Cytological Analysis of Uterine Body Endometrioid Adenocarcinoma Cells in Peritoneal Cavity : Cytological Findings and Histological Background

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Objective: We analyzed uterine-body endometrioid adenocarcinoma (EMA) cells appearing in the peritoneal cavity with special reference to squamous metaplastic-like cells (SMCs).
Methods: Cases were 33 EMA specimens surgically resected from 2000 to 2006 and consisting of 17 G1 (51.5%), 13 G2 (39.4%), and 3 G3 (9.1%). Focusing on SMCs using immunohisto/cytochemical tests, we analyzed cytohistological findings and their association with pathological conditions such as histological grade, depth, tumor size, and location.
Results: Peritoneal cytological findings were as follows: large to small papillary G1 or G2 clusters and scattered G3 patterns. With Papanicolaou staining, SMCs showed slight atypia and were polygonal to oval cells that had central nuclei. These cells also showed an increase of fine granular chromatin and had small nuclei. The tumor cells had abundant cytoplasm, which was densely stained with a light green, and their boundaries were well defined. The prevalence of SMCs relative to adenocarcinoma cells was 1+ in 33.3% (6 patients), 2+ in 55.6% (10 patients), and 3+ in 11.1% (2 patients). These features were found not to be related to invasion depth or tumor size.
Conclusions: SMCs appears to be a hallmark in peritoneal uterine-body EMA cytology, especially in G1 or G2 diagnosis.

Key words: endometrioid adenocarcinoma, squamous metaplastic-like cells, peritoneal cytology

INTRODUCTION

Endometrioid adenocarcinoma has the highest incidence among all malignant tumors of the uterine corpus. In recent years, endometrial cancer has demonstrated an upward trend in Japan [1–3], and increased use of peritoneal cytology is expected to expand the opportunities for detecting endometrioid adenocarcinoma in the future. The International Federation of Gynecology and Obstetrics (FIGO) [4] guidelines state that the presence or absence of endometrioid adenocarcinoma should be specified when tumor cells are detected by peritoneal cytology.

In our experience, atypical cells showing metaplasia (squamous metaplastic cells: SMCs) are also detected in addition to adenocarcinoma cells in patients with endometrioid adenocarcinoma. However, there have been few cytological reports on SMCs [5, 6]. This report presents the results of our investigation into the cytopathological and pathological features of endometrioid adenocarcinoma originating from the uterine corpus.

MATERIALS AND METHODS

The subjects were 33 patients with endometrioid adenocarcinoma that was histologically confirmed by examination of resected specimens between 2000 and 2006, in which the presence of tumor cells was detected in ascites and peritoneal washings ("positive peritoneal cytology"). The mean age at diagnosis was 51.5 years (range: 38–64 years). The tumor was classified as grade 1 (G1) in 17 patients, G2 in 13 patients, and G3 in 3 patients. For the detection of tumor cells in the peritoneal cavity, cytology specimens with Papanicolaou staining were subjected to immunocytochemistry with the following primary antibodies: MOC-31 (Dako, Carpinteria, CA, USA, 1:50 dilution), CK34βE12 (Dako, Carpinteria, CA, USA, 1:100 dilution), calretinin (Nichirei Bioscience, Tokyo, Japan, predilution antibody), and CD10 (Nichirei Bioscience, Tokyo, Japan, predilution antibody). Staining was done by an indirect technique (Simple Stain MAX-PO MULTI, Nichirei Bioscience, Tokyo, Japan). The primary tumors were also examined to assess the features of tumor cells. The results thus obtained were used to evaluate the cytopathological and pathological features (1, tumor grade; 2, histological depth of

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invasion; 3, tumor localization; and 4, tumor diameter) of adenocarcinoma cells and SMCs (by the chi-square test). The presence of SMCs was expressed as the percentage of 500 tumor cells per peritoneal cavity, and was semiquantitatively classified (by Student's t-test) according to the following criteria: negative, 0%; 1+, < 30%; 2+, 30–60%; and 3+, > 60%. Differences of $P < 0.05$ were considered to be statistically significant.

This study complies with the principles of the Declaration of Helsinki and all patients gave informed consent (oral).

RESULTS

Cytological findings

Adenocarcinoma cells were detected in the peritoneal cavity as large or small clusters in patients with G1 and G2 tumors, while small clusters or scattered cells were seen in G3 patients. G1/G2 endometrioid adenocarcinomas exhibited slight cytologic atypia, whereas marked atypia was observed in G3 tumors (Fig. 1). In addition to adenocarcinoma cells, SMCs were found sporadically or intermingled with the tumor cells. With Papanicolaou staining, SMCs showed slight atypia and were polygonal to oval cells that had central nuclei. These cells also showed an increase of fine granular chromatin and had small nuclei. The tumor cells had abundant cytoplasm, which was densely stained with a light green color, and their boundaries were well defined (Fig. 2).

The prevalence of SMCs relative to adenocarcinoma cells was 1+ in 33.3% (6 patients), 2+ in 55.6% (10 patients), and 3+ in 11.1% (2 patients) (Fig. 3).

Histopathologic findings

Of the patients with positive peritoneal cytology, 51.5% (17/33 patients) histologically showed minute squamous metaplastic-like areas scattered on the surface of the primary tumor, in addition to the typical features of endometrioid adenocarcinoma (Fig. 4). Of these 17 patients, 70.6% (n=12) had SMCs in the peritoneal cavity.

Immunocytochemical findings

The adenocarcinoma cells and SMCs in the peritoneal cavity were positive for MOC-31 and CK34βE12 expression, but negative for expression of calretinin, in all patients (Fig. 5). In specimens from the primary tumor, on the other hand, adenocarcinoma cells were positive for expression of MOC-31 and negative or expression of CD10, whereas SMCs were negative for expression of both MOC-31 and CD10. CK34βE12 expression was consistent with its expression profile in cells showing squamous metaplasia, regardless of whether the specimens were from the peritoneal cavity or the primary tumor (Figs. 6 and 7; Table 1).

Pathological findings

a) Tumor grade

When all 33 patients with positive peritoneal cytology were classified according to tumor grade, 51.5% (17/33 patients) had G1 tumors, 39.4% (13/33 patients) had G2 tumors, and 9.1% (3/33 patients) had G3 tumors. Among them, 54.4% (18/33 patients) had SMCs, including 61.15% (11/18 patients) of those with G1 tumors, 33.4% (6/18 patients) of those with G2 tumors, and 5.5% (1/18 patients) of those with G3 tumors (Figs. 8 and 9).

b) Histological depth of invasion, tumor localization, and tumor diameter

The patients with positive peritoneal cytology were also classified according to the histological depth of tumor invasion, tumor localization, and tumor diameter. As a result, 18.2% (n=6) were 'Depth a,' 45.5% (n=15) were 'Depth b,' and 36.3% (n=12) were 'Depth c' for depth of invasion. With respect to tumor localization, 69.7% (n=23) had diffuse tumors and 30.3% (n=10) had localized tumors. The mean tumor diameter was 49.8 × 37.2 mm (range: 8 × 7 mm to 110 × 95 mm). In patients with SMCs, on the other hand, the corresponding results were as follows: 16.7% (n=3) showed 'Depth a,' 61.1% (n=11) showed 'Depth b,' and 22.2% (n=4) showed 'Depth c' for invasion. In addition, 72.7% (n=13) had diffuse tumors and 27.8% (n=5) had localized tumors. The mean tumor diameter was 49.4 × 33.3 mm (range: 8 × 7 mm to 110 × 80 mm) (Table 2).

DISCUSSION

When the prevalence of SMCs among tumor cells is compared with the level of cellular differentiation, it tends to be higher in tumors with differentiation [7, 8].

This finding reflects the fact that most adenocarcinomas of the uterine corpus are endometrioid adenocarcinomas and the majority of such patients have G1 or G2 tumors.

The present investigation also disclosed that SMCs were frequently detected in addition to adenocarcinoma cells. According to the immunocytochemical findings of the primary tumors and intraperitoneal cells, although there was a difference in expression between cytological and tissue specimens related to the fixation technique, tumor cells showed positive expression of MOC-31 [9–11], while the expression of CK34βE12 was consistent with its expression profile in squamous metaplasia regardless of whether a cytological or tissue specimen was examined. On the other hand, intraperitoneal SMCs and some adenocarcinoma cells were positive for the expression of MOC-31 and CK34βE12 [12, 13], but negative for expression of calretinin [14–16]. This finding is not incompatible with the concept that SMCs detected in the peritoneal cavity are squamous metaplastic cells originating from endometrioid adenocarcinoma.

When peritoneal cytology reveals SMCs with this morphology, differentiation from reactive mesothelial cells is required. The cytological features of reactive mesothelial cells [17–25] include the following: 1) polygonal to oval morphology; 2) oval nuclei; 3) abundant cytoplasm and dense staining with a light green color, and 4) microvilli at cell borders. On the other hand, our investigation showed that SMCs have the following cytological features: 1) polygonal to oval morphology; 2) oval nuclei with an increased content of chromatin; 3) abundant cytoplasm with strong light-green staining; 4) no microvilli at the borders; and 5) proximity to clusters of adenocarcinoma cells (Table 3). Because it is not easy to clearly distinguish between

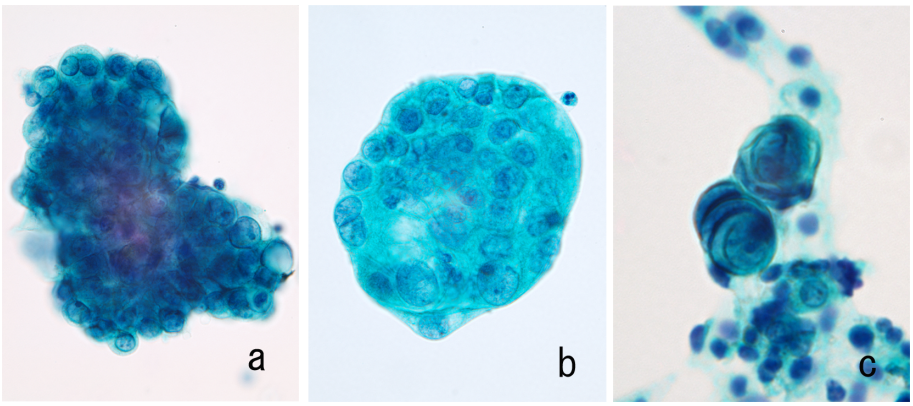

Fig. 1 Cytological findings for endometrioid adenocarcinoma in peritoneal cytology a: G1; b: G2; c: G3; Pap. staining × 100.

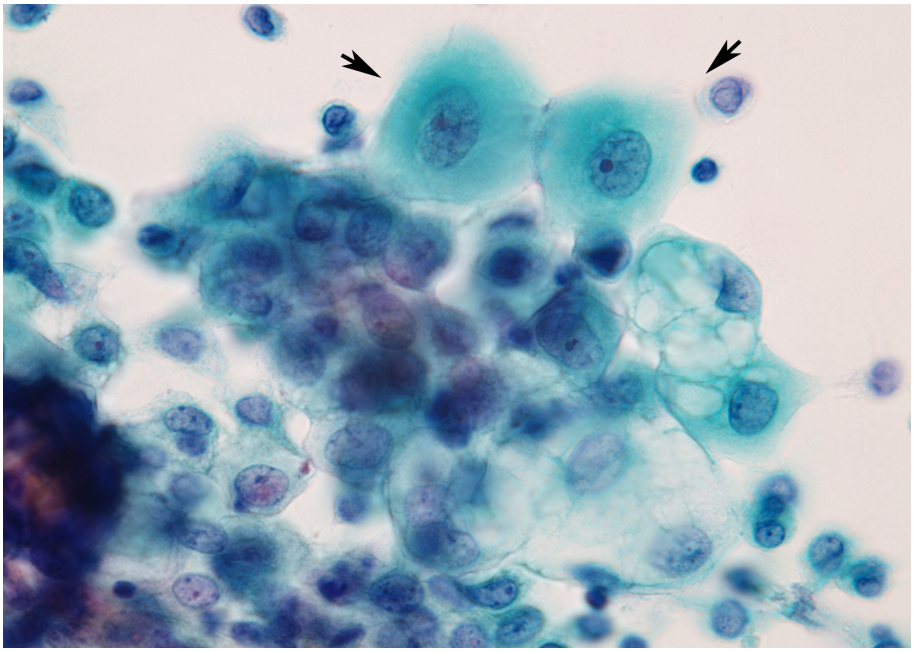

Fig. 2 Cytological SMC findings in peritoneal cytology (arrow) (Pap. staining × 100).

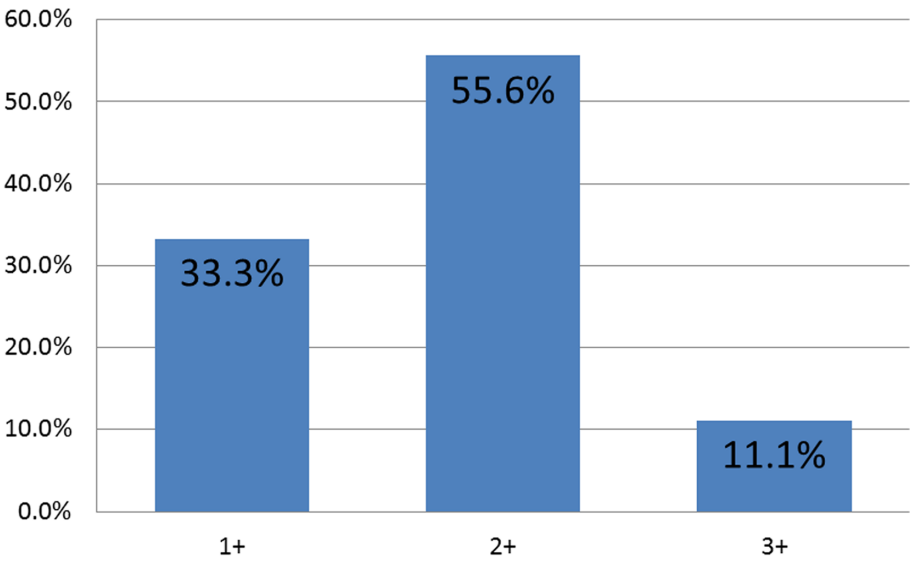

Fig. 3 Comparison of SMC in peritoneal cytology.

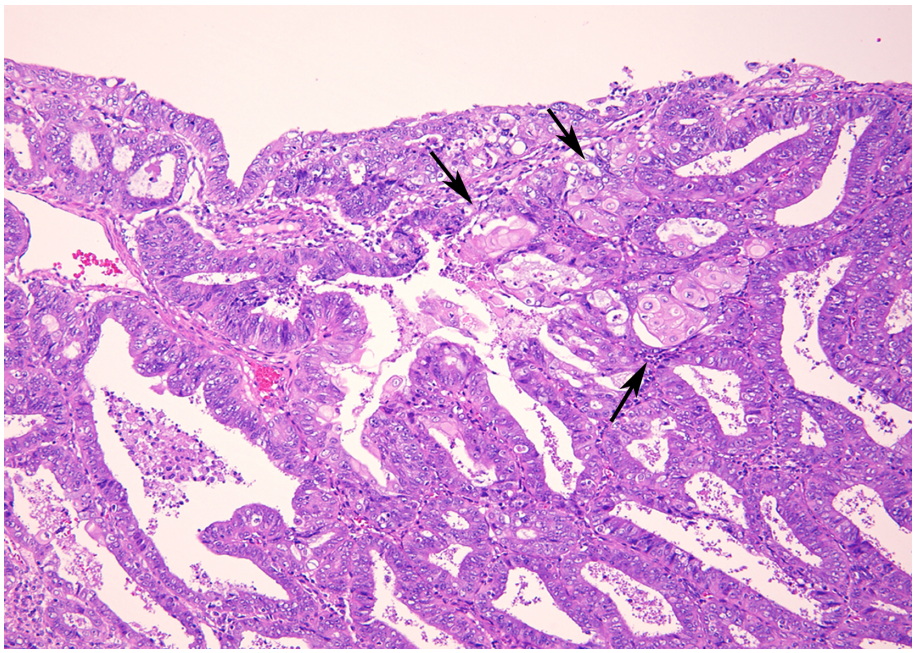

Fig. 4 Histological findings for squamous metaplasia in well-differentiated endometrioid adenocarcinoma (arrow) : HE staining × 20.

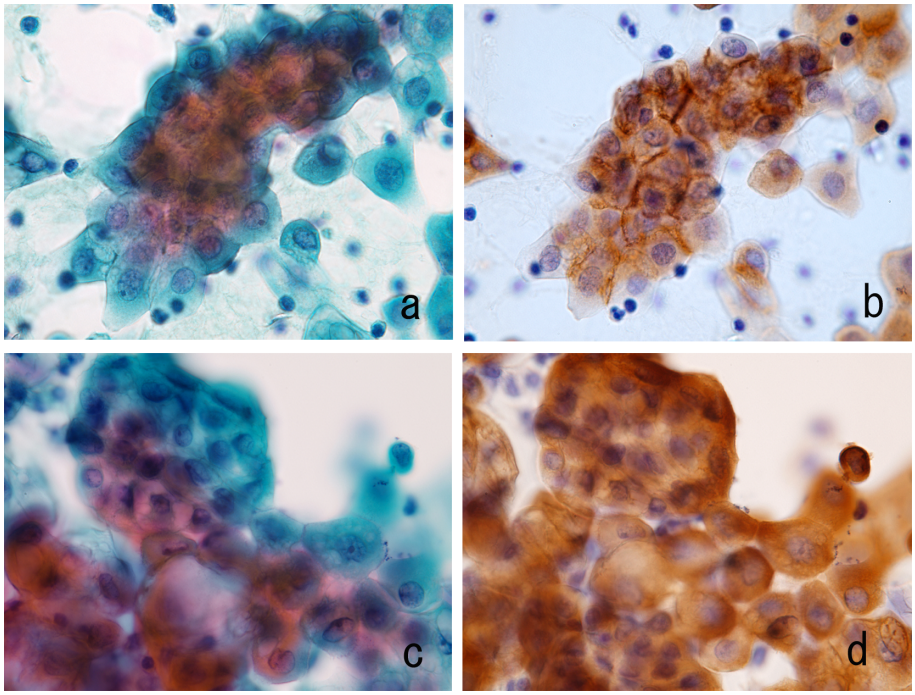

Fig. 5 Immunohistochemical findings for endometrioid adenocarcinoma with SMC in peritoneal cytology. a: Pap. staining × 100; b: MOC31 × 100; c: Pap. staining × 100; d: CK34βE12 × 100.

the two cell types, it may be of importance to pay close attention to the staining of the nuclei and cytoplasm as well as the presence or absence of microvilli at the cell borders.

According to a previous histopathological study of metaplasia in endometrioid adenocarcinoma, [26, 27] mucous cell and oxyphilic cell metaplasia are the most common, with squamous metaplasia being next in incidence. In the present histological investigation of patients with positive peritoneal cytology, squamous metaplasia was observed at a high incidence on the surface of the tumors, especially in patients with G1 or G2 tumors. Also among the SMCs appearing in the peritoneal cavity, patients with G1 tumors had an increased incidence of squamous metaplasia. As mentioned above, detection of SMCs in the peritoneal cavity was related to the extent of differentiation of the primary endometrioid adenocarcinoma and the prevalence of SMCs was higher in patients with well-differentiated tumors. In addition, even when

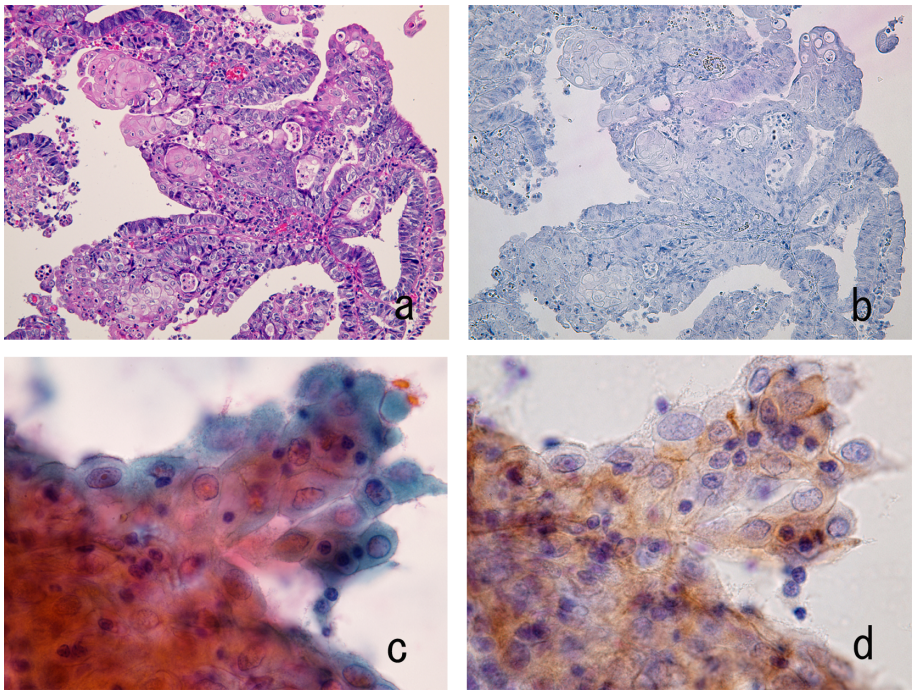

Fig. 6 Immunohistochemical findings for endometrium. a: HE staining × 20; b: MOC31 × 20; c: Pap. staining × 100; d: MOC31 × 100.

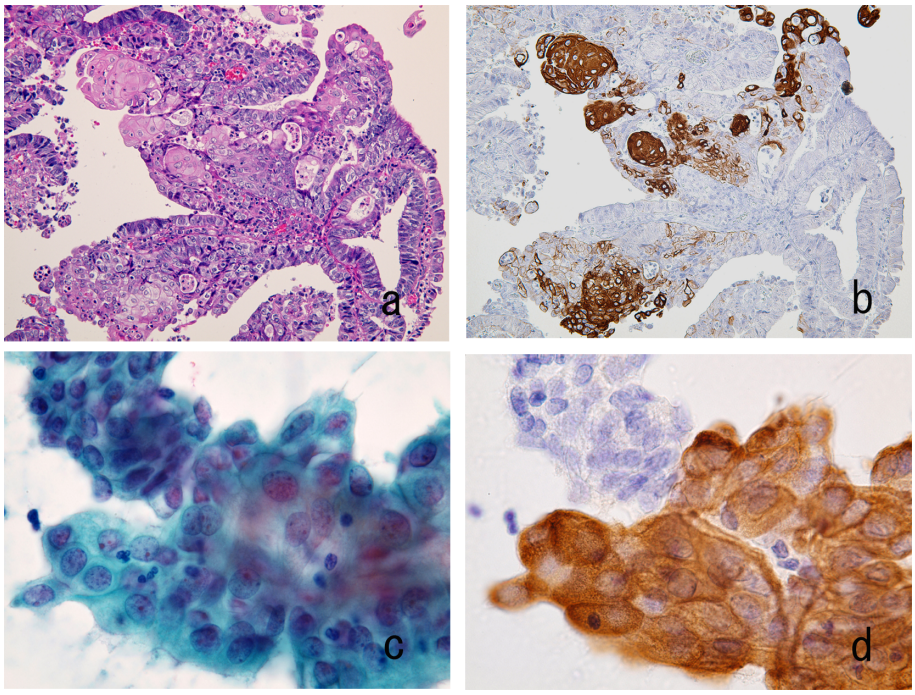

Fig. 7 Immunohistochemical findings for endometrium. a: HE staining × 20; b: CK34βE12 × 20; c: Pap. staining × 100; d: CK34βE12 × 100.

Table 1 Immunohistochemical findings for endometrioid adenocarcinoma.

	Peritoneal cytology		Cytological findings for endometrium		Histological findings for endometrium	
	SMC [*1]	AD [*2]	SMC	AD	SMC	AD
MOC-31	+	+	+	+	−	−
Ck34βE12	+	+/−	+	−	+	−
CD10	−	−	−	−	−	−
calretinin	−	−	/	/	/	/

[*1] SMCs : squamous metaplastic-lile cells
[*2] AD : adenocarcinoma

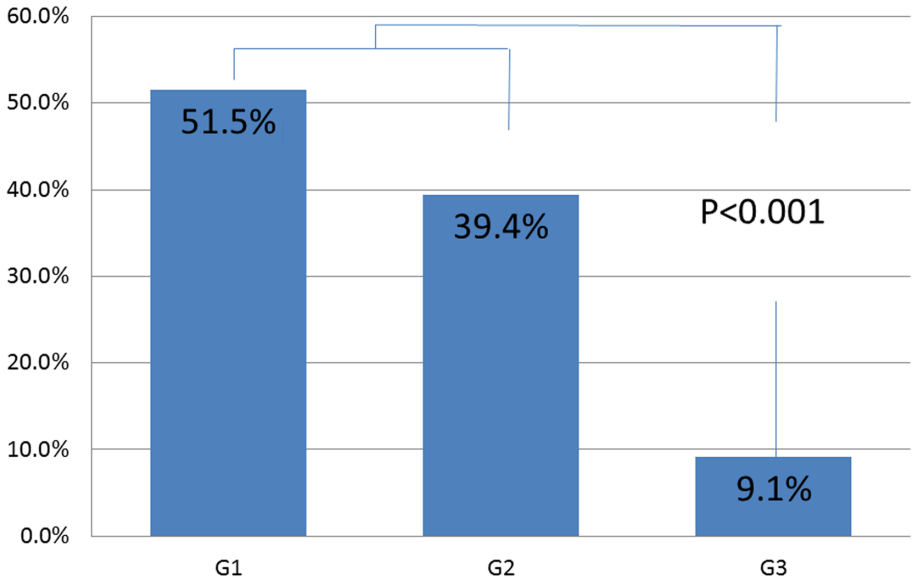

Fig. 8 Comparison of histological grade in positive peritoneal cytology group.

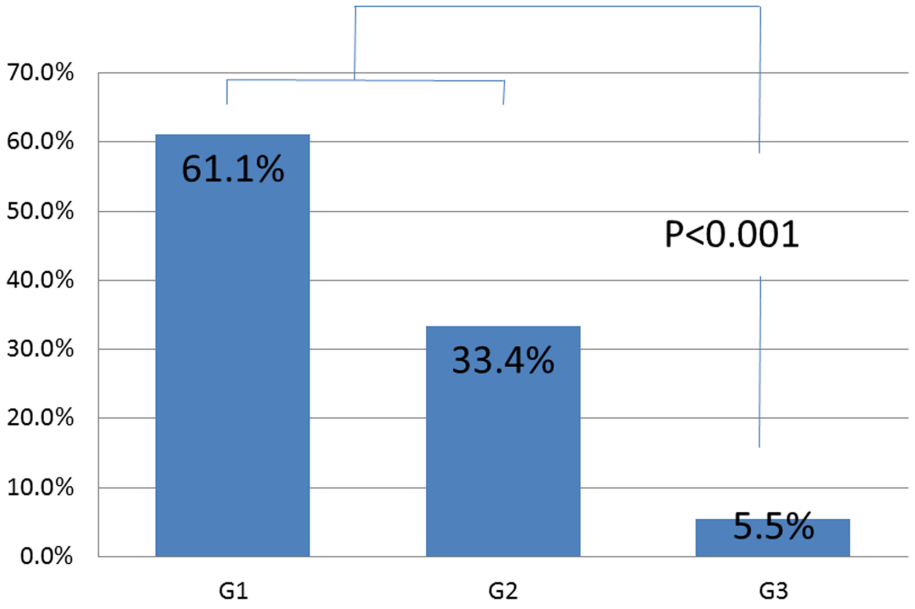

Fig. 9 Comparison of histological grade in positive SMA group.

we could not confirm the presence of squamous metaplasia histologically, intraperitoneal SMCs were detected in some patients. These findings suggest that endometrioid adenocarcinoma is a tumor in which squamous metaplasia is actually more likely to occur than was demonstrated by our observations. In addition, when the appearance of intraperitoneal tumor cells (including adenocarcinoma cells) was examined in relation to the pathological findings, there was no tumor invasion extending to the uterine serosa in any of the patients and the majority of patients had diffuse tumors. It was also suggested that detection of tumor cells in the peritoneal cavity, which was unrelated to tumor diameter or depth of myometrial invasion, reflected shedding of cells into the uterine cavity and transfer via the fallopian tubes.

In the present investigation, the cytological features of endometrioid adenocarcinoma cells in the peritoneal cavity were characterized by the detection of well-differentiated adenocarcinoma cells and the concomitant appearance of SMCs. When endometrial cancer is diagnosed preoperatively or perioperatively, endometrioid adenocarcinoma is likely to be present. Histological examination should therefore be conducted while keeping in mind the possibility that endometrioid adenocarcinoma cells will be found in the peritoneal cavity. Furthermore, when SMCs are detected by peritoneal cytology, these cells have many similarities to reactive mesothelial cells. It is therefore uncertain that identification of SMCs has been performed accurately in the past. It is necessary to have a thorough understanding of the cytological features of SMCs. The combined use of peritoneal cytology and immunostaining offers considerable promise for yield-

Table 2 Clinicopathological profiles for positive peritoneal cytology group.

Squamous metaplastic-like cells	positive [*1] % (cases)	negative [*2] % (cases)	total % (cases)
Histological grade			
G1	61.1 (11)	39.9 (6)	51.5 (17)
G2	33.4 (6)	46.6 (7)	39.4 (13)
G3	5.5 (1)	13.3 (2)	9.1 (3)
Myometrial invasion			
None (Depth a)	16.7 (3)	20.0 (3)	18.2 (6)
< 1/2 (Depth b)	61.1 (11)	26.7 (4)	45.5 (15)
> 1/2 (Depth c)	22.2 (4)	53.3 (8)	36.3 (12)
Cervical involment			
None	88.9 (16)	86.7 (13)	87.9 (29)
Mucosa	11.1 (2)	13.3 (2)	12.1 (4)
Stroma	0 (0)	0 (0)	0.00 (0)
Tumor location			
Diffuse	72.2 (13)	66.7 (10)	69.7 (23)
Local	27.8 (5)	33.3 (5)	30.3 (10)
Tumor size (mm)			
Max	110.0 × 80.0	100.0 × 95.0	110.0 × 95.0
Min	8.0 × 7.0	18.0 × 13.0	8.0 × 7.0
Ave	44.4 × 33.3	56.4 × 41.9	49.8 × 37.2

No statistically significant difference
[*1]SMCs positive: squamous metaplastic-like cells positive
[*2]SMCs negative: squamous metaplastic-like cells negative

Table 3 Comparison of cytological findings for SMC and reactive mesothelial cells.

	SMCs[*1]	RMCs[*2]
Cell cluster pattern	papillary, scattered	papillary, scattered, sheet-like
Nucleus		
form	round	round
chromatin	hyperchromasia	no hyperchromasia
nucleoli	unclear (small)	unclear (small)
Cytoplasm	light green deep staining	light green staining
Microvilli	none	present

[*1]SMCs : squamous metaplastic-like cells
[*2]RMCs：reactive mesothelial cells

ing useful information concerning the detection of SMCs.

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