The clinicopathological significance of SPC18 in colorectal cancer: SPC18 participates in tumor progression

Takuya Hattori, Kazuhiro Sentani, Oue Naohide, Naoya Sakamoto and Wataru Yasui

Department of Molecular Pathology, Hiroshima University Institute of Biomedical and Health Sciences, Hiroshima, Japan

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Correspondence

Wataru Yasui, MD, PhD, Department of Molecular Pathology, Hiroshima University Institute of Biomedical and Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
Tel: +81-82-257-5145;
Fax: +81-82-257-5149;
E-mail: wyasui@hiroshima-u.ac.jp

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Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. In order to identify novel prognostic markers or therapeutic targets for CRC, we searched for candidate genes in our comprehensive gene expression libraries, and focused on *SEC11A*, which encodes the SPC18 protein. SPC18 plays a key role in the endoplasmic reticulum-Golgi secretory pathway and presumably regulates the secretion of various secretory proteins. An immunohistochemical analysis of SPC18 in 137 CRC tissue samples demonstrated that 79 (58%) CRC cases were positive for SPC18. SPC18-positive CRC cases were more advanced in terms of *N* classification (*P* = 0.0315) and tumor stage (*P* = 0.0240) than SPC18-negative CRC cases. Furthermore, the expression of SPC18 was an independent prognostic classifier for CRC patients. The cell growth and invasiveness of SPC18 siRNA-transfected CRC cell lines was less than that of the negative control siRNA-transfected cell lines. The levels of phosphorylated epidermal growth factor receptor, Erk and Akt were lower in SPC18 siRNA-transfected CRC cells than in control cells. The expression of SPC18 was colocalized with β-catenin nuclear localization and MMP7 at the invasive front. An immunohistochemical analysis of human colorectal polyp specimens revealed a sequential increase in the expression of SPC18 through the conventional adenoma-carcinoma pathway, while SPC18 was not expressed or was expressed to a lesser extent in serrated pathway-related tumors. These results suggest that SPC18 is involved in tumor progression, and is an independent prognostic classifier in patients with CRC.

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide.[1] Although the survival rate of early-stage CRC patients has increased, the long-term survival rate still remains very poor, mainly due to local relapse and distant metastases.[2,3] It would be valuable to identify new therapeutic markers and to supplement standard clinicopathological staging using molecular markers to more precisely define the subset of patients at highest or lowest risk of recurrence following CRC surgery. In the search for new therapeutic or diagnostic markers, it is generally accepted that genes expressed at high levels in tumors and at very low levels in normal tissues are ideal diagnostic or therapeutic molecules.[4,5] We previously reported that Reg IV, olfactomedin 4, claudin-18 and h-prune are prognostic makers for CRC.[6–9] In the present study, we searched for the candidate genes that met these conditions in our comprehensive gene expression libraries, and focused on *SEC11A*, which encodes the SPC18 protein.[10,11] To the best of our knowledge, the expression and function of SPC18 have not been investigated in human cancers with the exception of GC.

SPC18 is one of the subunits of the signal peptidase complex (SPC). SPCs participate in the endoplasmic reticulum (ER)-Golgi secretary pathway and it is considered that the overexpression of the SPC18 protein can induce the secretion of growth factors, including TGF-α.[11–14] TGF-α can phosphorylate the epidermal growth factor receptor (EGFR), which subsequently stimulates multiple signaling pathways involved in cell proliferation. We investigated the clinicopathological significance and biological function of SPC18 in CRC.

The conventional adenoma-carcinoma pathway and the serrated pathway are considered to be the main colorectal carcinogenesis pathways.[15,16] It was reported that the activation of Wnt/β-catenin signaling occurred via distinct mechanisms in both pathways.[17] Furthermore, there are several immunohistochemical markers that can assist in the conventional morphological diagnosis in tumors that develop via the colorectal carcinogenesis pathway, including, but not limited to p53, β-catenin, claudin-18, MLH1 and MSH2.[9,18] In the present study, we analyzed the significance of SPC18 in pre-cancerous lesions of both colorectal carcinogenesis pathways.

Materials and Methods

Tissue samples and cell lines. In a retrospective study design, 137 primary tumors were collected from patients diagnosed with CRC who underwent surgery at Hiroshima University Hospital (Hiroshima, Japan). All patients underwent curative resection. Only patients without preoperative radiotherapy or chemotherapy were enrolled in the study. The study population included 46 men and 87 women. The mean age was 63 years (range, 29–89 years). 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-up was deemed necessary. Chest X-rays, chest computed

tomography scans and serum chemistry analyses were performed at every follow-up visit. Recurrence was evaluated from the records at Hiroshima University Hospital. Archival formalin-fixed, paraffin-embedded tissues were used for the immunohistochemical analysis. The histological classifications were determined based on the World Health Organization system. Tumor staging was performed according to the TNM stage grouping system. Ninety-two primary colorectal polyps were collected from patients who underwent endoscopic or surgical resection at Hiroshima University Hospital or its affiliated hospitals. The distribution of colorectal polyps was as follows: conventional adenoma low grade (CALG), $n = 24$ (tubular adenoma, $n = 17$ and villous or tubulovillous adenoma, $n = 7$); conventional adenoma high grade (CAHG), $n = 29$ (tubular adenoma, $n = 26$ and villous or tubulovillous adenoma, $n = 3$); HP (hyperplastic polyp), $n = 20$, TSA (traditional serrated adenoma), $n = 31$; sessile serrated adenoma/polyp (SSA/P) $n = 18$ and non-neoplastic mucosa, $n = 20$. Written informed consent was not obtained. Thus, for strict privacy protection, all of the identifying information associated with the samples was removed before the analysis; this procedure is in accordance with the Ethical Guidelines for Human Genome/Gene Research that were enacted by the Japanese Government.

Archival formalin-fixed, paraffin-embedded tissues from 137 patients who had undergone the surgical excision of CRC and from 97 patients who had undergone surgical or endoscopic resection were used for the immunohistochemical analysis. The histological classifications of CRC were based on the World Health Organization system. Tumor staging was performed according to the TNM stage grouping system.

Human colon cancer-derived cell lines, WiDr, CCK-81, DLD-1, COLO-201, COLO-320 and LoVo were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). All of the cell lines were maintained in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum (FBS, Whittaker, Walkersville, MD, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Western blotting. Tumor cells were lysed for Western blotting as described previously.[19] The lysates (40 μg) were solubilized in Laemmli sample buffer by boiling and then subjected to 12% SDS-polyacrylamide gel electrophoresis and then electrotransferred onto a nitrocellulose filter. The filter was incubated with the primary antibody against SPC18. The SPC18 antibody was a polyclonal antibody that had been raised in our laboratory, the specificity of which has been characterized in detail.[11] Peroxidase-conjugated anti-mouse IgG was used in the secondary reaction. Immunocomplexes were visualized with an ECL Western Blot Detection System (Amersham Biosciences). β-actin antibody (Sigma Chemical, St. Louis, MO, USA) was also used as a loading control.

Immunohistochemistry. One or two representative tumor blocks, including the tumor center, invading front, and the tumor-associated non-neoplastic mucosa, from each patient were examined by immunohistochemistry. In cases of large, late-stage tumors, two different sections were examined to include representative areas of the tumor center as well as of the lateral and deep tumor invasive front. The immunohistochemical analysis was performed with a Dako Envision+ 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 with 3% H_2O_2-methanol for 10 min, and the sections were incubated with normal goat serum (Dako Cytomation) for 20 min to block nonspecific antibody binding sites. Sections were incubated with a rabbit polyclonal anti-SPC18 antibody (dilution 1:50) for 1 h at room temperature, followed by incubation with Envision+ anti-mouse peroxidase for 1 h. The sections were incubated with the DAB Substrate-Chromogen Solution (Dako Cytomation) for 10 min for the color reaction. The sections were counterstained with 0.1% hematoxylin. Negative controls were created by omission of the primary antibody.

The expression of SPC18 in CRC was scored in all tumors as positive or negative. When more than 50% of tumor cells were stained, the immunostaining was considered positive for SPC18 (according to the median cut off values rounded off to the nearest 50%). The expression of SPC18 in pre-cancerous lesions was scored in all tumors as negative (score 0), mild (score 1), moderate (score 2) or strong (score 3) according to their immunostaining intensity. The expression of Matrix metalloproteinase 7 (MMP7), β-catenin nuclear localization at the invasive front and p53 was scored in all tumors as positive or negative. When more than 10% of tumor cells were stained, the immunostaining was considered to be positive for each molecule. Using these definitions, two surgical pathologists (TH and KS), who had no knowledge of the clinical or pathological parameters, or the patients' outcomes, independently reviewed the immunoreactivity of each specimen. Interobserver differences were resolved by consensus review at a double-headed microscope after independent review.

RNA interference. To knockdown endogenous SPC18, RNAi was carried out as described previously.[20] siRNA oligonucleotides for SPC18 and a negative control were purchased from Invitrogen. Transfection was performed using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. Briefly, 60 pmol of siRNA and 10 μL of Lipofectamine RNAiMAX were mixed in 1 mL of RPMI medium (10 nmol/L final siRNA concentration). After 20 min of incubation, the mixture was added to the cells and these were plated on dishes for each assay. In all of the experiments, the cells were analyzed at 48 h after transfection.

Cell growth and *in vitro* invasion assays. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to examine cell growth.[21] The cells were seeded at a density of 2000 cells per well in 96-well plates. Cell growth was monitored after 1, 2 and 4 days. Modified Boyden chamber assays were performed to evaluate the invasiveness. The cells were plated at 10 000 cells per well in RPMI 1640 medium plus 1% serum in the upper chamber of a Transwell insert (pre diameter, 8 μm; Chemicon, Temecula, CA, USA) coated with Matrigel. Medium containing 10% serum was placed in the bottom chamber. After 1 and 2 days, the cells in the upper chamber were removed by scraping, and the cells remaining on the lower surface of the insert were stained with CyQuant GR dye to assess the number of cells. We performed three different experiments and calculated the mean and standard deviation (SD) in each of the MTT assays and the Modified Boyden chamber assays.

Statistical analysis. Correlations between the clinicopathological parameters and the expression of SPC18 were analyzed using the χ^2 test. Kaplan–Meier survival curves were constructed for SPC18-positive and SPC18-negative patients. The survival rates of the SPC18-positive and SPC18-negative groups were compared. Differences between the survival curves were tested for statistical significance by the Log-rank test. Univariate and multivariate Cox regression analyses were

used to evaluate the associations between clinical covariates and survival. The SPSS software program (SPSS Inc., Chicago, IL, USA) was used for all of the statistical analyses. The hazard ratio (HR) and 95% confidence interval (CI) were estimated from Cox proportional hazard models. Age was treated as a categorical variable (>65 years *vs* ≤65 years). All of the variables that were found to be moderately associated ($P < 0.10$) with survival by a univariate analysis were included in the final multivariate Cox regression models. *P*-values of < 0.05 were considered to indicate statistical significance.

Results

Expression and distribution of SPC18 in CRC and its relationship with clinicopathological parameters. We used immunohistochemistry to investigate the expression of SPC18 in 137 human CRC samples. In the non-neoplastic colonic mucosa, the staining of SPC18 was either weak or absent in epithelial and stromal cells, whereas corresponding CRC tissue showed relatively stronger and more extensive staining (Fig. 1a). SPC18 staining was observed in the cytoplasm of tumor cells (Fig. 1b). In total, 79 (58%) of the 137 CRC cases were positive for SPC18. We found that the cancer cells that robustly expressed SPC18 were preferentially located in the deeper invasive region of the tumors (Fig. 1c). Next, we analyzed the relationship between the expression of SPC18 and various clinicopathological characteristics. SPC18-positive CRC cases showed a more advanced *N* classification ($P = 0.0315$), and tumor stage ($P = 0.0240$) than SPC18-negative CRC cases (Table 1). The expression of SPC18 was not associated with age, sex, M classification or budding grade.

The relationship between the expression of SPC18 in CRC and the prognosis. We performed a Kaplan–Meier analysis to investigate the association between the expression of SPC18 and the prognosis in order to further elucidate the clinical impact of SPC18 on CRC in our 137 patients. The expression of SPC18 was significantly associated with a poorer prognosis ($P = 0.0055$, Log-rank test, Fig. 1d). Univariate and multivariate Cox proportional hazards analyses were used to further evaluate the association between the expression of SPC18 and survival in CRC patients ($n = 137$, Table 2). In the univariate analysis, the expression of SPC18 (hazard ratio [HR] 2.74; 95% confidence interval [CI] 1.28–6.50; $P = 0.0078$), tumor stage (HR 5.08; 95% CI 2.39–12.04; $P < 0.0001$) and budding grade (HR 3.41; 95% CI 1.69–7.31; $P = 0.0006$) were found to be associated with survival. We also performed a multivariate analysis using a model that which included the expression of SPC18, tumor stage and budding grade. The expression of SPC18 and tumor stage were found to be independent prognostic predictors for survival in CRC patients (HR 2.47; 95% CI 1.06–5.44; $P = 0.0336$).

Effect of SPC18 inhibition on the cell growth and invasive activity of CRC cells. We performed a biological study of SPC18 using CRC cell lines. Western blotting revealed that all

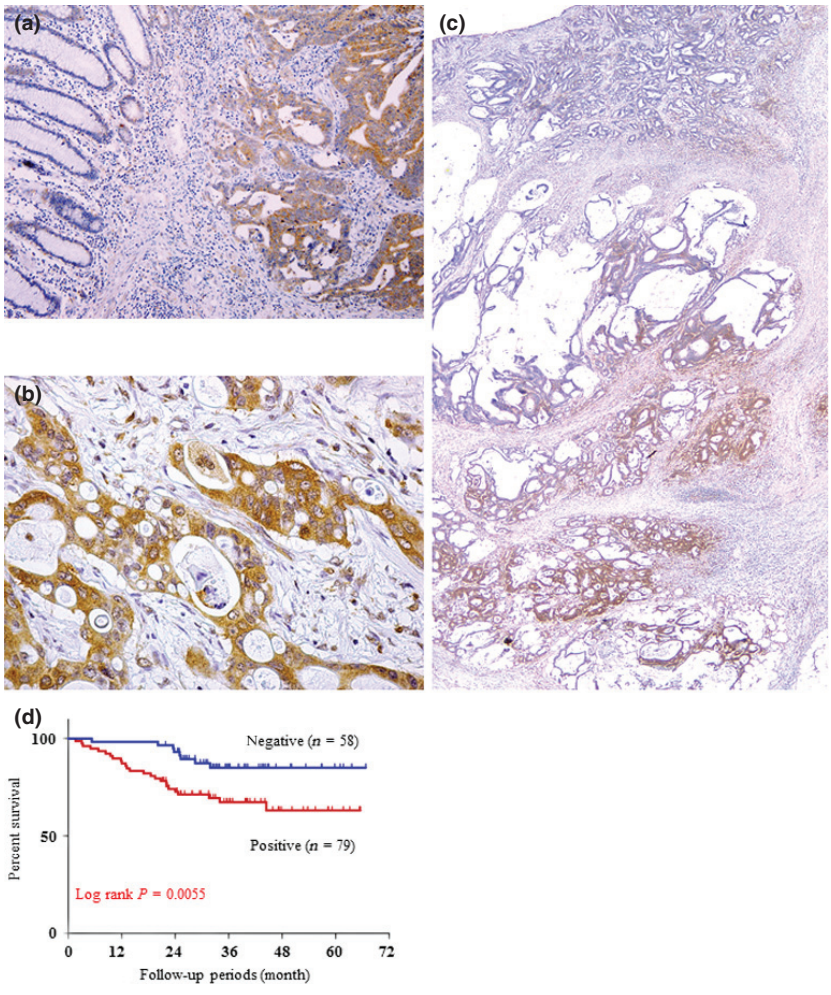

Fig. 1. The immunohistochemical analysis of signal peptidase complex 18 (SPC18) in colorectal cancer (CRC) tissue specimens. (a) SPC18 immunostaining in the non-neoplastic colonic mucosa and CRC. SPC18 staining was not observed in the non-neoplastic colonic mucosa, whereas SPC18 staining was observed in CRC cells (original magnification ×40). (b) SPC18 immunostaining in CRC (original magnification ×400). SPC18 staining was observed in CRC cells in the cell cytoplasm. (c) SPC18 immunostaining in CRC (original magnification ×20). SPC18 staining was observed to be stronger in the deeper invasive region of the tumors of CRC. (d) Kaplan–Meier plot of the survival of CRC patients.

Table 1. Relationship between SPC18 expression and clinicopathological characteristics in 137 patients with colorectal cancer

	SPC18 expression		
	Positive	Negative	*P*-value
Age			
≤65	37 (56%)	28	NS
>65	42 (58%)	30	
Sex			
Female	52 (60%)	35	NS
Male	27 (46%)	23	
T classification			
T1/2	24 (50%)	24	NS
T3/4	55 (62%)	34	
N classification			
Negative	40 (50%)	40	0.0315
Positive	39 (68%)	18	
M classification			
Negative	65 (55%)	54	NS
Positive	14 (78%)	4	
Tumor stage			
Stage I/II	38 (49%)	39	0.0240
Stage III/IV	41 (68%)	19	
Location			
Right	17 (65%)	9	NS
Left	62 (56%)	49	
Budding grade			
Low (Grade 1)	46 (56%)	36	NS
High (Grade 2/3)	33 (60%)	22	
Histologic classification			
Well/moderate	77 (59%)	54	NS
Poor/mucinous	2 (33%)	4	

P-values were calculated by Fisher's exact test. NS, not significant.

Table 2. Univariate and multivariate analysis of factors influencing survival in 137 patients with colorectal cancer

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age				
≤65	1 (Reference)	NS		
>65	1.37 (0.69–2.77)			
Sex				
Female	1 (Reference)	NS		
Male	1.27 (0.63–2.73)			
Tumor stage				
Stage I/II	1 (Reference)	<0.0001	1 (Reference)	0.0041
Stage III/IV	5.08 (2.39–12.04)		3.34 (1.44–8.36)	
Budding Grade				
Low (Grade 1)	1 (Reference)	0.0006	1 (Reference)	NS
High (Grade 2/3)	3.41 (1.69–7.31)		1.86 (0.87–4.26)	
Histlogic classification				
Well/ moderate	1 (Reference)	NS		
Poor/ mucinous	1.54 (0.249–5.101)			
SPC18 expression				
Negative	1 (Reference)	0.0078	1 (Reference)	0.0336
Positive	2.74 (1.28–6.50)		2.47 (1.06–5.44)	

P-values were calculated by Fisher's exact test. NS, not significant.

six CRC cell lines expressed SPC18 at various levels (Fig. 2a). The highest expression of SPC18 was detected in DLD-1 followed by LoVo. The other four remaining cell lines had expressed SPC18 at moderate or low levels. Next, we examined the transition of the expression of SPC18 by Western blotting using DLD-1 and LoVo cell lines that had been transfected with three *SEC11A*-specific siRNAs (siRNA1, 2, and 3) because the highest expression of SPC18 was detected in both of these cell lines (Fig. 2b). The expression of the SPC18 protein in DLD-1 and LoVo was strongly suppressed by treatment with siRNA 1 and 3. Thus, we used siRNA1 and 3 in the following experiments to knockdown the endogenous SPC18. To investigate the possible anti-proliferative effects of SPC18 knockdown, we performed an MTT assay at 4 days after the transfection of siRNA. The viability of the SPC18 siRNA1 and the 3-transfected DLD-1 cells was significantly in comparison to negative control siRNA-transfected DLD-1 cells (Fig. 2c). We performed the same assay in LoVo cells, and similar results were obtained (Fig. 2d). Next, we performed a transwell invasion assay to determine the possible role of SPC18 in the invasiveness of CRC cells (Fig. 2e,f). On day 2, the invasiveness of both DLD-1 and LoVo with SPC18 knockdown was <30% that of the negative control siRNA-transfected DLD-1 cells.

Effect of SPC18 inhibition on the phosphorylation of EGFR, AKT and ERK in CRC cells. We hypothesized that decreasing the SPC18 activity through *SEC11A* knockdown could reduce the secretion of TGF-α because we previously revealed that SPC18 contributes to the progression of GC via the secretion of TGF-α.[11] It is well-known that TGF-α can phosphorylate the EGF receptor (EGFR), which subsequently stimulates the multiple signaling pathways involved in cellular proliferation, anti-apoptosis and other processes, including the Ras-Mek-Erk and Akt-PI3k pathways.[22,23] To confirm the SPC18-induced activation of EGFR signaling in CRC, we analyzed the phosphorylation of EGFR, Akt and Erk in CRC cells with SPC18 inhibition. The levels of phosphorylated EGFR, Erk and Akt in the DLD-1 and LoVo cells transfected with SPC18 siRNA1 or SPC18 siRNA3 were lower than that with negative control siRNA (Fig 3a,b). These data suggest that SPC18 could contribute to tumor progression in CRC.

Analysis of the correlation between the expression of SPC18 and CRC-related molecules. We revealed that SPC18 could contribute to tumor progression in CRC and the distribution of the expression of SPC18 in the deeper invasive region of tumors. We next investigated the relationship between the expression of SPC18 and CRC-related major molecules, including β-catenin nuclear localization, MMP7 and p53 (Fig. 4a–d). We revealed that the expression of SPC18 was colocalized with β-catenin nuclear localization ($P = 0.0251$) and the expression of MMP7 ($P = 0.0218$) at the invasive front (Table 3). These data indicated the possibility of a correlation between SPC18 and Wnt/β-catenin signaling.

The analysis of the expression of SPC18 in pre-cancerous lesions in each colorectal carcinogenesis pathway. Finally, we performed an immunohistochemical analysis of SPC18 in 122 human colorectal polyp specimens, including CALG, CAHG, TSA and SSA/P (Fig. 5a–f). Staining of strong or moderate intensity was more frequently observed in CAHG than in CALG ($P = 0.0002$). There was no significant difference between

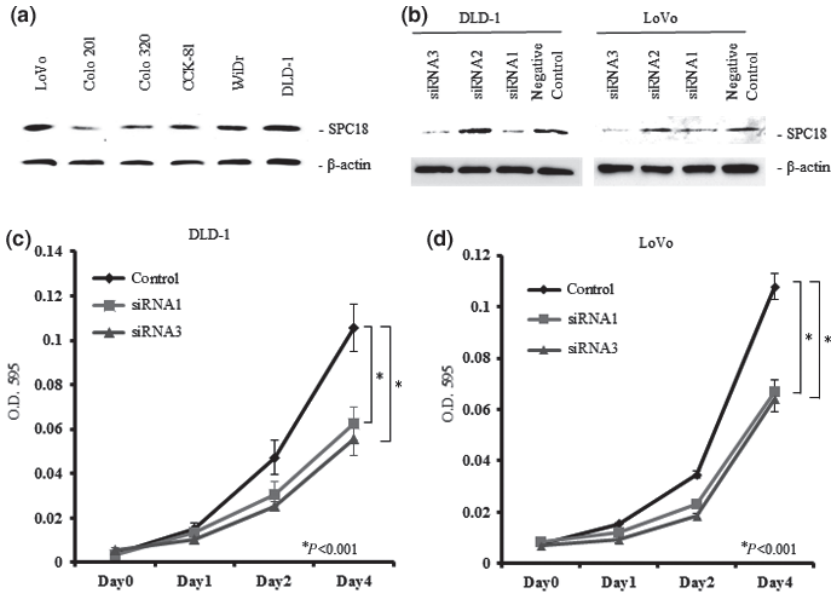

Fig. 2. The effects of the inhibition of signal peptidase complex 18 (SPC18) on cell growth and invasive activity in colorectal cancer (CRC) cells. (a) The Western blotting of SPC18 in the cell lysates from six CRC cell lines. (b) The Western blotting of SPC18 in cell lysates from DLD-1 and LoVo transfected with SPC18 siRNA or negative control siRNA. β-Actin was included as a loading control. (c, d) The effect of SPC18 knockdown on the cell growth of DLD-1 (c) and LoVo (d). Cell growth was assessed by an MTT assay at 1, 2 and 4 days after seeding on 96-well plates. The mean (bars) and standard deviation (SD; error bars) of three independent experiments are shown. (e, f) The effect of the knockdown of SPC18 on cell invasion in DLD-1 (e) and LoVo (f) transfected with SPC18 siRNA (siRNA1 and 3) or negative control siRNA that were incubated in Boyden chambers. After 1 and 2 days, the invading cells were counted. NS, not significant.

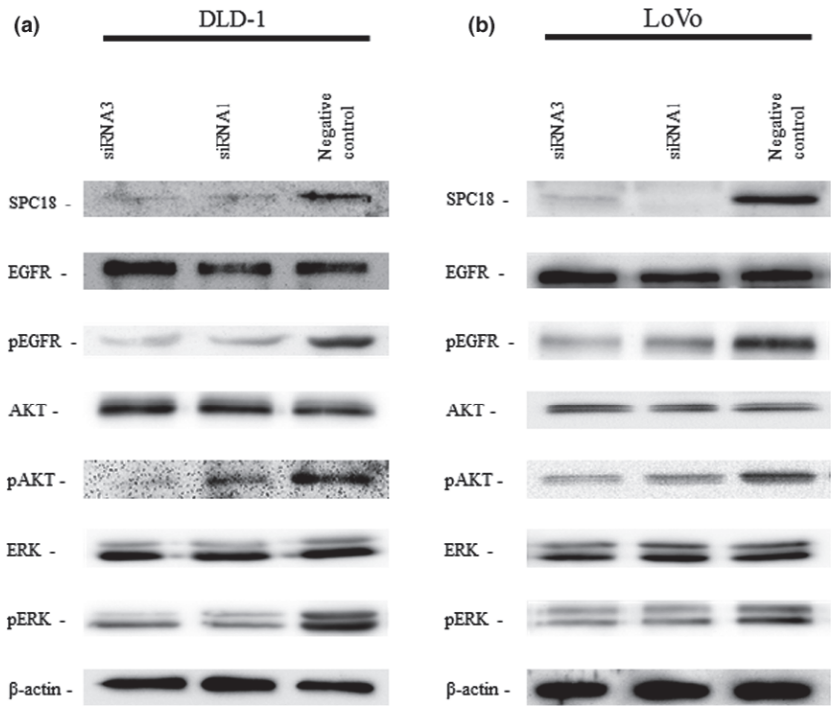

Fig. 3. The effect of the downregulation of signal peptidase complex 18 (SPC18) on the epidermal growth factor receptor (EGFR) signaling pathway. (a, b) Western blotting of SPC18, EGFR, phospho-EGFR (pEGFR), Erk1/2, phospho-Erk1/2 (pErk1/2), Akt, and phospho-Akt (pAkt) in cell lysates from DLD-1 (a) and LoVo (b) transfected with *SEC11A* siRNA or negative control siRNA. β-Actin was included as a loading control.

tubular adenoma component and villous component. Furthermore, the expression of SPC18 was significantly stronger in conventional adenoma-carcinoma pathway-related tumors (CALG and CAHG) than in serrated pathway related tumors (HP, TSA and SSA/P) ($P < 0.001$) (Fig. 5g). Our results imply that the expression of SPC18 is correlated with the progression

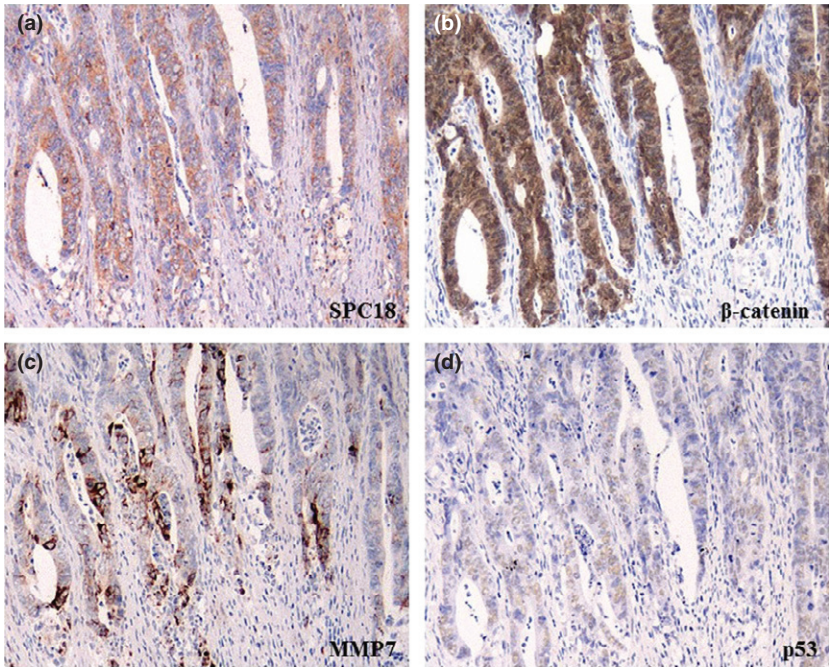

Fig. 4. The analysis of the correlation between the expression of signal peptidase complex 18 (SPC18) and colorectal cancer (CRC)-related molecules in the invasive front. (a) The expression levels of SPC18 (b–d) CRC-related molecules, including β-catenin, Matrix metalloproteinase 7 (MMP7) and p53, were examined. A serial section showed that the MMP7 and β-catenin nuclear localization at the invasive front was partially adjacent to the area in which SPC18 was expressed.

Table 3. Relationship between SPC18 expression and CRC related molecules at the invasive frontin 137 patients with colorectal cancer

	SPC18 expression		
	Positive	Negative	*P*-value
β-catenin (nuclear localization)			
Positive	58 (66%)	29	0.0251
Negative	21 (54%)	25	
MMP7			
Positive	29 (72%)	11	0.0218
Negative	50 (52%)	47	
p53 expression			
Positive	41 (58%)	29	NS
Negative	38 (56%)	29	

P-values were calculated by Fisher's exact test. NS, not significant.

of CRCs that take the conventional adenoma-carcinoma pathway. We also performed an immunohistochemical analysis of SPC18 in microsatellite instability-high (MSI-H) CRC, which is considered to be a serrated pathway related tumor.[24–26] Notably, the expression of SPC18 was only detected in one (10%) of 10 MSI-H CRC specimens (Fig. 5h). There is a possibility that SPC18 is involved in the pathogenesis of the conventional adenoma-carcinoma pathway-related colorectal tumor rather than serrated pathway-related colorectal tumors.

Discussion

In the current study, we analyzed the clinicopathological significance of SPC18 in CRC. Our immunohistochemical analyses demonstrated that 58% of CRC cases were positive for SPC18. The distribution of SPC18-positive CRC cells was more frequently detected in the deeper invasive region of the tumors. Moreover, the expression of SPC18 was associated with the *N* classification and tumor stage. The expression of SPC18 also served as an independent prognostic classifier of patients with CRC. The histological features of CRC differ widely from area to area within the same tumor due to tumor heterogeneity. The most useful clinicopathological features and molecular signatures, including the budding grade, can be deduced from the invasive front of the tumor, where the most transformed and presumably most aggressive cells reside.[27,28] Although the expression of SPC18 was not significantly correlated with the budding grade, SPC18 tended to be observed at the invasive front. Thus, SPC18 is likely to promote tumor progression through several secretion proteins, especially at the invasive region in SPC18 positive cases. Indeed, the expression of SPC18 was correlated with β-catenin nuclear localization and the expression of MMP7.

Previous studies have shown that the increased SPC activity caused by the overexpression of SPC18 protein induced tumor progression through the secretion of TGF-α, to phosphorylate EGFR and stimulate the multiple signaling pathways involved in cellular proliferation, anti-apoptosis and other processes.[11,23,24] The increased expression of TGF-α in colonic polyps and cancers has also been reported.[29] Indeed, the present study showed that the knockdown of *SEC11A* by RNA interference inhibited cancer cell proliferation and invasiveness in CRC cell lines. Moreover, we showed that the levels of phosphorylated EGFR and its downstream molecules, including Erk and Akt, were lower in SPC18 siRNA-transfected CRC cells than in control cells. It was reported that the phosphorylation of Erk and Akt results in inhibition of apoptosis and contribute to tumor progression, including metastasis.[30,31] Thus, these results suggest that SPC18 participates in malignant behavior, including the cell growth, metastasis and invasion of CRC cells via several growth factors, including at least TGF-α.

The main colorectal carcinogenesis pathways are the conventional adenoma-carcinoma pathway and the serrated pathway.[15,16] The present study demonstrated a sequential increase in the expression of SPC18 through the conventional adenoma-carcinoma pathway. The conventional adenoma-carcinoma pathway is well known as a multistep carcinogenesis mechanism that is associated with the activation of Wnt/β-catenin signaling.[16,24]

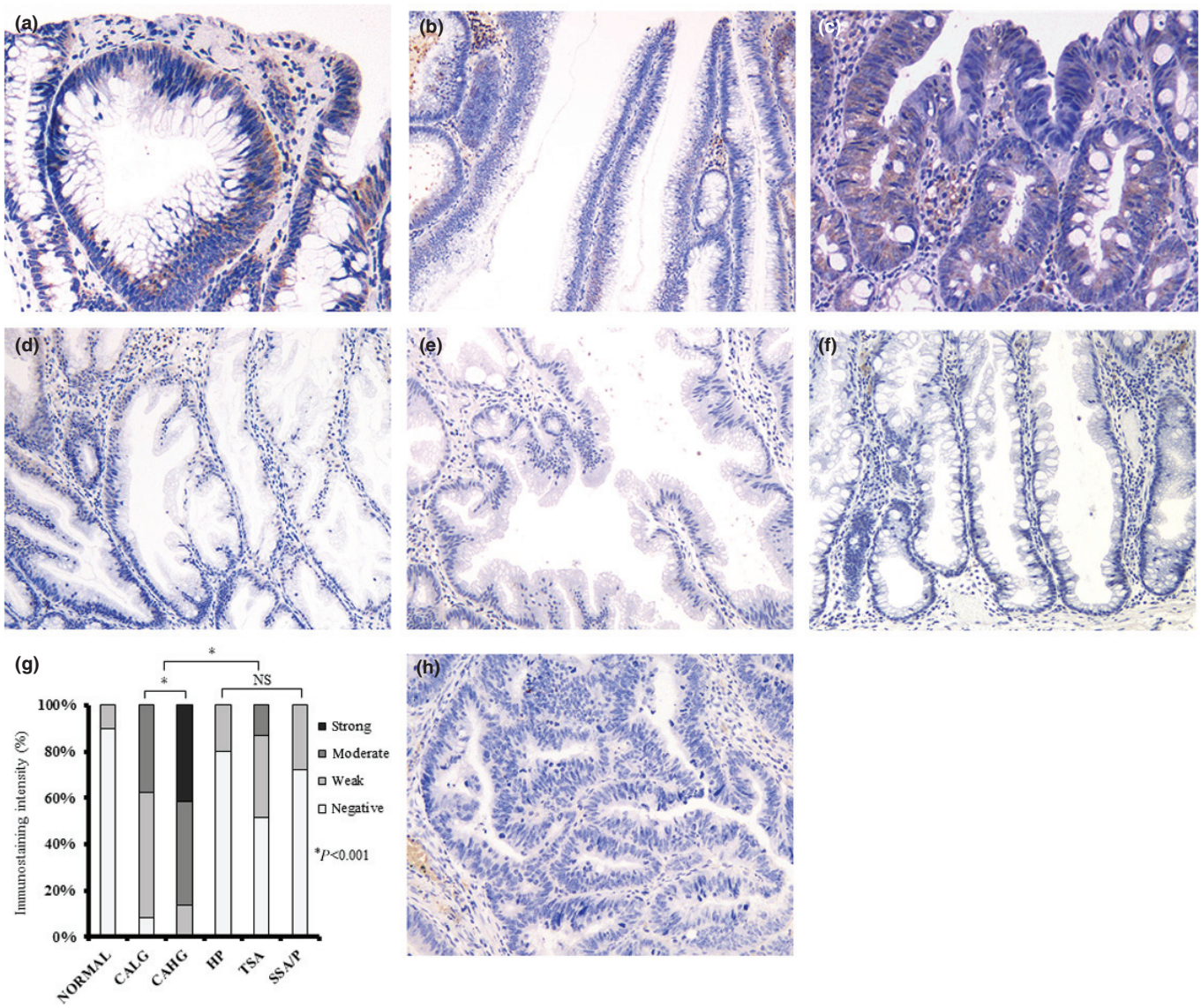

Fig. 5. The analysis of the expression of signal peptidase complex 18 (SPC18) in pre-cancerous lesions of each colorectal carcinogenesis pathway. (a–f) The immunostaining of SPC18 in colorectal polyps (CRPs). (a) Conventional adenoma low-grade: tubular adenoma component, $n = 17$; (b) Conventional adenoma low-grade: villous component, $n = 7$ (c) conventional adenoma high-grade (CAHG), $n = 29$; (d) hyperplastic polyp. $n = 20$; (e) traditional serrated adenoma (TSA), $n = 31$; and (f) sessile serrated adenoma/polyp (SSA/P), $n = 18$. (g) The SPC18 immunostaining scores in CRPs. The graph indicates the percentage of sections with different scores (negative, weak, moderate and strong). (h) A representative image of the expression of SPC18 in MSI-H serrated pathway-related CRC. NS, not significant.

It is also well known that the activation of Wnt/β-catenin signaling promotes tumor malignancy and induces the expression of MMP7.[32] In the present study, SPC18 expression was distributed extensively in the whole tumor and was unexceptionally observed at the invasive front. Although the relationship between SPC18 expression and T classification was not statistically significant, SPC18 might contribute to tumor progression. Moreover, β-catenin nuclear localization was also observed at the invasive front due to Wnt signaling activation.[33] Co-localization of these molecules at the invasive front suggests that β-catenin nuclear localization might activate SPC18 expression as well as Wnt pathway signaling. A detailed function analysis should be performed using methods such as siRNA-knock down or an *in vivo* analysis in order to further our understanding of the relationship between SPC18, β-catenin and MMP7.

In summary, we revealed that CRC with the expression of SPC18 was independently associated with a poor prognosis. SPC18 regulates the phosphorylation of EGFR and modulates downstream targets. Thus, SPC18 has potential as a therapeutic target and predictive biomarker for the survival of patients with CRC.

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Disclosure Statement

The authors declare no conflicts of interest in association with the present study.

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