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ORIGINAL ARTICLE

Association between Morphological Patterns of Myometrial Invasion and Cancer Stem Cell Markers in Endometrial Endometrioid Carcinoma

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Abstract In endometrial endometrioid adenocarcinoma (EEC), the depth of myometrial invasion (MI) is an important parameter for determining whether additional treatment is warranted. The present study investigated the association between MI patterns, cancer stem cell (CSC) phenotypes, and their clinicopathological significance in EEC. A total of 73 cases of EEC with MI were examined in this study. Haematoxylin and eosin-stained tissue specimens were analysed for MI pattern, which was categorised as infiltrating; expansile; adenomyosis (AM)-like; or microcystic, elongated, and fragmented (MELF)-type. The expression of CSC markers such as cluster of differentiation (CD)44, CD133, and Nanog1, as well as oestrogen receptor (ER) and progesterone receptor (PR) was examined by immunohistochemistry. Clinicopathological features including age, DOI, MI pattern, LVI, lymph node (LN) metastasis, disease progression, and survival outcome were recorded. Most examined cases (45/73) were International Federation of Gynecology and Obstetrics (FIGO) stage I. MI showed infiltrating (49.3%), AM-like (26.3%), MELF (15.1%), and expansile (9.6%) patterns. Tumours with the infiltrating pattern were associated with high FIGO grade ($P = 0.002$), reduced ER and PR, and CD44 expression ($P = 0.014$, 0.026, and 0.030, respectively); those with a MELF pattern showed LN metastasis ($P < 0.001$), lymphovascular invasion ($P = 0.011$), and reduced ER, CD44, and CD133 expression ($P = 0.036$, 0.006, and 0.016, respectively). EEC with infiltrating/MELF patterns of MI is associated with worse prognosis. These results suggest that CSC expression profiles are an unfavourable indicator of EEC.

Keywords Cancer stem cell marker · Epithelial-to-mesenchymal transition · Endometrioid carcinoma · Myometrial invasion

Introduction

Endometrial endometrioid carcinoma (EEC) is the most common primary gynaecological malignancy in the western countries, with approximately 40,000 new cases diagnosed each year in the US [1]. Clinicopathological parameters that have been traditionally used to predict prognosis include myometrial invasion (MI) depth and tumour cell histological grade [2]. Tumour size, lower uterine segment (LUS) involvement, cervical involvement, lymphovascular invasion (LVI), and MI pattern are further proposed as potential indicators of extrauterine progression [2]. MI morphological patterns include diffusely infiltrating irregular glands; broad front (or pushing border); adenoma malignum; adenomyosis (AM)-like; microcystic, elongated, and fragmented (MELF)-type glands [1], and can be useful for assessing the depth of invasion (DOI), extent of tumour spread, and presence of LVI [1].

Cancer stem cells (CSCs) have distinct characteristics including self-renewal, and expression of specific markers that enable their isolation [3]. CSCs have been detected in various malignancies and are involved in the resistance of cancers to conventional treatments such as radio-, chemo-, and hormonal therapy and molecular inhibitors [4–10]. Therefore,

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identifying CSCs is important for preventing cancer relapse and metastasis.

Stromal alterations associated with invasion are important for tumour progression, given that stromal cells and extracellular matrix tumour components can influence neoplastic cell proliferation, adhesion, and migration [11–13]. For instance, CSC induction is mediated by microenvironmental factors such as hypoxia/anoxia or epithelial-to-mesenchymal transition (EMT); conversely, a CSC phenotype that includes cluster of differentiation (CD)133 expression has been shown to induce EMT and promote tumour invasion, metastasis, and drug resistance in various malignancies including breast and ovarian cancers [14–20].

The present study investigated the association between morphological patterns of MI and immunohistochemical profiles related to CSC and EMT phenotypes, and evaluated their clinicopathological significance in EEC.

Methods

Materials

Archived specimens (from 2008 to 2013) from the Department of Pathology of Kyungpook National University Hospital were analysed, including only primary EEC specimens with no prior treatment (chemotherapy and/or radiotherapy); totalling 73 cases. Corresponding clinical characteristics were obtained from the hospital medical database and included age, procedure, status of post-operative adjuvant treatment, follow-up, and disease progression (recurrence or metastasis), and survival outcome. The pathological stage was determined according to the American Joint Committee on Cancer Staging Manual for Carcinoma of Corpus Uteri, 7th edition. This study was approved by the hospital institutional review board (KNUMCBIO_14–1008).

Histopathological Analysis

For each case, all available haematoxylin and eosin (H&E)-stained sections of resected specimens were examined for MI pattern (n = 5–16).Diagnostic criteria for MI patterns were as follows [1]: (1) infiltrating irregular gland pattern, defined as infiltrating dispersed glands throughout the myometrium with or without desmoplasia (Fig. 1a); (2) broad front (or pushing border) pattern, characterised as MI showing a large swath of neoplastic glands that appear to push into the underlying myometrium (Fig. 1b); (3) adenomyosis (AM)-like pattern, which can be difficult to distinguish from carcinoma-associated AM (Fig. 1c); and (4) microcystic, elongated, and fragmented (MELF)-type glands, characterised by infiltrating glands exhibiting MELF features and commonly associated with fibromyxoid stromal changes and inflammation (Fig. 1d).

Pathological parameters included tumour size, DOI, International Federation of Gynecology and Obstetrics (FIGO) grade, presence and proportion of tumour necrosis, presence of LVI, and lymph node involvement (LNI) status.

Immunohistochemistry

Consecutive whole sections from each specimen containing representative MI patterns at the most invaded areas were evaluated by immunohistochemistry using primary antibodies against the following proteins: oestrogen receptor (ER) (1:100, clone 6F11), progesterone receptor (PR) (1:100, PGR-312), and OCT3/4 (1:100, N1NK) (all from Novocastra, Newcastle, UK); E-cadherin (1:50, 18–0223) and β-catenin (1:2000, 18–0226) (both from Zymed, San Francisco, CA, USA); CD44 (1:200, M7082; Dako, Glostrup, Denmark); CD133 (1:100, AC133; Miltenyi Biotec, Bergisch Gladbach, Germany), Nanog1 (1:100, ab80892; Abcam, Cambridge, UK); and Sal-like (Sall)4 (1:100, CM384; Biocare, Concord, CA, USA).

Immunohistochemistry was carried out on formalin-fixed, paraffin-embedded tissue sections using the Ventana Benchmark XT Immunostainer Autosomal Platform system (Roche, Tucson, AZ, USA). Briefly, 3-μm-thick sections were transferred to adhesive slides and dried at 62°C for 30 min. After heat-induced epitope retrieval for 60 min in EDTA (pH 8.0), samples were incubated in the autostainer with primary antibodies, followed by incubation with biotinylated anti-mouse IgG, peroxidase-conjugated streptavidin (LSAB kit; Dako), and 3,3′-diaminobenzidine. Appropriate positive and negative controls were used throughout. Sections were counterstained with Harris haematoxylin (Ventana Medical Systems, Tucson, AZ, USA).

The localisation of effective staining was defined as follows: nuclear positivity for ER, and PR; cytoplasmic positivity for CSC markers; and cytoplasmic-membranous positivity for β-catenin and E-cadherin. ER and PR immunoreactivity was evaluated according to the guidelines [21]. The CSC phenotype was defined as a positive expression for any CSC marker (CD44, CD133, Nanog1, OCT3/4, and Sall4) in the EEC advancing area, discounting scattered positive cells within the non-advancing area. For the other antibodies, staining intensity was scored as follows: 0 = no staining, 1 = mild, 2 = moderate, and 3 = strong intensity. The extent of positive staining was initially measured as a percentage, and then classified as follows: 0, no stained cells; 1, <20%; 2, ≥20% to <50%; and 3, ≥50% of tumour cells stained. Immunoreactivity was categorised as negative upon no or only mild staining in <20% tumour cells, and

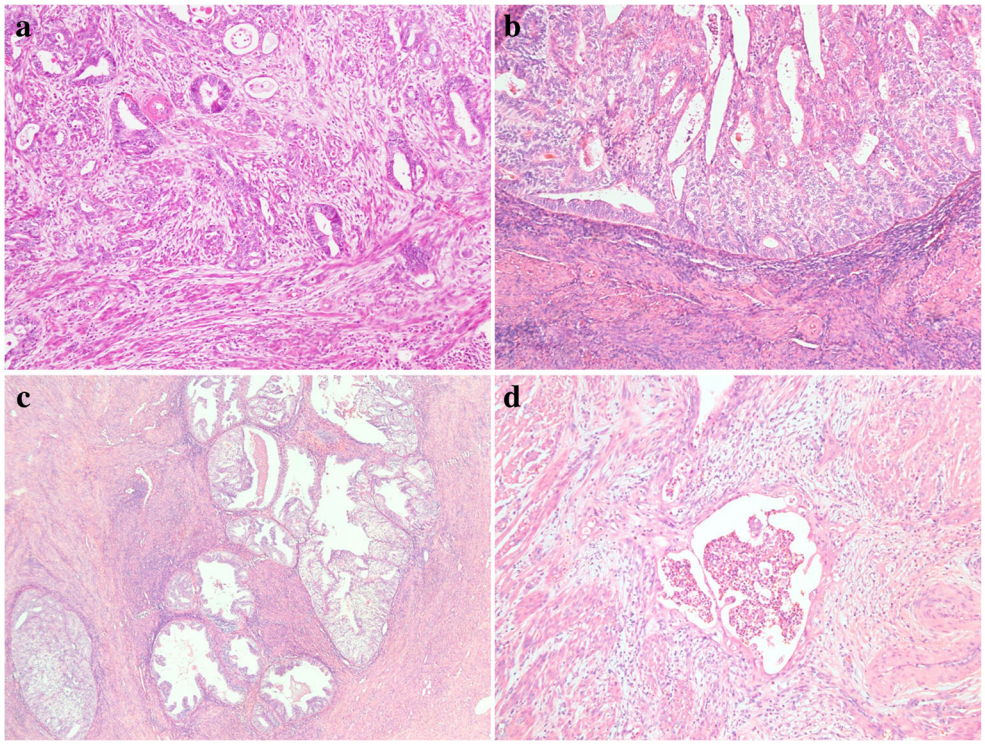

Fig. 1 Various patterns of myometrial invasion (MI) in endometrial endometrioid carcinoma. Infiltrating glandular pattern showed irregularly dispersed glands throughout the myometrium with or without desmoplasia (**a**). Expansile pattern characterised a large swath of neoplastic glands pushing into the underlying myometrium (**b**). Adenomyosis (AM)-like pattern exhibited a group of neoplastic glands that invade the myometrium confusing with adenomyosis involved by carcinoma (**c**). Microcystic, elongated, and fragmented (MELF)-type pattern defined as infiltrating neoplastic glands characterising MELF features with fibromyxoid stromal change and inflammation (**d**). Criteria for MI were referred to the paper by Cole AJ et al. [1]. These Sections were stained with H&E (a and b, × 100; c, × 40; d, ×200)

positive upon mild or moderate staining in ≥50% or strong staining in ≥20% tumour cells.

Histological and immunohistochemical analyses were independently carried out by two pathologists (JYP and JYP*), and cases with equivocal staining results were repeatedly reviewed until a consensus was reached.

Statistical Analyses

The χ^2 or Fisher's exact test was used to evaluate the correlation between clinicopathological parameters and MI pattern. Survival outcome was defined as time from surgery until death associated with disease or time of the last follow-up; disease progression was determined as time from surgery to detect any evidence of recurrence, relapse, or distant metastasis through the imaging investigations including computed tomography (CT) scan, magnetic resonance imaging (MRI), positron emission tomography-computed tomography (PET-CT), and/or a confirmative tissue biopsy. Survival curves were analysed by the Kaplan-Meier method and differences between them were estimated with the log-rank test. Uni- and multivariate Cox proportional hazards analyses were carried out to evaluate prognostic impact for survival outcome and disease progression. All statistical analyses were performed using SPSS v.20.0 for Windows software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Clinicopathological Characteristics

The mean age of patients at the time of diagnosis was 55.6 years old (standard deviation [SD], 8.1 years; range, 31–77 years). Patients had been treated primarily by total hysterectomy (n = 73, 100%) and bilateral (n = 70) or unilateral (n = 3) salpingo-oophorectomy. Pelvic LN dissections were performed in 71 patients (97.3%) and paraaortic LN dissections in 23 (31.5%).

The mean tumour size was 2.9 cm (1.9 cm; 1.0–6.0 cm). The DOI ratios ranged from 4% to 100% of the entire myometrial thickness and were <25% in 28 cases (38.4%); 26%–50% in 22 (30.1%); 51%–75% in nine (12.3%); and >75% in 14 (19.2%). The LVI and LNI were detected in 28 (38.4%) and 22 (30.1%) cases, respectively. The most common MI pattern was infiltrating (n = 36,

Table 1 Clinicopathological characteristics in endometrial endometrioid carcinoma according to growth patterns of myometrial invasion

Parameters		All EECs (n = 73)	Infiltrating (n = 36)	Expansile (n = 7)	AM-like (n = 19)	MELF-type (n = 11)	P value
Age (years, mean ± SD)		55.6 ± 8.1	54.7 ± 7.9	54.9 ± 13.3	57.1 ± 7.2	57.6 ± 6.0	*0.219*
Depth of invasion	< half	50 (68.5%)	22 (61.1%)	5 (71.4%)	18 (94.7%)	5 (45.5%)	***0.021***
	≥ half	23 (31.5%)	14 (38.9%)	2 (28.6%)	1 (5.3%)	6 (54.5%)	
FIGO grade	1	39 (53.4%)	13 (36.1%)	4 (57.1%)	17 (89.5%)	5 (45.5%)	***0.006***
	2	20 (27.4%)	12 (33.3%)	1 (14.3%)	2 (10.5%)	5 (45.5%)	
	3	14 (19.2%)	11 (30.6%)	2 (28.6%)	0 (0.0%)	1 (9.1%)	
Cervical involvement	–	60 (82.2%)	29 (80.6%)	6 (85.7%)	17 (89.5%)	8 (72.7%)	*0.685*
	+	13 (17.8%)	7 (19.4%)	1 (14.3%)	2 (10.5%)	3 (27.3%)	
LN metastasis	–	51 (69.9%)	25 (69.4%)	6 (85.7%)	18 (94.7%)	2 (18.2%)	***<0.0001***
	+	22 (30.1%)	11 (30.6%)	1 (14.3%)	1 (5.3%)	9 (81.8%)	
Lymphovascular invasion	–	45 (61.6%)	19 (52.8%)	6 (85.7%)	17 (89.5%)	3 (27.3%)	***0.002***
	+	28 (38.4%)	17 (47.2%)	1 (14.3%)	2 (10.5%)	8 (72.7%)	
Follow-up (months, mean ± SD)		28.1 ± 20.9	25.6 ± 20.6	36.3 ± 23.5	34.5 ± 18.9	27.5 ± 20.5	*0.938*

Abbreviations: *SD* standard deviation, *EEC* endometrial endometrioid adenocarcinoma, *AM* adenomyosis, *MELF* microcystic, elongated, and fragmented, *FIGO* International Federation of Gynecology and Obstetrics, *LN* lymph node

* Variables with statistically significant differences ($P < 0.05$) regarding the biochemical recurrence are indicated in bold letters

49.3%), followed by AM-like (n = 19, 26.3%), MELF-like (n = 11, 15.1%), and expansile (n = 7, 9.6%).

At initial diagnosis, seven patients (7/73, 9.6%) had ovarian metastasis, including four cases (5.5%) of unilateral and three (4.1%) of bilateral involvement; 13 (17.8%) had LUS involvement, and 22 (30.1%) had LNI. There were 45 cases (61.7%) of FIGO stage I (IA, n = 40 and IB, n = 5); two (2.7%) stage II; 25 (34.4%) stage III (IIIA, n = 2; IIIB, n = 2; and IIIC, n = 21); and one (1.4%) stage IVA. A total of 21 patients (28.8%) received adjuvant

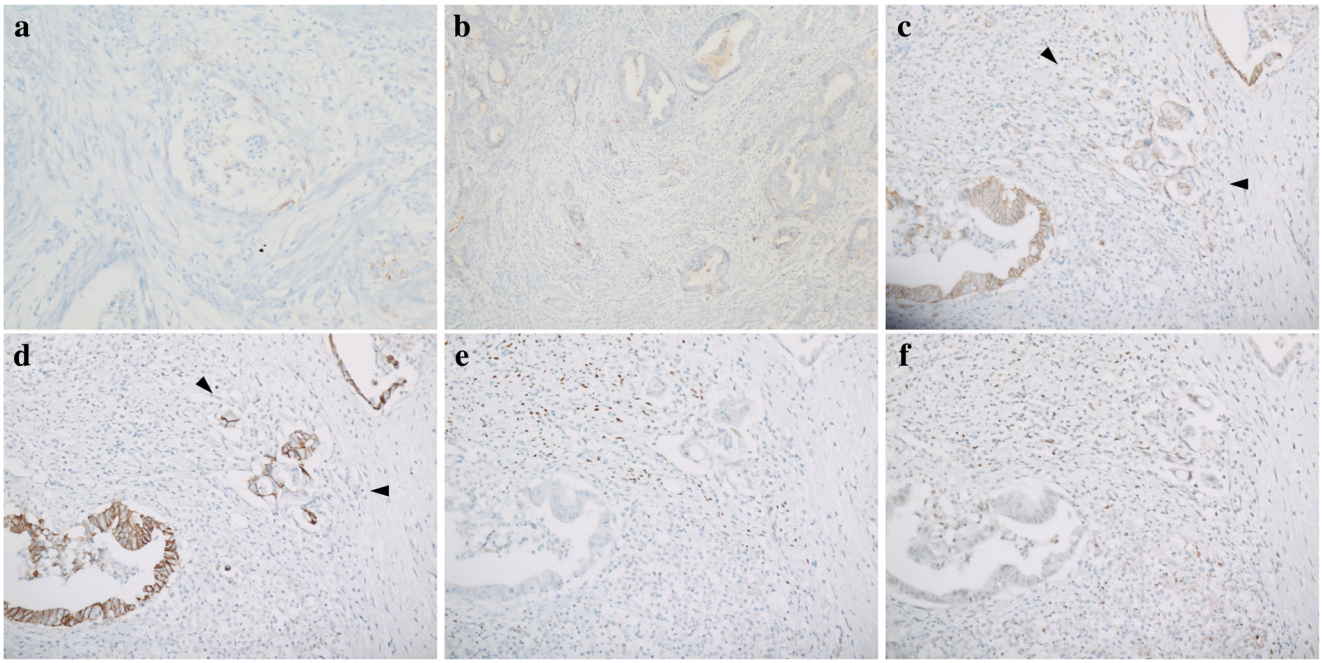

Fig. 2 Expression of cancer stem cell (CSC), epithelial-mesenchymal transition (EMT) biomarkers, and hormonal receptor in endometrial endometrioid carcinoma. CSC marker CD44 expressed in neoplastic glands exhibiting MELF-type features (**a**). A tumour showing infiltrating growth pattern showed CD133 expression (**b**), downregulation of both β-catenin (**c**, arrow heads) and E-cadherin (**d**, arrow heads) expression, and loss of ER (**e**) and PR (**f**) expression. Sections were stained by immunohistochemistry, and counterstained with Harris haematoxylin (a, × 100; b, × 40; c–f, × 100)

radiation therapy to the pelvis and 23 (31.5%) received postoperative chemotherapy; of these, seven (9.6%) received concurrent chemo-radiation therapy. Clinical and pathological characteristics are summarised in Table 1.

Expression of CSC and EMT Biomarkers Associated with MI Pattern

CD44 expression was detected in 32 patients (32/73, 43.8%), typically in cytoplasmic membranes with weak (n = 11, 15.1%) or moderate (n = 21, 28.7%) staining intensity. CD44 was predominantly expressed in an infiltrative pattern (16/36, 44.4%; P = 0.030), followed by MELF-type (9/11, 81.8%%; P = 0.006) (Fig. 2a), and other patterns. CD133 was expressed in 14 cases (14/73, 19.2%), primarily in tumours showing infiltrative (5/36, 13.9%; P = 0.054) (Fig. 2b) or MELF-type (5/11, 45.5%; P = 0.016) patterns. Tumours with an AM-like pattern had low CD133 expression (4/19, 21.1%); none with an expansile pattern exhibited CD133 expression (0/7). Nanog1 was expressed in 22 of 67 cases (30.1%) and was mostly associated with an infiltrating (16/36, 44.4%; P = 0.009), MELF-like (3/11, 27.3%), or AM-like (3/19, 30.1%) pattern. Sall4 expression was observed in five cases (5/73, 6.8%); four with infiltrative and one with MELF-like patterns. OCT3/4 was not detected in any of the samples.

To identify the propensity for EMT, we determined that E-cadherin expression was lost in 31 tumours (42.5%) with infiltrating (19/36, 52.8%; P = 0.023) and MELF-type (10/11, 90.9%; P < 0.0001) patterns however maintained in tumours exhibiting expansile (6/7, 85.7%) or AM-like (18/19, 94.7%) patterns. Aberrant β-catenin expression was detected in eight cases (8/73, 11.0%) and of both E-cadherin (Fig. 2c) and β-catenin (Fig. 2d) in four (5.4%) including infiltrative (n = 3) or MELF-like (n = 1) patterns.

MI pattern correlated with hormone receptor levels: infiltrating pattern tumours showed frequent ER (21/36, 58.3%; P = 0.014) (Fig. 2e) and PR (20/36, 55.6%; P = 0.026) loss (Fig. 2f), and MELF-type pattern tumours showed ER (8/11, 72.7%, P = 0.036) expression loss. In contrast, expansile pattern tumours showed intact ER expression (7/7, 100.0%, P = 0.014), and AM-like pattern tumours also maintained ER (16/19, 84.2%, P = 0.004) and PR (17/19, 89.5%, P = 0.001) expression. ER downregulation was associated with high CD133 (P = 0.021) and CD44 (P = 0.059) levels and E-cadherin expression loss (P = 0.010). Additionally, PR loss was related to CD44 expression (P = 0.002) and E-cadherin loss

Table 2 Immunohistochemical profiles of cancer stem cell (CSC), epithelial-mesenchymal transition (EMT) markers, and hormonal receptor in endometrial endometrioid carcinoma

Biomarkers		All EECs (n = 73)	Infiltrating (n = 36)	P value	Expansile (n = 7)	P value	AM-like (n = 19)	P value	MELF-type (n = 11)	P value
ER	Intact	41 (56.2%)	15 (41.7%)	***0.014***	7 (100.0%)	***0.016***	16 (84.2%)	***0.004***	3 (27.3%)	***0.036***
	Loss	32 (43.8%)	21 (58.3%)		0 (0.0%)		3 (15.8%)		8 (72.7%)	
PR	Intact	42 (57.5%)	16 (44.4%)	***0.026***	5 (71.4%)	*0.434*	17 (89.5%)	***0.001***	4 (36.4%)	*0.123*
	Loss	31 (42.5%)	20 (55.6%)		2 (28.6%)		2 (10.5%)		7 (63.6%)	
CD44	–	41 (56.2%)	20 (55.6%)	***0.030***	5 (71.4%)	*0.392*	14 (73.7%)	*0.074*	2 (18.2%)	***0.006***
	+	32 (43.8%)	16 (44.4%)		2 (28.6%)		5 (26.3%)		9 (81.8%)	
CD133	–	59 (80.8%)	31 (86.1%)	*0.054*	7 (100.0%)	*0.175*	15 (78.9%)	*0.809*	6 (54.5%)	***0.016***
	+	14 (19.2%)	5 (13.9%)		0 (0.0%)		4 (21.1%)		5 (45.5%)	
Nanog1	–	51 (69.9%)	20 (55.6%)	***0.009***	7 (100.0%)	*0.068*	16 (84.2%)	*0.113*	8 (72.7%)	*0.822*
	+	22 (30.1%)	16 (44.4%)		0 (0.0%)		3 (15.8%)		3 (27.3%)	
OCT3/4	–	73 (100.0%)	36 (100.0%)	–	7 (100.0%)	–	19 (100.0%)	–	11 (100.0%)	–
	+	0 (0.0%)	0 (0.0%)		0 (0.0%)		0 (0.0%)		0 (0.0%)	
Sall4	–	68 (93.2%)	32 (88.9%)	*0.155*	7 (100.0%)	*0.451*	19 (100.0%)	*0.169*	10 (90.9%)	*0.749*
	+	5 (6.8%)	4 (11.1%)		0 (0.0%)		0 (0.0%)		1 (9.1%)	
β-catenin	Intact	65 (89.0%)	31 (86.1%)	*0.429*	5 (71.4%)	*0.117*	19 (100.0%)	*0.075*	10 (90.9%)	*0.830*
	Aberrant	8 (11.0%)	5 (13.9%)		2 (28.6%)		0 (0.0%)		1 (9.1%)	
E-cadherin	Intact	42 (57.5%)	17 (47.2%)	*0.079*	6 (85.7%)	*0.113*	18 (94.7%)	***<0.0001***	1 (9.1%)	***<0.0001***
	Loss	31 (42.5%)	19 (52.8%)		1 (14.3%)		1 (5.3%)		10 (90.9%)	

Abbreviations: *EEC* endometrial endometrioid adenocarcinoma, *AM* adenomyosis, *MELF* microcystic, elongated, and fragmented, *ER* oestrogen receptor, *PR* progesterone receptor

* Variables with statistically significant differences (P < 0.05) regarding the biochemical recurrence are indicated in bold letters

($P < 0.0001$). Table 2 summarises the CSC and EMT marker immunoreactivity profiles in EEC.

Clinicopathological Significance of MI Pattern

The EEC infiltrating irregular gland pattern was associated with higher FIGO grade ($P = 0.002$). EEC with the MELF-type pattern had frequent LNI ($P < 0.0001$) and LVI ($P = 0.011$) whereas those with an AM-like pattern had lower DOI ($P = 0.004$), differentiated histology ($P < 0.0001$), and lower incidence of LNI ($P = 0.005$) and LVI ($P = 0.004$). AM-like pattern was related to better histology ($P < 0.0001$), lower DOI ($P = 0.005$), and low incidence of LVI ($P = 0.004$). Expansile pattern tumours showed no relationship to these parameters.

Prognostic Impact of MI Pattern and Biomarker Expression

The mean follow-up period was 45.3 months (SD, 21.7 months; range, 17.5–86.8 months). Five patients experienced disease progression (peritoneal recurrence in three patients and LN metastasis to the neck in two patients) although none died owing to their disease.

We analysed patient outcomes according to clinicopathological parameters, focusing on MI pattern and CSC/EMT marker expression. Survival analyses revealed that higher FIGO grades ($P = 0.003$) and infiltrative MI pattern ($P = 0.014$) were associated with disease progression. In univariate analyses, higher FIGO grade and an infiltrative tumour growth pattern were poor prognostic factors for disease progression (Table 3). Greater DOI and presence of LVI or LNI were, to a lesser degree, prognostic factors for disease progression. CSC or EMT marker expression had no prognostic impact with respect to disease progression. We also did not observe an independent prognostic significance of clinicopathological parameters and CSC or EMT biomarker expression in multivariate analyses.

Discussion

Endometrial cancer, the most common malignancy of the female reproductive tract in developed countries, usually comprises low-grade/stage endometrioid carcinomas with relatively favourable outcome versus other gynaecologic malignancies [2, 22]. However, tumour staging remains important for determining the appropriate treatment and assessing patient prognosis; recently, the MELF-type growth MI pattern has been associated with poor EEC prognosis [2]. Here, we showed that MI histological patterns are important determinants of EEC patient prognosis. Specifically, an infiltrating pattern was associated with reduced tumour cell differentiation, whereas the MELF-type pattern was linked to LNI and LVI. In addition, greater DOI, an infiltrative growth pattern, and higher FIGO grade were associated with disease progression.

We also observed that the CSC markers CD44, CD133, Nanog1, and Sall4 were mainly expressed in infiltrative or MELF-type pattern tumours. CD133 (prominin-1), considered a reliable CSC marker in EEC, is a membrane glycoprotein and prominin family member [20]. CD133-positive populations within EEC cell lines form floating spheres and colonies arising from clonal proliferation [23], and CD133 expression is associated with higher EEC tumour

Table 3 Univariate and multivariate analyses for prognostic significances in endometrial endometrioid carcinoma

Clinicopathological parameters		Univariate analysis			Multivariate analysis		
		HR	95% CI	*P* value	HR	95% CI	*P* value
FIGO grade	1		Reference				
	2	2.019	1.126–32.338	**0.046**			
	3	8.283	0.861–79.715	0.067	1.213	0.103–14.270	0.087
Depth of invasion	< half vs. ≥ half	9.883	0.014–47.641	0.074	6.690	0.000–62.914	0.642
LVI	< 4 vs. ≥4	8.517	0.949–76.462	0.056	1.493	0.126–17.662	0.075
LN metastasis	– vs. +	5.130	0.850–30.955	0.075	1.147	0.181–7.264	0.088
Pattern of MI	Infiltrative	7.697	0.064–11.426	**0.022**	7.195	0.018–22.711	0.093
	Expansile	0.039	0.000–264.467	0.568			
	AM-like	0.030	0.000–112.839	0.403			
	MELF-like	1.272	0.000–44.716	0.588			

Abbreviations: *HR* hazard ratio, *CI* confidence interval, *LVI* lymphovascular invasion, *LN* lymph node, *MI* myometrial invasion, *AM* adenomyosis, *MELF* microcystic, elongated, and fragmented

* Variables with statistically significant differences ($P < 0.05$) regarding the biochemical recurrence are indicated in bold letters

relapse and lower survival [14, 23, 24]. CD44 is an adhesion molecule implicated in tumour cell invasion and metastasis and serves as a CSC marker in endometrial cancer cell lines [14]. CD44 expression is associated with EEC proliferation, higher tumour grade, and LVI, although these findings are controversial [25]. Our results showed that CD133 or CD44 expression closely associated with disease progression and poor prognosis, suggesting their utility as biomarkers for predicting EEC progression.

We further identified reduced E-cadherin expression in EEC with infiltrating and MELF-type patterns; specifically, all cases of aberrant β-catenin combined with reduced E-cadherin expression also showed these patterns. E-cadherin downregulation was shown to play a role in tumour cell invasion and metastasis in various malignancies including breast, ovarian, and endometrial cancers, correlating with poor prognosis and lower overall survival [22, 26]. E-cadherin, β-catenin, and hormone receptor expression is downregulated in MELF-type glandular areas in EEC [27]; furthermore, EMT features in MELF-type pattern tumours were associated with frequent LVI and poor prognosis [26]. Permanent EMT marker expression in endometrial carcinoma has also been linked to sarcomatous element development in uterine carcinosarcomas [14]. Therefore, tumour cells with decreased E-cadherin involve the process of EMT, thus increasing cell motility, facilitating invasiveness, and contributing to MI in endometrial carcinoma.

Notably, we found that infiltrating and MELF-type pattern tumours were more likely to exhibit ER and PR or ER expression loss, respectively. Wik et al. [28] reported that ER loss in endometrial carcinoma was linked to EMT activation through increased EMT-related transcription factor and E-cadherin repressor expression. Previous studies also showed that correlated reduction in ER and E-cadherin expression in EEC was related to tumour invasion and disease progression [29]. Similarly, PR loss was correlated with tumour invasiveness and E-cadherin downregulation in both endometrial cancer cell lines and tissue samples [30–32]. Thus, reduced hormone receptor expression might also contribute to MI via the EMT process in EEC.

In this study, ER loss was associated with CD44 and CD133 immunoreactivity and decreased E-cadherin expression, whereas PR loss was associated with CD44 positivity and decreased E-cadherin expression. These findings are consistent with the notion that the CSC population of tumours have the necessary plasticity to undergo EMT [16, 29, 33, 34].

In conclusion, we demonstrated that MI histological pattern could be a significant parameter of EEC patient prognosis; in particular, infiltrating and MELF-type MI patterns in EEC were associated with poor histological differentiation, CSC marker expression, and frequent LN metastasis and LVI. In addition, these tumours showed changes related to EMT phenotypes and hormone receptor downregulation. Therefore, the accurate identification of each MI histological patterns could characterise the biological behaviour of EEC so that this would be helpful in selecting patients who need early and more aggressive treatment compared with those who do not need additional treatment.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations *AM*, adenomyosis; *CD*, cluster of differentiation; *CSC*, cancer stem cells; *DOI*, depth of invasion; *EEC*, endometrial endometrioid carcinoma; *EMT*, epithelial-mesenchymal transition; *ER*, oestrogen receptor; *FIGO*, International Federation of Gynecology and Obstetrics; *LN*, lymph node; *LNI*, lymph node involvement; *LUS*, lower uterine segment; *LVI*, lymphovascular invasion; *MELF*, microcystic, elongated, and fragmented; *MI*, myometrial invasion; *PR*, progesterone receptor

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