STEAP1 Inhibits Breast Cancer Metastasis and Is Associated With Epithelial–Mesenchymal Transition Procession

Jie Xie,[1] Yan Yang,[2] Jiali Sun,[1] Zhi Jiao,[1] Haozheng Zhang,[3] Jie Chen[1]

Abstract

To evaluate the role of 6-transmembrane epithelial antigen of prostate 1 (STEAP1) in breast cancer, we tested the expressions of STEAP1 in breast cancer and normal tissues and cells, as well as the functions of STEAP1 in breast cancer cell invasion and proliferation. Results revealed that STEAP1 was down-regulated in breast cancer, inhibited metastasis of breast cancer, and hampered the levels of epithelial–mesenchymal transition markers.

Purpose: Six-transmembrane epithelial antigen of prostate 1 (STEAP1) is a cell surface antigen overexpressed in multiple cancers and is associated with malignancy and disease prognosis. The aims of this study were to evaluate STEAP1 expression in breast cancer and to determine the mechanisms involved. **Methods:** STEAP1 expression was compared in normal breast tissue (n = 40), benign fibroadenoma (n = 52), and primary breast cancer (n = 211) using immunohistochemistry. Quantitative real-time polymerase chain reaction, Western blot analysis, and immunocytochemistry were used to evaluate STEAP1 expression in 3 breast cancer cell lines and in a normal mammary epithelial cell line. STEAP1 expression and its prognostic value in breast cancer were verified using the Oncomine and Kaplan-Meier Plotter databases. Transfection of cells to up-regulate or knock down STEAP1 expression was used to determine the effect of STEAP1 on cell invasion and proliferation, and to evaluate its relationship to epithelial–mesenchymal transition (EMT) progression. **Results:** STEAP1 expression was lower in breast cancers cells, and low expression was associated with a malignant phenotype and poor prognosis. Analysis of public databases supported our conclusions. Knockdown of STEAP1 expression enhanced cellular invasion and migration abilities, increased expression of EMT-related genes *MMP2*, *MMP9*, *MMP13*, *VIM*, and *CDH2*, and decreased *CDH1* expression. Enhanced STEAP1 expression significantly inhibited cellular invasion and migration abilities, decreased expression of the EMT-related genes, and increased *CDH1* expression. Up-regulation or knockdown of STEAP1 had little effect on cellular proliferation. **Conclusion:** STEAP1 was down-regulated in breast cancer, inhibited metastasis of breast cancer, and hampered the levels of EMT markers, which thus implicated STEAP1 in the suppression of EMT.

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Introduction

Breast cancer is a highly heterogeneous disease and is the second leading cause of death for women worldwide, accounting for 25% of all new cancer cases in women.[1,2] Previous research has revealed that breast cancer is a multistage and complex process that is influenced by many factors, including age, genetics, environment, lifestyle, and birth-relevant factors.[3,4] Because of the complex histologic and molecular subtypes of breast cancers, intervention still often results in unsatisfactory outcomes.

General breast cancer diagnostics are based on the expression of therapy-predictive biomarkers, such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. In addition, there are biomarkers that may be used to predict therapy response and cancer recurrence, such as those analyzed by the Oncotype DX test.[5] Prognosis for breast cancer patients currently relies primarily on classical clinicopathologic features, such as tumor size, histologic grade, and lymph node metastases.[6] However, it

[1]Department of Maternal and Child Health, School of Public Health
[2]Health Examination Center, Qilu Hospital, Shandong University, Jinan, China
[3]Research Institute of Pediatrics, Qilu Children's Hospital of Shandong University, Shandong University, Jinan, China

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Address for correspondence: Jie Chen, PhD, Department of Maternal and Child health, School of Public Health, Shandong University, Jinan, 250012, China
E-mail contact: 77chenjie@sdu.edu.cn

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remains a challenge to accurately diagnose and predict disease outcome on the basis of these parameters. The initiation and promotion of cancers are driven by changes at the genomic level; therefore, gene expression can provide important prognostic or predictive information.[7] Early diagnostic markers as well as middle and late stage treatment targets have gradually developed to become a focus of study.

The human 6-transmembrane epithelial antigen of prostate (STEAP) family are cell surface molecules with 6 transmembrane domains; it consists of STEAP1, STEAP2, STEAP3, and STEAP4.[8] Recent studies have revealed that it participates in intercellular communication through molecular trafficking, serves as channel or transporter proteins, and potentially functions in cell adhesion.[9] STEAP1 is a cell surface antigen expressed primarily in human prostate tissues, and in multiple cancers, including prostate, Ewing, bladder, colon, and lung cancers, it is barely expressed in neuronal tissues and is restrictively expressed in nonneuronal tissues.[10-13]

As a membrane-bound channel protein, STEAP1 is possibly involved in tumor intercellular communication by mediating the transfer of small molecules between adjacent cells.[14] Experiments demonstrate that high expression levels of sodium ion channels can promote the invasiveness of cancers in vitro.[15,16] Because of its specific membrane-bound localization and its high expression levels in cancers, STEAP1 is considered to be a tumor-associated antigen. Recent studies have shown STEAP1 to be an effective antigen for T-cell–based immunotherapy.[17,18] In addition, antibody immunotherapy based on STEAP1 has been used in the treatment of prostate cancer, including in STEAP1-specific antibody therapy and in the preparation of a cancer vaccine.[19] Monoclonal antibodies inhibit STEAP1-mediated intercellular transport in vitro, and they exhibit significant efficacy in inhibiting the growth of prostate and bladder tumors in vivo.[20] Roles for STEAP1 in tumor malignancy remain unconfirmed; however, STEAP1 may represent a potential target for therapeutic strategies.

With respect to breast cancer, studies have rarely provided evidence of a role for STEAP1. The aims of the current study were to evaluate STEAP1 expression in breast cancer and to determine any mechanism in which STEAP1 may be involved.

Methods

Cell Lines

The human breast cancer cell lines MCF-7, BT549, and MDA-MB-468, and the normal mammary epithelial cell line HBL-100 were used in this study; they were all purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All cells were cultured in Dulbecco modified Eagle medium (DMEM)/F-12 medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% antibiotics (penicillin–streptomycin solution; Gibco), and maintained at 37°C and 5% CO_2.

Tumor Tissues Samples

After informed consent was obtained from the patients, breast tissue specimens were collected at Qianfo Hill Hospital of Shandong province between 2001 and 2016. All specimens were reviewed by 2 pathologists. A total of 303 specimens were obtained and included 40 samples of normal breast tissue, 52 of breast fibroadenoma, and 211 of breast cancer. All patients with breast cancer were diagnosed according to the tumor, node, metastasis staging system (TNM) and had not received preoperative chemotherapy or any other treatment before the time of tissue collection. The breast cancer specimens included 23 classified as stage I, 58 cases classified as stage II, 76 cases classified as stage III, and 54 cases classified as stage IV disease. All patients received standard follow-up. At the end of December 2016, a total of 8 patients had been lost to follow-up, and 25 patients had died. This study was approved by the institutional medical ethics committee of Shandong University. All methods were performed in accordance with all relevant guidelines and regulations.

Immunohistochemistry

Immunohistochemistry was performed on 4 μm sections of formalin-fixed, paraffin-embedded tissues. All tissue sections were deparaffinized in xylene and rehydrated by passing the samples through graded alcohols. Antigen retrieval was performed in a steam pressure cooker at 125°C for 30 seconds in citrate buffer. The tissue was blocked with normal goat serum. The slides incubated with mouse anti-STEAP1 antibody (1:400 dilution, ab207914; Abcam) overnight at 4°C, then incubated for 30 minutes with biotin-conjugated anti-mouse secondary antibody. Finally, staining development was achieved by incubation with the enzyme substrate 3′,3-diaminobenzidine tetrahydrochloride (Sigma-Aldrich). On the basis of staining intensity and the percentage of positively stained cells, STEAP1 immunoreactivity was scored. The staining intensity was classified as 0 to 2 (0 = negative staining, 1 = low staining intensity, 2 = high staining intensity). The percentage of positively stained cells was scored as 0 (no cells staining positive), 1 (up to 25% of cells staining positive), 2 (25%-50% of cells staining positive), and 3 (> 50% of the cells staining positive). Final scores were derived by multiplying the score for the percentage of stained cells by the score for staining intensity. The final score values were grouped according to the demonstration of low (scores 0-3) or high (scores 4-6) immunoreactivity.

Total RNA extraction and Real-Time Quantitative PCR

Total RNA was extracted from the 4 cell lines using the RNAiso Plus extraction reagent (Takara). For the generation of complementary DNA, 2 μg total RNA was used as template in a reaction using a PrimeScript RT reagent Kit with genomic DNA Eraser (Takara). Real-time quantitative PCR (qPCR) analysis was performed using a LightCycler 480 system (Roche) according to the manufacturer's instructions. The qPCR mixture volume was 20 μL and included 10 μL TB Green Premix Ex Taq II (Takara), 2 μL complementary DNA, 0.8 μL forward primer (10 μM), 0.8 μL reverse prime (10 μM), and 6.4 μL sterilized water. The specific primers were designed and synthesized by Takara. The primers are listed in Supplemental Table 1. The relative gene expression levels were analyzed using the $\Delta\Delta C_t$ method.

Immunocytochemistry

Cultured cell lines were treated with trypsin and centrifuged to recover the cells. The cells were suspended and seeded into 24-well plates, then coverslipped. The cells were cultured in DMEM/F-12 medium with 10% FBS at 37°C, 5% CO_2. After 24 hours, the cells attached to the coverslips were subjected to streptavidin–biotin–peroxidase complex–based immunocytochemistry staining of STEAP1. The expression level of STEAP1 was

determined according to the intensity of staining and the percentage of stained cells. The classification method was as described for immunohistochemistry.

Western Blot Analysis

Cells were lysed on ice using radioimmunoprecipitation assay (RIPA) lysis buffer with phenylmethylsulfonyl fluoride (PMSF) as a serine protease inhibitor (RIPA:PMSF = 100:1). Protein samples (20 μg) were separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, then transferred to polyvinylidene difluoride membranes. The membranes were blocked for 1 hour with Tris-buffered saline with Tween 20 containing 5% bovine serum albumin, and then the membranes were incubated with a mouse anti-STEAP1 primary antibody (ab207914) or with anti−β-actin primary antibody, each at a 1:2000 dilution, overnight at 4°C. The membranes were then incubated with the corresponding secondary antibody at room temperature for 1 hour. Finally, positive labeling of the proteins on the membranes was visualized by enhanced chemiluminescence using a Bio-Rad ECL kit (Solarbio) and imaged with the ChemiDoc XRS+ Imaging System (Bio-Rad).

Analysis of Publicly Available Databases

Publicly available gene expression data were obtained from the Oncomine database to compare the expression of STEAP1 between normal breast tissues and breast cancers. In addition, gene expression and clinicopathologic information available in the Kaplan-Meier Plotter database (http://kmplot.com/analysis/) was used for analysis of the relationship between STEAP1 expression and the prognosis for breast cancer patients.

Knockdown and Up-Expression of STEAP1 *Gene*

STEAP1 knockdown and up-expression vectors, as well as a negative control, were purchased from GeneChem. The transfections were performed according to the manufacturer's recommendations at a multiplicity of infection of 100. After 12 hours, the transfection mixture was replaced with DMEM/F-12 medium with 10% FBS in order to avoid cell toxicity. The small interfering RNA sequence for STEAP1 was 5′-AGAAGACGATTATTTGCAT-3′. Transfection efficiency was monitored by fluorescence microscopy after 72 hours and confirmed by qPCR and Western blot analyses.

Boyden Chamber Migration and Invasion Assay

For Boyden chamber migration assays, cultured cells were suspended in 200 μL serum-free DMEM/F-12 medium and seeded into the upper Boyden chamber with 8.0 μm pore size membranes (BD Biosciences). For the invasion assays, cell suspensions in serum-free medium were placed into the upper chamber and covered with 50 μL Matrigel (BD Biosciences). After 24 hours, the nonmigrated or noninvaded cells in the upper membrane chamber were removed. The cells in the lower chamber were fixed with 4% paraformaldehyde, stained with crystal violet, and quantitated by counting the cells from 5 microscopic fields in each quadrant per membrane. Each experiment was performed in triplicate.

Cell Cycle Assay

Cells were collected and washed with phosphate-buffered saline (PBS) 3 times, fixed with 75% ethanol at −20°C for 5 hours, and then washed with PBS. The fixed cells were treated with RNase and stained with propidium iodide in the dark for 30 minutes, and analyzed by flow cytometry using a Muse Cell Analyzer (Merck Millipore).

Growth Curve

Cells were seeded into 24-well plates at 1×10^4 cells per well. The cells were cultured for 7 days and counted each day. All culture conditions were performed in duplicate for 3 separate time points. Growth curves were generated according to the number of cells counted for each day.

Statistical Analysis

Statistical analyses were performed by SPSS 17.0 software (IBM). Pearson chi-square or Fisher exact tests were used to compare differences in the proportions of STEAP1 expression levels between groups. The quantitative data were assessed by ANOVA. $P < .05$ was considered statistically significant. The differences in survival between the groups were estimated by the Kaplan-Meier method and the log-rank test.

Results

Expression of STEAP1 in Breast Cancer

Immunohistochemistry showed that the staining of STEAP1 was mainly concentrated in the cytoplasm and cell membrane (Figure 1A). STEAP1 expression was positive in 11.8% (25/211) of the breast cancer cases, which was statistically lower than the 85% (34/40) positivity observed for the cases of normal breast tissue, and 84.6% (44/52) in the fibroadenoma cases ($\chi^2 = 151.88$, $P < .001$). Patient characteristics according to STEAP1 status are listed in Table 1. STEAP1 expression demonstrated a significant negative correlation with TNM ($P = .001$), tumor grade ($P = .023$), and lymph node involvement ($P = .01$). Factors independent of STEAP1 expression included patient age, pathologic type, estrogen receptor status, and progesterone receptor status ($P > .05$). Kaplan-Meier analysis was used to detect an association between STEAP1 expression and patient outcome. The results showed that patients with high STEAP1 expression had a better prognosis (Figure 1B); thus, high STEAP1 expression was associated with good clinicopathologic features and a good prognosis for patients with breast cancer.

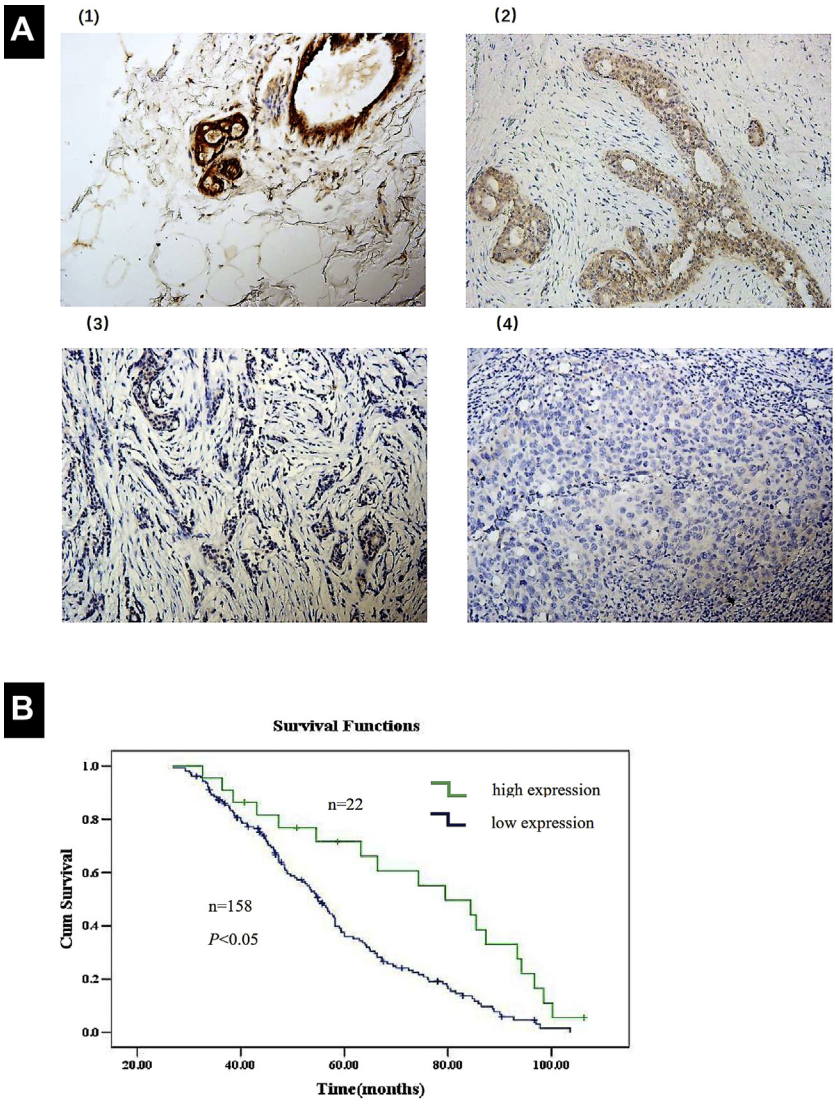
Expression of STEAP1 in Mammary Epithelial Cells and Breast Cancer Cell Lines

qPCR showed that the expression of STEAP1 messenger RNA (mRNA) in mammary epithelial cells (HBL-100) was significantly higher than that expression in breast cancer cell lines (BT549, MDA-MB-468, and MCF-7). The differences were statistically significant ($P < .05$; Figure 2A). Western blot and immunocytochemical analyses showed that the expression of STEAP1 protein in the mammary epithelial cell line HBL-100 was increased relative to the STEAP1 protein levels in breast cancer cell lines MDA-MB-468, MCF-7, and BT549 (Figure 2B and C).

Analysis of Publicly Available Databases

Analysis of the Oncomine database showed that STEAP1 mRNA levels were down-regulated in breast cancer compared to

Figure 1 Expression of STEAP1 in Breast Tissue and Kaplan-Meier Analysis. (A) STEAP1 Expression in Breast Tissue: (1) Normal Breast Tissue, (2) Fibroadenoma Tissue, (3) Breast Cancer Tissue (High Differentiation), and (4) Breast Cancer Tissue (Low Differentiation) (Original Magnification, ×200). (B) Kaplan-Meier Analysis of 2 Groups, Classified According to STEAP1 Expression



Abbreviation: STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

the levels in normal breast tissues reported in the Cancer Genome Atlas (TCGA) and Curtis databases (Figure 3A; Supplemental Table 2). Analysis using the publicly available Kaplan-Meier Plotter also confirmed our result that higher expression of STEAP1 correlated with better outcome (Figure 3B), with a hazard ratio of 0.86 (0.77-0.96) and a log-rank test demonstrating statistical significance ($P = .006$).

Determination of STEAP1 Transfection Efficiencies

Transfections using lentiviruses were performed to increase STEAP1 expression in the breast cancer cell line MCF-7 and to decrease STEAP1 expression in the normal mammary epithelial cell line HBL-100. The efficiencies of transfection were determined using fluorescence microscopy (Figure 4A). Western blot and qPCR analyses confirmed that HBL-100 cells transfected with STEAP1 short hairpin RNA to silence gene expression had significantly lower expression levels of STEAP1 at both the transcription and protein levels compared to the negative controls and nontransfected HBL-100 cells. Western blot and qPCR analyses also confirmed that lentivirus construct LV-STEAP1—transfected MCF-7 cells had significantly higher expression levels of STEAP1 than did negative controls and nontransfected MCF-7 cells at both the mRNA and protein levels (Figure 4B and C).

Table 1 Relationship Between Expression of STEAP1 and Clinicopathologic Parameters

Characteristic	N	STEAP1, N (%)		χ^2	*P*
		High	Low		
Age, y					
<50	59	10 (16.9)	49 (83.1)	0.710	.400
≥50	152	19 (12.5)	133 (87.5)		
Node Status					
N0	72	16 (22.2)	56 (77.8)	6.627	.010
N+	139	13 (9.4)	126 (90.6)		
TNM					
I-II	81	19 (23.5)	62 (76.5)	10.462	.001
III-IV	130	10 (7.7)	120 (92.3)		
Pathologic Type					
Nonspecific invasive breast carcinoma	104	13 (12.5)	91 (87.5)	5.274	.607
Invasive lobular breast carcinoma	76	9 (11.8)	67 (81.2)		
Medullary carcinoma	13	2 (15.4)	11 (84.6)		
Mucinous carcinoma	9	3 (33.3)	6 (66.7)		
Tubular carcinoma	9	2 (22.2)	7 (77.8)		
Histologic Grade					
I	65	15 (23.1)	50 (76.9)	7.508	.023
II	77	9 (11.7)	68 (88.3)		
III	69	5 (7.2)	64 (92.8)		
Molecular Subtype					
Basal-like	21	4 (19.0)	17 (81.0)	1.366	.714
Luminal A	46	5 (10.9)	41 (89.1)		
Luminal B	119	18 (15.1)	101 (84.9)		
HER2 positive	25	2 (8.0)	23 (92.0)		
ER					
Positive	130	16 (12.3)	114 (87.7)	.589	.443
Negative	81	13 (16.0)	68 (84.0)		
PR					
Positive	159	21 (13.2)	138 (86.8)	.157	.692
Negative	52	8 (15.4)	44 (84.6)		

Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor; STEAP1 = 6-transmembrane epithelial antigen of prostate 1; TNM = tumor, node, metastasis classification system.

Effects of STEAP1 on Breast Cancer Cell Migration and Invasion Abilities

The migration and invasion assays showed that increased expression of STEAP1 inhibited the invasion and migration abilities of breast cancer cells, while knockdown of STEAP1 expression enhanced the invasion and migration abilities of normal mammary epithelial cells (Figure 5).

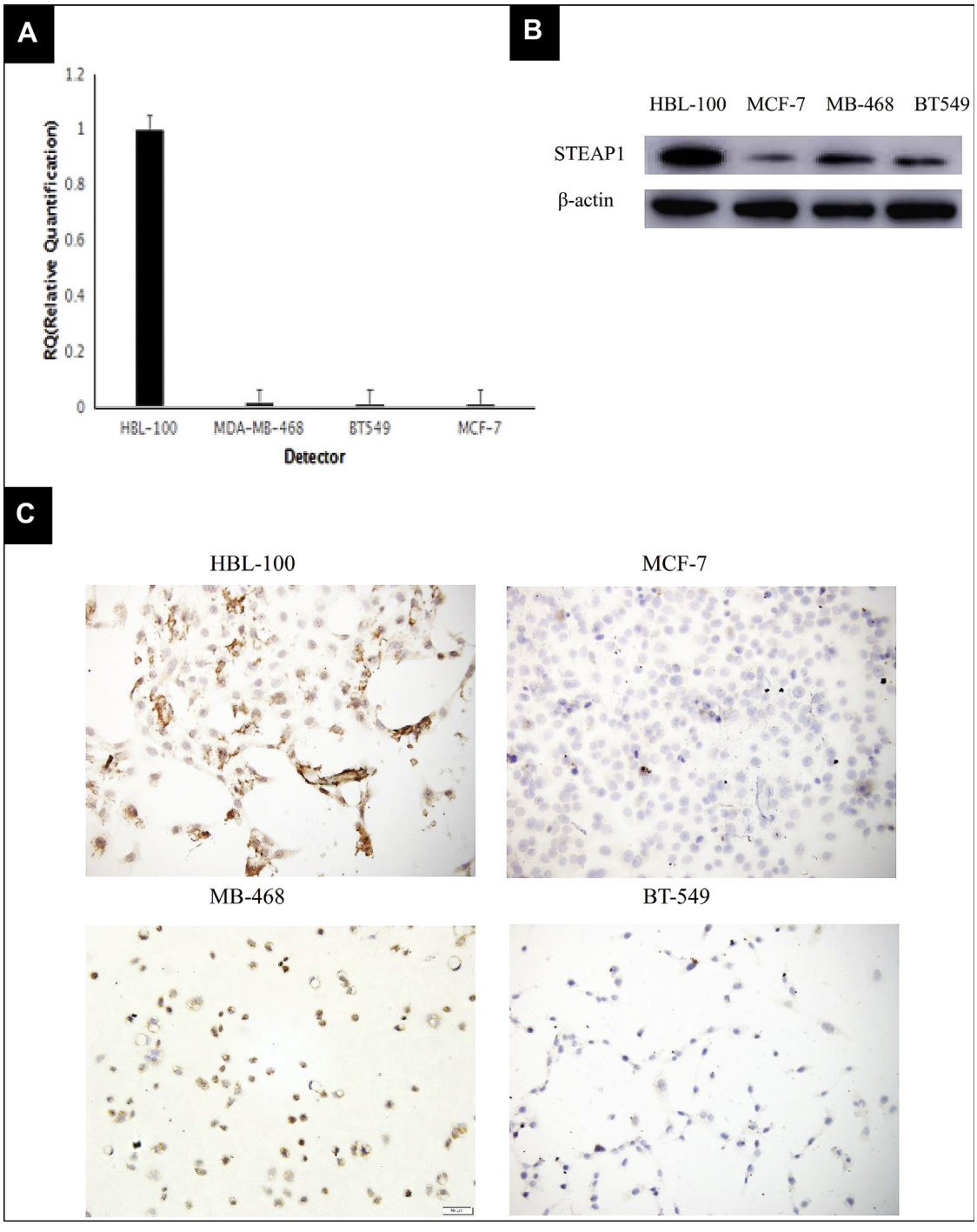
Effects of STEAP1 on Breast Cancer Cell Proliferation

Our results showed that increased expression of STEAP1 in the breast cancer cell line MCF-7 and the decreased expression of STEAP1 in the normal mammary epithelial HBL-100 cells had little influence on cell growth or the cell cycle (Figure 6). On the basis of our results, we concluded that STEAP1 has no influence on cell proliferation.

Effects of STEAP1 on Epithelial–Mesenchymal Transition Genes

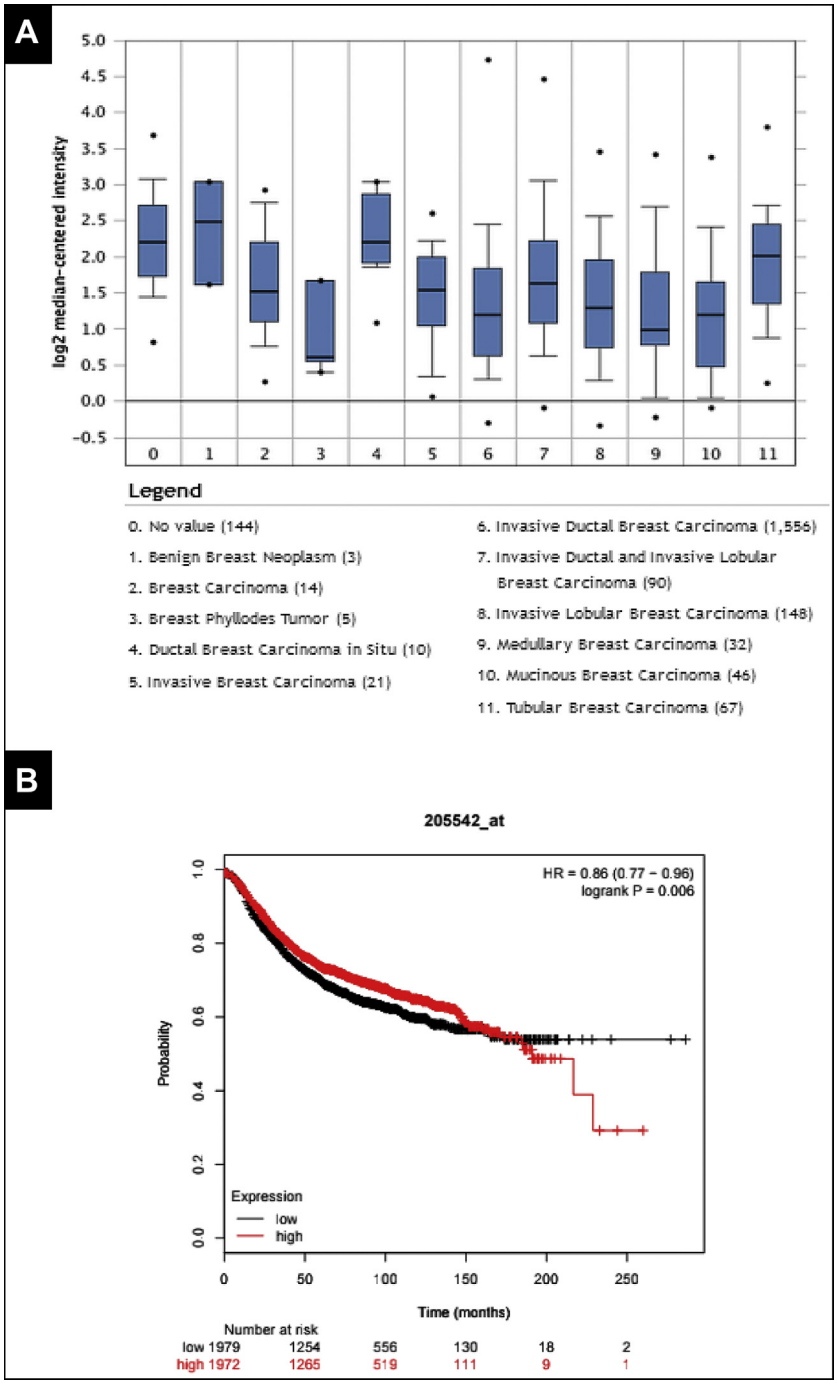
Because of evidence showing that epithelial–mesenchymal transition (EMT) is correlated with cancer invasion and metastasis, we suspected that there may be associations between STEAP1 and EMT. Analysis by qPCR showed that increased expression of STEAP1 in the breast cancer cell line MCF-7 significantly decreased mRNA levels of matrix metalloproteinases (MMPs) MMP2, MMP9, MMP13, vimentin (VIM), and cadherin (CDH)-2, and increased CDH1 mRNA levels, while knockdown of STEAP1 expression in the normal mammary epithelial cells HBL-100 increased mRNA levels of MMP2, MMP9, MMP13, VIM, and CDH2, and decreased CDH1 mRNA levels (Figure 7). We conclude that STEAP1 inhibited the EMT process in breast cancer cells.

Figure 2 Expression of STEAP1 in Breast Epithelial Cells and Breast Cancer Cells. (A) qPCR Was Performed to Quantitatively Detect Expression of STEAP1 mRNA in Breast Epithelial Cells and 3 Breast Cancer Cell Lines. (B) STEAP1 Expression in Breast Cells Was Evaluated by Western Blot Analysis. β-Actin Protein Was Used as Internal Positive Control. (C) STEAP1 Expressions in Breast Cells Were Determined by Immunocytochemistry



Abbreviations: mRNA = messenger RNA; qPCR = real-time quantitative PCR; STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

Figure 3 Analysis of Publicly Available Databases. (A) STEAP1 mRNA Expression in Breast Cancer Tissues Was Compared to Expression in Normal Breast Tissues Based on Curtis Data Set in Oncomine Database. (B) Evaluation of STEAP1 Expression and Prognosis for Breast Cancer Patients Using Kaplan-Meier Plotter Online Survival Analysis Website

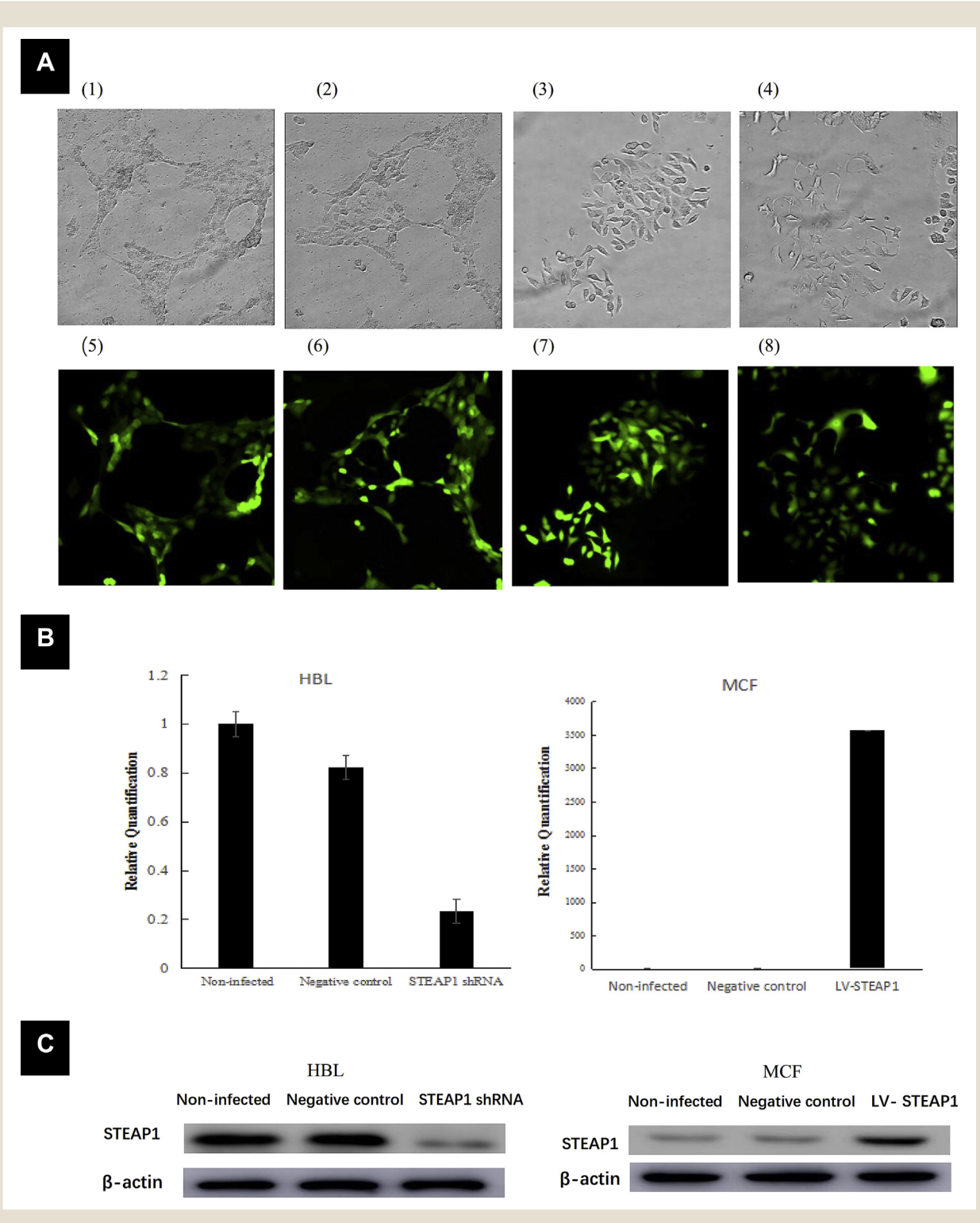


Abbreviations: mRNA = messenger RNA; STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

Discussion

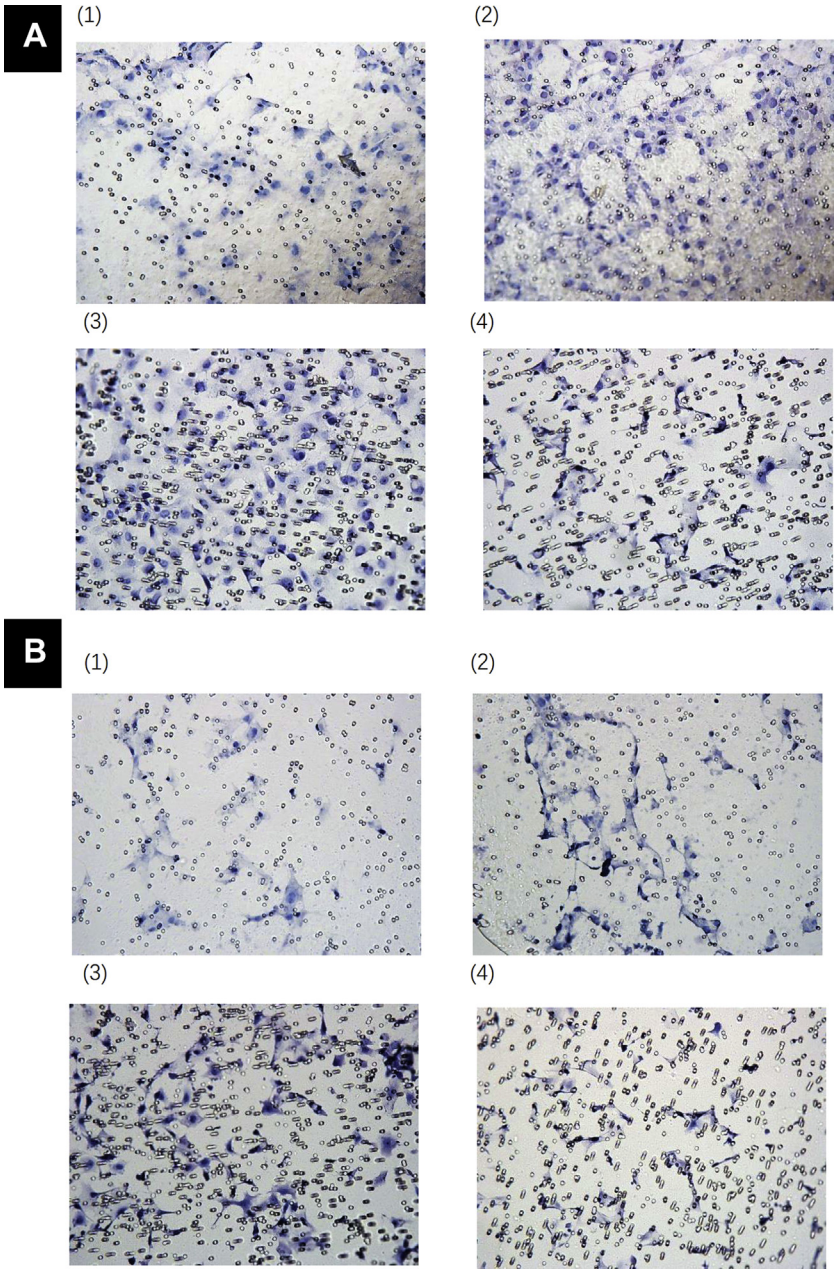
Although it has been previously shown that STEAP1 is expressed in multiple cancers, the relationship between STEAP1 expression and breast cancer is still unclear. Our results showed that the expression of STEAP1 was lower in breast cancer tissue compared to expression in normal breast tissues, and that STEAP1 was associated with lymph node metastasis, cell differentiation, and histologic grading. In addition, analysis using the Oncomine databases, which

Figure 4 Determination of STEAP1 Transfection Efficiencies. (A) (1) HBL-100 Cells Transfected With STEAP1 shRNA. (2) HBL-100 Cells Transfected With Negative Control Sequences. (3) MCF-7 Cells Transfected With Lentivirus Construct LV-STEAP1. (4) MCF-7 Cells Transfected With Negative Control Sequences. (5-8) Fluorescence Images of Representative Transfection Levels. (B) qPCR Analysis Was Conducted to Quantitatively Detect Expression Level of STEAP1 mRNA to Confirm Efficiencies of Transfection at Transcription Level; **P* < .05 Compared to Untreated Cells. (C) STEAP1 Protein Expression Was Measured by Western Blot Analysis to Confirm Efficiencies of Transfection at Protein Level



Abbreviations: mRNA = messenger RNA; qPCR = real-time quantitative PCR; shRNA = short hairpin RNA; STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

Figure 5 Effects of STEAP1 on Migration and Invasion Abilities. (A) Cell Migration Was Evaluated by Boyden Chamber Migration Assay. (B) Cell Invasion Was Evaluated by Boyden Chamber Invasion Assay. For (A) and (B), (1) HBL-100 Cells Were Treated With Negative Control Sequence, (2) HBL-100 Cells Were Treated With STEAP1 shRNA, Which Knocked Down Expression of STEAP1, (3) MCF-7 Cells Treated With Negative Control Sequence, and (4) MCF-7 Cells Treated With Lentivirus Construct LV-STEAP1, Which Enhanced Expression of STEAP1

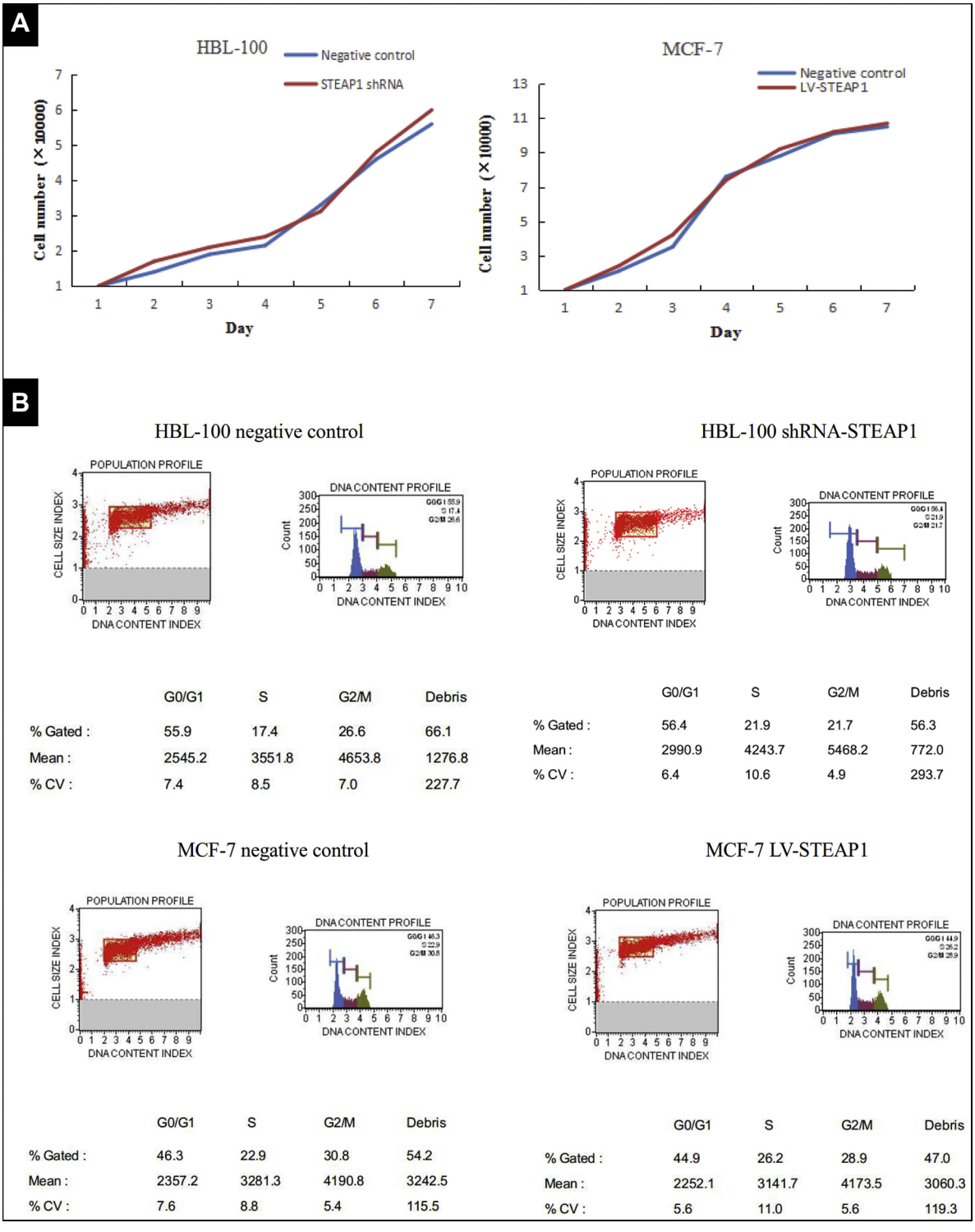


Abbreviations: shRNA = short hairpin RNA; STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

provide large-sample data, supported our conclusion that breast cancer patients demonstrate a lower expression level of STEAP1.

STEAP1 has been shown to be highly expressed in a variety of malignant tumors. The expression level of STEAP1 is positively correlated with the Gleason score in prostate cancer.[10] STEAP1 knockdown reduced Ewing tumor proliferation, and decreased growth and metastasis in in vivo experiments. This research supports the conclusion that STEAP1 expression is correlated with oxidative stress responses and elevated levels of reactive oxygen species, thus making it easy to speculate that STEAP1 may promote the development of the Ewing tumor process by increasing reactive oxygen species activity.[12] The high expression level of

Figure 6 Effects of STEAP1 on Cell Proliferation. (A) Cell Growth Assay Was Used to Generate Growth Curve, Which Shows That Knockdown or Up-regulation of STEAP1 Had Almost No Influence on Cell Proliferation, (B) Flow Cytometry Analysis Indicated That Knockdown or Up-regulation of STEAP1 Had Almost No Influence on Cell Cycle



	G0/G1	S	G2/M	Debris
% Gated :	55.9	17.4	26.6	66.1
Mean :	2545.2	3551.8	4653.8	1276.8
% CV :	7.4	8.5	7.0	227.7

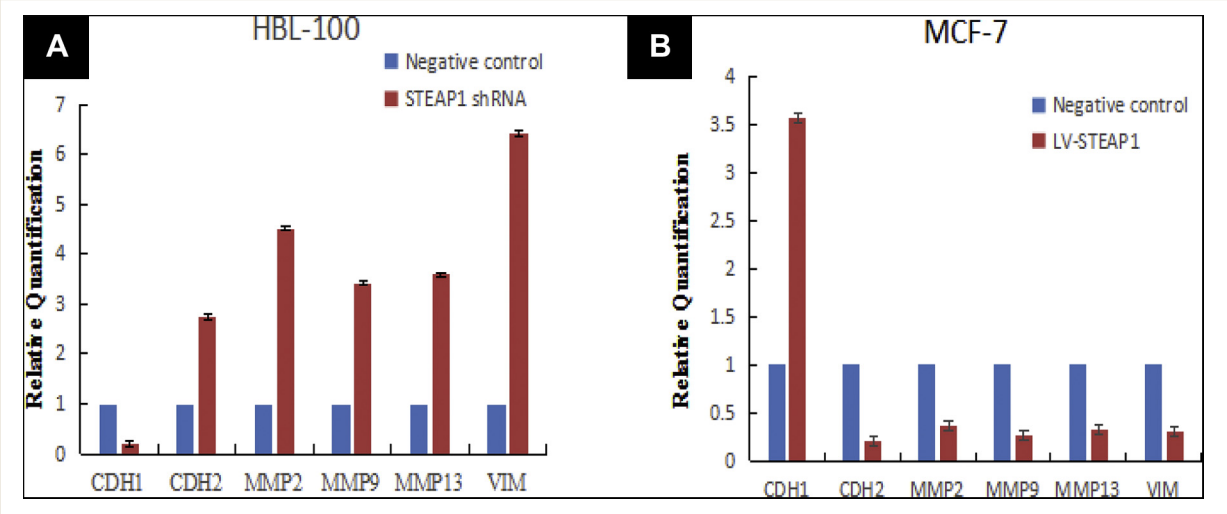
	G0/G1	S	G2/M	Debris
% Gated :	56.4	21.9	21.7	56.3
Mean :	2990.9	4243.7	5468.2	772.0
% CV :	6.4	10.6	4.9	293.7

	G0/G1	S	G2/M	Debris
% Gated :	46.3	22.9	30.8	54.2
Mean :	2357.2	3281.3	4190.8	3242.5
% CV :	7.6	8.8	5.4	115.5

	G0/G1	S	G2/M	Debris
% Gated :	44.9	26.2	28.9	47.0
Mean :	2252.1	3141.7	4173.5	3060.3
% CV :	5.6	11.0	5.6	119.3

Abbreviation: STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

Figure 7 qPCR Analyses to Detect Relationship Between STEAP1 and EMT-Associated Genes. (A) qPCR Was Performed to Quantitatively Detect Expression of EMT-associated Genes in HBL-100 Cells That Were Treated With STEAP1 shRNA or Negative Control Sequence. (B) qPCR Assay Detected Expression of EMT-associated Gene Expression in MCF-7 Cells Treated With Lentivirus Construct LV-STEAP1 or Negative Control Sequence



Abbreviations: EMT = epithelial–mesenchymal transition; qPCR = real-time quantitative PCR; shRNA = short hairpin RNA; STEAP1 = 6-transmembrane epithelial antigen of prostate 1.

STEAP1 was associated with poor prognosis in Ewing tumor; however, a survival analysis suggested the opposite result in that patients with high expression of STEAP1 have a better prognosis.[11,21] In colon cancer, STEAP1 expression is not significantly associated with age, tumor staging, or TNM staging, while the higher expression level of STEAP1 tends to correlate with a better prognosis.[13] STEAP1 is also highly expressed in vascular epithelial cells of lung cancer, and tumor cell invasion and migration are inhibited by reduced STEAP1 expression.[22] There is currently only one report demonstrating that STEAP1 is overexpressed in breast cancer, based on the comparison of 2 cases of normal breast tissue with 59 breast tumors cases. This study had a small number of samples.[23] In the current study, we analyzed 40 cases of normal breast tissue and 211 breast cancer cases by immunohistochemistry. In addition, we also examined STEAP1 expression by qPCR and Western blot analyses. Results from the immunocytochemistry, qPCR, and Western blot analysis were all consistent with STEAP1 being expressed at higher levels in normal mammary epithelial cells than in breast cancer cell lines. The high heterogeneity of STEAP1 observed in cancers may be accounted to the influence of a separate but related gene, *STEAP1B*.[24] This gene is located on the same chromosome as STEAP1, and it shares 88% amino acid identity with STEAP1. The *STEAP1B* gene encodes for 2 transcripts, STEAP1B1 and STEAP1B2. Experiments show that STEAP1 and STEAP1B2 are overexpressed in prostate cancer cells, while STEAP1B1 has no differential expression when comparing normal prostate cells and prostate cancer cells.

EMT appears to be a key regulator of cancer progression. The activation of the EMT program promotes tumor cell invasion and metastasis.[25] EMT is viewed as the loss of the epithelial phenotype and the gain of the mesenchyme-like phenotype.[26] A characteristic feature of EMT is the down-regulation of E-cadherin, a major epithelial marker coded by CDH1, which leads to the destabilization of adherens junctions.[27,28] Overexpression of N-cadherin coded by CDH2 and VIM are both mesenchymal markers of the EMT.[29,30] Our results showed that the expression of STEAP1 suppressed the migration and invasion abilities of breast cancer cells, and was associated with key EMT-related genes. It is believed that STEAP1 acts as an ion channel or transporter protein in cell junctions or cell adhesion, taking part in intercellular communication. STEAP1 may affect intercellular communication by altering intracellular ion concentrations, which in turn regulates gap or adherens junction activities, and participates in cancer cell proliferation and invasion.[9] It is speculated that STEAP1 may affect the balance between E-cadherin and N-cadherin by regulating the concentration of calcium ions in the body. The loss of E-cadherin expression is believed to be a primary cause for disruption of tight epithelial cell–cell contacts and the release of invasive tumor cells from the primary tumor, while the expression of N-cadherin in tumor cells induces scattered tumor morphology and improved motility.[31,32] VIM enhances the migration activity of breast cancer cells by regulating *Axl* and *Scrib* stability.[33] MMPs are crucial for the microenvironment during tumor progression, invasion, and metastasis, and they also serve as common EMT markers.[34,35] Studies on other types of tumors also show that STEAP1 is associated with metastasis. RNA interference of STEAP1 reduces the invasion and migration of tumor cells in lung cancer.[22] STEAP1 knockdown decreases Ewing tumor growth and metastasis in vivo.[12] However, the underlying mechanism between STEAP1 and EMT is still unknown, and further investigation is needed. A better

understanding of the contribution of the STEAP1 in the EMT process will undoubtedly contribute to the exploration of new strategies for breast cancer prevention and treatment.

In conclusion, our results were congruent with the public database analysis in that STEAP1 expression is down-regulated in breast cancer and is closely correlated to EMT markers. These results may have implications for STEAP1 contributing to an inhibitory role regarding EMT progression. More studies are required to clarify the underlying molecular mechanism of STEAP1 in breast cancer.

Clinical Practice Points

- Our results were congruent with a public database analysis in that STEAP1 expression was down-regulated in breast cancer, and its low expression was associated with a malignant phenotype and poor prognosis.
- STEAP1 has potential use in the development of a novel prognostic factor and possible therapeutic strategies for breast cancer patients.

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Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2018.08.010.

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Supplemental Table 1 Primer Sequences

Primer Name	Direction	Sequence (5′–3′)
STEAP1	Forward	TGGCACCCAGCACAATGAA
	Reverse	CTAAGTCATAGTCCGCCTAGAAGCA
MMP2	Forward	TGGCACCCAGCACAATGAA
	Reverse	CTAAGTCATAGTCCGCCTAGAAGCA
MMP9	Forward	ACGCACGACGTCTTCCAGTA
	Reverse	CCACCTGGTTCAACTCACTCC
MMP13	Forward	TCCTGGGCCAAATTATGGAG
	Reverse	GGGTCCTTGGAGTGGTCAAGA
CDH1	Forward	GGATTGCAAATTCCTGCCATTC
	Reverse	AACGTTGTCCCGGGTGTCA
CDH2	Forward	CGAATGGATGAAAGACCCATCC
	Reverse	GCCACTGCCTTCATAGTCAAACACT
VIM	Forward	AACCTGGCCGAGGACATCA
	Reverse	TCAAGGTCAAGACGTGCCAGA

Supplemental Table 2 Differential Analysis of STEAP1 Messenger RNA Expression in Normal Breast and Breast Carcinoma Tissue in Oncomine Data Sets

Normal vs. Carcinoma	Reference	Sample Number	Median	Fold Change	*P*
Normal vs. invasive breast carcinoma	TCGA	61, 76	0.710, −0.234	−2.088	1.12E-8
	Curtis	144, 21	2.197, 1.537	−1.773	3.13E-5
Normal vs. invasive ductal and lobular carcinoma	TCGA	61, 3	0.816, −0.076	−1.809	6.77E-7
	Curtis	144, 90	2.197, 1.640	−1.431	3.79E-6
Normal vs. invasive ductal breast carcinoma	TCGA	61, 389	0.710, −1.082	−3.133	4.07E-27
	Curtis	144, 1556	2.197, 1.196	−1.923	8.32E-40
Normal vs. invasive lobular breast carcinoma	TCGA	61, 36	0.710, −0.281,	−2.211	7.89E-6
	Curtis	144, 148	2.197, 1.296	−1.800	9.83E-20
Normal vs. medullary breast carcinoma	Curtis	144, 32	2.197, 0.978	−1.968	1.48E-6
Normal vs. mucinous breast carcinoma	Curtis	144, 46	2.197, 1.191	−2.143	4.90E-11

Abbreviations: Curtis = Curtis database; STEAP1 = 6-transmembrane epithelial antigen of prostate 1; TCGA = The Cancer Genome Atlas.