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Effusion cytodiagnosis of carcinosarcoma derived from the female genital tract: immunohistochemical features of MMP-7 and Ki-67 and immunofluorescence double staining analyses of eight cases

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Abstract

Objective. Expression of Matrix metalloproteinase 7 (MMP-7) and Ki-67 by carcinoma components (CCs) and sarcoma components (SCs) in carcinosarcoma of the female reproductive organs has been investigated by conventional methods, but analysis with immunohistochemical staining of multiple antigens has not been reported. We report the profiles of expression of MMP-7 and Ki-67 in carcinosarcoma determined with immunohistochemical staining techniques.

Methods. We used antibodies against epithelial antigen (EA), epithelial membrane antigen (EMA), and vimentin for immunofluorescence double staining of ascitic fluid in eight cases of carcinosarcoma of female reproductive organs. We also used immunohistochemical triple staining to compare MMP-7 and Ki-67 expression between CCs and SCs in the primary site of carcinosarcoma.

Results. Immunofluorescence analysis revealed that all neoplastic cells in the ascitic fluid were positive for EA or EMA, indicating that these cells were CCs. Immunohistochemical analyses of the primary organ of carcinosarcoma revealed that MMP-7 was expressed on CCs in four of eight cases of carcinosarcoma, whereas MMP-7 was not expressed on SCs. The average Ki-67 labeling index (LI) in CCs and SCs was 51.8% and 28.6%, respectively. The difference in Ki-67 LI between CCs and SCs was statistically significant (t test for paired samples, $P = 0.0173$).

Conclusions. This is the first study to examine carcinosarcoma of the female reproductive organ by immunohistochemical staining for multiple antigens, which allows analysis of mixed tumor elements. In addition, we found that expression of MMP-7 and the average Ki-67 LI differ between CCs and SCs in carcinosarcoma. The predominance of CCs as the malignant cells in the ascitic fluid may be due to cytological differences between CCs and SCs of carcinosarcoma.

Keywords: Carcinosarcoma; Immunohistochemistry; Immunofluorescence; Multiple staining; Effusion; Ki-67; MMP-7; Cytology

Carcinosarcoma is a rare neoplasm and occurs in diverse locations including uterus, ovary, breast, and lung. The histological and cytological characteristics of carcinosarcoma are the appearance of biphasic components: carcinoma components (CCs) and sarcoma components (SCs). With respect to the histogenesis of carcinosarcoma, the combination tumor hypothesis, which states that both CCs and SCs arise from a single precursor cell, is presently accepted. This hypothesis is based on results of studies with cultured cell lines [1] and analyses of p53 mutations and loss-of-heterozygosity (LOH) [2,3]. However, it has been reported that some cases of carcinosarcoma arise as a collision tumor that consists of multiple clones [4].

The metastatic process is complicated and involves several events including weak adhesion between cells, degradation of the basement membrane, and invasion into blood vessels and lymphatic ducts. Matrix metalloproteinases (MMPs) dissolve type IV collagen, laminin, and proteoglycans, which are components of the basement membrane [5]. Many MMPs are expressed at high

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levels by neoplastic cells, suggesting that MMPs are involved in metastasis of cancer. MMP-7 (matrilysin) is a member of the MMP family, for its deduced amino acid sequence contains the conserved catalytic domain of MMPs although it lacks the hemopexin-like carboxyl terminal domain characteristic of these enzymes. MMP-7, the smallest molecule among MMPs, digests the extracellular matrix and also is involved in the vasculogenesis [6].

The number of mitoses and proliferative capacity are crucial factors for tumor grading and metastasis. Ki-67 antigen is a nonhistone protein that was first described in 1983 and is expressed in cells in G1, S, and G2 phases and during mitosis, allowing estimation of the fraction of proliferative cells with only a small number of cells. Ki-67 nuclear antigen may be used as an alternative to mitotic counts in grading schemes or as a single parameter for assessment of fine-needle aspiration biopsies or surgical specimens, and Ki-67 is expressed throughout the cell cycle. Staining with a monoclonal antibody against Ki-67 is a reliable method to evaluate fractions of rapidly growing cells in normal and neoplastic populations of human cells [7,8].

Immunohistochemistry is a superior method to examine several antigens localized on the same cell [9,10]. Ikeda et al. developed a novel immunoenzymatic triple-staining method [9], and Suzuki et al. reported that an advanced boiling procedure can be applied to immunofluorescent analysis of cytospecimens to permit visualization of multiple antigens [10].

In the present study, we used multiple immunofluorescence staining for analysis of ascitic fluid cytology and histopathology of carcinosarcoma derived from female reproductive organs and compared MMP-7 and Ki-67 expression by CCs and SCs.

Materials and methods

Tissue samples

Surgical specimens of the primary tumor of ovarian or uterine corpus carcinosarcoma (malignant mixed mullerian tumor or malignant mixed mesodermal tumor) were obtained from eight patients with cancerous ascites who underwent surgery between April 1994 and April 2003 at Showa University Fujigaoka Hospital. Histopathologic examinations showed that six of the eight cases were of homologous carcinosarcoma, and the remaining two were of heterologous carcinosarcoma.

Immunohistochemistry (IHC)

IHC was performed with a highly sensitive indirect immunoperoxidase technique (Simple Stain MAX-PO, Nichirei, Tokyo, Japan). The primary antibodies, dilutions, and pretreatments are as follows:

- Ki-67 (clone MIB-1, 1:100, Dako Cytomation Ltd., Copenhagen, DK),
- MMP-7 (clone 141-7B2, 1:500, Daiichi Fine Chemical Ltd., Takaoka, JPN),
- EA (clone Ber-Ep4, 1:100, Dako Cytomation Ltd., Copenhagen, DK),
- EMA (clone E29, 1:100, Dako Cytomation Ltd., Copenhagen, DK),
- Vimentin (clone V9, 1:400, Dako Cytomation Ltd., Copenhagen, DK),
- CD10 (clone 56C6, 1:50, Novocastra Laboratories Ltd., Newcastle, UK),
- Desmin (clone D33, 1:100, Dako Cytomation Ltd., Copenhagen, DK).

Table 1
Clinicopathologic features of the 8 cases of carcinosarcoma in the female genital tract

Case no.	Age (years)	Tumors	Clinical stage	Patient outcome, D or A (months)	Histopathologic classification	
					Carcinoma	Sarcoma
1	77	Ovary	IIIc	unknown	Serous papillary adenoca.	Malignant fibrous histiocytoma
2	54	Ovary	Ic	11, D	Endometrioid adenoca.	Stromal sarcoma
3	78	unknown	IV	3, D	Endometrioid adenoca. and serous papillary adenoca.	Chondrosarcoma and uterine endometrial stromal sarcoma
4	66	unknown	IV	2, D	Endometrioid adenoca.	Uterine endometrial stromal sarcoma
5	65	Ovary	IIIc	1, D	Clear cell adenoca.	Malignant fibrous histiocytoma
6	55	Ovary	IV	11, D	Serous papillary adenoca.	Rhabdomyosarcoma
7	70	Ovary	Ic	24, D	Serous papillary adenoca. and clear cell adenoca.	Fibrosarcoma
8	34	Ovary	Ic	9, A	Mucinous adenoca. and endometrioid adenoca.	Fibrosarcoma

D, dead; A, alive; adenoca., adenocarcinoma.