Lung Adenocarcinoma With Bronchioloalveolar Carcinoma Component Is Frequently Associated With Foci of High-Grade Atypical Adenomatous Hyperplasia

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Key Words: Atypical adenomatous hyperplasia; AAH; Bronchioloalveolar carcinoma

Abstract

We assessed the occurrence of atypical adenomatous hyperplasia (AAH) in whole lung lobes with primary cancer lesions. Following surgical resection, tissue specimens were sliced to a thickness of 4 mm (3,641 specimens from 61 cases; mean = 59.7 specimens per case). A total of 119 AAH foci were found and an association was evident in 25 (57%) of 44 adenocarcinomas, 3 (30%) of 10 squamous cell carcinomas, and 2 (29%) of 7 other lung cancers. Histologic evaluation showed that 108 AAH foci were categorized as low-grade and the other 11 as high-grade AAH. These 11 foci of high-grade AAH were present in 7 patients with adenocarcinoma, and in 1 patient there was a synchronous double primary lung adenocarcinoma. High-grade AAH was closely associated with bronchioloalveolar carcinoma (BAC) type adenocarcinoma, and low-grade AAH with non-BAC adenocarcinoma. The mean ± SD Ki-67 labeling index in high-grade AAH (3.5% ± 2.9%) was significantly higher than for the low-grade index (1.4% ± 1.6%). We propose that foci of high- but not low-grade AAH may be potential precursor lesions of lung adenocarcinoma, especially with the BAC component.

Generally, atypical adenomatous hyperplasia (AAH) has been detected by chance during histopathologic studies on surgically resected lung specimens or for autopsy. AAH is a solitary alveolar lesion with proliferation of type II alveolar pneumocyte-like and/or Clara cell–like cells with a varied cellular atypia. Miller et al1 suggested that AAH might be an adenomatous lesion as there was an analogy to colonic tumor, and subsequent studies supported this hypothesis.2-11 AAH may be a precursor lesion of peripheral lung adenocarcinoma, especially of bronchioloalveolar carcinoma (BAC), based on morphologic2 and morphometric studies,3,10 flow cytometry,11 immunohistochemical assessment of abnormal oncogene and tumor suppressor gene expression,4,8,9 and K-ras gene mutation5,6 in cases of resected lungs in patients with adenocarcinoma. Therefore, in the current lung tumor classification of the World Health Organization, AAH is now categorized as a precursor lesion to adenocarcinoma.12

It is difficult to accurately identify AAH macroscopically as AAH foci are lesions usually smaller than 5 mm in diameter.2 The frequency of AAH found in lungs with adenocarcinoma has been reported to range from 9.3% to 30%13-18; however, these studies were done using more than 5 mm of the sliced lung, a portion near the main cancer, or alveolar lesions identified macroscopically. For a more precise examination to characterize AAH, using thinner and whole resected lung specimens seems preferable. Since a 5-mm diameter has been considered one of the critical points to differentiate AAH and adenocarcinoma,10,13 we chose to examine 4-mm intervals of thickness for each surgically resected lung specimen. We then compared the incidence of AAH and the clinicopathologic background including age, sex, habits, and prognosis to clarify the significance of AAH as a precursor of lung
adenocarcinoma. Our findings indicate that AAH is likely to be more frequent than noted in previous studies. We also suggest that high-grade AAH may be a potential precursor lesion of lung adenocarcinoma, especially with the BAC component.

Materials and Methods

Cases and Tissue Preparation

The 61 cases we examined consisted of 44 adenocarcinomas, 10 squamous cell carcinomas, 2 small cell carcinomas, 2 large cell carcinomas, 1 adenosquamous carcinoma, and 2 mucoepidermoid carcinomas. These Japanese patients underwent surgery at Kyushu University Hospital, Fukuoka, Japan, between 1997 and 1998. The 61 surgically resected lung specimens were obtained by lobectomy in 55 cases, pneumonectomy in 4 cases, and segmentectomy in 2 cases. The patients were not prescribed chemotherapy or irradiation before surgery. The mean ± SD age was 67.9 ± 8.6 years, and the male/female ratio was approximately 1.3. According to the TNM staging system of the International Union Against Cancer,19 36 patients were in pathologic stage I, 7 in stage II, and 18 in stage III. The resected specimens were fixed by the intrabronchial instillation of 10% buffered formalin to avoid alveolar collapse and incompleteness of fixation; then they were inflated using the same formalin. After fixation for a few days, the whole resected lungs were sliced at 4-mm-thick intervals. Lung blocks were obtained from alternate sliced specimens, and the remainder of the specimens were preserved for use in other examinations. Of the 3,641 blocks, the mean ± SD value was 59.7 ± 25.7 specimens per case. The 4-µm-thick sections obtained from the paraffin-embedded lung blocks were stained with H&E.

The histologic diagnosis of AAH was based on the following criteria:2,3,7,10,13,20: (1) localized and well-defined boundary; (2) consisted of atypical epithelial cells that were cuboidal to low columnar or peg-shaped, and either type II pneumocytes or Clara cells were proliferating along the slightly thickened alveolar wall with mild chronic inflammation but without scar formation; and (3) cell atypia of the epithelium in AAH was apparent, but nuclear size and atypia were less prominent than in cases of adenocarcinoma.

AAH was classified into 2 grades based on histologic atypia:2,21,22 Low-grade AAH consisted of a single layer of intermittent or rather uniform and continuous proliferation of mildly atypical cells lining the alveolar septa. High-grade AAH had more increased cellularity and cellular atypia than did low-grade AAH, such as enlarged and hyperchromatic nuclei and an increased nuclear/cytoplasmic ratio. A “heaping-up” formation of atypical cells and multinucleation were focal. Cellular polymorphism, cell density, and a nuclear area of high-grade AAH were less than in cases of adenocarcinoma.

The histopathologic classification of primary cancer was done based on the criteria of the World Health Organization but with some modifications in adenocarcinoma, as described,12,23 and was determined by 3 pathologists (Drs Koga, Matsuo, and Sueishi). The diagnosis of multiple primary lung cancer was based on documented criteria.24 Patients with a history of tobacco use were divided into smokers, including current smokers and those with a history of smoking, and nonsmokers with no history of tobacco use.

Immunohistochemical Analysis

To evaluate the growth potential of AAH, we used a mouse monoclonal anti–Ki-67 antibody (MIB-1, Immunotech, Marseille, France) on tissue sections obtained from alternate sliced specimens, and the remainder of the specimens were preserved for use in other examinations. Of the 3,641 blocks, the mean ± SD value was 59.7 ± 25.7 specimens per case. The 4-µm-thick sections obtained from the paraffin-embedded lung blocks were stained with H&E.

The Ki-67 labeling indices of AAH cells were estimated among all the AAH cells in each focus by counting the number of cells with a positive nucleus.

Statistical Analysis

All values are expressed as mean ± SD. To estimate the correlation between the histologic type and the frequency of AAH, the chi-square, the Fisher exact probability test, and the Mann-Whitney U test were used. All P values were based on 2-hypothesis testing, and statistical significance was assumed at a level of P less than .05. Survival curves were obtained using the Kaplan-Meier method, and the statistical significance of differences was calculated using the log-rank test.

Results

The AAH lesion was evident in 25 (57%) of 44 adenocarcinomas, 3 (30%) of 10 squamous cell carcinomas, 1 (33%) of 3 large cell carcinomas, and 1 adenosquamous carcinoma (Table 1). The AAH lesion was absent in 2 small cell carcinomas and 1 mucoepidermoid carcinoma. We found 96 AAH foci (85 in low-grade and 11 in high-grade AAH; Images 1A and 1B, respectively) in lung adenocarcinoma, 3 foci in squamous cell carcinoma, 2 foci in large cell carcinoma, and 18 foci in adenosquamous carcinoma (Table 1).
The number of AAH lesions per case with AAH was 3.8 ± 2.5 in adenocarcinoma, 1.0 ± 0.0 in squamous cell carcinoma, and 10.0 ± 11.3 in other lung cancers (Table 1). All 11 high-grade AAH foci were detected only in adenocarcinoma-bearing lungs. There was no statistical difference in the incidence of AAH between adenocarcinoma and squamous cell carcinoma or between adenocarcinoma and other histologic types. However, there was a statistically significant difference in the mean number of AAH lesions between adenocarcinoma and squamous cell carcinoma (P < .04, Table 1), but not between adenocarcinoma and other histologic subtypes, including large cell carcinoma and adenosquamous carcinoma (Table 1). The age of patients with AAH was 69 ± 7 years and without AAH was 65 ± 12 years. The male/female ratio was 17:13 in the group with AAH and 17:14 in patients without AAH. Of 30 patients with AAH, 20 were smokers, as were 24 of 31 without AAH (Table 2). There was no statistical difference in age, male/female ratio, or smoking history with regard to the presence or absence of AAH (Table 2). The mean diameters of low- and high-grade AAH were 1.71 ± 1.3 and 1.79 ± 1.3 mm, respectively (no statistical significance).

Six (86%) of 7 cases with high-grade AAH were associated with BAC type adenocarcinoma-bearing lungs, and conversely, 12 (67%) of 18 cases with low-grade AAH were associated with non-BAC type adenocarcinoma (P < .03, Table 3). The incidence of AAH-bearing lungs with pure BAC was significantly higher than that of BAC accompanied by invasive growth (P < .04; 7/8 [88%] and 5/13 [38%], respectively; data not shown). There was no statistical difference in incidence in comparison with BAC-type adenocarcinomas and non-BAC type adenocarcinomas (Table 3).
Multiple primary lung cancers were noted in 2 (3%) of 61 specimens. The histologic diagnosis was adenocarcinoma, one synchronous and the other metachronous. Synchronous lung adenocarcinomas were found in the right upper lobe as BAC with invasive growth and in the left upper lobe as a solid adenocarcinoma. Three low-grade AAH lesions and 2 high-grade AAH lesions were noted simultaneously in the right upper lobe. In the case of metachronous adenocarcinoma, both tumors observed at an interval of 9 years were BAC with invasion, and the AAH lesion was absent in the resected lung tissue.

The Ki-67 labeling index was 1.4% ± 1.6% in low-grade AAH and 3.5% ± 2.9% in high-grade AAH.

Discussion

We attempted to determine the precise incidence of AAH in surgically resected whole specimens of the lung, and we unexpectedly found that AAH was more frequent in cases of adenocarcinoma and other histologic subtypes than previously reported. We noted the following: (1) the presence of high-grade AAH only in lungs with lesions and 2 high-grade AAH lesions were noted simultaneously in the right upper lobe. In the case of metachronous adenocarcinoma, both tumors observed at an interval of 9 years were BAC with invasion, and the AAH lesion was absent in the resected lung tissue.

The Ki-67 labeling index was 1.4% ± 1.6% in low-grade AAH and 3.5% ± 2.9% in high-grade AAH.

As the degree of atypia advanced, the Ki-67 labeling index increased significantly ($P < .05$). In all 61 cases, a survival analysis revealed no statistical significance between patients with and without AAH ($P = .84$).

**Table 1**

<table>
<thead>
<tr>
<th>Histologic Type of Primary Site</th>
<th>No. of Cases</th>
<th>No. (%) of Cases With AAH</th>
<th>P†</th>
<th>No. of AAH Foci‡</th>
<th>Average No. of AAH Foci§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>44</td>
<td>25 (57)</td>
<td>—</td>
<td>85</td>
<td>3.8</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>10</td>
<td>3 (30)</td>
<td>.28</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Others⁵</td>
<td>7</td>
<td>2 (29)</td>
<td>.33</td>
<td>20</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>30 (49)</td>
<td></td>
<td>108</td>
<td>4.0</td>
</tr>
</tbody>
</table>

AAH, atypical adenomatous hyperplasia.

† Fisher exact probability test; adenocarcinoma vs squamous cell carcinoma and adenocarcinoma vs others.

§ Total number of AAH foci/number of total cases with AAH lesion.

‡ Vs adenocarcinoma: Mann-Whitney $U$ test.

|| Includes 3 large cell carcinomas, 1 adenosquamous carcinoma, 1 mucoepidermoid carcinoma, and 2 small cell carcinomas.

**Table 2**

<table>
<thead>
<tr>
<th>AAH Present</th>
<th>Absent</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of cases</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>No. (%) who smoke*</td>
<td>20 (45)</td>
<td>24 (55)</td>
</tr>
<tr>
<td>No</td>
<td>10 (59)</td>
<td>7 (41)</td>
</tr>
<tr>
<td>Mean ± SD age (y)</td>
<td>69 ± 7</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>17/13</td>
<td>17/14</td>
</tr>
</tbody>
</table>

AAH, atypical adenomatous hyperplasia.

* Percentages are based on total numbers for “Yes” and “No” rows.

† Chi-square test.

‡ Mann-Whitney $U$ test.

**Table 3**

<table>
<thead>
<tr>
<th>Histologic Subtype of Primary Site</th>
<th>No. of Cases</th>
<th>Low*</th>
<th>High*</th>
<th>P‡</th>
<th>Subtotal Absent</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC type§</td>
<td>21</td>
<td>6 (33)</td>
<td>6 (86)</td>
<td>&lt;.03</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Non-BAC type¹</td>
<td>23</td>
<td>12 (67)</td>
<td>1 (14)</td>
<td></td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>18</td>
<td>7</td>
<td></td>
<td>25</td>
<td>19</td>
</tr>
</tbody>
</table>

AAH, atypical adenomatous hyperplasia; BAC, bronchioloalveolar carcinoma.

† Low indicates cases with low-grade AAH; high, cases with high-grade AAH and with or without low-grade AAH. Data are given as number (percentage of column total).

‡ Fisher exact probability test for subtype of primary site and atypia grade of AAH.

§ Chi-square test for subtype of primary site and presence or absence of AAH.

¹ BAC type includes pure BAC and BAC with invasion.

² Non-BAC type consists of acinar, papillary, and solid adenocarcinomas.
adenocarcinoma but not in lungs with other histologic types, while low-grade AAH was ubiquitous; (2) high-grade AAH frequently was associated with BAC-type adenocarcinoma, with or without invasive growth; and (3) the Ki-67 labeling index, a cellular proliferative activity, was significantly higher in high- than in low-grade AAH, even when the mean diameter was similar. Based on these findings, we propose that (1) high-grade AAH should be distinguished from low-grade AAH, and (2) high-grade AAH is closely associated with the oncogenesis of lung adenocarcinoma, especially that with BAC. The biologic and clinicopathologic significance of low-grade AAH remains controversial.

Peripheral lung carcinomas are marked by a propensity to be multifocal. Reports from other workers stated that the frequency of synchronous multifocal carcinoma ranged from 0.2% to 2.0%. We also noted multiple cancers in 2 (3%) of 61 lung carcinoma specimens. Of the lung adenocarcinomas, BAC possibly arose from AAH in a manner of stepwise progression; thus, together with the tendency toward multifocality of cancer foci, there may be a correlation between the presence of BAC and the incidence of AAH in the same lung lobe. However, there is a paucity of information regarding the relationship between the histopathologic features of primary adenocarcinoma and AAH in each case. We found high-grade AAH to be frequent in lungs bearing BAC-type adenocarcinoma, but conversely, low-grade AAH was more frequent in lungs without BAC (P < .05). This observation supports the notion of a stepwise progression in that it implies that BAC originates from AAH, especially in cases of high-grade atypia.

The conflicting results, including the size of AAH foci, may relate to the methods used. Our data revealed that the mean size of low- and high-grade AAH was less than 2 mm, while other workers stated that the mean size was 3 to 5 mm. Therefore, the lesion size may not be a relevant marker for differentiating the grade of atypia in cases of AAH.

Studies have suggested that high-grade AAH is likely to be a premalignant lesion. In addition to morphologic characteristics such as lesion size, nuclear size, and AAH area, immunohistochemical expression of p53 protein and the Ki-67 labeling index tended to increase in a stepwise manner from low- to high-grade AAH. As indicated in previous reports, high-grade AAH might have a more precancerous phenotype, similar to the adenoma-carcinoma sequence hypothesis proposed in cases of colon cancer. Our ongoing studies will address the following: (1) genetic and epigenetic evidence as to whether high-grade AAH arises from low-grade AAH or from de novo tumorigenesis; (2) whether high-grade AAH can develop into BAC or other histologic types of adenocarcinomas; and (3) the clinicopathologic significance of low-grade AAH, namely, hyperplastic or neoplastic lesion leading to the occurrence of cancer.

Our findings indicate that high-grade AAH frequently is associated with lung adenocarcinoma, especially in cases of BAC-type adenocarcinoma; hence, high-grade AAH may be a significant precursor lesion of lung adenocarcinoma.

<table>
<thead>
<tr>
<th>Table 48</th>
<th>Comparison of Ki-67 Labeling Index of AAH Between Low- and High-Grade AAH Found in Lungs With Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypia Grade of AAH</td>
<td>No. of Cases</td>
</tr>
<tr>
<td>Low</td>
<td>85</td>
</tr>
<tr>
<td>High</td>
<td>11</td>
</tr>
</tbody>
</table>

AAH, atypical adenomatous hyperplasia. * Mann-Whitney U test. Low- vs high-grade AAH.

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References


