Case Report

A case of microscopic, multiple sclerosing pneumocytoma

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Sclerosing pneumocytoma is a rare tumor of the lung, commonly affecting middle-aged women, and is mostly isolated. Although this tumor is thought to be derived from primitive respiratory epithelial cells, the characteristics of the precursor cells are still unknown. A 19-year-old woman presented with multiple nodules in the right lung. Partial resection of the right middle lobe was performed, and seven sclerosing pneumocytomas, including four that were microscopic, were detected. The latter showed a simple papillary pattern, and three of them consisted of only round cell-like cells (single population). Interestingly, these round cell-like cells were positive for both p63 and TTF-1, but totally negative for SP-A. On the other hand, the tumor cells of the other four sclerosing pneumocytomas showing a papillary pattern with a dual population, were diffusely positive for TTF-1 and focally positive for SP-A (only in surface cells), but negative or very focally positive for p63. It has been reported that p63-positive stem cell-like cells are present in the distal airway and have potential to differentiate into type II pneumocytes. The immunohistochemical features of these multiple microscopic lesions suggest that the p63-TTF-1 double-positive cells are candidate precursor cells of sclerosing pneumocytoma.

Key words: distal airway stem cell, microscopic, p63, sclerosing pneumocytoma, SP-A, terminal respiratory unit, TTF-1

Sclerosing pneumocytoma is a rare tumor of pneumocytic origin with a dual population of surface cells resembling type II pneumocytes and round cells, with slightly different histogenetic profiles.1–5 Combinations of various histological features (papillary, solid, sclerotic and hemorrhagic patterns) are also characteristic. Sclerosing pneumocytoma usually appears as an isolated solid nodule and never metastasizes. Sometimes, however, multiple forms of sclerosing pneumocytoma may be encountered.5–9 Noguchi et al. have reported multiple minute and microscopic sclerosing pneumocytomas that tended to be composed of only epithelial cells arranged in papillary pattern, and suggested that the papillary lesions might represent sclerosing pneumocytoma at a very early stage.6 Although it has also been suggested that sclerosing pneumocytoma might be derived from primitive respiratory epithelial cells, the characteristics of its precursor cells and details of its molecular tumorigenesis are still unknown.10–18

We have experienced a unique case of multiple sclerosing pneumocytoma in a 19-year-old woman. Seven sclerosing pneumocytomas, including four that were microscopic, were present. All of the microscopic tumors exhibited a solely papillary pattern. One of them had a dual population of surface cells and round cells, but the other three were composed solely of round cell-like cells that were positive for both TTF-1 and p63. We hypothesized that the tumor cells showing double positivity for TTF-1 and p63 were the precursor cells of sclerosing pneumocytoma.

CLINICAL SUMMARY

An asymptomatic 19-year-old Japanese woman with unremarkable family and medical histories presented for a medical checkup in 2014, and chest X-ray demonstrated multiple coin lesions in her right lung. A computed tomography scan of the chest demonstrated dozens of small nodules up to 18 mm in diameter in the right upper and middle lobes. Clinically, granulomatous disease was suspected. To confirm the diagnosis, partial resection of the right middle lobe was performed by video-assisted thoracic surgery.

PATHOLOGICAL FINDINGS

The resected materials were fixed with 10% buffered formalin for 2 days and embedded in paraffin. For immunostaining,
These primary antibodies were used: TTF-1 (mouse monoclonal, 8G7G3/1, diluted; Dako, Glostrup, Denmark), SP-A (mouse monoclonal, PE10, 1:500; Dako) and p63 (mouse monoclonal, Dak-p63, diluted; Dako). The immunohistochemical processes were performed with a histostainer (Nichirei Biosciences, Tokyo, Japan). We evaluated the degree of positivity as follows: negative (−, 0%); very focally positive (+, <25% in hot spots); focally positive (+++, <50% in hot spots); diffusely positive (++++, >50% and a uniform pattern). For immunofluorescence, these primary antibodies were used: TTF-1 (rabbit monoclonal, ab76013, 1:250; Abcam, Cambridge, UK) and p63 (mouse monoclonal, M731729, 1:10; Dako). The secondary antibodies used were Thermo Fisher Scientific Goat anti-rabbit IgG (Alexa Fluor, 594, red, 1:50) and Thermo Fisher Scientific Mouse anti-mouse IgG (Alexa Fluor, 488, green, 1:50; Thermo Fisher Scientific, Waltham, MD, USA). Visualization was performed using a KEYENCE HS ALL-in-one microscope (BZ-900, KEYENCE, Osaka, Japan) with BZ-II observation and analysis application. The clones of p63 antibodies (both for immunostaining and immunofluorescence) are Dak-63, which recognize p63 isoforms both Tap63 and Np63(p40).

Macroscopically, there were three well-circumscribed tumors (tumor no. 1-A, 1-B, and 1-C, Table 1), the largest measuring 16 × 16 × 15 mm (Table 1). The surface of the largest tumor was gray-white and medullary (Fig. 1a). Microscopically, the largest tumor showed three histological patterns: papillary, sclerotic and solid (Fig. 1b). The papillary area contained a dual population of surface cells, and single population (no.1-D, 1-F, and 1-G, Table 1) and they were distributed at random. All of the microscopic tumors showed only a papillary pattern histologically (Fig. 2a,c). Although one microscopic tumor No.1-E was composed of surface cells and round cells (Fig. 2b), the other three (tumors no.1-D, 1-F and 1-G, Table 1) consisted of only round cell-like cells (single population) (Fig. 2d). Alveolar elastic fibers totally disappeared in the three macroscopic pneumocytomas, whereas microscopic pneumocytomas maintained some amount of alveolar elastic fibers inside. It might suggest that these lesions were very early stage of macroscopic pneumocytomas.

The microscopic tumor showing a dual population (no.1-E) was positive for TTF-1 (diffuse), SP-A (only surface cells), and negative for p63. The others, consisting of only round cell-like cells, were diffusely positive for both TTF-1 and p63 (Fig. 3a–c), but negative for SP-A. All macroscopic and microscopic lesions (both dual population and single population) were negative for CK5/6 (data not shown).

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**Table 1** Histology and immunohistochemical profile about eight cases of sclerosing pneumocytomas (15 tumors). This case is No. 1 (including seven tumors)

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor</th>
<th>Size (mm)</th>
<th>Histology</th>
<th>TTF-1</th>
<th>SP-A</th>
<th>p63</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>This case</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>19</td>
<td>1-A</td>
<td>16 × 16 × 15</td>
<td>SO &gt; PA &gt; SC</td>
<td>(+++)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>1-B</td>
<td></td>
<td></td>
<td></td>
<td>5 × 5</td>
<td>SO &gt; PA &gt; HE</td>
<td>(+++)</td>
<td>(+)</td>
<td>(–)</td>
</tr>
<tr>
<td>1-C</td>
<td></td>
<td></td>
<td></td>
<td>5 × 5</td>
<td>SO &gt; PA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>1-D</td>
<td></td>
<td></td>
<td></td>
<td>1.5 × 1.2</td>
<td>PA</td>
<td>(+)</td>
<td>(–)</td>
<td>(++)</td>
</tr>
<tr>
<td>1-E</td>
<td></td>
<td></td>
<td></td>
<td>0.4 × 0.4</td>
<td>PA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>1-F</td>
<td></td>
<td></td>
<td></td>
<td>0.4 × 0.4</td>
<td>PA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>1-G</td>
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<td></td>
<td></td>
<td>0.3 × 0.3</td>
<td>PA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td><strong>Others cases</strong></td>
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</tr>
<tr>
<td>2</td>
<td>F</td>
<td>73</td>
<td>3-A</td>
<td>20 × 15</td>
<td>SO &gt; SC &gt; PA</td>
<td>(+++)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>3</td>
<td>F</td>
<td>67</td>
<td>3-B</td>
<td>3 × 1.3</td>
<td>SO &gt; SC</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>63</td>
<td>4</td>
<td>15 × 13</td>
<td>SC &gt; SO &gt; PA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>52</td>
<td>5</td>
<td>13 × 11</td>
<td>HE &gt; SC &gt; SO</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>60</td>
<td>6</td>
<td>16 × 13</td>
<td>SC &gt; HE &gt; SO</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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<td>F</td>
<td>67</td>
<td>7</td>
<td>12 × 10</td>
<td>SC &gt; HE &gt; SO</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>50</td>
<td>8</td>
<td>5 × 5</td>
<td>PA &gt; SC</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

* papillary pattern consists of only round- cell-like cells (without dual population).

(–), 0%; (+), <25%; (++) , 25–50%; (+++), >50%.

SO, solid pattern; PA, papillary pattern; SC, sclerotic pattern; HE, hemorrhagic pattern.

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Figure 1  (a–d) Sclerosing pneumocytoma in a 19-year-old woman. (a) Macroscopically, the surface of the largest sclerosing pneumocytoma is gray-white and medullary. (b) Histologically, the largest sclerosing pneumocytoma shows papillary (**), solid (**) and sclerotic patterns. (c) In the papillary area, a dual cell population is evident (→: surface cell, ⇒: round cell). (d–f) Immunohistochemical staining for TTF-1(d), SP-A (e) and p63 (f).

Figure 2  (a–d) Microscopic lesions. (a, b) Tumor No.1-E shows a dual cell population. (c, d) Tumor No.1-D consists of only round cell-like cells and show a single cell population.
To determine whether the round cells in the microscopic lesions without a dual population were double-positive for both p63 and TTF-1, we performed an immunofluorescence study, and found that the tumor cells were double-positive for p63 (green) and TTF-1 (red) (Fig. 3d–f). To compare the immunohistochemical profiles of sclerosing pneumocytomas, we reviewed a total of 15 tumors from eight cases (including the present one) diagnosed as sclerosing pneumocytoma at Tsukuba University Hospital and Mito Kyodo Hospital between 1996 and 2016, and examined their positivity for TTF-1, SP-A and p63. The results were shown in Table 1. All of the sclerosing pneumocytomas were diffusely positive for TTF-1. Among the macroscopic tumors, positivity for SP-A and p63 varied, but p63-positive cells were detected only very focally (0–25%, hot spot). For reference, we also examined the expression of TTF-1, SP-A, and p63 of normal terminal bronchial tissue. Most of the surface epithelial cells were positive for p63 and several p63-positive surface epithelial cells were also positive for TTF-1 (double-positive) (Fig. 3g,h). On the other hand, CK5/6-positive surface epithelial cells were not detected (data not shown).

**DISCUSSION**

We experienced a 19-year-old female patient with seven sclerosing pneumocytomas, including four microscopic tumors. This case had two unique features: the patient was younger than usual and had several microscopic tumors. Several cases of multiple sclerosing pneumocytoma of the lung have been reported,6–9 and some of them also included microscopic lesions.6 Noguchi et al. reported multiple sclerosing pneumocytomas containing innumerable lesions varying in size from microscopic to 3.7 cm in diameter.6 They examined 24 tumors microscopically and found that small lesions, particularly minute ones, tended to be composed of only epithelial cells arranged in papillary patterns and round cells in solid areas. They concluded that both of these cell types were the main components of sclerosing pneumocytoma. In our case, we also found four microscopic tumors showing just a papillary pattern, and EVG staining showed some amount of elastic fibers remaining (tumors no.1-D, 1-E, 1-F and 1-G, Table 1). These findings suggested that the papillary lesions represented the very early stage of sclerosing pneumocytoma. In this...
case, one of the microscopic tumors, no.1-E, showing a papillary pattern was composed of surface cells and round cells (i.e., dual population), and the other three, no.1-D, 1-F and 1-G, were composed of round cell-like cells (single population).

Recently, Kumar et al. reported that p63- and CK5/6-positive cells were present in the distal airway of the lung in mouse models, and suggested that these cells are of pneumocyte lineage.19 Zuo et al. showed that p63- and CK5/6- positive distal airway stem cells proliferate and differentiate into type I and type II pneumocytes in response to influenza-induced lung damage in mouse models.20 These reports suggested that p63-positive distal airway stem cells have the potential to differentiate into type II pneumocytes.

In our reference sample, a proportion of epithelial cells in the terminal respiratory unit (TRU) were also positive for both TTF-1 and p63 (Fig. 3g,h), but negative for CK5/6 (data not shown). It might indicate that the mouse stem cells were positive for CK5/6, but human stem cells were not. In other words, in humans, the cells positive for both TTF-1 and p63 could be regarded as stem cells differentiating pneumocytes.

Recently, Zhang J et al. reported that over 75% of TTF-1 positive cuboidal cells in sclerosing pneumocytomas were positive for p63 (16/18 cases)16. And Wu J et al. reported that p40 was positive for TTF-1 positive cuboidal cells.17 They suggested sclerosing pneumocytoma was originated from pluripotent original respiratory epithelial cells. In our case, the microscopic sclerosing pneumocytomas consisted of p63-TTF-1 double-positive cells, whereas macroscopic lesions had less proportion of p63-TTF-1 double-positive cells. This result corresponded to these previous two reports.

In order to examine the expression profiles of pneumocytes stem cell markers and differentiation markers in sclerosing pneumocytomas, especially microscopic lesions, we performed immunostaining for p63, TTF-1 and SP-A (Table 1). In our cases, three microscopic tumors (tumor 1-D, 1-F and 1-G) showed diffuse (over 75%) positivity of p63 and TTF-1. All of them showed just papillary pattern with single population, composed of round cell-like cells, not cuboidal cells, and totally negative for SP-A. On the other hand, the tumor cells in three macroscopic tumors and one microscopic tumors with dual population were diffusely positive for TTF-1, focally positive for SP-A and p63. p63 positive cells were less numerous than those positive for TTF-1 and two of the four tumors were totally negative for p63. Our findings showed the difference of p63 positivity in sclerosing pneumocytomas is related to the size, histological feature and expression of pneumocyte differentiation markers. These results indicated that the round cell-like cells forming the microscopic tumors had the characteristics of differentiating pneumocytes but might be still immature in comparison to those forming the macroscopic tumors.

Recently, Jung SH et al. examined 44 cases of sclerosing pneumocytoma and found non-silent and recurrent mutations of AKT1 (19 of 44, 43.2%), CTNNB1 (b-catenin) (2 of 44) and ARID1B (2 of 44).18 All of the AKT1 mutations were localized to the pleckstrin homology domain, which were thought to be associated with tumor proliferation. The biological relationship between AKT1 mutation and sclerosing pneumocytoma is still unknown, but the AKT1 activation might be associated with the oncogenesis. Although there are many technical problems to use formalin fixed and microdissected materials for genetic analysis, this case may be suitable for the investigation of the oncogenesis and multiplicity of multiple sclerosing pneumocytomas.

In the present case, we found that multiple sclerosing pneumocytomas including some microscopic tumors, and we revealed the biological characteristics of the tumor cells using immunohistochemistry. The tumor cells of microscopic, single-population tumors showed double positivity

**Figure 4** Scheme suggesting the role of p63-TTF-1 double-positive cells. Hypothesis to explain the progression of sclerosing pneumocytoma.

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for p63 and TTF-1, these microscopic tumors seemed to be precursor lesions of sclerosing pneumocytoma, differentiating and acquiring the characteristics of pneumocytes in the course of tumor progression (Fig. 4).

DISCLOSURE STATEMENT

None declared.

REFERENCES


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