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Low Expression of CD44 Is an Independent Factor of Poor Prognosis in Ovarian Mucinous Carcinoma

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Abstract. *Aim: To determine whether CD44, which is associated with tumor growth and metastasis, is related to carcinogenesis and prognosis in ovarian mucinous carcinomas (MACs). Materials and Methods: Tissue blocks from 71 patients with benign mucinous ovarian tumors were used in the study: 35 were from patients with borderline mucinous ovarian tumors, and 60 from patients with MACs. Immunochemical analysis was performed to evaluate the expression of CD44 and examine its association with tumorigenesis and survival. Results: Compared to benign tumors, borderline tumors had high CD44 expression levels (p=0.047). Conversely, MACs had lower expression than borderline tumors (p=0.032). Progression-free and overall survival of patients with MAC with low CD44 expression were worse than those of patients with high expression (p=0.04 and p=0.02, respectively). Conclusion: Malignant transformation of mucinous tumors is associated with changes in CD44 expression, with low expression level being a prognostic factor in MAC.*

The incidence of epithelial ovarian carcinoma (EOC) has been increasing, and the prognosis of patients with advanced-stage disease remains poor despite aggressive treatment (1, 2). Ovarian mucinous carcinoma (MAC) accounts for approximately 10% of histological subtypes of all EOCs (3). In most patients, MAC is diagnosed at an early stage (4). Compared to serous carcinoma, the prognosis of patients with MAC of stage I is favorable (5), but it is worse in those with advanced-stage MAC (6). Furthermore, patients with MAC show a weaker response to platinum-based chemotherapy than individuals with serous carcinomas (7).

MAC develops through an adenoma–carcinoma sequence, originating from cystadenomas and mucinous borderline tumors (8). *KRAS* mutations represent an early genetic defect in the development of MACs, and their frequency gradually increases as the lesion progresses from benign to borderline and malignant (9).

CD44, a transmembrane protein, is ubiquitous in epithelial and normal tissues and is associated with tumor growth and metastasis (10). At least 10 variants of CD44 are expressed because of alternative splicing of nuclear RNA (11). The roles of CD44 in MAC have not yet been examined.

In this study, we used immunohistochemistry to analyze expression of CD44 in benign, borderline, and malignant mucinous ovarian tumors and investigated whether tumorigenesis of MAC is associated with CD44. Furthermore, we studied the correlation between CD44 and clinicopathological characteristics and evaluated survival according to the level of CD44 in MAC.

Materials and Methods

Patients and tissue microarray. Tissue blocks from 71 patients with benign mucinous ovarian tumors, 35 patients with borderline mucinous ovarian tumors, and 60 patients with MACs who underwent surgery at the National Defense Medical College between 1984 and 2008 were obtained. We prepared a tissue microarray (TMA) as follows. Cores of 1.5 mm were punched from donor blocks and inserted into a recipient block. All specimens were cut into 4-μm-thick sections. None of the patients had received chemotherapy before surgery. This research was approved by the Ethics Committee of the National Defense Medical College, Tokorozawa, Japan.

Immunochemistry. We used a mouse monoclonal antibody against CD44 (clone 2C5; R&D Systems, Abingdon, UK). Tissue microarray slides were deparaffinized in xylene, hydrated with

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Key Words: Ovarian mucinous carcinoma, CD44, prognosis, carcinogenesis.

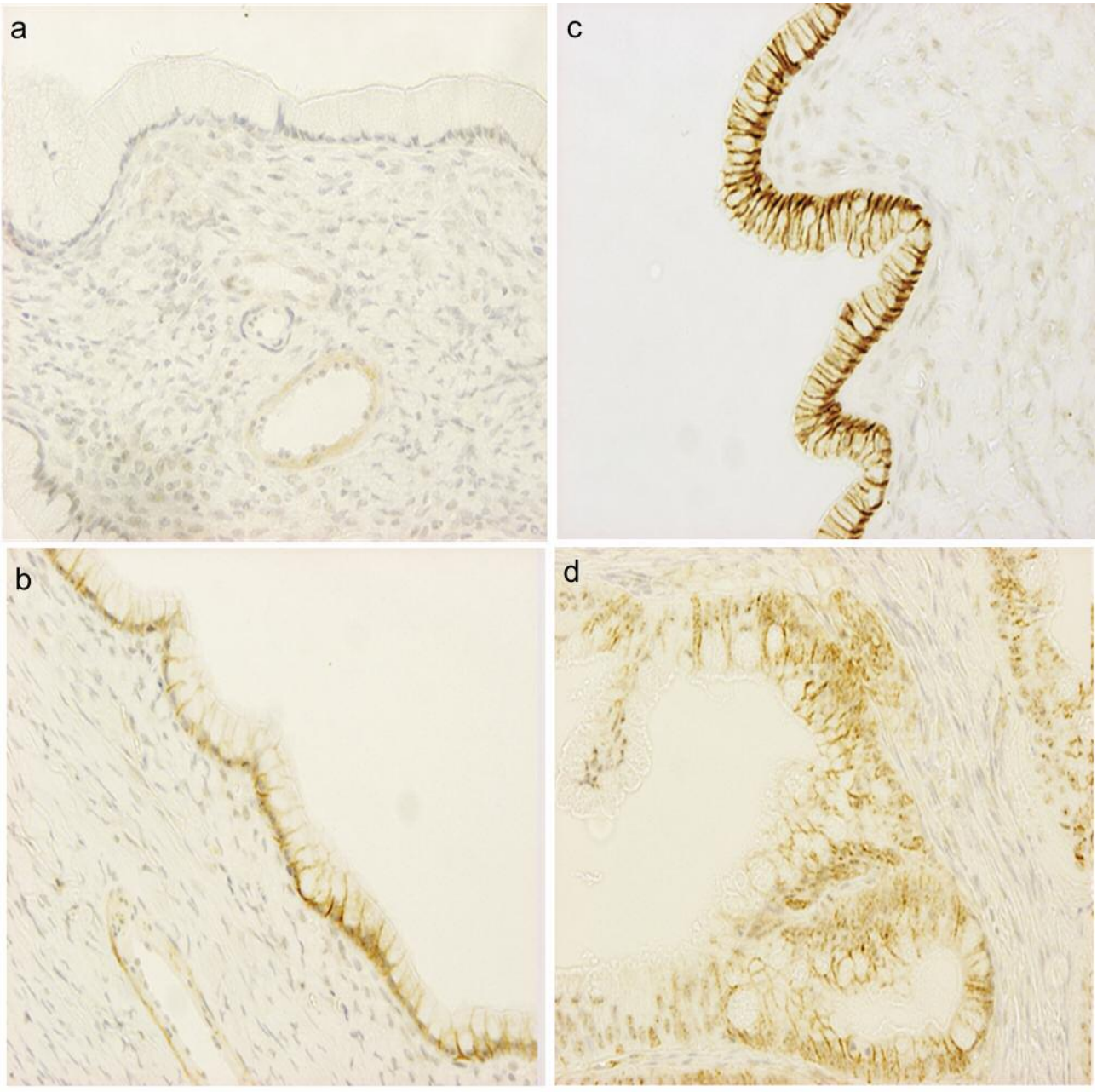

Figure 1. *Continued*

alcohol, incubated in an autoclave at 121˚C for 15 minutes in citrate buffer (0.01 M, pH 6.0), and allowed to cool at room temperature. Endogenous peroxidase was blocked by 0.3% H_2O_2/methanol. The slides were incubated at 4˚C overnight with primary antibodies, followed by an incubation with the DAKO EnVision+ system horseradish peroxidase labeled polymer with secondary antibodies (DAKO, Carpinteria, CA, USA) for 30 minutes at room temperature. Specific antigen-antibody reactions were visualized with 0.2% diaminobenzine tetrahydrochloride and hydrogen peroxide, and the slides were counterstained with Mayer hematoxylin. For each antibody, a negative control was prepared without the primary antibody. No significant staining was observed in the negative control sections. Serous ovarian carcinomas are known to be stained with this CD44 antibody (12).

Two investigators blinded to the clinical data independently evaluated and interpreted the results of immunohistochemical staining. The results were interpreted as negative, weak, or strong. Negative and weak cases were then defined as having low expression, whereas those scored as strong were defined as having high expression.

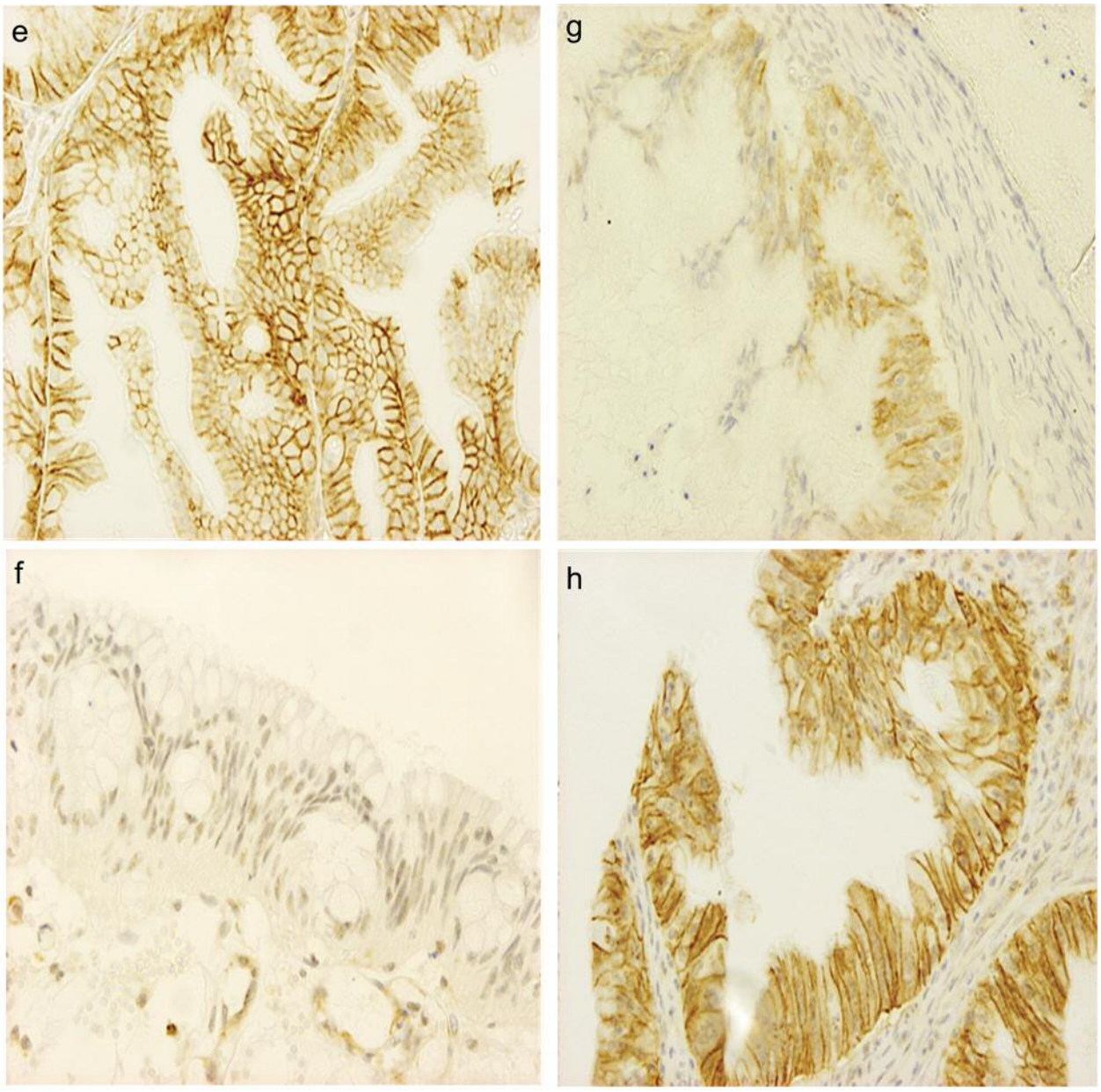

Figure 1. *CD44 in ovarian mucinous tumors analyzed by tissue-microarray-based immunohistochemistry. Tissue microarray images corresponding to negative (a and f), weak (b, d, and g), and strong (c, e, and h) CD44 staining. The images show staining in benign mucinous adenomas (a, b, and c), borderline mucinous tumors (d and e), and mucinous carcinomas (f, g, and h). Magnification is ×20 in all cases.*

Statistical analysis. The Stat View software, version 5.0, (SAS Institution Inc., NC, USA) was used for statistical analysis. Progression-free survival (PFS) was defined as the interval between first treatment and death or the date of disease progression. Overall survival (OS) was defined as the interval between first treatment and death. Staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) system (13). Performance status was evaluated using the World Health Organization criteria (14). Response rate was estimated based on the Response Evaluation Criteria in Solid Tumors (RECIST) (15). The chi-squared test, Fisher exact test, and Mann-Whitney *U*-test were used to evaluate the expression level of CD44 in several tumor subtypes and associations between expression of CD44 and clinicopathological parameters. PFS and OS curves were generated using the Kaplan–Meier method. Comparisons of survival distributions were made with the log-rank test. A Cox proportional hazards model was used for multivariate analysis of PFS and OS. The level of acceptable statistical significance was set at $p<0.05$.

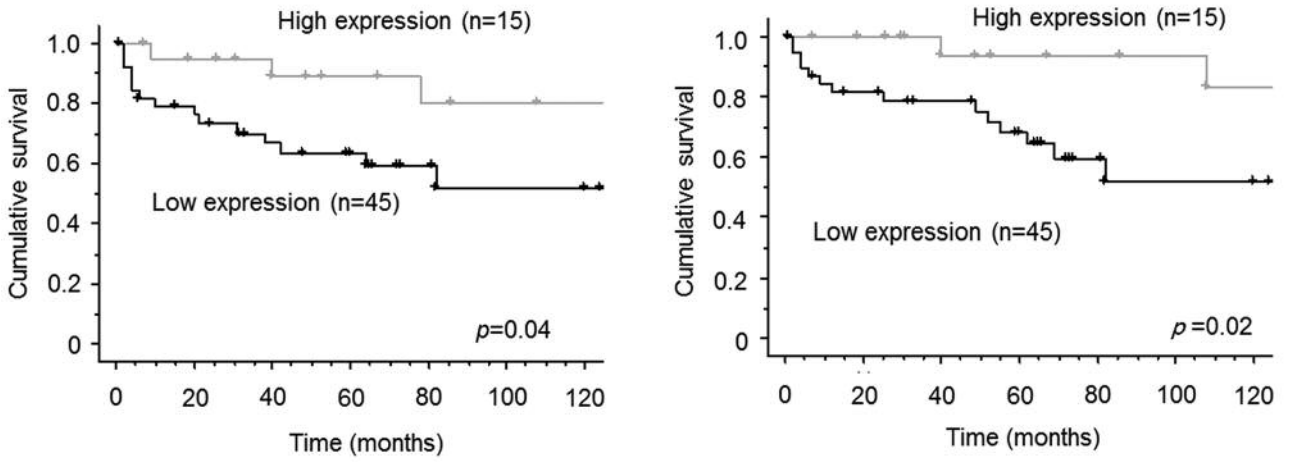

Figure 2. *Progression-free (a) and overall (b) survival curves of the 60 patients with ovarian mucinous carcinoma according to expression level of CD44. Low expression: Cases with negative or weak expression; high expression: cases with strong expression.*

Table I. *Correlation between expression level of CD44 and lesion type.*

Tumor type	N	Negative	Weak	Strong	*p*-Value
Mucinous adenoma	71	6 (9%)	40 (56%)	25 (35%)	
Borderline mucinous tumor	35	0	15 (43%)	20 (57%)	0.047*
Mucinous carcinoma	60	6 (10%)	33 (55%)	21 (35%)	0.03**

*Compared to mucinous adenomas; **compared to borderline mucinous tumors.

Results

Representative images of immunostaining are shown in Figure 1. Table I shows expression levels of CD44 in benign, borderline, and malignant mucinous tumors. Compared with benign mucinous tumors, there were significantly more cases of borderline mucinous tumor (p=0.047) with strong expression. Conversely, there were fewer cases of MAC with strong expression compared with borderline tumors (p=0.032).

Table II shows the correlations between CD44 expression levels and clinicopathological features in 60 patients with MAC. There were no statistically significant differences in age, performance status, stage, presence of residual tumors, and presence of chemotherapy between groups with high and low expression of CD44. PFS and OS of patients with MACs with low expression of CD44 were worse than those of patients with high expression (Figure 2a: PFS, p=0.04; Figure 2b: OS, p=0.02). Table III shows the results of multivariate analysis of 60 patients with MACs. Low expression of CD44, presence of residual tumors, and performance status were prognostic factors of PFS. Similarly, low expression of CD44, performance status, and presence of residual tumors were prognostic factors of OS.

Table II. *Characteristics of 60 cases with mucinous ovarian carcinomas.*

	CD44 expression		
Variable	Low (n=39)	High (n=21)	*p*-Value
Age, years			
Median (range)	53 (19-78)	43 (18-82)	0.25
Performance status, n (%)			
0	36 (92%)	19 (90%)	0.99
1	3 (8%)	2 (10%)	
Stage, n (%)			
I/II	29 (74%)	17 (81%)	0.54
III/IV	10 (26%)	4 (19%)	
Residual tumor, n (%)			
None	29 (75%)	17 (81%)	0.75
<1 cm	4 (10%)	0	
≥1 cm	6 (15%)	4 (19%)	
Chemotherapy, n (%)			
Platinum-based	36 (92%)	16 (76%)	0.11
None	3 (8%)	5 (24%)	
Response rate, n (%)			
CR/PR	5 (50%)	2 (50%)	0.99
SD/PD	5 (50%)	2 (50%)	

Low expression: Cases with negative or weak expression; high expression: cases with strong expression; CR/PR: complete/partial response; SD/PD: stable/progressive disease.

Table III. *Multivariate analysis of progression-free and overall survival in 60 patients with mucinous ovarian carcinomas.*

		Progression-free survival			Overall survival		
Variable	Comparison	Hazard ratio	95% CI	*p*-Value	Hazard ratio	95% CI	*p*-Value
Age	<55 *vs.* ≥55 years	0.51	(0.17-1.51)	0.22	0.48	(0.13-1.76)	0.27
Performance status	1 *vs.* 0	6.65	(1.55-28.5)	0.01	13.9	(2.78-69.1)	<0.01
FIGO stage	I/II *vs.* III/IV	0.35	(0.03-1.29)	0.11	0.23	(0.05-1.01)	0.051
Residual tumor	≥1 cm *vs.* <1 cm	4.61	(1.10-19.2)	0.04	8.77	(1.65-45.5)	0.01
CD44	Low *vs.* high expression	8.54	(2.01-37.0)	<0.01	26.3	(3.51-200)	<0.01

CI: Confidence interval; FIGO: Federation of Gynecology and Obstetrics.

Discussion

Most studies dedicated to the prognostic role of CD44 expression level in ovarian carcinomas included either all histological subtypes (12, 16-20) or only serous carcinomas (21). There have been no reports focusing on ovarian mucinous carcinomas. To our knowledge, this study is the first to investigate the role of CD44 in tumorigenesis and prognosis of MACs.

Generally, CD44 plays the main role in many cell–cell and cell–matrix interactions, including cell adhesion and migration (22). CD44 was found to be up-regulated during the development of ovarian carcinomas but subsequently down-regulated during their progression (16). Our study showed a higher rate of strong CD44 expression in borderline mucinous tumors than in benign mucinous tumors, whereas the rate of strong CD44 expression in MACs was lower than that in borderline mucinous tumors. These results suggest that CD44 expression increases in the process of malignant transformation and decreases during the invasive process corresponding to the development of MAC *via* an adenoma–carcinoma sequence (3), which is consistent with a previous report (16).

Most studies identified an association between low CD44 expression and advanced cancer stage (12, 16, 17). The opinions about whether CD44 expression is related to prognosis are divided (12, 16-21). A study of a large number (N=307) of ovarian carcinomas demonstrated low expression of CD44 to be a factor of poor prognosis (12). Nevertheless, a definitive conclusion has not been reached. We found no relation between CD44 expression level and FIGO stage. This result, however, is somewhat weakened by the small sample size. Nonetheless, it is important that low expression of CD44 in MAC was able to predict poor prognosis. Although most MACs are diagnosed at stage I (6), such patients, despite incomplete surgery, might include those with up-staged disease. Recently, a retrospective analysis suggested that fertility-sparing surgery is feasible in patients with stage I MACs (23). Because metastasis may be ongoing in pw stage I MAC with low CD44 expression, we should not perform fertility-sparing surgery in such cases.

In conclusion, malignant transformation and invasion by mucinous tumors were associated with CD44 expression level, and low CD44 expression was an independent prognostic factor in patients with MACs. Future studies should develop treatment strategies according to the level of CD44 expression.

Conflicts of Interest

The Authors declare no potential conflicts of interest in regard to this study.

Acknowledgements

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