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ORIGINAL ARTICLE

Tumor Budding is a Valuable Diagnostic Parameter in Prediction of Disease Progression of Endometrial Endometrioid Carcinoma

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Abstract

Recently, tumor budding (TB) found at the invasive margin has been related to lymph node involvement (LNI), local recurrence, and poor prognosis in various cancers. We assessed the presence of TB in endometrial endometrioid carcinoma (EEC), and examined the immunohistochemical (IHC) profiles to define its clinicopathological significance. Ninety-six EECs were obtained from 2008 to 2013. During the follow-up, ten patients experienced disease progression; of these, three patients succumbed to the disease. All hematoxylin and eosin-stained slides were scrutinized for the presence of TB. IHC stainings for estrogen receptor (ER), progesterone receptor (PR), β-catenin, and E-cadherin were performed. All cases were grouped as FIGO grade (G) 1 (47.9%), G2 (29.2%), and G3 (22.9%). The distribution for depth of invasion (DOI) was 68.5% with a DOI of less than half and 31.5% with a DOI of more than half. Myometrial invasion was characterized as infiltrating pattern (52.1%), adenomyosis-like (20.8%), microcystic, elongated, and fragmented (17.7%), or expansile (9.4%). TB was identified in 63 cases (65.6%). Lymphovascular invasion (LVI) and LNI were identified in 47 and 37 cases, respectively. TB was associated with deep DOI ($p = 0.001$), higher FIGO grade ($p = 0.006$), LVI ($p < 0.0001$), and LNI ($p < 0.0001$). TB showed loss of ER ($p < 0.0001$) and PR ($p < 0.0001$), reduced E-cadherin ($p < 0.0001$) expression, and aberrant β-catenin expression ($p = 0.042$). In EECs, TB was associated with deep DOI, less-differentiated histology, frequent LVI, and LNI; furthermore, TB was closely related to epithelial-mesenchymal transition phenotype and downregulation of hormonal receptors. Therefore, TB might be a determinant histologic clue for prediction of disease progression in EECs.

Keywords Endometrial endometrioid carcinoma · Tumor budding · Prognosis · Lymphovascular invasion · Lymph node involvement

Introduction

Endometrial cancer is the most common malignancy of the female reproductive tract in developed countries, and overall survival is usually better than other gynecologic malignancies [1, 2]. Most of these tumors are low-grade, low-stage, endometrial endometrioid adenocarcinomas (EECs) [3].

Parameters traditionally used to predict the outcomes of patients with endometrioid adenocarcinoma include histologic grade and depth of myometrial invasion (MI) [3]. In recent studies, other parameters, including tumor size, involvement of the low uterine segment (LUS) or uterine cervix, lymphatic or vascular invasion, and growth pattern of MI are proposed as potential predictive indicators of extrauterine disease [3].

Tumor budding (TB) is a histopathological feature that can be identified by usual routine pathologic examination in various cancers. Several studies assumed that TB is related to lymph node status, local recurrence, and poor prognosis, particularly in colorectal [4, 5], esophageal [6], and laryngeal [7] carcinomas. For endometrial adenocarcinomas, the TB-like feature has been described as an isolated single cancer cell or microscopic small cluster of cells found outside of the invasive margin of a tumor [3]. In addition, TB-like feature was proposed to be associated with advanced cancer stage and decreased survival outcomes in EECs [3, 8].

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Furthermore, expression of the progesterone receptor (PR) has been described as important in the prognosis and treatment of endometrioid adenocarcinoma; PR expression is usually associated with successful treatment using medroxyprogesterone acetate (MPA), while loss of PR is associated with a more invasive phenotype and a low response to MPA [9–11].

However, there are only limited studies about the clinicopathologic characteristics and biological impacts of TB in endometrioid adenocarcinoma. Therefore, we assessed the prevalence of TB in the invasive front of endometrioid adenocarcinoma and examined the immunohistochemical profiles to evaluate the clinicopathological and biological implications in EECs.

Materials and Methods

Case Selection

After approvals were obtained from the institutional review board from each of institutes, archived specimens between 2008 and 2013 from the Department of Pathology of Kyungpook National University Hospital and Daegu Catholic University Medical Center were analysed, including only primary EEC specimens with no prior treatment (chemotherapy and/or radiotherapy). A total of 96 EECs were finally selected. Clinical characteristics were collected 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.

Histopathological Analysis

For each case, all available hematoxylin and eosin (H&E)-stained sections of resected specimens (number of slides, 5–16) were examined for hotspot of TB. TB was defined as an isolated single cancer cell or small cell clusters composed of <5 tumor cells found in the EEC advancing area [8]. Hotspots of TB along the leading area of tumor invasion were evaluated, and cut-off values of 5 TB/high power filed (HPF) were used to categorize as a presence of TB. The number of TB was counted using a × 20 objective lens (Olympus, BX-53) [12]. Furthermore, diagnostic evaluation for MI patterns was referred to the description of Cole et al. (1): (a) Infiltrating irregular gland pattern; (b) Broad front (or pushing border) pattern; (c) Adenomyosis (AM)-like pattern; and (d) Microcystic, elongated, and fragmented (MELF)-type glands.

Pathological parameters included tumour size, depth of invasion (DOI), International Federation of Gynecology and Obstetrics (FIGO) grade, cervical involvement, presence of lymphovascular invasion (LVI), and lymph node involvement (LNI).

Immunohistochemical Staining and Assessment

Consecutive whole sections from each specimen containing representative TB feature at the invading areas were prepared for immunohistochemical (IHC) staining. IHC stainings for estrogen receptor (ER) (1:100, clone 6F11, Novocastra, Newcastle, UK), progesterone receptor (PR) (1:100, PGR-312, Novocastra), P53 (1:1500, DO-7, Dako, Glostrup, Denmark), E-cadherin (1:50, 18–0223, Zymed, San Francisco, CA, USA) and β-catenin (1:2000, 18–0226, Zymed) were performed using the Ventana Benchmark XT Immunostainer Autosomal Platform system (Roche, Tucson, AZ, USA) on whole sections of formalin-fixed, paraffin-embedded tissues. 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 hematoxylin (Ventana Medical Systems, Tucson, AZ, USA).

IHC stains were analysed using the standard H score method [13]. The standard H score was assessed along a continuous scale of 0–300, based on the staining intensity and the extent of TB features. The staining intensity was scored as follows: 0 = no staining, 1 = mild, 2 = moderate, and 3 = strong. The staining extent was measured on a positive percentage. The IHC H score was finally divided as negative (H score < 200) or positive (H score ≥ 200) for each IHC markers. Histological and immunohistochemical analyses were conducted by two pathologists (J-Y, Park and JY, Park), and cases with equivocal results were repeatedly reviewed and consulted for a consensus.

Statistical Analyses

The chi-square or Fisher's exact test was used to evaluate the correlation between the presence of TB and each clinicopathological parameter. Survival rates were analysed by the Kaplan-Meier method and the differences were estimated with the log-rank test. Uni- and multivariate Cox proportional hazards analyses were conducted to evaluate prognostic impact for worse outcome. 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

Clinical Characteristics

The average age of patients at diagnosis was 56.1 ± 8.0 years old (median, 56.0 years; range, 31–77 years). Patients were treated initially with total hysterectomy ($n = 96$, 100%) and bilateral ($n = 92$) or unilateral ($n = 4$) salpingo-oophorectomy. Pelvic lymph node (LN) dissection was conducted in 93 patients (96.9%), and para-aortic LN sampling was conducted in 30 patients (31.3%).

At the initial diagnosis, eleven patients (11/96, 11.4%) had ovarian metastasis, including five cases (5.2%) of unilateral and six (6.2%) of bilateral involvement. Twenty patients (20.8%) showed cervical (LUS) involvement, and 37 patients (39.8%) had LN metastasis. Characterization of FIGO stage showed 50 cases (52.1%) of stage I (IA, $n = 40$ and IB, $n = 10$), 3 cases (3.1%) of stage II, 39 cases (40.7%) of stage III (IIIA, n = 4; IIIB, $n = 0$; and IIIC, $n = 35$), and 4 cases (4.2%) of stage IVA. A total of 34 patients (35.4%) received adjuvant radiation therapy to the pelvis and 39 patients (40.6%) received postoperative chemotherapy. Of these, thirteen (13.5%) received concurrent chemo-radiation therapy.

Histopathological and Immunohistochemical Features

The average tumor size was 2.9 ± 1.6 cm (median, 2.8 cm; range, 1.0–6.0 cm). The DOI ranged from 4% to 100% of the entire myometrial thickness; <25% in 28 cases (29.2%), 26%–50% in 22 cases (22.9%), 51%–75% in 18 cases (18.8%), and > 75% in 28 cases (29.2%). The LVI were noted in 47 (49.0%). The common MI patterns were infiltrating ($n = 50$, 52.1%), followed by AM-like ($n = 20$, 20.8%), MELF ($n = 17$, 17.7%), and expansile ($n = 7$, 9.4%). Cases according to the FIGO grade were grouped as grade (G) 1 ($n = 46$, 47.9%), G2 ($n = 28$, 29.2%), and G3 ($n = 22$, 22.9%).

TB was identified in 65.6% of cases ($n = 63$). TB was significantly associated with deep DOI ($p = 0.001$), higher FIGO grade ($p = 0.006$), LN metastasis ($p < 0.0001$), and presence of LVI ($p < 0.0001$). Moreover, TB was positively correlated with an infiltrative pattern of MI ($p < 0.0001$) and MELF pattern ($p = 0.046$) (Fig. 1); while being negatively correlated with an AM-like pattern of MI ($p < 0.0001$). Clinical and pathological characteristics were summarized in Table 1.

TB showed losses of ER (38/63; $p < 0.0001$), PR (37/63; $p < 0.0001$), and E-cadherin (41/63; $p < 0.0001$) expression, and aberrant β-catenin expression (11/63; $p = 0.042$) (Fig. 2). Loss of ER expression was closely related loss of PR ($p < 0.0001$), and loss of E-cadherin expression ($p = 0.002$). Loss of PR showed a strong correlation to loss of E-cadherin expression ($p < 0.0001$). A marginal significance between loss of E-cadherin and aberrant β-catenin expression was identified ($p = 0.056$); while, there were no obvious correlations with loss of ER or PR expression and β-catenin ($p = 0.589$ and $p = 0.757$, respectively). All cases showed a wild phenotype of TP53 expression.

Clinicopathological Significance of Tumor Budding (TB) in EECs

The mean follow-up period was 43.8 ± 21.7 months (median, 46.4 months; range, 17.5–91.7 months). Ten patients experienced disease progression; the common metastatic site includes the lung, liver, lymph nodes, and peritoneum. Of these, three patients succumbed to the disease.

Survival curves with log-rank test analyzed that deeper DOI ($p = 0.028$), infiltrative pattern of MI ($p = 0.036$), presence of LVI ($p = 0.035$), and LN metastasis ($p = 0.007$) were associated with unfavorable outcomes (Fig. 3a–d). There appeared to be a weak correlation between the presence of TB and patients' survival ($p = 0.092$) (Fig. 3e). Deeper DOI ($p < 0.0001$), infiltrative pattern of MI ($p = 0.001$), presence of LVI ($p = 0.008$), and LN metastasis ($p = 0.036$) were associated with disease progression; while, TB feature had a marginal association with disease progression ($p = 0.056$) (Fig. 3f). More to the point, TB feature was frequently identified in the patients proven disease progression (80%, 8/10);

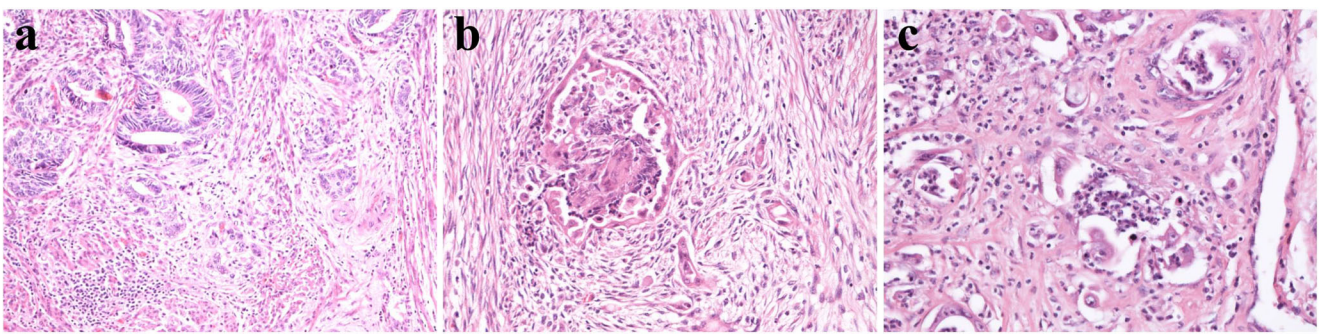

Fig. 1 Representative histological features of tumor budding (TB) in endometrial endometrioid carcinoma (EEC) (a – c). TB was significantly correlated with an infiltrative pattern of MI (**a**) and MELF pattern (**b**); TB was defined as the single cells or isolated small clusters of tumor cells at the leading area of cancer invasion (**c**). H&E stains, (**a**–**c**). Original magnification ×200 (**a**–**b**) and ×400 (**c**)

Table 1 Clinicopathological characteristics in endometrial endometrioid carcinoma according to tumor budding (TB) features

Clinicopathological parameters		All EECs ($n = 96$)	TB (−) ($n = 33$)	TB (+) ($n = 63$)	p value
Age (years, mean ± SD)		56.1 ± 8.0	57.7 ± 7.6	55.2 ± 8.2	0.219
Depth of invasion	< half	50 (52.1%)	25 (75.8%)	25 (39.7%)	**0.001**
	≥ half	46 (47.9%)	8 (24.2%)	38 (60.3%)	
FIGO grade	1	46 (47.9%)	24 (72.7%)	22 (34.9%)	**0.006**
	2	28 (29.2%)	8 (24.2%)	20 (31.7%)	
	3	22 (22.9%)	1 (3.0%)	21 (33.3%)	
Pattern of MI	Infiltrative	50 (52.1%)	9 (27.3%)	41 (65.1%)	**<0.0001**
	AM-like	20 (20.8%)	18 (54.5%)	2 (3.2%)	
	MELF	17 (17.7%)	2 (6.1%)	15 (23.8%)	**0.046**
	Expansile	9 (9.4%)	4 (12.1%)	5 (7.9%)	
Cervical involvement	–	76 (79.2%)	27 (81.8%)	49 (77.8%)	0.428
	+	20 (20.8%)	6 (18.2%)	14 (22.2%)	
LN metastasis	–	56 (60.2%)	27 (87.1%)	29 (46.8%)	**<0.0001**
	+	37 (39.8%)	4 (12.9%)	33 (53.2%)	
Lymphovascular invasion	–	49 (51.0%)	27 (81.8%)	22 (34.9%)	**<0.0001**
	+	47 (49.0%)	6 (18.2%)	41 (65.1%)	
Follow-up (months, mean ± SD)		43.8 ± 21.7	56.2 ± 7.6	37.5 ± 19.4	0.938

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

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

furthermore, all of those tumors demonstrated deep DOI, infiltrative MI, presence of LVI, and LN metastasis.

We subsequently analyzed the prognostic significance of TB, focusing on the unfavorable prognostic factors for EECs. In univariate analyses for LVI prediction, deeper MI, higher FIGO grade, LN metastasis, presence of TB, and loss of hormone receptor expression were negative prognostic factors. Multivariate analyses confirmed that deeper DOI ($p = 0.010$), presence of TB ($p = 0.004$), and loss of PR ($p = 0.026$) were independent prognostic factors for LVI. For a

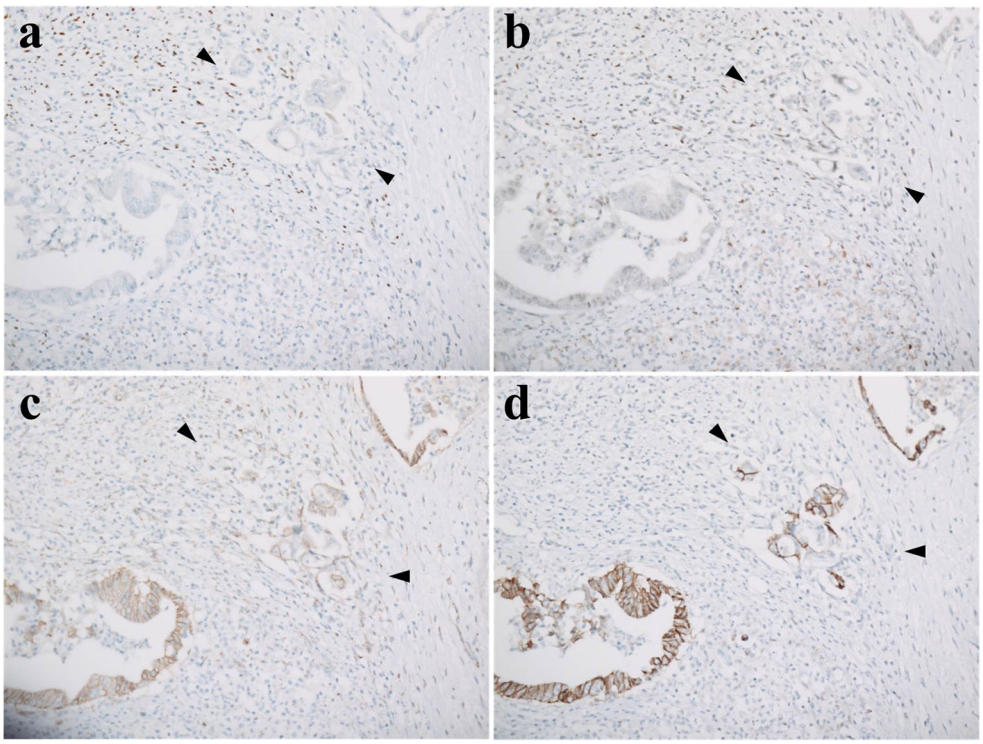

Fig. 2 Representative immunohistochemical features of tumor budding (TB) (**a**–**d**). TB showed loss of ER (**a**) and PR (**b**), aberrant β-catenin expression (**c**) and loss of E-cadherin expression (**d**). Original magnification ×200 (**a**–**d**, arrow head)

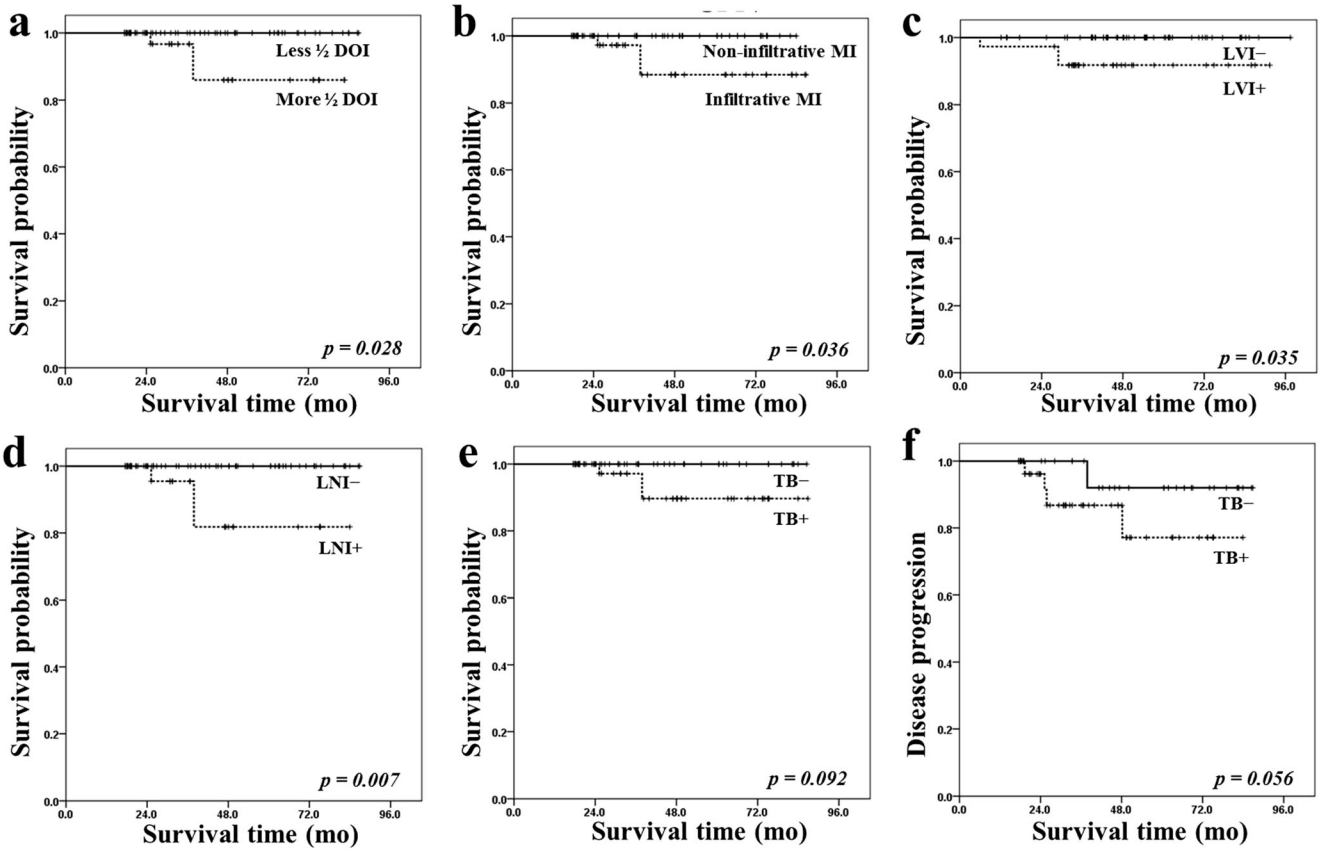

Fig. 3 Survival analyses (**a**–**f**). Survival curves with log-rank test demonstrated that deeper DOI (**a**), infiltrative pattern of MI (**b**), presence of LVI (**c**), and LNI (**d**) were associated with unfavorable outcomes. TB showed marginal significances associated with worse survival (**e**) and disease progression (**f**)

prediction of LN metastasis, deeper MI, high FIGO grade, presence of LVI, TB, and loss of ER or PR expression were unfavorable parameters in univariate analyses. Multivariate analyses ascertained that deeper DOI ($p = 0.022$) and presence of LVI ($p < 0.0001$) were worse prognostic parameters for LN involvement. Tables 2 and 3 summarized univariate and multivariate significances of the TB feature for prediction of LVI or LNI in EECs.

In contrast, several parameters indicated a favorable outcome; there were no involvement of LN (hazard ratio [HR], 1.543; $p = 0.05$; 95% confidence interval [CI], 1.001–2.386), no LVI (HR, 1.999; $p = 0.002$; 95% CI, 1.296–3.081), no TB feature (HR, 2.444; $p < 0.0001$; 95% CI, 1.536–3.888), and expression of E-cadherin (HR, 1.573; $p = 0.053$; 95% CI, 0.994–2.489).

Discussion

Endometrial cancer is usually comprised of low-grade and low-stage endometrioid carcinomas, and has favorable outcomes compared to other gynecologic malignancies [3, 8]. Although tumor staging remains important for determining the appropriate treatment and assessing patient prognosis, additional histologic parameters, such as TB and pattern of MI, have been proposed as important prognostic factors in recent studies [3]. In this study, we showed that the presence of TB feature could be an important determinant for the prognosis of EEC. TB was associated with deeper DOI, and higher FIGO grade, suggesting reduced histologic differentiation, LVI and LNI. The presence of TB was an independent parameter for prediction of LVI in multivariate analysis, and was a significant histologic factor for the prediction of LN metastasis in univariate analysis.

Currently, lymphadenectomy in patients with EEC should be performed as a surgical staging for assessing diagnostic and therapeutic advantages; but, it is not required in patients at low risk for recurrence, i.e., patients with ≤2 cm, grade 1–2 endometrioid tumors, and < 50% myometrial invasion [14, 15]. Previous nomograms and risk-scoring systems stratifying the risk of lymph node metastasis have included various clinicopathologic parameters, such as age, stage, tumor diameter, DOI, LVI, grade, histologic subtypes, serum CA125 level etc. [16, 17]; however, there remains a debate on their utilities for the surgical management of early stage EEC. Notably, our results showed that TB features had a marginal significance

Table 2 Univariate analyses for lymphovascular invasion (LVI) and lymph node involvement (LNI) in endometrial endometrioid carcinoma

Clinicopathological parameters		LVI			LNI		
		HR	95% CI	*p* value	HR	95% CI	*p* value
Depth of invasion	< half vs. ≥ half	5.997	2.893–12.433	**<0.0001**	7.120	2.959–17.130	**<0.0001**
FIGO grade	1&2 vs. 3	2.322	1.250–4.312	**0.008**	2.012	1.019–3.973	**0.044**
Pattern of MI	Infiltrative vs. non-infiltrative	1.785	0.978–3.188	0.059	1.180	0.617–2.258	0.617
LNI	– vs. +	5.927	3.031–11.589	**<0.0001**	–	–	–
LVI	– vs. +	–	–	–	12.710	4.481–36.046	**<0.0001**
Tumor budding	– vs. +	6.657	2.766–16.022	**<0.0001**	7.715	2.688–22.1451	**<0.0001**
ER	– vs. +	1.646	1.919–5.945	**0.003**	2.127	1.077–4.198	**0.030**
PR	– vs. +	2.767	1.479–5.176	**0.001**	4.104	1.874–8.986	**<0.0001**
E-cadherin	– vs. +	2.603	1.431–4.733	**0.002**	3.337	1.645–6.770	**0.001**
β-catenin	– vs. +	1.089	0.485–2.444	0.836	1.428	0.623–3.272	0.339

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

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

for lymph node metastasis and disease progression in the subgroup analysis of stage I EECs ($p = 0.071$ and $p = 0.082$, respectively; data not shown); at least, TB features would be considered as a not-low risk parameter for presuming a clinical decision of pelvic- and/or para-aortic lymphadenectomy in EECs.

Interestingly, TB features were closely associated with infiltrative pattern or MELF pattern of MI. TB was often observed at the leading border of invasion and was accompanied by stromal alterations, such as loose fibrous or fibromyxoid change, with or without inflammatory cell infiltration. In our study, infiltrative or MELF pattern of MI were frequently observed within the similar stromal changes and were significantly correlated with disease progression. These stromal alterations associated with cellular invasion might provide a fitting background for cancer progression in various malignancies; in other words, a close interaction between the stromal cells and extracellular matrix components could influence neoplastic cell proliferation, adhesion, and migration.

Furthermore, we identified that the TB feature was associated with reduced expression of hormone receptors (ER and PR) and aberrant β-catenin expression combined with loss of E-cadherin expression. In particular, E-cadherin downregulation has been known to play a crucial role in cellular invasion or metastasis in various cancers, and it is often associated with a worse prognosis and lower overall survival of the tumors. Previous studies have shown that reduced expression of hormone receptors were correlated with decreased E-cadherin immunoreactivity, increased expression of E-cadherin transcription repressors, such as Zinc finger protein SNAI1

Table 3 Multivariate analyses for lymphovascular invasion (LVI) and lymph node involvement (LNI) in endometrial endometrioid carcinoma

Clinicopathological parameters		LVI			LNI		
		HR	95% CI	*p* value	HR	95% CI	*p* value
Depth of invasion	< half vs. ≥ half	3.058	1.314–7.117	**0.010**	3.005	1.173–7.770	**0.022**
FIGO grade	1&2 vs. 3	1.500	0.723–3.113	0.277	1.243	0.562–2.747	0.591
LNI	– vs. +	1.977	0.900–4.340	0.089	–	–	–
LVI	– vs. +	–	–	–	7.661	2.515–23.335	**<0.0001**
Tumor budding	– vs. +	5.432	1.708–17.276	**0.004**	2.051	0.567–7.428	0.274
ER	– vs. +	1.427	0.982–2.001	0.050	1.639	0.264–3.545	0.320
PR	– vs. +	2.772	1.128–6.812	**0.026**	1.805	0.799–4.074	0.155
E-cadherin	– vs. +	1.250	0.561–2.788	0.585	1.306	0.568–3.003	0.530

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

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

(SNAIL), Zinc finger protein SNAI2 (SLUG), Zinc finger E-box-binding homeobox 1 (ZEB1), and Twist-related protein (TWIST), and enhanced tumor invasiveness in endometrial cancer cell lines and tissue samples [18–21]. In addition, loss of progesterone signaling might play a role in the induction of the epithelial-mesenchymal transition (EMT) [22]. MPA-treated PR signaling-modulated endometrial cancer cell lines resulted in inhibition of migration, suppression of mesenchymal markers, and down-regulation of epidermal growth factor, insulin-like growth factor-1, platelet-derived growth factor, transforming growth factor-β, vascular endothelial growth factor, and Wnt/β-catenin signaling associated with the EMT pathway [20, 22]. Therefore, reduced hormone receptor expression might also contribute to the EMT process in EECs and TB feature could be a specific phenotype of EMT in epithelial tumor progression. These findings were consistent with a prior other study. Euscher et al. [3] described that MELF pattern had a close relationship with single-cell/cell-cluster invasion (SCI), and suggested that SCI could represent an evolving, more aggressive, variant of MELF; therefore, TB features could be a crucial histologic clue for prediction a worse prognosis in EECs.

Since there was low number of events (10 disease progression and 3 death with disease, respectively) in this study, there were marginally significant differences by the presence of TB in the survival outcome or disease progression. Nevertheless, the presence of TB could be a crucial histological clue for both LVI and LNI. When TB feature was identified, more careful examination should be performed to clarify worse prognostic parameters in EEC.

In summary, we ascertained that TB features in EEC were associated with deeper DOI, reverse histological differentiation, infiltrative or MELF pattern of MI, frequent LVI, and LN metastasis. Furthermore, they were closely related to EMT phenotypes and downregulation of hormone receptors expression. Our findings indicated that TB feature could be a determinant morphology for prediction of disease progression or worse outcome, and for discrimination of patients requiring early and assertive treatment in EEC. Therefore, when identified, more careful examination is necessary to provide a clinically useful perspective for the patients with EEC.

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Compliance with Ethical Standards

Ethical Approval and Informed Consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research board (KNUMCBIO_14–1008) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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

Abbreviations *AM*, adenomyosis; *DOI*, depth of invasion; *EEC*, endometrial endometrioid carcinoma; *EMT*, epithelial-mesenchymal transition; *ER*, estrogen receptor; *FIGO*, international federation of gynecology and obstetrics; *IHC*, immunohistochemical; *LN*, lymph node; *LNI*, lymph node involvement; *LUS*, lower uterine segment; *LVI*, lymphovascular invasion; *MELF*, microcystic, elongated, and fragmented; *MI*, myometrial invasion; *MPA*, medroxyprogesterone acetate; *PR*, progesterone receptor

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