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Relation of squamous differentiation in endometrioid carcinoma with MELF pattern to a high ratio of lymph node metastasis

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ABSTRACT

One of the known histological patterns of endometrioid carcinoma (EC) in uterine corpus cancer is MELF (microcystic, elongated, and fragmented). MELF is associated with lymphovascular invasion and lymph node metastasis. Besides MELF, it is also known that squamous differentiation (SD) often occurs in EC. SD is known to be no significant difference in the frequency of lymph node metastasis in EC. However, there have been no previous reports on the association between MELF and SD. In this research, we investigated the presence of SD in MELF using an antibody to CK5. We examined 28 cases of EC with MELF pattern, in which 15 cases showed SD. Moreover, the relation of lymph node metastasis to SD was examined. Lymph node dissection was performed in 27 out of 28 cases. Among them, 12 cases showed lymph node metastasis. The ratio of lymph node metastasis was significantly higher in EC with SD (64.3 %, 9 in 14 cases) than EC without SD (23.1 %, 3 in 13 cases). In this study, we first showed the association between SD and MELF and that MELF with SD is associated with a high ratio of lymph node metastasis. It is clinically relevant to recognize that MELF with SD is aggressive with a high ratio of lymph node metastasis.

1. Introduction

In uterine corpus cancer, one of the known histological patterns of endometrioid carcinoma (EC) is MELF. MELF is an acronym for microcystic, elongated, and fragmented [1]. In EC, the more solid components without glands are considered aggressive. However, MELF is an aggressive histological pattern that occurs predominantly in cancers without solid components, and is associated with lymphovascular invasion and lymph node metastasis [1]. MELF shows unique expression pattern of genes distinct from usual EC [2–6]. Besides MELF, it is also known that squamous differentiation (SD) often occurs in EC. Zaino et al. reported that SD was identified in about 25 % of EC and that EC with SD showed no significant difference in the frequency of lymph node metastasis as compared to EC without SD [7]. Cells with SD have abundant acidophilic cytoplasm. In the MELF pattern, especially in elongated glands in invasive front, we often find abundant acidophilic cytoplasm. However, there have been no previous reports on the association between MELF and SD. In this research, we investigated the presence of SD in MELF. Moreover, we examined the relationship between SD in MELF and lymph node metastasis.

2. Materials and methods

2.1. Patients

We examined patients undergoing surgery for EC of the uterine corpus at Osaka University Hospital between 2018 and 2023. Among them, 28 cases of EC with MELF pattern were found. Resected specimens were fixed in 10 % solution of formalin neutral buffer and processed for embedding in paraffin. The specimens were stored at room temperature in a dark room. Specimens for evaluation were cut into sections of 4 μm thick and stained with hematoxylin and eosin (H&E). This study was approved by the Ethics Review Board of the Graduate School of Medicine, Osaka University (No. 15234). Written informed consent was obtained from all patients.

Abbreviations: CK, cytokeratin; EC, endometrioid carcinoma; EMT, epithelial-mesenchymal transition; H&E, hematoxylin and eosin; IHC, immunohistochemistry; MELF, microcystic elongated and fragmented; MM, morular metaplasia; SD, squamous differentiation.

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2.2. Immunohistochemistry for CK5, CK14, p40 and E-cadherin

Immunohistochemistry (IHC) was performed using a mouse monoclonal antibody anti-cytokeratin (CK) 5 (clone XM26, Abcam, Cambridge, UK) at a dilution of 1/200, anti-CK14 (clone LL002, Abcam) at a dilution of 1/1600, anti-p40 (clone BC28, Biocare Medical, Pacheco, CA, USA) at a dilution of 1/100, and anti-E-cadherin (clone NCH-38, Agilent, Santa Clara, CA, USA) at a dilution of 1/100. Immunostaining operations were performed by Dako Autostainer Link 48 (Agilent). We evaluated the proportion of the CK5-positive tumor cells in the surface area and in the invasive front area, respectively. Andrade et al. adopted 10 % as the criterion for the presence or absence of SD in EC, which we also adopted [8]. Furthermore, we assessed the expression of E-cadherin in invasive front area. Histological score (H-score) of E-cadherin was calculated using the following formula: [1× (% tumor cells of 1 +)+ 2× (% tumor cells of 2 +)+ 3× (% tumor cells of 3 +)].

2.2.1. Statistical analysis

We used JMP Pro 17 software (SAS Institute, Cary, NC, USA) to conduct statistical analyses. Chi-square test and Student's t test were used for the analysis, in which $p < 0.05$ was considered statistically significant.

3. Results

3.1. CK5 is an adequate marker of SD in EC

In IHC, p40 and CK5/6 are known markers of squamous epithelium [9]. As for antibody to CK5/6, the most commonly used clone is D5/16 B4. However, the target of D5/16 B4 is only CK5 and as a marker for CK5, clone XM26 is superior to D5/16 B4 [10]. Therefore, we used CK5 recognized by clone XM26 instead of CK5/6 as a marker of SD. To verify the usefulness of the markers, we performed IHC of p40 and CK5 on one case of EC with prominent SD and one case of EC with prominent morular metaplasia (MM), which arises from immature squamous epithelial cell differentiation. The area of SD recognizable by H&E staining coincided with the positive area of p40 or CK5 (Figs. 1a-1c). On the other hand, tumor cells with MM were focally and weakly positive for CK5 and negative for p40 (Figs. 1d-1f). We could distinguish SD from MM by using IHC of p40 or CK5. However, while CK5 was diffusely positive in SD, the pattern was a mixture of strong and weak positive signals in IHC of p40, and that made difficult in quantification. Therefore, we chose CK5 for evaluating SD in MELF.

3.2. SD is often found in MELF

Of the 28 cases of EC with MELF, 15 cases (53.6 %) were positive for CK5, demonstrating almost half cases showed SD (Table 1). Among 15 cases with SD, CK5 positivity was detected at both surface and invasive front areas in 9 cases (Fig. 2a), at only surface area in 3 cases (Fig. 2b) and at only invasive front area in 3 cases (Fig. 2c). CK5-positive cells were arranged in sheet-like patterns at surface area, while CK5 were positive mainly in elongated glands at invasive front area (Figs. 2b, 2c). As for CK14 immunostaining, the distribution of positive cells was generally similar to that of CK5 immunostaining, although CK14-positive cells were fewer than CK5-positive cells (Figure S1).

3.3. SD of MELF exhibits a prominent EMT and is related to a high ratio of lymph node metastasis

The intensity of E-cadherin expression was examined as an indicator of the degree of epithelial-mesenchymal transition (EMT). The typical staining patterns were shown in Fig. 3a. We compared H-score of E-cadherin in invasive front area between MELF with CK5-positive (15 cases) and MELF with CK5-negative (13 cases). H-score of E-cadherin was significantly lower in MELF with CK5-positive than MELF with CK5-negative (Fig. 3b). Moreover, lymph node dissection was performed in

Table 1
Presence and location of SD.

Presence of SD	Present			Absent
Location of SD	Both surface and invasive front areas	Surface area only	Invasive front area only	
No. of cases	9	3	3	13

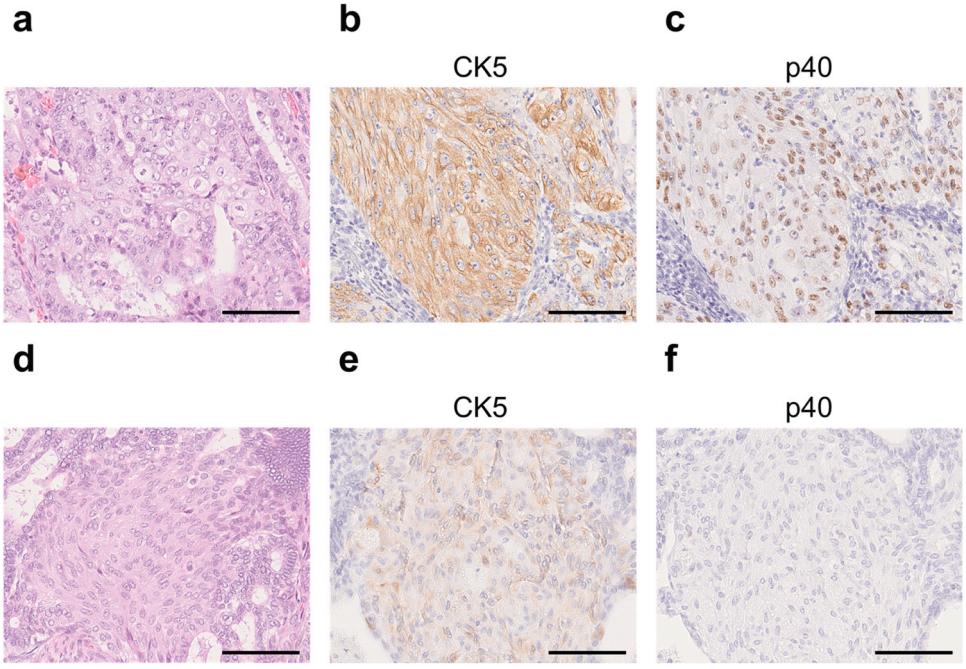

Fig. 1. Histological images of endometrioid carcinoma with prominent squamous differentiation (SD) and morular metaplasia (MM). (a-c) Representative images of SD of H&E stain (a), IHC for CK5 (b), IHC for p40 (c). (d-f) Representative images of MM of H&E stain (d), IHC for CK5 (e), IHC for p40 (f). Scale bars: 100μm (a-f).

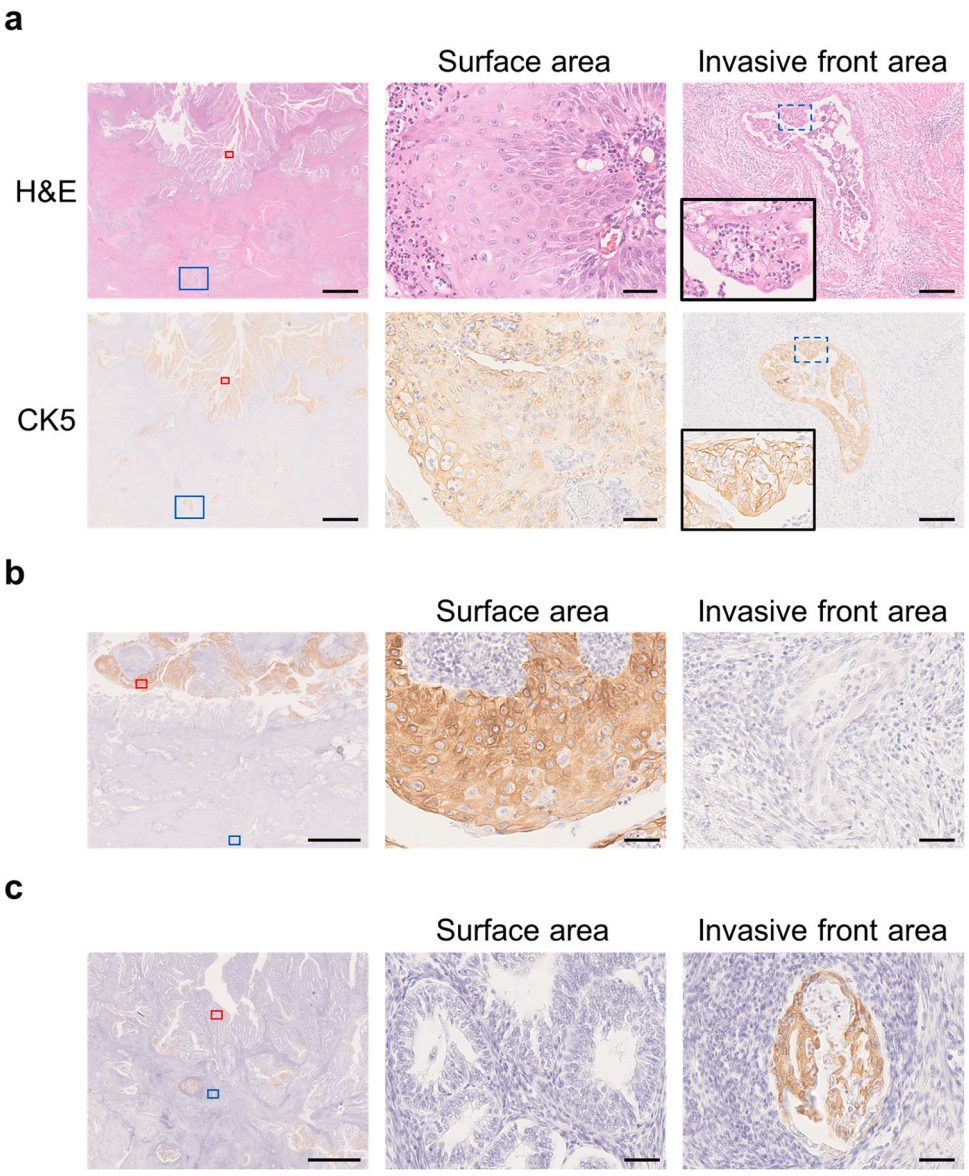

Fig. 2. CK5 expression in MELF. (a) Cases with CK5-positive both in the surface and the invasive front area. The upper figures show H&E staining and the lower show CK5 immunostaining. From left to right: loupe image, the figure showing the area with solid red line is enlarged (surface area), the figure showing the area with solid blue line is enlarged (invasive front area), including the inset containing an enlarged version of the dotted blue area. (b, c) CK5 immunostaining images of cases with CK5-positive only in the surface area (b) and CK5-positive only in the invasive front area (c). From left to right: loupe image, the figure showing the area with red line is enlarged (surface area), the figure showing the area with blue line is enlarged (invasive front area). Scale bars: (a) from left to right: 2 mm, 50 μm, 200μm, (b, c) from left to right: 2 mm, 50 μm, 50 μm.

27 out of 28 cases, in which 12 cases showed lymph node metastasis. Then, the relation of lymph node metastasis to SD was examined. The ratio of lymph node metastasis was significantly higher in MELF with SD (64.3 %, 9 in 14 cases) than MELF without SD (23.1 %, 3 in 13 cases) (Table 2), indicating that SD in MELF was related to high ratio of lymph node metastasis (chi-square 4.646, $p = 0.031$). Next, we examined the relation of lymph node metastasis to location of SD. The ratio of lymph node metastasis was 77.8 % (7 in 9 cases), 33.3 % (1 in 3 cases), 50.0 % (1 in 2 cases) at MELF with SD in both surface and invasive front areas, MELF with SD in surface area, and MELF with SD in invasive front area, respectively (Table 3). Location of SD did not appear to be related to the ratio of lymph node metastasis (chi-square 2.143, $p = 0.3420$).

4. Discussion

It is well known that EC is often accompanied by SD. This is easy to determine when keratinization is present or when SD is widely spread, but can be difficult to determine when this is not the case. Using the antibody against CK5, the area of SD was easily recognized. Moreover, MM is known to arise from immature squamous epithelial cell differentiation. Travaglino et al. reported that CK5/6 was focally positive in the typical MM areas and diffusely positive in the SD areas [11]. We obtained similar results by immunohistochemistry of CK5 (Figs. 1b, 1e). We confirmed that CK5 is an appropriate marker for SD of EC. Then IHC of CK5 was performed against MELF. CK5-positive cells with a sheet-like pattern in the superficial area was similar to that of SD in normal EC, but the positivity of CK5 in the elongated gland of the invasive front area was unique to MELF. Although it is difficult to consider SD from the point of view of gland formation, cells with abundant acidophilic cytoplasm had the characteristics of SD. It has not been previously noted that MELF is accompanied by SD, but by using IHC, we found that it is accompanied by SD in this study. SD is identified in approximately one

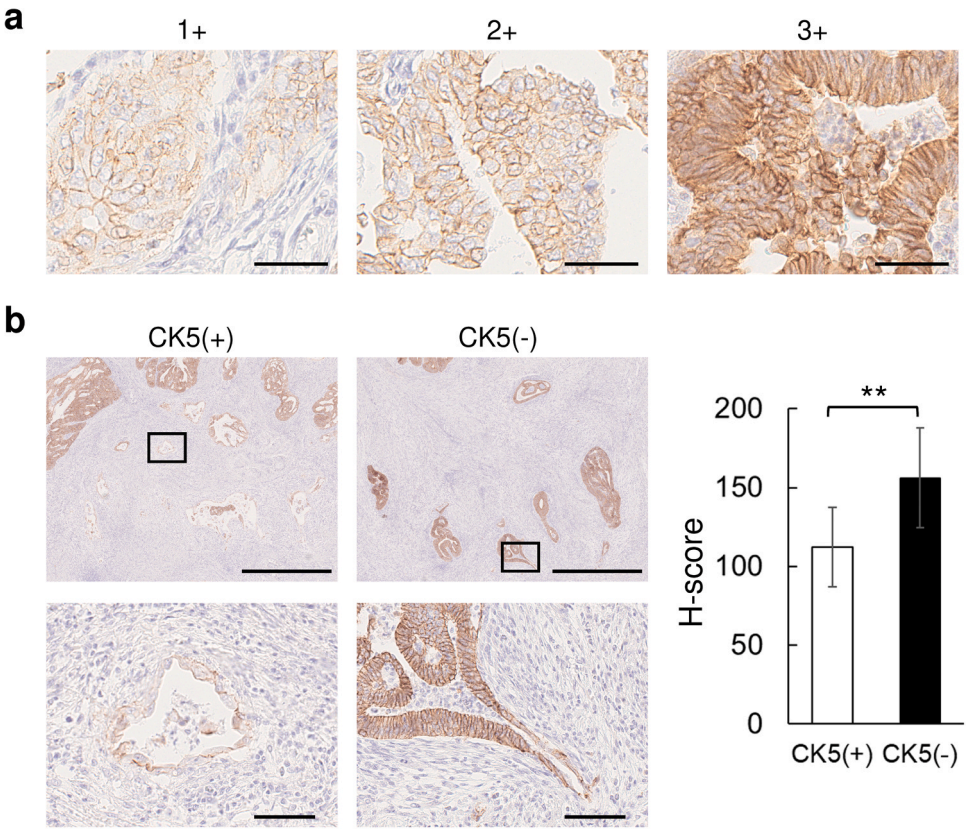

Fig. 3. E-cadherin expression in MELF with CK5-positive and CK5-negative. (a) Representative immunohistochemical images of E-cadherin are shown with intensity classified as weak (1 +), moderate (2 +), or strong (3 +). (b) Representative images and comparison of H-scores between MELF with CK5-positive (n = 15) and CK5-negative (n = 13). The lower picture is an enlargement of the area circled in the upper picture. Asterisks indicate significant differences as determined by Student's *t*-test (**$P<0.01$). Scale bars: (a) 50 μm, (b) 1 mm in upper pictures and 100 μm in lower pictures.

Table 2
Relationship between presence of SD in MELF and lymph node metastasis.

Presence of SD	Present	Absent
No. of cases with lymph node metastasis	9	3
No. of cases without lymph node metastasis	5	10
Ratio of cases with lymph node metastasis	64.3 %	23.1 %

Table 3
Relationship between location of SD and lymph node metastasis.

Location of SD	Both surface and invasive front areas	Surface area only	Invasive front area only
No. of cases with lymph node metastasis	7	1	1
No. of cases without lymph node metastasis	2	2	1
Ratio of cases with lymph node metastasis	77.8 %	33.3 %	50.0 %

fourth of usual EC [7], but half of EC with MELF showed SD, indicating that the potential of SD was higher in EC with MELF than in usual EC. These findings suggested that EC with MELF possessed the potential of aberrant differentiation other than differentiation to endometrial glands.

It has been reported that SD does not influence poor prognostic factors such as lymph node metastasis in usual EC. Zaino et al. analyzed 456 cases of typical EC and 175 cases of EC with SD, and reported that the presence of SD per se was not associated with a statistically significant difference in the frequency of lymph node metastasis [7]. Ocak et al. reported that in an analysis of a study group consisting of 272 EC patients with low and intermediate-risk, SD did not significantly affect recurrence [12]. However, SD has been associated with an increased incidence of lymph node metastasis in EC with MELF. This suggests that SD may serve as a marker of aggressiveness in EC with MELF. EC with MELF is known to have a higher propensity for metastasis, and the metastatic potential appears to be exacerbated in the presence of SD. One of the candidates for molecular mechanism underlying the relationship between SD and lymph node metastasis is EMT. The EMT process generates poorly differentiated and potentially pluripotent cells, and this intermediate phenotype is linked to the expression of basal cytokeratins such as CK5 and CK14 [13]. In fact, in most cases of MELF with SD, not only CK5 but also CK14 was expressed (Figure S1). MELF is known to be involved in EMT [14]. Our experiments showed that MELF with SD had a more marked decrease in E-cadherin expression than MELF without SD, resulting in more significant EMT. EMT cells are associated with lymph node metastasis by hijacking the immune system [15]. Therefore, we speculated that EMT is associated with lymph node metastasis of MELF with SD.

It is clinically useful to recognize that MELF with SD is aggressive with a high ratio of lymph node metastasis. If this can be noted during preoperative biopsy or intraoperative rapid diagnosis, it would help to determine whether and to what extent lymph node dissection should be performed at surgery. It is sometimes difficult to recognize SD, especially in the invasive front area, but it is helpful to understand the morphology of cells with an abundant acidophilic cytoplasm.

Taken together, we first showed the association between SD and MELF and that MELF with SD is associated with a high ratio of lymph node metastasis in the current study.

CRediT authorship contribution statement

Eiichi Morii: Writing – review & editing, Supervision. **Kansuke Kido:** Data curation. **Kazuaki Sato:** Data curation. **Shinichiro Tahara:** Writing – original draft, Methodology, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2024.155804.

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