Sclerosing Hemangioma of the Lung

Immunohistochemical Demonstration of Mesenchymal Origin Using Antibodies to Tissue-Specific Intermediate Filaments

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A case of pulmonary sclerosing hemangioma of the lung was studied by light microscopy and indirect immunofluorescence using tissue-specific antibodies against intermediate filament subunits. All the tumor cells stained positively and exclusively with antivimentin antibodies thus indicating their mesenchymal origin. In addition, positive staining with cytokeratin antibodies was observed in cells lining cystic spaces and elongated slit-like spaces were occasionally encountered throughout the tumor, disclosing residual epithelial elements. Using brightfield microscopy, the keratin-positive areas were identified as distorted alveolar spaces lined by hyperplastic respiratory epithelium entrapped within the tumor. It is proposed that these entrapped epithelial elements may account for the conflicting results obtained by different investigators in previous attempts to determine the histogenesis of this tumor.

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SCLEROSING HEMANGIOMA OF THE LUNG was first described by Liebow and Hubbel as a distinct entity in 1956.[1] Although the clinicopathological features of this tumor have been well characterized and a reasonable consensus regarding its microscopic diagnosis has been achieved,[2,3] its histogenesis still remains a matter of debate. The original contention that the tumor was derived from vascular endothelium has been recently challenged on the basis of negative immunoperoxidase staining for Factor VIII-related antigen,[4] a cell marker considered specific for endothelial cells. Most of the current disagreement, however, centers on whether this neoplasm is derived from mesenchymal or epithelial elements. Electron microscopic, histochemical, tissue culture, and other studies have so far failed to settle the issue, and in some instances have further obscured the problem due to the conflicting results obtained by different investigators.

We have examined a case of sclerosing hemangioma of the lung presenting as an asymptomatic coin lesion in a 62-year-old woman by the application of an indirect immunofluorescence technique using tissue-specific antibodies against intermediate filament subunits of the cytokeratin, vimentin, and desmin types. Our results demonstrated that the tumor cells labeled extensively and exclusively with anti-vimentin antibodies, thus supporting a mesenchymal origin for this neoplasm.

Materials and Methods

Tissues

Small pieces of tumor with surrounding normal lung were frozen at −20°C in the cryostat. Parallel sections of tumor were fixed in buffered formalin, embedded in paraffin and routinely stained with hematoxylin and eosin (H & E).

Preparation of Frozen Sections

Frozen sections of 4 to 5 μm were cut in a cryostat (Cryocut, American Optical Co., Buffalo, NY). The slides were fixed with acetone at −20°C for 20 minutes, then air dried and labeled for immunofluorescence with antibodies against keratin, vimentin, and desmin.

Immunochemical Reagents

Murine monoclonal cytokeratin antibodies were derived from mouse immunized with bovine epidermal prekeratin.[5] The monoclonal antibody used (KG 8-13.2) binds to polypeptides Nos. 1,5,6,7,8 (basic cytokeratin subfamily) as well as to a few acidic cytokeratins, namely Nos. 10,11, and 18 according to the classification of Moll *et al.*[6] Characterization of the antibodies indicated that they were reactive with all epithelial tissues and cultured epithelial cells examined.[5]

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Vimentin antibodies were raised in rabbits by injection of pure vimentin from baby hamster kidney (BHK) cells as previously described.[7,8] This antibody reacted specifically with mesenchymal cells or with purified vimentin as determined by immunoprecipitation or immunoblotting analysis.

Antibodies to desmin were prepared in rabbits by injections of pure chicken gizzard desmin as previously described.[9] This antibody reacted with all types of muscle as well as with intermediate filaments of cultured smooth muscle cells. Immunoblot analysis indicated exclusive reactivity with desmin.

For secondary antibodies we used affinity-purified goat antibodies against rabbit or mouse IgG labeled with lissamine-rhodamine sulfonyl chloride as previously described.[10]

Immunofluorescent labeling

Indirect immunofluorescence was performed as follows: Tissue sections were covered with the primary antibodies to cytokeratin, vimentin, and desmin for 30 minutes at room temperature. The sections were rinsed with phosphate-buffered saline (PBS) and incubated for 30 minutes with rhodamine-labeled goat antimouse or goat antirabbit IgG. The sections were rinsed with PBS, dehydrated in ethanol, and mounted in Entellan (Diagnostica, Merck, Darmstadt, FRG). Fluorescence microscopy was performed using a Zeiss photomicroscope III (Carl Zeiss, Oberkochen, FRG) equipped with epi-illuminators and filter sets for selective observation of fluorescin and rhodamine.

Results

Light Microscopy

Histologic examination of the tumor disclosed a variegated morphology with four major architectural patterns: solid, papillary, hemorrhagic, and sclerotic. The predominant pattern seen in our case was papillary (Fig. 1A), followed by solid, sclerosing (Fig. 1B), and hemorrhagic (Fig. 1C). The papillary formations were composed of two cell types: a small uniform round cell population containing pale cytoplasm that was located within the cores of the papillae, and smaller round cells with dark staining nuclei lining the papillary structures (Fig. 1D). No atypia or mitotic figures were observed in any of the two cell types. The solid areas were composed predominantly of monotonous-appearing round cells with a clear cytoplasm identical to those located within the cores of the papillae. Areas of hemorrhage in various stages of organization were also present in addition to prominent foci of blood vessel hyalinization. Other, less commonly observed features were aggregates of foamy macrophages, deposits of hemosiderin, and occasional psammoma bodies. An additional important finding was the identification of entrapped alveolar spaces lined by hyperplastic cuboidal epithelial cells. These were particularly prominent in the periphery of the tumor where they retained a round to oval elongated shape and showed obvious circumscription from the surrounding neoplastic cells (Figs. 2A and 2B). A few random entrapped alveolar spaces could also be occasionally identified in deeper portions of the tumor where they adopted a slit-like configuration and appeared to merge with the surrounding papillae, thus making it difficult, and at times virtually impossible, to differentiate them from interpapillary spaces by light microscopy.

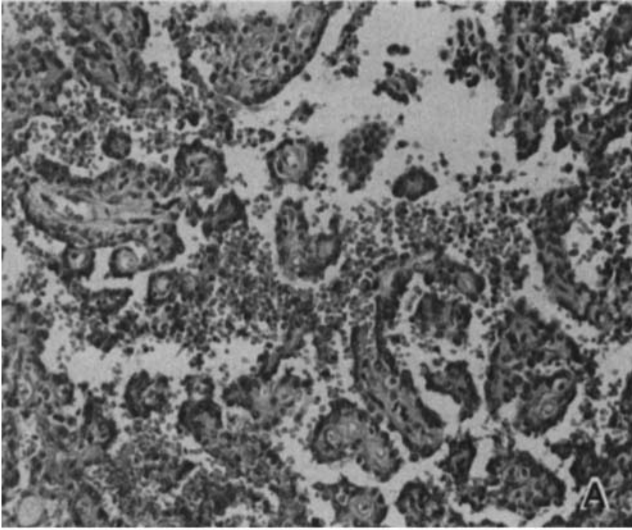
Immunofluorescence Microscopy

All the tumor cells including the two morphologically dissimilar cell types present in the papillae stained uniformly positive with antivimentin antibodies (Figs. 3A and 3B). No reaction was evidenced against desmin and cytokeratin in these cells (Fig. 3B). Staining with anticytokeratin antiserum was strongly positive, however, in the cells lining the round to oval cystic spaces in the periphery of the tumor as well as in occasional elongated slit-like spaces lying within the deeper areas of the specimen (Figs. 4A and 4B).

Discussion

Sclerosing hemangioma of the lung is an uncommon benign neoplasm with a distinctive well-defined histologic appearance. The diagnostic features as originally described by Liebow and Hubbel[1] include the presence of dilated blood vessels with a tendency to undergo sclerosis, papillary projections, hemorrhages in different stages of organization, and infiltration of the stroma and air spaces by sheets of round cells resembling histiocytes. An admixture of these features in varying proportions coupled with the proper clinical setting invariably permits the diagnosis. As has been pointed out in several recent reviews[4,11] and single case reports,[12-14] the histogenetic origin of this tumor has not yet been unequivocally demonstrated. Different methods have been applied in the past to approach this problem including electron microscopy,[11,12,14-17] histochemistry,[4,17] immunocytochemistry,[4,18] tissue culture,[17] and biochemical analysis.[4] In many instances, contradictory results have been obtained by different investigators even when the same method of study was employed. Electron microscopic examination for example has yielded conflicting interpretations with opinions divided between those who favor an epithelial origin[12,15,17,19,20] and those who favor an endothelial derivation.[14,16] The speculations generated over the alleged cell of origin have thus given rise to a profusion of new designations, which have only further contributed to the confusion in nomenclature of this entity.

The indirect immunofluorescence technique using anti-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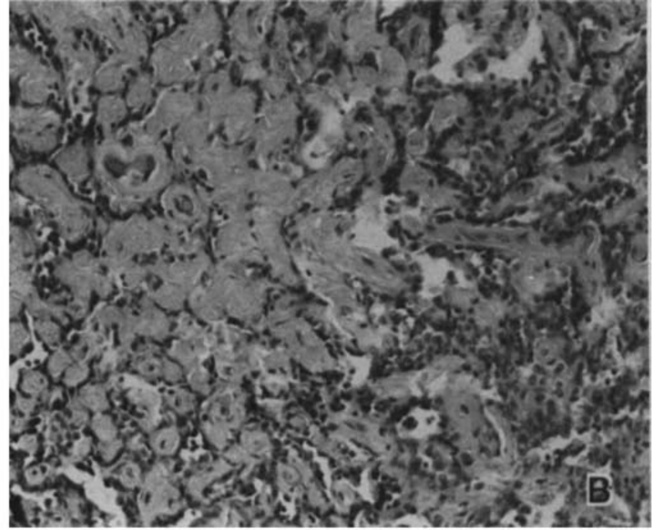
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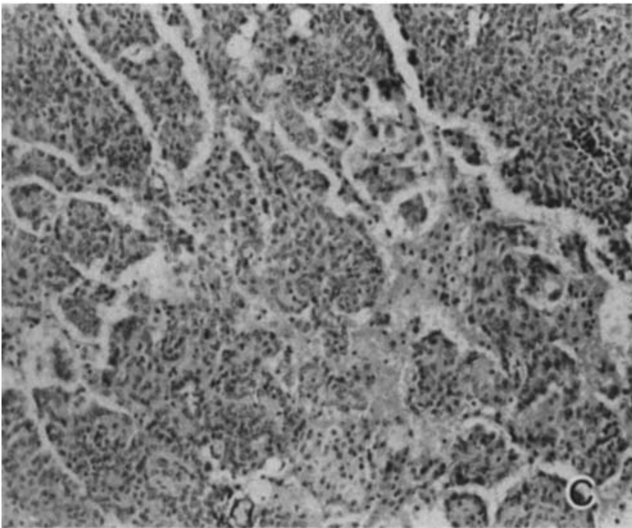
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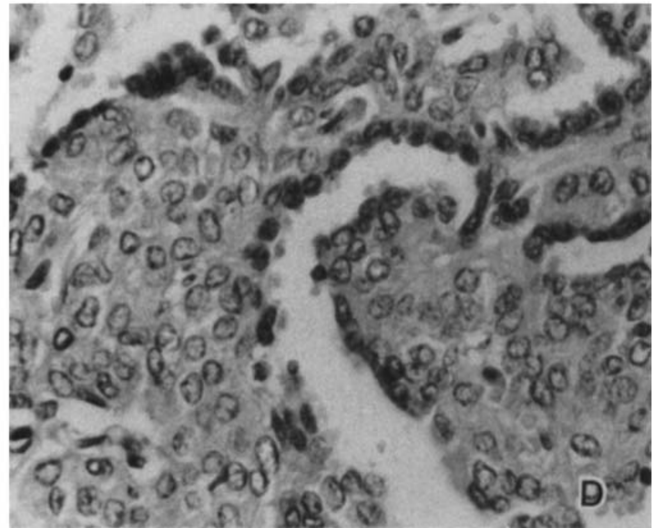
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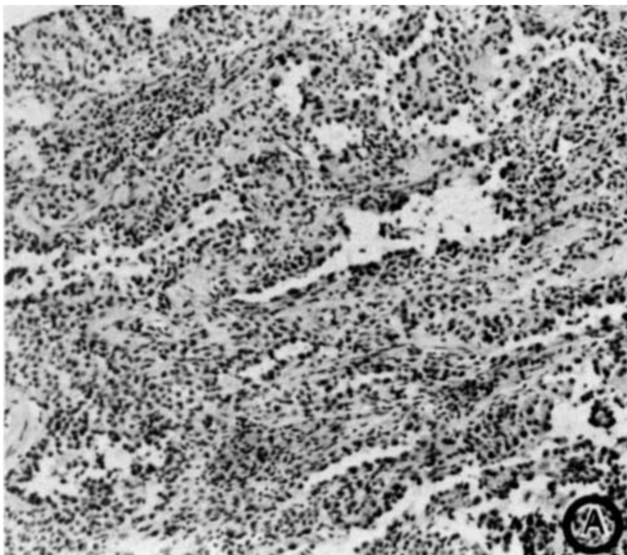
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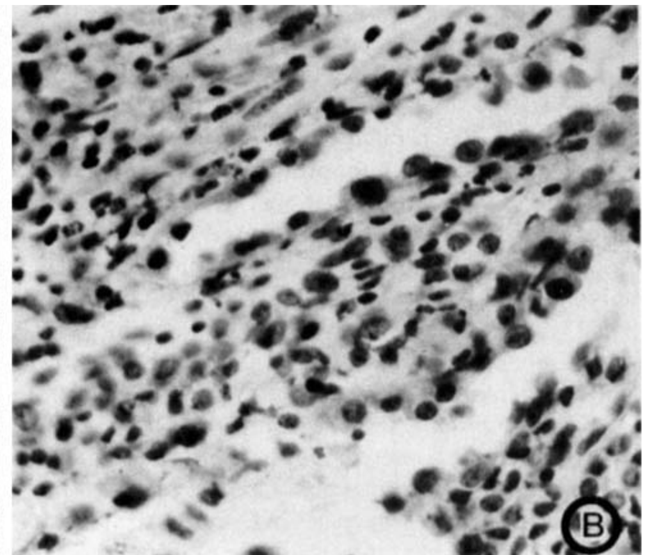
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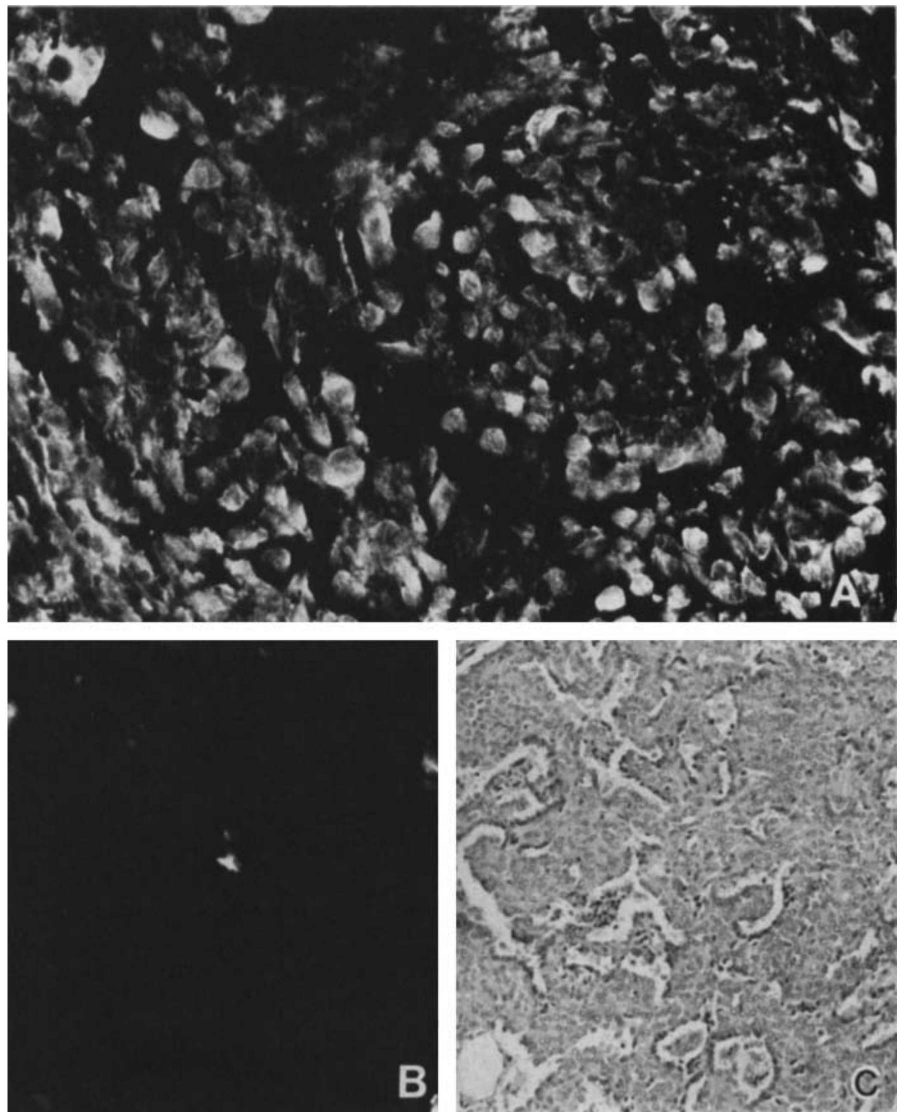
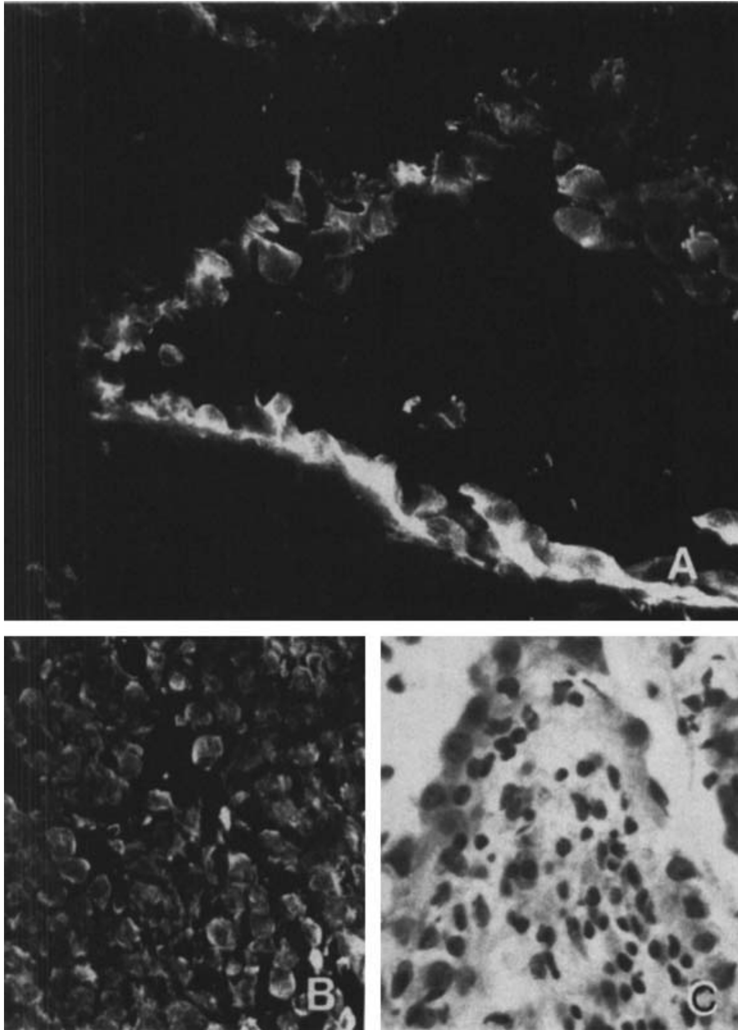
FIGS. 1A–1D. Sclerosing hemangioma of the lung showing papillary areas (A), sclerosing (B), and hemorrhagic areas (C). High-power view of papillae showing two cell types: small uniform round cells with clear cytoplasm within the cores and round cells with dark staining nuclei lining the papillae (D) (H & E ×100 original magnification, A–C; ×400 original magnification, D).

FIGS. 2A AND 2B. Area from the periphery of the tumor showing spaces lined by large cuboidal epithelial cells (H & E, ×100 original magnification, A; ×400 original magnification, B).

bodies against tissue-specific intermediate filaments on frozen sections or aldehyde fixed tissues employed in this study is a sensitive method presently available for the demonstration of cellular markers at the submicroscopic level on account of its maximal preservation of antigenicity and the avoidance of the tissue sampling error that may frequently be encountered in electron microscopic examination.[21,22] Recent studies have demonstrated that the intermediate filament composition of cells undergoing neoplastic transformation remains largely unchanged.[23,24] On the basis of their protein subunits, five major classes of intermediate filaments have been distinguished: cytokeratins pertain to nearly all epithelial cells; vimentin is present in non-muscle tissue of mesenchymal derivation; desmin is present in smooth, skeletal, and cardiac muscle; neurofilaments are present in neurons; and glial fibrillary acidic protein are expressed in astrocytes.[6,7,24,25] Immunolabeling with antibodies against intermediate filaments



FIGS. 3A–3C. Section taken from the central portion of the tumor labeled with antivimentin antibody showing uniformly positive staining of the two cell types in the papillae with this antibody (A). Center of the previous field stained with antiprekeratin antibody demonstrating negative staining of the papillary formations (B). H & E stained section of the preceding areas (same field as in A and B) (C) (Immunofluorescent stain ×400, A,B; H & E ×100, C).



FIGS. 4A–4C. Section taken from the peripheral portions of the tumor showing hyperplastic alveolar cells which are labeled positively with antiprekeratin antibody (A) while the cells surrounding them positively stain for vimentin (B). H & E stained section of the preceding area (C) (Immunofluorescent staining ×400, A,B; H & E ×400 original magnification, C).

may thus allow the positive identification of the specific line of differentiation along these broad categories expressed by the tissues examined.

The results obtained in our case support the existence of a mesenchyme-derived line of differentiation for this tumor. Strong positive labeling with antivimentin antibodies was uniformly demonstrated in the cytoplasm of all the tumor cells including the two morphologically dissimilar cell populations observed in the papillary component. Although it may be difficult to extrapolate from the findings in a single study, the overwhelming vimentin positivity observed in our case strongly argues in favor of a mesenchymal derivation for this neoplasm. An additional feature of the tumor that was revealed by the current study was the positive staining with anticytokeratin antibodies in cells lining cystic spaces in the periphery of the tumor as well as in occasional slit-like spaces located deeper within the tumor. We interpret this finding as indicative of the presence of entrapped epithelial elements, most likely representing distorted alveolar spaces. This latter feature may account for some of the apparently contradictory results reported by previous studies. Entrapment of alveolar spaces within the tumor has been frequently suggested in sclerosing hemangioma of the lung as well as in other tumors of the lungs.[26] Because of the variegated nature of this neoplasm, different elements have been defined as "entrapped" by some authors and as "neoplastic" by others,[4,11-17] and a clear cut distinction between the two may not be easily rendered on the basis of light microscopy. This is particularly true of the deeper areas of the tumor where compression artifact may cause distortion of alveolar spaces, or in areas where the elongated slit-like air spaces begin to merge into the papillary areas of the tumor. Although by light microscopy the distorted air spaces may be easily confused with the spaces separating the papillary formations, immunofluorescence staining will clearly differentiate between the two by showing positive staining of the tumor cells with antivimentin antiserum in the latter and positive staining with anticytokeratin in the former. In the current case, the location predominantly in the periphery of the tumor of the keratin-positive structures, their elongated slit-like

appearance suggestive of compression artifact, and their overall sparcity as compared with the general mass of the tumor are features that support the contention that they represent residual epithelial elements that have been encased within the advancing borders of the lesion. Random sampling from such areas could therefore explain some of the conflicting observations previously reported by other authors.

The findings from this particular case, however, can still not rule out several alternate possibilities for the origin of the tumor. Such alternatives include origin from vascular endothelium,[14,16] or from primitive undifferentiated pulmonary germ cells.[2,12] In light of recent reports pointing out the unreliability of Factor VIII-related antigen determination using the immunoperoxidase technique in the diagnosis of vascular endothelium-derived tumors,[22,27] this latter possibility cannot be categorically excluded until further studies are made available using more sensitive markers. Also, in view of the occasional foci of cytokeratin-positive cells demonstrated by our study, the possibility that this tumor may arise from primitive stem cells bearing the capability to differentiate along multiple cell lines (as has been recently demonstrated for certain lung tumors and other neoplasms[28-32]) also has to be strongly considered. Another theory that has been proposed and which deserved mention is that of Hill and Eggleston[12] who postulated that the entity known as sclerosing hemangioma may be comprised of a spectrum of lesions showing differing degrees of vascular and epithelial proliferation. Finally, the possibility should be entertained that the tumor cells in this neoplasm may be expressing combinations of differentiated features different from those encountered in normal cells, therefore rendering all speculation about the "cell of origin" futile.

A full understanding of the true nature of this neoplasm therefore awaits further study. The development of more sensitive and specific tumor cell markers may hold the key to this problem. Until then, the term "sclerosing hemangioma," which claims historical precedence, remains in our opinion the most adequate designation for this neoplasm.

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