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Localized fibrous tumour of serosal surfaces

Immunohistochemical and ultrastructural evidence for a type of mesothelioma *

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Summary. It is uncertain whether localized lesions of serosal membranes have a kinship to mesotheliomas or are truly fibromatous in nature. Ultrastructural and immunohistochemical investigations were carried out on 12 localized benign and malignant pleural and peritoneal tumours from 10 patients. Electron microscopic findings, including the consistent and non-fibroblastic cellular organization of localized neoplasms, the presence of some form of intercellular junctions in 7 of 10 cases, basal lamina deposition in 3 cases, and polarized microvilli in one case indicated a form of mesothelial differentiation. Using monoclonal and polyclonal antibodies, positive immunostaining of tumour cells for cytokeratin peptides was detected in one case, while antibody to vimentin stained four cases. Light microscopic, ultrastructural and immunohistochemical features of one benign localized serosal tumour, with a unique blend of epithelial and spindle cells, provided further evidence for a histogenic link between localized serosal tumours and diffuse epithelial mesotheliomas. On the basis of the current findings and reports in the literature, it would appear that the majority of localized tumours of serosal membranes are a subset of mesothelioma, while a minority are fibromas.

Key words: Mesothelioma – Serous membrane – Localized tumour – Histogenesis – Ultrastructure

Introduction

Localized tumours of serosal surfaces are generally benign, well circumscribed spindle cell neoplasms. Although many such tumours were described in earlier literature, it was not until 1931 when Klemperer and Rabin re-

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ported on five localized lesions of pleura that these neoplasms became established as a distinct entity. They classified these tumours with others that arose on serosal surfaces and their classification scheme was much the same as that currently in use. However, controversy continues over the cellular origin of these localized tumours and their histogenic link with other tumours of serosal surfaces.

Two principal hypotheses are in vogue with respect to the histogenesis of serosal tumours and such designations as localized mesothelioma and solitary fibrous tumour of pleura emphasize the opposing viewpoints. Though both theories postulate origin from submesothelial cells, one implicates a specialized cell capable of differentiation to surface mesothelium (Dalton et al. 1979; Scharifker and Krancko 1979; Poroshin and Krylou 1981), while the other suggests derivation from a nonmesothelial or fibroblast-like cell (Luse and Spjut 1964; Hernandez and Fernandez 1974; Alvarez-Fernandez and Diez-Nau 1979). Ultrastructural and immunohistochemical observations with conflicting interpretations have been reported. Some authors find cells in localized serosal tumours with some surface mesothelial cell characteristics (Kay and Silverberg 1971; Osamura 1977; Kawai et al. 1978; Bolen and Thorning 1980; Benisch et al. 1981; Briselli et al. 1981; Dardick et al. 1984a), while others report cases composed of less differentiated spindle cells usually interpreted as being fibroblasts (Luse and Spjut 1964; Wang 1973; Hernandez and Fernandez 1974; Briselli et al. 1981; Bürrig and Kastendieck 1984; Said et al. 1984). Such investigations require reassessment in light of reports of diffuse mesotheliomas displaying a spectrum of tumour cell differentiation from epithelial cells with mesothelial characteristics to spindle cells with fibroblast-like features (Suzuki et al. 1976; Bolen and Thorning 1980; Dardick et al. 1984a). Furthermore, lack of typical mesothelial cell characteristics ultrastructurally does not eliminate mesothelial derivation for a tumour of serosal membranes (Dardick et al. 1984b).

During an ultrastructural and histochemical review of 12 localized mesothelial tumours of the pleura and peritoneum, we have noted fine structural features indicating a spectrum of cellular differentiation with some cells exhibiting transitional characteristics of both surface mesothelial cells and subsurface fibroblast-like cells. An evaluation of these features and their conceptual histogenic interpretation form the basis of this report.

Materials and methods

Materials for a histochemical and ultrastructural study of localized mesotheliomas were obtained from the files of the Electron Microscope Unit of the Department of Pathology at the Toronto General Hospital, Toronto, Ontario and from the Canadian Tumour Reference Centre, Ottawa, Ontario. A total of ten cases (12 tumours; one case had the biopsy and resection specimen of the original lesion and two of three recurrences examined ultrastructurally) were available from 1975 to 1984. The operative and surgical pathology reports were reviewed to establish the exact distribution and growth characteristics of the lesions.

Representative portions from all formalin-fixed tumours were processed in a routine manner for light microscopy. Sections were stained with haematoxylin and eosin, Hale's colloidal iron (with and without previous hyaluronidase treatment), and mucicarmine.

Table 1. Clinicopathologic profiles

Case	Age	Sex	Location	Gross features	Microscopic pattern	Mitoses /10 HPF	Follow-up (years)
1	68	F	Visceral Pleura (RUL)	Solid, white (5.5 cm)	Diffuse sheets of spindle cells	6	10
2	38	M	Visceral Pleura (RUL)	Solid, yellow (3.5 × 3.0 cm)	Interlacing fascicles of spindle cells	0	9
3	56	F	Visceral Pleura (RUL, RLL)	Solid, white (5.0 × 1.0 cm & 8.5 × 7.0 cm)	Interlacing fascicles of spindle cells	8	7 (Recurrences resected at 2, 4 and 7 y)
4	38	F	Visceral Pleura (RML)	Solid, whorled (23.0 × 15.0 cm)	Interlacing fascicles of spindle cells	0	5
5	51	M	Visceral Pleura (LUL)	Solid, pedunculated (13.0 × 8.0 cm)	Spindle cells with peripheral epithelial lined clefts	0	4
6	57	F	Visceral Pleura (LUL)	Solid, white, pedunculated (6.0 × 4.0 cm)	Interlacing fascicles of spindle cells	0	4
7	42	M	Visceral Pleura (LML)	Pedunculated, whorled (4.0 × 3.0 cm)	Interlacing fascicles of spindle cells	0	4
8	64	F	Visceral Pleura (LML)	Pedunculated, lobulated (13.0 × 7.0 cm)	Interlacing fascicles of spindle cells	0	6
9	47	M	Peritoneal Serosa (stomach)	Multiple, sessile, hard white nodules (0.6 cm each)	Sparse spindle cells and abundant collagen	0	8
10	26	F	Visceral Pleura (RUL)	Intrapulmonary, subpleural firm, white (2.8 cm)	Biphasic; spindle cells admixed with a few epithelial zones	0	5

All 10 cases were examined by immunoperoxidase staining, performed on formalin-fixed, paraffin-embedded tissue sections. The method of Sternberger et al. (1970) was used in conjunction with antibodies to cytokeratin (CK), both epidermal (ECK) and Mallory body (MBCK), and to vimentin. ECK was isolated from human plantar scrapings by the method of Sun and Green (1978). An anti-ECK antibody was prepared in rabbits, as previously described (Kahn et al. 1982; Kahn et al. 1984). Mallory bodies were isolated from human autopsy liver and an anti-MBCK antibody was prepared in rabbits (Kimoff and Huang 1981). This antibody was a gift of Dr. S-N Huang, Sunnybrook Medical Centre, Toronto, Ontario. Using antibodies to ECK and MBCK and CK proteins extracted from skin and colon, immunoblotting was performed using the method of Towbin et al. (1979). The antibody to ECK reacted with molecular weight keratins in the range of 55 to 60 kilodaltons, which correspond to CKs 4, 5, and 6 of Moll et al. (1982). The antibodies to MBCK reacted with molecular weight

keratins of 45 and 53 kilodaltons, which correspond to CKs 8 and 18 (Moll et al. 1982). Positive controls for CK staining were performed on sections of normal skin and alcoholic liver. To detect the intermediate filament, vimentin, a commercially available anti-vimentin antibody (Ortho Diagnostics, Raritan, NJ, USA) was utilized. We have found consistent staining only of fibroblasts and endothelial cells in the dermis of normal skin and in the stromal tissues of placenta when using formalin-fixed and paraffin-embedded portions of these tissues. For negative controls, normal rabbit serum was substituted for anti-CK and anti-vimentin antibodies.

Minced portions of tissue from all of the tumours had been fixed in Karnovsky's solution, post-fixed in osmium tetraoxide, dehydrated in graded alcohols and embedded in Epon-Araldite. Appropriate areas for electron microscopy were selected from toluidine blue-stained semithin sections. Thin sections were cut, stained with uranyl acetate and lead citrate and examined with a Philips EM 301 electron microscope.

Results

Clinical profiles

Examination of Table 1 illustrates the uniform clinical presentation of localized serosal tumours. The age of the patients ranged from 38 to 68 years, with the peak incidence occurring in the sixth and seventh decades. Six patients were female and four were male. A history of asbestos exposure or other significant environmental toxic agent could not be elicited in any case. The most common mode of presentation was an asymptomatic mass discovered on routine chest x-ray. None of the patients had documented hypoglycaemia, occasionally reported in association with these tumours (Dalton et al. 1979). All patients are alive and well, for variable periods of time, following excision; the average post-operative interval was 6.3 years with a range of 4 to 10 years. However, one patient (case 3), required further surgery for a recurrent tumour on three separate occasions (Table 1). This latter case and case 1 were considered malignant because of the presence of numerous mitoses in both cases, and in case 1 tumour growth encompassed nerve fascicles.

Pathology

The gross examination of all tumours was not too dissimilar (Table 1). All pleural lesions, except case 10, arose on the visceral surface and were solitary and well circumscribed. Case 10 was unique in being localized within the pulmonary parenchyma of the right upper lobe, subjacent to, but not demonstrably in contact with, the pleural surface. At least four were pedunculated, with the remainder sessile. The size of the tumours varied widely from a case with small 0.6 cm nodules to a large, bulky, 23.0×15.0 cm neoplasm weighing 1,750 grams. The cut surface usually demonstrated solid grey-white to tan-yellow tissue, generally described as having a whorled or lobulated appearance. The capsules were smooth and glistening. No areas of haemorrhage or necrosis were noted in any of the tumours.

All tumours were composed of round to spindle-shaped cells arranged in interlacing fascicles (Fig. 1). The cellularity varied from tumour to tumour

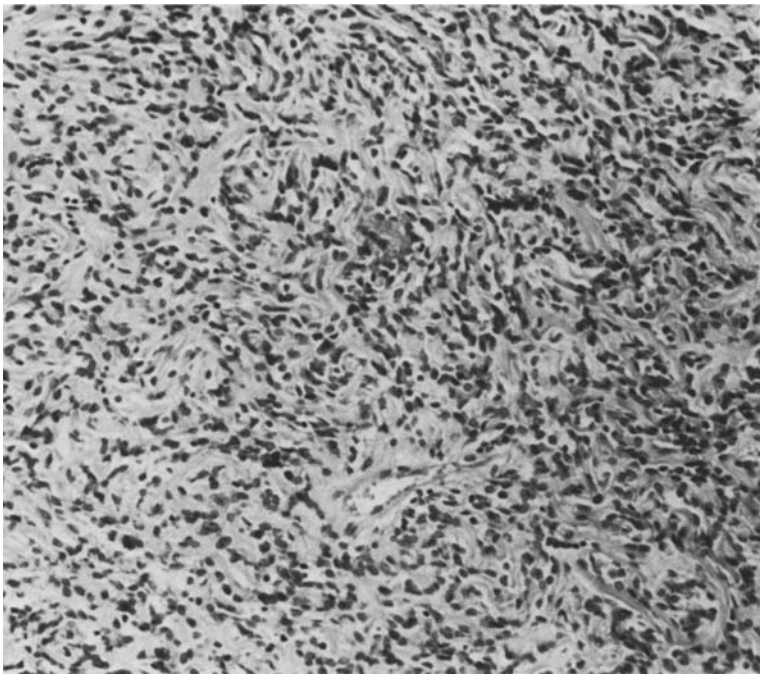

Fig. 1. Case 1. Representative section of a localized mesothelioma showing compact narrow fascicles of spindle cells alternating with bundles of collagen. (H & E, × 400)

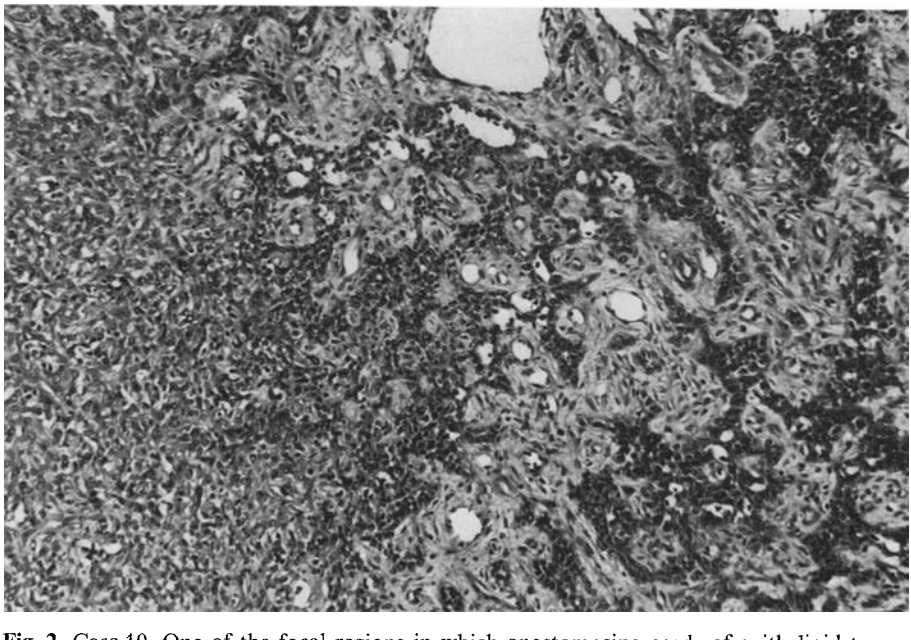

Fig. 2. Case 10. One of the focal regions in which anastomosing cords of epithelioid tumour cells gradually merge with the major spindle cell component on the left. The more obvious vascularized stroma on the right also blends with the collagen bundles between the spindled tumour cells. (H & E, × 120)

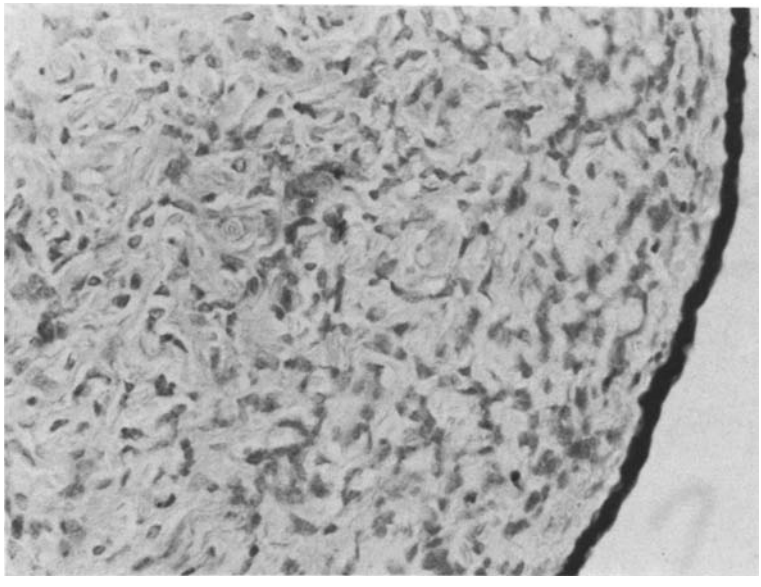

Fig. 3. Case 3. Localized mesothelioma stained with antibodies to MBCK using the immunoperoxidase technique. Positive staining is confined to the mesothelial cells overlying the tumour. (PAP with heamatoxylin counterstain, × 350)

Table 2. Ultrastructural and immunohistochemical profiles in localized tumours of serosal surfaces

Case	Cell to cell contacts	Micro-villi	Tono-filaments	Junctions	Basal lamina	CK	Vimentin
1	+	−	+	+	+	−	−
2	+	−	−	+	−	−	+
3	+	−	−	+	−	−	−
4	+	−	−	+	−	−	−
5	+	−	−	+	−	−	−
6	+	−	−	+	−	−	+
7	+	−	−	−	−	−	−
8	+	−	−	−	−	−	+
9	+	−	−	−	+	−	−
10	+	+	+	+	+	+	+

with some having only occasional spindle cells alternating with large areas of hyalinized collagen. The majority, however, showed alternating bundles or rows of spindle cells between thick bands of collagen. The cells had elongated nuclei, inconspicuous nucleoli and indistinct cytoplasm. Although no recognizable neoplastic epithelial components forming nests or glands were seen in cases 1 to 9, the microscopic evaluation of case 10 presented a somewhat unusual biphasic morphology (Fig. 2). Here, the major spindle

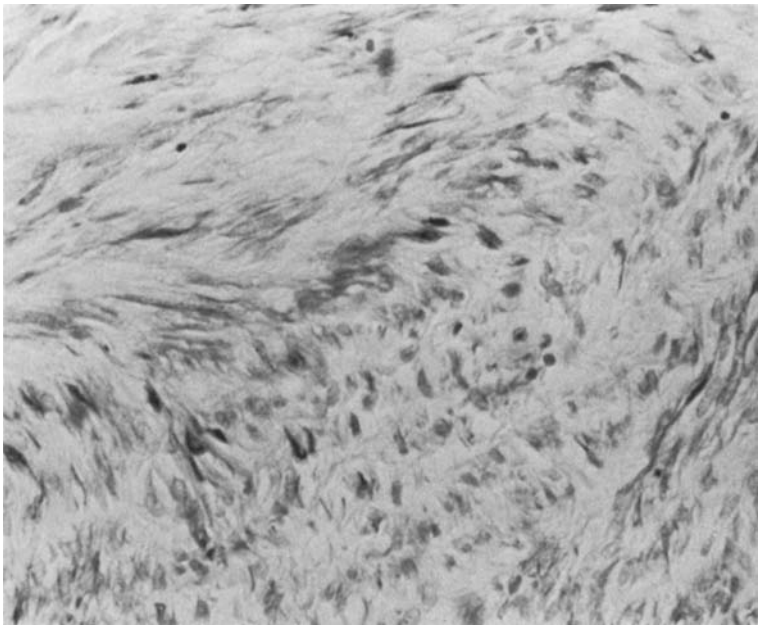

Fig. 4. Case 6. Immunostaining using antibody directed against vimentin shows positive staining of the cytoplasm of some tumour cells (PAP with haematoxylin counterstain, ×350)

cell population appeared adjacent to and merged with a small number of focal nests and compressed cords of more epithelioid, polygonal cells. Again, neither tubular nor glandular structures were evident (Fig. 2). An occasional tumour was lined on the outer surface by a flattened to cuboidal single layer of mesothelial cells (Fig. 3). Entrapped alveolar epithelium was not apparent in any of the tumours examined.

Histochemistry

Special stains were performed in a search for epithelial mucopolysaccharide production. In all tumours, with Hale's colloidal iron stain (with and without pretreatment with hyaluronidase), the neoplastic spindle cell population showed no areas of positive staining. Mucicarmine and periodic acid-Schiff stains were negative as well.

Immunohistochemistry

Immunostaining with antibodies directed against both ECK and MBCK was uniformly negative in cases 1–9 (Table 2). Although the neoplastic spindle cell population stained negatively in these cases, cases 1, 3, and 5 did show positive cytoplasmic staining of the non-neoplastic mesothelial cells covering the surface of the tumor (Fig. 3) and forming cleft-like spaces

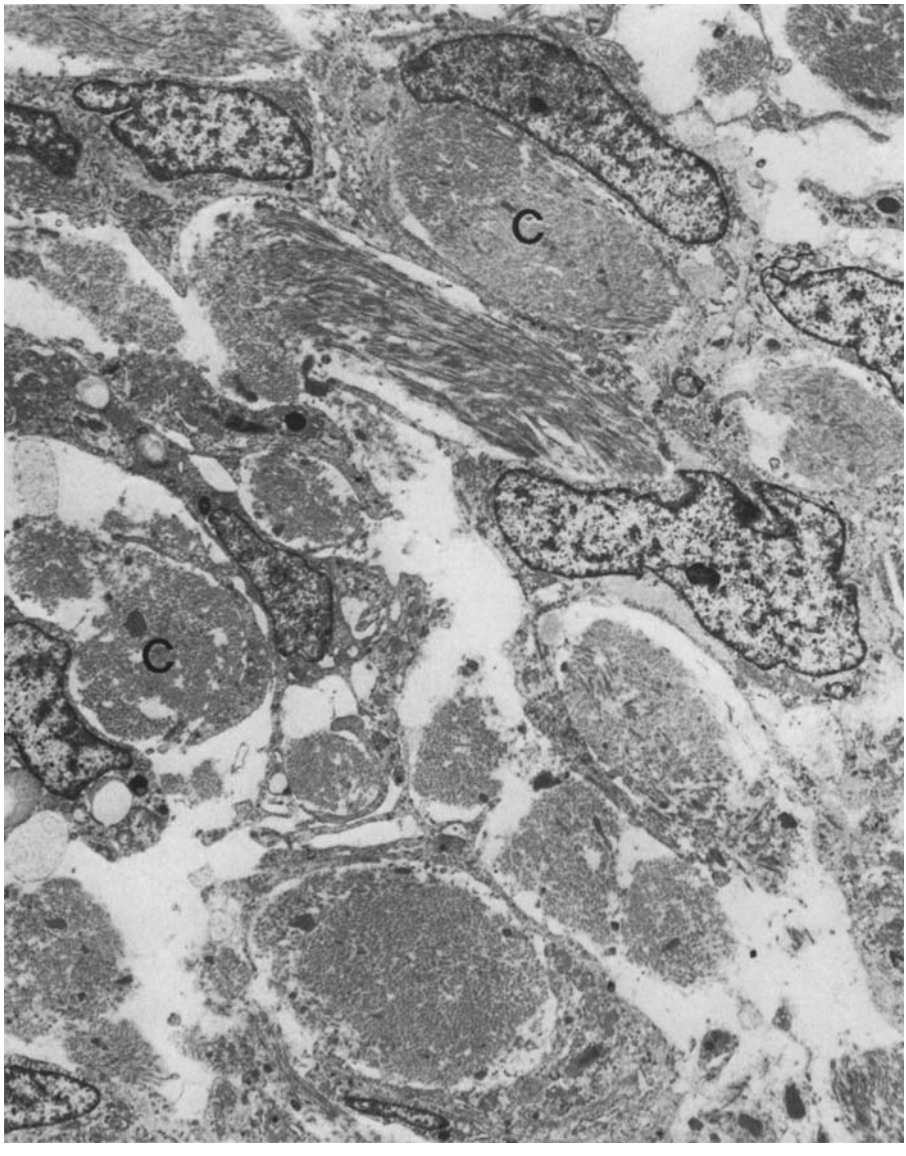

Fig. 5. Case 4. Compact bundles of orderly arranged collagen fibers (*C*) are partially surrounded by narrow cytoplasmic processes that also tend to interconnect adjacent tumour cells. (× 3,900)

in case 5. However, immunostaining of case 10 showed strong positive staining for both ECK and MBCK localized only to the cytoplasm of the epithelioid cell aggregates. Evaluation of the neoplasms using an antibody directed against the intermediate filament vimentin showed diffuse positive cytoplasmic staining in cases 6 (Fig. 4) and 8, and focal positivity in case 2 (Table 2). Areas of positive staining for vimentin in case 10 appeared to be localized

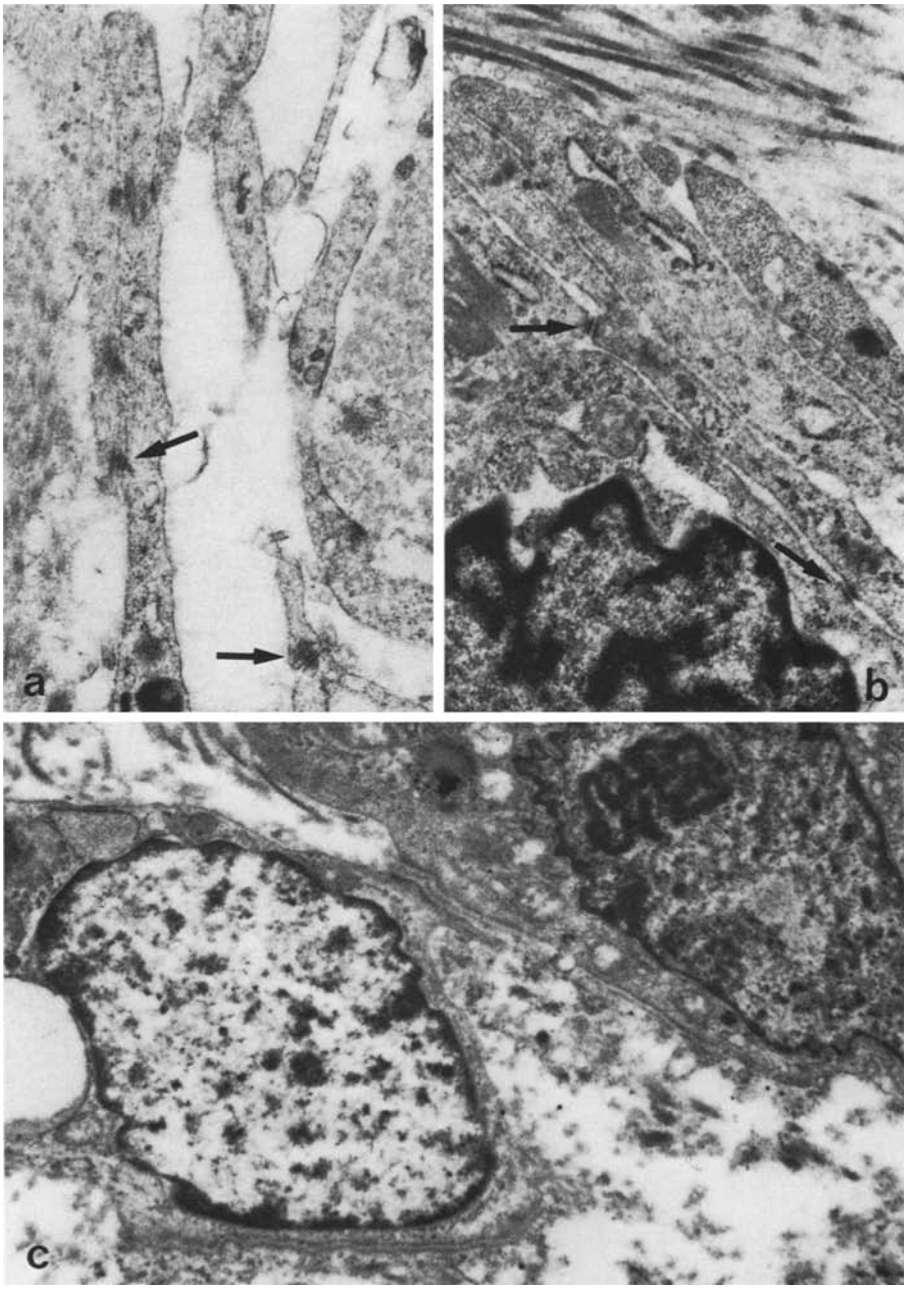

Fig. 6a–c. Localized mesotheliomas with (**a**) small tonofilament-associated desmosomes (*arrows*) joining narrow cytoplasmic processes in case 4, (**b**) intercellular junctions of subplasmalemmal densities (*arrows*) in case 2, and (**c**) focal segments of external lamina on isolated groups of tumour cells in case 1

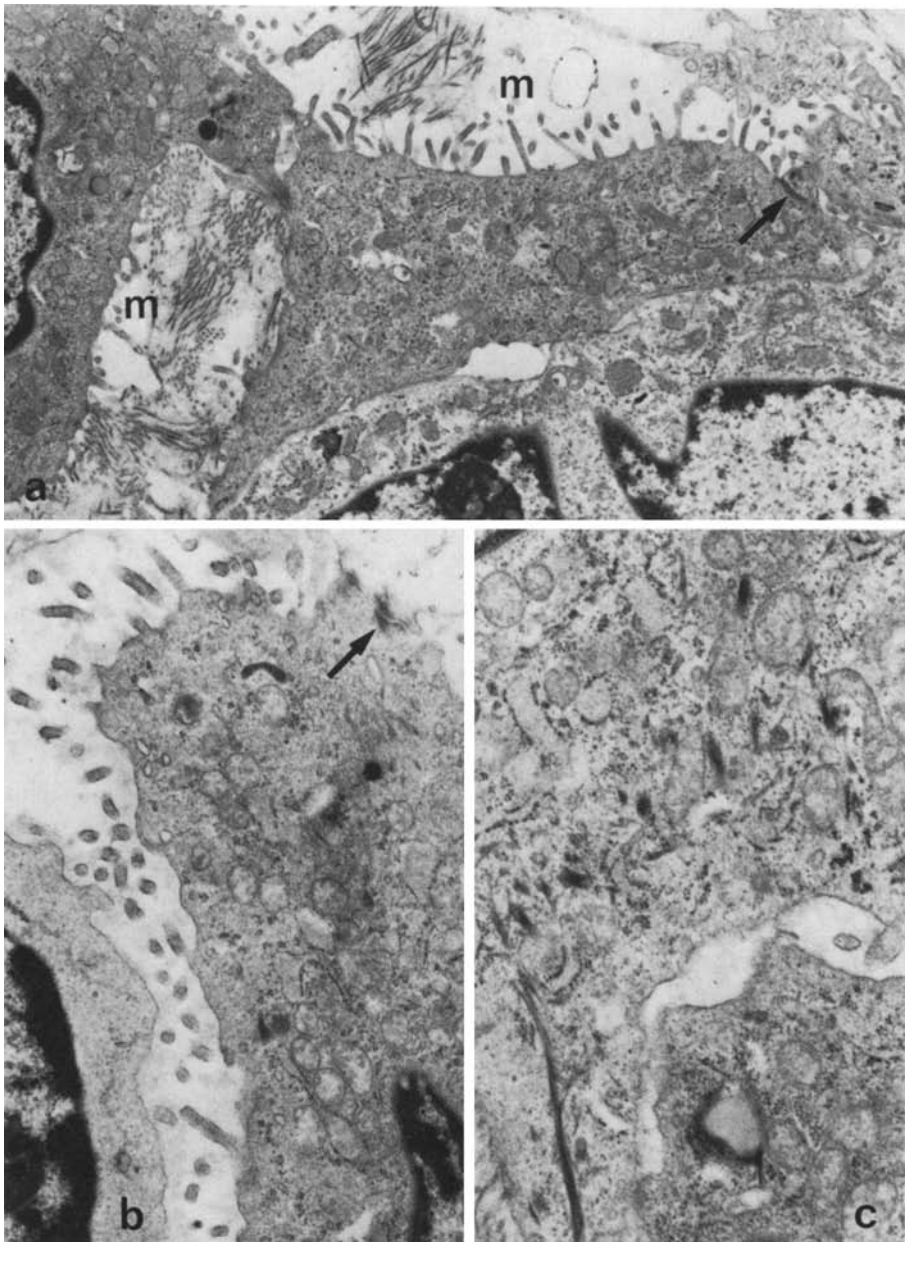

Fig. 7a–c. Case 10. Groups of tumour cells have (**a**) microvilli (*m*) on certain cell surfaces and a few occludens-type intercellular junctions (*arrow*), (**b**) tonofilament-associated junctions (*arrow*), and (**c**) a number of tonofilament bundles of variable length. (**a**, ×9,000; **b**, ×13,000; **c**, ×24,600)

to the spindle cell component. The antibody to vimentin failed to stain the surface mesothelium.

Ultrastructural observations

All 10 cases were examined ultrastructurally and displayed a uniform cellular organization, and an association of tumour cells with compact, well defined bundles of mature collagen (Fig. 5). A constant feature was the presence of narrow extensions of cell cytoplasm that encircled the bundles of collagen and interacted with similar cytoplasmic processes of adjacent tumour cells (Figs. 5 and 6). Generally there were no specific nuclear or cytoplasmic features within tumour cells and the amount and prominence of rough endoplasmic reticulum not only varied between individual cases but also between tumour cells in each case.

Although the majority of the spindle-shaped tumour cells were unspecialized, a careful search revealed some localized serosal tumours with occasional evidence of fine structural differentiation unexpected in fibroblastic neoplasms (Table 2 and Fig. 6). The most common specialization was some form of intercellular junction seen in 7 of the 10 cases (Table 2). Such junctions were generally small and inconspicuous and either consisted of tonofilament-associated desmosomes (Fig. 6a), maculae occludentes, or opposing subplasmalemmal densities (Fig. 6b). In three cases, isolated groups of tumour cells showed focal external lamina formation (Fig. 6c). As is evident from Table 2, only two cases (case 1 and case 10), had more than one indicator of non-fibroblastic differentiation. Case 10 was unique in exhibiting clusters of closely associated and epithelioid-appearing tumour cells with microvilli polarized along a specific surface that occasionally involved a series of adjacent cells (Fig. 7). Neighbouring cells were joined by intercellular junctions, either reminiscent of portions of zonulae occludentes (Fig. 7a) or desmosome-like (Fig. 7b). Scattered cells in case 10 contained distinct tonofilament bundles (Fig. 7c). Such fine structural features were evident in spindle-cell regions, which were the only areas available for examination in case 10.

Discussion

Arguments continue as to the histogenesis of localized serosal tumours. Based on the ultrastructural findings in the 12 localized tumours of pleura and peritoneum in this report, and the majority of the 32 additional localized serosal lesions with electron microscopic data in the literature (Luse and Spjut 1964; Kay and Silverberg 1971; Wang 1973; Hernandez and Fernandez 1974; Osamura 1977; Kawai et al. 1978; Bolen and Thorning 1980; Briselli et al. 1981; Kawai et al. 1981; Benisch et al. 1981; Said et al. 1984; Bürrig and Kastendieck 1984), many features of these tumours are analogous to certain aspects of diffuse epithelial mesotheliomas. The organization and degree of differentiation of tumour cells in the "stromal" or sarcomatoid regions of diffuse mesotheliomas is reflected in localized fibrous serosal

tumours (Bolen and Thorning 1980; Dardick et al. 1984; Bolen et al. 1986). Unlike fibroblasts, tumour cells in all 12 examples of localized serosal tumours evaluated in this study had many cytoplasmic processes forming numerous intimate contacts with adjacent tumour cells, or the cells had a more epithelioid character and formed nest-like aggregates (case 10). In addition, only two of the cases failed to exhibit some form of cytoplasmic organelle or membrane specialization such as tonofilaments, microvilli, intercellular junctions and basal lamina, features not evident in typical fibroblastic lesions. These characteristics alone suggest that the term "localized mesothelioma" may be appropriate for most fibrous tumours of serosal surfaces.

Some ultrastructural studies of diffuse epithelial mesotheliomas (Suzuki et al. 1976; Suzuki 1980; Bolen and Thorning 1980; Dardick et al. 1984a; Bolen et al. 1986) reveal a spectrum of cell types with epithelial cells, fibroblast-like cells and numerous transitional forms, all having varying degrees of the fine structural characteristics or normal and reactive mesothelial cells. Indeed, the "stromal" regions of diffuse epithelial mesotheliomas and the spindle cell areas of biphasic and sarcomatoid mesotheliomas also contain a proportion of transitional cells (Bolen and Thorning 1980; Dardick 1984; Dardick et al. 1984a; Bolen et al. 1986). As has been demonstrated recently, an absence of microvilli, desmosomes and tonofilaments does not exclude a diagnosis of mesothelioma in serosal lesions with the characteristic clinical presentation, and the gross appearance and distribution of this tumour at surgery or autopsy (Dardick et al. 1984b).

Despite differences in pathogenesis, gross organization, histology and biological behaviour, it is still possible that solitary and diffuse serosal membrane tumours are closely related. There is accumulating evidence that their variety may reflect the potential of a population of subsurface cells in serosal membranes to differentiate as mesothelium. Experimentally, direct injury to or asbestos exposure of serosal tissues results in proliferation of spindle cells in the submesothelial compartment (Raftery 1973a, b; Ryan et al. 1973; Davis 1974). In the former case, migration of stimulated submesothelial cells results in reconstitution of surface mesothelium (Raftery 1973a, b), while in the latter case epithelial mesotheliomas develop (Davis 1974). Knowledge of the differentiation capability of subserosal cells has expanded recently, on the basis of correlative ultrastructural and immunohistochemical investigations of serosal membranes responding to injury (Bolen et al. 1986). Certain subserosal cells are specialized and specifically programmed as evidenced by conversion from solely vimentin-expressing cells when quiescent, to vimentin and low molecular weight cytokeratin co-expressing spindle-shaped cells during response to the repair process of serosal membranes (Bolen et al. 1986). Subsequent phases of differentiation of such specialized, fibroblast-like subserosal cells to surface mesothelial cells involves acquisition of high molecular weight cytokeratins and loss of vimentin (Bolen et al. 1986). The fact that the above two morphological and functional phases of serosal cell differentiation are reflected in the fine structural and immunohistochemical findings in desmoplastic or sarcomatoid mesothelio-

mas on the one hand, and diffuse epithelial mesotheliomas on the other (Bolen et al. 1986), has important histogenetic implications for serosal membrane tumours in general. Notwithstanding the above evidence for repair of serosal tissues from subsurface cells, it is important to appreciate that more than one mechanism may be active. Perhaps, in certain specialized sites such as the ovary (Nicosia et al. 1985) and tunica vaginalis (Whitaker and Papadimitriou 1985), regeneration of surface tissue involves differentiated mesothelial cells.

Do findings in the current series of localized tumours of serosal membranes fit within the above concepts? Certainly the ultrastructural features and the sole expression of vimentin in some examples of cases 1–9 reflect the morphological and functional characteristics of the unactivated but specialized subsurface cell of normal serous surfaces (Bolen et al. 1986). Case 10 has interesting and somewhat unusual features. While maintaining the clinical presentation and gross morphology of the remainder of the localized tumours, its microscopic and histochemical features are unusual for a localized mesothelioma, and resemble to some degree features found in the diffuse mesothelioma group. Its biphasic cellular pattern on light microscopy, ultrastructural features resembling those of normal mesothelium or diffuse mesothelioma, and the finding of cells staining in epithelial regions for CK while spindle-cell areas stained for vimentin, suggest that this lesion may show a pattern of differentiation intermediate between the localized fibrous tumour and diffuse mesothelioma groups. Similar features have been observed in biphasic mesotheliomas (Bolen et al. 1986). Thus, this particular localized lesion serves as an important morphogenetic and histogenetic link between purely spindle-cell localized serosal tumours and diffuse epithelial mesotheliomas. Taken together, the results of the current study suggest that at least some forms, and possibly the majority, of localized lesions of serosal membranes are mesotheliomas.

Review of electron microscopic descriptions and illustrations of 32 examples of localized serosal tumours in the literature (Luse and Spjut 1964; Kay and Silverberg 1971; Wang 1973; Hernandez and Fernandez 1974; Osamura 1977; Kawai et al. 1978; Bolen and Thorning 1980; Kawai et al. 1981; Briselli et al. 1981; Benisch et al. 1981; Said et al. 1984; Bürrig and Kastendieck 1984) reveals that many exhibit the same organizational and cellular developmental features detailed in this report. In fact, only three of these appear to be composed of fibroblasts (Luse and Spjut 1964; Hernandez and Fernandez 1974; Briselli et al. 1981), and may represent a much less frequent nonmesothelial type of tumour of serosal membrane, a true fibroma. In the present study, those cases which exhibit diffuse cytoplasmic staining with anti-vimentin antibodies (case 6 and case 8) could represent examples of such a tumour. However, both of these vimentin-positive tumours had electron microscopic arrangement of tumour cells differing from fibroblasts, and one had intercellular junctions (Table 2).

Said et al. (1984) have interpreted the absence of staining for CK in localized serosal tumours as divorcing this type of tumour from the mesothelioma family. However, it is possible that tumours arising from or represent-

ing the counterpart of the non-CK filament-containing specialized submesothelial cells might not express CK intermediate filaments in the majority of cases. In fact, not all malignant mesotheliomas stain positively for CK (Holden and Churg 1984). Thus, the negative staining by CK antibodies in localized serosal tumours must be assessed in the context of the ultrastructural findings, features that are frequently more indicative of a mesothelial genesis rather than a fibroblastic or nonmesothelial one.

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