The morphological diversity of small lung adenocarcinoma with mixed subtypes is associated with local invasiveness and prognosis

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Abstract

Objective: Under the current World Health Organization (WHO) classification, 'lung adenocarcinoma with mixed subtypes' is the most frequent type, even in small lung adenocarcinomas, with a diameter of 3 cm or less. For this type of lung adenocarcinoma, it has been reported that the high ratio of the peripheral bronchioloalveolar carcinoma (BAC)/lepidic growth (LG) component was a favorable prognostic factor. On the other hand, the central solid components of lung adenocarcinoma with mixed subtypes have not been focused on in the past. In this study, we took note of the histological features in central solid components of lung adenocarcinoma with mixed subtypes and evaluated whether the morphological diversity of these tumors is associated with local invasiveness and prognostic implication.

Methods: A total of 103 surgically resected peripheral lung adenocarcinomas were reviewed. All the tumors were 3 cm or less in diameter and histologically diagnosed as lung adenocarcinoma with mixed subtypes, containing a BAC/LG component at the peripheral lesion of the tumor. The tumors were classified into two groups, according to the number of histological subtypes in the tumor, using the modified WHO classification (including the micropapillary subtype); group A (n = 76) has two or three histological subtypes, and group B (n = 27) has four or five subtypes in the tumor, respectively. Then, we evaluated the differences in clinicopathological factors and prognosis between these two groups.

Results: Group B was significantly associated with positive lymphatic and vascular invasion, lymph node metastasis, and advanced pathological stage, compared with group A. The 5-year survival rates of all patients were 91.4% for group A and 43.3% for group B, respectively, with a significant difference (p < 0.01). Multivariate analysis showed that the group classification by the number of histological subtypes was an independent prognostic factor in stage IA patients (p < 0.01). Conclusions: The morphological diversity of small lung adenocarcinoma with mixed subtypes is an independent prognostic factor and is associated with tumors' local invasiveness and patients' prognosis.

Keywords: Lung adenocarcinoma; Morphological diversity; Prognosis; Micropapillary subtype

1. Introduction

Lung cancer is currently the leading cause of cancer death in the world [1]. Among non-small-cell lung cancers (NSCLCs), adenocarcinoma is the most common in Japan and appears to be increasing in prevalence owing to the increased use of computed-tomography (CT) screening and the improvement in accuracy of high-resolution CT (HRCT) [2–4]. These image-diagnostic technologies have brought significant benefits in the form of early detection of the pure ground-glass opacity (GGO)-type lung adenocarcinomas. This type of tumor is a likely candidate for limited surgery in the future because it shows almost histologically noninvasive behavior, such as atypical adenomatous hyperplasia (AAH) or pure bronchioloalveolar carcinoma (BAC), and has a distinctly favorable prognosis [5,6]. On the other hand, it is also clear that there is a population of small lung adenocarcinoma, which has a relatively unfavorable prognosis. Although the classification of lung adenocarcinoma with a diameter of 2 cm or less proposed by Noguchi and colleagues [7] is quite adequate for prognostic prediction and is widely used, especially in Japan, it is necessary to find other various prognostic predictors of histological features to identify this unfavorable population and provide appropriate care, including postoperative adjuvant therapy.

Under the current World Health Organization (WHO) classification, lung adenocarcinoma is mainly classified into five subtypes. Among them, 'lung adenocarcinoma with mixed subtypes' is the most frequent type, accounting for approximately 80% of all lung adenocarcinomas [1]. This heterogeneous subtype is also frequent even in small lung adenocarcinomas with a diameter of 3 cm or less, although its
degree of morphological diversity varies. Almost all of these tumors contain a BAC/lepidic growth (LG) component in the peripheral area and a solid component with other subtypes or fibrotic foci in the central area of the tumor [8]. Regarding this type of tumor, there have been many studies demonstrating that the ratio of peripheral BAC/LG components was associated with prognostic implication [9–13], whereas there have been few reports about the relationship between histological features of the central solid component and clinicopathological factors or prognosis [14,15]. Therefore, we focused on the solid component of lung adenocarcinoma with mixed subtypes and hypothesized that its morphological diversity is directly associated with tumor invasiveness and patients’ prognosis.

Moreover, although the histological evaluation and positioning for the subtype ‘micropapillary’ in lung adenocarcinoma have been controversial, this component is often found in various proportions, especially in peripheral lesions of the tumor, which leads to unfavorable prognosis [16–19]. In this retrospective study, we classified all subjects into two groups using the modified WHO classification including the micropapillary subtype, and evaluated the relationship between the morphological diversity of small lung adenocarcinoma with mixed subtypes and clinicopathological factors or prognostic implication.

2. Materials and methods

2.1. Study population

We retrospectively reviewed 103 patients with lung adenocarcinoma, who underwent a curative operation in Tottori University Hospital and its affiliate hospital between January 1997 and December 2007. The study was approved by the Institutional Review Board and informed consent was obtained from all patients for specimen collection. Histological specimens had been reviewed by the lead author (T.H.) and the coauthors of qualified pathologists (K.S. and T.S.) at diagnosis. All tumors were 3 cm or less in diameter and were histologically diagnosed as lung adenocarcinoma with mixed subtypes, which contained a BAC/LG component at the peripheral lesion of the tumor. Routinely, neutral buffered formalin (pH 7.4)-fixed and paraffin-embedded tumor tissue samples were sectioned in 3-μm slices. Specimens were stained by hematoxylin–eosin (HE) and Elastica-van-Gieson (EvG) stain. The presence of BAC/LG, papillary, and micropapillary components was confirmed by a review of both HE- and EvG-stained sections. Mucin production was confirmed by a review of both HE- and EvG-stained sections. Mucin production was confirmed by a review of HE- and EvG-stained sections. Mucin production was confirmed by a review of HE- and EvG-stained sections. Mucin production was confirmed by a review of HE- and EvG-stained sections. Mucin production was confirmed by a review of HE- and EvG-stained sections. Mucin production was confirmed by a review of HE- and EvG-stained sections.

2.2. Statistical analysis

Data were analyzed using StatView version 5.0 (SAS Inc., Cary, NC, USA). To estimate the correlation between the two groups and clinicopathological data, Fisher’s exact probability test and Mann–Whitney U test were used. A p-value < 0.05 was considered to be significant in statistical analyses. The cancer-related survival rates were estimated with the Kaplan–Meier method, and statistical analyses were carried out using the log-rank test. Univariate and multivariate Cox regression analyses were used to evaluate the contribution of various factors to the cancer-related survival of 78 stage IA patients. Variables for which p < 0.05 in univariate analysis were inserted into the multivariate analysis.

3. Results

3.1. Group classification and subtypes of the tumors in each group

According to this method of group classification, all 103 tumors were divided into 76 (74%) in group A and 27 (26%) in group B. The subtypes, which composed of the tumors in each group and the number of subtypes in the tumor, are listed in Table 1. All tumors showed a BAC/LG histological subtype. Acinar, solid with mucin, and micropapillary subtypes were more frequent in group B with significant differences (p < 0.01). Figs. 1 and 2 show the representative cases of groups A and B, respectively. There is a BAC/LG component in the peripheral lesion, and papillary (Fig. 1(a)) and acinar...
(Fig. 1(b)) components in the central lesion in Fig. 1. On the other hand, there is a BAC/LG component and a micropapillary component (Fig. 2(a)) in the peripheral lesion, and papillary (Fig. 2(b)), acinar (Fig. 2(c)), and solid with mucin components (Fig. 2(d)) in the central lesion as shown in Fig. 2.

### 3.2. Association between groups of histological subtypes and clinicopathological factors

Table 2 shows the association between the group classification and clinicopathological factors. There were significant differences between groups A and B with regard to gender ($p = 0.02$), smoking status ($p = 0.02$), lymphatic and vascular invasion ($p < 0.01$ and $p = 0.01$, respectively), lymph node metastasis ($p < 0.01$), and pathological stage ($p < 0.01$), whereas no significant differences in age, tumor size, and pleural involvement were observed.

### 3.3. Prognosis and univariate and multivariate analysis of prognostic factors in stage IA patients with small lung adenocarcinoma with mixed subtype

For all patients, the cancer-related 5-year survival rates were 91.4% for group A and 53.2% for group B, respectively, with a significant difference between these two groups ($p < 0.01$; Fig. 3). For the 78 stage IA patients, the cancer-related 5-year survival rates were 95.6% for group A and 58.3% for group B, respectively, with a statistically significant difference between groups ($p < 0.01$; Fig. 4). Univariate analysis revealed that vascular invasion and group classification were significant prognostic factors for survival of the 78 stage IA patients. In multivariate analysis, group classification was shown to be a significant prognostic factor ($p < 0.01$; hazard ratio (HR) = 22.8), as significant as vascular invasion ($p = 0.01$; HR = 12.4) (Table 3).

### 4. Discussion

In this retrospective study, we clearly demonstrated that the morphological diversity of small lung adenocarcinoma with mixed subtypes (3 cm or less in size) was associated with lymphatic and vascular invasiveness, lymph node metastasis, pleural involvement, and advanced pathological stage. Furthermore, the morphological diversity of small lung adenocarcinoma with mixed subtypes was a significant prognostic factor for survival.

Recent investigations into the pathogenesis of lung adenocarcinoma have revealed that some genetic abnormalities such as epidermal growth factor receptor (EGFR) mutation and EGFR amplification cause sequential morphological changes that lead AAH to progress to localized BAC without central collapse or fibrotic lesions, and then to BAC.
with central fibrotic foci or an invasive component [21—25]. Although these investigations have clarified the molecular mechanism of the development of invasiveness in early and noninvasive lung adenocarcinoma to some extent, the development of the morphological diversity in the invasive component of lung adenocarcinoma, which is expected to develop sequentially thereafter, has not been elucidated yet. It is expected that these invasive components are formed by more complicated molecular mechanisms. To give a more precise histological aspect to the research in this field, we think that a proposal of more detailed histological classifications of lung adenocarcinoma with invasive components is expected. From this point of view, it seems that the classification by the number of subtypes is relatively easy to understand and could be a useful parameter for clinical practice.

As expected, there were significant differences between groups A and B with regard to some clinicopathological factors such as lymphatic and vascular invasion, lymph node metastasis, and pathological stage. In addition, the cancer-related survival rate was more favorable in group A than in group B with a significant difference, and multivariate analysis indicated that group classification was an independent prognostic factor in the case of stage IA patients. Most tumors in group B have more diverse subtypes, especially in the central solid component, than those of group A. As for the association between the solid component (non-BAC/LG component) and the invasiveness or the prognosis of lung adenocarcinoma, Suzuki and colleagues [14] revealed that the size of central fibrosis was associated with vascular invasion and lymph node metastasis, and was a significant prognostic factor in peripheral lung adenocarcinoma. In addition, Sakao and colleagues [15] suggested that not only the tumor size but also the tumor subtype and the size of the non-BAC/LG component should be considered to evaluate the impact of a new treatment strategy such as limited surgery or adjuvant chemotherapy. These previous reports and our results implied the importance of focusing on the solid component (non-BAC/LG component) of lung adenocarcinoma with mixed subtypes as well as the peripheral BAC/LG component.

Among the subjects of this present study, the micropapillary subtype was found at the peripheral lesion more frequently in group B than in group A with a significant difference. The micropapillary subtype has not been identified as an independent histological subtype yet, being described as a supplementary subtype in the current WHO criteria of lung adenocarcinoma [1]. The detailed pathogenesis of this subtype has yet to be elucidated fully in spite of...
many studies on the clinicopathological significance of the micropapillary subtype. However, it is an obvious fact that small lung adenocarcinomas often contain this aggressive subtype, which is an unfavorable prognostic factor [18,19]. Considering these important features of the micropapillary subtype, it might be appropriate that this subtype will be incorporated into the next criteria and become established as the independent subtype of lung adenocarcinoma.

The preoperative diagnostic imaging of lung adenocarcinoma generally plays a crucial role because it is difficult to make a definite diagnosis for small peripheral lung adenocarcinoma histologically. Almost all lung adenocarcinomas with mixed subtypes show mixed GGO lesions, which consist of a GGO component in the peripheral lesion and a solid component in the central lesion of the tumor on HRCT [5]. Suzuki and colleagues [14] emphasized that it is possible to evaluate preoperatively the central fibrosis as a focal area of consolidation; however, it is very difficult to predict the diversity of the invasive component or presence of a micropapillary subtype even on the current HRCT. Concerning these points, it seems that the limited surgery (segmentectomy or wedge resection) for early lung adenocarcinoma should, for now, be performed only in cases that have a small, solitary, and pure GGO or mixed GGO with minimal solid component on HRCT. It is hoped that the diagnostic imaging technology for small lung cancer will evolve to become more sophisticated, along with the surgical techniques.

There are some limitations to this study. First, the retrospective nature of this study might have introduced biased information. It was a fact that there were some differences of patients’ background including gender, smoking status, and operative procedures between groups. Second, the sample size was small and insufficient to reach a definitive conclusion. Third, there were some histological diagnostic biases because we evaluated the tissue samples pathologically only for the maximal cut surface, not for all cut surfaces. In addition, there was no definite line of demarcation among subtypes in the tumor, and not all pathologists in this study had the diagnostic consensus for all cases. Further detailed investigation including molecular biological analysis will be necessary to elucidate the mechanisms of the morphological diversity of lung adenocarcinoma.

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References


Table 3. Univariate and multivariate analysis of prognostic factors in stage IA patients of lung adenocarcinoma with mixed subtypes.

<table>
<thead>
<tr>
<th>Variables</th>
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Ref: reference; CI: confidence interval.