We report the morphological characteristics of 30 cases of sclerosing hemangioma (SH) of the lung and explore the histological origin of the major cells in these tumors. In addition to routine light and electron microscopy, immunohistochemistry was performed by using 12 monoclonal primary and 5 polyclonal primary antibodies. These included surfactant protein B (SP-B), thyroid transcription factor-1 (TTF-1), mast cell trypsin, CD68, epithelial antigen markers (high molecular weight cytokeratin, low molecular weight cytokeratin [CK-L], epithelial membrane antigen [EMA], cancer embryonic antigen), mesothelial antigen, neuroendocrine markers (neuron-specific enolase [NSE], chromogranin A, synaptophysin, calcitonin, adrenocorticotropic hormone, human growth hormone [hHG]), vimentin, and CD34. Surface cuboidal cells have short microvilli and have lamellar bodies in their cytoplasm. They can sometimes merge into multinuclear giant cells. Immunohistochemical results showed that these cells are strongly positive for SP-B, TTF-1, CK-L, EMA, and cancer embryonic antigen, whereas polygonal cells, previously also described as round or pale cells, were strongly positive for vimentin and TTF-1, and positive or weakly positive for 2 to 3 kinds of neuroendocrine markers. Sparse neuroendocrine granules and abundant microfilaments were observed in their cytoplasm. Some cell clusters in the solid regions were positive for SP-B and EMA. Mast cells existed sparsely in almost every field. Both cuboidal and polygonal cells were negative to CD34 and mesothelial antigen staining. We conclude that cuboidal cells of SH originate from reactive proliferation of type II pneumocytes, which can fuse into multinuclear giant cells. Polygonal cells, as true tumor cells, likely originate from multipotential primitive respiratory epithelium and possess the capability for multipotential differentiation. The antibodies of SP-B, TTF-1, vimentin, and CK-L are very helpful to diagnosis and differential diagnosis of SH.

Key words: so-called sclerosing hemangioma of the lung, tumor of the lung, immunohistochemistry.

Abbreviations: SH, sclerosing hemangioma; SP-B, surfactant protein B; TTF-1, thyroid transcription factor-1; MCT, mast cell trypsin; CK-L, low molecular weight cytokeratin; CK-H, high molecular weight cytokeratin; EMA, epithelial membrane antigen; CEA, cancer embryonic antigen; VT, vimentin; NSE, neuron-specific enolase; MC, mesothelial antigen.

MATERIALS AND METHODS

Thirty diagnosed cases of SH were collected for this study at the Pathology Diagnostic Center of the First Affiliated Hospital of China Medical University from 1990 to 2002. The patients included 9 men and 21 women (male-female ratio, 1:2.3). The age range was 23 to 54 years, and the mean age was 41.9 years. Twenty-one patients were asymptomatic and had been diagnosed during routine examinations. The clinical symptoms of the other 9 patients included cough (6), chest pains (2), and blood in the sputum (1). The typical results from chest radiograph and computed tomography scan showed a peripheral round, solitary, highly dense nodule with a clear margin. The tumors were located in the right inferior lung lobe (14 cases), left inferior lung lobe (8), right superior lung lobe (6), and left superior lung lobe (2). All the patients had been followed up since operation with routine chest radiographs, which show an entirely benign course. So far no recurrence or metastasis has occurred. Specimens were fixed with 4% neutral formalin or 0.05% glutaraldehyde in phosphate-buffered saline. The paraffin sections were sliced
into 4-μm-thick samples for light microscopy, and thin slides were made for electron microscopy. In combination with light microscopy on hematoxylin-eosin samples, immunohistochemistry was performed with 12 kinds of monoclonal primary antibodies. They included surfactant protein B (SP-B), thyroid transcription factor-1 (TTF-1), mast cell trypsin (MCT), CD68, low molecular weight cytokeratin (CK-L), high molecular weight cytokeratin (CK-H), epithelial membrane antigen (EMA), cancer embryonic antigen (CEA), vimentin (VT), neuron-specific enolase (NSE), CD34, and mesothelial antigen (MC). Five polyclonal primary antibodies were also applied, which included adrenocorticotropic hormone, synaptophysin, human growth hormone, chromogranin A, and calcitonin. All the antibodies and the peroxidase detection kit were purchased from the American Maxim (San Francisco, CA) and Neomarker Biological Technique Company (Fremont, CA). All the specimens underwent antigen recovery with heat-induced epitope retrieval, except for those stained with MCT, which was pretreated with trypsin. The immunoreaction was visualized by demonstration of conjugated peroxidase with DAB as the substrate. The slides were counterstained with hematoxylin after DAB staining. For the negative control, primary antibodies were omitted. Endogenous peroxidase reactions were quenched with 2% H₂O₂ in phosphate-buffered saline.

Semiquantitative evaluations of immunohistochemistry results were performed according to the percentages of positive cells: no positive cells (−), positive cells <10% (+), >10%–50% (++), >50%–75% (+++), and >75% (++++)

RESULTS

Gross and Histological Features

Tumours were located in lung parenchyma. The nodules were 1.5 to 5.0 cm in diameter and were well circumscribed with or without capsule. They were medium soft and often had a pale-brown region caused by hemorrhage. Under light microscopy, the tumors displayed four histological patterns: solid, papillary, hemorrhagic, and sclerotic. Four of the 30 cases contained primarily solid pattern, whereas 3 were of the papillary pattern. The majority of tumors showed a mixed pattern of histology (23 cases). Even in an individual tumor, mixed histological patterns often can be seen. Tumor cells in solid and papillary regions were often

FIGURE 2. The immunohistochemical staining of low molecular weight cytokeratin. Note the strong positivity in cuboidal cells. Original magnification ×100.
polygonal and uniform in shape. Clear or eosinophilic cytoplasm and a round or oval nucleus were noted. In other studies, the cells have also been described as round or pale cells. The ratio of nucleus to plasma was in the reference range. Mitotic figures were rarely seen, and no pathological mitotic figure was seen. Papillary processes and hemorrhagic cleft spaces were covered by flattened endothelial-like cuboidal cells (Figs 1 and 2). In the sclerosing regions, both cuboidal cells and multinuclear giant cells were seen in the cleft spaces (Fig 2A). In the stroma was fibrotic proliferation, hyaline degeneration, infiltration with both foam cells and lymphocytes, calcification, and hemosiderin sediment.

**Immunohistochemical and Ultrastructure Markers**

All immunohistochemistry results are summarized in Table 1. Epithelial markers were all positive in cuboidal cells of all patterns (Fig 2). In the solid regions, some cell clusters showed positive immunoreactivity with CK-L. The results of SP-B staining were identical to the results seen with epithelial markers in the cuboidal cells. The multinuclear giant cells derived from cuboidal cells were also positive to SP-B staining but negative to CD68 (Fig 3A). Some cuboidal cells remaining in the solid region of SH also expressed the surfactant proteins (Fig 3B). However, both epithelial markers and SP-B staining were negative in polygonal cells, which lined the stroma (Fig 3). Both cuboidal cells and polygonal cells in the nucleus were strongly positive for TTF-1 (Fig 4). The results of electron microscopy revealed a few short microvilli on the cuboidal cell surface. The cuboidal cells also had abundant rough endoplasmic reticulum, mitochondria, and typical lamellar bodies for pneumocytes type II (Fig 5A). However, no neuroendocrine granule was seen in the cuboidal cells. Some polygonal cells contained sparse neuroendocrine granules and abundant microfilaments besides abundant rough endoplasmic reticulum and mitochondria (Fig 5B). On the contrary, no lamellar body was seen in these cells. In addition, 2 to 3 neuroendocrine markers, such as NSE, were positive or
dispersed positive in the polygonal cells of every pattern (Fig 6). Other neuroendocrine markers were faintly positive in polygonal cells, including adrenocorticotropic hormone, synaptophysin, human growth hormone, and chromogranin A. Vimentin staining was positive in all cases (Fig 7). All of the tumor cells were negative for calcitonin. CD34 expressed positive results only in the endothelial cells of small vessels and was negative in cuboidal cells and polygonal cells. MC was negative in all cases. Some positive cells for MCT were seen sparsely in papillary, solid, and sclerosing regions (Fig 8).

**DISCUSSION**

The histogenesis of the major types of cells in SH is a controversial and important issue in pulmonary tumor pathology. In the present study we found that inner covering cells in the hemangioma region of SH, surface cuboidal cells, glandular epithelium, and cellular clusters in the solid region all share similarities in cell morphology and antigen expression. They all expressed CK-L, CK-H, EMA, CEA, SP-B, and TTF-1. It is plausible to speculate that they had the same origin—type II pneumocytes, because SP-B was expressed only in type II alveolar epithelium, and TTF-1 was expressed not only in adult type II pneumocytes but also in embryonic nonciliated epithelial nuclei. Ultrastructural features of that particular lamellar body appearing in cuboidal cells also support this notion. Another important and disputed issue in SH pathology is the cell of origin of the polygonal cells. In addition to the hypotheses of endothelium, mesothelium, pneumocyte, or neuroendocrine origin, a new theory has recently been postulated: Polygonal cells may originate from respiratory multipotential primitive epithelium. Because CD34 and mesothelium antigen are both negative, and epithelium antigens are negative or dispersed weakly positive, our results do not support the theory of mesothelial and epithelial origin. SP-B, which was expressed in type II pneumocytes, was negative in the polygonal cells, and no lamellar body was found under the electron microscope, which indicated that polygonal cells do not originate from pneumocytes. Although polygonal cells in all cases were positive for 2 to 3 kinds of neuroendocrine markers, all were just weakly positive except for NSE. By electron microscopy we found that only a small portion of polygonal cells contained some neuroendocrine granules. Therefore, it is unlikely that they originate from neuroendocrine ancestors. However, TTF-1 was strongly positive in these cells, which is consistent with results from another study. Those in-

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**TABLE 1.** List of 17 Kinds of Antibodies and Immunohistochemical Findings in 30 Sclerosing Hemangioma Patients

NOTE. Semiquantitative evaluations were done according to the percentage of positive cells: no positive cells (–) and positive cells <10% (±), >10%–50% (+), >50%–75% (++), and >75% (+++).

Abbreviations: CK-L, cytokeratin low molecular weight; CK-H, cytokeratin high molecular weight; EMA, epithelial membrane antigen; MC, mesothelial antigen; CEA, cancer embryonic antigen; SP-B, surfactant protein B; VT, vimentin; TTF-1, thyroid transcription factor-1; MCT, mast cell trypsin; NSE, neuron-specific enolase; ACTH, adrenocorticotropic hormone; Sy, synaptophysin; hGH, human growth hormone; Ch-A, chromogranin A; CT, calcitonin.

**FIGURE 5.** Ultrastructural differences in cuboidal and polygonal cells. The typical lamellar bodies and microvilli are clearly seen in the cuboidal cells. Transmission electron microscopy, ×24,000. The neuroendocrine granules, rough endoplasmic reticulum, and microtubules were observed in polygonal cells. Transmission electron microscopy, ×10,000.
vestigators found that TTF-1 could be detected in fetal lung epithelial cell nuclei, mainly type II epithelial cells. The expression of TTF-1 in embryonic multipotential respiratory epithelium indirectly suggests that polygonal cells in SH may originate from primitive multipotential cells. This may also explain why polygonal cells are strongly positive for vimentin and have neuroendocrine and epithelial differentiation (CK-H and EMA). An earlier report also favored the multipotential differentiation of SH. It reported that ultrastructurally the tumor is comprised of a mixture of epithelial and mesenchymal elements in varying stages of differentiation, including type II pneumocytes and primitive mesenchymal cells. Another potential explanation for disputation on SH histogenesis is tumor transdifferentiation as hypothesized by Zhang et al. SH cells may commit to particular specialization changes, which result in transition between neoplastic epithelia and neuroendocrine cells, between neoplastic epithelia and mesenchyme, as well as transition between nonneuroectodermal and neuroectodermal cells. In addition to clear morphological differences in the major SH cells, we also observed the transitional phenomenon between cuboidal cells and glandular cells. The results of immunohistochemistry suggest that these cells have similar antigen expression. When a few of the cuboidal cells disperse in solid regions, it is hard to distinguish them from surrounding polygonal cells.

In addition, both types of cells are positive to epithelial markers. Under these circumstances it is readily misconstrued that polygonal cells originate from epithelial tissue. Although 4 histological patterns of SH can exist in a single case with different arrays and proportions, transitional patterns often appear. The formation of 4 histological patterns may occur through several steps under the influence of certain factors. They may first...
cause hemorrhage in the alveolar cavity and alveolar duct and stimulate the proliferation of type II pneumocytes. Although polygonal cells, as the true tumor cells in alveolar walls, also proliferate, alveolar walls became thicker, and a so-called hemangioma region can form. When polygonal cells further proliferate, cell clumps may protrude into the tumor cavity and finally form a papillary pattern. At the same time, a layer of cuboidal cells may form on the papillary surface. These cells are of the same type as the inner covered cells in the hemangioma pattern. With the progressive proliferation, polygonal cells may merge into cell patches, which form a solid pattern. Then erythrocytes may be squeezed into small glandular cavities or solid areas in the region. In this way many cell clusters appear and polygonal cells spread in solid pattern areas.

On the basis of our histological observation of all the tumors, we speculate that SH formation is a progressive process, moving from a hemangioma to a papillary to a solid and finally to a sclerosing pattern. The histological patterns can appear in a mixed form. Alternatively, only 1 to 2 patterns may predominate. The formation of a sclerosing pattern may be related to fibrous tissue hyperplasia, hyaline degeneration, or dispersing mast cells at the late stage of SH. Large amounts of mast cells in SH tissues could be another important histocytological characteristic of SH, which may partially explain why this tumor was previously named inflammatory pseudotumor of lung. Regarding the clinicopathologic implications of all the immunomarkers used in this study, we believe that the combined application of SP-B, CK, TTF-1, vimentin, and NSE will provide helpful information in diagnosing SH and distinguishing the tumor from lung inflammatory pseudotumor, bronchioloalveolar adenoma, bronchioloalveolar carcinoma, carcinoid tumors, and paraganglioma. However, use of standard light microscopy and clinicopathologic experience remains the best approach to making these distinctions.

In conclusion, cuboidal cells and polygonal cells in SH do not have the same origin. The cuboidal cells originate from type II pneumocytes and are the result of responsive proliferation in the tumorigenesis. The polygonal cells are true tumor cells and possibly originate from respiratory primitive epithelium. However, this conclusion requires further studies.

REFERENCES