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# Tumor Budding Correlates With the Protumor Immune Microenvironment and Is an Independent Prognostic Factor for Recurrence of Stage I Lung Adenocarcinoma

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**BACKGROUND:** Immune cell infiltration associated with tumor capsule disruption and tumor budding has been shown to reflect invasiveness, metastasis, and unfavorable prognosis in colorectal cancer. We investigated the influence of tumor budding on prognosis and its association with the immune microenvironment in lung adenocarcinoma.

**METHODS:** Tumor slides from resected stage I lung adenocarcinomas were reviewed (n = 524 and n = 514, for training and validation cohorts, respectively) for assessment of tumor budding. $CD3^+$ and forkhead box $P3^+$ ($FoxP3^+$) lymphocytes, $CD68^+$ macrophages, IL-7 receptor, and IL-12 receptor β2 were analyzed using tissue microarrays constructed from tumor and stroma. Probability of recurrence was calculated using the competing risks method.

**RESULTS:** In the training cohort, risk of recurrence for high-grade tumor budding was higher than it was for low-grade tumor budding (32% vs 12%, $P < .001$), which was confirmed in the validation cohort ($P = .005$). Tumor budding stratified the risk of recurrence for acinar-predominant (22% vs 9%, $P < .001$), papillary-predominant (22% vs 13%, $P = .045$), and solid-predominant (39% vs 19%, $P = .022$) tumors. Tumor budding was associated with higher stromal $FoxP3^+$ lymphocyte infiltration, higher stromal FoxP3/CD3 risk index, higher tumoral and stromal CD68+ macrophage infiltration, and IL-7 receptor overexpression ($P < .001$, all associations). Tumor budding remained independently associated with recurrence on multivariate analysis (hazard ratio, 1.61; $P = .008$).

**CONCLUSIONS:** Tumor budding is an independent prognostic factor of stage I lung adenocarcinoma and correlates with the protumor immune microenvironment. Our findings advocate investigating tumor-immune cell interactions at the invading edge as a biologic driver of tumor aggressiveness.

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**ABBREVIATIONS:** CIR = cumulative incidence of recurrence; FoxP3 = forkhead box P3; H&E = hematoxylin and eosin; HPF = high-power field; IASLC/ATS/ERS = International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society; IL-7R = IL-7 receptor; IL-12Rβ2 = IL-12 receptor β2; MSK = Memorial Sloan Kettering Cancer Center; OS = overall survival

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Adenocarcinoma is the most common histologic type of lung cancer, and the rate of adenocarcinoma has increased during the last decade.[1,2] Following results of previous randomized trials assessing low-dose CT screening for lung cancers,[3-5] it is anticipated that there will be an increase in the number of patients diagnosed with early-stage lung adenocarcinoma. The present TNM staging system is the most reliable prognostic tool for lung cancers.[6] Additionally, prognostic significance of histologic subtypes—based on the 2011 International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society (IASLC/ATS/ERS) lung adenocarcinoma classification system[7]—has been validated in large, independent cohorts spanning multiple countries.[8-12] A limitation of the IASLC/ATS/ERS classification is that the majority (> 40%) of adenocarcinoma cases are classified as intermediate-grade, acinar or papillary subtype, and these patients also displayed a wide spectrum of clinical outcomes.[8-12] Thus, it would be helpful to identify significant prognostic factors that can further stratify patients within the intermediate-grade group. In this study, we investigated whether tumor budding can predict patient disease recurrence and can be used to further stratify patients with histologically intermediate-grade tumors into prognostic subgroups.

Tumor budding, which is defined as the presence of isolated small tumor nests (composed of < 5 tumor cells) in the stroma at the outer edge of the tumor, has been shown to reflect tumor invasive behavior and is an unfavorable prognostic factor of colorectal cancers.[13,14] In their attempts to unravel the biologic significance of tumor budding, investigators have noted that tumor budding may be associated with epithelial mesenchymal transition, thereby increasing cancer cell migration and invasion.[15-18] In breast cancer, immune-induced responses have been shown to promote epithelial mesenchymal transition.[19-21] More importantly, studies have demonstrated that tumor-associated macrophages—especially those of the tumor-promoting M2 phenotype—are frequently found within regions of tumor budding[22] and that they have contributed to induction of cancer cell epithelial mesenchymal transition at the tumor invasive front.[23-25]

Prognostic significance of tumor budding and its correlation with immune factors have yet to be investigated in early-stage lung adenocarcinoma. We demonstrated that stromal forkhead box P3 (FoxP3)/CD3 lymphocyte risk index, tumoral IL-7 receptor (IL-7R) overexpression, and loss of tumoral IL-12 receptor β2 (IL-12Rβ2) expression were independent prognostic factors of stage I lung adenocarcinoma.[26] In this study, we investigated whether tumor budding correlated with tumor-infiltrating immune cells ($CD3^+$ or $FoxP3^+$ lymphocytes and $CD68^+$ macrophages) and immune markers (IL-7R and IL-12Rβ2).

## Materials and Methods

### *Patients*

This retrospective study (WA0269-08) was approved by the institutional review board at Memorial Sloan Kettering Cancer Center (MSK) and was designed in accordance with REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines (Fig 1).[27] We reviewed patients with pathologic stage I solitary lung adenocarcinoma who had undergone surgical resection at MSK between 1995 and 2009. Tumor slides from 1,038 patients were available for histologic evaluation and were included in this study. Clinical data were collected from our prospectively maintained lung adenocarcinoma database. Of the 1,038 patients with available tumor slides, 585 were tested for *EGFR* and *KRAS* mutation status. Disease stage was assigned based of the seventh edition of the *American Joint Committee on Cancer Staging Manual*.[28]

### *Histologic Evaluation*

All available hematoxylin and eosin (H&E)-stained tumor slides were reviewed by two pathologists (K. K. and W. D. T.), both of whom were unaware of patients' clinical outcomes, using an Olympus BX51 microscope (Olympus Corporation) with a standard 22-mm diameter eyepiece. Discrepancies in histologic evaluation between the pathologists were later resolved by consensus using a multihead microscope. Tumors were classified according to the IASLC/ATS/ERS classification[7] and were grouped into three architectural grades on the basis of histologic subtype: (a) low-grade (adenocarcinoma in situ, minimally invasive adenocarcinoma, and lepidic predominant); (b) intermediate-grade (papillary-predominant and acinar-predominant); and (c) high-grade (micropapillary-predominant, solid-predominant, invasive mucinous, and colloid-predominant).[29-32]

After reviewing the entire set of tumor slides at intermediate-power fields of ×100 magnification, we assessed tumor budding at the most invasive area with the maximal number of the smallest tumor nest (Fig 2A). Tumor budding was defined as small tumor nests composed of fewer than five tumor cells (Fig 2B) and was quantified by counting 10 high-power fields (HPFs) at ×200 magnification.[13] During evaluation using 10 HPFs, the maximum number of tumor buds per one HPF was considered the number of buds for each tumor. We then classified tumor budding as grade 0 (zero buds per HPF), grade 1 (one to four) (Fig 2C), grade 2 (five to nine), or grade 3 (≥ 10) (Fig 2D).[14,16,17]

Nuclear atypia was identified in the area with the highest degree of atypia and was graded as mild, moderate, or severe.[29,33] Mitoses were

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