Tumoral CD10 Expression Correlates with High-Grade Histology and Increases Risk of Recurrence in Patients with Stage I Lung Adenocarcinoma

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ABSTRACT

Objective: CD10 (neutral endopeptidase) is expressed in various normal and tumor cells, and its biological function can be controlled through enzymatic activity and signaling pathways. We investigated whether CD10 expression predicted disease recurrence and whether it correlated with histologic subtypes of stage I lung adenocarcinoma.

Material and Methods: We reviewed tumor slides of resected pathologic stage I lung adenocarcinomas (1995–2009). Tumors were classified according to the IASLC/ATS/ERS classification. CD10 immunohistochemistry was performed using tissue microarrays (n = 915). We combined the intensity (0–3) and distribution scores (0–2) for CD10 to create a total score (0–5). Risk of recurrence was estimated using competing risks methods.

Results: In the training cohort (n = 313), risk of recurrence of patients with high tumoral CD10 (score > 1, n = 57) was significantly higher (5-year cumulative incidence of recurrence [CIR], 37%) than in those with low CD10 (score ≤ 1; n = 256; 5-year CIR, 16%; $P < 0.001$); this finding was confirmed in the validation cohort (n = 602, $P = 0.036$). High tumoral CD10 was associated with higher risk of recurrence in acinar ($P = 0.007$) and papillary predominant tumors ($P = 0.022$). High tumoral CD10 was most frequently identified in micropapillary predominant (41%) and solid predominant tumors (34%). On multivariate analysis of intermediate-grade tumors, high tumoral CD10 remained a significant independent risk factor of recurrence (hazard ratio, 1.88; $P = 0.025$).

Conclusion: In stage I lung adenocarcinoma, tumoral CD10 correlated with high-grade histology and was an independent predictor of recurrence in intermediate-grade tumors.

KEYWORDS

adenocarcinoma, lung, CD10, histology, recurrence

ABBREVIATIONS

ATS = American Thoracic Society

CIR = Cumulative incidence of recurrence

CT = computed tomography

EGFR = epidermal growth factor receptor

ERS = European Respiratory Society

HPF = high-power fields

HR = hazard ratio

IASLC = International Association for the Study of Lung Cancer

MSK = Memorial Sloan Kettering Cancer Center

NSCLC = non-small cell lung carcinoma

TNM = tumor-node-metastasis

1. INTRODUCTION

In many countries, adenocarcinoma is the most common histologic type of lung cancer and the rate of adenocarcinoma has increased over the past decade.¹ To date, the tumor-node-metastasis (TNM) staging system has been the most reliable prognostic indicator of lung adenocarcinoma.² Based on the 2011 International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS) lung adenocarcinoma classification,³ predominant histologic subtypes have been shown to be prognostic factors; this has been validated in independent, large cohorts across multiple countries.⁴⁻⁶ With recent randomized trials that have assessed low-dose computed tomography (CT) screening for lung cancer,^{7,8} it has been anticipated that an increasing number of patients will be diagnosed with early-stage lung adenocarcinoma. Therefore, identifying this group's prognostic markers is of great importance to improving patient care. The IASLC/ATS/ERS adenocarcinoma classification uses histologic subtypes to stratify patients with stage I disease into 3 prognostic groups—low-risk, intermediate-risk, and high-risk.^{4,5} One limitation of this classification is that the majority (>40%) of patients who were classified in the intermediate-risk group (acinar or papillary subtypes) displayed a wide spectrum of favorable and unfavorable clinical behaviors.⁴⁻⁶ Thus, it will be helpful to identify significant prognostic factors that can further stratify patients in this group.

CD10 (neutral endopeptidase) is a zinc-dependent metalloproteinase that is expressed in various normal tissues⁹ and is capable of efficiently degrading various peptides and cytokines.^{10,11} CD10 is upregulated in malignant tumors and has been identified as a predictor of tumor biological aggressiveness through extracellular enzymatic degradation and intracellular

signaling crosstalk.¹²⁻¹⁹ Although CD10 has been known to upregulate in lung cancers, its prognostic significance for early-stage lung adenocarcinoma remains unknown.²⁰⁻²²

In this study, we investigated whether CD10 expression could be used to stratify patients, with respect to risk of recurrence, and whether it correlates with histologic subtypes and clinicopathologic factors in patients with stage I lung adenocarcinomas.

2. MATERIAL AND METHODS

2.1 Patients

This retrospective study (WA0269-08) was approved by the Institutional Review Board at Memorial Sloan Kettering Cancer Center (MSK). We reviewed patients with pathological stage I solitary lung adenocarcinoma who had undergone surgical resection at MSK between 1995 and 2009. Tumor slides from 1038 patients were available for histologic evaluation and were included in this study. Clinical data were collected from our prospectively maintained lung adenocarcinoma database. Tumor slides and blocks from 944 patients were available for tissue microarray construction. Of all, 499 were tested for epidermal growth factor receptor (*EGFR*) and *KRAS* mutation status. Disease stage was assigned on the basis of the seventh edition of the American Joint Committee on Cancer Staging Manual.²³

2.2 Histologic evaluation

All available hematoxylin and eosin stained tumor slides were reviewed by 2 pathologists (K.K. and W.D.T.), both of whom were unaware of patient clinical outcomes, using an Olympus BX51 microscope (Olympus Optical Co., Tokyo, Japan) with a standard 22-mm diameter eyepiece. Tumors were classified according to the IASLC/ATS/ERS classification³ and were grouped into

3 architectural grades based on histologic subtype—low-grade (adenocarcinoma in situ, minimally invasive adenocarcinoma, and lepidic predominant), intermediate-grade (papillary-predominant and acinar-predominant), or high-grade (micropapillary-predominant, solid-predominant, invasive mucinous, and colloid-predominant).²⁴

Mitoses were evaluated in 50 high-power fields (HPF) at $\times 400$ magnification (0.237 mm² field) in areas with highest mitotic activity and were counted as the average number of mitotic figures per 10 HPFs.²⁴ Visceral pleural invasion, lymphovascular invasion, and tumor necrosis were also investigated.

2.3 Immunohistochemical analysis and scoring of CD10 expression

We briefly deparaffinized 4 μ m-thick sections from the previously constructed tissue microarray blocks.^{25,26} Each case had 2–6 tumoral cores and 2–3 stromal cores; each core was 0.6 mm in diameter. Using the standard avidin-biotin-complex peroxidase technique, sections were immunostained for anti-CD10 antibody (56C6; Vector Laboratories, Burlingame, CA; diluted at 1:50) with a Ventana Discovery XT automated immunohistochemical stainer (Ventana Medical Systems, Inc., Tucson, AZ) according to manufacturer's guidelines.

As expression of CD10 was heterogeneous in distribution for each case, intensity (0: no expression; 1: mild; 2: intermediate; and 3: strong) and distribution scores (0: 0%, 1: >0–50%, and 2: >50–100%) for CD10 expression (in cytoplasm and membrane) were summed into a total score (range, 0–5) for each tumoral and tumor-associated stromal core.^{26,27} The average of total scores was used as the score for each case and was classified into: 0, >0–1, >1–2, >2–3, >3–4 and >4 (Fig. 1A–G). CD10 positive granulocytes were counted in each tumoral and stromal core (Fig. 1H). The average number of positive granulocytes was used as the number for each case

and was classified into: 0, 1–2, 3–9, 10–29, and ≥ 30 /core. Of all, 915 cases had adequate tumor cores available for CD10 analysis.

2.4 Statistical analysis

Associations between variables were analyzed using Fisher's exact test (for categorical variables) and the Wilcoxon test (for continuous variables). Cumulative incidence of recurrence (CIR) was estimated using competing risks methods that accounted for death without documented recurrence as a competing event.²⁸ Follow-up was calculated from date of surgery to date of any first recurrence, death from any cause, or last follow-up. Differences in CIR between groups were tested using Gray's method (for univariate nonparametric analysis) and the Fine-Gray competing risk model (for multivariate analysis).²⁸ All clinicopathologic factors associated either with CIR or with high CD10 were considered for inclusion in the multivariate model. If two or more factors were highly correlated (for example, pleural, lymphatic, vascular invasion and necrosis), only one of them was included in the model in order to avoid multicollinearity. For the purpose of categorizing CD10 expression into two categories (high vs. low), the entire cohort was chronologically split into a training cohort (n = 313; 1995–2002) and a more recent validation cohort (n = 602; 2003–2009). Once binary categorization was determined, all other analyses were performed on the entire cohort.

A two-sided P -value < 0.05 was considered statistically significant. Statistical analyses were conducted using SAS v9.2 (SAS Institute, Inc., Cary, NC) and R v3.0.1 (R Development Core Team), including the “survival” and “cmprsk” packages.

3. RESULTS

3.1 Patient demographics and their associations with disease recurrence

Median patient age was 69 years (range, 23–96 years) and most patients were women (62%) and had stage IA disease (70%). With regard to surgical procedure, 78% had undergone lobectomy and 22% had undergone limited resection (segmentectomy [n = 66] and wedge resection [n = 132]). Patient characteristics are shown in Table 1.

During the study period, 14% (n = 129) of patients experienced recurrence and 15% (n = 136) died from any cause without documented recurrence. Median follow-up period for patients who did not experience recurrence was 38.5 months (range, 2.6–160.1 months). The univariate associations between clinicopathologic factors and CIR are summarized in Table 1.

3.2 CD10 expression associations with disease recurrence

In the training cohort, the risk of recurrence of patients with tumoral CD10 total scores >1–2, >2–3, and >3–4 (5-year CIR, 44%, 29%, and 37%, respectively) was higher than those with scores 0 and >0–1 (16% and 15%, respectively; $P < 0.001$). Based on these results, tumoral CD10 expression was divided into high (total score > 1) and low (total score ≤ 1) groups. In the training cohort, CIR of patients with high tumoral CD10 expression was significantly higher (n = 57; 5-year CIR, 37%) than those with low tumoral CD10 expression (n = 256; 5-year CIR, 16%; $P < 0.001$) (Fig. 2). This finding was confirmed in the validation cohort; CIR of patients with high tumoral CD10 expression was significantly higher (n = 124; 5-year CIR, 21%) than those with low tumoral CD10 (n = 478; 5-year CIR, 14%; $P = 0.036$).

Stromal CD10 expression (total score > 1) and CD10-positive granulocytes infiltration ($\geq 10/\text{core}$) in tumors were not associated with disease recurrence ($P = 0.14$ and $P = 0.22$,

respectively). The risk of recurrence appeared elevated in patients with high CD10-positive granulocyte infiltration in stroma ($\geq 10/\text{core}$; $n = 41$, 5-year CIR, 30%) compared with low infiltration ($< 10/\text{core}$; 5-year CIR, 17%), but this difference was not statistically significant ($P = 0.068$).

Subgroup analyses confirmed that high tumor CD10 was associated with increased risk of recurrence for each stage and type of surgical procedure. CIR of patients with high tumoral CD10 expression was significantly higher than in those with low CD10 expression in stage IA (5-year CIR, 20% [$n = 110$] vs. 12% [$n = 529$]; $P = 0.013$) and stage IB (36% [$n = 71$] vs. 24% [$n = 205$]; $P = 0.014$) (Figs. 3A & 3B). Similarly, CIR of patients with high tumoral CD10 expression was significantly higher than in those with low CD10 expression in the lobectomy group (5-year CIR, 24% [$n = 138$] vs. 13% [$n = 579$]; $P = 0.001$) and in the limited resection group (34% [$n = 43$] vs. 25% [$n = 155$]; $P = 0.030$) (Figs. 3C & 3D). In subgroup analysis by architectural grade, CIR of patients with high tumoral CD10 expression was significantly higher than in those with low CD10 expression in intermediate-grade tumors (5-year CIR, 24% [$n = 111$] vs. 12% [$n = 469$]; $P = 0.001$). In subgroup analysis by predominant subtype, CIR of patients with high tumoral CD10 expression was significantly higher than in those with low CD10 expression in acinar predominant tumors (5-year CIR, 23% [$n = 87$] vs. 11% [$n = 276$]; $P = 0.007$) and papillary predominant tumors (29% [$n = 24$] vs. 14% [$n = 193$]; $P = 0.022$) (Figs. 3E & 3F). However, tumoral CD10 expression was not associated with recurrence in patients with other predominant subtypes.

On multivariate analysis, the association of high tumoral CD10 with CIR did not reach statistical significance after adjustment of significant confounders (hazard ratio [HR], 1.43; $P = 0.083$) (Table 2A). On multivariate analysis of patients with intermediate architectural grade

tumors, high tumoral CD10 remained an independent factor of increased risk of recurrence (HR, 1.88; $P = 0.025$) (Table 2B).

3.3 Tumoral CD10 associations with patient clinicopathologic factors

Tumors with high tumoral CD10 were more frequent in men ($P = 0.019$), patients with smoking history ($P < 0.001$), higher pathologic stage ($P = 0.003$), higher architectural grade ($P < 0.001$), pleural invasion ($P = 0.001$), lymphatic invasion ($P < 0.001$), vascular invasion ($P < 0.001$), and tumor necrosis ($P < 0.001$) (Table 1). Additionally, high tumoral CD10 was more frequently identified in tumors with *KRAS* wild type (85%) than those with *KRAS* mutation (73%; $P = 0.016$). However, tumoral CD10 was not associated with *EGFR* mutations ($P = 0.25$).

3.4 Tumoral CD10 associations with predominant histologic subtypes and mitotic count

Percentages of tumors with high tumoral CD10 in each histologic subtype are presented in Figure 4A. High tumoral CD10 was most frequently identified in micropapillary predominant tumors (41%; $P < 0.001$ [vs. other subtypes]) and solid predominant tumors (34%; $P < 0.001$ [vs. other subtypes]). By contrast, high tumoral CD10 was rarely identified in minimally invasive tumors (4%), lepidic predominant tumors (5%), and invasive mucinous tumors (3%). Mitotic count was significantly higher in tumors with high tumoral CD10 (median, 5; range, 0–43) than in those with low tumoral CD10 (median, 1; range, 0–76; $P < 0.001$) (Fig. 4B).

4. DISCUSSION

We have demonstrated that, in patients with pathologic stage I lung adenocarcinomas, high tumoral CD10 was more frequently identified in high architectural grade (solid predominant and

micropapillary predominant) tumors and it was associated with an increased risk of recurrence, especially in patients with intermediate-grade (acinar predominant and papillary predominant) tumors.

Previous studies have identified CD10 as a predictor of biological aggressiveness in various malignant tumors.¹²⁻¹⁹ In breast carcinoma, CD10 expression in tumor-related stroma was correlated with worse survival and with a greater risk of lymph node metastasis and higher tumor grade.^{12,13} In colorectal carcinoma, tumoral CD10 expression was correlated with a greater risk of liver metastasis.¹⁴ Additionally, CD10 expression was observed in tumor-infiltrating granulocytes and was correlated with worse survival.¹⁵ In prostate carcinoma, tumoral CD10 expression was used to identify subsets of patients with poor prognoses for survival, higher risk of lymph node metastases, and higher Gleason grades.^{16,17} By contrast, in hematopoietic tumors such as malignant lymphoma, tumoral CD10 expression was correlated with better survival.^{18,19}

In non-small cell lung carcinoma (NSCLC), prognostic value of tumoral CD10 was controversial because previous studies used small cohorts that were heterogeneous in histology and/or TNM stage.²⁰⁻²² Tokuhara et al reported tumors with high CD10 and low CD13—another type of metallopeptidase—were associated with favorable prognoses using gene expression analysis of NSCLCs (n = 132).²¹ Gurel et al reported that in lung squamous cell carcinomas (n = 31) both tumoral and stromal CD10 expression were unfavorable prognostic factors, while in non-squamous cell carcinomas (n = 35) tumoral CD10 expression was a favorable prognostic factor.²⁰ In a study conducted by Kristiansen et al, tumoral CD10 expression was not associated with prognosis in NSCLCs (n = 114).²² Ono et al reported that neither tumoral nor stromal CD10 expression correlated with prognosis in stage I lung squamous cell carcinomas (n = 142).²⁹ Recently, Leithner et al reported that high CD10 expression, based on gene expression analysis,

was associated with unfavorable prognoses in lung adenocarcinomas but not in non-adenocarcinomas.³⁰ However, it is unclear whether this result was based on tumoral or stromal CD10 expression because localization of CD10 was not confirmed via immunohistochemistry. Therefore, the prognostic value of tumoral CD10 for early-stage lung adenocarcinoma remains unknown. In our study, which analyzed a large cohort (n = 915) of patients with stage I lung adenocarcinomas, we determined that tumoral CD10 was a risk factor of recurrence and that it can stratify patients with intermediate architectural grade tumors into two distinct prognostic subgroups for recurrence. Moreover, tumoral CD10 remained prognostic in subgroup analyses of patients with stage IA and IB disease, and in the lobectomy and limited resection groups. CD10 was also expressed in tumor-related stroma and tumor-infiltrating immune cells (granulocytes) in stage I lung adenocarcinomas however, these findings did not have prognostic significance in our study.

Although the prognostic significance of CD10 has been reported in various malignancies,¹²⁻¹⁹ its central role in cancer progression is undetermined. In addition to the peptidase activity of CD10,^{10,11,31} it also has elastase activity that degrades elastic fibers.³² One possibility is that the elastase activity of CD10 may facilitate tumor invasiveness, thereby degrading elastic fibers in the lung parenchyma. Additionally, Maguer-Satta et al suggested that upregulation of CD10 enzymatic activity may inhibit epithelial differentiation of stem cells into mature cells or maintain cancer stem cells by cleaving peptides for differentiating signaling.³¹ Our study demonstrated that CD10 expression, based on mitotic count, was positively associated with tumor cell proliferation. Methionine-enkephalin—an endogenous opioid peptide—has an antiproliferative effect on tumor cells and is degraded by CD10.¹⁴ Interestingly, it was reported that CD10 enhanced tumor proliferation and liver metastasis by degrading the antitumor effect of

methionine-enkephalin in colorectal carcinomas.¹⁴

Our study demonstrated that tumoral CD10 was associated with the IASLC/ATS/ERS histologic classification. High tumoral CD10 presented more frequently in high-grade tumors (>30% in tumors with micropapillary and solid subtypes) and less frequently in low-grade tumors (≤5% in minimally invasive and lepidic subtypes). Because of this strong association between CD10 expression and histologic grade, CD10 might not be an independent prognostic factor for recurrence on multivariate analysis, including histologic grade as a confounding variable. Moreover, CD10 was upregulated in a subset of acinar predominant (24%) and papillary predominant (11%) tumors, and it was associated with higher risk of recurrence. A limitation of this study was that CD10 negative cases using tissue microarray analysis might have been focally positive if stained using whole tissue blocks.

Presently, adjuvant cisplatin-based chemotherapy is recommended for routine use in patients who had undergone completely resection (R0 resection) of stage II–IIIA NSCLCs.^{33,34} Using cell lines from other solid malignancies, CD10 was determined to be a novel marker of cisplatin resistance and cancer stem cells.³⁵ Therefore, further research is warranted to determine the role of CD10 as a potential marker for chemotherapy sensitivity and resistance to cancer treatment, and to select lung adenocarcinoma patients who are eligible for adjuvant cisplatin-based chemotherapy.

5. CONCLUSIONS

In conclusion, for patients with stage I lung adenocarcinomas, tumoral CD10 correlated with high-grade histologies (micropapillary and solid subtypes) and was an independent predictor of recurrence in intermediate-grade tumors (acinar and papillary subtypes). CD10 may help

clinicians select patients who are at high risk of recurrence as candidates for adjuvant therapy following surgical resection of early-stage lung adenocarcinomas.

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FIGURE LEGENDS

Figure 1. CD10 immunohistochemical analysis using tissue microarrays (original magnification, A–F: $\times 100$, G and H: $\times 400$)

(A) Tumor cells negative for CD10 (total score 0). (B) Tumor cells weakly and focally positive for CD10 (total score 2). (C) Tumor cells weakly and diffusely positive for CD10 (total score 3). (D) Tumor cells moderately and diffusely positive for CD10 (total score 4). (E) Tumor cells strongly and diffusely positive for CD10 (total score 5) in solid pattern. (F) Tumor cells strongly and diffusely positive for CD10 (total score 5) in micropapillary pattern. (G) Tumor-related stroma positive for CD10. (H) CD10-positive granulocytes infiltration in stroma.

Figure 2. Cumulative incidence of recurrence (CIR) analyses of tumoral CD10 expression in the training cohort

CIR of patients with high tumoral CD10 expression was significantly higher (score > 1 ; $n = 57$; 5-year CIR, 37%) than in those with low tumoral CD10 expression (score ≤ 1 ; $n = 256$; 5-year CIR, 16%; $P < 0.001$).

Figure 3. Subgroup analyses of cumulative incidence of recurrence (CIR) of tumoral CD10 expression in each pathologic stage, surgical procedure group, and histologic subtype

(A and B) CIR of patients with high tumoral CD10 expression was significantly higher than in those with low tumoral CD10 expression in stage IA (5-year CIR, 20% [$n = 110$] vs. 12% [$n = 529$]; $P = 0.013$) and stage IB (36% [$n = 71$] vs. 24% [$n = 205$]; $P = 0.014$). (C and D) CIR of patients with high tumoral CD10 expression was significantly higher than in those with low tumoral CD10 expression in the lobectomy group (5-year CIR, 24% [$n = 138$] vs. 13% [$n = 579$];

$P = 0.001$) and the limited resection group (34% [n = 43] vs. 25% [n = 155]; $P = 0.030$). (E and F) CIR of patients with high tumoral CD10 expression was significantly higher than in those with low tumoral CD10 expression in acinar predominant tumors (5-year CIR, 23% [n = 87] vs. 11% [n = 276]; $P = 0.007$) and papillary predominant tumors (29% [n = 24] vs. 14% [n = 193]; $P = 0.022$).

Figure 4. Tumoral CD10 associations with predominant histologic subtypes and mitotic count

(A) High tumoral CD10 expression was most frequently identified in micropapillary predominant tumors (41%; $P < 0.001$ [vs. other subtypes]), followed by solid predominant tumors (34%; $P < 0.001$ [vs. other subtypes]), while high tumoral CD10 expression was less frequently identified in minimally invasive (4%), lepidic predominant (5%), and invasive mucinous tumors (3%). (B) Mitotic count was significantly higher in tumors with high tumoral CD10 expression (median, 5; range, 0–43) than in those with low (median, 1; range, 0–76).

Figure 1

Figure 2

Figure 3

Figure 4

A

B

TABLES

Table 1. Clinicopathologic associations with recurrence and tumoral CD10 expression

Variables	N	(%)	5-yr CIR	P	Tumoral CD10, N (%)				P
					Low		High		
Age, years				0.82					0.17
≤65	331	(36)	18%		69 ^a		68 ^a		
>65	584	(64)	17%		23–96 ^b		43–87 ^b		
Sex				0.008					0.019
Female	565	(62)	14%		467	(64)	98	(54)	
Male	350	(38)	23%		267	(36)	83	(46)	
Smoking status				0.5					<0.001
Never	152	(17)	16%		137	(19)	15	(8)	
Former/Current	763	(83)	17%		597	(81)	166	(92)	
Surgery				<0.001					0.44
Lobectomy	717	(78)	15%		579	(79)	138	(76)	
Limited resection	198	(22)	26%		155	(21)	43	(24)	
Pathologic stage				<0.001					0.003
IA	639	(70)	13%		529	(72)	110	(61)	
IB	276	(30)	27%		205	(28)	71	(39)	
Architectural grade				<0.001					<0.001
Low	113	(12)	5%		108	(15)	5	(3)	
Intermediate	580	(63)	15%		469	(64)	111	(61)	
High	222	(24)	30%		157	(21)	65	(36)	
Pleural invasion				<0.001					0.001
Absence	761	(83)	15%		626	(85)	135	(75)	
Presence	154	(17)	29%		108	(15)	46	(25)	
Lymphatic invasion				<0.001					<0.001
Absence	623	(68)	13%		531	(72)	92	(51)	
Presence	292	(32)	26%		203	(28)	89	(49)	
Vascular invasion				<0.001					<0.001
Absence	682	(75)	13%		573	(78)	109	(60)	
Presence	233	(25)	29%		161	(22)	72	(40)	
Necrosis				<0.001					<0.001
Absence	761	(83)	13%		634	(86)	130	(72)	
Presence	151	(17)	36%		100	(14)	51	(28)	

Significant *P*-values (<0.05) are shown in bold.

^amedian; ^brange

CIR, cumulative incidence of recurrence

Table 2. Multivariate analysis for recurrence**(A) In all patients**

Variables		HR	95% CI	<i>P</i>
Tumoral CD10	(high vs. low)	1.43	0.95–2.13	0.083
Sex	(male vs. female)	1.33	0.92–1.92	0.12
Surgery	(limited resection vs. lobectomy)	2.42	1.65–3.54	<0.001
Pathologic stage	(IB vs. IA)	2.27	1.58–3.27	<0.001
Architectural grade	(intermediate vs. low)	4.31	1.07–17.33	0.040
	(high vs. low)	10.58	2.60–43.05	<0.001
Lymphatic invasion	(presence vs. absence)	1.51	1.04–2.2	0.032

(B) In patients with intermediate grade (acinar and papillary subtypes) tumors

Variables		HR	95% CI	<i>P</i>
Tumoral CD10	(high vs. low)	1.88	1.08–3.25	0.025
Sex	(male vs. female)	1.46	0.87–2.46	0.15
Surgery	(limited resection vs. lobectomy)	2.59	1.54–4.37	<0.001
Pathologic stage	(IB vs. IA)	2.44	1.50–3.99	<0.001
Lymphatic invasion	(presence vs. absence)	1.27	0.75–2.16	0.38

Significant *P*-values (<0.05) are shown in bold.

CI, confidence interval