学位論文

Doctoral Thesis

CD44 variant 6 is involved in peritoneal dissemination and poor prognosis in patients with advanced epithelial ovarian cancer

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腹膜播種および予後不良に関与している）

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2015年9月

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1. Abstract of the thesis

Cancer stem cells (CSCs) drive tumor metastasis in several types of human cancer. In epithelial ovarian cancer, the CSCs contribution of peritoneal metastasis has remained unclear. The cell adhesion molecule CD44 has been identified as a major marker for CSCs in solid tumors, including ovarian cancer. CD44 exists as a standard form (CD44s) and also as numerous variant isoforms (CD44v) generated by alternative mRNA splicing. Here I show that disseminated ovarian tumors in the pelvic peritoneum contain highly enriched CD44v6-positive cancer cells, which drive tumor metastasis. Clinically, an increased number of CD44v6-positive cancer cells in primary tumors was associated with a shortened overall survival in stages III–IV ovarian cancer patients. In addition, a subpopulation of CD44v6-positive cancer cells manifested the ability to initiate tumor metastasis in the pelvic peritoneum in an *in vivo* mouse model, suggesting that CD44v6-positive cells show the potential to serve as metastasis-initiating cells. Taken together, the peritoneal disseminated metastasis of ovarian cancer is initiated by the CD44v6-positive subpopulation, and CD44v6 expression is a biomarker for the clinical outcome of advanced ovarian cancer patients. Given that a distinct subpopulation of CD44v6-positive cancer cells plays a critical role in peritoneal metastasis, definitive treatment should target this subpopulation of CD44v6-positive cells in ovarian cancer.

2. Reference Article

2-1. Companion article

Tjhay F, Motohara T, Tayama S, Narantuya D, Fujimoto K, Guo J, Sakaguchi I, Honda R, Tashiro H, and Katabuchi H. CD44 variant 6 is correlated with peritoneal dissemination and poor prognosis in patients with advanced epithelial ovarian cancer. ***Cancer Science*** 106: 1421-1428, 2015.

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I would also like to thank my Molecular Oncology Group member and staffs in the Department of Obstetrics and Gynecology, Kumamoto University. I would especially like to thank the physicians, nurses, and officers at Kumamoto University. All of you have been there to support me when I collected patient data for my study. I would like to thank the Alumni of Department of Obstetrics and Gynecology, Kumamoto University for giving me the research fellowship scholarship in my research fellowship time. I would like to thank to the Hashiya Foundation for their financial support granted through the Hashiya Scholarship. I would like to thank the Embassy of Republic of Indonesia staff and Atma Jaya Catholic University of Indonesia colleagues for supporting my study. A special thanks to my family and friends. Your prayers and support for me was what has sustained me. This thesis could not have been completed without all the great support that I have received from all of you over the years.

To anyone that I may have forgotten to mention here, I apologize and thank you as well. No words can express how grateful I am to all of you.

4. List of Abbreviations

APC:	Allophycocyanin
CD44s:	CD44 standard
CD44v6:	CD44 variant 6
CSCs:	Cancer stem cells
DAB:	Diaminobenzidine peroxidase
ELDA:	Extreme limiting dilution analysis
EMT:	Epithelial-mesenchymal transition
FACS:	Fluorescence-activated cell sorting
FBS:	Fetal bovine serum
FIGO:	International Federation of Gynecology and Obstetrics
HR:	Hazard ratio
PBS:	Phosphate-buffered saline
RT-PCR:	Real-time reverse transcriptase-polymerase chain reaction
SD:	Standard deviation
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide
WHO:	World Health Organization

5. Research background

5-1. Epithelial ovarian cancer

Epithelial ovarian cancer is the leading cause of death from gynecological malignancies[1-4]. Because most patients with ovarian malignancies are generally asymptomatic until the cancer has progressed and metastasized, more than two-thirds of tumors are diagnosed at an advanced stages III–IV with multiple disseminated tumors in the pelvic peritoneum[5](Fig. 1). Ovarian cancer can spread through lymphatics to nodes and through blood vessels to the parenchyma of the liver, lung, and brain. Especially, small clusters of ovarian cancer cells shed by the ovary metastasize to the peritoneal surface, forming numerous disseminated nodules. Once the ovarian cancer cells have detached as single cells or clusters from the primary ovarian tumors, they have metastasized through a passive mechanism, carried by the physiological movement of peritoneal fluid to the peritoneum. Furthermore, peritoneal cells are known to express and secrete several extracellular matrix proteins, adhesion molecules that provide an important microenvironment to aid the implantation of ovarian cancer cells. Thus, microenvironment of peritoneum and interaction between ovarian cancer cells and peritoneal cells are believed to critical for tumor initiation, tumor spread, metastasis in the pelvic peritoneal cavity.

The clinical outcomes for women diagnosed with advanced ovarian cancer are poor even after treatment with extirpative surgery and intensive chemotherapy. Although the cancer may respond to primary therapy, chemoresistant residual cancer cells can persist in a dormant state for many months in the pelvic peritoneum, leading to relapse[6,7]. Therefore, elucidating the molecular events that control peritoneal metastasis may provide potential molecular targets for the treatment of advanced ovarian cancer with multiple peritoneal disseminated tumors.

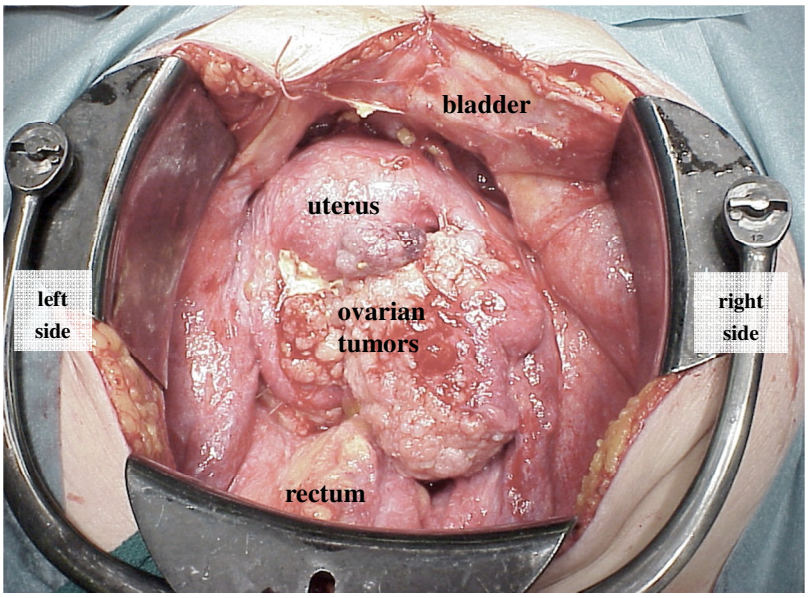

Fig 1. Gross aspect of the pelvic cavity in patients with advanced ovarian cancer.

5-2. CD44

CD44 cell adhesion molecule is a membrane glycoprotein that binds hyaluronic acid and contributes to tumor growth and metastasis[8-10]. CD44 exists as a standard form (CD44s) and also as numerous variant isoforms (CD44v) generated by alternative mRNA splicing of up to 10 variant exons that encode parts of the extracellular domain[9-13](Fig. 2). Among CD44v isoforms, CD44v6 was initially found to promote the metastatic potential of a rat pancreatic adenocarcinoma cell line[14]. In addition, several previous studies supported the premise that CD44v6 plays a key role in cancer proliferation, migration, and invasion in a variety of human cancers, such as colorectal, breast, lung, and ovarian cancers[15-18]. In ovarian cancer, it is known that CD44v6 promotes tumor metastasis by binding hyaluronic acid on peritoneal mesothelial cells[19].

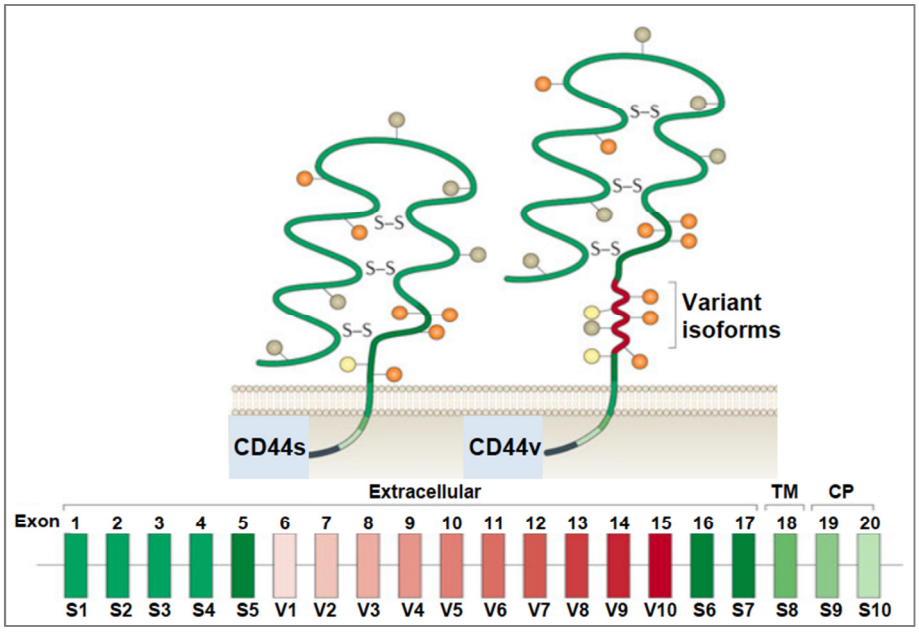

Fig. 2. CD44 consists of several exons, some of which are constant region exons that are used in every CD44 mRNA and protein and others are variant exons that are used in the CD44 variant proteins and are selected by alternative splicing.

5-3. Concept of cancer stem cells (CSCs)

The cancer stem cells (CSCs) theory has proposed that the bulk of tumor cells are generated by a rare population of tumor-initiating cells, conceptually termed CSCs[20-22](Fig. 3). CSCs possess the ability to self-renew and differentiate into a heterogeneous lineage of cancer cells and inherently drive the metastatic process[21]. CD44 has been identified as one of the major cell surface markers associated with CSCs in several types of epithelial tumors, including ovarian cancer[9,23-25]. Intriguingly, recent studies indicated that a subpopulation of CD44v6-positive cells shows a characteristic phenotype of CSCs in colorectal cancer, bladder cancer, and brain tumor[26-28]. These findings led me to hypothesize that CD44v6-positive ovarian cancer cells may possess CSCs traits and play a key role in tumor initiation and disseminated metastasis[29].

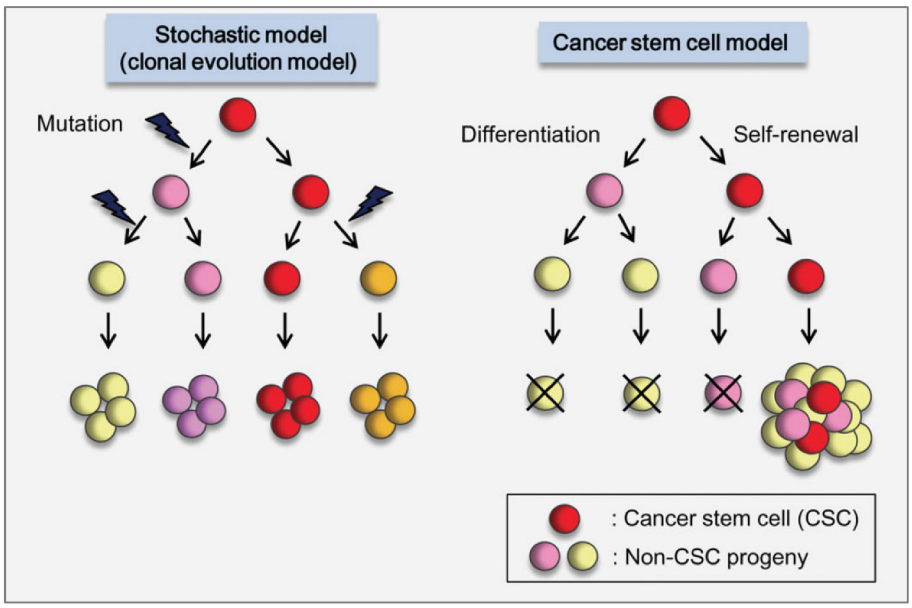

Fig. 3. The stochastic model and CSCs model are the two major models to account for heterogeneity of tumors. In the stochastic model, all tumor cells possess tumorigenic activity and are the product of clonal evolution. In the CSCs model, only a subpopulation of tumor cells possesses high tumorigenic activity, with these cells representing the top of a hierarchical organization similar to that of normal tissue. CSCs undergo self-renewal and generate differentiated progeny cells.

5-4. Aim of the study

Uncovering the molecular mechanisms underlying peritoneal metastasis is the final frontier in ovarian cancer biology. Even though ovarian CSCs have not been fully elucidated, these cells are thought to play a crucial role in disseminated metastasis and relapse at peritoneal metastatic sites[30]. Given that peritoneal dissemination is responsible for most cancer-related deaths in patients with advanced ovarian cancer, the elucidation of molecular biology of the peritoneal metastasis and the characteristics of ovarian CSCs is essential to eradicate ovarian cancer. In addition, although previous studies focused on the relationship of CD44v with ovarian cancer survival to address the prognostic values of CD44v, there is no unified view on this issue.

The present study was designated to evaluate the role of CD44v6 in peritoneal disseminated metastasis and the potential relevance of CD44v6 to the clinical outcome of patients with advanced ovarian cancer with long-term follow-up.

6. Materials and Methods

6-1. Patients and tissue preparation From January 2002 to December 2012, the clinical records of advanced ovarian cancer patients were reviewed retrospectively, and 59 patients with peritoneal disseminated tumors who underwent primary standard surgery followed by proper chemotherapy at Kumamoto University Hospital were selected in this study. The eligible patients were followed-up until December 2014.

Tumor tissues obtained surgically were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4-μm thickness for histological diagnosis. Sections were stained with hematoxylin and eosin, and histologic typing was performed according to the WHO classification of surface epithelial-stromal ovarian tumors[31]. All tumors were staged according to the FIGO criteria[32].

6-2. Immunohistochemical staining Immunohistochemical analysis was performed as described previously[33]. Briefly, the sections were washed with phosphate-buffered saline, subjected to antigen retrieval by heating in a microwave in 0.01 M sodium citrate buffer for 15 min, and exposed to 3% H_2O_2 in methanol before staining with the primary antibody. Immune complexes were detected with use of the avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA, USA) and diaminobenzidine (DAB) substrate (Vector Laboratories), and the sections were counterstained with hematoxylin. CD44v6 was detected with the mouse monoclonal antibody CD44v6 (2F10, R&D Systems, Minneapolis, MN, USA).

The expression level of CD44v6 was quantified as a percentage of the total number of stained cells. The primary ovarian tumors that contained at least 10% CD44v6-positive cancer cells were categorized as the "CD44v6-high" group, whereas the tumors that contained less than 10% CD44v6-positive cells were categorized as the "CD44v6-low" group. The percentage of CD44v6-positive cancer cells in primary tumors was evaluated by counting cells in at least three microscopic fields per slide.

6-3. Cell line An ovarian cancer cell line, ES-2, was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). ES-2 cells were maintained in RPMI-1640 medium (Wako Pure Chemical Industries. Ltd., Japan) supplemented with 10% FBS at 37°C in a 5% CO_2-containing atmosphere.

6-4. Flow cytometry and transplantation assay Cell sorting and flow cytometric analysis were performed with the use of a fluorescence-activated cell sorter (FACS) Aria II (BD Biosciences, San Jose, CA, USA). Cells were incubated with allophycocyanin (APC)-conjugated mouse monoclonal antibody CD44v6 (2F10, R&D Systems) for 30 min. FACS-sorted CD44v6-positive or -negative cancer cells were suspended in RPMI-1640 medium and injected into 7-week-old female BALB/c nude mice. Tumor-initiating frequencies were assessed with the use of ELDA software for limiting dilution analysis[(34)].

6-5. Statistical analysis Survival rates were calculated using the Kaplan–Meier method, and differences between curves were assessed with the log-rank test. Correlations between variables were evaluated with the χ^2 test, Fisher's exact test, Mann–Whitney *U* test, or Wilcoxon test. Data are presented as mean ± standard deviation (SD) and were analyzed with the Student's *t* test. Univariate and multivariate Cox proportional hazard model analyses were performed to calculate hazard ratios (HRs) using SPSS. In all analyses, a *P* value of <0.05 was

considered statistically significant.

7. Results

7-1. No significant correlation between the expression of CD44v6 and clinicopathological characteristics

The relationship between CD44v6 expression and the clinicopathological characteristics of the 59 patients was shown in Table 1. Thirteen (22.0%) cases belonged in the CD44v6-high group, and 46 (78.0%) cases in the CD44v6-low group. The median age of all patients at diagnosis was 57 years (range of 37-82 years). There was no significant difference in the median age between the CD44v6-high and CD44v6-low groups. Additionally, no significant correlation was observed between the immunohistochemical expression of CD44v6 and clinicopathological characteristics, such as tumor histological type, tumor marker CA125, and tumor size. Adjuvant systematic chemotherapy was administered as clinically indicated in accordance with standard practices, and almost all of patients received paclitaxel-carboplatin as first-line adjuvant chemotherapy. No significant difference was recorded in the distribution of the number of cycles of chemotherapy between CD44v6-high and CD44v6-low groups (Table 1).

Table 1. Association between the CD44v6 expression pattern and clinicopathological characteristics in patients with stages III–IV ovarian cancer

	All cases	CD44v6-high group	CD44v6-low group	*P* value
All cases	59	13	46	
All cases (mean ± SD)	57 (37-82)	59 (43-82)	56 (37-77)	0.840
<50 years	18 (30.5%)	3 (23.1%)	15 (32.6%)	0.510
≥50 years	41 (69.5%)	10 (76.9%)	31 (67.4%)	
Histological type				
serous	42 (71.2%)	7 (53.8%)	35 (76.1%)	0.120
clear	3 (5.1%)	2 (15.4%)	1 (2.2%)	
endometrioid	5 (8.5%)	1 (7.7%)	4 (8.7%)	
mucinous	1 (1.7%)	1 (7.7%)	0 (0.0%)	
mixed	7 (11.8%)	1 (7.7%)	6 (13.0%)	
undifferentiated	1 (1.7%)	1 (7.7%)	0 (0.0%)	
CA125 (U/ml)				
<500	18 (30.5%)	6 (46.2%)	12 (26.1%)	0.165
≥500	41 (69.5%)	7 (53.8%)	34 (73.9%)	
Tumor size				
<10cm	40 (67.8%)	7 (53.8%)	33 (71.7%)	0.223
≥10cm	19 (32.2%)	6 (46.2%)	13 (28.3%)	
First - line chemotherapy				
Paclitaxel / carboplatin	57 (96.6%)	12 (92.3%)	45 (97.8%)	0.332
Other	2 (3.4%)	1 (7.7%)	1 (2.2%)	
No. of cycles of chemotherapy				
<2	41 (69.5%)	8 (61.5%)	33 (71.7%)	0.481
≥3	18 (30.5%)	5 (38.5%)	13 (28.3%)	
Residual tumor size				
< 1cm (optimal surgery)	41 (69.5%)	6 (46.2%)	35 (76.1%)	0.038
≥ 1cm (suboptimal surgery)	18 (30.5%)	7 (53.8%)	11 (23.9%)	

7-2. Highly enriched CD44v6-positive cancer cells in peritoneal disseminated tumors

To investigate whether CD44v6-positive cancer cells are associated with peritoneal metastasis, I compared the average number of CD44v6-positive cells among the 59 samples of primary ovarian tumors to that in samples of peritoneal disseminated tumors taken from the same patients. Representative immunohistochemical staining patterns for CD44v6 in primary and disseminated tumors were shown in Fig. 4A and Fig. 4B. Immunohistochemical analysis revealed a significantly higher percentage of CD44v6-positive cells detected in peritoneal disseminated tumors than in corresponding primary ovarian tumors ($P< 0.01$; Fig. 4C). These findings indicated that CD44v6-positive cells are correlated with peritoneal dissemination, and the pelvic peritoneum may have the potential to form a part of niche microenvironment involved in tumor initiation and metastasis.

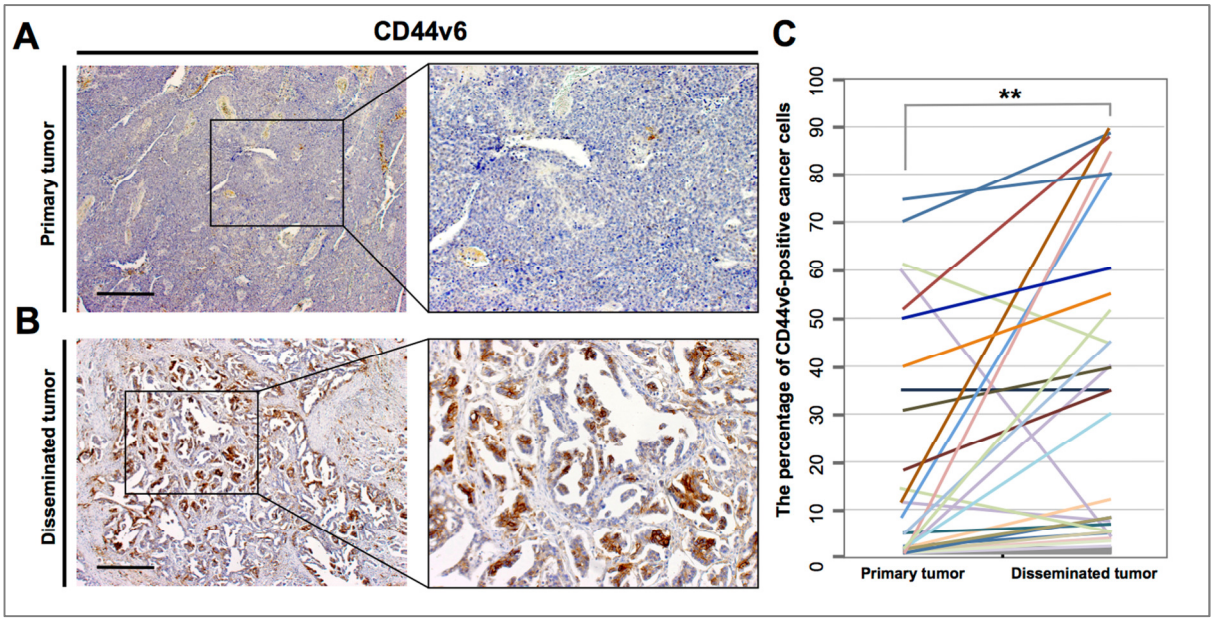

Fig. 4. (A) Immunohistochemical analysis with an anti-CD44v6 antibody in primary ovarian tumors. Scale bar: 500 μm. (B) Immunohistochemical staining with an anti-CD44v6 antibody in peritoneal disseminated tumors. Scale bar: 500 μm. (C) The percentage of CD44v6-positive cancer cells in primary and disseminated tumors. Peritoneal disseminated tumors contained significantly higher percentages of CD44v6-positive cells than primary tumors (Mann–Whitney *U* test, $^{**}P < 0.01$).

7-3. Prognostic impact of CD44v6 expression in advanced ovarian cancer patients

Given that a subpopulation of CD44-positive cancer cells in hierarchically organized ovarian cancer manifests CSCs properties[23], I hypothesized that CD44v6 expression would correlate with aspects of ovarian cancer survival. To address this issue, I performed Kaplan–Meier analysis of overall- and progression-free survival between the CD44v6-high and CD44v6-low groups. Representative immunohistochemical staining patterns for CD44v6 in CD44v6-high and CD44v6-low groups were shown in Fig. 5A and Fig. 5B. In the evaluation of the sites of primary lesions, the 5-year overall survival rates were 18.0% [95% confidence interval (CI), 0.0–40.2] in the CD44v6-high group and 59.6% (95% CI, 44.3–74.8) in the CD44v6-low group. Significant differences were observed in overall survival between the CD44v6-high and

CD44v6-low groups for patients with stages III–IV ovarian cancer (P= 0.0059; Fig. 5C). On the other hand, no significant differences were observed in progression-free survival between the CD44v6-high and CD44v6-low groups (P = 0.4290; Fig. 5D). These findings suggested that CD44v6-positive cancer cells in primary tumors play an important role in the survival of advanced ovarian cancer patients.

Univariate and multivariate analysis of various clinocopathological factors in relation to overall survival were shown in Table 2. Immunohistochemical expression of CD44v6 proved to be a highly predictive factor based on the univariate Cox proportional hazards model (P = 0.007; HR. 2.930; 95% CI, 1.334-6.436) and the multivariate Cox proportional hazards model (P = 0.022; HR, 2.568; 95% CI, 1.149-5.738). In addition, surgical debulking status also significantly correlated with overall survival based on the univariate Cox proportional hazards model (P = 0.011; HR, 2.568; 95% CI, 1.247-5.288) and the multivariate Cox proportional hazards model (P = 0.028; HR, 2.283; 95% CI, 1.091-4.775).

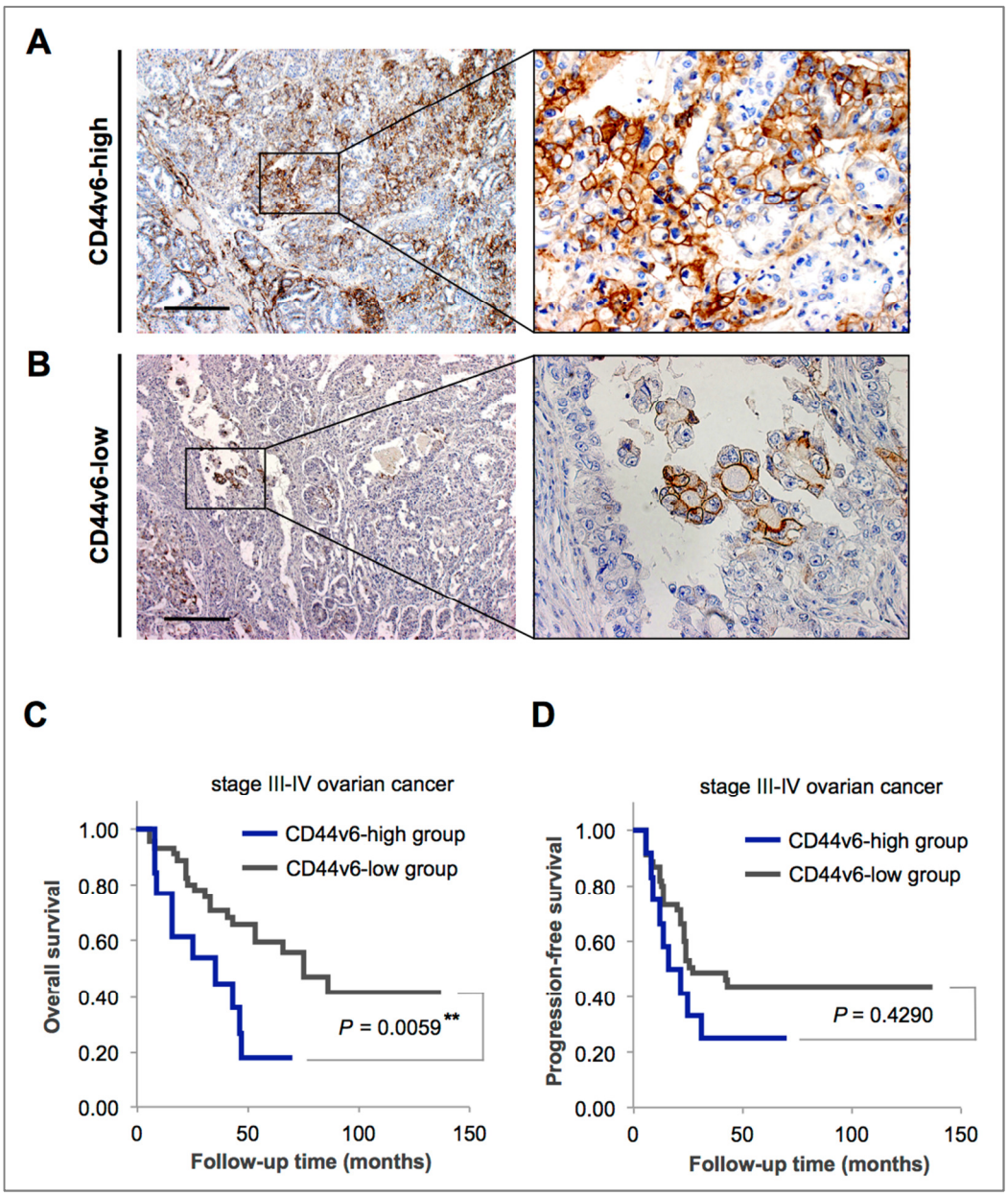

Fig. 5. (A) Immunohistochemical analysis with an anti-CD44v6 antibody in primary ovarian tumors. The tumors that contained at least 10% CD44v6-positive cancer cells were categorized as the CD44v6-high group. Scale bar: 500 μm. (B) The tumors that contained less than 10% CD44v6-positive cancer cells were categorized as the CD44v6-low group. Scale bar: 500 μm. (C) Kaplan–Meier analysis of overall survival in patients with stages III–IV ovarian cancer according to the expression of CD44v6. There were significant differences in overall survival between the CD44v6-high and CD44v6-low groups (**P= 0.0059). (D) Kaplan–Meier analysis of progression-free survival in stages III–IV ovarian cancer patients according to the expression of CD44v6. Progression-free survival was not significantly different between the CD44v6-high and CD44v6-low groups (P = 0.4290).

Table 2. Hazard ratios using univariate and multivariate Cox proportional hazard model in relation to overall survival

	Univariate analysis		
	HR	**95% CI**	***P* value**
All cases (mean ± SD)			
<50 years			
≥50 years	1.286	0.574 - 2.879	0.542
CA125 (U/ml)			
<500			
≥500	1.060	0.487 – 2.306	0.884
Tumor size			
<10cm			
≥10cm	1.063	0.498 – 2.267	0.874
First-line chemotherapy			
Paclitaxel / carboplatin			
Other	0.905	0.122- 6.727	0.923
Surgical debulking status			
Optimal surgery			
Suboptimal surgery	2.568	1.247 – 5.288	0.011
CD44v6 expression			
Low group			
High group	2.930	1.334 - 6.436	0.007

	Multivariate analysis		
	HR	**95% CI**	***P* value**
Surgical debulking status			
Optimal surgery			
Suboptimal surgery	2.283	10.91 – 4.775	0.028
CD44v6 expression			
Low group			
High group	2.568	1.149 – 5.738	0.022

7-4. High metastatic ability in a subpopulation of CD44v6-positive cancer cells

Given that CD44v6-positive cancer cells showed high metastatic potential in patients with advanced ovarian cancer, I next examined the relevance of peritoneal metastasis in a subpopulation of CD44v6-positive cells in an *in vivo* mouse model. To compare the peritoneal metastatic abilities of CD44v6-positive and -negative cancer cells, I sorted CD44v6-positive

and -negative cells from the ES-2 ovarian cancer cell line (Fig. 6A) and serially transplanted them intraperitoneally into nude mice. Limiting-dilution assay revealed that CD44v6-positive cells had a greater tumor initiating ability than CD44v6-negative cells, suggesting that a subpopulation of CD44v6-positive cells is highly efficient at metastatic dissemination (Table 3). CD44v6-positive cells generated extensive disseminated tumors, resulting in massive abdominal distension by hemorrhagic ascites, within 5 weeks of inoculation, whereas CD44v6-negative cells showed little ability to form disseminated tumors in the peritoneal cavity (Fig. 6B). The total weight of peritoneal disseminated tumors formed by CD44v6-positive cells was significantly greater than that of those formed by CD44v6-negative cells ($P< 0.05$; Fig. 6C). In addition, transplantation of CD44v6-positive cells caused a significant increase in the ascitic volume in comparison with that resulting from transplantation of CD44v6-negative cells ($P< 0.05$; Fig. 6D). A representative immunohistochemical staining pattern for CD44v6 in peritoneal disseminated tumors generated by CD44v6-positive cancer cells was shown in Fig. 6E. These results suggested that CD44v6-positive cells play a crucial role in the formation of disseminated tumors in the pelvic peritoneum and have the potential to contain specialized metastasis-initiating cells.

I next compared the tumor invasiveness between CD44v6-positive and –negative cells by wound healing assay, and found that CD44v6-positive cancer cells resulted in a significance increase of invasiveness in ovarian cancer cells, than CD44v6-negative cancer cells (Fig. 7).

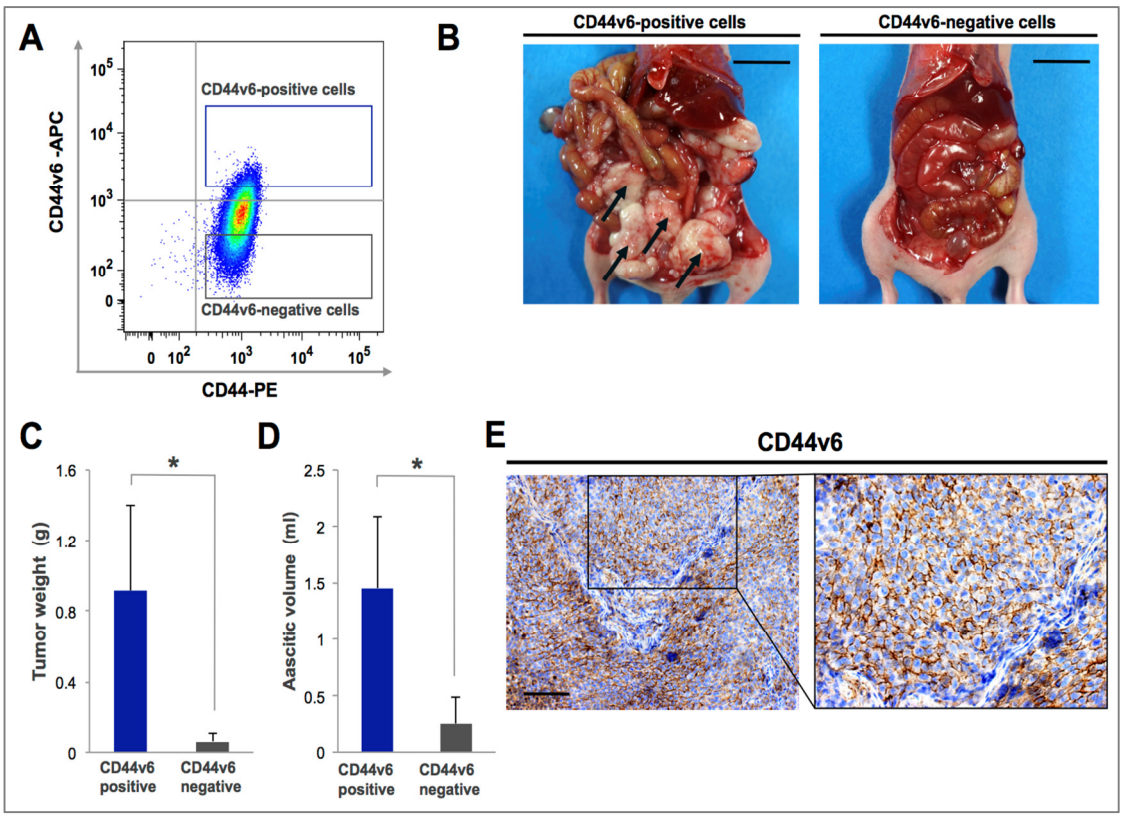

Fig. 6. (A) Flow cytometric analysis of CD44v6 expression in ES-2 ovarian cancer cells. (B) Macroscopic appearance of disseminated tumors at 35 days after cell transplantation. CD44v6-positive cells generated more extensive disseminated tumors in the peritoneal cavity than CD44v6-negative cells. Scale bars: 20 mm. (C) Total weight of peritoneal disseminated tumors determined at 35 days after cell injection. Quantitative data were presented as mean ± SD for five mice. *P< 0.05. (D) Ascitic volume determined at 35 days after transplantation. Quantitative data are presented as mean ± SD for five mice. *P< 0.05. (E) Immunohistochemical analysis with antibody to CD44v6 in peritoneal disseminated tumors in a mouse model. Paraffin-embedded sections of disseminated tumors generated by CD44v6-positive cancer cells were subjected to immunohistochemical staining with an anti-CD44v6 antibody. Scale bar: 200 μm.

Table 3. *In vivo* tumorigenicity of CD44v6-positive and -negative cells.

***In vivo* tumorigenicity of CD44v6-positive and CD44v6-negative cells**				
	No. of transplanted cells			Frequency of metastasis-initiating cell (95% CI)
	10,000	**1,000**	**100**	
CD44v6-positive cells	**6/6**	**6/6**	**4/5**	**62.6** (21.4-185.0)
CD44v6-negative cells	**3/12**	**1/12**	**0/12**	**29,211.0** (10,813.7-78,910.0)

CD44v6-positive and -negative cancer cells were separated by FACS as in Fig. 6A, and the indicated numbers of cells were transplanted into nude mice. The incidence of tumor formation within 8 weeks was scored. Tumorigenic cell frequencies were estimated with the use of ELDA software for limiting dilution analysis.

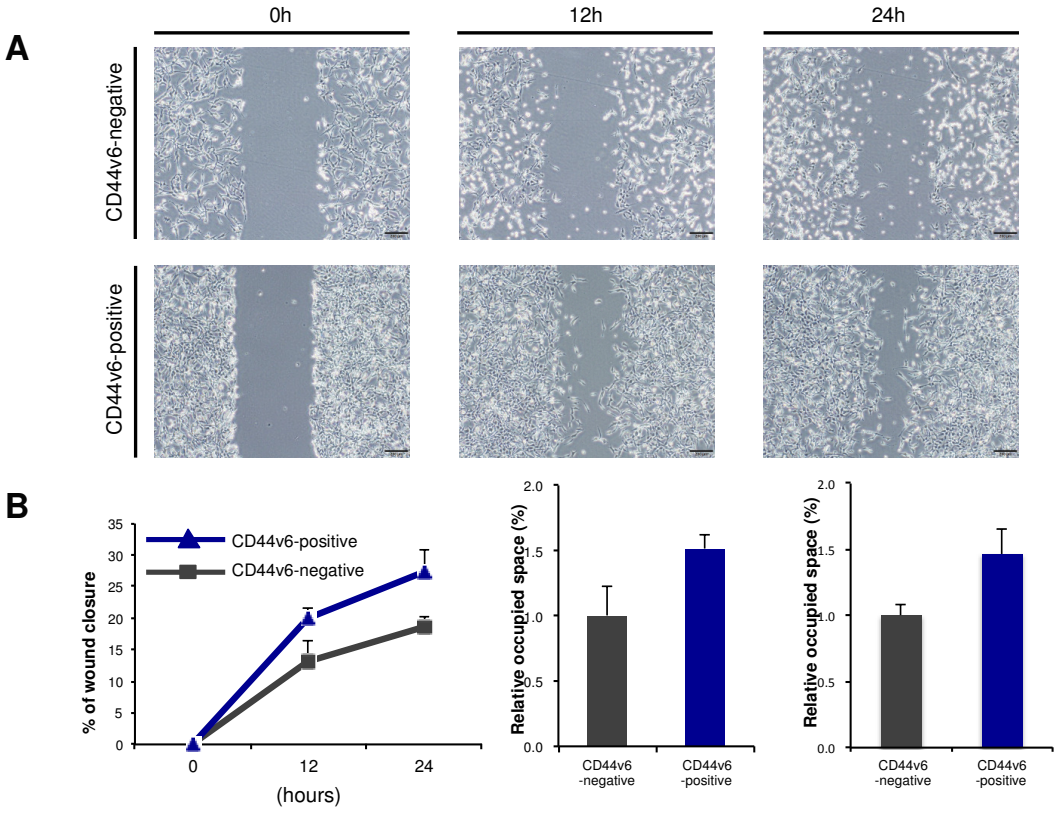

Fig. 7. CD44v6-positive cells induced the invasiveness of ovarian cancer cells in a scratch wound healing assay. Cell invasiveness was quantified according to a wound healing assay, shown by representative images (A), and by quantification (B). **P*<0.05, n=3, Statistics: Student's t-test. The speed of wound closure was monitored at 12 and 24 h by measuring the

ratio of the distance of the wound from 0 h (A). Images were taken at 0, 12 and 24 h with a Nikon Eclipse E600 microscope (Japan) (B) at ×40 magnification and the percentage of migrating cells was quantified using image JMP software Version 5.1 for Windows (SAS Institute Japan, Tokyo, Japan).

8. Discussion

Here, I identified that disseminated tumors in the pelvic peritoneum are highly enriched in CD44v6-positive ovarian cancer cells, which contribute to peritoneal metastasis of advanced epithelial ovarian cancer. Of particular interest in this study was that an increased number of CD44v6-positive cancer cells were associated with a shortened overall survival. Additionally, I demonstrated that a subpopulation of CD44v6-positive ovarian cancer cells possesses a strong ability to initiate tumor metastasis in the pelvic peritoneum in an *in vivo* mouse model, suggesting that CD44v6-positive cells have the potential to serve as metastasis-initiating cells.

Given that peritoneal disseminated metastasis is responsible for most cancer-related deaths in patients with advanced epithelial ovarian cancer, the elucidation of molecular mechanisms underlying the peritoneal metastasis and the characteristics of ovarian CSCs is essential to combat ovarian cancer. Although CD44v6 plays a key role in the tumor growth and metastasis of several types of tumors[26, 27], the functions of CD44v6 have not been completely characterized in ovarian cancer metastasis. In the current study, I demonstrated that CD44v6 expression was increased in tumor tissues at the peritoneal metastasis sites compared with those at the corresponding primary tumors, indicating that CD44v6 is clinically correlated with the peritoneal disseminated metastasis.

Even though previous studies have focused on the potential correlation of CD44v with ovarian cancer survival to address the diagnostic and prognostic values of CD44v, there was no unified view on this issue[35, 36]. Some authors suggested that the expression of the CD44v6 was

not correlated with tumor development and prognosis of ovarian cancer[15], whereas others showed that CD44v6 expression levels were involved in ovarian cancer progression, metastasis, and relapse[37]. Taken together, several questions regarding the relationship between CD44v6 expression and prognosis remain to be resolved. In the light of these unanswered questions, I evaluated the association between CD44v6 expression and overall and progression-survival. As a result, the tumors containing at least 10% CD44v6-positive cancer cells showed significantly poorer prognosis in terms of overall survival than those containing less than 10% CD44v6-positive cells. Furthermore, the multivariate Cox proportional hazards model demonstrated that the expression of CD44v6 is an independent prognostic factor for the overall survival of patients with advanced epithelial ovarian cancer.

Emerging evidence has provided support for the existence of CSCs in various cancers, including ovarian cancer[20]. Even though previous studies demonstrated that a CD44v6-positive cell population possesses CSCs properties in several types of tumors[26, 27], the correlation between CD44v6-positive cells and ovarian CSCs remained unclear. To investigate whether a subpopulation of CD44v6-positive cancer cells manifest highly metastatic activity, I compared the tumorigenic and peritoneal metastatic potential of CD44v6-positive and -negative cells in an *in vivo* mouse model. Consistent with my clinical observations, I demonstrated that a subpopulation of CD44v6-positive cells is prominently involved in peritoneal metastasis in a mouse model. In a set of experiments, I also demonstrated that CD44v6 expression demarcates a highly tumorigenic ovarian CSCs population with peritoneal metastatic potential and CD44v6-positive cells possess the potential to serve as metastasis-initiating cells. Recent evidence indicates the existence of a “CSC niche,” a specialized microenvironment that regulates CSCs properties and contributes to

tumor-initiation, growth, and metastasis[38-40]. The present study revealed the close relationship between CD44v6 expression and the pelvic peritoneum and thereby, raises the possibility that the microenvironment of the pelvic peritoneum forms a possible CSC niche for ovarian cancer.

My observations indicated that CD44v6-targeted therapy might impair the ability of CD44v6-positive ovarian CSCs, which contribute to peritoneal dissemination of advanced epithelial ovarian cancer and thereby sensitize tumors to currently available cancer treatments, including chemo- and radiotherapy.

9. Conclusion

In conclusion, CD44v6-positive cancer cells may be a potential molecular therapeutic target for eliminating ovarian CSCs. The finding that a distinct subpopulation of CD44v6-positive CSCs plays a critical role in peritoneal metastasis suggests that definitive treatment should target the CD44v6-positive cell population in ovarian cancer.

10. References

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