

ARTICLE



Grading of endocervical adenocarcinoma: a novel prognostic system based on tumor budding and cell cluster size

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A novel 3-tiered grading system based on tumor budding activity and cell nest size has been validated to be highly prognostic in organ-wide squamous cell carcinomas. In this study, we applied a similar grading system with slight modification to assess the prognostic value in an institutional cohort of well annotated endocervical adenocarcinomas (EAC) consisting of 398 consecutive cases with surgical resection, no neoadjuvant chemotherapy, and higher than stage pT1a. Each case was reviewed by the International Endocervical Adenocarcinoma Criteria and Classification (IECC) and Silva pattern classification, and scored on tumor budding activity and cell cluster size to form the basis of a novel grading system. High budding activity, small tumor cell cluster size, and novel grade 3 were more frequently associated with a decreased overall survival time and tumor recurrence time ($p < 0.001$), and several other clinicopathologic factors including HPV-independent adenocarcinoma, lymphovascular invasion, lymph node metastasis, advanced FIGO stage, and Silva pattern C ($p < 0.05$). Moreover, the novel grading system was helpful in stratifying overall survival in HPV-associated adenocarcinoma ($p = 0.036$) and gastric-type adenocarcinoma ($p = 0.033$). On multivariate analysis, novel grade 3 was an adverse indicator for overall survival and tumor recurrence independently of age and FIGO stage ($p < 0.05$). By comparison, Silva pattern C was only associated with tumor relapse ($p = 0.020$) in HPV-associated adenocarcinomas whereas the conventional FIGO system was not associated with overall survival and tumor recurrence in EAC ($p > 0.05$). In conclusion, our study demonstrates that the grading system based on tumor budding activity and cell cluster size is robust in prognostic assessment that outperforms the conventional FIGO grading and Silva pattern classification in EAC. The novel grading system, if further validated, could be applicable in routine pathologic descriptions of EAC by providing useful information in clinical decision-making.

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INTRODUCTION

Cervical carcinoma remains the fourth leading cancer in women worldwide.¹ Endocervical adenocarcinoma (EAC) is the second most common histotype following squamous cell carcinoma (SCC), accounting for 10–25% of all cervical carcinomas. EAC usually has a poorer prognosis than SCC despite the same treatment strategies in both cancers.² EAC shows a highly variable disease course, not only ranging from early stage to advanced stage with wide dissemination and metastasis, but it is also a heterogeneous group in terms of its subtypes. Valid prognostic indicators are critically required for tailored treatment of EAC.

There have been significant advances in our understanding of the prognostic assessment of EAC in recent years, such as revised tumor classification and pattern-based systems.^{3,4} The International Endocervical Adenocarcinoma Criteria and Classification (IECC) scheme classified EAC into HPV-associated adenocarcinoma (HPVA) and HPV-independent adenocarcinoma (HPVI), based on etiology (hrHPV infection) and related surrogate morphology.³ The IECC approach appears to be valuable for prognostic assessment in EAC⁵; therefore, it has been adopted by the recently published

fifth edition of the World Health Organization (WHO) classification of female genital tumors.⁶ The Silva Pattern Classification subdivides HPVA into 3 prognostic groups, correlating the presence and extent of destructive stromal invasion, nuclear grade and structure with lymph node metastasis and clinical outcomes.⁴

Several pathological parameters are helpful in the assessment of prognosis in cervical cancers, such as histotype, tumor size, depth of invasion, and lymphovascular invasion (LVI).^{7,8} Tumor grade is a conventional prognostic factor in many cancers. However, in EAC, tumor grading, including grading criteria and its clinical significance, has received scant coverage in the literature. Recently, the International Society of Gynecological Pathologists (ISGyP) suggested the revised International Federation of Gynecology and Obstetrics (FIGO) system for uterine endometrioid carcinoma for EAC grading based on the available evidence and its common application in clinical settings.⁹ There is no consensus on the grading criteria and clinical significance in EAC; therefore, the issue remains unresolved to date. On account of an expectation from clinicians, tumor grading remains as a

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Table 1. Algorithm for determining novel grading system based upon tumor budding and cell cluster size in EAC*.

	Score
Tumor budding (10 HPF)	
0	1
<15	2
≥15	3
Tumor cell cluster size	
>15 cells or pure glands (no clusters)	1
5–15 cells	2
2–4 cells	3
Single cell	4
Grading	Total score
G1 (well differentiated)	2–3
G2 (moderately differentiated)	4–5
G3 (poorly differentiated)	6–7

*The algorithm is slightly modified from those reported previously.^{15–20}

recommended (non-core) but not a required (core) element in the recent International Collaboration on Cancer Reporting data set for reporting cervical SCC and EAC.¹⁰ It is an important requirement to develop a novel grading system with prognostic value and potential universal application.

Tumor budding, defined as isolated cells or small tumor cell clusters consisting of <5 neoplastic cells that “bud” into the peritumoral stroma, conceptually represents a common form of destructive stromal invasion.^{11,12} Data have accumulated to indicate that increased tumor budding is a strong adverse prognostic factor in many cancers.^{12–14} Based on the combination of tumor budding activity and cell nest size, a second parameter that qualitatively measures the capability of cancer cell dissociation, a novel grading approach with substantial clinical significance has been established for SCC from several anatomic sites including uterine cervix.^{15–20} High tumor budding count has been found to be associated with decreased disease-free and cancer-specific survival in patients with early-stage EAC recently.²¹ Nevertheless, the clinical value of cell nest size (the term, “cluster” is preferred in EAC) and the novel grading approach has not been investigated in EAC yet.

In this study, we compared the clinical significance between the novel grading approach based on tumor budding and cell cluster size and the revised FIGO grading system in a large cohort of EAC patients from a major Chinese university women’s hospital. We also analyzed the relationship between histological grading and other histopathologic and molecular parameters, such as IECC histotypes, Silva pattern, and p53 staining. Our specific aim is to evaluate its applicability and prognostic performance in EAC.

MATERIALS AND METHODS

Case selection

Consecutive cases of cervical adenocarcinomas in a resected specimen were retrieved from the archives of the Department of Surgical Pathology, Women’s Hospital, School of Medicine, Zhejiang University, PR China, between January 2004 and December 2019. We excluded patients with biopsy only, neoadjuvant chemotherapy, pT1a1 EAC, and the serous type and the endometrioid type if they had concurrent carcinomas in the upper female genital tract. The clinical data were extracted from the electronic medical records before de-identification. Tumor stage was re-assessed according to the 2018 FIGO cervical cancer staging system.²² Histologic slides and follow-up data were available for review. The hospital’s Institutional Review Board approved this study (IRB: 20170139).

Histologic assessment

Two authors (SH and YL), who were blinded to the clinical data and follow-up, independently reviewed the archival H&E slides for detailed morphological analysis. Cases resulting in interpretational disagreement between the two authors were submitted to the third pathologist (LB). All tumors were graded by evaluating the additive scores of tumor budding and cell nest size [Table 1]. A similar approach with slight modification has been reported in SCC from various organs previously.^{15–20} Briefly, tumor budding activity was recorded in the tumor area having the highest budding activity (hotspot) after assessment throughout the whole tumor area. Tumor budding activity was scored as 1, 2 and 3 for 0, 1–14 (low tumor budding activity) or ≥15 budding foci (high tumor budding activity) in 10 high power fields (HPFs) with a total area of 2.37 mm² (Leica DMR 2000; the field diameter of a 10× objective: 22 mm), respectively. Cell cluster size was evaluated according to the minimal size of invasive tumor cell nests. Cell cluster size comprising 2–4 (small), 5–15 (intermediate), and >15 tumor cells (large) had scores of 3, 2 and 1, while single tumor cells detached within the stroma received a score of 4. Representative images are illustrated in Fig. 1. The number of tumor cell clusters with minimal size did not influence the cell cluster size scoring. Tumors with pure glands (no tumor cell clusters or single cells) were frequently present in EAC. Cell cluster size score 1 was designated for these tumors given that they were well differentiated by conventional grading whereas cell clusters lacking glandular formation were believed to be poorly differentiated.²³ The sum of tumor budding and cell cluster size scores generated a final grading score ranging from 2 to 7. A grading score of 2–3, 4–5, and 6–7 were defined as grade 1 (G1, well differentiated), 2 (G2, moderately differentiated), and 3 (G3, poorly differentiated), respectively. This grading approach based on tumor budding will be referred to as “novel grade” in the following sections of the manuscript.

The tumors were reclassified according to the IECC.³ The Silva pattern classification was applied in HPVA.⁴ All cases were also graded in accordance with the revised FIGO system recommended from ISGYP.⁹ Tumors with ≤10%, 11–50%, and >50% solid growth were designated as grade 1, 2, and 3, respectively. Tumors were upgraded if marked nuclear atypia was present in the majority (>50%) of the tumor cells. The FIGO grading was not recommended for HPV, particularly gastric-type adenocarcinoma (GAC), because of their aggressive clinical behavior even in cases with deceptively bland (low grade) histology.^{9,24} Nevertheless, in this study, we retained FIGO grading in GAC for comparison with the novel grading scheme.

Immunohistochemistry

Immunohistochemistry (IHC) was carried out on 4-μm thick sections from neutral-buffered formalin-fixed, paraffin-embedded tissue blocks. The antibodies included p16 (G175-405; BD Bioscience, San Jose, CA; 1:100), MUC6 (MRQ-20; Cell Marque, Rocklin, USA; ready-to-use), and p53 (DO-7; Thermo Fisher Scientific, Waltham, USA; 1:300). An EnVision immunostaining procedure (DAKO, Carpinteria, USA) was performed according to the manufacturer’s protocols. Strong, diffuse nuclear staining (>80% cells with deep staining) in EAC and null expression in HPV were defined as aberrant p53 expression; otherwise, as wild-type expression. Two patterns of p16 immunoreactivity were identified – widespread, diffuse staining (block staining) and patchy positivity. Block p16 staining and positive MUC6 staining (>10% positive cells) was applied to aid the diagnosis of HPVA and GAC, respectively.

Statistics

Statistical analysis was performed using the SPSS 16.0 software package (SPSS, Inc., Chicago, IL, USA). Non-parametric tests (Wilcoxon test or Mann–Whitney *U* test), χ^2 test, and one-way analysis of variance were used to determine the clinicopathologic significance among tumors with different histologic grade. The Kaplan–Meier method was performed to compare mortality and recurrence between groups, and the log-rank test was used to analyze the statistical significance of the differences between groups. Multivariate survival analysis was carried out by the Cox’s proportional regression hazard (HR) method. We adjusted multiple parameters that were found to be significant in the univariate model. The statistical threshold was set at 0.05 (two-sided).

RESULTS

General clinicopathologic features

The study cohort had 398 EAC cases after excluding adenosquamous carcinoma, endometrioid and serous carcinoma from the

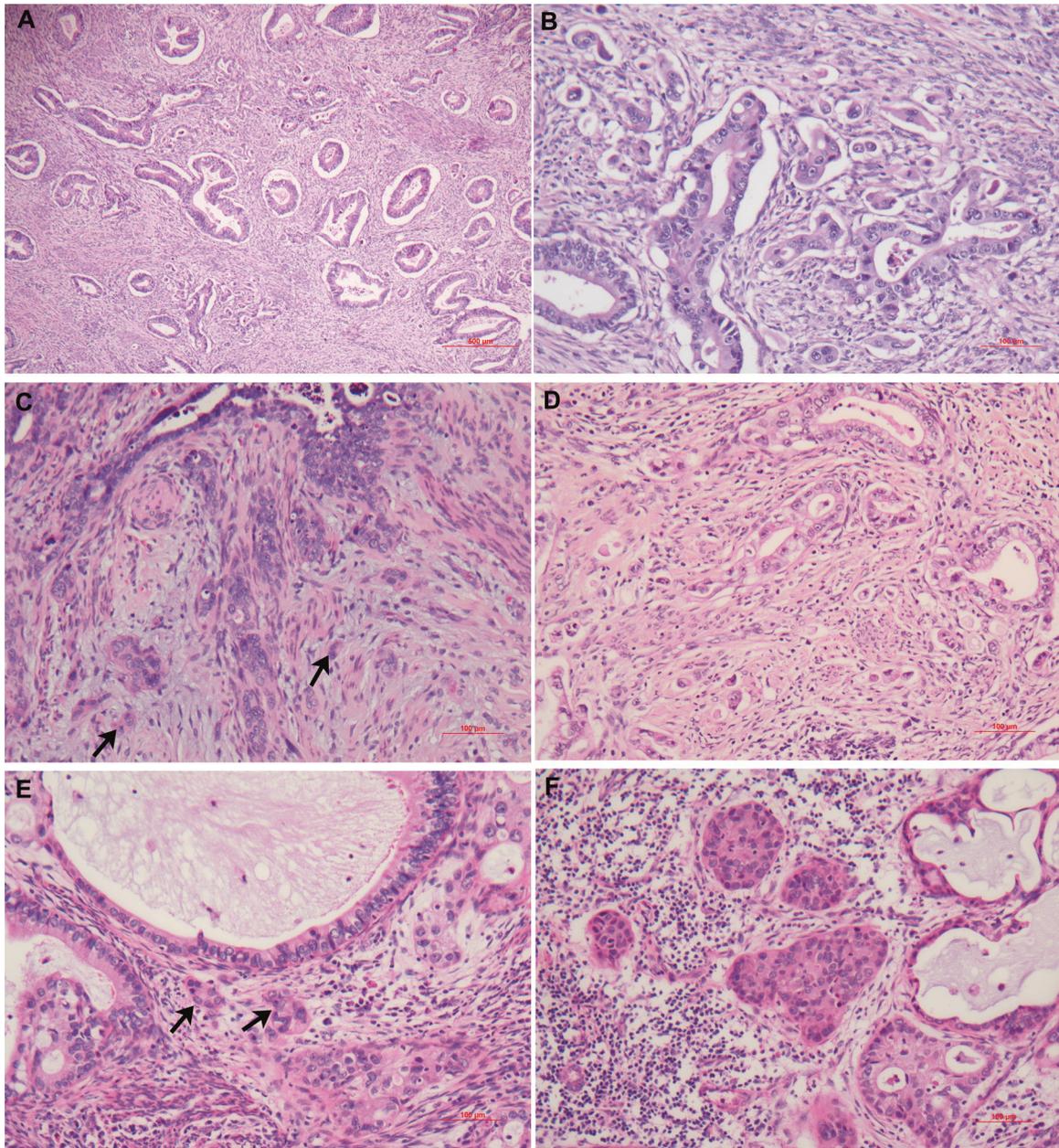


Fig. 1 Tumor budding and cell cluster size in EAC. Tumor budding (A–C): overview (A \times 50) and details (B \times 200) of an EAC with high tumor budding activity, indicated by the branching of numerous small tumor clusters of $<$ 5 cells into the surrounding tissue; details of low tumor budding activity with 2 clusters of tumor cells, each showing budding (arrows) into the tumor stroma (C \times 200). Cell nest size (D–F): single cell invasion (D \times 200), intermediate (E \times 200) and large (F \times 200) sized cell clusters.

upper genital tract. The age of the patients ranged from 19 to 77 years (median 46 years, mean 46.6 ± 9.9 years). Most patients ($n = 356$) underwent radical abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymph-node dissection. Thirty-three patients were at FIGO stage IA, 274 at stage IB, 32 at stage II, 48 at stage III, 7 at stage IV and 4 unstaged. The patients were followed up for 3–185 months (median time: 26 months). Thirty-one patients recurred with a median time of 24 months (range: 1–142 months) post-operatively. Twenty patients died of disease (median survival time: 17 months; range: 3–144 months).

The IECC types included 294 HPVA (73.8%), 88 HPVI (22.1%) and 16 adenocarcinoma-not otherwise specified (4.1%). Usual-type EAC (224/294, 76.2%) was the main histotype in HPVA while GAC (73/88, 83.0%) was predominant in HPVI. The other HPVI subtypes included clear cell carcinoma ($n = 10$), endometrioid carcinoma

($n = 4$) and mesonephric adenocarcinoma ($n = 1$). In HPVA, the Silva pattern included pattern A in 58 patients (19.7%), pattern B in 46 cases (15.6%) and pattern C in 190 cases (64.6%). LVI and nerve involvement (NI) were present in 165 (41.5%) and 29 (7.3%) cases, respectively. Aberrant p53 expression was observed in 48 of 357 (13.4%) cases. Representative morphology and IHC are shown in Fig. 2A–D.

Distribution of tumor budding, minimal cell cluster size and novel grading

The distribution of tumor budding activity included 264 (66.3%) cases without any activity, 52 (13.1%) cases with low activity ($<$ 15/10HPF) and 82 (20.6%) with high activity (\geq 15/10HPF). The assessment of minimal tumor cell cluster size indicated that large clusters ($>$ 15 cells or pure glands) were present in 217 (54.5%)

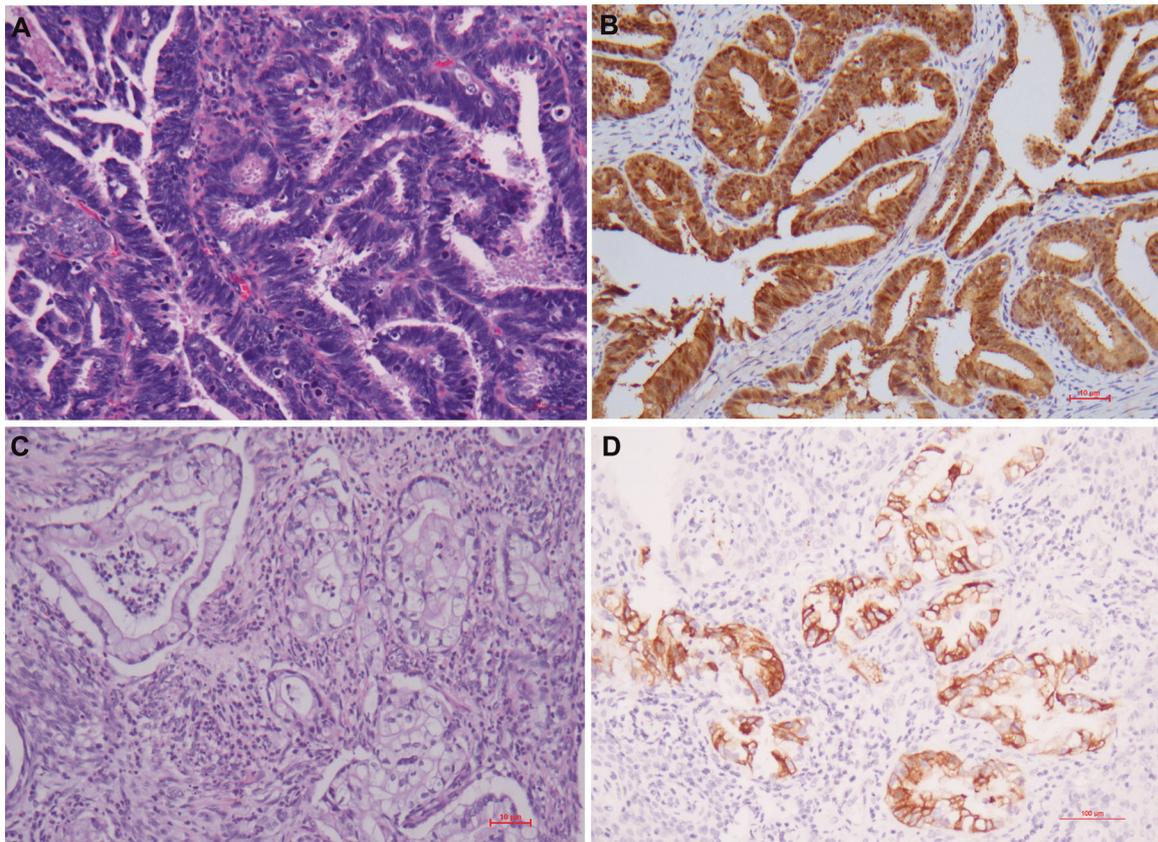


Fig. 2 Major histotypes in EAC. Representative examples of usual-type EAC (**A**, H&E $\times 200$; **B**, p16 IHC $\times 200$) and GAC (**C** H&E $\times 200$; **D**, MUC6 IHC $\times 200$).

cases, intermediate clusters (5–15 cells) in 47 (11.8%), small clusters (2–4 cells) in 48 (12.1%), and single cell invasion in 86 (21.6%) tumors. High budding activity was positively correlated with small cell cluster size ($r = 0.984$, $p < 0.001$). The novel grade based on the combination of tumor budding activity and tumor cell cluster size scores identified that 264 (66.3%) cases were well differentiated (G1), 28 (7.0%) moderately differentiated (G2), and 106 (26.7%) poorly differentiated (G3).

Correlation of tumor budding, minimal cell cluster size and novel grade with clinicopathologic parameters and prognosis

The correlation of tumor budding, minimal cell cluster size and novel grade with clinicopathologic parameters is summarized in Table 2. Briefly, high budding activity, small tumor cell cluster size and single cells, plus novel grade 3 were more frequently associated with HPVI, lymph node metastasis, ovarian involvement, LVI, PNI, invasion of deep cervical wall, advanced FIGO stage, and aberrant p53 expression in EAC as well as Silva pattern C in HPVA ($p < 0.05$).

Univariate survival analysis indicated that tumor budding, cell cluster size and novel grade were significantly associated with overall survival and tumor recurrence in EAC (all $p < 0.001$, Table 3). High budding activity, small cluster size and single cells, and high novel tumor grade showed a decreased overall survival time and tumor recurrence time (Fig. 3A–F). Moreover, novel grade 3 was an adverse indicator for overall survival in HPVA ($p = 0.036$) and GAC ($p = 0.033$) (Fig. 3G, H) as well as early tumor recurrence in HPVA ($p = 0.004$) while Silva pattern C was only associated with tumor relapse ($p = 0.020$) in HPVA. Multivariate analysis demonstrated that the novel grading system was significantly associated with overall survival and tumor recurrence independently of age and FIGO stage in EAC patients ($p < 0.05$, Table 4).

Correlation of revised FIGO grading system with clinicopathologic parameters and prognosis

By revised FIGO grading,⁹ 73.1% ($n = 291$) of EAC cases were well differentiated (G1), 14.8% ($n = 59$) were moderately differentiated (G2), and 12.1% ($n = 48$) were poorly differentiated (G3). The revised FIGO system did not significantly correlate with the novel grading system ($p = 0.398$, $r = -0.057$). Among the clinicopathologic parameters with potential prognostic value, the revised FIGO G3 was the only one positively associated with LVI and deep invasion of the cervical wall in EAC, and Silva pattern C in HPVA ($p < 0.05$, Table 2). Survival analysis indicated that the revised FIGO system was not associated with overall survival or tumor recurrence in EAC ($p > 0.05$, Table 3).

DISCUSSION

The histopathologic grading system based on tumor budding and cell nest size has been validated as prognostically useful in patients with SCC from several anatomic sites, including lung,¹⁵ esophagus,¹⁶ larynx/hypopharynx,¹⁸ and oral cavity.¹⁷ Two recent studies on uterine cervical SCC have indicated that the novel grading system can provide more powerful prognostic information than the WHO-based grading system.^{19,20} These studies have unanimously concluded that the combination of tumor budding activity and cell nest size can stratify organ-wide SCC patients into subgroups with good, intermediate and poor prognosis. Moreover, the grading system had minimal methodological variations across studies, a high concordance among pathologists, and easy applicability in clinical practice. The grading approach based on tumor budding has demonstrated considerable clinical utility in prognostic assessment, and may potentially contribute to future decision-making in SCC from many anatomic sites.

Table 2. Association of tumor budding, cell cluster size, novel grade, and FIGO grade with clinicopathological parameters in EAC*.

	Tumor budding score				Tumor cell cluster size score				Novel grade			FIGO grade			P	
	1 n = 264	2 n = 52	3 n = 82	P	1 n = 217	2 n = 47	3, n = 48	4 n = 86	P	G1 n = 264	G2 n = 28	G3 n = 106	G1 n = 291	G2 n = 59		G3 n = 48
Age				0.165				0.451	0.323							0.857
≤46 yr	154	28	37		127	27	24	41		154	14	51	162	32	25	
>46 yr	110	24	45		90	20	24	45		110	14	55	129	27	23	
WHO type				<0.001					<0.001							0.554
HPVA	220	38	36		180	40	36	38		220	22	52	220	40	34	
HPVI	32	14	42		29	3	12	44		32	6	50	71	10	7	
Silva pattern**				<0.001					<0.001							<0.001
A	58	0	0		58	0	0	0		58	0	0	56	2	0	
B	43	3	0		43	0	3	0		43	3	0	45	1	0	
C	127	30	33		83	44	27	36		127	14	49	119	39	32	
Lymph node metastasis				<0.001					<0.001							0.076
Yes	15	7	32		8	7	9	30		15	3	36	31	15	8	
No	208	41	43		170	38	38	46		208	24	60	210	44	38	
Ovarian involvement				<0.001					0.002							0.56
Yes	2	2	8		2	0	2	8		2	2	8	10	2	0	
No	241	49	71		197	44	45	75		241	25	95	259	57	45	
Lymphovascular invasion (LVI)				<0.001					<0.001							0.024
Yes	56	33	76		30	26	33	76		56	15	94	109	33	23	
No	208	19	6		187	21	15	10		208	13	12	182	26	25	
Perineural involvement (PNI)				<0.001					<0.001							0.516
Yes	1	7	21		0	1	6	22		1	4	24	21	6	2	
No	263	45	61		217	46	42	64		263	24	82	270	53	46	
Depth of invasion of cervical wall				<0.001					<0.001							0.001
≤1/3	76	2	0		72	4	2	0		76	2	0	69	6	3	
>1/3, ≤2/3	122	12	16		108	14	9	19		121	5	24	110	20	20	
≥ 2/3	65	37	64		36	29	37	64		65	22	79	108	33	25	
FIGO stage				<0.001					<0.001							0.155
I	236	38	33		201	35	30	41		236	20	51	230	43	34	
II-IV	25	14	48		13	12	18	44		25	8	54	57	16	14	
Aberrant p53 expression				<0.001					<0.001							0.162
Yes	12	8	28		11	1	5	31		12	3	33	37	9	2	
No	213	42	54		174	39	41	55		213	23	73	216	48	45	

Annotation:

*The algorithm for novel grade and related scores is given in Table 1.

**Silva pattern was only analyzed in HPVA.

Table 3. Association of tumor budding, cell cluster size, novel grade, and FIGO grade with overall survival and tumor recurrence in EAC (univariate analysis)*.

Total	Overall survival (OS)			Tumor recurrence		
	Events	Mean ± SD (mo.)	P	Events	Mean ± SD (mo.)	P
Tumor budding score			<0.001			<0.001
1	228	4	176.0 ± 5.2	8	171.4 ± 5.7	
2	46	3	144.8 ± 5.7	5	135.8 ± 8.2	
3	63	13	119.6 ± 12.6	18	110.3 ± 11.7	
Cell cluster size score			<0.001			<0.001
1	188	2	174.3 ± 7.5	6	168.5 ± 8.0	
2	40	2	162.1 ± 6.8	2	162.6 ± 6.5	
3	43	3	125.3 ± 8.8	4	121.4 ± 9.5	
4	66	13	113.9 ± 14.6	19	102.8 ± 13.2	
Novel grade			<0.001			<0.001
G1	227	4	176.0 ± 5.2	8	171.3 ± 5.7	
G2	27	1	134.9 ± 5.0	1	134.9 ± 5.1	
G3	83	15	122.9 ± 11.5	22	111.7 ± 10.7	
FIGO grade			0.568			0.420
G1	254	13	170.3 ± 4.3	21	163.3 ± 4.8	
G2	45	3	162.3 ± 8.8	3	161.4 ± 9.4	
G3	43	4	135.8 ± 6.5	6	126.5 ± 8.5	

*Annotation: The algorithm for novel grade and related scores is given Table 1.

In this study, we investigated the prognostic significance of the novel grading scheme incorporating tumor budding and tumor cluster size from a large cohort of resected EAC. By analogy with SCC,^{15–20} we confirmed that tumor budding activity and cell cluster size were powerful histology-based prognostic factors, and the combination of both parameters resulted in an easily applicable, 3-tiered grading system of major prognostic significance. The grading system is also helpful to stratify HPVA and GAC patients in terms of overall survival. Notably, high tumor budding activity, small cell cluster size and novel grade 3 were additionally closely associated with several important prognostic variables, such as HPV, lymph node metastasis, LVI, invasion of the deep cervical wall, advanced FIGO stage, aberrant p53 expression and Silva pattern C.^{3–5,7,8,25} The biological basis for the prognostic performance of the novel grading system is not understood, but the prevailing hypothesis is that tumor budding and cell clusters may be associated with dissociation, plasticity, motility, invasiveness and epithelial-mesenchymal transition in cancer cells.^{11,12,26} The underlying molecular events may be linked to RAS oncogenic mutations, nuclear β -catenin and reduced E-cadherin expression.²⁶ In our study, we have found that increased tumor budding and minimal cell clusters correlated with mutant-type p53 expression, which may cause severe dysregulation of cellular growth and result in poor prognosis in cancers.²⁷

While the effects of the novel grading system have not been investigated in adenocarcinomas yet, high tumor budding activity or score has been well established as an indicator of poor prognosis in adenocarcinomas from several anatomic locations, such as colorectum,^{13,28} small intestine,²⁹ esophagus,³⁰ stomach,¹⁴ pancreas,³¹ breast,³² liver,³³ lung,³⁴ uterine corpus³⁵ and cervix.²¹ Recently, a large colorectal cancer cohort comprising 1004 cases has shown that histologic subtypes and tumor budding, but not WHO grade, are stage-independent prognosticators for patient survival; therefore, tumor budding is superior to WHO grade in prognostic prediction in colorectal cancers.²⁸ Satabongkoch et al.²¹ found that usual-type EAC patients with a high tumor bud count had a significant decrease in both disease-free survival and cancer-specific survival on univariate analysis, but did not on

multivariate analysis. Poorly differentiated clusters, defined as solid cancer cell nests comprising five or more cancer cells and lacking glandular formation, have been validated to be a highly reproducible and relevant factor for predicting the prognosis and metastatic risk in patients with colorectal cancers,²³ and may provide more robust prognostic information than tumor budding and conventional grading.^{36,37} In EAC, the prognostic value of tumor cluster size has not been specifically investigated yet. In our study, we slightly adjusted the algorithm of cell nest size proposed for SCC by designating cell cluster size score 1 for EAC with pure glandular formation (no cell clusters or detached single cells) in contrast with the poor differentiation represented by cell clusters lacking glandular formation.^{23,29,36,37} We found that small cell cluster was a significant indicator for poor overall survival and tumor recurrence in EAC. Despite the presence of strong correlation between tumor budding activity and tumor cell cluster size, we believe that the combination of both parameters can more precisely reflect the malignant potential of EAC than a single factor, generating a highly prognostic grading system in EAC. Likewise, Jesinghaus et al.¹⁹ suggested that the two factors may assess the ability for cellular dissociation in cancers from different angles: cell nest size qualitatively describing the degree of cellular discohesion, and tumor budding being a quantitative parameter to measure the amount of dissociative growth within a given cancer.

Conventional histologic grading of EAC according to the revised FIGO system of uterine endometrioid carcinoma uses the combination of solid architecture and cytologic features.⁹ A few studies have evaluated the clinical outcomes against conventional histologic grading in EAC. Baalbergen et al.³⁸ found that FIGO stage, grade and lymph node metastasis were significant independent predictors of 5-year survival in 305 cervical adenocarcinomas of mixed types. Another study on 129 HPVA indicated that grade 3 histology was the only independent predictor of decreased disease-free and cancer-specific survival on multivariate analysis.²¹ In contrast, other studies did not find that histologic grade was an independent prognostic factor.^{5,39} GAC, the predominant histotype of HPV, is a clinically aggressive tumor

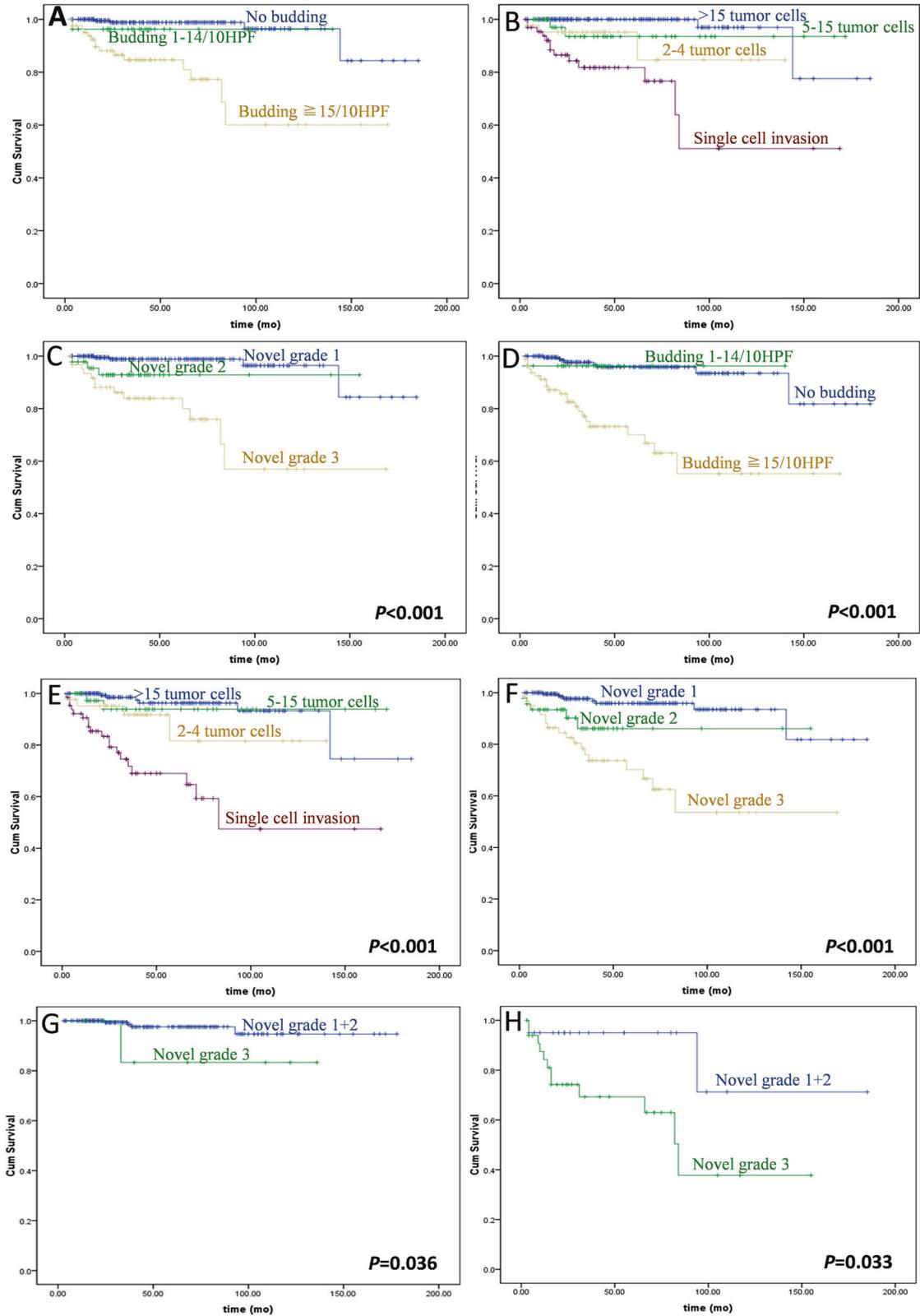


Fig. 3 Association of tumor budding activity, cell cluster size and novel grade with overall survival and tumor recurrence in EAC. Overall survival: (A) tumor budding activity, (B) cell cluster size, and (C) novel grade; Tumor recurrence: (D) tumor budding activity, (E) cell cluster size and (F) novel grade; Novel grading in stratifying overall survival in HPVA (G) and GAC (H).

Table 4. Multivariate analysis for overall survival and tumor recurrence, including age, novel grade and FIGO stage in EAC*.

	Exp(B) (95% CI)	P
Overall survival		
Age (>46 yr vs ≤46 yr)	3.655 (1.194–11.20)	0.023
Novel grade (G1/2 vs G3)	1.899 (1.055–3.421)	0.033
FIGO stage (II–IV vs I)	2.435 (1.391–4.260)	0.002
Tumor recurrence		
Age (>46 vs ≤46 yr)	2.061 (0.934–4.550)	0.073
Novel grade (G1/2 vs G3)	1.787 (1.138–2.805)	0.012
FIGO stage (II–IV vs I)	2.368 (1.529–3.666)	<0.001

*Annotation: The algorithm for novel grade and related scores is given in Table 1.

with poor prognosis, irrespectively of degree of differentiation.²⁴ In accordance with a recent study using IECC criteria in 205 EAC,⁵ our study indicated that conventional histologic grade was not statistically significant with regard to prognosis in HPVA, HPVI, or both. The findings support the concept that conventional tumor grading is a questionable major prognostic factor in EAC and has limited influence on treatment algorithms guiding patient management.⁹

The Silva pattern classification is based on pattern of growth and invasion, and takes into account tumor architecture and nuclear grade. It is capable of stratifying HPVA into subgroups with no (pattern A), minimal (pattern B) and high risk (pattern C) of lymph node metastasis.⁴ Importantly, the reproducibility and prognostic value of the Silva and IECC classifications have been well documented.²⁵ However, the Silva pattern system is suitable only for HPVA rather than HPVI. A combination of Silva pattern classification and IECC tumor type emerged to form the basis for prognostic stratification of EAC, with the Silva pattern acting as a surrogate for tumor grade: pattern A and B tumors being well to moderately differentiated and pattern C tumors having poor differentiation architecturally.⁹ In our study, we found that Silva pattern C was associated with tumor recurrence by univariate analysis, but was not an independent variable by multivariate analysis. By comparison, the novel grade was significantly associated with overall survival and tumor recurrence independently of age and FIGO stage. Moreover, it can stratify GAC, the major type of HPVI, into two subgroups with different prognosis—thereby, potentially contributing to decision-making in the treatment of this uncommon aggressive cancer. Collectively, in terms of prognostic assessment, the novel grade appears to outperform both the Silva pattern scheme and conventional histologic grade.

Our retrospective study is limited by relatively short follow-up time, a small number of rare subtypes, and a restriction to resected specimens that impacts on the transferability of this novel grading to biopsy specimens. Further multi-institutional investigation of a larger number of tumors, both resected and biopsy specimens, with longer follow-up time is critically required to consolidate the prognostic significance of the novel grading system in EAC and its subtypes. As in SCC, an organ-wide study may also expand the potential clinical application of the tumor grade scheme.

In conclusion, our retrospective study on a large case series has verified that a 3-tiered grading system based on tumor budding activity and cell cluster size is a prognostic indicator that appears to be superior to the conventional FIGO grading and Silva pattern classification in EAC. The novel grading system can be applicable in the prognostic stratification of various EAC subtypes including HPVA and GAC. Our findings validate previous studies that proposed tumor budding and poorly differentiated clusters for

prognostic stratification in pancreatic and colorectal adenocarcinomas as well as the similar grading system for organ-wide SCC patients. If further confirmed, the novel grading system would be applied in pathologic descriptions of EAC routinely, with the potential to enhance prognostic accuracy and influence treatment algorithms in EAC patients in the future.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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AUTHOR CONTRIBUTIONS

H.S. and Y.L. performed the study and data collection. H.S. also wrote the paper. W.L. performed data analysis, and review and revision of the paper. B.L. performed study concept and design, and approved the final submission. All authors read and approved the final paper.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board, Women's Hospital, School of Medicine, Zhejiang University (IRB: 20170139). The study was performed in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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