

Expression of Adhesion Molecules and the Proliferative Activity of Carcinosarcoma of the Ovary

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Abstract. *Background:* To clarify the mechanism underlying the formation of a sarcomatous component of ovarian carcinosarcoma, we investigated the expression of adhesion molecules and the proliferative activity of carcinosarcomas. *Materials and Methods:* We immunohistochemically examined the expression of E-cadherin and β -catenin, and the Ki-67 labeling index (Ki-67 LI) in six carcinosarcomas containing endometrioid carcinoma as a carcinomatous component. *Results:* The sarcomatous components of the carcinosarcomas did not express E-cadherin or β -catenin. All carcinomatous components expressed these molecules but the expression was reduced compared to that in endometrioid ovarian carcinomas. In five of the six carcinosarcomas, the Ki-67 LI of the sarcomatous component was less than that of the carcinomatous component. *Conclusion:* The present results suggest that a carcinomatous component transforms more easily than an ordinary endometrioid carcinoma from the viewpoint of the cell adhesion, and cells in a carcinomatous component continuously transform into sarcomatous cells during the growth of carcinosarcoma.

Carcinosarcoma of the ovary, composed of both carcinomatous and sarcomatous components, is synonymous with malignant mixed Müllerian tumor (MMMT). It is an aggressive tumor and the prognosis of patients with carcinosarcoma is generally poor (1).

E-cadherin is a 120-kDa transmembrane protein involved in cell-cell adhesion. The extracellular domain of E-cadherin binds to that of an adjacent cell initiating cell adhesion (2-

4), while the intracellular domain binds to the cytoskeleton, through α - or β - catenin (2-4). A decrease in the expression of E-cadherin and β -catenin reduces cell adhesion. Consistently, reduced expression levels of these adhesion molecules has been shown to be associated with metastasis and invasion of carcinoma cells and a poor survival rate in patients with various types of carcinomas (5, 6).

Carcinosarcoma of the ovary is a tumor composed of endometrioid carcinoma as a carcinomatous component and a sarcomatous component originally derived from a carcinomatous component. To clarify the mechanisms underlying the formation of a sarcomatous component of carcinosarcoma, we investigated the expression of the adhesion molecules, E-cadherin and β -catenin, and the proliferative activity in both carcinomatous and sarcomatous components of carcinosarcoma of the ovary.

Materials and Methods

Materials. The samples used in the present study included six carcinosarcomas of the ovary and eight endometrioid carcinomas of the ovary as a control. The carcinosarcomas and endometrioid carcinomas were diagnosed according to the criteria of the 2012 WHO classification of tumors (1). Each tumor was cut into several blocks that were fixed in 0.01M phosphate-buffered 10% formalin (pH 7.4) and embedded in paraffin. Tumor sections (5 μ m in thickness) were made from each tumor block. Some sections were used for hematoxylin-eosin staining and some were used for immunohistochemistry. The present study was approved by the Ethics Committees of the affiliated hospitals.

Immunohistochemistry. The antibodies used for immunohistochemistry, their sources and the dilutions used are presented in Table I. Immunohistochemical staining was performed using an avidin-streptavidin immunoperoxidase method. Antigen retrieval was performed before immunohistochemistry with all antibodies. Antigen retrieval was performed by incubation of deparaffinized sections in cell condition 1 solution at the standard degree and immunohistochemical staining was performed using an automated Benchmark system (Ventana Medical System, Tucson, AZ, USA), according to the manufacturer's instructions.

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Table I. Antibodies used, their sources and dilutions.

Antigen	Clone	Dilution	Source
E-cadherin	36B5	×50	Novocastra Laboratorie, Newcastle, UK
β-catenin	β-Catenin-1	×80	Dakocytomation, Glostrup, Denmark
Ki-67	ki67	×100	Dakocytomation, Glostrup, Denmark
Vimentin	V9	Diluted	Dakocytomation, Glostrup, Denmark

Table II. Clinicopathological data of patients with carcinosarcoma of the ovary.

Case	Age	Location	p-stage	Histology of sarcomatous component	Chemotherapy	Follow-up (months)
1	51	Left	Ib	Spindle cell	(+)	DOD(24)
2	67	Left	IIIc	Spindle cell + cartilage	(+)	Unknown
3	74	Left	IIb	Spindle cell	(+)	DOD(6)
4	83	Right	Ib	Spindle cell + giant cell	(+)	DOD (60)
5	45	Left	III	Spindle cell + giant cell	(+)	Alive (57)
6	72	Right	Ia	Spindle cell + giant cell + cartilage	(+)	Alive (101)

DOD, Death of disease.

The evaluation of the immunohistochemical staining for E-cadherin, β-catenin or vimentin was carried out according to the proportion of positive cells (p) as follows: (-); $p < 1\%$, (1+); $1 \leq p < 25\%$, (2+); $25 \leq p < 50\%$, (3+); $50 \leq p < 75\%$, (4+); $p \geq 75\%$. Staining grades 1+ and 2+ were regarded as indicating expression. The Ki-67 labeling index (Ki-67 LI) was determined by examining more than 500 tumor cells in areas with the highest labeling index in each component, using the Win Roof program (Minani Co, Tokyo, Japan).

Statistical analyses. The frequencies of the findings in the two groups were analyzed using the χ^2 test. The Ki-67 LIs of each group are presented as the means±S.D. The Ki-67 LIs of the carcinomatous and sarcomatous components in each carcinosarcoma were analyzed using the Student's paired *t*-test, while the Ki-67 LIs of two groups were analyzed by the Student's unpaired *t*-test. A *p*-value < 0.05 was considered to be significant.

Results

The clinicopathological information for the patients with carcinosarcoma of the ovary is presented in Table II. The carcinomatous components of all of the carcinosarcomas included in the present study were endometrioid carcinoma (Figure 1A). The sarcomatous components of carcinosarcomas mainly consisted of spindle-shaped cells (Figure 1B). In two carcinosarcomas, a few giant cells were found among these spindle-shaped tumor cells (Figure 1C). In addition, neoplastic cartilage was observed in sarcomatous components in two carcinosarcomas (Figure 1D). All patients with carcinosarcoma received chemotherapy. Out of the five patients who could be followed-up, three patients died within five years after surgery (Table II).

Table III shows the expression levels of E-cadherin, β-catenin and vimentin, and the Ki-67 LI in the carcinomatous and sarcomatous components. None of the sarcomatous components expressed E-cadherin or β-catenin. All endometrioid carcinomas (used as a control for the endometrioid carcinoma components in the carcinosarcomas) expressed E-cadherin (Figure 2A) and β-catenin at a grade of 3+ or 4+, while the carcinomatous component expressed E-cadherin at a grade of 3+ in only one of the six carcinosarcomas (Figure 2B) and the carcinomatous components expressed β-catenin in only 3 carcinosarcomas (Figure 2C) at grade of 3+ or 4+. The expression of β-catenin in the nuclei was found in both carcinomatous and sarcomatous components in one carcinosarcoma (Figures 2D and E). Vimentin was expressed in the sarcomatous components of all carcinosarcomas (Figure 2F), while a carcinomatous component of one carcinosarcoma (Figure 2G) and three endometrioid carcinomas (Figure 2H) expressed vimentin.

The Ki-67 LIs (means±S.D.) of the carcinomatous and sarcomatous components of carcinosarcomas and the control endometrioid carcinomas were $31.8 \pm 15.6\%$, $21.8 \pm 15.6\%$ and $50.1 \pm 21.2\%$, respectively; no significant differences were found among these groups. However, the Ki-67 LI of a carcinomatous component was higher than that in a sarcomatous component in five out of six carcinosarcomas, while the Ki-67 LI of the carcinomatous components was significantly higher than that of the sarcomatous components when the data were analyzed using the paired Student's *t*-test ($p < 0.05$) (Table IV).

Table III. Immunohistochemical results of each component of carcinosarcoma of the ovary.

Case	E-cadherin		β-catenin		Vimentin	Ki-67	
	Car comp	Sar comp	Car comp	Sar comp	Car comp	Car comp	Sar comp
Carcinosarcoma							
1	(2+)*	(-)	(2+)*	(-)	(-)	28	4
2	(2+)*	(-)	(2+)*	(-)	(-)	31	22
3	(2+)*	(-)	(4+)	(-)	(3+)	47	41
4	(2+)*	(-)	(2+)*	(-)	(-)	19	27
5	(3+)	(-)	(3+) &N	(3+) &N	(-)	51	33
6	(1+)*	(-)	(3+)	(-)	(-)	10	3
Endometrioid carcinoma							
1	(4+)		(4+)		(-)	33	
2	(4+)		(4+)		(-)	80	
3	(4+)		(4+)		(-)	73	
4	(3+)		(4+)		(4+)	22	
5	(4+)		(4+)		(4+)	53	
6	(4+)		(4+)		(-)	29	
7	(4+)		(4+)		(-)	52	
8	(4+)		(4+)		(4+)	60	

Car comp, carcinomatous component; sar comp, sarcomatous component; N, nuclear expression. Staining grade was determined according to the proportion of positive cells (p) as follows: (-); $p < 1\%$, (1+); $1 \leq p < 25\%$, (2+); $25 \leq p < 50\%$, (3+); $50 \leq p < 75\%$, (4+); $p \geq 75\%$.

Table IV. Comparison of the expression of E-cadherin, β-catenin, vimentin and Ki-67 labeling index.

	Carcinosarcoma (n=6)			Endometrioid carcinoma (n=8)
	Sarcomatous component	Carcinomatous component		
Reduced E-cadherin expression*	6/6 (100%)	5/6 (83.3%)	$p < 0.05$	0/8 (0%)
		NS		
Reduced β-catenin expression*	6/6 (100%)	3/6 (50%)	$p < 0.05$	0/8 (0%)
		NS	NS	
Vimentin expression	6/6 (100%)	1/6 (16.7%)	$p < 0.05$	3/8 (37.5%)
		NS	NS	
Ki-67 labeling index	21.8±15.6	31.8±15.6	$p < 0.05$	50.1±21.2
		NS	NS	

*Staining grades 1+ and 2+ were regarded as the reduced expression.

Discussion

E-cadherin and β-catenin were not expressed in the sarcomatous components of carcinosarcoma of the ovary. On the other hand, E-cadherin and β-catenin were expressed in

the carcinomatous components but their expression was reduced compared to that of the control endometrioid carcinomas of the ovary. The cells in a sarcomatous component originally derive from the cells in a carcinomatous component. Therefore, it is conceivable that

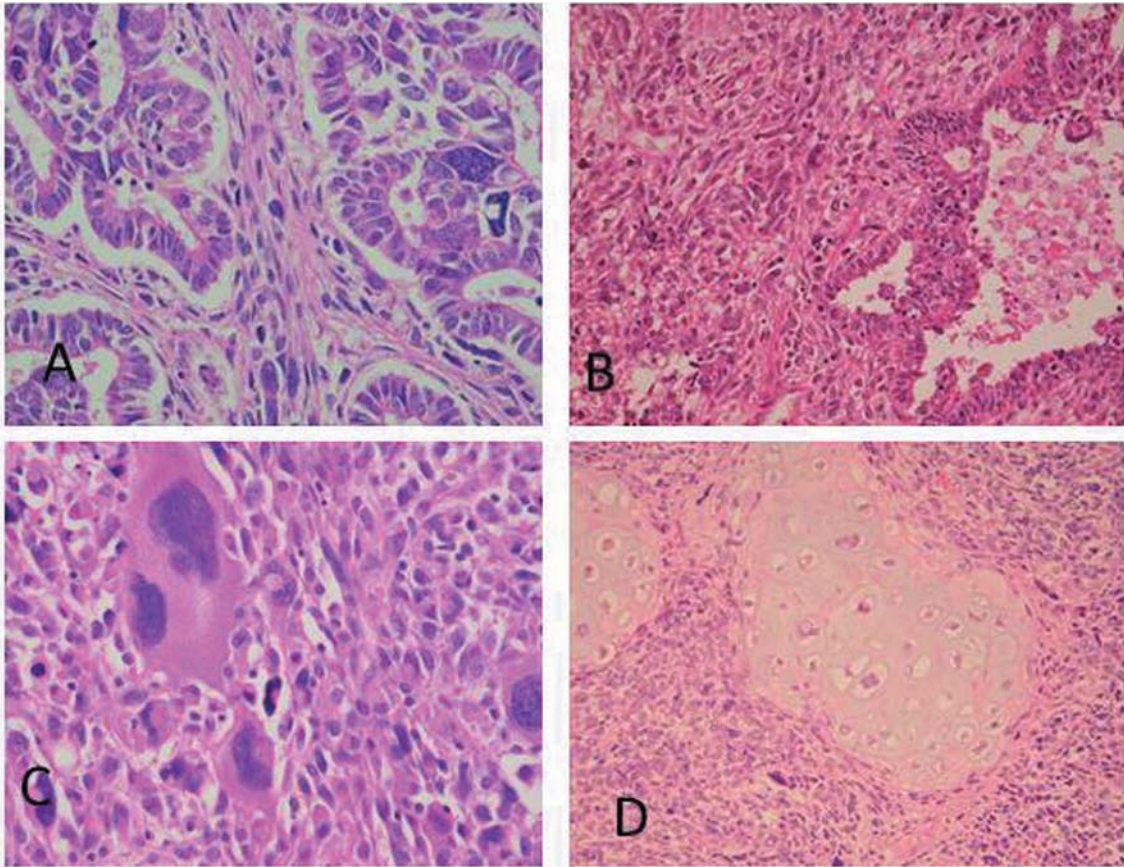


Figure 1. Representative histological findings of carcinosarcoma of the ovary: (A) An endometrioid carcinoma component (carcinosarcoma, case 4), (B) Sarcomatous and carcinomatous components (carcinosarcoma, case 2), (C) Giant cells in a sarcomatous component (carcinosarcoma, case 4), (D) Neoplastic cartilage in a sarcomatous component (carcinosarcoma, case 2).

the cells in the carcinomatous components of carcinosarcoma more easily transform into sarcomatous cells than endometrioid carcinoma from the viewpoint of cell adhesion.

In five out of six carcinosarcomas in the present study, the Ki-67 LI of a sarcomatous component was lower than that of the carcinomatous components, suggesting that a proliferative activity of the carcinomatous components exceeds that of their sarcomatous counterparts. However, an enlarged carcinosarcoma generally contains a large amount of sarcomatous component. It can be inferred from the results of the Ki-67 LI that the sarcomatous component would disappear or remain very small in such cases if there was no continuous sarcomatous transformation. Therefore, we believe that the cells in a carcinomatous component continuously transform into sarcomatous cells during the growth of carcinosarcoma.

The prognosis of carcinosarcoma of the ovary is worse than ordinary carcinomas of the ovary (7, 8). However, the Ki-67 LIs of carcinomatous and sarcomatous components were not significantly greater than those of endometrioid carcinomas. These results suggest that the proliferative activity of

carcinosarcoma is not responsible for its poor prognosis. On the other hand, the sarcomatous components expressed neither E-cadherin nor β -catenin, thus suggesting the loss of cell adhesion in the sarcomatous components. The reduction or loss of cell adhesion facilitates the invasion and metastasis of tumor cells. Therefore, the loss of cell adhesion may be associated with the poor prognosis of carcinosarcoma (9, 10).

β -Catenin, in concert with E-cadherin, plays an important role in cell adhesion (9). However, when the Wnt signaling pathway is activated, β -catenin translocates into the nuclei and participates in regulating gene expression. In the cells of some cancers, β -catenin accumulates in the nuclei because of the activation of the Wnt signaling pathway due to mutation(s) of protein(s) involved in the Wnt signaling pathway or because of mutations in the β -catenin gene affecting, thus, gene expression relevant to tumor growth promotion (10-13). In the present study, nuclear β -catenin expression was found in one carcinosarcoma. This suggests that this carcinosarcoma had Wnt signaling activation or mutant β -catenin.

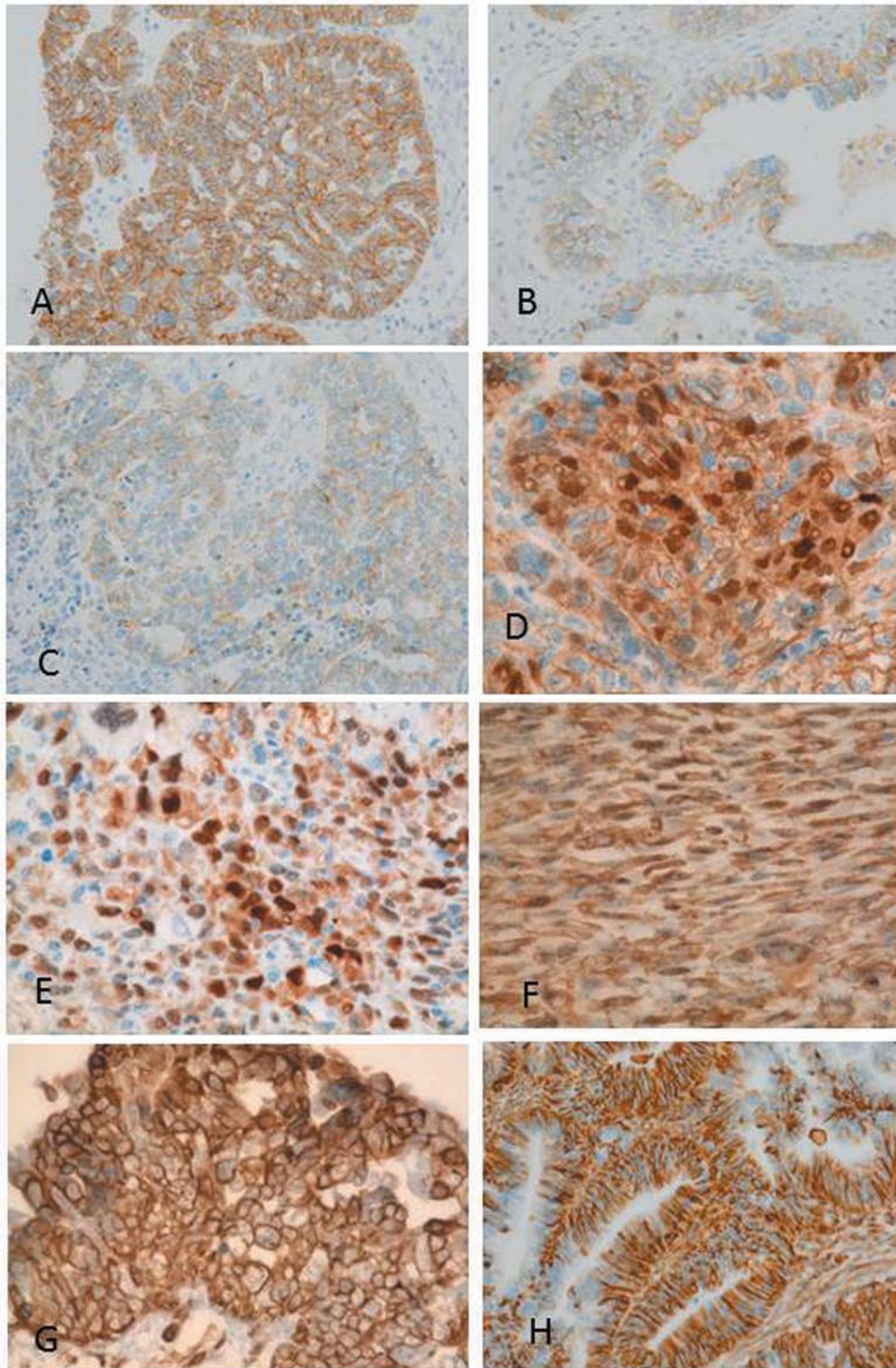


Figure 2. The results of the immunohistochemical staining of E-cadherin, β -catenin and vimentin: (A) E-cadherin expression (grade 4+) in an endometrioid carcinoma (endometrioid carcinoma, case 1), (B) E-cadherin expression (grade 2+) in a carcinomatous component (carcinosarcoma, case 4), (C) β catenin expression (grade 2+) in a carcinomatous component (carcinosarcoma, case 2), (D) Nuclear expression of β -catenin in a sarcomatous component (carcinosarcoma, case 5), (E) Nuclear expression of β -catenin in a carcinomatous component (carcinosarcoma, case 3), (F) Vimentin expression (grade 4+) in a sarcomatous component (carcinosarcoma, case 3), (G) Vimentin expression (grade 3+) in a carcinomatous component (carcinosarcoma, case 3), (H) Vimentin expression (grade 4+) in an endometrioid carcinoma (endometrioid carcinoma, case 8).

Vimentin, a mesenchymal cell marker, is one of the intermediate filament proteins (14-16). It was consistently expressed in the mesenchymal component of carcinosarcoma in the present study and was also expressed in three endometrioid carcinomas and a carcinomatous component of one carcinosarcoma. It has been previously reported that vimentin is expressed in the epithelial cells covering the cavity of, or epithelial cells derived from, the Müllerian duct (14). Endometrioid carcinoma of the ovary derives from the surface epithelium of the ovary (8). Therefore, the expression of vimentin in endometrioid carcinoma may be related to its origin.

In conclusion, the results presented herein suggest that the cells in a carcinomatous component of carcinosarcoma easily transform into sarcomatous cells from the view point of cell adhesion and that the proliferative activity of the carcinomatous components exceeds that of the sarcomatous components. Therefore, it is conceivable that the cells in a carcinomatous component of carcinosarcoma continuously transform into sarcomatous cells during the growth of carcinosarcoma.

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