

Cytological Study of Grade 3 Endometrioid Adenocarcinoma of Endometrial Origin: Cytoarchitecture and Features of Cell Clusters Assessed With Endometrial Brushing Cytology

— Focusing on a comparison with endometrioid adenocarcinoma Grade 1, 2 —

Naruaki MATSUI^{*1}, Hiroshi KAJIWARA^{*2}, Akihiro MORISHITA^{*3},
Hitomi TSUKADA^{*4}, Kazumi NAKAZAWA^{*4}, Masaki MIYAZAWA^{*5}, Mikio MIKAMI^{*5},
Naoya NAKAMURA^{*2} and Shinkichi SATO^{*1}

^{*1} Department of Pathology, Tokai University Oiso Hospital

^{*2} Department of Pathology, Tokai University School of Medicine

^{*3} Department of Pathology, Chigasaki Municipal Hospital

^{*4} Department of Obstetrics and Gynecology, Tokai University Oiso Hospital

^{*5} Department of Obstetrics and Gynecology, Tokai University School of Medicine

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Objectives: Aim of study was to clarify the cytological characteristics of grade 3 endometrioid adenocarcinoma of endometrial origin (G3 EA) by endometrial brushing cytology.

Methods: The subjects were 11 patients in whom G3 EA was diagnosed by review of preoperative cytological specimens obtained at our hospital and related institutions between 2000 and 2010. These patients were investigated with respect to the preoperative cytological diagnosis, background changes, cell cluster patterns, and individual cellular findings. Background changes were classified as inflammatory or tumorous, while cell clusters were classified as overlapping cell cluster, sheet-like cell cluster, clump of high dense gland, papillary, or other cell cluster. Cellular findings were investigated by comparing the incidence of squamous and clear cell metaplasia, the nuclear rounding rate, and the nuclear area with the findings in a control group (35 patients with G1-2 EA).

Results: Background changes were classified as inflammatory in 63.6% and necrotic in 36.4%. The cell clusters were classified as overlapping cell cluster in 44.8%, cell cluster in 21.7%, clump of high dense gland in 10.0%, papillary in 4.0%, and other cell cluster in 19.5%. The incidence of squamous and clear cell metaplasia was 27.2% and 18.1%, respectively. The mean nuclear rounding rate was 0.97, and the mean nuclear area was 55.98 μm^2 .

Conclusion: Investigation of the cytoarchitecture of G3 EA with endometrial brushing cytology revealed overlapping cell cluster and tumor cells of a relatively uniform size. These findings suggest that it is necessary to recognize that there are differences between the cytological findings of G3 EA and the usual features of G1-2 EA.

Key words: Endometrial cytology, Grade 3 endometrioid adenocarcinoma, Cytoarchitecture, Features of Cell Clusters, Endometrial brushing cytology

INTRODUCTION

Endometrioid adenocarcinoma of endometrial origin is classified into types 1 and 2, and each of these types have characteristic clinical and histological findings [1-4]. Grade 1 and grade 2 endometrioid adenocarcinoma (G1-2EA) are classified as type 1 endometrial cancer, which is based on endometrial hyperplasia and has a favorable prognosis. On the other hand, grade 3 (G3 EA), clear cell adenocarcinoma, and serous adenocarcinoma are classified as type 2 endometrial cancer. These tumors are not closely related to the ordinary risk factors for endometrial cancer and have an unfavorable prognosis. By cytodiagnosis, it is relatively easy to determine whether G3 EA is malignant or not, but it is difficult to assess the histological type. In the present study, we evaluated the preoperative cytology

of G3 EA and assessed its cytological characteristics by endometrial brushing cytology (using Softcyto, SOFT MEDICAL CO., LTD, Japan).

MATERIALS AND METHODS

The subjects were 11 patients in whom G3 EA was diagnosed by review of preoperative endometrial cytology at our hospital and related institutions between 2000 and 2010. These patients were investigated with respect to 1) preoperative cytology, 2) background changes, 3) the pattern of tumor cell growth, and 4) the features of individual tumor cells. Then each finding was compared between the G3 EA group and a control group of 35 patients with G1-2 EA.

Background changes were classified as tumorous or inflammatory. The tumor cell cluster pattern was classified as overlapping cell clusters (OLC), sheet-like

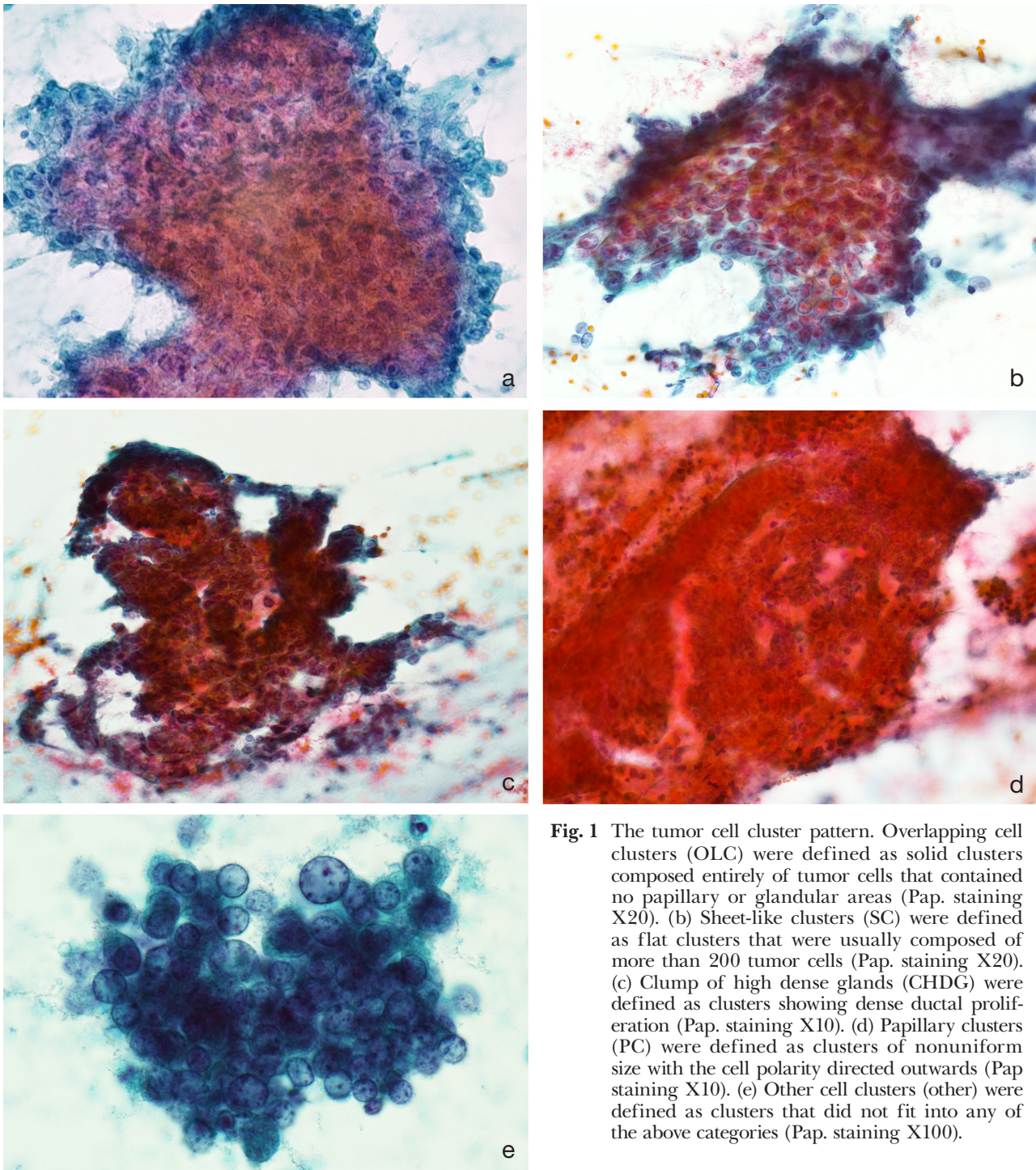


Fig. 1 The tumor cell cluster pattern. Overlapping cell clusters (OLC) were defined as solid clusters composed entirely of tumor cells that contained no papillary or glandular areas (Pap. staining X20). (b) Sheet-like clusters (SC) were defined as flat clusters that were usually composed of more than 200 tumor cells (Pap. staining X20). (c) Clump of high dense glands (CHDG) were defined as clusters showing dense ductal proliferation (Pap. staining X10). (d) Papillary clusters (PC) were defined as clusters of nonuniform size with the cell polarity directed outwards (Pap. staining X10). (e) Other cell clusters (other) were defined as clusters that did not fit into any of the above categories (Pap. staining X100).

clusters (SC), clump of high dense glands (CHDG), papillary clusters (PC), or other cell clusters (other). OLC were defined as solid clusters composed entirely of tumor cells that contained no papillary or glandular areas. SC were defined as flat clusters that were usually composed of more than 200 tumor cells, while SHDG were defined as clusters showing dense ductal proliferation. PC were defined as clusters of nonuniform size with the cell polarity directed outwards. Finally, other were defined as clusters that did not fit into any of the above categories (Fig. 1). The incidence of each type of cluster in each sample obtained from each patient was calculated and expressed as percentage.

Tumor cells were also investigated to determine the incidence of squamous metaplasia [5-7] and clear cell

metaplasia (Fig. 2) [8], as well as the nuclear rounding rate and nuclear area. The tumor cell nuclear rounding rate and nuclear area were measured for 200 tumor cells in each sample. In all patients, cells were collected with an endosoft and measurements were made using Nikon NIS-Elements Ver. 3.1 (Fig. 3). For statistical analysis, Student's t-test was employed and $P < 0.05$ was considered to be statistically significant.

This study was approved by the Ethics Committee of the Institutional Review Board (IRB) for Clinical Research at Tokai University School of Medicine (14R-265). Informed consent was provided in compliance with the Helsinki Declaration. Patient anonymity was preserved.

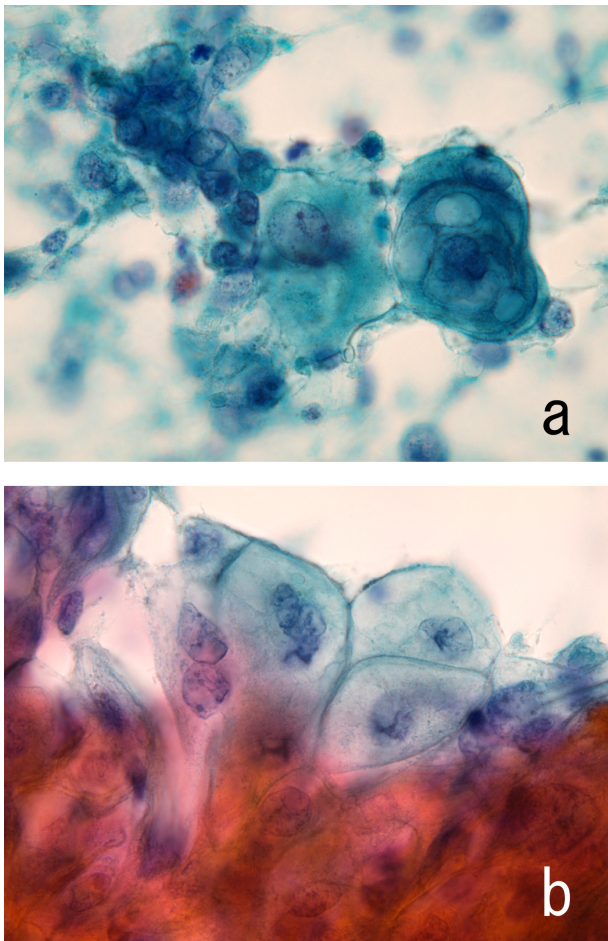


Fig. 2 Cytological findings of endometrial metaplasia in grade3 endometrioid adenocarcinoma. (a) squamous metaplasia (Pap. staining X100). (b) clear cell metaplasia (Pap. staining X100).

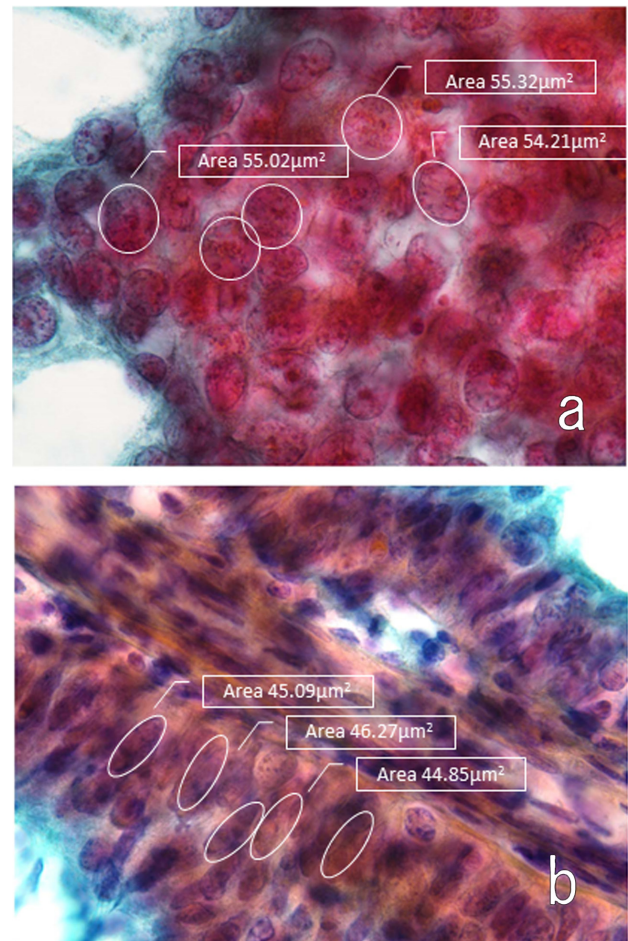


Fig. 3 Comparison of nuclear findings in grade 3 and grade 1-2 endometrioid adenocarcinoma. Nuclear measurement image of grade3 (a) and grade 1-2(b) endometrioid adenocarcinoma. The tumor cell nuclear rounding rate and nuclear area are measured by Nikon NIS-Elements Ver. 3.1. (a, b, Pap. staining X100).

RESULTS

1) Preoperative cytological diagnosis

In the G3 EA group, the mean age of the patients was 66 years (range: 52-83 years) and 8 of the 11 patients were postmenopausal women. Preoperative endometrial cytology revealed malignancy in all 11 patients. However, G3 EA was diagnosed accurately in only 6/11 patients (54.5%). On the other hand, the mean age of the patients was 55 years (range: 33-79 years) and 14 of the 35 patients were postmenopausal women in the G1-2 EA group. Preoperative endometrial cytology revealed malignancy in 28 out of 35 patients (80.0%). G1-2 EA was diagnosed accurately in 23/28 patients (82.1%) (Table 1).

2) Background changes

In the G3 EA group, an inflammatory background that mainly featured infiltration of neutrophils was noted in 7/11 patients (63.6%), while a tumorous background that mainly featured necrosis was noted in 4/11 patients (36.4%). On the other hand, in the G1-2 EA group, an inflammatory background that mainly featured infiltration of neutrophils was noted in 18/35 patients (51.4%), while a clean background was noted

in 13/35 patients (37.2%), and tumorous background that mainly featured necrosis was noted in only 4/35 patients (11.4%) (Table 2). The mixture cases of the necrotic and inflammatory background are divided by the amount of significant findings.

3) Tumor cell cluster pattern

In the G3 EA group, OLC, SC, CHDG, PC, and other accounted for 44.8%, 21.7%, 10.0%, 4.0%, and 19.5% of the cell clusters, respectively. On the other hand, in the G1-2 EA group, OLC, SC, CHDG, PC, and other accounted for 0.0%, 24.0%, 27.8%, 35.4%, and 12.8% of the cell clusters, respectively (Fig. 4).

4) Tumor cell findings

a) Incidence of squamous and clear cell metaplasia

In the G3 EA group, the incidence of squamous metaplasia was 27.2% (3/11 patients) and it was 18.1% (2/11 patients) for clear cell metaplasia. On the other hand, the incidence of squamous metaplasia was 82.9% (29/35 patients) in the G1-2 EA group and clear cell metaplasia was not seen (Table 3).

b) Nuclear rounding rate and nuclear area

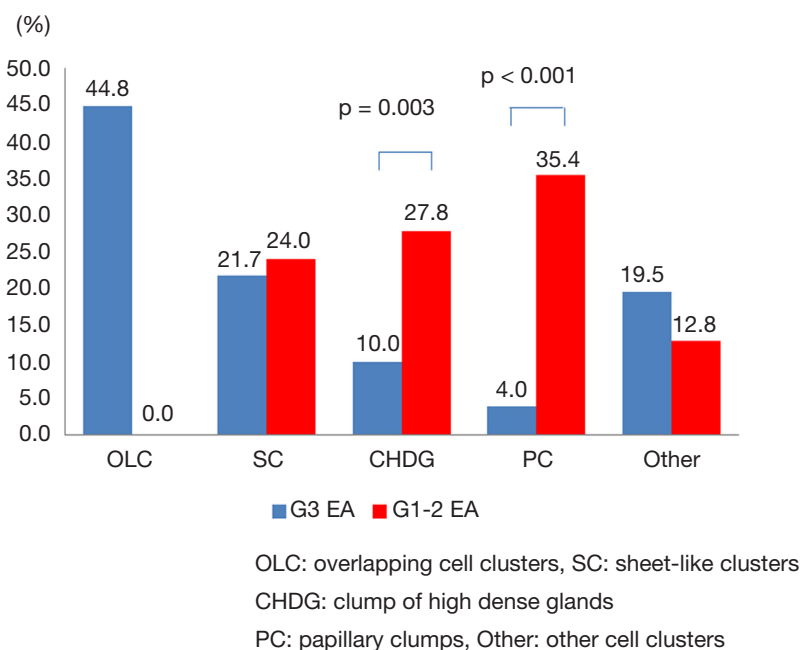
In the G3 EA group, the mean nuclear rounding

Table 1 Preoperative profiles

	G3 EA (n = 11)	G1-2 EA (n = 35)
Age	66(52-83)	55(33-79)
Complaint % (cases)		
Genital bleeding	72.7(8)	74.2(26)
Other	27.3(3)	25.8(9)
Cytological diagnosis % (case)		
Malignancy	100(11)	80.0(28)
Indeterminate	0(0)	20.0(7)
Histological type % (case)		
Suspicious of G3 EA (n = 11) or G1-2 EA (n = 28)	54.5(6)	82.1(23)
Histological finding % (case)		
T stage		
1	45.4(5)	77.1(27)
2	36.4(4)	14.3(5)
3	18.2(2)	8.6(3)
Depth		
<1/2	36.4(4)	68.6(24)
>1/2	63.6(7)	31.4(11)

Table 2 Comparison of cytological background

	G3 EA % (cases)	G1-2 EA % (cases)
Inflammation	63.6(7)	51.4(18)
Necrosis	36.4(4)	11.4(4)
Clean	0 (0)	37.2(13)

**Fig. 4** Comparison of cell cluster ratio in grade 3 and grade 1-2 endometrioid adenocarcinoma.

rate was 0.97 (maximum: 1.00, minimum: 0.49), and each cell had an oval nucleus with a longitudinal/lateral ratio of nearly 1. The mean nuclear area was $55.98 \mu\text{m}^2$ (maximum: 180.47, minimum: 19.01), showing a wide distribution, but assessment of the nuclear area in each patient suggested that the clusters were

composed of tumor cells with a relatively uniform size. On the other hand, the mean nuclear rounding rate was 0.74 (maximum: 1.00, minimum: 0.31) in the G1-2 EA group and the mean nuclear area was $46.18 \mu\text{m}^2$ (maximum: 95.91, minimum: 17.19) (Fig. 5, 6).

Table 3 Frequency of endometrial metaplasia in grade 3 and grade 1-2 endometrioid adenocarcinoma

	G3 EA %(cases)	G1-2 EA % (cases)
Squamous metaplasia	27.2(3)*	82.9(29)*
Clear cell metaplasia	18.1(2)	0(0)

*p <0.001

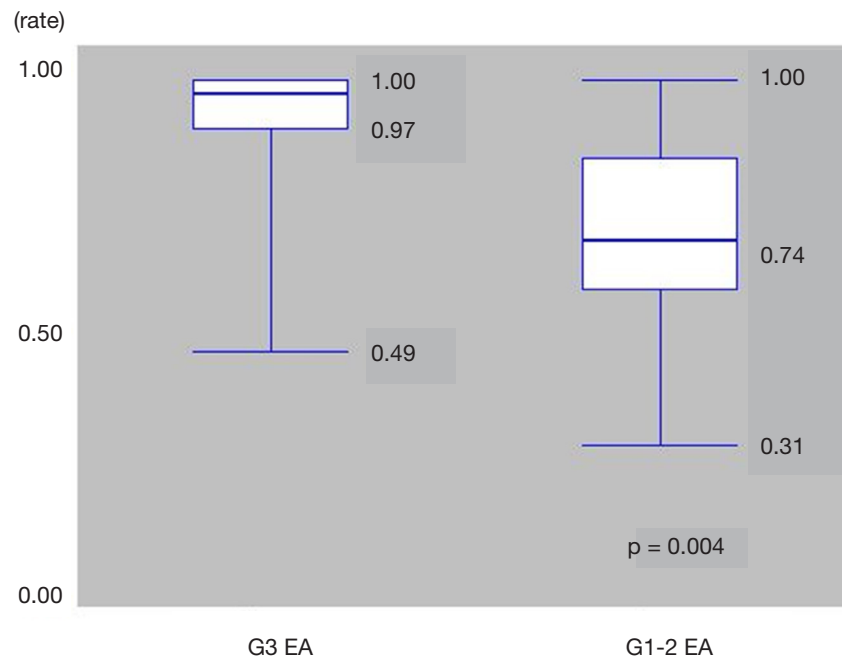


Fig. 5 Comparison of nuclear rounding rate in grade 3 and grade 1-2 endometrioid adenocarcinoma.

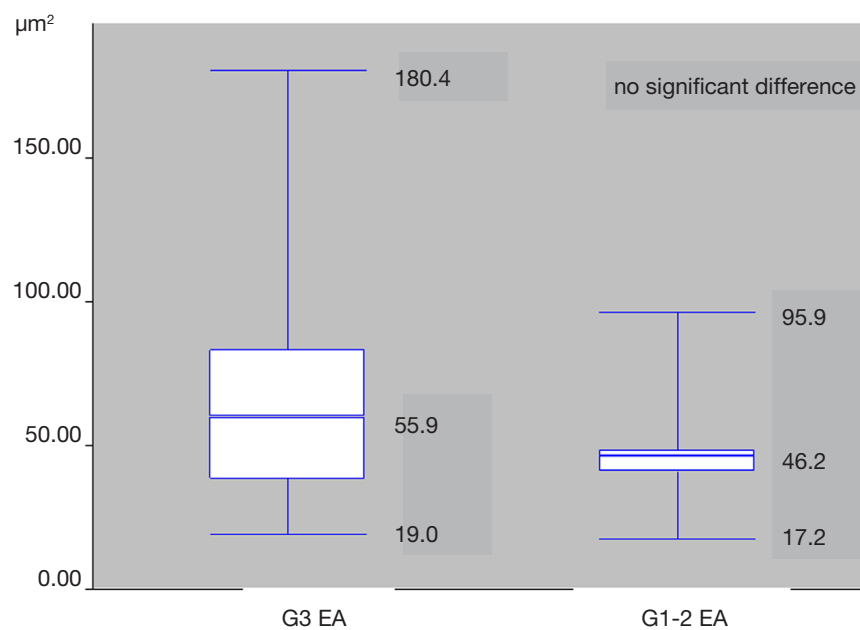


Fig. 6 Comparison of nuclear area in grade 3 and grade 1-2 endometrioid adenocarcinoma.

DISCUSSION

Although there have been many reports concerning well-differentiated endometrioid adenocarcinoma of endometrial origin [9–23], there have been few detailed investigations of G3 EA [24–26]. This suggests that sufficient importance has not been attached to G3EA because cytologically distinguishing between benign and malignant disease is easier for G3 than for G1–2 EA. However, detection of G3 EA by preoperative cytology is the most important determinant of the range of lymph node dissection at laparotomy. Because G3 EA does not have characteristic cytological findings, it is not necessarily easy to accurately assess its histological type. In the present study, whether malignancy or benign was diagnosed preoperatively in all patients, but whether G3 EA or G1–2 EA was only identified in half of them.

The following features of G3 EA [24–26] have been reported previously: 1) a tumorous background, 2) scattered cells, 3) tumor cells with marked variation in size, 4) a high N/C ratio, and 5) relatively prominent nucleoli. In addition to these findings, we identified some characteristics with respect to the background changes, pattern of growth, and cellular features.

The background was either an inflammatory background that mainly featured infiltration of neutrophils or a tumorous background. An inflammatory background featuring neutrophils is usually more common for G1–2 EA [6]. In G3 EA, extensive necrosis has often occurred due to disease progression at the time of detection. The stage was T2 or higher in 6 of our 11 patients, and the background was tumorous in 3 of them. Thus, it is suggested that both an inflammatory background featuring neutrophils and a tumorous background can be observed as the background changes in G3 EA.

Investigation of the tumor cell cluster pattern revealed that OLC accounted for approximately 50%, followed by sheet-like clusters. It seems likely that OLC of various sizes appeared as a result of loss of ducts due to a decrease in the degree of differentiation, while the appearance of SC was presumably ascribable to separation of cell clusters from the OLC aggregates.

Investigation of the nuclear area and nuclear rounding rate of the tumor cells showed that most of the nuclei were relatively uniform in size and oval in shape. In general, as adenocarcinoma becomes less differentiated, variation in nuclear size becomes more marked [27–30], resulting in a decrease of intercellular adhesion. In G3 EA, we detected both cell clusters in which cohesiveness was maintained and cell clusters composed of tumor cells with a relatively uniform size. Thus, there were differences in the pattern of tumor growth between G3 EA and ordinary poorly differentiated adenocarcinoma.

In endometrioid adenocarcinoma of endometrial origin, both type 1 and 2 endometrial cancer are classified as the same histological type. However, it is important to distinguish two types, because the prognosis depends on the degree of differentiation. According to various reports concerning G1–2 EA that have been published previously (6, 9, 15–21), papillary clusters are most common and followed by clusters with dense

ducts. The incidence of squamous metaplasia is approximately 80% [6, 7], and no clear cell metaplasia is noted. In G1–2 EA, the mean nuclear rounding rate was reported to be 0.74 (maximum: 1.00, minimum: 0.31), and the nuclei were oval to tall cylindrical in shape. Therefore, to achieve cytological differentiation of G3 EA from G1–2 EA, it seems to be necessary to focus on the features of cell clusters, the nuclear findings, and the incidence of metaplastic cells.

In the present cytological study of G3 EA, the following characteristics were confirmed in addition to those reported previously: 1) both inflammatory and tumorous backgrounds were observed, 2) the incidence of OLC aggregates and SC was high, 3) a few clear cell metaplasia was noted, 4) the tumor cells were relatively uniform in size, and 5) nuclei were oval with no appreciable notches or constrictions. These cytological features of G3 EA are different from those usually associated with poorly differentiated adenocarcinoma. It is uncertain whether clear cell metaplasia is nonspecific or diagnostic findings, however the results in our present study suggest that the presence of clear cell metaplasia is one of the characteristic change seen in high grade malignancy. Although this was a limited study in a small number of subjects, it is considered to have clarified some of the cytological characteristics of G3 EA. If attention is focused on these findings, it might be possible to make a more accurate cytological diagnosis in patients who are suspected to have adenocarcinoma or type 2 endometrial cancer. Importance has recently been attached to identification of structural atypia in the observation of well-differentiated endometrioid adenocarcinoma. When endometrial cytology is performed, it seems to be important to carefully observe not only the appearance of tumor cells but also the above-mentioned findings in order to identify G3 EA.

CONFLICT OF INTEREST STATEMENT

None declared.

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