SEC11A Expression Is Associated with Basal-Like Bladder Cancer and Predicts Patient Survival

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Keywords

SEC11A · EGFR · Bladder cancer · Basal-like bladder cancer

Abstract

Objectives: Bladder cancer (BC) is a common malignancy worldwide. Signal peptidase complex 18 (SPC18) protein, which is encoded by the *SEC11A* gene, is one of the subunits of the signal peptidase complex and induces transforming growth factor- α secretion. In the present study, we analyzed the expression and function of SPC18 protein in human BC. **Methods:** Expression of SPC18 was analyzed by immunohistochemistry. RNA interference was used to inhibit *SEC11A* expression in BC cell lines. For constitutive expression of the *SEC11A* gene, a *SEC11A* expression vector was transfected into BC cell lines. To examine cell viability, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Modified Boyden chamber assays were used to examine cell invasiveness. **Results:** SPC18 was up-regulated in 54% of 81 BC cases. SPC18 expression served as an independent prognostic classifier of patients with BC. SPC18-positive BC cases frequently expressed cytokeratin 5/6, a marker of basal-like BC. Cell growth and invasiveness

were inhibited by *SEC11A* knockdown and enhanced by forced expression of *SEC11A*. **Conclusion:** These results indicate that SPC18 plays an important role in the progression of BC. Specific inhibitors of SPC18 may be promising anticancer drugs for patients with basal-like BC. © 2019 S. Karger AG, Basel

Introduction

Bladder cancers (BCs), the majority of which are urothelial carcinomas, are a common malignancy worldwide. The prognosis of patients with BC is poor when the disease includes muscle invasion. Metastatic BC is usually fatal. However, progress in systemic therapies for muscle-invasive BC has been stagnant for decades with few new systemic therapies being evaluated until recently [1]. Thus, there is an urgent need for new potential therapeutic targets in BC.

Transforming growth factor (TGF)- α is a mitogenic polypeptide that has a wide range of biological activities [2]. TGF- α phosphorylates the epidermal growth factor receptor (EGFR) and stimulates multiple signaling path-

ways involved in cell proliferation, anti-apoptosis, and other processes [3]. Overexpression of TGF- α has been reported in BC [3]. However, the detailed mechanisms have not been analyzed. Previously, we reported that signal peptidase complex 18 (SPC18) induces TGF- α secretion in gastric cancer [4]. SPC18 protein, which is encoded by the *SEC11A* gene, is one of the subunits of the signal peptidase complex. Most secretory proteins contain amino terminal or internal signal peptides that direct their sorting to the endoplasmic reticulum (ER) [5]. From the ER, proteins are transported to either the extracellular space or plasma membrane. The ER signal peptides are then cleaved by the signal peptidase complex. It has been reported that the signal peptidase complex purified from canine microsomes has 5 distinct subunits [6]. Two of these subunits, SPC18 and SPC21, are presumed to have catalytic activity [7]. Therefore, TGF- α secretion can be decreased by SPC18 inhibition. However, expression of SPC18 has not been examined in BC.

TGF- α phosphorylates the EGFR. It has been reported that basal-like BC exhibits activation of the EGFR pathway linked to frequent EGFR overexpression and activation of an EGFR autocrine loop [8]. Furthermore, the prognosis of patients with basal-like BC has been reported to be poor [8]. Because SPC18 induces TGF- α secretion [4], SPC18 expression may be frequent in basal-like BC. However, SPC18 expression has not been examined in basal-like BC.

In the present study, we analyzed the expression and distribution of SPC18 protein in human BC by immunohistochemistry and examined the relationship between SPC18 protein expression and clinicopathologic characteristics. Furthermore, the association between SPC18 expression and basal-like BC was analyzed by immunostaining of cytokeratin 5/6 (CK5/6), a basal-type marker [9]. We also analyzed the effect of inhibiting SPC18 expression by RNA interference (RNAi) on cell growth and invasiveness.

Materials and Methods

Tissue Samples

Using a retrospective study design, 89 primary tumors were collected from patients diagnosed with BC, who underwent surgery between 2003 and 2011 at the Hiroshima University Hospital (Hiroshima, Japan). All patients had undergone curative resection. Only patients without preoperative radiotherapy or chemotherapy were enrolled in the study. Operative mortality was defined as death within 30 days of patient discharge. These patients were excluded from the analysis. Postoperative follow-up was scheduled every 1, 2, or 3 months during the first 2 years after surgery and

every 6 months thereafter unless more frequent follow-ups were necessary. Chest X-ray, chest computed tomography, and serum chemistries were performed at each follow-up visit. Patients were followed by their physician until death or the date of the last documented contact. Tumor staging was performed according to the TNM (tumor-node-metastasis) classification system [10].

For quantitative reverse transcription polymerase chain reaction (qRT-PCR), we used 8 urothelial carcinoma (UC) samples. The samples were frozen immediately in liquid nitrogen and stored at -80°C until use.

For immunohistochemical analysis, we used archival formalin-fixed, paraffin-embedded tissues from 81 patients who had undergone surgical excision for UC. All 81 patients with UC were treated by cystectomy.

qRT-PCR Analysis

Total RNA was extracted with an RNeasy mini kit (Qiagen, Valencia, CA, USA), and 1 μg total RNA was converted to cDNA using a first-strand cDNA synthesis kit (Amersham Biosciences, Piscataway, NJ, USA). Quantitation of *SEC11A* mRNA levels was performed by real-time fluorescence detection as described previously [11]. PCR was conducted using a SYBR green PCR core reagents kit (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Real-time detection of the emission intensity of SYBR green bound to double-stranded DNA was performed with an ABI PRISM 7700 sequence detection system (Applied Biosystems). *ACTB*-specific PCR products were amplified from the same RNA samples and served as an internal control.

Immunohistochemistry

Immunohistochemical analysis was performed with a Dako Envision+ rabbit or mouse peroxidase detection system (Dako Cytomation, Carpinteria, CA, USA). Antigen retrieval was performed by microwave heating in citrate buffer (pH 6.0) for 30 min. Peroxidase activity was blocked in sections by incubation with 3% H_2O_2 in methanol for 10 min, followed by incubation with normal goat serum (Dako Cytomation) for 20 min to block nonspecific antibody binding sites. Sections were incubated with an anti-SPC18 antibody [4], anti-CK5/6 antibody (1:50; Dako Cytomation), or anti-EGFR antibody (1:20; Novocastra, Newcastle, UK) for 1 h at room temperature, followed by incubation with Envision+ anti-rabbit or anti-mouse peroxidase for 1 h. For color development, sections were incubated with DAB substrate-chromogen solution (Dako Cytomation) for 10 min. Sections were counterstained with 0.1% hematoxylin. Negative controls were prepared by omission of the primary antibody.

Expression of SPC18, CK5/6, and EGFR was scored in all tumors as positive or negative. When $>10\%$ of tumor cells were stained, the immunostaining was considered as positive. Using this definition, 2 surgical pathologists (N.O. and K.S.) without knowledge of the clinical and pathological parameters or the patient outcomes independently reviewed the immunoreactivity in each specimen. Interobserver differences were resolved by consensus review under a double-headed microscope after independent review.

Cell Lines

We used 2 cell lines derived from human BC (T24 and KMBC-2). Both cell lines were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). The cell lines were maintained in RPMI 1640 (Nissui Pharmaceutical Co, Ltd,

Tokyo, Japan) containing 10% fetal bovine serum (BioWhittaker, Walkersville, MD, USA) at 37°C in a humidified atmosphere with 5% CO₂.

Western Blot Analysis

Cells were lysed as described previously [12]. The lysates (40 µg protein) were solubilized in Laemmli sample buffer by boiling and then subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by electrotransfer onto a nitrocellulose filter. Western blot procedures were performed as described previously [12]. An anti-SPC18 antibody was prepared as described previously [4]. Anti-EGFR, anti-phosphorylated EGFR, anti-ERK1/2, anti-phosphorylated ERK1/2, anti-AKT, and anti-phosphorylated AKT antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG were used in the secondary reaction. Immunocomplexes were visualized with an ECL Western blot detection system (Amersham Biosciences).

RNAi, Expression Vector, and Transfection

Short interfering RNA (siRNA) oligonucleotides targeting *SEC11A* and a negative control were purchased from Invitrogen (Carlsbad, CA, USA). We used 2 independent *SEC11A* siRNA oligonucleotide sequences. Transfection was performed using Lipofectamine RNAiMAX (Invitrogen) as described previously [13]. Briefly, 60 pmol siRNA and 10 µL Lipofectamine RNAiMAX were mixed in 1 mL RPMI 1640 medium (10 nmol/L final concentration). After 20 min of incubation, the mixture was added to the cells, and then the cells were plated in culture dishes. At 48 h after transfection, the cells were analyzed.

For constitutive expression of the *SEC11A* gene, cDNA was PCR amplified and subcloned into pcDNA 3.1 (Life Technologies, Carlsbad, CA, USA). The *SEC11A* expression vector was transfected into T24 and KMBC-2 cells with FuGENE6 (Roche Diagnostics, Indianapolis, IN, USA), according to the manufacturer's instructions. Stable transfectants were selected after 2 weeks of culture with 100 µg/mL G418 (Life Technologies).

Cell Growth and Invasion Assays

Cell growth was monitored after 1, 2, and 4 days by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [14]. Three independent experiments were carried out. The mean ± standard error (SE) was calculated for each experiment.

A modified Boyden chamber assay was performed to examine cell invasiveness. Cells were plated at 10,000 cells per well in RPMI 1640 medium plus 1% serum in the upper chamber of a Transwell insert (8-µm pore diameter; Chemicon, Temecula, CA, USA) coated with Matrigel. Medium containing 10% serum was added to the bottom chamber. After 2 days, cells in the upper chamber were removed by scraping, and cells on the lower surface of the insert were stained with CyQuant GR dye to assess the number of cells. Three independent experiments were carried out. The mean ± SE was calculated for each experiment.

Statistical Methods

Associations between clinicopathological parameters and SPC18 expression were analyzed by Fisher's exact test. Kaplan-Meier survival curves were constructed for SPC18-positive and -negative patients. Differences between survival curves were tested

for statistical significance by the log-rank test. Univariate and multivariate Cox regressions were used to evaluate the associations between clinical covariates and cancer-specific mortality. The hazard ratio and 95% confidence interval were estimated from Cox proportional hazard models. A *p* value <0.05 was considered to be statistically significant. Differences between the two groups (*SEC11A* siRNA- and negative control siRNA-transfected cells) were tested by the Student *t* test.

Results

Expression of *SEC11A* in BC

We first analyzed *SEC11A* mRNA expression in 8 BC tissue samples and a nonneoplastic mucosa sample by qRT-PCR (Fig. 1a) and observed overexpression of *SEC11A* (tumor/nonneoplastic mucosa ratio >2) in 6 (75%) of the 8 BC tissue samples.

Next, immunohistochemistry of SPC18 was performed in 81 BC tissue samples. In nonneoplastic mucosa, weak or no staining of SPC18 was observed in the epithelial and stromal cells. In contrast, BC tissue showed stronger, more extensive staining (Fig. 1b). SPC18 was detected in the cytoplasm of tumor cells (Fig. 1c). Many BC cases showed heterogeneity of SPC18 staining, and the percentage of SPC18-stained BC cells ranged from 0 to 80%. A tendency for upregulation of SPC18 was not observed at the invasive front. We next examined the relationship of SPC18 expression with clinicopathological characteristics (Table 1). When at least 10% of tumor cells were stained, the immunostaining was considered positive for SPC18. In total, 44 (54%) of 81 BC cases were positive for SPC18. Expression of SPC18 was associated with vascular invasion, histological grade, histological, T, and N classification, and tumor stage. We found that SPC18 expression was significantly associated with overall survival (*p* = 0.003, log-rank test; Fig. 1d). Univariate analysis indicated that lymphatic invasion, vascular invasion, T, N, and M classification, tumor stage, and SPC18 expression were associated with overall survival. In the multivariate model, tumor stage and SPC18 expression were independent predictors of BC patient survival. These results suggest that SPC18 is a promising biomarker to identify patients with a poor prognosis.

SPC18 Expression in Basal-Like BC

To analyze the association between SPC18 expression and basal-like BC, immunostaining of CK5/6 was performed in 81 BC tissue samples. When at least 10% of tumor cells were stained, the immunostaining was considered positive for CK5/6. In total, 39 (48%) of 81 BC cases were positive for CK5/6. Expression of CK5/6 was

not associated with any clinicopathological characteristics. Furthermore, CK5/6 expression was not associated with overall survival (data not shown). In contrast, SPC18 staining was observed in CK5/6-positive BC cells (Fig. 2). SPC18-positive BC cases had a significantly increased frequency of CK5/6 positivity ($p < 0.0001$; Table 2).

Table 1. Relationship between SPC18 expression and clinicopathological characteristics of bladder cancer

	SPC18 expression		p (χ^2 test)
	positive	negative	
Age			
<65 years	12 (43%)	16	0.132
≥65 years	32 (60%)	21	
Sex			
Male	36 (54%)	31	0.815
Female	8 (57%)	6	
Lymphatic invasion			
Positive	22 (58%)	16	0.5436
Negative	22 (51%)	21	
Vascular invasion			
Positive	15 (79%)	4	0.0112
Negative	29 (47%)	33	
Histological grade			
G1/G2	6 (33%)	12	0.0420
G3	38 (60%)	25	
Histological classification			
Pure UC	36 (50%)	36	0.0180
UC/SD	8 (89%)	1	
T classification			
Ta/is/1	12 (32%)	25	0.0002
T2/3/4	32 (73%)	12	
N classification			
N0	33 (48%)	36	0.0023
N1	11 (92%)	1	
M classification			
M0	41 (53%)	37	0.0525
M1	3 (100%)	0	
Stage			
Stage 0/I/II	22 (44%)	28	0.0166
Stage III/IV	22 (71%)	9	

UC, urothelial carcinoma; SD, squamous differentiation.

Fig. 1. Expression of *SEC11A* in BC. **a** qRT-PCR analysis of *SEC11A* mRNA in normal bladder and 8 bladder cancer (BC) tissue samples. **b** Immunostaining of SPC18 in nonneoplastic mucosa and BC. Original magnification, $\times 40$. **c** High magnification image of the field indicated by the box in **b**. Original magnification, $\times 400$. **d** Kaplan-Meier plot of BC patient survival according to SPC18 expression.

Fig. 2. Immunostaining of SPC18, cytokeratin 5/6 (CK5/6), and epidermal growth factor receptor (EGFR). Original magnification, $\times 100$.

Next, we analyzed EGFR expression by immunohistochemistry and confirmed that all SPC18-positive BC cases were positive for EGFR (Fig. 2). These results indicate that the EGFR pathway could be activated in SPC18-positive BC cases.

Effect of SEC11A Inhibition or Overexpression on Cell Growth and Invasion

Previously, we showed that inhibition of *SEC11A* by siRNA reduces cell growth, and the levels of phosphorylated EGFR and AKT are lower in *SEC11A* siRNA-transfected gastric cancer cells than in control cells [4]. However, the biological function of *SEC11A* has not been investigated in BC cells. Therefore, we examined the effect of *SEC11A* inhibition on cell growth using 2 different siRNA sequences. T24 BC cells were selected, which expressed detectable levels of SPC18 protein by Western blotting (Fig. 3a). The SPC18 expression in T24 cells was substantially suppressed by treatment with siRNA1 and

siRNA2 as shown by Western blotting (Fig. 3b). Next, cell growth was analyzed by MTT assays. *SEC11A* siRNA1- and siRNA2-transfected T24 cells showed significantly reduced cell growth relative to negative control siRNA-transfected T24 cells (Fig. 3c).

To further investigate the function of SPC18, KMBC-2 cells were stably transfected with pcDNA-SEC11A. Clones were selected in G418 and examined for SPC18 protein expression by Western blotting. Two clones, stable lines 1 and 2, expressed SPC18 at significantly higher levels than KMBC-2 cells transfected with the empty vector (Fig. 3b). To investigate the effect of SPC18 on cell growth, we performed MTT assays. Cell growth was significantly enhanced in pcDNA-SEC11A-transfected cells compared with empty vector-transfected cells (Fig. 3c). These results indicate that SPC18 promotes tumor growth.

In the present study, immunohistochemical analysis demonstrated that expression of SPC18 was associated

Table 2. Univariate and multivariate Cox regression analyses of SPC18 expression and survival of bladder cancer patients

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Age				
≤65 years	1 (ref.)			
>65 years	1.58 (0.75–3.6)	0.2324		
Sex				
Male	1 (ref.)			
Female	0.72 (0.25–1.73)	0.4930		
Lymphatic invasion				
Negative	1 (ref.)		1 (ref.)	
Positive	2.79 (1.35–6.19)	0.0051	2.40 (0.89–6.74)	0.0823
Vascular invasion				
Negative	1 (ref.)		1 (ref.)	
Positive	2.38 (1.12–4.84)	0.0247	0.93 (0.38–2.24)	0.8896
Grade				
G1/G2	1 (ref.)			
G3	2.20 (0.86–7.44)	0.1052		
Histological subtype				
Pure UC	1 (ref.)			
UC/SD	2.29 (0.91–5.03)	0.0757		
T classification				
Ta/Tis/T1	1 (ref.)		1 (ref.)	
T2/T3/T4	2.46 (1.17–5.61)	0.0163	0.48 (0.13–1.64)	0.2436
N classification				
N0	1 (ref.)		1 (ref.)	
N1	5.14 (2.22–11.32)	0.0003	1.36 (0.49–3.90)	0.5528
M classification				
M0	1 (ref.)		1 (ref.)	
M1	5.25 (1.22–15.63)	0.0291	1.50 (0.31–5.46)	0.5799
Stage				
0/I/II	1 (ref.)		1 (ref.)	
III/IV	4.17 (2.04–8.77)	<0.0001	3.05 (1.01–10.00)	0.0476
SPC18 expression				
Negative	1 (ref.)		1 (ref.)	
Positive	3.14 (1.47–7.48)	0.0027	3.11 (1.15–9.34)	0.0250
HR, hazard ratio; CI, confidence interval.				

with vascular invasion and the T classification. Thus, we analyzed the possible role of SPC18 in the invasiveness of BC cells. We performed modified Boyden chamber assays using T24 cells. On day 2, the invasiveness of *SEC11A* siRNA1- and siRNA2-transfected T24 cells was less than that of negative control siRNA-transfected cells (Fig. 3d). We also performed invasion assays using KMBC-2 cells. On day 2, invasiveness was significantly enhanced in pcDNA-*SEC11A*-transfected cells compared with empty vector-transfected cells (Fig. 3d). These results indicate that SPC18 also enhances invasiveness.

Effect of SEC11A Inhibition or Overexpression on EGFR Signaling

Because EGFR activates RAS-MEK-ERK1/2 and AKT-PI3K pathways leading to cancer cell proliferation and survival [15], we analyzed the effect of *SEC11A* inhibition on EGFR signaling. The levels of phosphorylated EGFR, AKT, and ERK1/2 were lower in *SEC11A* siRNA1- and siRNA2-transfected T24 cells than in negative control siRNA-transfected cells (Fig. 3e). We also analyzed the levels of phosphorylated EGFR, AKT, and ERK1/2 in KMBC-2 cells. The levels of phosphorylated EGFR, AKT, and ERK1/2 were higher in pcDNA-*SEC11A*-transfected cells compared

Fig. 3. Effect of *SEC11A* inhibition or overexpression on BC cell lines. **a** Western blot analysis of SPC18. MKN-45 is a gastric cancer cell line and served as a positive control. **b** Western blot analysis of SPC18 in T24 cells transfected with negative control siRNA or *SEC11A* siRNAs (left panel). Western blot analysis of SPC18 in KMBC-2 cells stably transfected with pcDNA-*SEC11A* or the empty vector (right panel). **c, d** Bars and error bars indicate the mean and SE, respectively, of 3 independent experiments. **c** Cell viability was assessed by MTT assays on days 1, 2, and 4 after seeding in 96-well plates. Effect of *SEC11A* knockdown on T24 cell growth (left panel) and effect of *SEC11A* overexpression on KMBC-

2 cell growth (right panel). **d** Effect of *SEC11A* knockdown on T24 cell invasiveness (left panel) and effect of *SEC11A* overexpression on KMBC-2 cell invasiveness (right panel) evaluated by the modified Boyden chamber assay. **e** Western blot analysis of SPC18, epidermal growth factor receptor (EGFR), phosphorylated EGFR (pEGFR), AKT, and phosphorylated AKT (pAKT), ERK1/2, and phosphorylated ERK1/2 (pERK1/2) in lysates of T24 cells transfected with the negative control siRNA or *SEC11A* siRNAs (left panel) and in lysates of KMBC-2 cells stably transfected with pcDNA-*SEC11A* or the empty vector (right panel).

with empty vector-transfected cells (Fig. 3e). We also analyzed expression of CK5/6 by immunostaining in BC cell lines because SPC18-positive BC cases had a significantly increased frequency of CK5/6 positivity in BC tissue specimens. However, expression of CK5/6 was not significantly changed in *SEC11A* siRNA-transfected T24 cells and pcDNA-*SEC11A*-transfected KMBC-2 cells. These results indicate that SPC18 activates EGFR signaling.

Next, we examined epithelial-mesenchymal transition in BC cell lines because EGFR signaling is involved in epithelial-mesenchymal transition [16]. Expression of *CDH1* and *VIM* was measured by qRT-PCR. However,

expression of *CDH1* and *VIM* was not significantly changed in *SEC11A* siRNA-transfected T24 cells and pcDNA-*SEC11A*-transfected KMBC-2 cells.

Discussion

Previously, we reported overexpression of SPC18 in gastric and colorectal cancers. SPC18 expression is associated with tumor progression and patient survival in both gastric and colorectal cancers [4, 17]. In the present study, we analyzed the expression and distribution of SPC18 pro-

tein in human BC by immunostaining. In nonneoplastic mucosa, weak or no staining of SPC18 was observed in epithelial and stromal cells, whereas BC tissue showed stronger, more extensive staining. We found that SPC18 was upregulated in 54% of 81 BC cases. SPC18 expression was associated with vascular invasion, histological grade, histological classification, T and N classification, and tumor stage. These results indicate that SPC18 plays a major role in the progression of BC. Furthermore, SPC18 expression was correlated with overall survival and served as an independent prognostic classifier of patients with BC. These results suggest that SPC18 immunostaining is a clinically useful method to predict BC patient survival.

SPC18 protein is one of the subunits of the signal peptidase complex and has catalytic activity of signal peptidase [7]. Increased activity of the signal peptidase complex caused by SPC18 protein overexpression may induce secretion of several kinds of growth factors. In fact, we have reported that SPC18 induces TGF- α secretion in gastric cancer [4]. In the present study, we showed that cell growth was inhibited by *SEC11A* knockdown and enhanced by forced expression of *SEC11A*, suggesting that secretion of several kinds of growth factors, including TGF- α , could be activated by SPC18 expression. We confirmed that the levels of phosphorylated EGFR, AKT, and ERK1/2 were reduced by *SEC11A* knockdown and enhanced by forced expression of *SEC11A*. These results indicate that SPC18 plays a major role in BC cell growth.

In the present study, SPC18-positive BC cases were frequently positive for CK5/6. CK5/6 is a basal-type marker [9], and CK5/6-positive BC is classified as basal-like BC. It has been reported that the EGFR pathway is activated in basal-like BC [8]. In our analysis, SPC18 expression induced phosphorylation of EGFR, AKT, and ERK1/2 in BC cell lines, indicating that SPC18 is involved

in the activation of the EGFR pathway in basal-like BC. Although clinical trials could not demonstrate the effectiveness of anti-EGFR therapy for BC, blockade of the EGFR pathway may be effective for basal-like BC [18]. In addition to EGFR, SPC18 may be a therapeutic target.

In summary, we found SPC18 overexpression in BC. We also showed that *SEC11A* knockdown inhibited tumor cell growth and EGFR signaling. Because SPC18 staining was an independent prognostic indicator, immunostaining of SPC18 may be a suitable method to identify patients with a poor prognosis. SPC18-positive BC cases were frequently found to have basal-like BC. Specific inhibitors of SPC18 may be promising anticancer drugs for patients with basal-like BC.

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Statement of Ethics

This study was approved by the Ethics Committee for Human Genome Research of the Hiroshima University (Hiroshima, Japan). Because written informed consent was not obtained, for strict privacy protection, identifying information for all samples was removed before analysis.

Disclosure Statement

The authors declare no conflict of interest.

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