Ultrastructural Findings in Metastatic Bronchioloalveolar Carcinoma

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Abstract. This study was prompted by the recent revision of the definition of bronchioloalveolar carcinoma (BAC) that defines BAC, light microscopically, as a non-invasive carcinoma. Doubt has been raised whether BACs retain certain specific microscopic features after becoming invasive or metastatic. We studied 7 cases of metastatic, non-mucinous BAC by electron microscopy. Of these cases, 5 showed Clara cell granules and 1 revealed lamellar bodies. The remaining case did not show ultrastructural features of BAC. These findings suggest that most BACs retain some of their ultrastructural features after becoming metastatic neoplasms. (received 12 May 2003; accepted 15 May 2003)

Keywords: Metastatic bronchioloalveolar carcinoma, invasive bronchioloalveolar carcinoma, electron microscopy, Clara cell granules, lamellar inclusions

Introduction

Bronchioloalveolar carcinoma (BAC) is cytologically, histologically, ultrastructurally, and by molecular composition, a distinct pulmonary adenocarcinoma [1-7]. BAC was first described by Malassez in 1876 [8]. In 1960, Liebow [9] defined BAC as a pulmonary carcinoma that consists of cuboidal or columnar epithelial cells growing on preexisting alveolar septae [9]. For nearly 4 decades this definition received worldwide acceptance by pathologists and oncologists. In addition, it has been recognized during the past decade, more than ever before, that BAC is an unique neoplasm, with frequent multifocal and multicentric presentations and a disproportionate occurrence in young patients, particularly women and nonsmokers [10-12].

In 1999, a new World Health Organization (WHO) definition, based on light microscopic features, was introduced whereby BAC with invasion cannot be classified as BAC [13]. In 2002, we reported ultrastructural evidence that BACs retain their ultrastructural phenotypes after becoming invasive (solid) carcinomas [7].

As a further step, the present study was undertaken to investigate whether the ultrastructural features of BAC are retained in extrapulmonary metastases.

Materials and Methods

Seven consecutive cases of metastatic, non-mucinous BAC were selected for ultrastructural examination. After histopathologic review of all available microscopic slides, the paraffin embedded tissue blocks containing the metastatic carcinoma were identified. In 6 cases, the region of the block containing the carcinoma was excised, rehydrated, and prepared for electron microscopic study. In 1 case, simultaneously with the light microscopic diagnosis of metastatic BAC, the fresh tumor sample was fixed in 0.1 M cacodylate buffered glutaraldehyde, post-fixed in osmium-tetroxide, and prepared for ultrastructural study by standard methods.

In all cases, sections (1 µm) of toluidine blue-stained tissue were examined by light microscopy to identify the tumor cell populations. Appropriate areas were selected and sectioned for examination with a transmission electron microscope (JEOL JEM 100 CXII).
Results

Of the 7 patients, 5 were women and 2 were nonsmokers. Four patients were <50 yr old at time of diagnosis. (Table 1). The primary tumors ranged from 1 to 5 cm and were commonly situated in the upper lobes of the lungs. In 1 case, the primary neoplasm was multifocal; in another case, there was multicentric presentation in 2 lobes of both lungs. Follow-up information and pertinent clinicopathological findings are listed in Table 1.

All 7 primary BACs showed 2 distinct growth patterns by light microscopy. The dominant pattern was that of a solid, poorly-differentiated, and invasive adenocarcinoma without recognizable light microscopic histologic features of BAC. However, there were substantial and readily visible areas of well-differentiated, invasive, and non-invasive BAC with typical alveolar features, as well as atypical bronchioloalveolar hyperplasia.

At sites of metastases, the neoplasms were all composed of solid nests of adenocarcinoma. In 5 cases, ultrastructural examination of the neoplastic cells showed intracytoplasmic Clara cell granules (Figs. 1 and 2). In 1 case, the neoplastic cells showed intracytoplasmic lamellar inclusions (Fig. 3). In the remaining case, the ultrastructural features of BAC were not detected.

Discussion

In this study, consistent with our prior study [7] and as reported by others [4,5,10-12], the patients with BAC were mostly female and were generally younger than most patients with lung cancer.

The commonly observed multifocal and multicentric presentation of BACs [11-12] was noted in 2 of our patients. Peripheral location of the tumors, modest size, and predilection for the upper lobes are well known features of BAC. All in all, the

<table>
<thead>
<tr>
<th>Case #, age, gender</th>
<th>Smoker/Non-smoker</th>
<th>Site, size (cm), specimen</th>
<th>Light microscopic findings</th>
<th>Metastases</th>
<th>Electron microscopic findings</th>
<th>Follow-up information</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 44 yr, M smoker</td>
<td>RLL,* 3x3, biopsy</td>
<td>pleural invasion</td>
<td>mediastinal &amp; right scalene lymph nodes</td>
<td>lamellar inclusions</td>
<td>died in 15 mo</td>
<td></td>
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<tr>
<td>(2) 38 yr, F smoker</td>
<td>LUL*, 4x3, wedge biopsy</td>
<td>pleural invasion</td>
<td>brain</td>
<td>none</td>
<td>no follow-up</td>
<td></td>
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<tr>
<td>(3) 49 yr, F non-smoker</td>
<td>RUL, 5x4, lobectomy</td>
<td>pleural invasion</td>
<td>peribronchial lymph nodes &amp; brain</td>
<td>Clara cell granules</td>
<td>Recurrence in brain; died in 6 mo</td>
<td></td>
</tr>
<tr>
<td>(4) 69 yr, M former smoker</td>
<td>LUL, 4x4, lobectomy</td>
<td>invasive</td>
<td>1 mediastinal lymph node</td>
<td>Clara cell granules</td>
<td>NED$ 9 mo</td>
<td></td>
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<tr>
<td>(5) 47 yr, F smoker</td>
<td>LUL, 2x1, lobectomy</td>
<td>invasive</td>
<td>2 hilar lymph nodes</td>
<td>Clara cell granules</td>
<td>NED 6 mo</td>
<td></td>
</tr>
<tr>
<td>(6) 66 yr, F non-smoker</td>
<td>RLL, multifocal, wedge biopsy</td>
<td>invasive</td>
<td>2 mediastinal lymph nodes</td>
<td>Clara cell granules</td>
<td>NED 11 mo</td>
<td></td>
</tr>
<tr>
<td>(7) 73 yr, F smoker</td>
<td>LLL, 3x2, lobectomy, RUL, 1x0.5 wedge biopsy</td>
<td>invasive pattern</td>
<td>2 hilar lymph nodes</td>
<td>Clara cell granules</td>
<td>no follow-up</td>
<td></td>
</tr>
</tbody>
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* RLL, right lower lobe; LUL, left upper lobe; \$ NED, no evident disease.
clinical profile and radiologic presentation of the cases in this study, without pathologic examination, would favor BAC.

According to the WHO’s recently proposed light microscopic classification of lung tumors [13], none of the tumors in this study would be classified as BAC, because of the presence of microscopic invasion. We showed earlier [7] that neoplastic cells of BAC retain their ultrastructural BAC phenotype after becoming invasive. The results of this study add to that observation by showing that BACs retain some ultrastructural features in neoplastic cells even in metastases. Our findings support the observations made >20 years ago [14,15] that neoplastic cells of metastatic BACs retain specific cytologic features.

Generous sampling and diligent ultrastructural examination are prerequisite for finding lamellar membranous inclusion bodies, indicative of type II pneumocytes differentiation, and Clara cell granules. This is particularly true as the tumor cells spread from the primary site to adjacent and distant tissues by metastasis. The matter is complicated by the fact that lamelliform inclusions are unlikely to be demonstrable in paraffin-retrieved material because the organic solvents extract lipids during processing of tissues.

In summary, we have found that metastatic malignant cells of invasive BACs, although not
recognizable as cells derived from BAC by light microscopy, retain some of the ultrastructural features of well-differentiated non-invasive BAC. Therefore, the idea [13] that loss of the characteristic light microscopic features of BAC when they become invasive (poorly differentiated or “dedifferentiated”) indicates that the neoplasm is not BAC should be reconsidered.

References


Fig. 2 (Case 7). Electron photomicrograph from a bronchioloalveolar carcinoma cell in a hilar lymph node, from material prepared from paraffin retrieved material as in Fig. 1. The tumor cell cytoplasm contains several Clara cell granules (G). Nucleus is at N; nucleolus is at NU. (magnification x 17,000).
Fig. 3 (Case 1). Electron photomicrograph of a group of malignant tumor cells, showing Type II pneumocyte-like features, from a bronchioloalveolar carcinoma metastasized to a scalene lymph node that was well preserved by primary fixation in glutaraldehyde. Note the numerous swirled membranous, lamellar inclusions (arrows) in the cytoplasm of the tumor cells and the cell surface lined by short, widely spaced microvilli (MV). A large prominent ribbon-like nucleolus (NU) is found in some profiles of tumor cell nuclei (N). (magnification x 4,500; inset x 15,000)

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