

Scinderin-knockdown inhibits proliferation and promotes apoptosis in human breast carcinoma cells

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Abstract. Previous studies have reported that scinderin (SCIN) affects multiple cellular processes, including proliferation, migration and differentiation in cancer. However, the specific role of SCIN in breast cancer (BC) cells is unknown. Immunohistochemistry was used to investigate SCIN expression in 46 BC and 21 mammary fibroadenoma or fibroadenomatoid hyperplasia tissue samples. SCIN expression was ablated in MDA-MB-231 and T-47D cells using lentivirus-mediated small interfering RNA technology. Cell proliferation was tested using Celigo and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. Cell apoptosis was analyzed by measuring Caspase 3/7 activity and annexin-V staining. The results of the present study demonstrated that SCIN expression was elevated in BC tissues compared with mammary fibroadenoma or fibroadenomatoid hyperplasia tissues. Specifically, higher SCIN expression was observed in Ki-67-positive BC tissues (78.6%) compared with Ki-67-negative BC tissues. Furthermore, knockdown of SCIN expression in the BC cell lines significantly suppressed cell proliferation and induced apoptosis. The data presented in the present study indicate that SCIN serves an important role in the development of breast cancer.

Introduction

Breast cancer (BC) is one of the most common malignancies in women worldwide (1) and accounts for 25% (1.7 million) of all cancer cases and 15% (521,900) of all cancer-related mortalities among females, based on data through 2012 (2).

The percentage of cancer-related mortalities among females in less developed countries is even higher, for example, female breast cancer mortality in Belize was 14.0%, while 29.7% in the Cayman Islands in 2013 (3). Despite recent advancements in BC diagnoses and treatments, prognosis outcomes remain unsatisfactory due to the highly complex and heterogeneous nature of this disease (2). Thus, identification of novel therapeutic targets is required to develop additional effective treatment strategies.

Scinderin (SCIN), which is also referred to as adseverin, belongs to the gelsolin super family of actin binding proteins (4,5). SCIN was originally identified in chromaffin cells of the adrenal medulla (6), but is typically expressed in a variety of tissues. For instance, high expression of SCIN was observed in the kidneys and intestines (7), but was expressed at lower levels in other tissue types (6,8). Earlier studies indicated that SCIN serves an important role in the regulation of various cellular processes, including proliferation, migration and differentiation. In human prostate cancer and lung carcinoma cells, SCIN suppression inhibited cell proliferation (9,10). Conversely, in megakaryoblastic leukemia, SCIN overexpression inhibited cell proliferation and tumorigenesis via activating the ras-related C3 botulinum toxin substrate/p21-activated kinase/mammalian mitogen-activated protein kinase kinase, mitogen-activated protein kinase kinase 4/c-jun n-terminal kinase/c-jun, and rapidly accelerated fibrosarcoma kinase/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signaling pathways (11). In addition, manipulation of SCIN in gastric cancer cells has been linked to increased cell invasion and metastasis *in vivo* (12), and has also been revealed to be involved in the differentiation of dental pulp cells (13). However, the role of SCIN in BC is largely unknown.

Thus, in the present study, the biological role of SCIN in BC was investigated by analyzing its expression in BC tissue samples, as well as in control mammary fibroadenoma or fibroadenomatoid hyperplasia tissue samples. In addition, SCIN expression was ablated in MDA-MB-231 and T-47D BC cells using lentivirus (LV)-mediated short hairpin (sh)RNA technology to understand the functional

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consequences of knocking down SCIN expression. The results of the present study indicate that SCIN expression serves an important role in inhibiting proliferation and promoting apoptosis in BC cells.

Materials and methods

Selection of tumor specimens. This study was approved by the Ethics Committee of the Second People's Hospital of Shenzhen (Shenzhen, Guangdong, China), and written consent was obtained from all study participants. A total of 46 paraffin-embedded BC specimens, with a median age of 47.54 years (range, 28-64 years) and 21 paraffin-embedded control specimens, with a median age of 29.95 years (range, 17-45 years) collected from patients with mammary fibroadenoma or fibroadenomatoid hyperplasia were obtained from the Center for Breast Disease Diagnosis and Treatment at the Second People's Hospital of Shenzhen between January 2017 and June 2017. All patients were women. Among the 46 cases, 36 were invasive ductal carcinoma (IDC), 6 were ductal carcinoma *in situ* (DCIS) and 4 were mucoid carcinoma. All diagnoses and classifications were made according to the World Health Organization criteria (14). These resected specimens were 2-3 mm thick, then fixed in 10% neutral buffered formalin (pH 7.4) at room temperature for 12 h, embedded in paraffin, and cut into 4- μ m sections.

Cell culture. Human BC MDA-MB-231, T-47D and MCF-7 cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). These cells were cultured in Dulbecco's modified Eagle medium (DMEM; Corning Incorporated, Corning, NY, USA) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere with 5% CO₂.

Immunohistochemistry (IHC). Selected paraffin-embedded specimens were cut into 4- μ m sections and stained using the Dako REAL Envision Detection system (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) per the manufacturer's protocols. 4- μ m sections were dewaxed by xylene and rehydrated in a graded ethanol series (100% ethanol for 5 min, 95% alcohol for 5 min, 90% alcohol for 3 min, 80% alcohol for 3 min, 70% alcohol for 3 min at room temperature), following washing 3 times with Phosphate Buffer Solution (PBS) for 10 min at room temperature. Sections were boiled in 0.01 mol/l citrate buffer (pH 6.0, MVS-0066, Maixin Biotechnology Development Co., Ltd, Fuzhou, China) at 100°C for 4 min, then cooled at room temperature for 1 h for antigen retrieval. The endogenous peroxidase activity was blocked with 3% H₂O₂ for 10 min at room temperature, followed by washing 3 times with PBS for 10 min at room temperature. Next, slides were immersed in 5% goat serum (YJ0130, Yanjing Biological Co., Shanghai, China) for 10 min at room temperature to block nonspecific binding. Following removal of goat serum, the slides were incubated with primary rabbit anti-SCIN monoclonal antibody (1:800 dilution; cat. no. ab199723, Abcam, Cambridge, MA, USA) and mouse anti-Ki67 monoclonal antibody (1:100 dilution; cat. no. MAB-0129, MXB Bio-company, Fuzhou, China) at 4°C overnight. The next day, the slides were re-heated at

room temperature for 1 h. Then washed 3 times with PBS for 10 min in room temperature, the sections were then incubated with poly-horseradish peroxidase (HRP) anti-rabbit/mouse IgG (cat. no PV-9000, Beijing ZSGB-Bio Company, Beijing, China) for 30 min at room temperature. DAB staining was performed and later semi-quantitative evaluation of the immunohistochemical signal was performed by two pathologists working independently in a blinded fashion with an Olympus BX-51 microscope (Olympus Corporation, Tokyo, Japan) at x200 magnification (15). The staining intensity of SCIN expression was scored as follows: 0, Negative (no cytoplasmic SCIN expression); 1, weak (1-25% staining); 2, moderate (26-50% staining); 3, strong (51-75% staining); and 4, high (76-100%) staining. The staining scores were analyzed using the X-tile software program (v3.6.1; Yale University School of Medicine, New Haven, CT, USA), and a score of 2 was designated as the cutoff value (16). Thus, samples having total scores of ≥ 2 were classified as having high SCIN expression, while scores of < 2 represented no or low SCIN expression. Samples that exhibited $< 14\%$ Ki-67 (proliferation marker) expression were grouped as Ki-67 negative (17).

SCIN expression in breast cancer cells from GEO. The information about SCIN expression in BC cells was extracted from GEO (<https://www.ncbi.nlm.nih.gov/geo/>). The term 'breast cancer cells' was entered and searched in the GEO datasets, and eligible and high quality profiling expression data was identified, 'SCIN' gene was then searched in the GDS4296 datasets and for the expression of SCIN in BC cells.

Generation of LV expressing SCIN, shRNA and target cell transfection. Several shRNAs were designed and tested to target the human SCIN gene in preliminary studies (NM_033128). The shRNA sequence (5'-CGAGATGAGCTGACAACAT-3') that optimally ablated SCIN expression was selected for the present study. A random shRNA sequence (5'-TTCTCCGAACGTGTCACGT-3') was used as a control. These shRNAs were subsequently ligated into the GV115 lentiviral vector with enhanced green fluorescent protein (GFP) cDNA (GeneChem). To generate LV, MDA-MB-231 and T-47D cells were transfected with GV115-SCIN or control shRNA using X-tremeGENE HP DNA Transfection Reagent (cat no. 6366546001, Roche Diagnostics, Basel, Switzerland), along with two helper plasmids, pHelper 1.0 and pHelper 2.0 (GeneChem), per the manufacturer's protocols. At 2 days post-transfection, the supernatant containing packaged LV was collected and filtered through a 0.45- μ m filter. To infect MDA-MB-231 and T-47D cells, LV particles, at a multiplicity of transfection of 20, were added to the plated cells in 6-well plates and then cultured at 37°C in an incubator. A transfection efficiency of $\sim 80\%$ was observed via GFP expression using fluorescence microscopy following 3 days of LV transfection. Subsequent experimentations were performed 72 h following transfection.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from BC MDA-MB-231, T-47D and MCF-7 cell lines respectively using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cDNA was

retro-transcribed from isolated RNA using the M-MLV kit (cat no. M1705, Promega Corporation, Madison, WI, USA). The RT-qPCR reaction conditions were at 95°C for 15 sec, at 95°C for 5 sec, then at 60°C for 30 sec, and the amplification was for 45 cycles using the Real-Time PCR Detection system (cat no. MX3000p; Agilent Technologies, Inc.) with SYBR Premix Ex Taq (cat no. DRR041B, Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocols. The primer sequences for SCIN gene amplification were as follows: Forward, AGGAAGGTCTGAACTAAT and reverse, CTGTTACTTATGTCTGCTAT. The primer sequences for GAPDH amplification were as follows: Forward, TGACTTCAACAGCGACACCCA and reverse, CACCTGTGCTGTAGCCAAA. The relative mRNA expression levels were determined by the cycle threshold (Ct) normalized to GAPDH expression, using the $2^{-\Delta\Delta C_q}$ formula (18).

Western blot analysis. The knockdown efficiency of SCIN shRNA in BC cells transfected with GV115-SCIN shRNA or control shRNA LV was determined. At 2 days post-LV transfection, the cells were collected and lysed in radioimmunoprecipitation lysis buffer (BioTeke Corporation, Beijing, China). Protein concentration was measured using the BCA Protein assay kit (Beyotime Institute of Biotechnology, Haimen, China), and 20 μ g protein lysates in each pane were separated by 10% SDS-PAGE, followed by transfer to polyvinylidene difluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). Subsequently, the PVDF membranes were blocked in 5% skimmed milk diluted with TBST (cat no. BD232100, Solarbio Co, Beijing, China) for 1 h at room temperature. The membranes were then incubated with primary rabbit anti-SCIN monoclonal antibody (mAb) (80 kDa, 1:200; cat no. HPA020518, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and GAPDH mouse mAb (37 kDa, 1:1,000; cat no. SC-32233, Santa Cruz Biotechnology, Dallas TX) at 4°C overnight. Thereafter, the membranes were incubated with secondary goat anti-rabbit IgG (1:2,000; cat. no. sc-2004, Santa Cruz Biotechnology, Inc.) and goat anti-mouse IgG (1:2,000; cat. no. sc-2005, Santa Cruz Biotechnology, Inc.) antibodies for 1.5 h at room temperature, respectively. The protein bands were visualized using the Pierce™ ECL Western Blotting Substrate kit (Thermo Fisher Scientific, Inc.).

Cell Celigo analysis. LV infected MDA-MB-231 and T-47D cells were seeded into 96-well plates at a concentration of 2,000 cells/well in incubator with 4°C and 5% CO₂ for 3 days. The cell nuclei were stained with Hoechst (2.6 μ g/ml; Invitrogen; Thermo Fisher Scientific, Inc.) for 5 min at room temperature to quantify absolute cell number; green fluorescence-tagged shRNA was measured by a fluorescence microscope at x100 magnification to quantify cellular siRNA uptake. All analyses were performed using a Celigo cytometer (Nexcelom Bioscience, Lawrence, MA, USA) over a period of 5 days. Gross quantitative analysis for each fluorescence channel was performed, including total counts of gated events.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. BC cells were seeded into a 96-well plate (2,000 cells/well) 3 days after LV transfection. The MTT assay

was performed per the manufacturer's protocols (cat no. JT343; Gen-view, Beijing, China). Briefly, at the indicated time points (1, 2, 3, 4 and 5 days), the MTT solution (5 mg/ml) was added to the wells and the cells were further incubated at 37°C for 4 h. The formazan dye was dissolved with 100 μ l dimethyl sulfoxide at 37°C for 2-5 min. The optical density of the dye was measured using a microplate reader at 490 nm absorbance and 570 nm as the wavelength reference.

Caspase-3/7 activity assay. Caspase-3/7 activity was determined using the Caspase-Glo 3 and 7 kit (cat no. G8091, Promega Corporation), according to the manufacturer's protocols, following plating of transfected cells in 96-well plates (in triplicate) for 72 h.

Cell apoptosis. Cell apoptosis was analyzed using the allophycocyanin (APC)/annexin V kit (cat no. 88-8007, eBioscience; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. After 5 days of transfection, the cells were stained for 10-15 min in 100 μ l cell suspension buffer, containing 5 μ l annexin V-APC stain, at room temperature in the dark. Cell apoptosis was analyzed by flow cytometry (EMD Millipore).

Statistical analysis. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference. Association between SCIN expression and clinicopathological features of patients with BC, as well as RT-qPCR experiments, were evaluated using the χ^2 test. All other data sets were evaluated using Student's t-test and expressed as the mean \pm standard error of the mean.

Results

SCIN expression is increased in breast cancer tissues. The IHC results revealed that SCIN protein expression was elevated in BC tissue samples compared with that in control mammary fibroadenoma or fibroadenomatoid hyperplasia tissue samples (Fig. 1). SCIN expression in the cytoplasm of BC ductal epithelial hyperplasia cells was primarily detected with varying intensity (Fig. 1A-D). However, very weak or no SCIN expression was observed in mammary fibroadenoma or fibroadenomatoid hyperplasia tissue samples (Fig. 1E and F). Quantification of SCIN expression in the BC tissue samples demonstrated that 60.9% of the BC tissue samples (28/46) revealed high SCIN expression compared with 28.6% of the mammary fibroadenoma or fibroadenomatoid hyperplasia tissue samples (6/21) ($P = 0.014$) (Table I; Fig. 1).

Further analysis of the association between SCIN expression and clinicopathological features demonstrated no significant differences between high and low expression of SCIN. The proportion of high SCIN-expressing Ki-67-positive BC tissue samples (78.6%, 22/28) was higher compared with the Ki-67-negative BC tissue samples (21.4%, 6/28); however, this difference was not statistically significant (Table II). This observation may indicate that SCIN expression in BC tissue is associated with Ki-67 expression, suggesting that changes in SCIN expression could affect BC cell proliferation.

Table I. Comparison of SCIN expression in breast fibroadenoma and breast cancer tissues.

Type of tissue	n	SCIN expression		χ^2	P-value
		None/low, n (%)	High, n (%)		
Fibroadenoma of the breast	21	15 (71.4)	6 (28.6)	6.017	0.014
Breast cancer	46	18 (39.1)	28 (60.9)		

SCIN, scinderin.

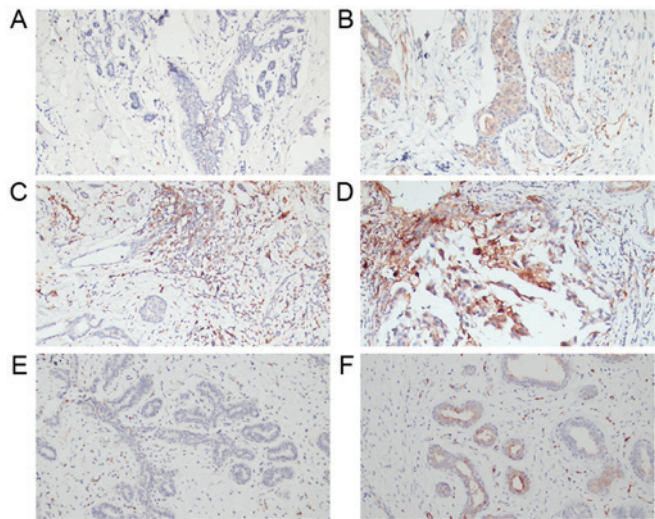


Figure 1. Immunohistochemistry analysis of SCIN expression in fibroadenoma and BC tissue samples. Representative images of (A) negative, (B) weak, (C) moderate and (D) strong SCIN staining in BC tissue, (E) negative and (F) and weak SCIN staining in fibroadenoma (original magnification, x200). SCIN, scinderin; BC, breast cancer.

SCIN is expressed in breast cancer cells. To investigate the functional contribution of SCIN in BC, the Gene Expression Omnibus database was initially utilized (GDS4296, Affymetrix Human Genome U133 Plus 2.0 Array; Affmetrix; Thermo Fisher Scientific, Inc. https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4296%3A22272_x_at&sortby=tissue) to determine SCIN levels in BC samples (19). Using this database, SCIN was revealed to be expressed in multiple BC cell lines, including BT-549, HS578T, MCF-7, MDA-MB-231 and T-47D. As presented in Fig. 2A, SCIN expression was higher in MCF-7 and T-47D cells. SCIN expression in BC cells was further validated using RT-qPCR (Fig. 2B). The MDA-MB-231 cell line is a highly invasive and metastatic, while T-47D and MCF-7 cell lines exhibit comparatively mild invasive and metastatic capabilities. Therefore, MDA-MB-231 and T-47D cells were selected to examine the effect of SCIN on different BC cells.

Suppressed SCIN expression inhibits breast cancer cell proliferation. In order to investigate the molecular function of SCIN in BC cells, SCIN expression was ablated using LV-mediated knockdown. Following 3 days of LV transfection, infection efficiency and good cell morphology were observed by fluorescent microscope with x200 magnification (Fig. 3A and B).

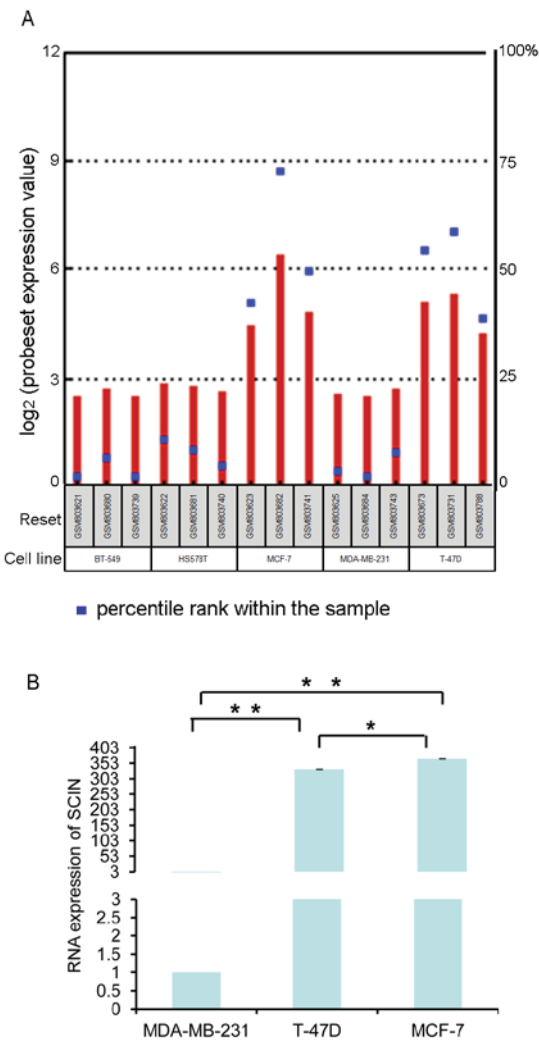


Figure 2. Assessment of SCIN expression using the GEO database and RT-qPCR. (A) Bioinformatics analysis of SCIN expression in five BC cell lines from the GEO database. (B) RT-qPCR analysis of SCIN expression in three BC cell lines; MDA-MB-231, T-47D and MCF-7. GAPDH expression was used as a control to normalize SCIN expression. SCIN, scinderin; BC, breast cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; GEO, Gene Expression Omnibus. **P<0.05, *P>0.05.

Then RT-qPCR analysis revealed that SCIN mRNA expression was significantly lower in MDA-MB-231 and T-47D cells in the shSCIN group compared with that in the shCtrl group (Fig. 3C and D). Similarly, a reduction in SCIN protein expression was confirmed in BC cells that were transfected with shSCIN (Fig. 3E and F).

Table II. Association between SCIN expression and clinicopathological features in patients with breast cancer.

Pathological variables	n	SCIN expression		χ^2	P-value
		None/low, n (%)	High, n (%)		
Pathological type				0.634	0.728
IDC	36	13 (36.1)	23 (63.9)		
DCIS	6	3 (50.0)	3 (50.0)		
Mucoid carcinoma	4	2 (50.0)	2 (50.0)		
ER				0.417	0.518
Negative	18	6 (33.3)	12 (66.7)		
Positive	28	12 (42.9)	16 (57.1)		
PR				0.225	0.635
Negative	25	9 (36.0)	16 (64.0)		
Positive	21	9 (42.9)	12 (57.1)		
HER2				0.004	0.949
Negative	36	14 (38.9)	22 (61.1)		
Positive	10	4 (40.0)	6 (60.0)		
Ki-67				0.004	0.949
Negative	10	4 (40.0)	6 (60.0)		
Positive	36	14 (38.9)	22 (61.1)		
Stage				0.48	0.488
\leq II	38	14 (36.8)	24 (63.2)		
\geq III	8	4 (50.0)	4 (50.0)		
Molecular characteristics				4.036	0.258
Luminal A	7	5 (71.4)	2 (28.6)		
Luminal B	21	7 (33.3)	14 (66.7)		
HER2 overexpression	10	4 (40.0)	6 (60.0)		
Triple-negative	8	2 (25.0)	6 (75.0)		

Ki-67 expression: $\leq 14\%$, Ki-67 negative staining; and $\geq 15\%$, Ki-67 positive staining. IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; SCIN, scinderin; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor.

Next, proliferation was assessed in MDA-MB-231 and T-47D cells using the *in vitro* Celigo assay 3 days after shSCIN or shCtrl transfection. A marked reduction in proliferation of MDA-MB-231 and T-47D cells was identified (Fig. 3G-L). A separate experiment using the MTT assay confirmed that cellular metabolic activity was decreased in the MDA-MB-231 when silencing SCIN expression (Fig. 3M and N). The lower cellular metabolic activity was also examined in the T-47D cells with knockdown of SCIN expression (Fig. 3O and P). Thus, these results indicate that SCIN-knockdown inhibits BC cell proliferation and cellular metabolic activity.

SCIN-knockdown induces cell apoptosis. To further investigate the mechanism by which SCIN-knockdown reduces cell proliferation, Caspase-3/7 activity in MDA-MB-231 and T-47D cells was analyzed following 72 h of LV transfection of shSCIN and shCtrl. SCIN-knockdown significantly enhanced Caspase-3/7 activity in the two cell types ($P < 0.01$; Fig. 4A and B). Flow cytometric analysis of cell apoptosis was also performed. As presented in Fig. 4C-F, apoptosis was significantly increased in the MDA-MB-231 and T-47D cells in the shSCIN group ($P < 0.01$) following 5 days of LV transfection.

Discussion

The majority of cases of BC-associated mortality occur due to metastasis, for which cell proliferation is the biggest contributor. Cell proliferation is directly and indirectly associated with BC prognosis (20). Multiple studies evaluating the role of numerous proliferation genes have added to the current understanding of BC metastasis (21-23), but the functional contribution of SCIN expression in BC proliferation has been relatively under-reported. Thus, in the present study, the role of SCIN in BC proliferation and apoptosis was investigated. The results demonstrated that SCIN expression is increased in BC cells at the protein (IHC) and mRNA (RT-qPCR) levels. Furthermore, knockdown of SCIN expression in MDA-MB-231 and T-47D cells, with LV-mediated gene silencing technology, suggested that SCIN expression regulates cell proliferation and apoptosis. Inhibition of proliferation and induction of apoptosis were observed in MDA-MB-231 and T-47D cells following SCIN-knockdown.

Uncontrolled cell proliferation is a central hallmark of all types of cancer. Since the actin cytoskeleton serves a prominent role in cancer cell cycle regulation (24), a number of cytoskeleton-associated proteins have been studied in reference

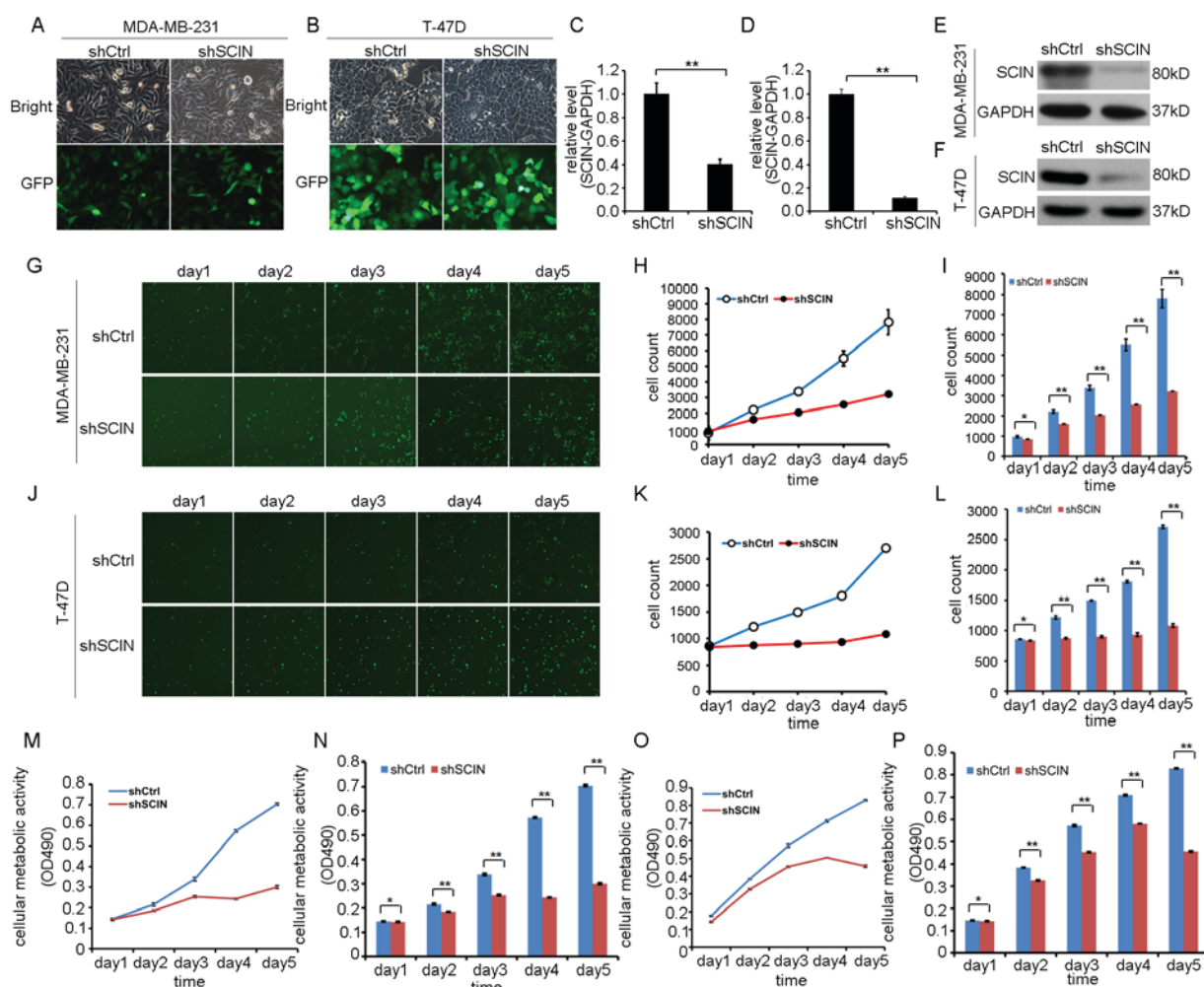


Figure 3. Knockdown of SCIN expression by lentivirus-mediated shRNA and its effect on cell proliferation. Representative images of SCIN-knockdown in (A) MDA-MB-231 and (B) T-47D cells, assessed by GFP expression using fluorescence microscopy (x200 magnification). SCIN RNA expression following knockdown in (C) MDA-MB-231 and (D) T-47D cells, assessed by reverse transcription-quantitative polymerase chain reaction analysis. GAPDH expression was used as an internal control. Data are presented as mean values \pm standard error of the mean of three independent experiments. ** $P < 0.01$ compared with the shCtrl group. Western blot analysis of SCIN-knockdown in (E) MDA-MB-231 and (F) T-47D cells. Representative blots of three separate experiments. (G) Representative images of Celigo assay (x100 magnification), (H) cumulative data of Celigo assay, and (I) bar graphs indicate the mean cell count values in MDA-MB-231 cells transfected with shCtrl or shSCIN were illustrated. (J) Representative images of Celigo assay (x100 magnification), (K) cumulative data of Celigo assay, and (L) bar graphs indicate the mean cell count values in T-47D cells transfected with shCtrl or shSCIN were presented. Cellular metabolic activity, assessed with MTT assay, of (M) MDA-MB-231 and (O) T-47D cells. Bar graphs indicate the mean cellular metabolic activity values in the MDA-MB-231 (N) and T-47D cells (P). All data represented are mean values \pm standard error of the mean from four independent experiments. Student's t-test was used for statistical analysis. ** $P < 0.01$. * $P > 0.05$ vs. control, SCIN, scinderin; BC, breast cancer; shCtrl, short hairpin control; GFP, green fluorescent protein; OD, optical density.

to BC (25-27). SCIN contains six gelsolin-like domains, three actin-binding sites and two calcium-binding sites (6,28). Previous studies have linked abnormal SCIN expression to various carcinomas, as well as to cell proliferation. In the present study, SCIN expression was revealed to be associated with Ki-67-positive BC cells in tissue samples. Although not significant, this trend does indicate that there may be an association between SCIN and Ki-67 expression in BC tissues. Since Ki-67 is a cellular marker of proliferation, the results of the current study indicate that SCIN may also be involved in the proliferation of BC cells. Consistent with this conclusion, SCIN-knockdown was revealed to inhibit breast carcinoma cell proliferation *in vitro* in the present study. This observation was in accordance with studies by Liu *et al* (10) and Wang *et al* (9), who revealed that SCIN overexpression enhanced human lung and prostate carcinoma cell proliferation. Therefore, as SCIN governs filamentous actin

(F-actin) cytoskeleton remodeling, it is possible that SCIN's effects on cell proliferation may be mediated through F-actin. This hypothesis requires further analysis, for example, observing whether the F-actin cytoskeleton is changed when SCIN expression is silenced in BC cells. Further, animal experiments should be performed to validate the proliferative ability of SCIN.

Apoptosis can significantly contribute to normal cell physiology. Apoptosis maintains absolute cell numbers, affects wound healing and regulates remodeling (29). Abnormalities in apoptosis lead to numerous diseases, including cancer, in which the normal mechanisms of cell cycle regulation become dysfunctional (30). Inducing apoptosis during carcinogenesis can contribute to the treatment and prevention of cancer development and progression (31,32). In the current study, SCIN-knockdown induced cell apoptosis. Therefore SCIN may serve an important role in the development and progression of BC.

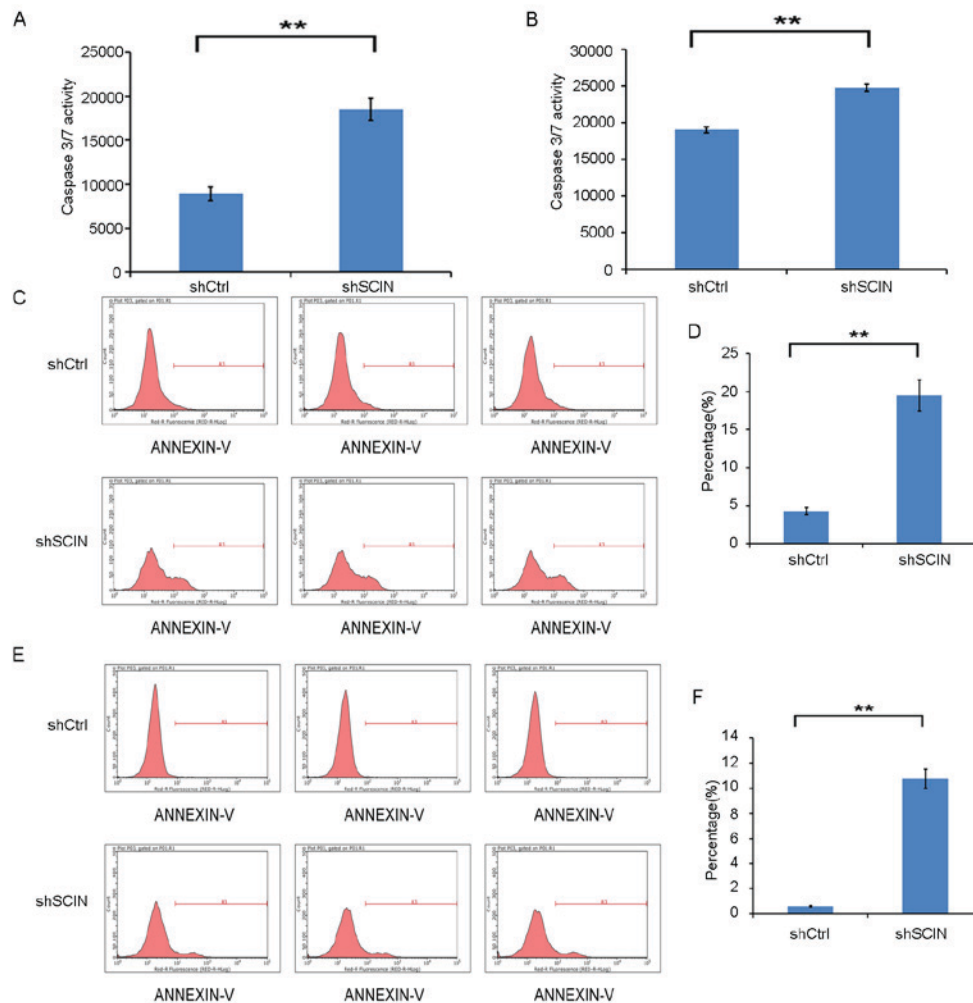


Figure 4. Effects of SCIN-knockdown on cell apoptosis. Caspase 3/7 activity in (A) MDA-MB-231 and (B) T-47D cells 3 days following shRNA transfection. (C) Cumulative data of annexin-V staining analyzed by flow cytometry in MDA-MB-231 cells transfected with shCtrl or shSCIN and (D) its representative histograms were presented. (E) Cumulative data of annexin-V staining analyzed by flow cytometry in T-47D cells transfected with shCtrl or shSCIN and (F) its representative histograms were presented. All data are presented as mean values \pm standard error of the mean from four independent experiments. Student's t-test was used for statistical analysis. ** $P < 0.01$. shCtrl, short hairpin control; SCIN, scinderin.

In summary, the results of the current study demonstrated that shRNA-mediated SCIN-knockdown inhibited breast cancer cell proliferation and induced apoptosis. This sheds new light on the novel role of SCIN in BC development and progression.

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Availability of data and materials

All data used during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WJ designed the study and wrote the manuscript. XZ, JW and YL performed the experiments. CH analyzed data. XW and RL also contributed to study design, data analysis and modified the manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Second People's Hospital of Shenzhen, and written consent was obtained from all study participants.

Patient consent for publication

Patients provided written consent for the publication of data and any associated images.

Competing interests

The authors declare that they have no competing interests.

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