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Ultrastructural observations on the histogenesis of localized fibrous tumours of the pleura (benign mesothelioma)

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Summary. Five localized fibrous tumours of the pleura (benign mesothelioma) were studied ultrastructurally in order to elucidate their histogenesis. The histological subtypes of this benign fibrous lesion of the visceral pleura, i.e. the cellular, the collagenous, and the hyaline, were separately analysed.

The tumours are composed of undifferentiated mesenchymal cells, intermediate and differentiated fibroblasts as well as collagenous interstitial tissue. The varying distribution of these cell elements account for the various histological subtypes. Morphological similarities between the mesenchymal tumour cells and the superficial mesothelial cells, which are always separated from the true tumour tissue by an intact basement membrane, were not observed.

The different cellular elements can be regarded as parts of a continuous spectrum of cytodifferentiation, in which the mature fibroblasts are derived via intermediate forms from the undifferentiated cells. It is concluded that the localized fibrous tumours of the pleura arise from immature mesenchymal stem cells, which seems to be normally found in the submesothelial layer of the visceral pleura.

Key words: Localized fibrous tumour of the pleura – Benign mesothelioma – Ultrastructure

Benign localized as well as malignant diffuse mesotheliomas are divided into three histological types: epithelial, biphasic, and fibrous (WHO 1981; Enzinger and Weiss 1983). The fibrous type is the most frequent variant of the localized tumours, which are nearly always found in the visceral pleura. Their clinico-pathological features have been well documented in recent surveys (Blanchon et al. 1978; Scharifker and Kaneko 1979; Briselli et al. 1981), but their histiogenesis has not been clearly established. Since

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in recent light and electron microscopic studies the mesothelial origin of these fibrous tumours has been questioned, it has been suggested that we should replace the term "benign mesothelioma" by the more indifferent term "localized fibrous tumour of the pleura" (LFP) (Hernandez and Fernandez 1974; Dalton et al. 1979; Scharifker and Kaneko 1979).

It was the purpose of the present study of five LFP to contribute further ultrastructural findings that might elucidate the unresolved histogenesis of these tumours.

Materials and methods

The material for this study include clinical histories, surgical specimens, and follow-up data of 5 cases with LFP.

For light microscopy, surgical specimens were fixed in phosphate buffered 10% formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin, van-Gieson, Gomori's method for reticulin, resorcin-fuchsine, periodic acid Schiff (PAS), and alcianblue. In addition, representative sections were immunostained using the peroxidase anti-peroxidase (PAP) technique (Sternberger 1979). Commercial antisera from rabbits to alpha-1-antitrypsin and lysozyme (Dakopatts A/S, Copenhagen, Denmark) were employed.

For electron microscopy, small tissue blocks were taken from the tumours surface, its center, and its base. The tissue blocks were fixed in 3% cacodylate-buffered glutaraldehyde, postfixed in osmic acid, processed routinely and embedded in Spurr's resin. In each case 12–16 blocks were studied and semi-thin sections were stained with toluidine-blue. Ultra-thin sections of corresponding areas were contrasted with uranyl acetate and lead citrate.

A histological subclassification of the studied LFPs was done accordingly to the criteria described previously (Bürrig et al. 1983).

Results

The clinical data and gross findings are summarized in Table 1.

Light microscopy

The five tumours show a distinct variation in their cellularity, extent of vascularisation, and amount of collagenous interstitial tissue, resulting in the different histological subtypes (Table 1). The common histological pattern consists of oval to fusiform cells resembling fibroblasts and reticulin as well as collagen fibers surrounding capillary blood vessels. Though sometimes arranged in interdigitating fascicles, whorls or storiform patterns, the cells are mostly randomly distributed. Large amounts of hyaline collagen occur in two tumours, and one of these displays numerous spherical calcifications. In the more cellular variants small myxoid areas are frequently seen in the vicinity of blood vessels. Mitoses are, in general, infrequent.

The free surface of the tumour is usually covered by a single layer of flattened mesothelial cells lacking signs of proliferative activity (Fig. 5a). Peripheral lung parenchyma forms the subjacent tissue (Fig. 6a). In some areas trapping of the bronchiolo-alveolar epithelium into the tumour tissue is noticed, sometimes mimicking a glandular pattern.

None of the five tumours is positively stained for alpha-1-antitrypsin or lysozyme.

Table 1. Summary of clinico-pathological data of 5 cases of localized fibrous tumour of the pleura

Age/sex	Initial manifestation	Localisation in the visceral pleura	Size (cm)	Gross findings	Histologic subtype	Follow-up
58 m	Routine X-ray examination	Left upper lobe	1.3:1:0.9	Firm, grey-whitish, sessile	Collagenous	Alive and well 2 years
47 m	Routine X-ray examination	Right lower lobe	2.2:1.8 5 g	Firm, grey-whitish, sessile	Hyaline	Alive and well 2 years
51 f	Routine X-ray examination	Left lower lobe	2.5:1.8:1.2	Firm, pale-grey, whorled cut surface, sessile	Hyaline	Alive and well $1^1/_2$ years
49 m	Routine X-ray examination	Left lower lobe	5.5:4:3.5 46 g	Firm, pale-grey, whorled cut surface, sessile	Cellular	Alive and well 1 year
57 f	Routine X-ray examination	Right lower lobe	4:4:2 15 g	Firm, pale-grey, pedunculated	Cellular	Alive and well $^1/_2$ year

Electron microscopy

At ultrastructural level, there are three different cell types: undifferentiated mesenchymal cells, intermediate and mature fibroblasts. Transitional forms between these cell types are, however, also present.

The undifferentiated cells are round to oval shaped, their nuclei are rounded with focal invaginations, finely dispersed chromatin, and inconspicuous nucleoli (Fig. 1a and b). The thin cytoplasmic rim contains only few poorly developed organelles. Because of their morphological pattern these cells are comparable to pluripotent mesenchymal stem cells. They are predominantly found in the more cellular tumour areas and are mostly seen in the surrounding of capillaries (Fig. 1a). In these areas the extracellular components consist of a amorphous ground substance and very few scattered collagen fibrils.

The intermediate fibroblasts are usually found in clusters being sometimes separated by delicate bundles of collagen fibrils. Frequently, they are intermingled with undifferentiated cells as well as mature fibroblasts (Fig. 2b). Though the intermediate cells are sometimes arranged in a seemingly epithelial pattern, true desomosomes or basement membrane material or microvilli are not present. Occasionally mitotic figures are observed in these cell clusters (Fig. 2b). Individual cells of this type vary in shape from oval to polygonal and are seldom as elongated as typical fibroblasts

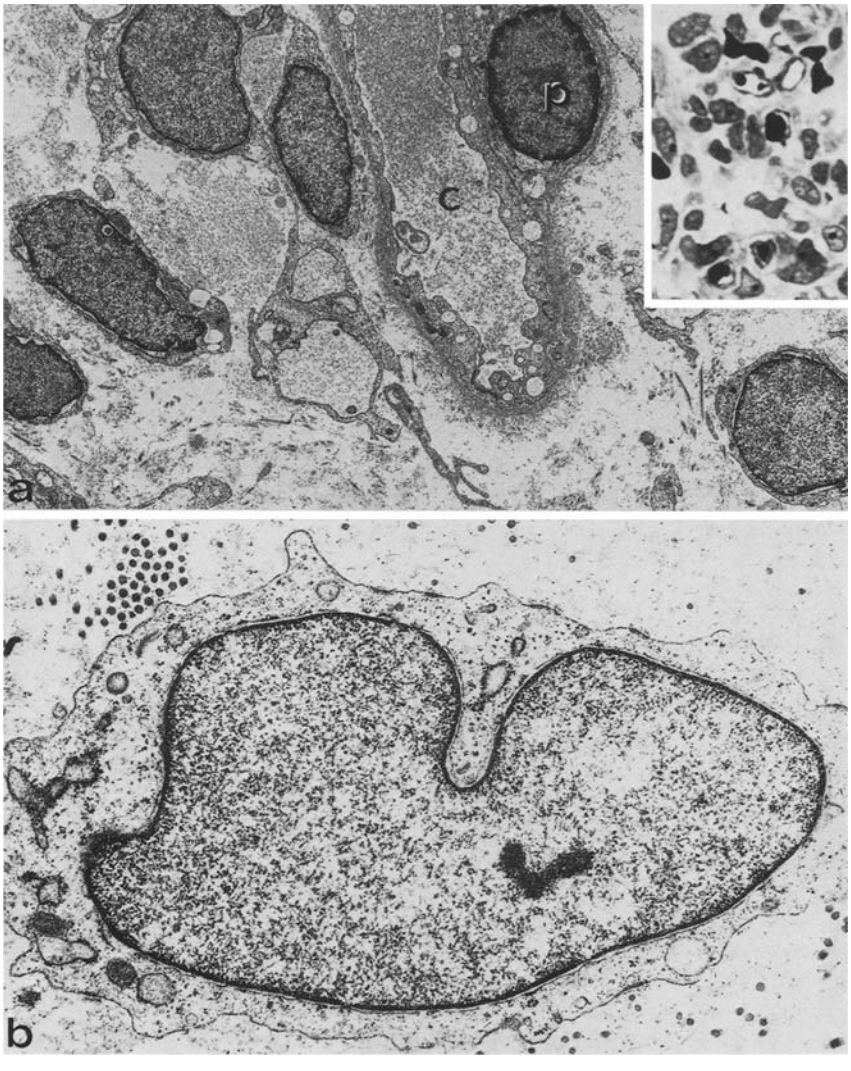

Fig. 1. Undifferentiated cells in the localized fibrous tumour of the pleura (LFP): **a** Tumour cells in the vicinity of a capillary (*c*: capillary lumen; *p*: pericyte). ×4,500. *Inset:* Semi-thin section of a corresponding area with small capillaries and randomly distributed tumour cells. Toluidine-blue, ×1,000. **b** Higher magnification of a undifferentiated cell with poorly developed cytoplasm and euchromatic nucleus. ×12,500

(Fig. 2a). The nuclei are oval having fairly homogenously chromatin and usually small nucleoli. The cytoplasmic components are better developed than those of the undifferentiated cells but signs of functional activity are mostly lacking.

The mature fibroblasts, the third cell type, are seen in various stages of functional activity.

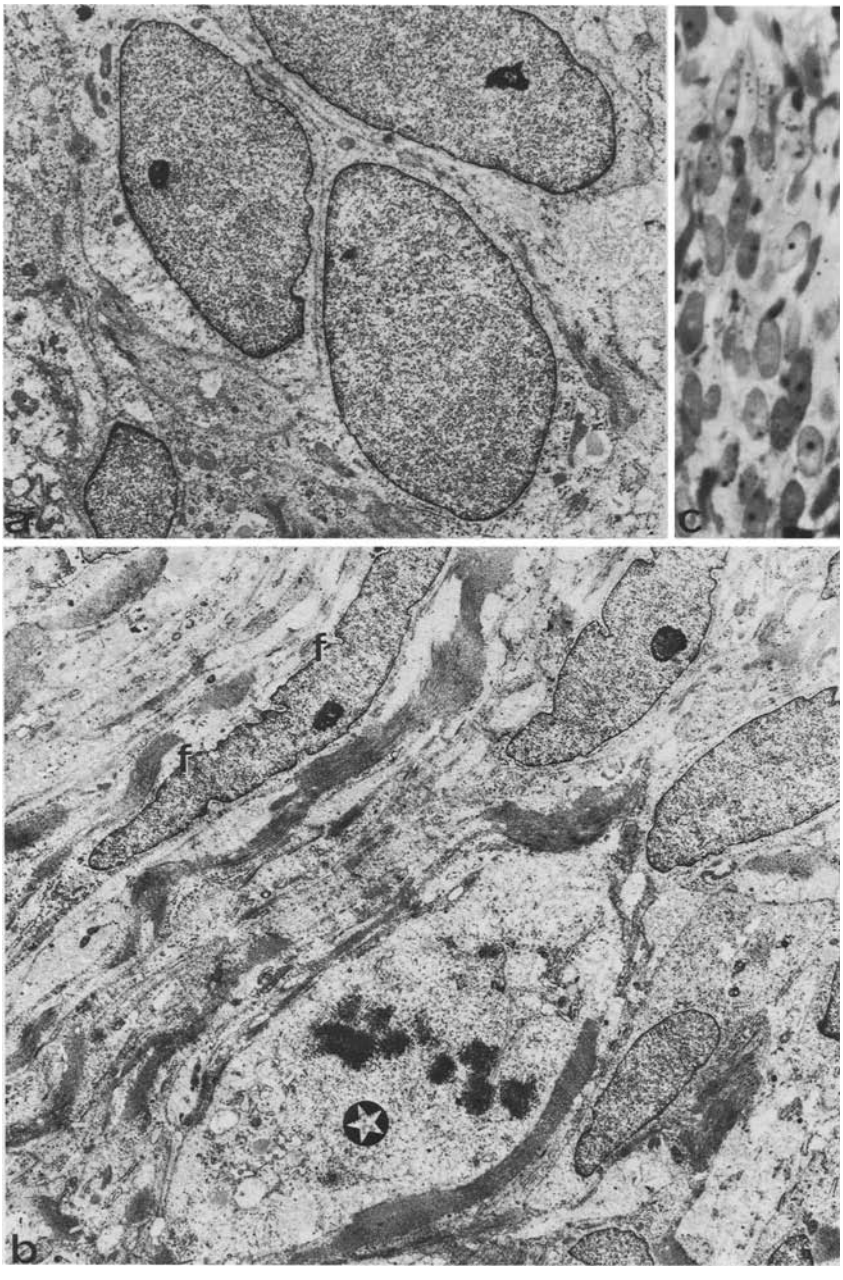

Fig. 2. Intermediate fibroblasts in the LFP: **a** Cluster of less differentiated tumour cells. ×11,000. **b** Tumour cells with increasing fibroblastic differentiation intermingled with a mature fibroblast (*f*). Mitotic figure (*star*). ×9,100. **c** Semi-thin section of clustered tumour cells in a cellular subtype of LFP. Toluidine-blue, ×1,100

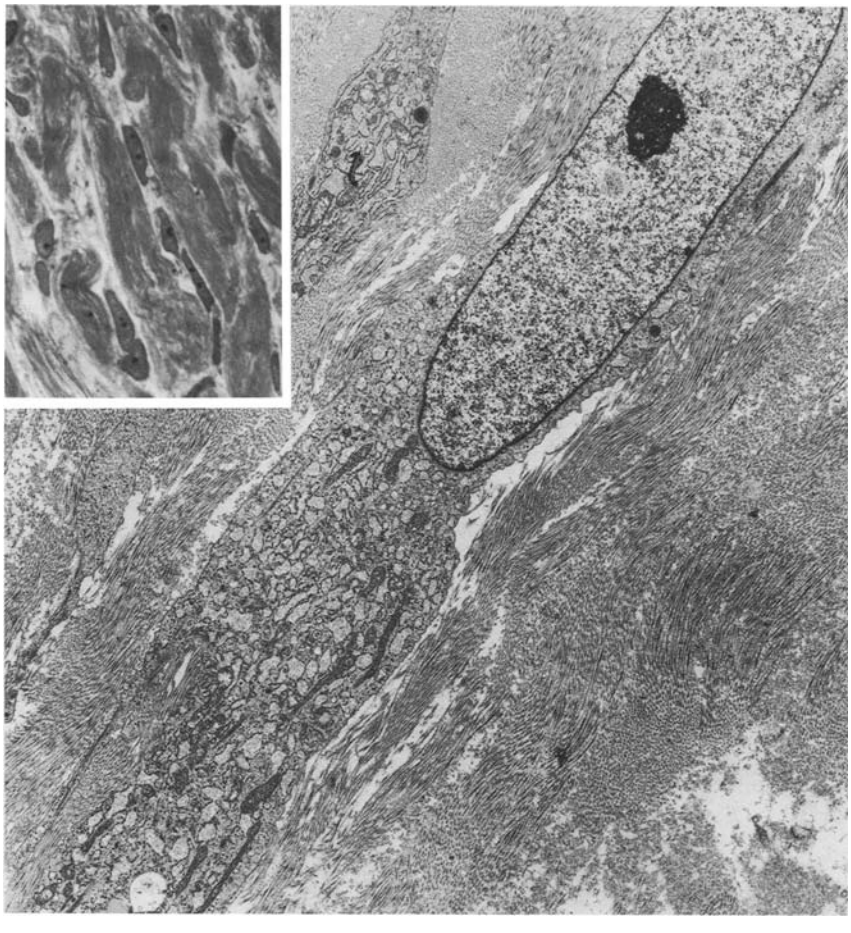

Fig. 3. Active fibroblast featuring abundance of rough endoplasmic reticulum and elongated mitochondria. ×9,200. *Inset:* Semi-thin section of a collagenous subtype of LFP with active looking fibroblasts. Toluidine-blue, ×1,100

The active looking fibroblasts have abundant cytoplasm arranged in polar extension or in a star-shape. The nuclei are oval or elongated with finely granular chromatin and prominent nucleoli, sometimes situated in marginal position. The most striking feature of the cytoplasm is the large amount of rough endoplasmic reticulum expanded into closely packed stalks of cisternae (Fig. 3). Furthermore, well developed Golgi zones and enlarged mitochondria are found. Mostly, these cells are randomly distributed and separated by bulky collagen fibers.

The inactive looking fibroblasts are characterized by a fusiform cytoplasm and nucleus (Fig. 4a). The latter shows a considerable amount of coarse heterochromatin. The cytoplasmic organelles are inconspiciuous, only the rough endoplasmic reticulum is sometimes irregularly dilated. These fibroblasts can be arranged in parallel (Fig. 4b) or in wide-meshed networks,

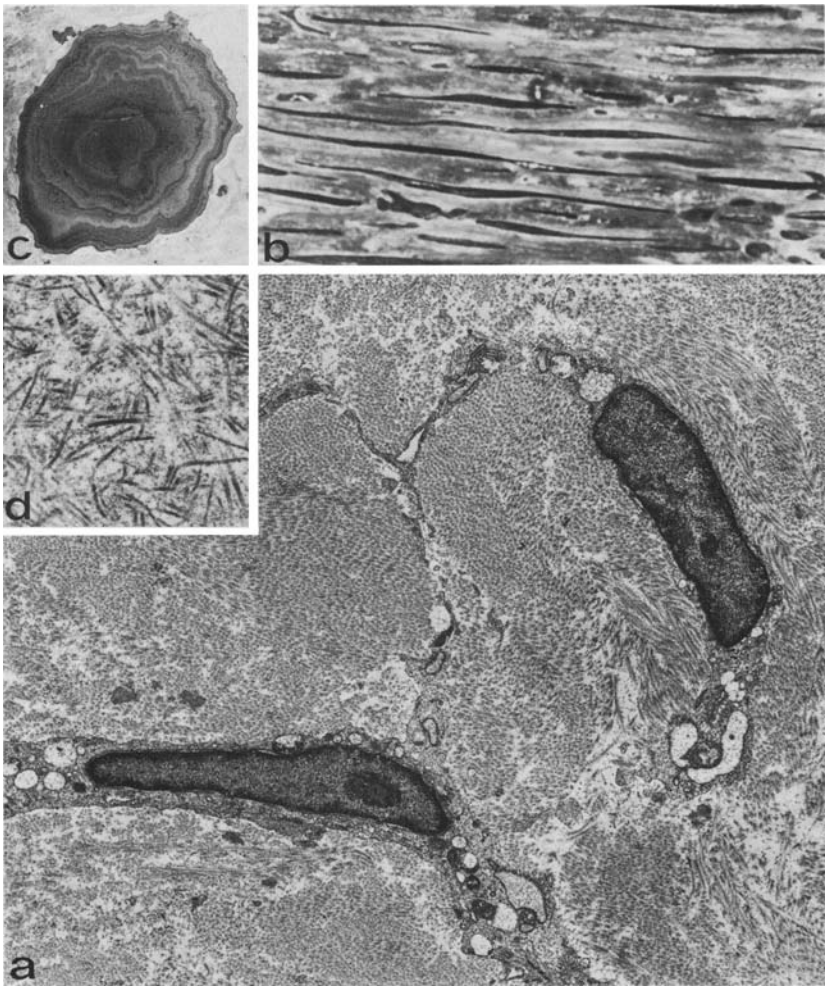

Fig. 4. **a** Inactive fibroblasts scattered in heavily collagenized interstitial tissue. ×4,800. **b** Semi-thin section showing parallely arranged fusiform fibroblasts in a hyaline subtype of LFP. Toluidine-blue, ×1,100. **c** Spherical calcification in a hyaline subtype. ×1,600. **d** Irregular meshwork of collagen fibrils in a hyaline subtype. ×12,500

but they are mostly scattered throughout large amounts of collagenous interstitial tissue. In the latter case, the collagen fibrils are often haphazardly arranged (Fig. 4d) and spherical calcifications can be observed (Fig. 4c).

The histological pattern and the special subtype of LFP, respectively, is dependent on the frequency in distribution of these cell types. Though, the cellular variant consists of immature cells and differentiated fibroblasts in approximately equal proportions. In the collagenous and hyaline subtype, however, the mature fibroblasts clearly prevail and inactive looking fibroblasts predominate in the latter.

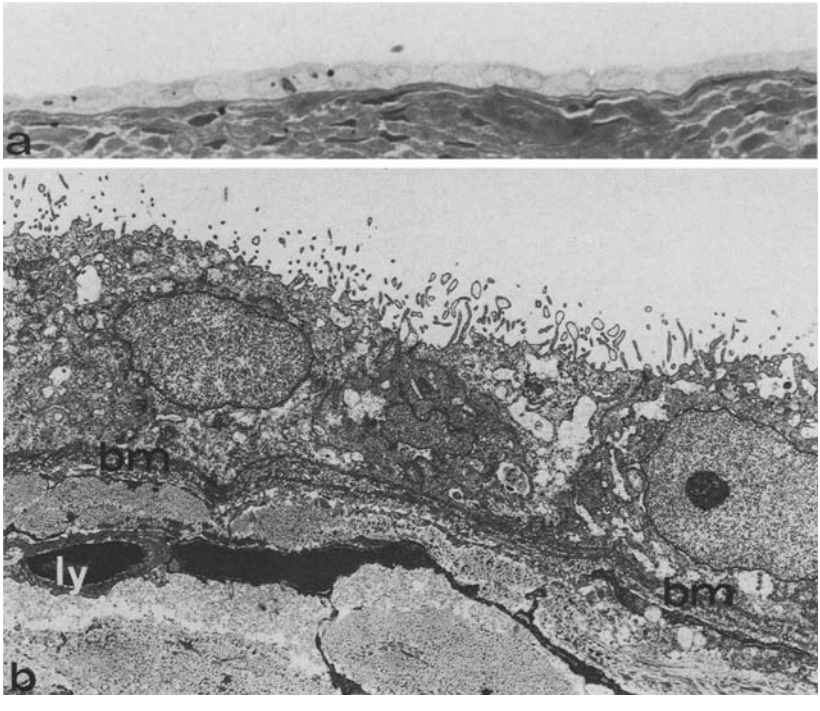

Fig. 5. **a** Semi-thin section of the tumour surface covered by a single layer of mesothelial cells. Toluidine-blue, ×400. **b** Ultrastructural details of **a** showing mesothelial cells with slender microvilli separated from the tumour tissue by a distinct basement membrane (*bm*) (*ly*: a lymphocyte within the tumour). ×4,200

In addition to the cells described above, sparsely spread inflammatory cells such as lymphocytes, plasma cells, and mast cells are observed.

In all cases the free surface of the tumour is covered by a single layer of mesothelial cells, which are separated from the true tumour by an always continuous basement membrane (Fig. 5b). Likewise, the bronchiolo-alveolar epithelium of the lung alveoli adjoining to the tumor base is outlined by a distinct basement membrane (Fig. 6b). In these areas, the bronchiolo-alveolar epithelium mainly consists of type 2 pneumocytes.

Discussion

A basic concept of the histogenesis of primary pleural tumours was provided first by Klemperer and Rabin (1931), who supposed that the diffuse ones arise from the mesothelial lining cells whereas the localized ones orginate from the submesothelial areolar tissue. Subsequently, the discussion on the histogenesis of localized pleural tumours has been complicated by a confusing terminology and greatly influenced by tissue culture studies, which led to the conclusion that all primary pleural tumours including localized fibrous ones arise from the surface mesothelial lining cells (Stout and Murray

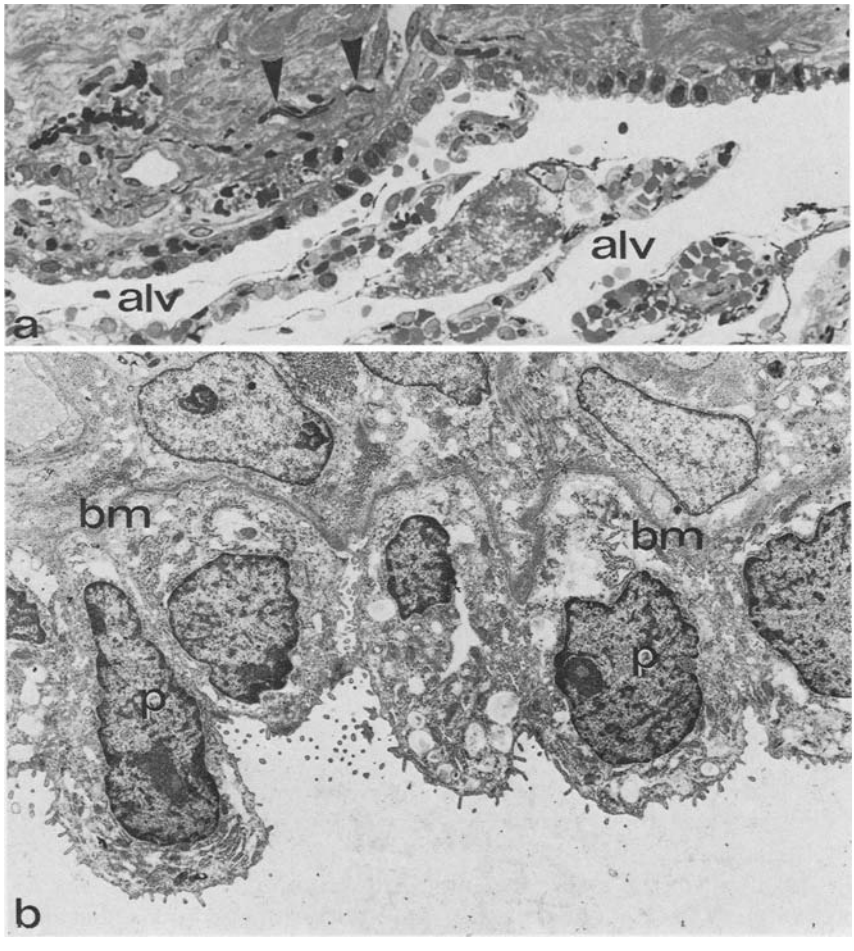

Fig. 6. a Peripheral lung parenchyma subjacent to the tumour base (*alv*: alveoli). Within the tumour rudimentary elastic lamellae of submesothelial areolar tissue are present (*arrowheads*). Semi-thin section, toluidine-blue, × 300. **b** Ultrastructural details of **a** showing adjoining bronchiolo-alveolar epithelium consisting of type 2 pneumocytes (*p*). A distinct basement membrane outlines the epithelium (*bm*). × 4,200

1942; Sano et al. 1950). These authors reported that cultured fragments of fibrous pleural tumours produced cells similar to mesothelial cells. In explanation of the fibroblastic character of these tumours, an earlier study of Maximow (1927) was cited, who had shown that mesothelial cells of peritoneal effusion could differentiate into fibroblast-like cells. This view has been generally accepted (Stout 1950; Stout and Himadi 1951; Godwin 1957; Luse and Spjut 1964; Osamura 1977; Donna and Betta 1981) and indeed, transitional forms between mesothelial and fibroblastic cells have been identified ultrastructurally in malignant diffuse mesotheliomas (Bolen and Thorning 1980; Suzuki 1981).

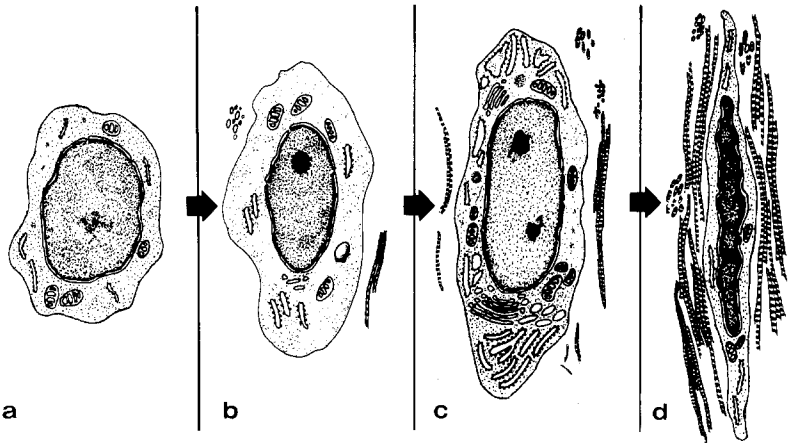

Fig. 7. Schematic drawing showing the individual steps of the suggested cytomorphogenesis of tumour cells in the LFP. **a** Undifferentiated mesenchymal cell representing the tumour stem cell. **b** Intermediate fibroblast. **c** Active fibroblast. **d** Inactive fibroblast

Furthermore, it has been claimed that tumour cells of LFP also display morphological characteristics of mesothelial cells (Luse and Spjut 1964; Kay and Silverberg 1971; Wang 1973; Osamura 1977; Briselli et al. 1981; Huu et al. 1983). However, the quoted structures such as blunt cytoplasmic processes, oligocilia, primitive junctions as well as glycogen particles are non-specific characteristics and are to be seen even in other fibroblastic lesions (Erlandson 1981; Ghadially 1982). Moreover, the suspected basement membrane material is either comparable with the external lamina of mesenchymal cells (Ghadially 1982) or entraped bronchiolo-alveolar epithelium has been misinterpreted as an epithelial component of the tumour (Luse and Spjut 1964). Finally, to our knowledge, unequivocal microvilli or true desmosomes have not been illustrated in LFP so far. In summary, as confirmed by our study, tumour cells of LFP lack the signs of mesothelial differentiation.

Since the histological pattern of LFP sometimes is closely similar to fibrohistiocytic lesions, a histiocytic nature of these tumours has been considered (Germouty et al. 1976). Histiocytic differentiation of the tumours in the present study can be largely excluded since no lysosomal activity or immunohistochemical markers such as alpha-1-antitrypsin or lysozyme were demonstrated (Kindblom 1982).

In the present study it is shown, that the LFP consist of mesenchymal cells with varying degrees of cytoplasmic differentiation. At the one end there are undifferentiated cells comparable to multipotent mesenchymal stem cells. At the other end there are mature fibroblasts, which can be distinguished from the intermediate fibroblasts by the development of their organelles, particularly the rough endoplasmic reticulum. These cell elements seem to be part of a continuous spectrum of cytodifferentiation, in which the more advanced stages develope from the immature stem cells (Fig. 7). The striking similarities between the different cell types of the presented

tumours and corresponding cells during the development of fibroblasts in connective tissue (Grillo 1963; Ross and Odland 1968) support this conclusion.

Varying proportions of the described cell elements account for the various subtypes of LFP and the biological behaviour of these various subtypes probably is a reflection of the supposed process of cytodifferentiation. When surgically removed, the collagenous and hyaline variants are usually smaller than the cellular ones (Briselli et al. 1981; Bürrig et al. 1983). In the latter immature cells featuring proliferative activity are frequently encountered. In contrast, the collagenous and hyaline variants are mainly composed of more differentiated cells.

The primary concept of submesothelial origin of localized pleural tumours (Klemperer and Rabin 1931) as far as LFPs are concerned, is reinforced by our findings and other ultrastructural studies (Hernandez and Fernandez 1974; Dalton et al. 1979). Further, a more recent tissue culture study showing only fibroblastic outgrowth of tumour cells of LFP support this concept too (Alvarez-Fernandez and Diez-Nau 1979) and furthermore evidence is given by the presence of a definite intact layer of mesothelial cells covering the tumours (Hernandez and Fernandez 1974; Scharifker and Kaneko 1979).

In this regard, it has been assumed that submesothelial fibroblasts are the origin of LFP (Hernandez and Fernandez 1974). Still submesothelial fibroblasts seem to be derived from primitive cells of the perivascular tissue (Ross and Odland 1968; Raferty 1973a and 1973b) and, as mentioned already, those primitive mesenchymal cells are often found in the LFP, especially in the cellular ones. Our conclusion is that these immature mesenchymal cells of the submesothelial areolar tissue are the cells from which the LFP arise.

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