressed in nearly all tissues, and Talin2 is expressed primarily in the heart, brain, testis and muscle (11-14). Talin1 is also known to regulate focal adhesion dynamics, cell migration and cell invasion (15-17). In a recent study, Talin2 knockdown was demonstrated to inhibit the migration of hepatocellular carcinoma cells (18). However, the role of the Talin2 isoforms in cell invasion and metastasis remains unclear.

In the present study, Talin2 expression was disrupted in MDA-MB-231 cells using short hairpin-RNA (shRNA)-mediated interference, to establish Talin2 knockdown stable cell lines and Talin2-specific functions were examined. The effects of Talin2 were further studied on MDA-MB-231 cell migration and invasion. In addition, the role of Talin2 in the occurrence and development of breast cancer was analyzed, and the underlying mechanisms were examined. The present study provides a novel method and basis for the diagnosis and treatment of breast cancer.

Materials and methods

Patients and samples. A total of 32 breast cancer tissue specimens from tumor and adjacent tissues were obtained from previously untreated female patients aged 36-73 with a mean age of 53 years who underwent surgical treatment at the Department of General Surgery, The First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) between January 2016 and June 2017. All samples were cut into two pieces. One piece was embedded in paraffin and processed for routine histopathological examination, while the other piece of tissue was frozen immediately in liquid nitrogen and stored at -80˚C for further studies. For all patients, the diagnosis of breast cancer was based on clinical and histopathological examination. Tumor stage was defined according to Union Internationale Contre le Cancer. Tumor pathological grade was defined based on the World Health Organization classification. The present study was approved by the Board and Ethical Committee of The First Affiliated Hospital of Wenzhou Medical University. All study participants provided written informed consent in accordance with the Declaration of Helsinki.

Reagents. Anti-Talin1 (1:1,000 dilution; cat. no. MCA4770) and anti-Talin2 [1:1,000 dilution in the western blot analysis and 1:25 dilution in the immunohistochemical (IHC) assay; cat. no. MCA4771GA] antibodies were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA); anti-poly ADP-ribose polymerase (PARP; 1:1,000 dilution; cat. no. AY3523) and anti-cleaved PARP (1:1,000 dilution; cat. no. CY3838) were obtained from Shanghai Abways Biotechnology Co., Ltd. (Shanghai, China); anti-cleaved Caspase-8 (1:1,000 dilution; cat. no. RLC0011) and anti-cleaved Caspase-3 (1:1,000 dilution; cat. no. RLC0004) were obtained from Suzhou Rui Sheng Biological Technology Co., Ltd. (Suzhou, China); anti-Caspase-8 (1:1,000 dilution, cat. no. 9746S), anti-Caspase-3 (1:1,000 dilution, cat. no. 9661S), anti-Tubulin (1:1,000 dilution, cat. no. 2148S) and anti-GAPDH (1:1,000 dilution, cat. no. 2188S) antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). Control shRNA TRC1/1.5 (5'-CCG GGCGCGATAGCGCTAATAATTTCTCGAGAAATTATT AGCGCTATCGCGCTTTTT-3'), Talin2 shRNA clones TRCN0000122990 (Talin2 90, 5'-CCGGCCATGTTAGAA GAGTCCGTTTCTCGAGAAACGGACTCTTCTAAC ATGGTTTTTG-3') and TRCN0000122992 (Talin2 92, 5'-CCGGCCATGCAGTTTGAACCATCTACTCGAGTA GATGGTTCAAACTGCGTTTTTG-3') were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:1,000; cat no. A0208) and DAB (0.05-0.03%) horseradish peroxidase color development kits (cat no. ST033) were obtained from Beyotime Institute of Biotechnology (Haimen, China). Lipofectamine™ 2000, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). SuperSignal™ West Pico PLUS Chemiluminescent substrate was purchased from Thermo Fisher Scientific, Inc. Penicillin G and streptomycin were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China).

Cell culture. MDA-MB-231 human breast cancer and 293T cells were obtained from the American Type Culture Collection (Manassas, VA, USA), and were maintained in DMEM containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were cultured at 37˚C in a humidified 5% CO_2 atmosphere incubator.

Preparation of viruses and cell infection. To produce the lentivirus, VSV-G-pseudotyped lentiviral vectors were produced by co-transfecting $6x10^6$ 293T cells with 2 pmol of the respective control shRNA, Talin2 90 (TRCN0000122990) and Talin2 92 (TRCN0000122992) along with 1 pmol pMDLg.RRE, 0.5 pmol pMD2.G and 0.5 pmol pRSV.REV. using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 8 h, the original medium [DMEM containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml)] was replaced with fresh medium, and the lentiviral supernatant was collected at 48 and 72 h post-transfection. After each collection, the supernatant was filtered through a cellulose acetate membrane (0.45 μm pore).

A total of $1x10^5$ MDA-MB-231 cells were plated into each well of a 6 well plate and infected with a total volume of 100 μl lentiviruses for 24 h. Cells that stably expressed lentiviral shRNAs were obtained by selecting the infected cells with 1 mg/ml puromycin for a period of 3-4 weeks at 37˚C in a humidified 5% CO_2 atmosphere incubator.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from MDA-MB-231 and MDA-MB-231 Talin2 knockdown cells using the PureLink RNA kit (Ambion; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. cDNA was synthesized at 42˚C for 50 min using the SuperScript First Strand Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) using 0.5-1.0 μg RNA samples according to the manufacturer's protocol. qPCR reactions were performed using SYBR Green PCR master mix reagents (cat no. 4309155; Invitrogen; Thermo Fisher Scientific, Inc.) on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). The relative quantification of gene expression for each sample was analyzed using the $2^{-\Delta\Delta Cq}$ method (19). The following primers were used: Talin1 forward, 5'-TGTTCC CCAGAGCCACCTGCC-3' and reverse, 5'-GAAGCCGCA CATCAGGGGC-3'; Talin2 forward, 5'-GGGGAATGTGTG GGGATTGCATCC-3' and reverse, 5'-GATGAGGCGATG CGGCAGGCA-3'; 18S rRNA forward, 5'-ACCTGGTTGATC CTGCCAGT-3' and reverse, 5'-CTGACCGGGTTGGTTTTG AT-3'. The thermocycling conditions were as follows: 20 sec at 95˚C; followed by 40 cycles of 5 sec at 95˚C and 30 sec at 60˚C. Each experiment was repeated three times in duplicate.

Colony formation assay. MDA-MB-231 cells were trypsinized, counted by an automatic cell counter (Invitrogen Countess; Invitrogen; Thermo Fisher Scientific, Inc.) and seeded for the colony formation assay in six-well plates at a density of $1x10^3$ cells/well, then incubated at 37˚C in a humidified 5% CO_2 atmosphere incubator. During colony growth, the culture medium was replaced every 3 days. A colony was only acknowledged if it contained >50% cells. After 2 weeks, cells were fixed for 15 min with 3.7% formaldehyde

at room temperature and stained using 0.1% crystal violet in 10% ethanol for 30 min at room temperature. The number of invaded cells per field was counted under a light microscope at a magnification of x400. The colony formation rate was calculated using the formula: (Number of colonies/number of seeded cells) x100%. Three independent experiments were performed for each group.

Cell migration assay. After cells had grown to 100% confluence in six-well culture plates, an artificial wound was created by scratching the cell monolayer with the tip of a 10-μl pipette. The wound area was inspected after 24 and 48 h using an inverted phase-contrast microscope with a digital camera (magnification, x400). The wound healing speed was calculated as the percentage of the initial wound at different time points (24, 36 and 48 h) until total wound closure.

Invasion assays. To examine the effect of Talin2 on cell invasion, 100 μl of Matrigel (0.35 mg/ml; 1:30 dilution in serum-free DMEM) was added to each Transwell polycarbonate filter (6-mm diameter; 8-μm pore size) and incubated with the filters at 37˚C for 6 h. Breast cancer cells MDA-MB-231 were trypsinized and washed three times with DMEM containing 1% FBS. The cells were resuspended in DMEM containing 1% FBS at a density of $5x10^5$ cells/ml. The cell suspensions (100 μl) were seeded into the upper chambers and 600 μl of DMEM medium containing 10% FBS was added to the lower chambers. The cells were allowed to invade for 12 h in a CO_2 incubator, then fixed, stained and quantified as described previously (20).

IHC staining. Representative tumor and adjacent regions were marked on the paraffin-embedded blocks. Paired cancer and normal cores were punched and transferred to a recipient block to make the tissue microarray (TMA) block. For each patient, one tumor core and one normal core were taken from the donor block. IHC staining was performed as described previously (21). Briefly, 4-μm thick TMA slides were baked for 1 h at 60˚C, deparaffinized, dehydrated and treated in citrate buffer (pH 6.0). The TMA slides were blocked using equine serum (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), and then incubated with 3% hydrogen peroxide at room temperature for 1 h, followed by overnight incubation with anti-Talin2 (1:25 dilution) at 4˚C. On the second day, the slides were incubated with secondary antibodies (goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase antibody (1:2,000; cat no. PV-6001; OriGene Technologies, Inc., Rockville, MD, USA) at 37˚C for 1 h. then stained using hematoxylin (cat no. H8070; Beijing Solarbio Science & Technology Co., Ltd.), dehydrated, cleared and mounted. The distribution area of Talin2 in different tissues was analyzed with ImagePro Plus software 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). The positive rate of stained tumor cells and the corresponding score were assigned as follows: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). The intensity of CDK5 staining was scored from 0 to 3, and the detailed standard was as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining, and observed under an Eclipse 50i/55i Microscope (Optical microscope) at a magnification of x400. Positive staining intensity: colorless, 0; light yellow, 1; pale brown, 2; and brown, 3.

Hoechst 33342 staining. Hoechst 33342 staining was performed to detect altered nuclear morphology in MDA-MB-231 and MDA-MB-231 Talin2 knockdown cells. Cells were plated on coverslips overnight; subsequently, the cells were stained with 10 μM Hoechst 33342 solution for 15 min at 37˚C. After staining, cells were washed three times with PBS, then viewed and counted by eye under a fluorescence microscope (magnification, x200) with standard excitation filters (Nikon Corporation, Tokyo, Japan). The excitation wavelength used was 346 nm and the emission wavelength was 460 nm.

Annexin V/fluorescein isothiocyanate (FITC) flow cytometric assay. The flow cytometric assay was performed with the Annexin V kit (BD Pharmingen; BD Biosciences, San Jose, CA, USA). Cells were seeded in six-well plates at a density of $1x10^5$ cells/well and incubated at 37˚C in a humidified 5% CO_2 atmosphere incubator. After 12 h, cells were harvested and pelleted via centrifugation (1,000 x g for 5 min) at 4˚C, immediately resuspended in binding buffer and subsequently stained with 5 μl FITC Annexin V and 5 μl propidium iodide (PI), according to the kit protocol. The mixture was placed on ice (4˚C) in the dark and analyzed using a FACS system with BD Accuri C6 Software (BD 1.0.264.21; BD Biosciences).

Western blot analysis. A total of $2x10^6$ cells (MDA-MB-231 or MDA-MB-231 Talin2 knockdown cells) were seeded overnight in six-well plates, cells were washed with ice-cold PBS and harvested using RIPA buffer (cat no. P0013C; Beyotime Institute of Biotechnology). The protein concentration was determined using the BCA method (cat no. P0011; Beyotime Institute of Biotechnology) and 20 μg of total protein was separated using 12% SDS-PAGE prior to western blot analysis. The proteins were transferred to a nitrocellulose membrane that was blocked in 5% milk for 1 h at room temperature. The expression levels of Talin1, Talin2, Caspase-3, cleaved Caspase-3, GAPDH and Tubulin were determined by incubating the membrane with the specific aforementioned primary antibodies (1:1,000 dilution in 5% milk) overnight at 4˚C. This was followed by incubation with a HRP-conjugated secondary antibody (Beyotime Institute of Biotechnology) for 1 h at room temperature prior to development using SuperSignal West Pico PLUS Chemiluminescent substrate. Tubulin or GAPDH was used as the loading control. All protein expressions levels were quantified using ImageJ software (ImageJ 1.43u/Java 1.6.0-10; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. All data were presented as the mean ± standard deviation and analyzed using single-factor analysis of variance (one-way ANOVA) for comparison between groups. Multiple comparisons were performed among groups using ANOVA followed by the least significant difference test. The software package SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. The results are representative of three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

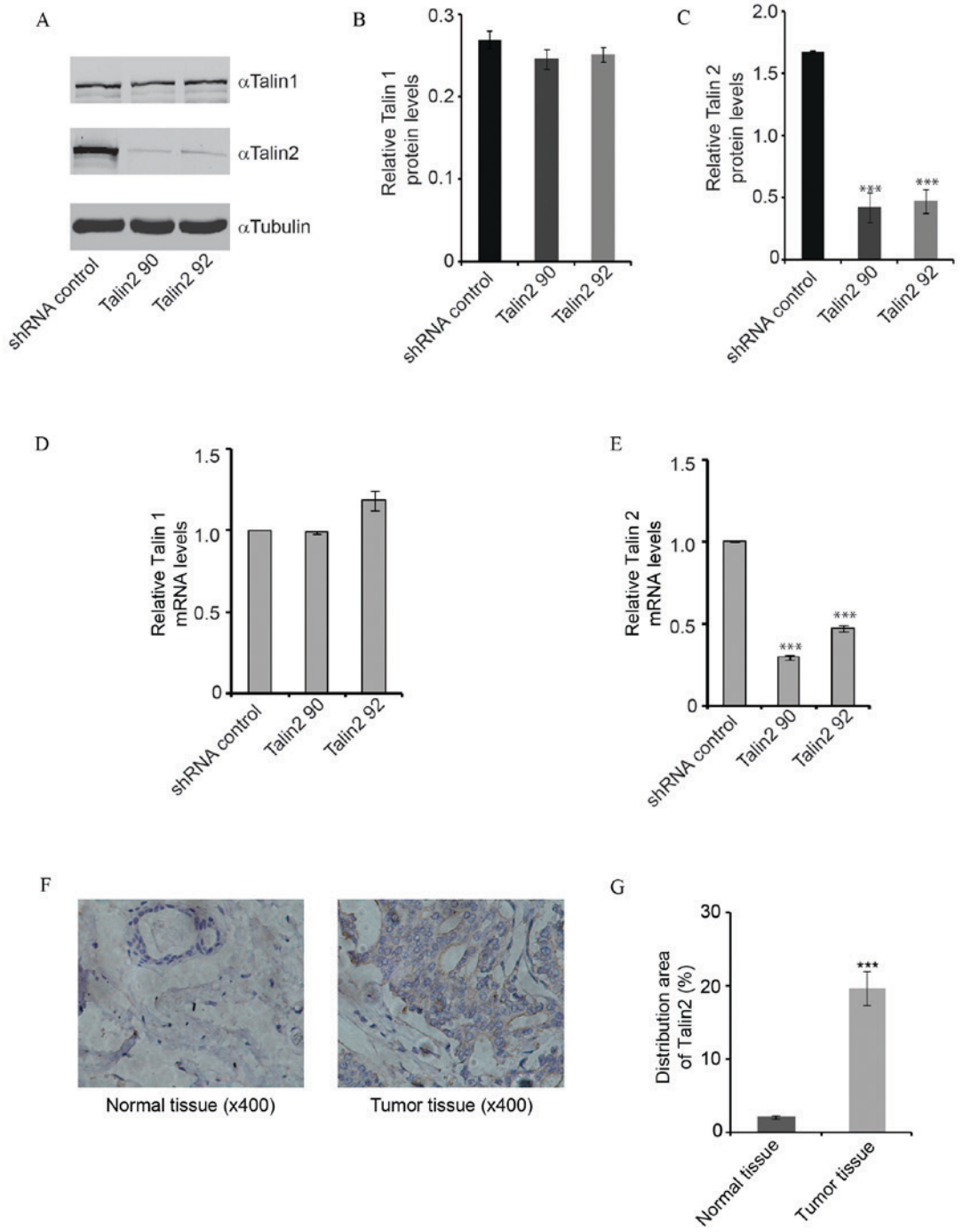

Figure 1. Expression levels of Talin2 in MDA-MB-231 cells and breast cancer tissues. (A) The protein levels of endogenous Talin1 and Talin2 in Talin2-depleted MDA-MB-231 cells were measured by western blotting. (B) The relative protein expression levels of Talin1 in MDA-MB-231 cells infected with control, Talin2 90 or Talin2 92 shRNA. (C) The relative protein expression levels of Talin2 in MDA-MB-231 cells infected with control, Talin2 90 or Talin2 92 shRNA. (D) The relative mRNA levels of Talin1 in MDA-MB-231 cells infected with control, Talin2 90 or Talin2 92 shRNA. ***P<0.001, Talin2 90/92 vs. shRNA control. (E) The relative mRNA levels of Talin2 in MDA-MB-231 cells infected with control, Talin2 90 or Talin2 92 shRNA. ***P<0.001, Talin2 90/92 vs. shRNA control. (F) The distribution of Talin2 in tumor tissues and adjacent tissues from patients with breast cancer (magnification, x400). (G) Quantification of immunohistochemistry assays. ***P<0.001, tumor tissue vs. adjacent tissue. shRNA, short hairpin RNA.

Results

Construction of stable Talin2 knockdown cells. To establish the stable Talin2 90 or Talin2 92 cell lines, Talin2 recombinant lentivirus infected MDA-MB-231 cells were screened with puromycin for 3-4 weeks and then amplified (Fig. 1). The protein and mRNA expression levels of Talin2 in the knockdown cell lines were significantly lower compared with that in the Talin2 knockdown groups (Talin2 90 and Talin2 92) and shRNA control group, with ~2-fold decreases in mRNA and protein levels (all P<0.001). However, no significant differences in the mRNA or protein expression levels of Talin1 were observed among the three groups (all P>0.05; Fig. 1A-F). The results revealed successful construction of stable Talin2 knockdown cell lines, which laid the foundation for the subsequent experiments.

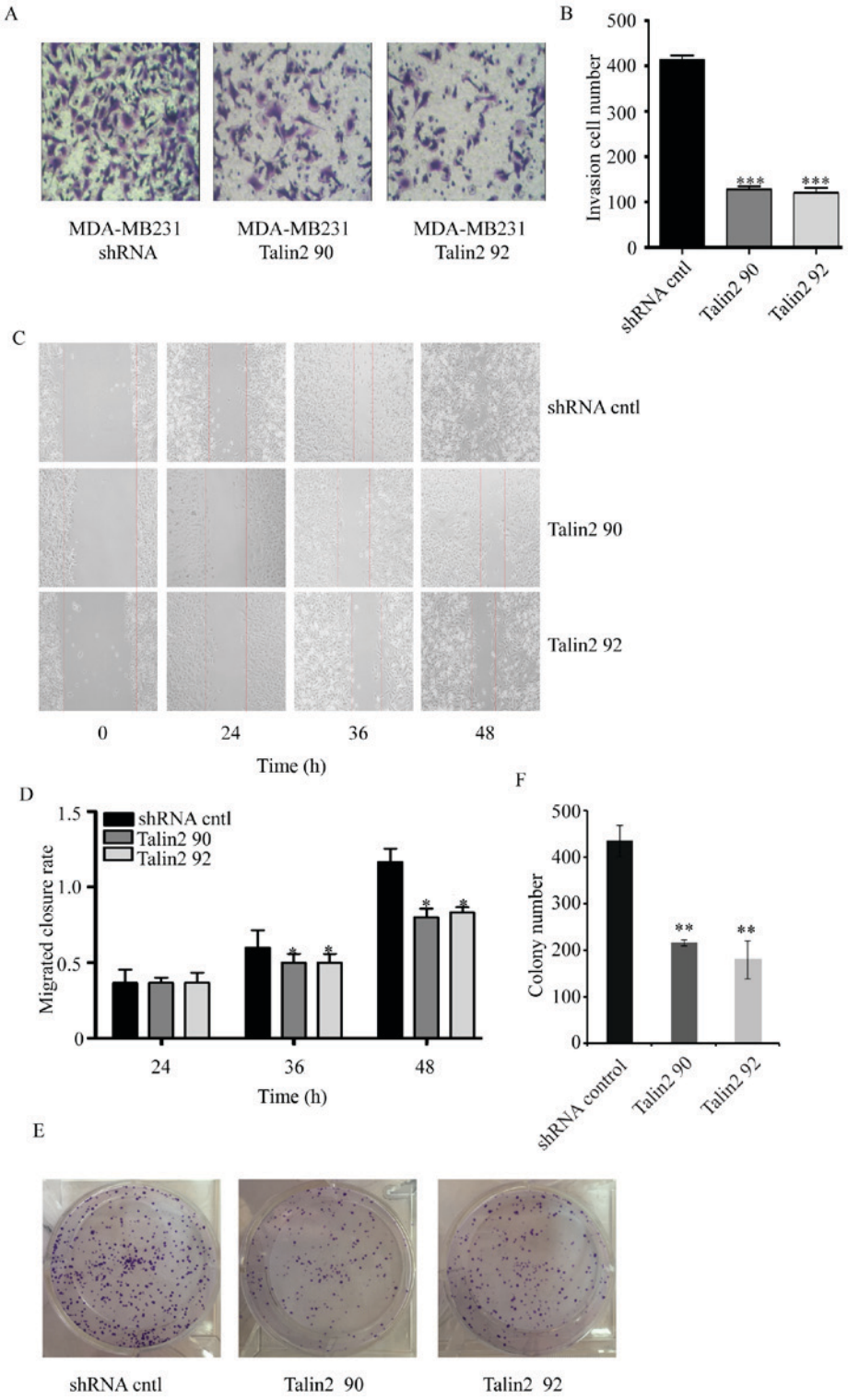

Figure 2. Talin2 knockdown inhibits the proliferation and invasive growth of MDA-MB231 cells. MDA-MB231 cells were infected with pLKO1 lentiviruses expressing shRNA control or Talin2 shRNA and then selected with puromycin. (A) Depletion of endogenous Talin2 inhibited the invasion of MDA-MB231 cells. (B) Quantification of invasion assays. (C) Wound healing assays of shRNA control group and Talin2 shRNA group (magnification, x400). (D) Quantification of the wound-healing assays. (E) Colony formation of MDA-MB231 cells was inhibited by Talin2 knockdown (magnification, x400). (F) Quantification of colony formation of MDA-MB231 cells that stably express shRNA control or Talin2 shRNA. *P<0.05, **P<0.01, ***P<0.001, Talin2 90/92 vs. shRNA control. shRNA, short hairpin RNA.

Talin2 regulates cancer cell invasion and migration. To examine the role of Talin2 in breast cancer cell invasion, MDA-MB-231 cells were infected with lentiviruses that expressed Talin2 shRNA or shRNA control. The invasive growth potential of these cells was measured by examining the functional capacity of the cells to penetrate through Transwell membranes coated with 0.35 mg/ml Matrigel (Fig. 2). The results indicated that the invasiveness of MDA-MB-231 cells was significantly reduced following Talin2 depletion (Talin2 90 and Talin2 92) as compared with that in the shRNA control

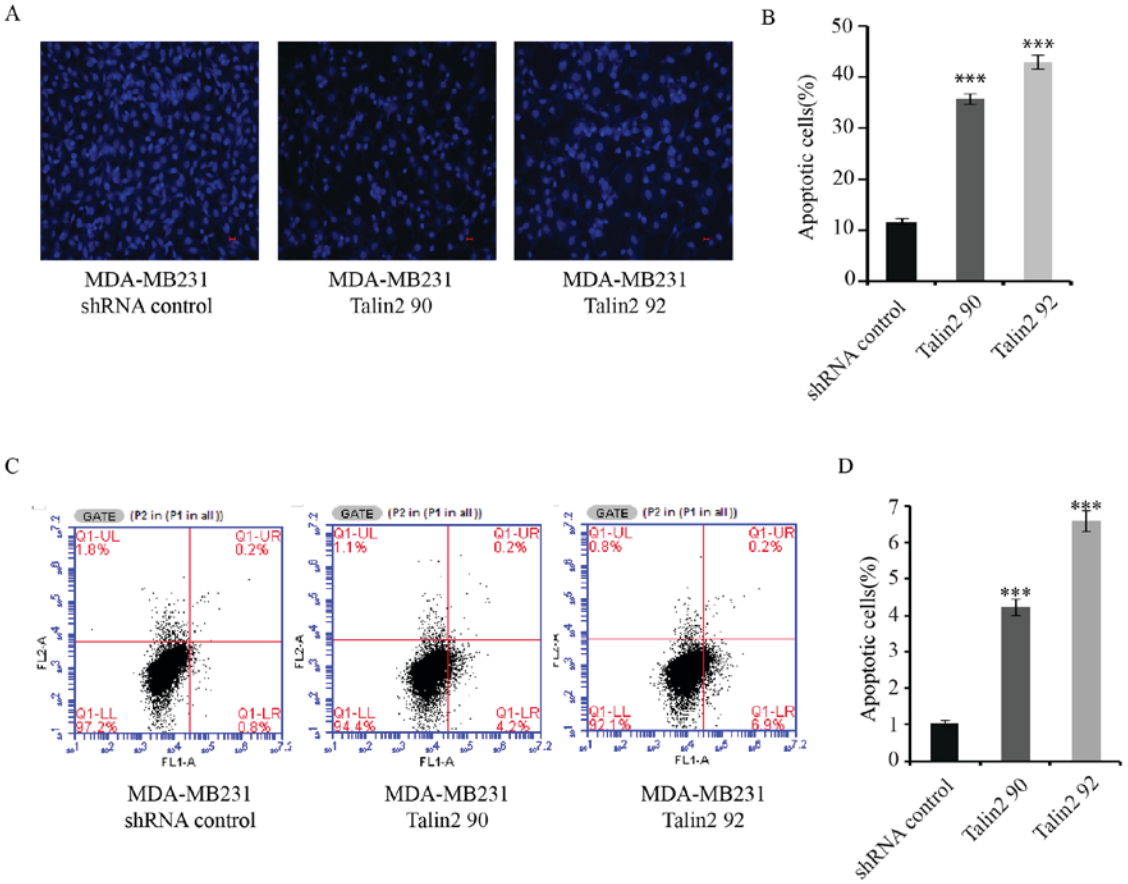

Figure 3. Talin2 shRNA induces apoptosis of MDA-MB231 cells. (A) Cells were seeded onto coverslips for 24 h and subjected to Hoechst 33342 staining. Scale bar, 100 nm. (B) Quantification of Hoechst staining data. (C) The shRNA control or Talin2-depleted MDA-MB231 cells were subjected to FACS. (D) Quantification of FACS. ***P<0.001, Talin2 90/92 vs. shRNA control. shRNA, short hairpin RNA.

group, whereby the rates were reduced by 70 in Talin2 92 and 58% in Talin2 90 (Fig. 2A and B).

Talin2 expression levels were also analyzed in different breast cancer tissues by IHC. As shown in Fig. 1F and G, the distribution area of Talin2 in tumorous tissue was 8.4 times greater compared with that in normal tissue (P<0.001). The results suggested that Talin2 expression is upregulated in breast cancer. To determine whether Talin2 regulates the migration of breast cancer cells, an artificial wound was created by scratching the cell monolayer of MDA-MB-231 cells with the tip of a 10-μl pipette. The wound area was examined after 24 and 48 h using an inverted phase-contrast microscope with a digital camera. The effect of Talin2 on the migratory ability of cells is shown in Fig. 2C and D. At 36 h, the wound healing rate of Talin2 knockdown (Talin2 90 and Talin2 92) cells was significantly reduced compared with that of cells in the blank control group (P<0.05). At 48 h, the migratory ability of Talin2 knockdown cells was 0.625 times (Talin2 90) and 0.7 times (Talin2 92) that of the control cells. These results indicated that Talin2 had a significant suppressive effect on the migratory ability of MDA-MB-231 cells. The colony formation assay also demonstrated that the proliferative ability of the Talin2 knockdown (Talin2 90 and Talin2 92) cells was significantly lower compared with that of cells in the control group (Fig. 2E and F).

Talin2 knockdown promotes apoptosis of MDA-MB-231 cells. The development of tumors is associated with the disruption of homeostasis between cell proliferation and apoptosis (22). The morphological changes in MDA-MB-231 cells with Talin2 knockdown were examined with Hoechst 33342 staining. In Talin2-depleted (Talin2 90 and Talin2 92) cells, the cell nuclei became agglutinated, were smaller in size and the chromatin was partially condensed into small spherical or crescent shapes, indicating that cells underwent apoptosis (Fig. 3A and B). In order to analyze the effect of Talin2 on the apoptosis of MDA-MB-231 cells, the cell apoptotic rate was detected by FACS analysis with AnnexinV-FITC and PI double labeling. As shown in Fig. 3C and D, the apoptotic rate of MDA-MB-231 cells was 1.03±0.088%, while the apoptotic rates of Talin2 knockdown cells were 4.23±0218 (Talin2 90) and 6.6±0.265% (Talin2 92). The difference between the Talin2 knockdown groups and the normal control group were statistically significant (both P<0.001). These data suggest that Talin2 knockdown promotes apoptosis of breast cancer MDA-MB-231 cells.

To further analyze the cause of Talin2-induced apoptosis, the effect of Talin2 on the tumor apoptosis signaling pathway was determined using western blot analysis. The protein expression levels of Caspase-8, Caspase-3 and PARP (a substrate of Caspase-3) were detected. The expression level of Caspase-8 is shown in Fig. 4A and B. The total expression levels of Caspase-8 in MDA-MB-231 cell and Talin2 knockdown (Talin2 90 and Talin2 92) cell were comparable; however, the level of cleaved Caspase-8 in Talin2 knockdown

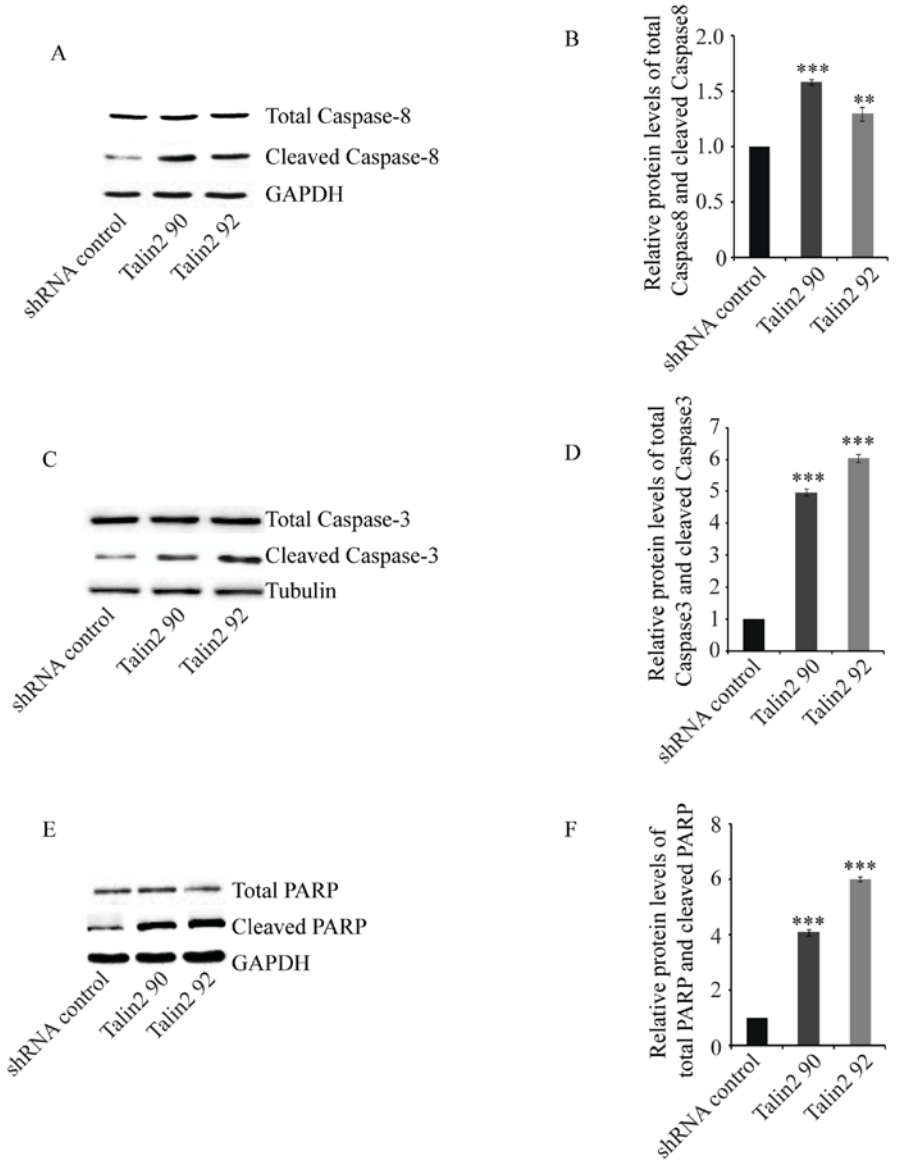

Figure 4. Expression levels of Caspase-8, Caspase-3 and PARP detected by western blotting in the shRNA control and Talin2 shRNA groups. (A) The expression levels of total Caspase-8 and cleaved Caspase-8 in Talin2-depleted MDA-MB-231 cells were measured by western blotting. (B) The relative protein expression levels of total Caspase-8 and cleaved Caspase-8 in MDA-MB-231 cells infected control, Talin2 90 or Talin2 92 shRNA. (C) The expression levels of total Caspase3 and cleaved Caspase-3 in Talin2-depleted MDA-MB-231 cells were measured by western blotting. (D) The relative protein expression levels of total Caspase-3 and cleaved Caspase-3 in MDA-MB-231 cells infected control, Talin2 90 or Talin2 92 shRNA. (E) The expression levels of total PARP and cleaved PARP in Talin2-depleted MDA-MB-231 cells were measured by western blotting. (F) The relative protein expression levels of total PARP and cleaved PARP in MDA-MB-231 cells infected control, Talin2 90 or Talin2 92 shRNA. **P<0.01, ***P<0.001, Talin2 90/92 vs. shRNA control. shRNA, short hairpin RNA; PARP, poly ADP-ribose polymerase.

cells was significantly higher compared with that in shRNA control-transfected MDA-MB-231 cells (P<0.001). Similarly, the total expression levels of Caspase-3 in MDA-MB-231 cells and Talin2 knockdown (Talin2 90 and Talin2 92) cells were comparable; however, the expression level of cleaved Caspase-3 in Talin2 knockdown cells was significantly higher compared with that in the shRNA control group (Fig. 4C and D; P<0.001). PARP, the cleaved substrate of Caspase, is considered to be an important indicator of cell apoptosis; it is also considered as an indicator of Caspase-3 activation. The results of western blot analysis revealed that the total expression of PARP was comparable between MDA-MB-231 cells and Talin2 knockdown (Talin2 90 and Talin2 92) cells; however, PARP in Talin2 knockdown cells was significantly activated (Fig. 4E and F). This indicated that Caspase-8 induced by Fas cell surface death receptor or tumor necrosis factor during apoptosis, directly or indirectly activated the downstream effector molecules Caspase-3, and that the activated Caspase-3, in turn, cleaved PARP which reactivated the endonuclease, resulting in DNA fragmentation and the promotion of apoptosis of tumor cells.

Discussion

The incidence of malignant tumors and the associated mortality rate has steadily increased in China from 2000 to 2011 (2). The invasive growth of cancer cells and metastasis are key impediments to the treatment of cancer

and a major contributor to cancer-associated mortality (4). The tumor microenvironment serves an essential role in tumor invasion and metastasis. The ECM is the primary component of the tumor microenvironment, and contains various growth factors and cytokines, that modulate tumor properties (23). Talin is a key molecule among the ECM, integrins and cytoskeleton (24). Vertebrates express two closely associated Talins encoded by separate genes. While it is well established that Talin1 serves an important role in cell adhesion and dissemination, little is known regarding the role of Talin2 (25).

To investigate the function of Talin2 in breast cancer cells, Talin2 knockdown stable expression cell lines were constructed and the functions of Talin2 were examined. The colony formation assay demonstrated that Talin2 knockdown significantly inhibited the proliferative ability of MDA-MB-231 cells. Wound healing and invasion assays were used to investigate the effects of Talin12 on MDA-MB-231 cell migration and invasion. The results of the wound healing assay revealed that the migratory rate of Talin2 knockdown cells was significantly slower compared with that of cells in the control group. At 48 h, the migratory ability of Talin2 knockdown cells was ~0.7 times that of cells in the control group. These results indicated that Talin2 regulates the migration of MDA-MB-231 cells. Furthermore, Talin2 knockdown reduced the invasive ability to ~60% of that observed in the control group, indicating that Talin2 knockdown significantly inhibited the invasiveness of MDA-MB-231 cells. In a recent study by Le *et al* (26), increased miR-194 expression was revealed to markedly reduce the expression levels of the cytoskeletal protein Talin2 and specifically inhibit the migration of breast cancer cells. The present study did not investigate the direct association between Talin2 and breast cancer metastasis, which is a limitation and a potential focus for our future research.

Apoptosis is the process of programmed cell death that is regulated by multiple genes and is required for the stability of the internal environment and the development of multiple systems (27). Evasion of apoptosis by tumor cells is a key challenge in the clinical treatment of tumors. In the current study, Hoechst 33342 staining demonstrated that the nuclei of Talin2 knockdown cells were agglutinated and smaller, and the chromatin was partially condensed into small spherical or crescent shapes. Furthermore, the results of FACS Annexin V/PI assay demonstrated that the apoptosis rate in the Talin2 knockdown group was significantly higher compared with that in the control group. The aforementioned results suggest that Talin2 knockdown promotes the apoptosis of MDA-MB-231 cells.

The apoptotic process is divided into two categories: Caspase dependent and non-Caspase dependent. The Caspase family is a mediator and executor of programmed cell death in mammals. Proapoptotic signals culminate in activation of different initiator Caspases that, in turn, activate effector Caspases through enzyme cascade pathways. Activated effectors cleave a set of substrates resulting in cellular disassembly. Caspase is synthesized and stored in the form of an inactive precursor under normal conditions, whereby apoptotic signaling then activates the Caspase cascade reaction. Caspase-3 is known as the 'executor', and Caspase-8 and Capase-9 are referred to as the 'initiators' (28). Caspase-3 serves a key role in the process of apoptosis. Upon activation, it triggers a cascade of activation reactions, which lead to cell apoptosis. Detection of Caspase-3 reflects cell apoptosis rate and is also a marker of initiation of programmed apoptosis in the cells. The western blot analysis results in the present study revealed that the protein levels of cleaved Caspase-8 and Caspase-3 in the experimental groups were significantly higher compared with that in the control group. PARP is a Caspase substrate and is considered to be an important indicator of cell apoptosis. The western blotting results demonstrated that PARP phosphorylation levels in the Talin2 knockdown groups were higher compared with those in the control group. Hence, we hypothesize that Talin2 knockdown activates Caspase-3, which then initiates a cascade reaction, leading to cell apoptosis.

In conclusion, the results of the present study suggest that Talin2 knockdown regulates MDA-MB-231 cell migration and invasion through apoptosis. Thus, these results may provide a novel target and basis for the diagnosis and treatment of breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL and YLL conceived and designed the experiments. YFL, HWC, LJ and XFX performed the experiments. XL, YFL, JFD, HWC analyzed the data. XL and YLL wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Board and Ethical Committee of the First Affiliated Hospital of Wenzhou Medical University. All study participants provided written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

All patients provided consent for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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