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ORIGINAL ARTICLE

Silencing of syndecan-binding protein enhances the inhibitory effect of tamoxifen and increases cellular sensitivity to estrogen

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ABSTRACT

Objective: Tamoxifen is used as a complementary treatment for estrogen receptor (ER)-positive breast cancer (BCa), but many patients developed resistance. The aim of this study was to examine the role of syndecan-binding protein (SDCBP) silencing in ER-positive BCa cells.

Methods: In MCF-7/T47D cells, the effects of SDCBP silence/overexpression on cell proliferation and estrogenic response were examined. Cell proliferation was examined using the MTT assay and cell cycle regulators were examined by Western blot. Estrogen response was examined from a luciferase activity and evaluation of transcript levels of pS2 and progesterone receptor (PR) upon estrogen administration. Samples of ER-positive BCa were stained with ERα, PR, and SDCBP antibodies, and their expression correlations were analyzed.

Results: We found that SDCBP silencing inhibited the proliferation of ER-positive BCa cells and arrested a greater number of cells in the G1 phase of the cell cycle compared to tamoxifen alone, while SDCBP overexpression limited the anti-cancer effects of tamoxifen. SDCBP silencing and overexpression also enhanced and attenuated the estrogenic response, respectively. Expression of SDCBP was negatively correlated with PR, ERα, and the PR/ERα ratio in ER-positive BCa tissue samples.

Conclusions: SDCBP may be involved in tamoxifen resistance in ER-positive BCa. Tamoxifen treatment combined with SDCBP silencing may provide a novel treatment for endocrine therapy-resistant BCa.

KEYWORDS Syndecan-binding protein (SDCBP); tamoxifen; breast cancer; endocrine-therapy resistance

Introduction

Breast cancer (BCa) is a heterogeneous disease, and approximately 75% of all BCa cases show overexpression of estrogen receptors (ER) and/or progesterone receptors (PR)[1]. The estrogen pathway affects the expression of hundreds of genes involved in proliferation, differentiation, survival, invasion, metastasis, and angiogenesis, all of which are particularly relevant to cancer[2].

Apart from surgery, endocrine therapy is considered a complementary treatment in most patients and has shown consistent clinical benefits, particularly for ER-positive patients with respect to inducing tumor remission[3]. Among all endocrine therapies, tamoxifen is the most extensively used drug and functions as a selective ER modulator[4] that competitively blocks estrogen binding[5]. However, many breast tumors show either primary resistance to endocrine therapies or develop secondary resistance after initial responsiveness[6-10]. Approximately 20%–30% of patients who received adjuvant tamoxifen experienced relapse, and most patients with advanced disease who showed an initially positive response to tamoxifen eventually experienced disease progression[11]. The mechanism of this resistance involves cross-talk between ER and alternative signaling pathways involved in cell survival and proliferation, such as those for epidermal growth factor receptor and human epidermal growth factor receptor 2[12-15].

Melanoma differentiation-associated gene 9 was discovered through screening of differentially expressed genes upon treatment of melanoma[16]. This protein, also known as syntenin, interacts with syndecan family members and is therefore also known as syndecan-binding protein

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(SDCBP). The syndecan family belongs to a group of cell surface molecules and is involved in cell–cell and cell–matrix adhesion. SDCBP has a total of 298 amino acids and contains two PDZ domains, PDZ-1 (amino acids 110–193) and PDZ-2 (amino acids 194–274)[17]. The PDZ domain is found in a family of proteins that controls diverse and central physiologic processes such as migration and lipid binding[18-20]. Through cross-talk with protein kinase C alpha via adhesion-mediated activation downstream of the fibronectin signal, SDCBP activates focal adhesion kinase to take part in cellular migration and invasive BCa development[21]. Moreover, activation of integrin β1 and extracellular signal-related kinase 1/2 was shown to be required for syntenin-mediated migration and invasion of BCa cells[22].

Our previous study showed that SDCBP expression was positively correlated with histologic grade and tumor staging, but negatively correlated with ERα expression. In ER-negative BCa cells, SDCBP silencing increased cell populations in G1 phase of the cell cycle and resulted in up-regulation of p21 and p27 while down-regulating cyclin E, thereby arresting the cell cycle and prohibiting cell proliferation[23]. In the present study, we examined the effects of SDCBP on ER- positive BCa cells. To determine the role of SDCBP expression in ER-positive BCa development and whether SDCBP down-regulation can be used as a targeted treatment, we evaluated the expression profile of SDCBP in ER-positive cases. Using the RNAi technique, we analyzed the mechanisms underlying the involvement of SDCBP in ER-positive BCa development and its correlation with the estrogen-signaling pathway as well as its impact on endocrine therapy.

Material and methods

Sample collection

ER-positive breast tissue samples (n = 99) were obtained from patients who underwent surgical excision at the Department of Breast Cancer Pathology and Research Laboratory at Tianjin Medical University Cancer Institute and Hospital (China) from January to March of 2010. These samples were used in our previous study[23].

Immunohistochemistry

Staining of ERα, PR, and SDCBP was performed as described in our previous publication[23]. **Table S1** lists information regarding the antibodies used.

The expression levels of ERα, PR, and SDCBP were semi-quantified using a modified scoring system, where the intensity score (0 = negative; 1 = low; 2 = medium; 3 = high) was multiplied by the percentage of cells that were stained. This scoring system gives a final score ranging from 0 to 300. In the presence of cytoplasmic staining, SDCBP status was classified according to this modified scoring system: negative (0–50), weak (51–100), moderate (101–200), or strong (201–300). ERα and PR status were categorized in the same manner as SDCBP signals in the presence of nuclear staining. All cases were evaluated by two pathologists independently and any discrepancy was resolved by group discussion. The PR/ERα ratio was calculated as the PR staining score/ERα staining score. The correlation between SDCBP status and pathologic features were analyzed using a non-parametric Spearman correlation test.

Cell culture

The human BCa cell lines MCF-7 and T47D were purchased from American Type Culture Collection (ATCC® HTB-22™ and ATCC® HTB-133 respectively, Manassas, VA, USA).

To deplete estrogen, cells were cultured in phenol red-free RPMI 1640 containing 2.5% HyClone Charcoal/Dextran-Treated Fetal Bovine Serum (SH30068.03, Thermo Scientific, Waltham, MA, USA) for 24 h. Next, 17-β estradiol (E2, Sigma-Aldrich, St. Louis, MO, USA) in ethanol was added to the culture medium at a final concentration of 0, 0.1, 1, or 10 nM, and the cells were cultured for another 24 h. Tamoxifen was purchased from Sigma and added to the culture medium at a final concentration of 2 μM.

Real-time quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA extraction was performed as previously reported[23]. Primers for pS2, PR, and SDCBP are listed in **Table S2** and β-actin was used as an internal control. The real-time qRT-PCR assay was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). pS2 and PR mRNA transcription levels were normalized against β-actin mRNA expression.

Establishment of SDCBP- silenced MCF-7 cells

The shRNA used to silence SDCBP and negative control shRNA are listed in **Table S3** [both were designed by Genepharma Co., Ltd (Shanghai, China)]. The procedures for screening the SDCBP-silenced stable MCF-7 cell line were

performed as previously reported[23]. Subcultures showing maximal SDCBP silencing were designated as "MCF-7 shRNA", while control shRNA-transfected subcultures were designated as "MCF-7 NC".

SDCBP-overexpression BCa cell line construction

SDCBP-overexpressing and control cell lines were constructed as described previously[24]. Corresponding exogenous protein overexpression was evaluated by Western blot after the cells were cultured for 8 and 6 weeks for MCF-7 and T47D cells, respectively, in the appropriate medium containing 0.5 mg/mL of G418 (Sigma-Aldrich).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The MTT assay was performed as previously reported[23], except that MCF-7 and T47D cells were seeded at 2,000 and 1,500 cells per well in a 96-well plate, respectively.

Flow cytometric cell-cycle analysis

Cell-cycle analysis was performed on a BD FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) as described previously[25].

Western blot assay

Holoproteins in cell lysates were extracted, quantified, and immunoblotted as previously described[23]. The information and usage of antibodies are listed in **Table S1**. Protein expression levels were quantified using intensities normalized to β-actin. The expression levels calculated from three repeated immunoblots of all groups followed a normal distribution and were presented as the mean ± standard deviation. Student's *t*-test was used to examine differences between groups.

Luciferase assay

Cells were co-transfected with the ERα luciferase reporter plasmid PGMER-Lu (Genomeditech Co., Ltd., Shanghai, China) and wide-type Renilla luciferase reporter gene control plasmid pGMR-TK in 24-well plates. Luciferase activities in cell lysates were measured using the Dual-Luciferase Reporter Assay in triplicate and normalized to Renilla luciferase activity. pGM-CMV-Lu-transfected cells were used as positive controls and the average relative luciferase activity of transfected MCF-7 NC/MCF-7 Neo/T47D Neo cells was defined as "1". Student's *t* test was used to examine the differences between these normally distributed groups.

Results

Silenced/overexpressed SDCBP influences the effects of tamoxifen on BCa proliferation

As shown in **Figure 1A**, SDCBP shRNA silenced most target proteins compared to MCF-7 NC in either the presence or absence of tamoxifen. However, SDCBP silencing alone did not affect cellular proliferation in the absence of tamoxifen, but rather enhanced the suppressive effect of tamoxifen (**Figure 1B**). Although SDCBP silencing did not affect MCF-7 cell-cycle kinetics in the absence of tamoxifen, it consistently contributed to the arrest of more cells in G1 in the presence of tamoxifen ($P < 0.001$, **Figure 1C**).

As shown in **Figure 1D** and **1G**, SDCBP was significantly overexpressed in both MCF-7 and T47D cells; SDCBP overexpression accelerated cellular proliferation in both the absence and presence of tamoxifen in both cell lines. Under conditions of SDCBP overexpression, the effect of tamoxifen on cell proliferation was significantly attenuated (**Figure 1E** and **1H**). Accordingly, in both cell lines, SDCBP overexpression reduced cells in G1 phase in both the absence and presence of tamoxifen and weakened the effects of tamoxifen on the cell cycle (**Figure 1F** and **1I**).

Effects of SDCBP silencing/overexpressing on cell-cycle regulators in MCF-7 cells in the presence of tamoxifen

In ER-positive MCF-7 cells, tamoxifen treatment alone significantly increased p21 levels but attenuated the levels of phosphorylated Rb and cyclin D1. However, SDCBP silencing alone did not influence levels of p21, p27, cyclin D1, cyclin E, or phosphorylated Rb. In contrast, SDCBP silencing significantly up-regulated the levels of p21 and p27, but down-regulated the levels of phosphorylated Rb and cyclin E beyond that of tamoxifen alone. However, SDCBP silencing failed to further decrease cyclin D1 compared to tamoxifen alone (**Figure 2A**–**2G**). SDCBP overexpression alone did not influence p21 levels, but significantly down-regulated p27. Tamoxifen treatment did not recover the levels of p27, but up-regulated the levels of p21 under conditions of SDCBP overexpression (**Figure 2H**–**2K**).

Effects of SDCBP silencing/overexpression on estrogen responsiveness in ER-positive BCa cell line

The luciferase assay suggested that SDCBP silencing enhanced the estrogenic response when E2 was administrated at concentrations between 0.1 and 10 nM compared to MCF-7 NC counterparts (P = 0.017, P = 0.020 and P = 0.002, respectively) (**Figure 3A**). qRT-PCR evaluation showed that SDCBP silencing up-regulated pS2 and PR by 40.0% and

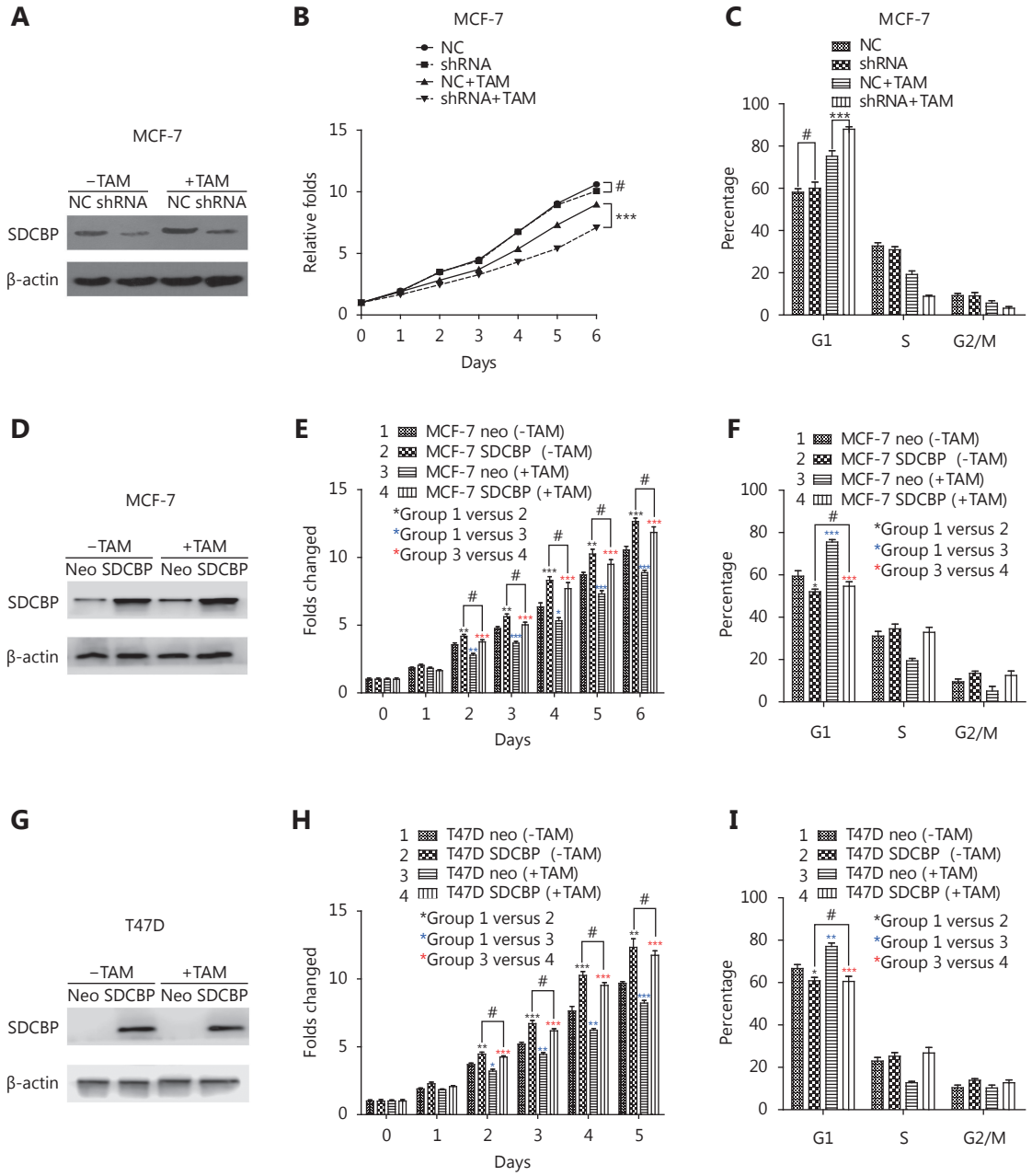

Figure 1 Effect of tamoxifen treatment and SDCBP silencing/overexpressing on proliferation of ER-positive breast cancer cells. (A, D, G) Expression of SDCBP in the absence or presence of 2 μM tamoxifen as shown by Western blot assay. β-actin was used as an internal reference. (B, E, H) Proliferation was examined by the MTT assay. (C, F, I) Cell-cycle progression was determined by flow-cytometric cell-cycle analysis in the absence or presence of 2 μM tamoxifen. Student's *t*-test was then used to compare differences (#P > 0.05, *P < 0.05, **P < 0.01 and ***P < 0.001).

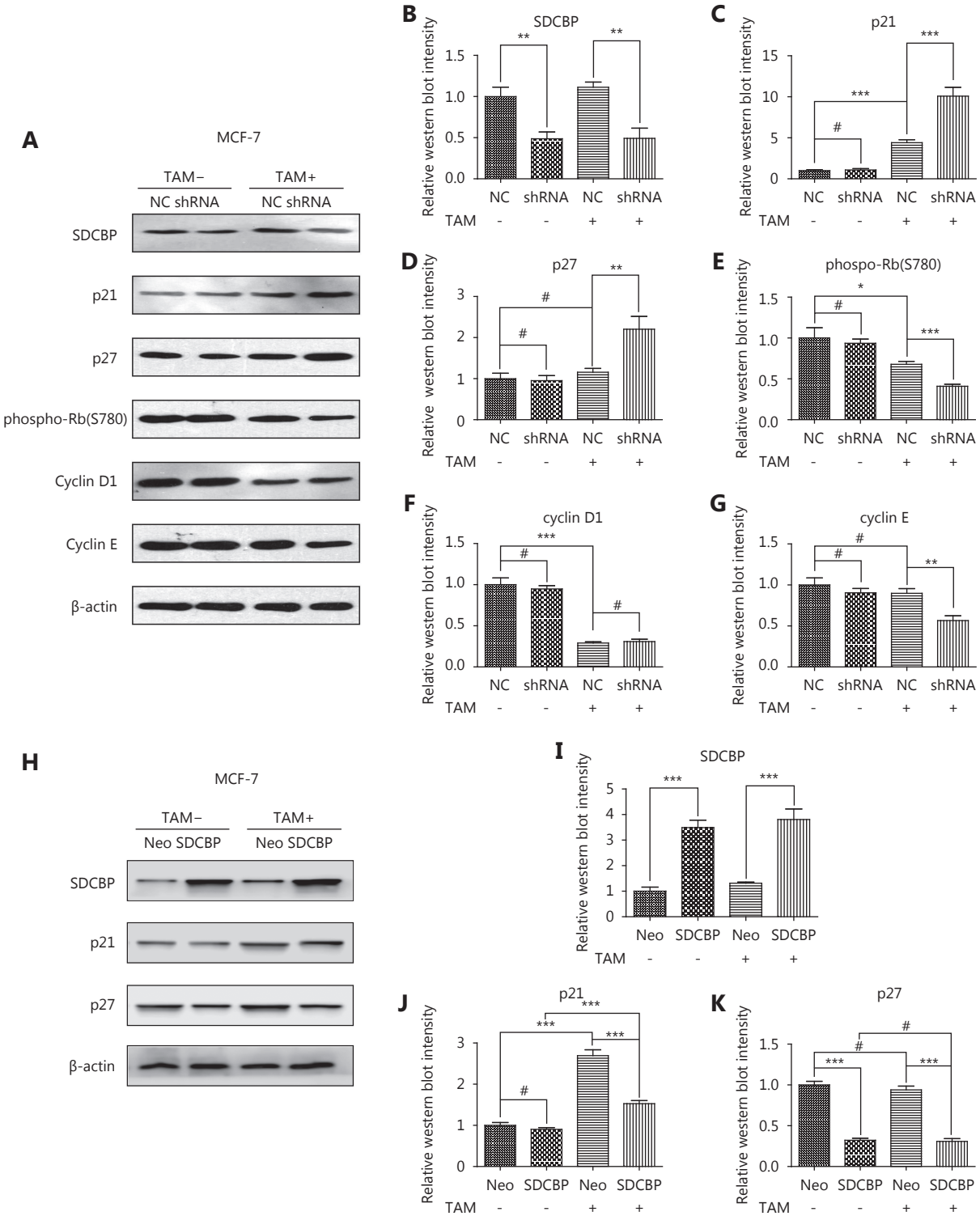

Figure 2 Effect of tamoxifen treatment and SDCBP silencing/overexpression on G1/S cell-cycle regulators. Western blot was conducted to examine the differential expression of p21, p27, phosphorylated Rb (phospho-Rb), cyclin D1, and cyclin E levels in MCF-7 cells with 2 μM tamoxifen treatment and/or SDCBP silencing (A)/overexpression (H). The ratios of Western blot intensities for the examined proteins to β-actin were calculated from triplicate experiments (B-G, I-K); Student's *t*-test was then used to compare differences (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, #*P* > 0.05).

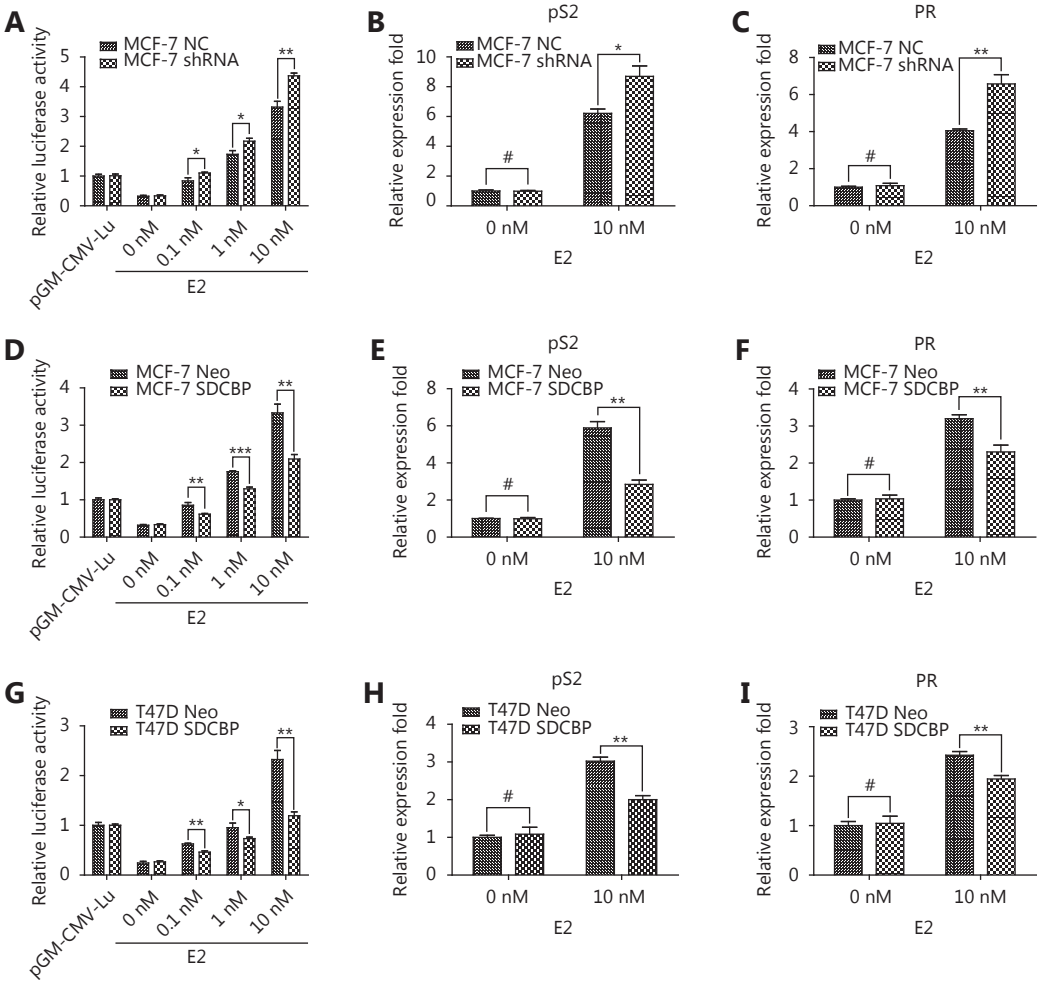

Figure 3 Effects of SDCBP silencing/overexpression on estrogenic responses. (A, D, G) Effects of SDCBP silencing/overexpression on expression of estrogen-response reporter genes after administration of 0, 0.1, 1, or 10 nM E2 were examined using the dual luciferase assay. pGM-CMV-Lu-transfected cells were used as positive controls and the average relative luciferase activity of transfected MCF-7 NC/MCF-7 Neo/T47D Neo cells was defined as "1"; Student's *t*-test was used to compare differences. Quantitative analysis of pS2 (B, E, H) and PR (C, F, I) transcription levels in MCF-7 shRNA/MCF-7 SDCBP/T47D SDCBP or MCF-7 NC/MCF-7 Neo/T47D Neo cells under steroid hormone deprivation or 10 nM E2 stimulation was performed by real-time quantitative reverse transcription-PCR. Transcription levels were normalized against β-actin. Each experiment was repeated three times and Student's *t*-test was used to compare differences (*P < 0.05, **P < 0.01, ***P < 0.001, #P > 0.05).

62.3% at the mRNA level, respectively, compared to those in MCF-7 NC cells incubated with 10 nM E2 (P = 0.026 and P = 0.0011, respectively) (**Figure 3B** and **3C**). The enhanced effect of SDCBP silencing on pS2 and PR transcription depended upon the presence of estrogen, as transcriptional levels were unaffected in the absence of estrogen (P = 0.847 and P = 0.413, respectively).

In contrast, the luciferase assay suggested that SDCBP overexpression in MCF-7 or T47D cells attenuated the estrogenic response compared to their MCF-7 and T47D Neo counterparts, respectively (**Figure 3D** and **3G**). qRT-PCR showed that SDCBP overexpression resulted in down-regulation of pS2 and PR in MCF-7 cells (51.6% and 28.1%, respectively) (**Figure 3E** and **3F**) and T47D cells (33.7% and 19.8%, respectively) (**Figure 3H** and **3I**) when incubated with 10 nM E2.

Clinical pathologic characters of ER-positive BCa cases and their correlations with SDCBP expression

Correlations between pathologic characters and SDCBP

expression were examined in ER-positive BCa tissues (n = 99). Among PR-negative tumors, 26.3% (5/19) demonstrated strong SDCBP staining (**Table 1**), while no tumors staining strongly positive for PR (0/26) showed strong positive staining for SDCBP (**Table 1**). Negative correlations between SDCBP expression and PR status or the PR/ERα ratio were also established (R_S = –0.37, P < 0.001; and R_S = –0.24, P = 0.017, respectively) (**Figure 4** and **Table 1**). This experiment also showed that SDCBP expression was negatively correlated with ERα (R_S = –0.29, P = 0.004) (**Table 1**). There were no significant differences among the different levels of SDCBP staining in lymph node involvement and pTNM stage (**Table 1**). Detailed information for each case is shown in **Table S4**.

Discussion

Tamoxifen is the most commonly used chemotherapeutic agent for patients with ER-positive BCa[26], and tamoxifen resistance poses great challenges to BCa treatment. Some patients have presented with intrinsic resistance regardless of showing high levels of ER, while other patients initially respond to tamoxifen but later develop acquired resistance[27].

Our previous study showed that expression of SDCBP can be attenuated by estrogen[23]; in the present study, we found that silencing of SDCBP enhances the inhibitory effect of tamoxifen with regard to cellular proliferation and cell-cycle progression in ER/PR-positive MCF-7 cells. This indicates that SDCBP drives cell proliferation and cell-cycle progression by up-regulating self-expression and activating alternative signaling pathways when estrogen signaling is inhibited. Under conditions of SDCBP overexpression, the function of tamoxifen on cell proliferation was significantly attenuated, suggesting that SDCBP overexpression leads to tamoxifen resistance in ER-positive BCa. Notably, SDCBP silencing alone did not affect cell proliferation or the expression of molecules that control the cell cycle in ER-positive MCF-7 cells; however, SDCBP overexpression

Table 1 Correlation of SDCBP expression with pathologic features in ER-positive breast cancers

Pathological features	Cases	Syndecan binding protein (%)				r_s	P*
		Negative	Weak	Moderate	Strong		
Lymph node status						0.08	0.441
Negative	52	15 (28.8)	24 (46.2)	8 (15.4)	5 (9.6)		
Positive	47	12 (25.5)	18 (38.3)	14 (29.8)	3 (6.4)		
pTNM stage						0.17	0.101
Ⅰ	33	10 (30.3)	14 (42.4)	5 (15.2)	4 (12.1)		
Ⅱ	47	14 (29.8)	24 (51.1)	8 (17.0)	1 (2.1)		
Ⅲ–Ⅳ	19	3 (15.8)	4 (21.1)	9 (47.4)	3 (15.8)		
PR status						–0.37	<0.001
Negative	19	2 (10.5)	5 (26.3)	7 (36.8)	5 (26.3)		
Weak	15	4 (26.7)	4 (26.7)	5 (33.3)	2 (13.3)		
Moderate	39	13 (33.3)	17 (43.6)	8 (20.5)	1 (2.6)		
Strong	26	8 (30.8)	16 (61.5)	2 (7.7)	0 (0.0)		
ERα status						–0.29	0.004
Weak	30	7 (23.3)	8 (26.7)	8 (26.7)	7 (23.3)		
Moderate	24	5 (20.8)	11 (45.8)	7 (29.2)	1 (4.2)		
Strong	45	15 (33.3)	23 (51.1)	7 (15.6)	0 (0.0)		
PR/ERα ratio[a]						–0.24	0.017
Cases	99	27	42	22	8		
		0.94 (0.72)	0.92 (0.60)	0.65 (1.02)	0.29 (0.85)		

*, P values were calculated by Spearman's rank-correlation test (n = 99).
[a], PR/ERα ratio: represented by median (inter-quartile range), i.e. M (QR).

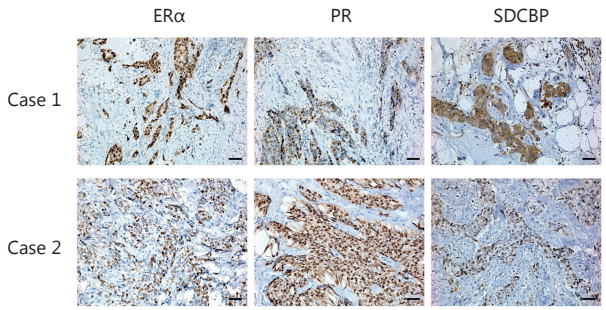

Figure 4 ERα, PR, and SDCBP expression in ER-positive breast cancer tissue. Case 1: the sample was stained with high ERα and SDCBP but low PR (H&E staining, 200 ×, respectively). Case 2: the sample was stained with moderate ERα and low SDCBP but high PR (H&E staining, 200 ×, respectively). Scale bar = 50 μm.

accelerated cellular proliferation in both the absence and presence of tamoxifen. This indicates that ER signaling counteracts some of the SDCBP-signaling lost in malignancy development.

In MCF-7 cells, under estrogen-signaling pathway activation, cellular proliferation and cell-cycle progression (including G1/S checkpoint-related regulators) showed no obvious changes when SDCBP expression was silenced by RNA interference. It has been reported that activated estrogen signaling can accelerate cell-cycle progression by limiting p21 expression, increasing phosphorylation levels of Rb protein, and promoting cyclin D1 expression[28], all of which are consistent with our results. In contrast, SDCBP silencing further up-regulated the expression of p21 and p27 while down-regulating phosphorylation levels of Rb and expression of cyclin E in MCF-7 cells in the presence of tamoxifen, similar to the effects of SDCBP silencing alone in triple-negative BCa cell lines. Interestingly, SDCBP silencing can either exert its effect in the presence of tamoxifen (such as by promoting p27 expression and inhibiting cyclin E expression) or augment the effect of tamoxifen (such as by further promoting p21 expression and inhibiting phosphorylation levels of Rb), but SDCBP silencing does not change cyclin D1 expression markedly beyond that of tamoxifen alone. SDCBP overexpressing alone did not influence the levels of p21 but significantly down-regulated p27. Tamoxifen treatment did not recover the levels of p27, but up-regulated the levels of p21 under conditions of SDCBP overexpression. This indicates that when the estrogen signal is restrained, SDCBP not only partially substitutes for the estrogen signal, but also is involved in some other regulating mechanism(s) of cell-cycle progression. In the absence of tamoxifen, because SDCBP silencing up-regulates the estrogenic response as shown in **Figure 3**, the up-regulation tendency of p21 and p27 may be counteracted by a larger estrogenic response; however, in the absence of tamoxifen, this counteraction was eliminated. This may partly explain why SDCBP silencing alone did not alter the levels of p21 and p27, while these levels were changed with tamoxifen. We also predicted that p21 levels depend more on the estrogen pathway than on SDCBP in ER-positive BCa cells; however, p27 levels may be closely related to the interaction between SDCBP and c-src as previously reported in triple-negative breast cancer cells[24]. In addition, SDCBP silencing enhances the effects of tamoxifen and may be useful as a targeted treatment in ER-positive BCa.

PR is an ER-regulated gene that mediates the effects of progesterone on the development of both the normal mammary gland and BCa[29]. Compared to ER/PR double-positive BCa, patients with BCa who are ER-positive but PR-negative suffered a poorer prognosis and were more prone to developing resistance against endocrine treatment[30-32]. The 21-gene recurrence score assay (Oncotype DX®) is a multigene assay used to predict the recurrence of tamoxifen-treated, node-negative BCa. In this scoring system, the ER group score is negatively correlated with cancer recurrence, and PR carries even more weight than ER in the ER group score[33]. The pS2 gene was originally identified as an estrogen-inducible transcript in the human BCa cell line MCF-7 and was shown to be a direct target of ERs[34,35]. Our study showed that SDCBP silencing enhanced the estrogen response of MCF-7 cells and further elevated the expression levels of PR and pS2 in response to estrogen, while its overexpression had the opposite effect in both MCF-7 and T47D cells, indicating a role for SDCBP in suppressing estrogenic responses. As indicated by immunohistochemistry analysis, the expression level of SDCBP was negatively correlated with PR status and the PR/ERα ratio. This supports that SDCBP negatively regulates the estrogenic response and may play an important role in developing resistance to endocrine treatment in ER-positive BCa. These results also suggest that SDCBP silencing can be applied as a targeted treatment in ER-positive BCa.

In conclusion, SDCBP promotes cell cycle progression in ER-positive BCa, particularly when the estrogen-signaling pathway is blocked. It also negatively regulates the estrogen response in ER-positive BCa, but its underlying molecular mechanism of action and related signaling pathway(s) remain unclear. Silencing of SDCBP or its downstream signal(s) may improve the therapeutic effect of endocrine treatment in ER-positive BCa, particularly in cases of primary or secondary resistance.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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Table S1 Detailed information for antibodies used in this work.

Name	Type	Supplier	Catalog #	Origin	Dilution ratio	
					For WB*	For IHC**
Cyclin D1 Antibody (H-295)	Rabbit polyclonal	Santa Cruz Biotechnology, Inc.	sc-753	Dallas, TX, U.S.A.	1:1000	
Cyclin E Antibody (M-20)	Rabbit polyclonal	Santa Cruz Biotechnology, Inc.	sc-481	Dallas, TX, U.S.A.	1:1000	
β-Actin Antibody (C4)	mouse monoclonal	Santa Cruz Biotechnology, Inc.	sc-47778	Dallas, TX, U.S.A.	1:1500	
p27 Kip1 (D69C12)	Rabbit monoclonal	Cell Signaling Technology, Inc.	#3686	Danvers, MA, U.S.A	1:1000	
p21 Waf1/Cip1 (12D1)	Rabbit monoclonal	Cell Signaling Technology, Inc.	#2947	Danvers, MA, U.S.A	1:1000	
ERα antibody (6F11)	mouse monoclonal	Thermo Fisher Scientific Inc.	MA5-13304	MA, U.S.A		1:50
PR antibody (SP2)	Rabbit monoclonal	Thermo Fisher Scientific Inc.	MA5-14505	MA, U.S.A		1:50
Syntenin-1 Antibody (N-20)	goat polyclonal	Santa Cruz Biotechnology, Inc.	sc-19379	Dallas, TX, U.S.A	1:500	1:75
Phospo-Rb (S780)	goat polyclonal	Santa Cruz Biotechnology, Inc.	sc-12901	Dallas, TX, U.S.A	1:500	

*, Westernblot**, Immunohistochemistry

Table S2 Primers for semi-quantitative and real-time quantitative reverse transcription-PCR.

Official symbol of target gene	Genebank No.	Amplified fragment length (bp)	Annealing temperature (°C)	Primer name	Sequence (from 5′ to 3′)
pS2	X00474	356	56.2	pS2 foward	CATGGAGAACAAGGTGATCTG
				pS2 reverse	CAGAAGCGTGTCTGAGGTGTC
PR	NM_001278456	320	55.6	PR foward	CCATGTGGCAGATCCCACAGGAGTT
				PR reverse	TGGAAATTCAACACTCAGTGCCCGG
β-actin	NM_001101	194	55.9	β-actin forward	GTCACCAACTGGGACGACAT
				β-actin reverse	AGCACAGCCTGGATAGCAAC

Table S3 The target sequence for short-hairpin RNA design of syndecan-binding protein (SDCBP)

Target site	Target sequence (from 5′ to 3′)
Negative control	AATTCTCCGAACGTGTCACGT
611#	GGGACCAAGTACTTCAGATCA

#: The numbers represents the position of the 5′ starting site of target sequences in syndecan binding protein mRNA (NM_001007067).

Table S4 Detailed features of 99 consecutive cases of estrogen receptor (ER)-positive breast cancer from January to March of 2010.

SDCBP status	PR scoring	PR status	ERα scoring	ERα status	PR/ER ratio	Lymph node status	pTNM staging
Negative	160	Moderate	55	Weak	2.91	Negative	I
Negative	65	Weak	60	Weak	1.08	Positive	II
Negative	150	Moderate	70	Weak	2.14	Negative	II
Negative	30	Negative	80	Weak	0.38	Negative	I
Negative	80	Weak	85	Weak	0.94	Positive	II
Negative	20	Negative	90	Weak	0.22	Positive	II
Negative	120	Moderate	100	Weak	1.2	Positive	II
Negative	270	Strong	135	Moderate	2	Positive	II
Negative	180	Moderate	140	Moderate	1.29	Negative	I
Negative	130	Moderate	150	Moderate	0.87	Negative	II
Negative	270	Strong	160	Moderate	1.69	Positive	III–IV
Negative	75	Weak	165	Moderate	0.45	Negative	I
Negative	270	Strong	210	Strong	1.29	Negative	I
Negative	270	Strong	210	Strong	1.29	Negative	II
Negative	180	Moderate	210	Strong	0.86	Positive	II
Negative	140	Moderate	210	Strong	0.67	Negative	I
Negative	120	Moderate	210	Strong	0.57	Negative	II
Negative	120	Moderate	210	Strong	0.57	Positive	II
Negative	270	Strong	225	Strong	1.2	Negative	II
Negative	240	Strong	225	Strong	1.07	Negative	I
Negative	120	Moderate	225	Strong	0.53	Positive	III–IV
Negative	95	Weak	225	Strong	0.42	Negative	I
Negative	240	Strong	240	Strong	1	Negative	I
Negative	190	Moderate	240	Strong	0.79	Positive	II
Negative	160	Moderate	255	Strong	0.63	Positive	II
Negative	270	Strong	285	Strong	0.95	Positive	III–IV
Negative	160	Moderate	285	Strong	0.56	Negative	I
Weak	165	Moderate	55	Weak	3	Negative	II
Weak	120	Moderate	55	Weak	2.18	Positive	II
Weak	140	Moderate	55	Weak	2.55	Negative	II
Weak	110	Moderate	60	Weak	1.83	Positive	II
Weak	0	Negative	75	Weak	0	Negative	I
Weak	90	Weak	80	Weak	1.13	Positive	II
Weak	25	Negative	80	Weak	0.31	Positive	II
Weak	55	Weak	90	Weak	0.61	Positive	II
Weak	70	Weak	110	Moderate	0.64	Negative	I
Weak	270	Strong	120	Moderate	2.25	Negative	II
Weak	60	Weak	125	Moderate	0.48	Negative	I

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Weak	255	Strong	135	Moderate	1.89	Positive	II
Weak	160	Moderate	135	Moderate	1.19	Negative	I
Weak	210	Strong	140	Moderate	1.5	Negative	I
Weak	180	Moderate	150	Moderate	1.2	Negative	II
Weak	50	Negative	150	Moderate	0.33	Positive	III–IV
Weak	225	Strong	160	Moderate	1.41	Negative	II
Weak	40	Negative	165	Moderate	0.24	Negative	II
Weak	120	Moderate	180	Moderate	0.67	Negative	I
Weak	270	Strong	210	Strong	1.29	Positive	III–IV
Weak	150	Moderate	210	Strong	0.71	Positive	II
Weak	240	Strong	210	Strong	1.14	Negative	I
Weak	0	Negative	210	Strong	0	Positive	III–IV
Weak	270	Strong	225	Strong	1.2	Positive	II
Weak	255	Strong	225	Strong	1.13	Positive	II
Weak	180	Moderate	225	Strong	0.8	Negative	I
Weak	150	Moderate	225	Strong	0.67	Positive	II
Weak	255	Strong	240	Strong	1.06	Positive	II
Weak	240	Strong	240	Strong	1	Positive	II
Weak	180	Moderate	240	Strong	0.75	Negative	I
Weak	180	Moderate	240	Strong	0.75	Negative	I
Weak	140	Moderate	240	Strong	0.58	Positive	II
Weak	240	Strong	255	Strong	0.94	Negative	II
Weak	140	Moderate	255	Strong	0.55	Negative	II
Weak	285	Strong	270	Strong	1.06	Negative	I
Weak	240	Strong	270	Strong	0.89	Negative	II
Weak	180	Moderate	270	Strong	0.67	Positive	II
Weak	160	Moderate	270	Strong	0.59	Positive	III–IV
Weak	285	Strong	285	Strong	1	Negative	II
Weak	210	Strong	285	Strong	0.74	Negative	I
Weak	130	Moderate	285	Strong	0.46	Negative	I
Weak	270	Strong	285	Strong	0.95	Negative	I
Moderate	40	Negative	60	Weak	0.67	Negative	II
Moderate	40	Negative	60	Weak	0.67	Negative	I
Moderate	0	Negative	60	Weak	0	Negative	I
Moderate	160	Moderate	75	Weak	2.13	Positive	II
Moderate	100	Weak	75	Weak	1.33	Positive	II
Moderate	20	Negative	80	Weak	0.25	Positive	III–IV
Moderate	0	Negative	85	Weak	0	Positive	III–IV

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Moderate	120	Moderate	90	Weak	1.33	Positive	III–IV
Moderate	120	Moderate	105	Moderate	1.14	Positive	III–IV
Moderate	150	Moderate	120	Moderate	1.25	Negative	II
Moderate	210	Strong	150	Moderate	1.4	Positive	III–IV
Moderate	90	Weak	150	Moderate	0.6	Positive	III–IV
Moderate	120	Moderate	160	Moderate	0.75	Positive	II
Moderate	0	Negative	165	Moderate	0	Negative	I
Moderate	270	Strong	180	Moderate	1.5	Positive	II
Moderate	90	Weak	210	Strong	0.43	Positive	III–IV
Moderate	80	Weak	225	Strong	0.36	Positive	II
Moderate	40	Negative	225	Strong	0.18	Positive	III–IV
Moderate	150	Moderate	240	Strong	0.63	Negative	I
Moderate	60	Weak	255	Strong	0.24	Positive	III–IV
Moderate	180	Moderate	270	Strong	0.67	Negative	II
Moderate	160	Moderate	285	Strong	0.56	Negative	I
Strong	120	Moderate	55	Weak	2.18	Negative	I
Strong	0	Negative	55	Weak	0	Negative	I
Strong	20	Negative	60	Weak	0.33	Positive	III–IV
Strong	65	Weak	65	Weak	1	Negative	I
Strong	0	Negative	75	Weak	0	Positive	III–IV
Strong	20	Negative	80	Weak	0.25	Negative	I
Strong	10	Negative	95	Weak	0.11	Negative	II
Strong	60	Weak	120	Moderate	0.5	Positive	III–IV