Virchows Archiv A Pathol Anat (1991) 419:513–518

Virchows Archiv A
Pathological Anatomy and Histopathology

Case report

Pulmonary blastoma with a topographic transition from blastic to more differentiated areas

An immunohistochemical assessment of its embryonic nature using stage-specific embryonic antigens

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Received December 27, 1990 / Received after revision April 30, 1991 / Accepted May 2, 1991

Summary. In order to investigate the probable embryonic nature of pulmonary blastoma, immunohistochemical studies were performed using stage-specific embryonic antigens (Le^y, Le^x, sialyl Le^x-i) in a case of pulmonary blastoma with a very wide spectrum of morphological features. The tumour presented a topographic transition from primitive blastic and embryonic areas to more differentiated areas showing diverse differentiation. Blastic areas composed of extremely immature cells were found in most peripheral parts of the tumour. Inside the blastic areas there were "embryonic" areas which morphologically resembled human embryo lungs in the pseudoglandular and canalicular stages. Most central parts of the tumour showed more differentiated features including chondrosarcomatous, leiomyosarcomatous and rhabdomyosarcomatous elements and the common type of adenocarcinomatous element. Electron microscopic observation suggested the blastic and embryonic nature of these immature cell elements. Le^y was expressed in the blastic and pseudoglandular areas. Le^x was expressed in the canalicular areas. These antigens were not expressed in the more differentiated areas. The topographic gradient in the tumour of morphology and antigen expression from the peripheral blastic areas to the central more differentiated areas suggests that the primitive cells gradually differentiated into more mature cells of various directions as the tumour grew in size.

Key words: Pulmonary blastoma – Stage-specific embryonic antigens – Immunohistochemistry

Introduction

Pulmonary blastoma is a rare malignant lung tumour comprising immature epithelial and mesenchymal elements, which morphologically resembles the human embryo lungs (Carter and Eggleston 1980; World Health Organization 1982; Francis and Jacobsen 1983; Snyder et al. 1985). Barnard (1952) first called it "embryoma of the lung". Spencer (1961) described 3 additional cases 10 years later and proposed the term "pulmonary blastoma". Since then, about 100 cases have been reported. Although the definition of the tumour depends on the morphological similarity to embryonic lung, no direct evidence has been reported that the morphological immature cells of the tumour are "embryonic" in nature.

In 1988, Miyake et al. described three carbohydrate antigens, Le^x (SSEA-1), Le^y (fucosyl SSEA-1) and sialyl Le^x-i (sialyl SSEA-1), which were stage-specific and characteristically localized in the development of human embryo lungs. These antigens are useful not only as tumour-related antigens but also as stage-specific markers of embryonic nature in pulmonary tumours.

We report an interesting case of pulmonary blastoma with special emphasis on the evidence for cell immaturity and differentiation in various directions, using antibodies against stage-specific embryonic antigens and immunohistochemical and electron microscopic studies.

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Case report

A 56-year-old male was admitted to Tsukuba University Hospital complaining of dull pain in his left arm and swelling on the anterior chest wall. Chest X-ray films revealed a large tumour shadow, $9 \times 15 \times 17$ cm in size, with heterogeneous densities in the left upper lung field. Computed tomography showed direct tumour invasion into the chest wall. No hilar node metastases were found. Cytological studies of his sputum presented atypical cells compatible with adenocarcinoma, but the needle-biopsy specimen was chondrosarcomatous. After a course of oncostatic chemotherapy for 1 month, left pneumonectomy with a partial resection of the chest wall was performed. The patient died suddenly of a heart attack a month after the operation. No autopsy was performed.

Table 1. Immunohistochemical expression of stage-specific embryonic antigens, epithelial and mesenchymal markers, and other antigens in the various areas and elements of pulmonary blastoma

Specificity	Source of antibodies	Histological areas and elements									
		BA		PA		CA		MDA			
		E	M	E	M	E	M	E	C	MY	F
Le^y	Ohtsuka (Tokushima, Japan)	+	−	++	−	−	−	−	−	−	−
Le^x	Ohtsuka	−	−	−	−	+	−	−	−	−	−
Sialyl Le^x-i	Ohtsuka	−	−	−	−	−	−	−	−	−	−
SC[a]	Dakopatts (Copenhagen, Denmark)	−	−	+	−	+	−	++	−	−	−
CK HMW	Enzo (New York, NY, USA)	−	−	−	−	−	−	++	−	−	−
LMW	Enzo	−	−	++	−	++	−	++	−	−	−
Vimentin	Sanbio (Uden, The Netherlands)	−	−	−	−	−	−	−	++	−	−
S-100[a]	Dakopatts	−	−	−	−	−	−	−	++	−	−
Desmin	Sanbio	−	−	−	−	−	−	−	−	++	−
Muscle actina	ICN Biomed (Lisle, IL, USA)	−	−	−	−	−	−	−	−	++	−
Myoglobin	Miles (Kankakee, IL, USA)	−	−	−	−	−	−	−	−	++	−
CEA	Dakopatts	−	−	−	−	−	−	+	−	−	−
AFP[a]	BioGenex (Dublin, CA, USA)	−	−	−	−	−	−	−	−	−	−

BA, Blastic area; PA, pseudoglandular area; CA, canalicular area; MDA, more differentiated area; E, epithelial element; M, mesenchymal element; C, chondrosarcomatous; MY, myosarcomatous; F, fibrosarcomatous; SC, secretory component; CK, cytokeratin; HMW, high molecular weight (58, 56.5, 56 kDa); LMW, low molecular weight (52.5 kDa); −, no expression; +, focal (<50%); ++, diffuse (≧50%)

[a] Polyclonal

Materials and methods

The surgically removed tumour was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (H&E), Masson's trichrome, periodic acid-Schiff (PAS) reaction with or without diastase, and silver impregnation.

For ultrastructural studies, small pieces from the tumour were initially fixed by 2% glutaraldehyde in 0.1 M phosphate buffer, and post-fixed by 1% osmium tetroxide in the same buffer. Ultrathin sections from Epon-embedded specimens were stained with uranyl acetate and lead citrate, and then examined with a JEM-1200EX electron microscope (Jeol, Tokyo).

Immunohistochemical studies were carried out on selected formalin-fixed and paraffin-embedded tissue sections by using the avidin-biotin-peroxidase method. Monoclonal antibodies (Ohtsuka Assay Lab., Tokushima, Japan) against carbohydrate antigens (Le^x, Le^y and sialyl Le^x-i) were used to investigate the embryonic nature of epithelial elements of the tumour. Additionally, various other antigens were also used to confirm the cell differentiation. The sources of these antibodies are summarized in Table 1.

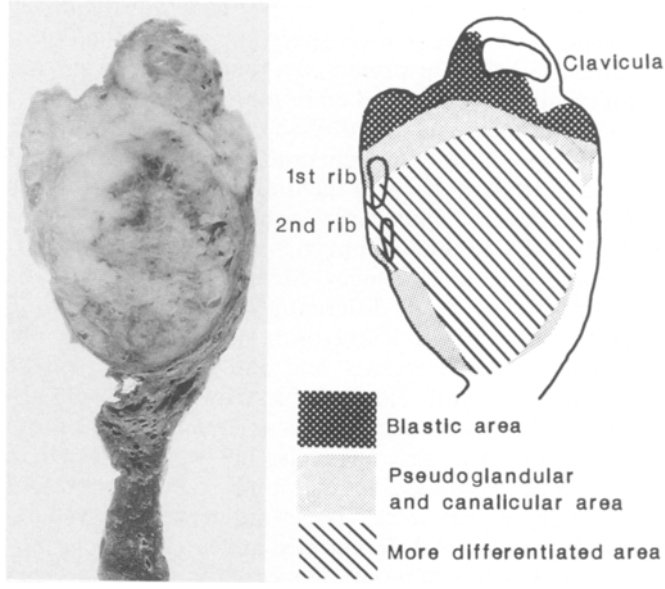

Fig. 1. Pulmonary blastoma. The solid tumour of the lung continuously invading the chest wall shows a topographic distribution of three histological areas: the peripheral blastic areas, the midzone of pseudoglandular and canalicular areas, and the central zone of more differentiated areas

Results

A large and well-encapsulated tumour, measuring 18 × 11 × 8 cm, occupied most of the left upper lobe of the lung (Fig. 1, left). It grew expansively in most parts and was clearly defined from the surrounding compressed lung parenchyma. A part of the tumour had invaded the anterior chest wall, destroying the first and second ribs. The surface of the central part of the tumour mainly showed a cartilaginous appearance mixed with necrotic and haemorrhagic foci. The bronchial tree was not involved. No lymph-node metastases were found.

Histologically, the tumour was mainly divided into three areas according to cell differentiation, though each area showed transition (Fig. 1, right). The first area, which was found in the most peripheral parts of the tumour, was composed of extremely primitive or blastic cells which were small cells with little cytoplasm. The blastic cells proliferated in solid pattern with many mitotic figures and were closely associated with myxomatous immature mesenchymal tissue. There were occasionally undefined borders between epithelial and mesenchymal cells, in which both elements appeared to be

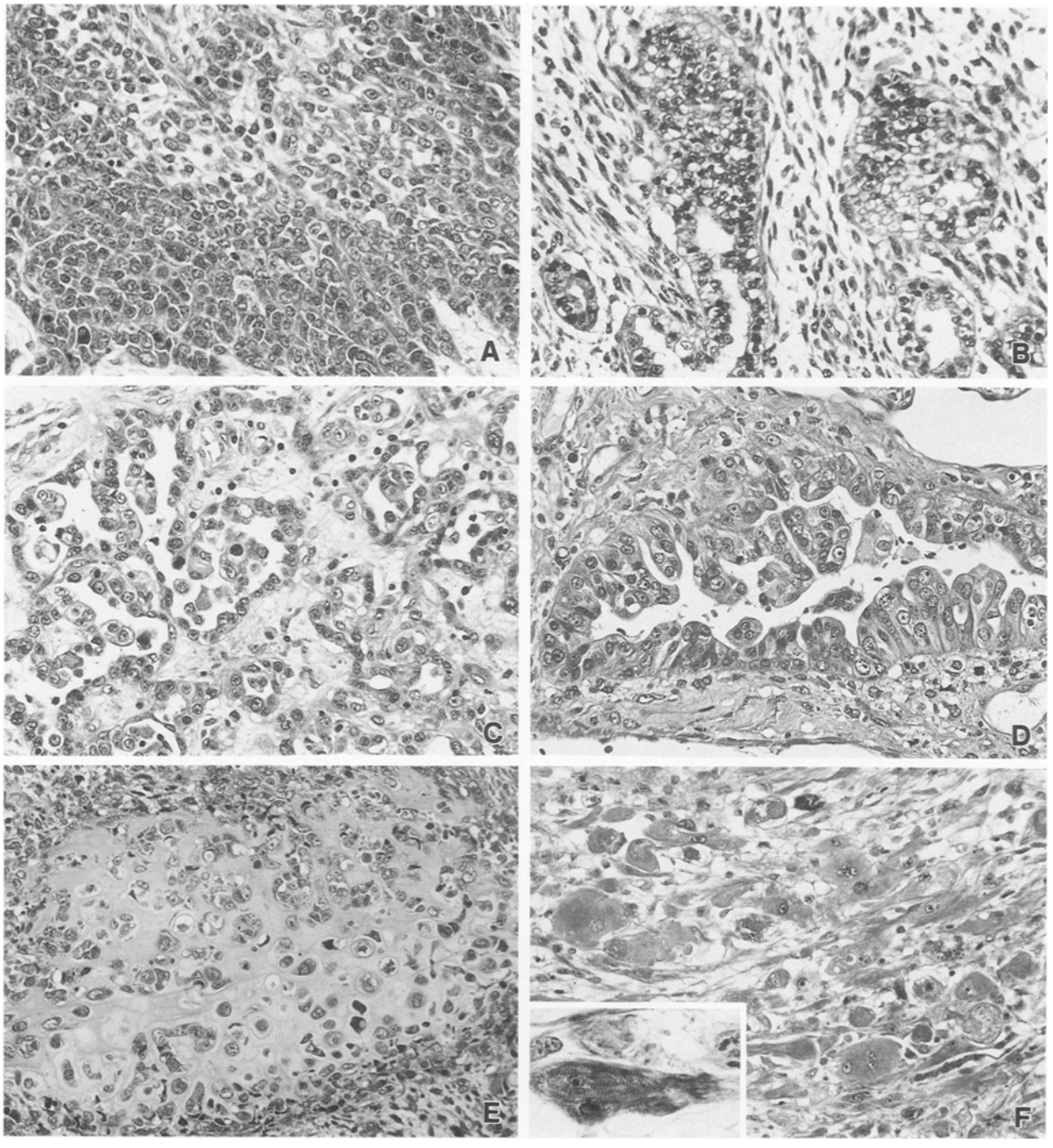

Fig. 2A–F. Variation in the epithelial and mesenchymal elements of the tumour. **A** Blastic area: the extremely primitive cells apparently showing epithelial features proliferate in a solid pattern, but in some areas they make the border unclear against the adjacent immature mesenchymal tissue; H&E, ×220. **B** Pseudoglandular area: the glandular structures lined by glycogen-rich, clear and highly columnar epithelial cells resembling embryo lungs in the pseudoglandular stage; H&E, ×140. **C** Canalicular area: the canalicular structures lined by cuboidal epithelial cells resembling embryo lung in the canalicular stage; H&E, ×220. **D** Epithelial element of more differentiated area: papillotubular growth of relatively larger cells with eosinophilic cytoplasm compatible with the histology of pulmonary adenocarcinoma of the common type; H&E, ×220. **E** Chondrosarcomatous appearance of the more differentiated area: atypical chondroblasts noted within the cartilage matrix; H&E, ×140. **F** Rhabdomyosarcomatous appearance of the more differentiated area: the polymorphic eosinophilic tumour cells with atypical nuclei occasionally containing striated myofilaments; H&E, ×220; *inset:* Masson's trichrome, ×440

transitional (Fig. 2A). The second area, which existed inside the blastic area, resembled human embryo lungs in the pseudoglandular and canalicular stages. The epithelial elements in the areas formed glandular or microcystic structures, which were lined by either glycogen-rich columnar cells or cuboidal cells with relatively monotonous nuclei (Fig. 2B, C). The mesenchymal tissue surrounding the structures was also immature. The cen-

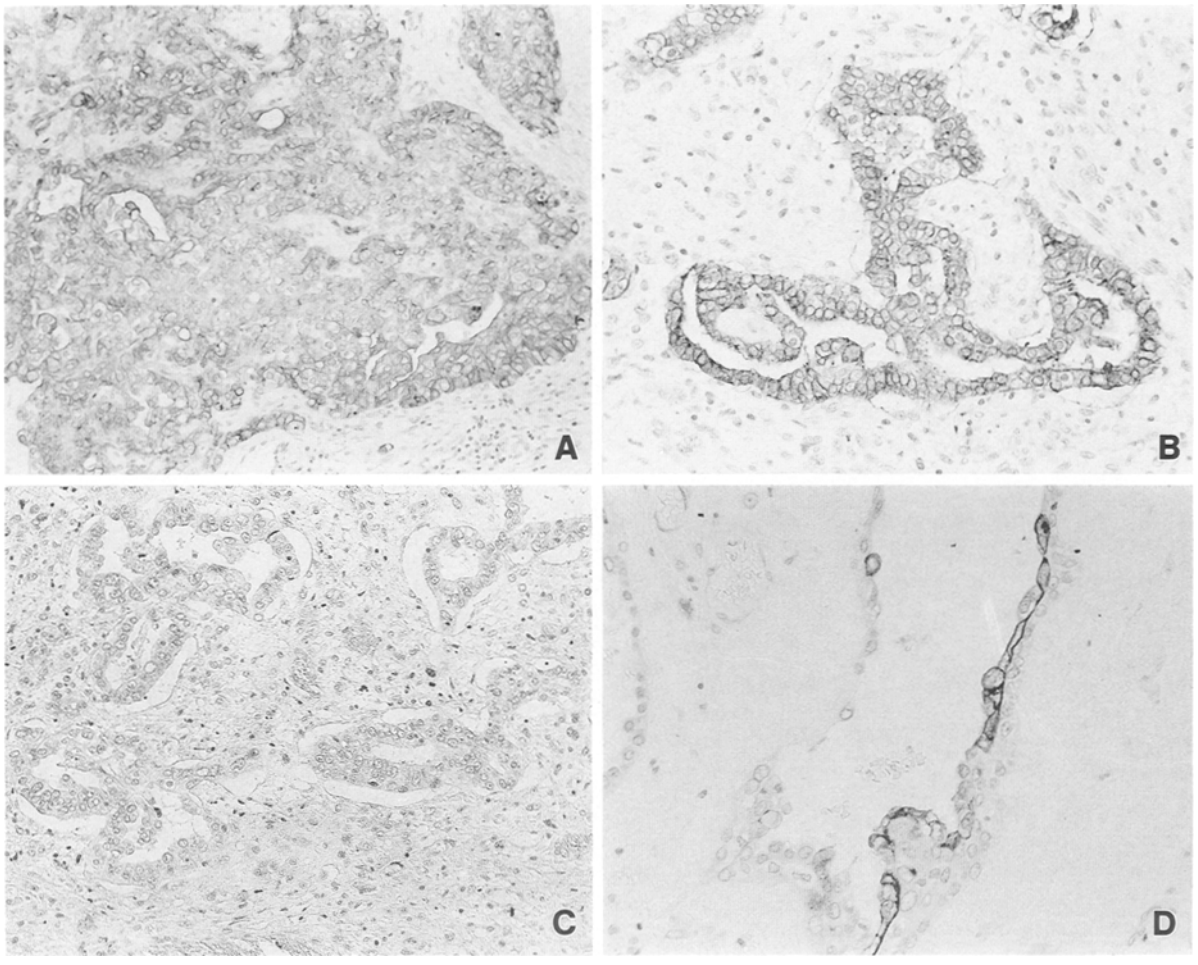

Fig. 3A–D. Immunohistochemical study of stage-specific embryonic antigens in pulmonary blastoma. Le^y antigen is much more positive in the pseudoglandular area (**B**) than in the blastic area (**A**). Immunoperoxidase, ×110. Le^x is negative in the pseudoglandular area (**C**), but clearly positive in the canalicular area (**D**). Immunoperoxidase, ×174

tral part of the tumour, which was the third area, showed various patterns of more differentiated features. The epithelial element consisted of larger cells that were more polymorphic than those of the above-described immature epithelial cells and were quite similar to those in adenocarcinoma of the common type of human peripheral lung (Fig. 2D). The mesenchymal element of this area was differentiated in various directions, which included chondrosarcomatous, rhabdomyosarcomatous, leiomyosarcomatous and fibrosarcomatous differentiation. Chondrosarcomatous parts contained various amounts of hyaline cartilage matrix, including atypical chondroblasts (Fig. 2E), and rhabdomyosarcomatous parts were composed of polymorphic cells with abundant eosinophilic cytoplasm. Striations were occasionally detected (Fig. 2F).

In electron microscopy, the cell nests of the blastic areas were mostly outlined by basal laminae, but there were some parts in which the defined basal laminae were absent between the epithelial and mesenchymal elements. These blastic cells contained a very small amount of rough-surfaced endoplasmic reticulum and a few mitochondria. Most blastic cells which histologically formed clusters were connected to each other by junctional complexes, though their intercellular spaces were relatively wide. The epithelial cells which proliferated in pseudoglandular or canalicular pattern contained a large pool of glycogen in their cytoplasm, but cytoplasmic organelles were few. The immature mesenchymal cells located in the loose mesenchymal matrix surrounding the blastic and embryonic epithelial cell nests were simple in feature and had few cytoplasmic organelles. In more differentiated areas, the epithelial cells had abundant rough-surfaced endoplasmic reticulum and mitochondria, and some cells contained electron-dense spherical bodies, which were comparable with secretory granules in normal non-ciliated bronchiolar cells (Clara cells). Sarcomatous cells showed a characteristic ultrastructure corresponding in differentiation with features of malignant chondroblasts and myogenous cells.

The immunohistochemical results are summarized in Table 1. Epithelial markers such as secretory component and cytokeratin of low molecular weight were identified in the epithelial elements of the pseudoglandular, canali-

cular and more differentiated areas, while they were negative in the blastic cells. Carcinoembryonic antigen-positive cells were found only in the cells of the adenocarcinomatous element. Vimentin and S-100 protein were strongly positive in the chondrosarcomatous tissue, and desmin, muscle actin and myoglobin were positive in the myosarcomatous tissue. These cytoplasmic proteins specific to mesenchymal cells were not demonstrated in the blastic and embryonic areas. The expression of stage-specific embryonic antigens mostly corresponded to the morphological features of the tumour cells. Le^y was expressed in some epithelial cells of the blastic areas and diffusely expressed in the pseudoglandular areas (Fig. 3A, B). Le^x was less positive than Le^y, but the epithelial cells simulating embryonic lung in the canalicular stage were definitely stained (Fig. 3C, D). These antigens were not expressed in the adenocarcinomatous cells of the more differentiated areas. Sialyl Le^x-i was not demonstrated in any of the tumour cells.

Discussion

This tumour contained not only blastic and immature components simulating human embryonic lung in different stages of development, but also a more differentiated component showing diverse differentiation. This is an extremely rare case in terms of the variety of cell differentiation seen, since the majority of cases of pulmonary blastoma were of a "pure" type, which shows a monotonous structure resembling the immature pseudoglandular stage of pulmonary organogenesis (Spencer 1961; Bauermeister et al. 1966; Karcioglu and Someren 1974; Carter and Eggleston 1980). Only cartilage and striated muscle were demonstrated among the immature elements in several cases (Barson et al. 1968; Stackhause et al. 1969; Iverson and Straehly 1973; McCann et al. 1976; Roth and Elguezabal 1978; Edwards et al. 1979; Heckman et al. 1988). The present case contained a more differentiated component, composed of the mixed structures of the common type of pulmonary adenocarcinomatous element and various types of sarcomatous elements. The carcinomatous cells contained Clara cell granules, which are characteristic secretory granules of non-ciliated bronchiolar epithelium and of peripheral adenocarcinoma of the lung (Ogata and Endo 1984).

Furthermore, the present case showed a topographic gradient from the peripheral zone of blastic areas to the central zone of more differentiated areas through a mid-zone of pseudoglandular and canalicular areas. This finding indicates the possibility that the blastic tumour cells gradually developed into more differentiated cells of various types as the tumour grew in size. The topographic growth pattern may be important in understanding the structural varieties within this tumour.

With regard to the stage-specific embryonic antigens, Miyake et al. (1988) demonstrated in their studies of human embryo lungs that Le^x, Le^y and sialyl Le^x-i were expressed successively in the budding epithelium in the developing stages. According to their findings, Le^y is the initial embryonic antigen which appears at lung buds rising from foregut in the approximately 38-day-old embryos and is maximally expressed in early bronchial buds in 50-day-old embryos (pseudoglandular stage); Le^x antigen next appears at branching bronchial buds and is maximal in 12-week-old embryos (canalicular stage); sialyl Le^x-i is the final embryonic antigen expressed at terminal buds and maximally expressed in 18-week-old embryos. Our present study demonstrated that the immature structures of a pulmonary blastoma expressed each embryonic antigen. Their expression patterns corresponded to those of the developmental stages of human lungs. These findings support the ideas that the immature cells of pulmonary blastoma are "embryonic" in nature, and that the tumour may develop in the same way that lung organogenesis occurs in human embryo.

Epithelial and mesenchymal markers were expressed in the pseudoglandular, canalicular and more differentiated components corresponding to the expected cell differentiation. These markers were negative in the blastic areas, though Van Muijen et al. (1987) reported that the expression of cytokeratin occurs earlier in development than the pseudoglandular state of embryo lungs and Samuel et al. (1990) demonstrated co-expression of vimentin and keratin in the blastematous elements. There is no immunohistochemical evidence suggesting a transition between the epithelial and mesenchymal elements, even though no defined borders between them were seen in the blastic areas on histology and electron microscopy. The results may reflect the extreme immaturity of the blastic cells of the present case.

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