Histopathologic Features of the Tumor Budding in Adenocarcinoma of the Lung

Tumor Budding As an Index to Predict the Potential Aggressiveness

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Introduction: The term tumor budding has been applied to single cells or small clusters of up to four cells within the stromal tissue at the invasive margin of colorectal cancers. This morphologic feature is increasingly being recognized as an adverse prognostic factor. The purpose of this study was to evaluate the clinicopathologic significance of tumor budding in adenocarcinomas of the lung.

Methods: We investigated the relationship between tumor budding and clinicopathologic parameters of adenocarcinomas of the lung and the prognostic significance of tumor budding by reviewing the cases of 201 consecutive patients who had undergone complete resection of adenocarcinoma of the lung measuring 30 mm or less in diameter. We examined immunohistochemical profile of budding cells (BCs) by immunohistochemical staining with 14 antibodies.

Results: Tumor budding was observed in 78 (43.1%) of the 181 cases with invasive adenocarcinoma. The presence of tumor budding was significantly associated with lymph node metastasis ($p = 0.005$), pathologic stage ($p < 0.001$), vascular invasion ($p = 0.003$), lymphatic invasion ($p = 0.009$), and pleural invasion ($p = 0.029$). Examination of the relation between the presence of tumor budding and the predominant histologic subtype revealed that the predominant papillary subtype was significantly associated with the presence of tumor budding ($p = 0.0023$), whereas the predominant bronchioloalveolar carcinoma subtype was significantly associated with the absence of tumor budding ($p < 0.001$). The overall 5-year survival rates of the group with budding and the group without budding was 67.5% and 88.3%, respectively, and difference was significant ($p = 0.0057$). Compared with cancer cells forming nests, BCs displayed reduced expression of cellular adhesion molecule, E-cadherin, and β-catenin ($p < 0.05$ and $p < 0.05$, respectively) and increased expression of laminin5-γ2 ($p < 0.05$). However, BCs displayed reduced expression of differentiation marker, surfactant protein A ($p < 0.05$). Multivariate analysis revealed that tumor budding was significant independent prognostic factor of the small-sized adenocarcinoma of the lung.

Conclusions: Our data showed that tumor budding in adenocarcinoma of the lung is a distinct morphologic feature that has biologic and prognostic significance.

Key Words: Tumor budding, Lung cancer, Small adenocarcinoma, Prognostic factor, Papillary subtype.

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Lung cancer is the leading cause of cancer deaths worldwide, and the prognosis is generally poor, even if surgery is successful. The incidence of one type of lung cancer, adenocarcinoma, has been increasing in recent years.[1] Although pure bronchioloalveolar carcinoma (BAC) has been reported to be the only subtype without any invasive features and to have an excellent prognosis,[2] in some of the patients with other subtypes of adenocarcinoma sometimes develop distant metastasis soon after complete surgical resection.[3] Even significant fraction of the patients with clinical stage IA (cT1N0M0) lung adenocarcinoma experience recurrence and die after curative resection.[4] A better understanding of the changes in tumor cell biology that result in a more aggressive neoplastic phenotype that have been completely resected may help identify patients with respectable adenocarcinoma at risk for recurrent disease and lead to the development of more effective therapeutic modalities.

The main characteristics of malignancy are invasive growth by tumor cells and a tendency to infiltrate and metastasize. Crucial steps in the invasive growth process are detachment at the intercellular junctions between tumor cells and active invasion of the surrounding stroma. The term "tumor budding" has been applied to these steps in colorectal cancer when single cells or small clusters are observed within the stromal tissue at the invasive margin.[5–7] Tumor budding can be evaluated semiquantitatively by various methods, and

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several study groups have found the results to be reproducible. Tumor budding is thought to be the morphologic expression of this crucial step in the invasive growth process, and, importantly, tumor budding has been shown to be an independent prognostic factor.[8]

We have often observed scattered single cells of small cell clusters in lung adenocarcinoma that are the equivalent of tumor budding, and we speculate that they are the morphologic expression of the crucial steps in the invasive growth process, including dedifferentiation and dissociation, the same as the budding in colorectal cancer. To examine the pathophysiologic significance of the budding process in small adenocarcinomas, we reviewed the adenocarcinomas of the lung measuring 30 mm or less in diameter. In addition, we elucidated the immunohistochemical characteristics in BCs by using tissue microarray (TMA).

PATIENTS AND METHODS

Patients

During the period from November 1993 to March 1998, a total of 665 patients with primary lung carcinoma were treated by surgical resection at the National Cancer Center Hospital East, Chiba, Japan. We reviewed the cases of the 201 consecutive patients in whom an adenocarcinoma of the lung measuring 30 mm or less in diameter had been completely resected as candidates for inclusion in this study. All patients signed an Institutional Review Board-approved written informed consent. The tumors were staged according to the tumor-node-metastasis classification of the International Union Against Cancer (UICC)[8] and were histologically subtyped and graded according to the third edition of the World Health Organization guidelines.[9] The median follow-up period for these patients was 10.0 years. Survival time was measured from the date of surgery.

Pathologic Review

All surgical specimens were fixed with 10% formalin and embedded in paraffin. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with hematoxylin and eosin (HE) and by the Alcian blue-periodic acid Schiff method to visualize cytoplasmic mucin production and the Verhoeff-van Gieson method to visualize elastic fibers. Lymphatic invasion and pulmonary metastasis were evaluated in sections stained with HE. Vascular and pleural invasion was evaluated by the Verhoeff-van Gieson method. Two observers (Y.Y. and G.I.) who are unaware of the clinical data independently reviewed all pathologic slides. The histologic diagnoses were based on the revised World Health Organization histologic classification. Tumor size was measured as the maximal diameter on the cut sections of the lung. The pathologic stage was determined according to the UICC.

Definition and Evaluation of Tumor Budding

Isolated single cancer cells and clusters composed of lesser than five cancer cells were defined as budding as previously described.[6] Such buddings are sometimes observed in the fibrosis and collapse at the tumor–stromal interface. To semiquantify budding, a field in which budding intensity seemed to be maximal was selected on the slide, and the number of budding in that field was counted using a 20× objective lens.[10] Discordant results were resolved by a joint review of the specimen through a multiheaded microscope. Budding counts of 1 to 4 were rated as grade 1, counts of 5 to 10 as grade 2, and counts of 11 or more were as grade 3 (Figure 1). This classification was based on a previously described classification.[10]

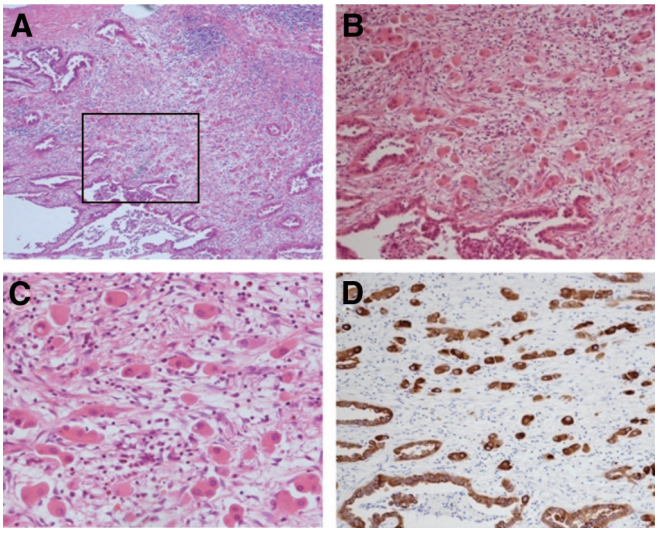

FIGURE 1. Histologic features of tumor budding. *A,* Tumor budding in the area of fibrosis (hematoxylin and eosin [HE] staining). *B,* Moderate magnification of the area boxed in *A*: budding cells (BCs) are observed in the fibrotic stroma (HE staining). *C,* Higher magnification of the area boxed in *A*. Tumor budding is visible in the form of isolated small clusters or single invading carcinoma cells with eosinophilic cytoplasm (HE staining). *D,* Cytokeratin AE1/3 immunostaining of BCs.

Construction of TMAs

We immunohistochemically stained the tumor of 20 patients whose tumors were with grade 3 budding during the period from November 2004 to March 2009. The most representative tumor areas were carefully selected and marked on the HE-stained slide of donor tissue for construction of microarrays. We evaluated immunohistochemical profiles of the following components: I, BCs, and II, cancer cells forming nests near budding cells (near BCs). TMAs were constructed with a manual tissue-arraying instrument (Azumaya, Tokyo, Japan) as previously described.[11] Specimens were routinely sampled by taking a core sample of each tumor from two different areas. A normal control TMA that included samples from nonmalignant specimens of various organs was used as a positive control.

Antibodies and Immunohistochemical Staining

In this study, the 14 antibodies used for immunohistochemical staining are listed in Table 1, and the immunochemical staining was performed as follows. TMA recipient blocks were cut into 4-μm sections and mounted on silane-