Published in final edited form as:

Am J Surg Pathol. 2010 October ; 34(10): 1417–1424. doi:10.1097/PAS.0b013e3181f0b05a.

Tumor Budding as a Strong Prognostic Indicator in Invasive Ampullary Adenocarcinomas

Nobuyuki Ohike, MD^{*}, Ipek Coban, MD^{*}, Grace E. Kim, MD[†], Olca Basturk, MD[‡], Takuma Tajiri, MD[§], Alyssa Krasinskas, MD^{||}, Sudeshna Bandyopadhyay, MD[¶], Toshio Morohoshi, MD[#], Yuki Shimada, MS^{**}, David A. Kooby, MD^{††}, Charles A. Staley, MD^{††}, Michael Goodman, MD, MPH^{‡‡}, and Nazmi Volkan Adsay, MD^{*}

^{*}Department of Pathology, Emory University Hospital, Atlanta, GA

^{††}Department of Surgery, Emory University Hospital, Atlanta, GA

^{**}Department of Mathematics, Georgia Institute of Technology, Atlanta, GA

^{‡‡}Department of Epidemiology, Emory University, Atlanta, GA

[†]Department of Pathology, University of California-San Francisco, San Francisco, CA

[‡]Department of Pathology, Memorial Sloan-Kettering Cancer Center, NY

^{||}Department of Pathology, University of Pittsburgh, PA

[¶]Department of Pathology, The Karmanos Cancer Institute and Wayne State University, MI

[§]Department of Pathology, Showa University, Fujigaoka Hospital, Yokohama

[#]Department of Pathology, Showa University, Tokyo, Japan

Abstract

Prognostication of invasive ampullary adenocarcinomas (AACs) and their stratification into appropriate management categories have been highly challenging owing to a lack of well-established predictive parameters. In colorectal cancers, recent studies have shown that tumor budding confers a worse prognosis and correlates significantly with nodal metastasis and recurrence; however, this has not been evaluated in AAC.

To investigate the prevalence, significance, and clinical correlations of tumor budding in AAC, 244 surgically resected, stringently defined, invasive AAC were analyzed for tumor budding—defined as the presence of more than or equal to 5 isolated single cancer cells or clusters composed of fewer than 5 cancer cells per field measuring 0.785 mm² using a 20 × objective lens in the stroma of the invasive front. The extent of the budding was then further classified as “high” if there were greater than or equal to 3 budding foci and as “low” if there were <3 budding foci or no budding focus.

One hundred ninety-four AACs (80%) were found to be high-budding and 50 (20%) were low-budding. When the clinicopathologic features and survival of the 2 groups were compared, the AACs with high-budding had larger invasion size (19 mm vs. 13 mm; $P < 0.001$), an unrecognizable/absent pre-invasive component (57% vs. 82%; $P < 0.005$), infiltrative growth (51%

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Correspondence: Nazmi Volkan Adsay, MD, Anatomic Pathology, Emory University Hospital, Department of Pathology and Laboratory Medicine, 1364 Clifton Road NE, Room H-180B, Atlanta, GA 30322 (volkan.adsay@emory.edu).

This study was presented in part at the annual meeting of the United States and Canadian Academy of Pathology in Washington, DC, March 2010.

vs. 2%; $P < 0.001$), nonintestinal-type histology (72% vs. 46%; $P < 0.001$), worse differentiation (58% vs. 10%; $P < 0.001$), more lymphatic (74% vs. 10%; $P < 0.001$), and perineural invasion (28% vs. 2%; $P < 0.001$); more lymph node metastasis (44% vs. 17%; $P < 0.001$), higher T-stage (T3 and T4) (42% vs. 10%; $P < 0.001$), and more aggressive behavior (mean survival: 50 mo vs. 32 mo; 3-year and 5-year survival rates: 93% vs. 41% and 68% vs. 24%, respectively; $P < 0.001$). Furthermore, using a multivariable Cox regression model, tumor budding was found to be an independent predictor of survival ($P = 0.01$), which impacts prognosis (hazard ratio: 2.6) even more than T-stage and lymph node metastasis (hazard ratio: 1.9 and 1.8, respectively).

In conclusion, tumor budding is frequently encountered in AAC. High-budding is a strong independent predictor of overall survival, with a prognostic correlation stronger than the 2 established parameters: T-stage and lymph node metastasis. Therefore, budding should be incorporated into surgical pathology reports for AAC.

Keywords

ampulla; ampullary; carcinoma; tumor; budding; prognostic parameter

Although ampullary carcinomas seem to have a better prognosis compared with pancreatic ductal adenocarcinomas or distal bile duct carcinomas, there is a wide range of survival rates (reported 5-year survival varying from 21% to 68%).¹⁹ This raises interest in better, more stringent definition of these tumors, discovery of other possible prognostic markers, and development of parameters that would help stratify patients into more meaningful management categories.

“Tumor budding,” initially named as “sprouting” by Imai,¹⁵ is a morphologic phenomenon observed at the advancing edge of neoplasms. It is characterized by isolated or small clusters of tumor cells that detached from the neoplastic epithelium and migrate a short distance into the neoplastic stroma,^{50,51} which may explain the more aggressive behavior. To date, several criteria have been proposed for evaluation of tumor budding in different tumor types and these studies indicate that it is a useful prognostic marker in colorectal adenocarcinomas^{10,18,22,27,31,33,46,47,49,51,52} and esophageal,^{20,24,37} and anal squamous cell carcinomas.³⁰ Tumor budding; however, has not been evaluated in ampullary adenocarcinomas (AAC).

In this study, AACs resected at the authors’ institutions were reviewed to identify those with tumor budding and to determine whether tumor budding correlates with known prognostic parameters and survival of AAC.

MATERIAL AND METHODS

The study was conducted in accordance with Institutional Review Board requirements.

Case Selection

About 1469 pancreatoduodenectomy specimens in the surgical pathology files of the authors’ institutions were reviewed to identify true primary AACs. A tumor was regarded as primary AACs only if:

1. its epicenter was located in the ampulla/major papilla. If more than 75% of the tumor was located outside the ampulla, it was regarded as nonampullary unless it could convincingly be characterized as having preinvasive component. The possibility of colonization or surface cancerization (i.e., secondary involvement by

an invasive carcinoma originating elsewhere) was considered carefully before classification of an intramucosal lesion as “preinvasive” in this location.

2. carcinoma did involve the papilla-Vater mucosa. Cases originating from the very distal common bile duct (CBD) and involving the common channel by forming a plaque underneath the papilla but typically sparing the mucosa of the papilla were excluded.

Among 317 cases originally classified as AAC, when these criteria were applied with the purist’s approach, only 249 cases qualified as AAC. In each case, a detailed analysis of the tumor was done to evaluate the preinvasive and invasive aspects of the tumor [All the tumor slides (range, 1 to 4; mean, 2.7) were reviewed for each case]. If there was any mucosa-confined component of the lesion, it was designated preinvasive (Fig. 1) and classified as recognizable or unrecognizable/absent.

The size of the invasive carcinoma was verified by microscopic examination. If the entire tumor was visibly invasive on microscopic examination, or if the size of the invasive component was reported separately in the original surgical pathology report, this measurement was verified and recorded as the size of invasive carcinoma. In cases with a small invasive component, the size of invasion was determined by measuring from the largest focus available on the slides. For cases with larger invasive carcinomas that did not fit onto individual slides and for which the invasion size was not mentioned in the report, the size of invasion was estimated by reconstructing and correlating the microscopic and gross findings. Only the cases with definitive invasive adenocarcinoma were included.

The patterns of invasion were classified as expansive or infiltrative as specified by Jass et al.^{16,17} That is, tumors with branching neoplastic glands and a sharply demarcated deep invasive margin were classified as expansive. In contrast, tumors those dissect the tunica muscularis with long stretched neoplastic glands, which dissolve into loosely arranged microglandular proliferations in the extramural adipose tissue (ie, an ill-defined invasive margin), were classified as infiltrative.

Cell lineage was determined based on predominant morphologic and cytologic criteria agreed upon by 3 of 5 authors (NO, GEK, IC, TT, NVA). Accordingly, the tumors were classified as intestinal-type if the tumor resembled intestinal-type adenocarcinoma (Fig. 2A) or as nonintestinal-type [gastric-like, pancreatobiliary (Fig. 2B), mucinous or signet ring cell-type adenocarcinomas]. Any secondary nonadenocarcinoma pattern (squamous, neuroendocrine, sarcomatoid, etc) was classified as focal (<30%) or extensive (≥30%). The cases with extensive nonadenocarcinoma component, such as neuroendocrine carcinoma (n = 4) and sarcomatoid carcinoma (n = 1), were excluded from the study because of their known dismal prognosis.

Evaluation and Quantification of Tumor Budding

Tumor budding was defined as the presence of more than or equal to 5 isolated single cancer cells or clusters composed of fewer than 5 cancer cells per field measuring 0.785 mm² using a 20 × objective lens in the stroma of the invasive front (Fig. 3) in concordance with the definition by Ueno et al.⁵¹ The extent of the budding was then classified as “high” if there were greater than equal to 3 budding foci and as “low” if there were <3 budding foci or no budding focus.

Correlative Parameters

Demographic data, age, and sex were recorded from surgical pathology reports and the patient’s charts.

Histologic grade, lymphovascular/perineural invasions, lymph node, and resection margin status were verified by histologic examination.

Pathologic staging was determined according to the guidelines of the American Joint Committee on Cancer (AJCC 2010).⁷

Follow-up information was obtained from prospectively maintained institutional AAC databases, from the patient's charts, by contacting the primary physicians, or through the Surveillance and Epidemiology End Results (SEER) database.

Statistical Analysis

Differences in age, male/female ratio, overall tumor size, size of invasive component, and the rate of lymph node metastasis between AACs with high-budding and AACs with low-budding were analyzed by an unpaired student *t* test or χ^2 tests. Overall survival was analyzed using the Kaplan-Meier method and differences in survival between patients whose tumors revealed high-budding and those whose tumors showed low-budding were assessed by log-rank test. A Cox proportional hazards regression model version 2.9.1 (open source statistical software) was used to identify factors independently associated with postresection survival. All the tests were 2-sided, and statistical significance was defined as a *P*-value <0.05.

RESULTS

Clinicopathologic Characteristics of AACs

The 244 patients ranged in age from 27 to 88 years (mean, 65 y). About 145 patients were male and 99 were female (male/female ratio = 1.5).

The overall size of the tumors ranged from 3 to 80 mm (mean, 26 mm; median, 17 mm).

Microscopically, 151 tumors (62%) were found to have a recognizable preinvasive component (Fig. 1). About 135 tumors (55%) showed an expansive growth pattern and 109 tumors were infiltrative. Eighty-two cases (34%) were classified as intestinal (Fig. 2A) and 162 as nonintestinal (Fig. 2B). Of note, 20 cases had an invasive micropapillary pattern (10 focal, 10 extensive) and 14 had a mucinous component (7 focal, 7 extensive). Twelve cases (19%) were also associated with a distinctive secondary nonadenocarcinoma component. Among these, 5 had a neuroendocrine component, 4 had sarcomatoid areas, and 3 had squamous cell changes. Lymphovascular invasion was identified in 155 AACs (64%) and perineurial invasion in 56 (23%). Resection margins were involved in 12 (5%). Out of 12, 10 were retroperitoneal margins, 1 was pancreatic neck margin and 1 was bile duct margin. Most of the margin-positive cases either represented metastatic deposits and extranodal extensions to the presumed margins of the specimens or as insidious invasions (presumably through nerves and vessels) forming distant microfoci at the reported margins. Regarding tumor grade, 126 tumors (52%) were well-differentiated and 118 were moderately to poorly differentiated adenocarcinomas.

T-stage was classified according to AJCC 2010 guidelines⁷; 67 patients were classified as stage T1, 90 as stage T2, 82 as T3, and 5 as T4. Regional lymph node metastasis was seen in 87/226 cases (38%). Lymph node information could not be obtained in 18 cases. The length of available clinical follow-up was 1 to 207 months (mean, 36 mo; median, 22 mo).

Tumor Budding and its Relationship With Clinicopathologic Findings (Table 1)

Among the 244 cases, low-budding and high-budding were found in 50 (20%) and 194 (80%) cases, respectively.

The mean age and male/female ratio of the patients with low-budding seemed to be very similar to those of patients with high-budding (64.6 vs. 64.9 and 1.6 vs. 1.4, respectively, $P = 0.88$, $P = 0.71$).

There was no significant difference between groups in terms of the overall tumor size. AACs with low-budding ranged from 3 to 80 mm (mean, 29 mm) and AACs with high-budding from 8 to 75 mm (mean, 26 mm). However, the size of the invasive component was significantly larger in the high-budding group (mean, 19 mm vs. 13 mm; $P < 0.001$). A recognizable preinvasive component was also less common in the high-budding group (57% vs. 82%; $P < 0.005$).

In addition, infiltrative growth (51% vs. 2%; $P < 0.001$), nonintestinal-type histology (72% vs. 46%; $P < 0.001$), worse (moderate/poor) tumor differentiation (58% vs. 10%; $P < 0.001$), and lymphatic (74% vs. 10%; $P < 0.001$) and perineural invasion (28% vs. 2%; $P < 0.001$) were more common in tumors with high-budding. More importantly, high-budding was more likely to be associated with higher (T3 or T4) T-stage (42% vs. 10%; $P < 0.001$) and nodal disease (44% vs. 17%; $P < 0.001$).

AACs with high-budding also had an extensive (>30%) invasive micropapillary carcinoma component in 10 cases (5%), which was not found in AACs with low-budding; however, the difference was not statistically significant owing to small number of the cases affected.

Association of Tumor Budding With Survival in Different Patient Subgroups

The overall survival of patients with high-budding was significantly worse than that of patients with low-budding. The mean overall survival of patients with high-budding was 50 months with 3-year and 5-year survival rates of 41%, and 24%, respectively, whereas for patients with low-budding, the overall survival was 32 months, with 3-year and 5-year survival rates of 93% and 68%, respectively ($P < 0.001$ Table 2 and Fig. 4). Differences in survival also applied to restricted groups, such as well-differentiated tumors ($P < 0.01$), expansive tumors ($P < 0.05$), lower (T1 and T2) stage tumors ($P < 0.05$), or higher (T3 and T4) stage tumors ($P < 0.05$).

In addition, intestinal-type AACs had a significantly better prognosis than nonintestinal-type AACs ($P < 0.05$, Fig. 5); however, when low-budding and high-budding groups were analyzed separately, histologic type was not found to impact prognosis ($P = 0.27$ in low-budding group and $P = 0.42$ in high-budding group).

Prognostic Factors for AAC (Tables 3, 4)

Univariate and multivariate analyses were done on 210 cases for which all the factors tested were available. In univariate analysis, a prominent preinvasive component ($P < 0.05$), growth pattern ($P < 0.01$), histologic type ($P < 0.05$), lymphatic invasion ($P < 0.005$), perineural invasion ($P < 0.001$), T-stage ($P < 0.001$), lymph node metastasis ($P < 0.001$), resection margin involvement ($P < 0.001$), and tumor budding ($P < 0.001$) were found to be the significant prognostic factors for patients with AAC (Table 3). A multivariate Cox regression analysis was done using these 9 factors also identified T-stage ($P = 0.012$), lymph node metastasis ($P = 0.021$), resection margin involvement ($P = 0.013$), and tumor budding ($P = 0.012$) as significant independent factors related to prognosis. Furthermore, among these 4 variables, tumor budding was found to impact prognosis (hazard ratio of 2.6) more than T-stage and lymph node metastasis (hazard ratio of 1.9 and 1.8, respectively; Table 4).

DISCUSSION

The ampulla's pathologic and clinical significance is out of proportion to its small size. Tumors arising at this location have the potential to obstruct 2 major organs often resulting in early detection and presumably contributes to better prognosis when compared with carcinomas of adjacent structures, particularly the pancreas.¹⁹ However, studies comparing stage-matched ampullary and pancreatic adenocarcinomas still maintain more favorable outcome for AACs,^{13,53} suggesting a biology difference.

Nevertheless, the large variance in AAC survival rates (reported 5-year survival varying from 21% to 68%),¹⁹ owing partly to the improper characterization of true AACs in the literature, is striking. Oncologists have been searching for pathologic indicators that predict clinical outcome more accurately and striving to develop appropriate management algorithms. So far, the factors most consistently reported to influence prognosis have been grade and stage.¹ In their study of the SEER (Surveillance Epidemiology End Results) database, Al-bores-Saavedra et al showed that (1) overall survival of AAC is directly related to histologic grade and high-grade tumors tend to show more aggressive behavior than lower-grade tumors and tend to present at deeper levels of mural infiltration with more nodal involvement and more likely metastatic spread (2) stage of the disease is the most important prognostic factor for survival. Patients with localized-stage disease have significantly more favorable 5-year survival rate (45%) than patients with regional (31%) or distant-stage disease (4%). Their findings are consistent with data from smaller series.^{5,21,25,26,29,36,54}

Other studies have reported a significant association of lymphovascular invasion,^{11,26,29,53,54} perineural invasion,^{42,53,54} nodal metastases^{6,11,25,26,36,42,48,53} and margin status² with patient survival. Although there is not much datum in the literature, MIB-1 index,⁴⁵ DNA ploidy^{40,41,44,45} and microsatellite instability³⁹ have also been proposed as prognostically relevant factors. Some even suggest that MIB-1 index and DNA ploidy are independent prognostic parameters with an impact on survival higher than the grade and stage.^{44,45} Although recently a more favorable survival rate for intestinal-type relative to pancreatobiliary-type AACs has been shown,^{1,5,8,38,55} it is unclear if this difference is independent of stage. In our study, intestinal-type AACs was found to have a significantly better prognosis than nonintestinal-type AACs ($P<0.05$).

Tumor budding shows increasing promise in clinicopathologic studies as a prognostic factor independent of stage, in colorectal carcinomas^{28,31,33,51} and in esophageal^{20,24,37} and anal squamous cell carcinomas³⁰; however, it had not been studied in AACs.

The mechanism of budding has not yet been fully clarified, but it is thought to occur as a feature of the dedifferentiation observed at the invasive margin, and to represent the initial phase of tumor invasion.⁵¹ Studies indicate that epithelial-mesenchymal transition has an important role in the process.³² In the early phase of epithelial-mesenchymal transition, tumor cells undergo reduced intercellular contacts mediated by e-cadherin and start cell-matrix contacts mediated by integrins leading to reorganization of actin cytoskeleton to form cytoplasmic protrusions. When new cell-matrix contacts are completed at the leading edge, migration follows. Recently, loss of the E-cadherin/beta-catenin complex was shown to be related to poor prognosis in ampullary cancer.¹⁴ Furthermore, matrix metalloproteinases and the urokinase plasminogen activator (uPA)/uPA receptor system initiate pericellular matrix degradation.^{3,4} Not surprisingly, studies that address tumor budding have also shown altered cohesiveness of budding cells and degradation of the extracellular matrix.^{9,12,23,32,34,35,43}

In this study, the prevalence, and possible prognostic significance of budding was analyzed in a large series of AAC. Cases were identified based on stringent criteria, which excluded tumors that secondarily invade the ampulla, whereas most surgical databases commonly

included these as “ampullary” cancer cases (see materials and methods). Hence, this study of 244 cases is by far the largest pathologically characterized series of AACs. In analyzing the budding in this series, a modified version of the method put forth by Ueno et al⁵¹ was used. The entire invasive tumor front was carefully evaluated given the observation of heterogeneous nature of tumor budding. Variance in the amount and degree of budding was noticed and categorized into 2 groups: AACs in which tumor budding was easily observed with patchy or diffuse distribution (high-budding) and those with minimal or no budding (low-budding). For this, we defined greater than or equal to 5 isolated single cancer cells or clusters composed of fewer than 5 cancer cells per $20 \times (0.785 \text{ mm}^2)$ as budding focus (Fig. 3), and the presence of greater than equal to 3 such foci as high-budding.

This study shows high-budding in AAC is strongly associated with larger invasion size, infiltrative growth pattern, worse differentiation, higher incidence of lymphatic and perineural invasion and lymph node metastasis, which suggests that tumor budding contributes to invasiveness, spread of AACs, and may be one of the key factors in lymph node metastasis. This may not be surprising, considering the biological significance of budding discovered recently in other organ systems. Most importantly, high-budding is an independent prognostic parameter with an impact (HR: 2.64) even greater than established outcome predictors such as T-stage and N-stage. In addition, when a restricted subset of the study population were analyzed independently, budding still served as a stratifying factor for both lower stage and well-differentiated categories, and cases with expansive growth.

In conclusion, tumor budding is frequently encountered in AAC. High-budding is a strong independent predictor of overall survival, with a prognostic correlation stronger than the established parameters of tumor stage and lymph node metastasis. Therefore, tumor budding should be incorporated into surgical pathology reports for AAC.

Acknowledgments

This study is supported in part by the National Cancer Institute Specialized Program in Research Excellence (SPORE) CA101936 in Pancreas Cancer (PAR-02-068) and in part by the Georgia Cancer Coalition Distinguished Cancer Clinicians and Scientists Program.

The authors thank Leslie Ducato and Rhonda Everett for their assistance in the preparation of this manuscript, and Ahnon Milham for her critical review of the manuscript.

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FIGURE 1.

One hundred fifty-one tumors were found to have recognizable preinvasive component growing within the ampullary channel.

FIGURE 2.

Ampullary adenocarcinomas were classified as intestinal type in 82 cases (A) and nonintestinal-type (pancreatobiliary shown here) in 162 cases (B).

FIGURE 3. The presence of ≥ 5 isolated single cancer cells (A) or clusters composed of fewer than 5 cancer cells (B) per field measuring 0.785 mm^2 (20 objective lens) in the stroma of the invasive front was defined as budding focus (C).

FIGURE 4. Kaplan-Meier survival curve comparing patients with low-budding and high-budding ampullary adenocarcinomas.

FIGURE 5.

Kaplan-Meier survival curve comparing patients with intestinal-type ampullary adenocarcinoma (AAC) and nonintestinal-type AAC.

TABLE 1

Comparison of the Clinicopathologic Characteristics of Low-budding and High-budding Groups

Tumor Budding	Low-budding	High-budding	P
Case number	50	194	—
Mean age (y)	64.6	64.9	0.88
Male/female ratio	1.6	1.4	0.71
Tumor size, mean (mm)	29	26	0.22
Size of invasion, mean (mm)	13	19	≤0.001
Recognizable preinvasive component (%)	82	57	≤0.005
Infiltrative histologic growth (%)	2	51	≤0.001
Nonintestinal histologic type (%)	46	72	≤0.001
Extensive micropapillary carcinoma (%)	0	5	0.10
Lymphatic invasion (%)	10	74	≤0.001
Vascular invasion (%)	6	14	0.11
Perineural invasion (%)	2	28	≤0.001
Moderate/poor histologic differentiation (%)	10	58	≤0.001
Advanced tumor staging (T3+T4) (%)	10	42	≤0.001
Lymph node involvement (%)	17	44	≤0.001

TABLE 2

Comparison of the Survival of Low-budding and High-budding Groups

Tumor Budding	Low-budding	High-budding	P
Case number	50	194	
Mean survival (mo)	32	50	≤0.01
Three-year survival rate (%)	93	41	≤0.001
Five-year survival rate (%)	68	24	≤0.001

Table 3

Univariate Analysis of Prognostic Factors of Invasive Ampullary Adenocarcinomas

Variables	Groups	P
Gender	Male vs. female	0.46
Age (y)	—	0.38
Tumor size (mm)	—	0.84
Size of invasion (mm)	—	0.07
Preinvasive component	Recognizable vs. minimal/absent	≤0.05
Histologic growth pattern	Infiltrative vs. expansive	≤0.01
Histologic type	Intestinal vs. nonintestinal	≤0.05
Extensive micropapillary carcinoma	Present vs. absent	0.12
Lymphatic invasion	Present vs. absent	≤0.005
Vascular invasion	Present vs. absent	0.08
Perineural invasion	Present vs. absent	≤0.001
Histologic differentiation	Moderate/poor vs. well	0.09
T stage	T3+T4 vs. T1+T2	≤0.001
Lymph node metastasis	Present vs. absent	≤0.001
Resection margin	Positive vs. negative	≤0.001
Budding	High vs. low	≤0.001

TABLE 4

Multivariate Analysis of Prognostic Factors of Invasive Ampullary Adenocarcinomas

Variables	Groups	HR	95% CI	P
Preinvasive component	0: recognizable 1: minimal/absent	0.84	0.54–1.32	0.46
Histologic growth	0: expansive 1: infiltrative	1.01	0.62–1.65	0.96
Lymphatic invasion	0: absent 1: present	1.01	0.59–1.71	0.98
Perineural invasion	0: absent 1: present	0.96	0.54–1.72	0.90
T stage	0: T1+T2 1: T3+T4	1.91	1.15–3.18	0.01
Lymph node metastasis	0: absent 1: present	1.78	1.09–2.92	0.02
Resection margin	0: negative 1: positive	3.06	1.26–7.42	0.01
Tumor budding	0: low 1: high	2.64	1.23–5.64	0.01