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Immunohistochemical Study of So-called Sclerosing Hemangioma of the Lung†

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= Abstract = **The origin of tumor cells composing sclerosing hemangioma of the lung has been extensively studied by many researchers, but it is still obscure. We analyzed seven cases of sclerosing hemangioma of the lung from the files of Seoul National University Hospital, Department of Pathology by immunohistochemical staining as well as histologic observation. The tumors are composed of two kinds of cells; the epithelial cells lining the papillary area are cytokeratin(+) and vimentin(−), while subepithelial tumor cells are cytokeratin(−) and vimentin(+). Two kinds of cells are separated by basement membrane, visualized by type IV collagen monoclonal antibody. The angiomatous and solid areas are devoid of cytokeratin(+) epithelial cells and basement membrane is not formed beneath the blood-filled cysts. The epitheial cells are frequently seen at the periphery of the tumor, where the epithelial cells maintain their pseudostratified and ciliated character of bronchiolar lining epithelium. The above findings suggest that the epithelial cells would not be tumor cells but an entrapped mucosa, and that the tumor cells be not of epithelial origin.**

Key Words: *Lung neoplasm, Sclerosing hemangioma, Basement membrane, Immunohistochemistry*

INTRODUCTION

Since the first report of sclerosing hemangioma in 1956 by Liebow and Hubbel, there are several hypotheses as to its histogenesis, including pulmonary epithelial (Chan *et al.* 1982; Hill and Eggleston 1972; Haimoto *et al.* 1985; Palacios *et al.* 1979; Jose *et al.* 1979; Singh *et al.* 1984), mesothelial (Katzenstein *et al.* 1983), and endothelial origin (Hass *et al.* 1972; Kay *et al.* 1977). The tumor is composed of characteristic round cells which are present predominantly in the solid area and flattened cells lining blood lakes or papillary fronds. Recently round cells arranged in sheets are considered to be neoplastic cells (Hass *et al.* 1972; Hill and Eggleston 1972; Katzenstein *et al.* 1983).

Tissue architecture is established by the interaction between cellular components and extracelluar matrix. Type IV collagen, one of the major components of extracellular matrix, is involved in the structural framework of basement membrane. The basement membrane lies between the interface of different compartments; epithelial, stromal, and endothelial. In this study we investigated the origin of tumor cells in sclerosing hemangioma by visualizing type IV collagen as well as intermediate filament immunohistochemistry.

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MATERIALS AND METHODS

Between the year of 1984 and 1992, seven patients were operated and diagnosed as sclerosing hemangioma of the lung at Seoul National University Hospital. All the specimens of the resected tumors were fixed in 10% formalin, embedded in paraffin, and diagnosed by means of hematoxylin and eosin stained sections using the criteria described by Katzenstein *et al.* (1980).

The avidin-biotin-peroxidase method with a Vector Elite Kit (Vector Lab. Inc, CA) was used to detect the antigens on formalin-fixed paraffin-embedded tissue. Primary antibody included anti-vimentin (monoclonal, from DAKO, Carpinteria, CA), anti-desmin (rabbit polyclonal, from DAKO), anti-type IV collagen (monoclonal, from DAKO), anti-epithelial membrane antigen (monoclonal, from DAKO) and anti-factor VIII-related antigen (rabbit, polyclonal from DAKO). Anti-cytokeratin monoclonal antibody was purchased from Zymed (San Francisco, CA), which is known to react with acidic keratin such as keratin 10 (56 kD), keratin 17 (46 kD), keratin 18 (45 kD) and keratin 19 (40 kD). Trypsinization was performed for staining of type IV collagen and cytokeratin. Colorization was done by diaminobenzidine with hydrogen peroxide.

RESULTS

The clinical features and locations of the seven cases are summarized in Table 1. One patient was male and six were females. The mean age of the patients was 43 years old, and age ranged from 20 to 62 years. Only one patient was presented with vague chest pain, and five patients were detected incidentally by routine chest X-ray. Operations were done under the impression of tuberculous granuloma or hamartoma of the lung. The size of the tumors ranged from 2.0 to 7.0 cm in diameter (average 3.8 cm). Most of the tumors were located in the lung parenchyma beneath the pleura, and the tumors in cases 4 and 7 were situated at the interlobar fissure.

Grossly, the tumors were well circumscribed, ovoid or round and surrounded by pseudocapsule. The cut surface usually showed a variegated appearance characterized by hemorrhagic zones, which were spongy-like, admixed with yellowish gray areas.

Table 1. Clinical summary of 7 cases of "sclerosing hemangiomas" of the lung

Case No.	Age	Presenting Sx	Clinical Dx	Location	Diameter(cm)	Operation
1	F/39	?	?	Left upper lobe	4 x 3	Lobectomy
2	F/30	Vague chest pain	Tbc granuloma R/O malignancy	Right upper lobe	3 x 3	Lobectomy
3	F/58	None	Tbc gronuloma Benign tumor R/O malignancy	Right upper lobe	3 x 3	Excision
4	F/41	None	Tbc granuloma Lung cancer Hamartoma	Major fissure	2 x 1.5	Excision
5	M/20	None	Bronchogenic cyst Hamartoma	Lingula segment	4 x 3.5	Segmentectomy
6	F/51	None	Lung cancer Hamartoma	Left upper lobe	3.5 x 3.5	Lobectomy
7	F/62	None	Tbc granuloma Hamartoma	Major fissure	7 x 6	Excision

Table 2. Summary of histological features of 7 cases of sclerosing hemangioma

Case	Solid	Papillary	Angiomatoid	Sclerosis	Foam cell	Calcification
1	+	+ +	±	±	−	+
2	+	+	+ +	−	+	−
3	+	±	+ +	±	+	−
4	+	+ +	±	+	+	−
5	+	+ +	−	+	±	−
6	+ +	±	+	±	−	−
7	+ +	±	+ +	±	−	+

Table 3. Immunohistochemical staining result of 7 cases of sclerosing hemangioma

Case	Cytokeratin S/L	Vimentin S/L	EMA S/L
1	−/+	±/−	−/+
2	−/±	+/−	±/+
3	−/±	+/−	±/+
4	−/+	+/−	±/+
5	−/+ +	+ +/−	−/+ +
6	−/±	+/−	±/+
7	−/±	+/−	±/+

(S : solid area, L : lining cell)

On histopathologic examination, four major histologic patterns - solid, papillary, hemorrhagic, and sclerotic - were encountered in varying proportions within these tumors (Table 2). The polygonal cells with moderate amounts of eosinophilic cytoplasm and round or oval nuclei were arranged in sheets in solid areas and in papillary stalks. In the hemorrhagic areas, variable sized honeycomb spaces containing red blood cells were lined by a single layer of flattened, cuboid or oval cells. The cleft-like spaces with projections of papillary structures were covered by a layer of cuboidal to low columnar cells, some with hobnail appearance. Sclerotic areas were composed of dense fibrous tissue and varying amounts of hyalinization. Mast cells and foamy histiocytes were scattered. Hemosiderin pigment and siderotic nodules were noted in some cases and calcification was occasionally present.

The results of the immunohistochemical observation are summarized in Table 3. The

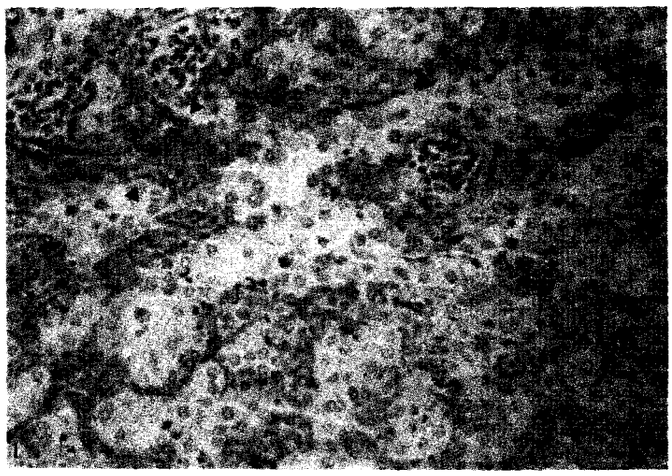

Fig. 1. Round cells arranged in sheets show marked cytoplasmic staining with anti-vimentin (arrow heads), whereas lining cells of papillary stalks and ductal space show an absence of vimentin. (Case 5, immunoperoxidase staining, vimentin)

neoplastic cells arranged in sheets in solid or papillary areas exhibited positive staining for vimentin, but cytokeratin was not stained in these cells (Fig. 1). In the hemorrhagic area, both lining cells or stromal cells showed the same staining pattern; positive for vimentin and negative for cytokeratin. Cuboid or oval cells lining the villous structure in the papillary areas showed positive staining for cytokeratin and negative staining for vimentin (Fig. 2). While, the stromal cells in the same area were negative for cytokeratin and positive for vimentin. .Type IV collagen was revealed between those two kinds of cell, especially in the papillary area (Fig. 3).

The basement membrane was not formed in solid or hemorrhagic areas. Endothelial cells

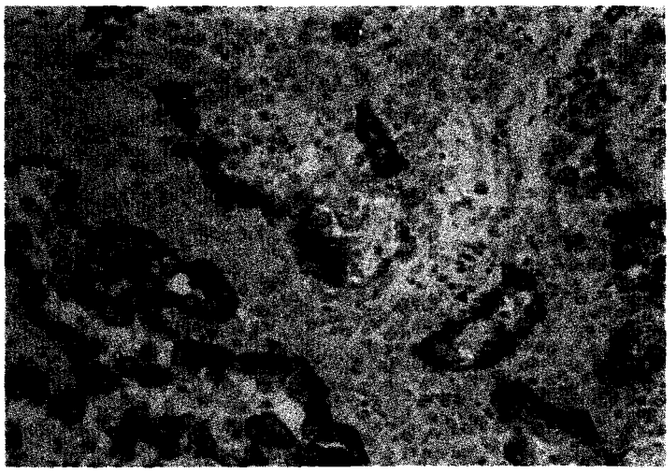

Fig. 2. Oval or cuboid cells lining the papillary stalks and ductal spaces are positively stained with anti-cytokeratin (arrow heads), whereas small clusters of round cells in the central stalks show negative staining. (Case 5, anti-cytokeratin)

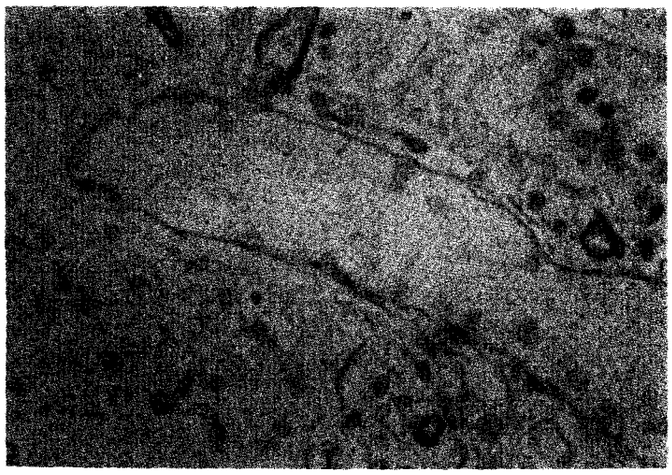

Fig. 3. Immunoperoxidase staining against type IV collagen reveals basement membrane beneath the lining cell of papillary projection. (Case 5, anti-type IV collagen)

of capillaries and small blood vessels in the tumor tissue exhibited positivity for factor VIII but tumor cells showed negative staining. Tumor cells were also negative for desmin.

DISCUSSION

The histologic features of sclerosing hemangioma of the lung are variable; papillary, sclerotic, solid, and hemorrhagic (Katzenstein *et al.* 1980). Most researchers agree that the prototype cell of this lesion is a round cell arranged in sheets which is most prominent in solid area (Hass *et al.* 1972; Hill and Eggleston 1972; Katzenstein *et al.* 1983).

It is very peculiar that the histogenetic origin of this tumor has not yet been unequivocally proved. Approaches to this problem include electron microscopy, immunohistochemistry, histochemistry, biochemical analysis and tumor cell culture. Electron microscopic interpretations have yielded different results indicating epithelial (Chan *et al.* 1982; Hill and Eggleston 1972; Palacios *et al.* 1979), endothelial (Hass *et al.* 1972; 1972; Kay *et al.* 1977) or mesothelial origin (Katzenstein *et al.* 1983). Immunohistochemistry results are more conflicting; epithelial origin (Haimoto *et al.* 1985; Singh *et al.* 1984), mesothelial origin (Katzenstein *et al.* 1983), and mesenchymal cell origin (Huazar *et al.* 1986).

Intermediate filaments have been used to define the histogenetic origin of tumor cells, because these filaments' composition is well preserved even after the neoplastic transformation. We applied three of them; cytokeratin, vimentin, and desmin and found that cytokeratin (+) cells and vimentin (+) cells are intermingled in this tumor but that they are not the same cells. Moreover, it was evident that cytokeratin (+) cells and vimentin (+) cells were separated by type IV collagen. This finding was most prominent in the papillary area.

The hemorrhagic area was almost entirely composed of vimentin (+) cells without basement membrane structure. It is very important that these two kinds of cells are divided by basement membrane, because the same kinds of cells are not usually compartmentalized by basement membrane.

We agree with the view of Huszar *et al.* (1986) who regarded the cytokeratin (+) cells as entrapped epithelial cells rather than neoplastic cells of this lesion. The fact that the cytokeratin (+) cells of well developed bronchiolar lumen are frequently seen at the periphery of the tumors favors our interpret-

ation. The entrapment of an epithelial component is prominent in several benign conditions such as chondroid hamartoma. We applied immmunohistochemical staining to chondroid hamartoma and obtained the same staining characteristics of entrapped epithelial cells. Chan *et al.* (1982) listed the causes of contradictory results concerning histogenesis on the basis of electron microscopy. First, some authors included lesions other than genuine sclerosing hemangioma. Second, limitation of sampling and third, interpretation of entrapment versus neoplastic. They analyzed 5 cases of sclerosing hemangioma by electron microscopy and described that all of the cells, whether lining channels or papilla, forming sheets, lying within the scleotric area had features of type II pneumonocyte.

It is our assumption that contradictory results are derived from different interpretations of the lining epithelium in the papillary structure. Some authors have insisted that the epithelium is neoplastic (Singh *et al.* 1984; Noguch *et al.* 1986), because minute sclerosing hemangioma is composed of epithelial cells without "round cells", and the cells in metastatic sclerosing hemangioma are positive for surfactant apoprotein. They regarded these "round cells" as an intermediate stage between differentiated epithelial cells and undifferentiated cells. We disagree with their opinion because the same kind of epithelial cells cannot be separated by basement membrane regardless of different degree of differentiation. Most of the researchers doing the electron microscopic observation concluded that sclerosing hemangioma of the lung was composed of type II pneumonocytes. Some considered the presence of lamella bodies as a hallmark of type II pneumonocytes, although lamella bodies are seen in varieties of normal and abnormal tissue. Singh *et al.* (1984) suggested that the sclerosing hemangioma is a lesion of dual cell proliferation; epithelial and stromal. Although this seems an attractive theory, the absence of an epithelial component in the hemorrhagic or sclerotic areas cannot be explained.

In this study, we were not able to define the origin of tumor cells in sclerosing hemangioma with absolute confidence. However, it was clearly visualized that the "round" cells are not of epithelial origin. It is our opinion that the tissue architecture established by the basement membrane should be considered to distinguish the origin of tumor cells.

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