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Leiomyosarcomas: Three cases with desmin positive tumour cells, lacking ultrastructural features of smooth muscle cells

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Summary. A combined study of light and electron microscopy and of immunolabelling of three pleomorphic spindle cell sarcomas is presented. The light and electron microscopic features of these sarcomas were most compatible with those described for malignant fibrous histiocytoma (MFH, pleomorphic-storiform subtype). Electronmicroscopically undifferentiated and fibroblast-like cells, fibrohistiocytes and multinucleated histiocytes were observed. Characteristics belonging to smooth muscle cells were absent.

By immunostaining, vimentin and desmin could be observed in tumour cells of all three cases, at least on frozen sections. Other markers such as alpha$_1$-antichymotrypsin, S-100 proteins, laminin, collagen IV and markers specific for skeletal muscle cells (myoglobin, actin and myosin specific for skeletal muscle) could not be demonstrated. These findings indicate that three MFH's are, in fact, poorly differentiated variants of smooth muscle tumours.

It is concluded that immunophenotyping is very useful for this type of neoplasm.

Key words: Malignant fibrous histiocytoma, Leiomyosarcoma, Desmin

Introduction

Leiomyosarcomas are formed by the malignant analogues of the leiomyogenic cell lineage (Enzinger and Weiss, 1983). Therefore cells of these neoplasms resemble normal smooth muscle cells to varying degrees. Malignant fibrous histiocytomas (MFH) are thought to be derived from primitive «fibrohistioblasts», fibroblast-like and undifferentiated cells (Fu et al., 1975; Enzinger and Weiss, 1983; Roholl et al., 1985a,b; Shirasuma et al., 1985; Brooks, 1986; Roholl et al., 1986; Wood et al., 1986).

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In terms of histology a distinction between these two kinds of neoplasms is however not always clear and even ultrastructurally the distinction between fibroblast and smooth muscle cell tumors may be blurred (Churg and Kahn, 1977). This distinction may be made even more difficult by the presence of myofibroblasts, which can form a quantitatively important component of soft tissue tumours (Lagacé et al., 1980).

Immunohistochemistry is being applied more and more and can be very useful as an aid in the diagnosis of soft tissue tumours (du Boulay, 1985; Roholl et al., 1985c). So far no markers have been described which are specific for the types of neoplasm mentioned above, and which could provide clues enabling clear distinctions to be made. By process of exclusion, however, indications for their correct diagnosis can be obtained. Immunoreactivity for desmin is for instance considered to be conclusive for a myogenic character of a tumour (Altmannsberger et al., 1981, 1985; Roholl et al., 1985c), while positive staining for only desmin and not for markers specific for skeletal muscle cells, strongly favours the diagnosis of leiomyosarcoma (De Jong et al., 1987).

This study forms part of a multiparametric investigation of soft tissue sarcomas. The cases presented here were chosen because the light and electron microscopic observations were not in accord with the results of immunostaining. Light microscopic and ultrastructural features showed fibroblastic, histiocytic and/or fibrohistiocytic characteristics, suggestive for MFH. Immunohistochemical findings however, proved the diagnosis of leiomyosarcoma. The consequences of these findings with regard to the diagnosis of these neoplasms and their histogenesis are discussed.

Materials and methods

Tumour tissue

Was obtained from the Department of Pathology of the Netherlands Cancer Institute (Amsterdam) and from

the Institute of Pathology of the Academic Hospital (Utrecht). Three sarcomas are described in detail. The first sarcoma was a recurrence of a myxoid MFH with a diameter of 6 cm. The diagnosis of the primary tumour was based on light microscopical features. The recurrence was located in the thorax wall of a 37-year-old woman. The second was a primary tumour with a diameter of 1 cm and was located intramuscularly in the buttock of a 24-year-old woman. The third was a recurrence of a tumour diagnosed as a fibrosarcoma and had a diameter of 3 cm. It was located in the neck of a 50-year-old man. For this study the three tumours were numbered 1, 2 and 3 respectively.

Tissue fragments for light microscopy were fixed in 4% formaldehyde and embedded in paraffin. Routine histological stains (HE, PAS and reticulin) were performed. Alcian blue staining was done according to Scott and Dorling (1965). Several tissue fragments for electron microscopy were fixed in Karnovsky-fixative, postfixed in osmium tetroxide, stained en bloc in uranyl acetate, dehydrated in ethanol, and embedded in Epon. Ultrathin sections were stained with lead citrate. Tissue fragments were also frozen in liquid nitrogen.

Immunoperoxidase studies

Were performed on sections of formaldehyde-fixed and paraffin-embedded material, and also on frozen sections. For paraffin sections the sensitive avidin-biotin-peroxidase complex (ABC) method as described in detail by De Jong et al. (1984) was applied, while for frozen sections an indirect peroxidase labelling method was used (Roholl et al., 1985a).

Reagents

The following antisera were used: rabbit anti-calf lens vimentin, anti-chicken gizzard desmin, and anti-human collagen IV (Organon Technica, Oss, The Netherlands), rabbit anti-bovine S-100 (Dakopatts, Demark), anti-human Factor-VIII-related-antigen and anti-human alpha$_1$-antichymotrypsin (Behringwerke, Germany), and anti-mouse laminin (Bethesda Research Laboratories, USA). The following mouse monoclonal antibodies were used: anti-vimentin (clone V9, Dakopatts) and anti-desmin (clone DE-U-10, Sigma Chemicals, St. Louis, MO).

The specificity of the rabbit anti-vimentin and anti-desmin antisera has been described by Ramaekers et al. (1983). The specificity of the mouse anti-vimentin and anti-desmin antibodies has been described by Osborn et al. (1984) and Debus et al (1983), respectively.

The antisera specific for skeletal muscle actin (rabbit anti-carper skeletal muscle actin) and myosin (rabbit anti-human skeletal muscle myosin) were prepared as described by De Jong et al. (1985, 1987). These antisera were only immunoreactive with skeletal and heart muscle cells.

Reagents for the ABC technique were obtained from Vector Laboratories (Vectastain, Burlingame, USA) and the antibodies for the indirect peroxidase method (swine anti-rabbit IgG conjugated with peroxidase and rabbit anti-mouse IgG conjugated with peroxidase) from Dakopatts.

Results

Light microscopy

The histology of the three tumours varied from cell-poor areas with a myxoid appearance to cell-dense areas with fibrous and pleomorphic aspects.

In the fibrous areas the cell formed so-called cartwheel patterns. In pleomorphic areas sheets of bizarre cells with abundant eosinophilic cytoplasm were seen. In all three cases multinucleated giant cells were regularly present. The number of mitotic figures for neoplasms 1, 2 and 3 were: 33, 30 and 10 per ten high power fields. Atypical mitotic figures were also observed.

The presence of PAS stainable material was negligible, whereas alcian-blue positive material was abundant in the intercellular space. The staining for alcian-blue disappeared after hyaluronidase treatment of the sections.

These tumours had a high degree of malignancy, as could be determined by their cellularity, marked pleomorphism and high number of mitotic figures. The light-microscopic features were in all three cases compatible with the diagnosis MFH (Figs. 1-3).

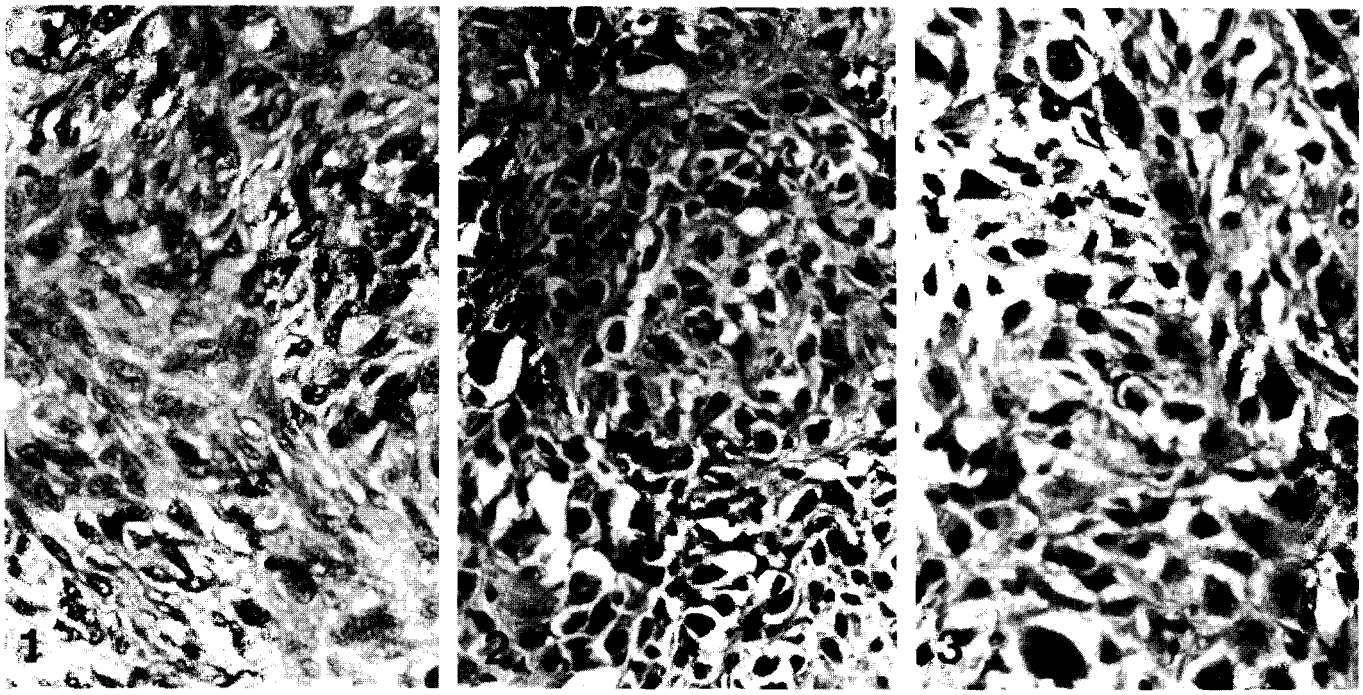
Immunostainings

The results of the immunostainings are shown in Table 1. The immunolocalization of desmin was striking (Figs. 4, 5a, 6). It could be detected in the various cell types in all cases, although its presence could only be seen on frozen sections of case 3 (Fig. 6). Desmin was absent in the multinucleated osteoclast-like giant cells of case 2 (Fig. 5b). In none of the cases could any myoglobin, skeletal muscle actin, skeletal muscle myosin, S-100 proteins, laminin and collagen type IV be found in or along the tumour cells. Alpha$_1$-antichymotrypsin was seen in the osteoclast-like giant cells, but was lacking in the other tumour cells.

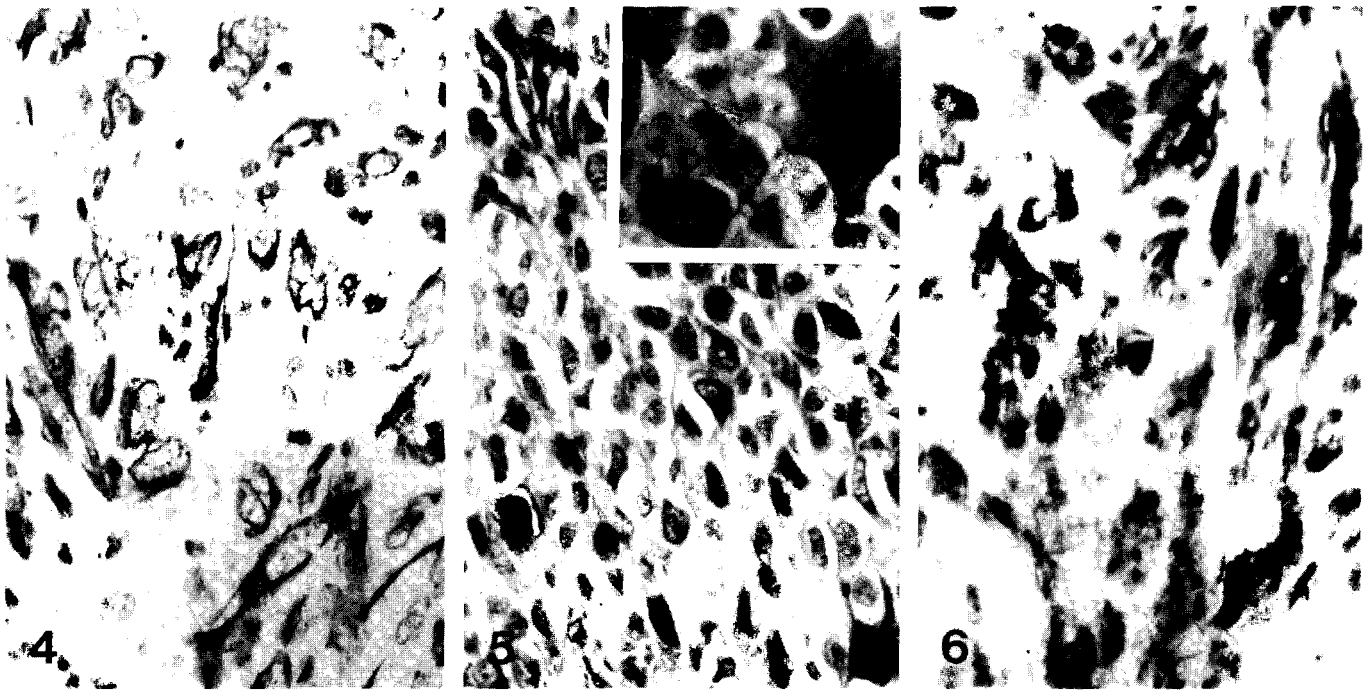
Table 1. Immunophenotypic characterization of tumour cells

Markers	Case 1	Case 2	Case 3
A: **paraffin sections**			
vimentin*	+	+	+
desmin*	+	+	–**
actin	+	+	+
actin and myosin (spec. for skeletal muscle) myoglobin, ACT, S-100, laminin, collagen IV	–	–	–
B: **frozen sections**			
vimentin, desmin*	+	+	+
F VIII rel. ag.	–	–	–

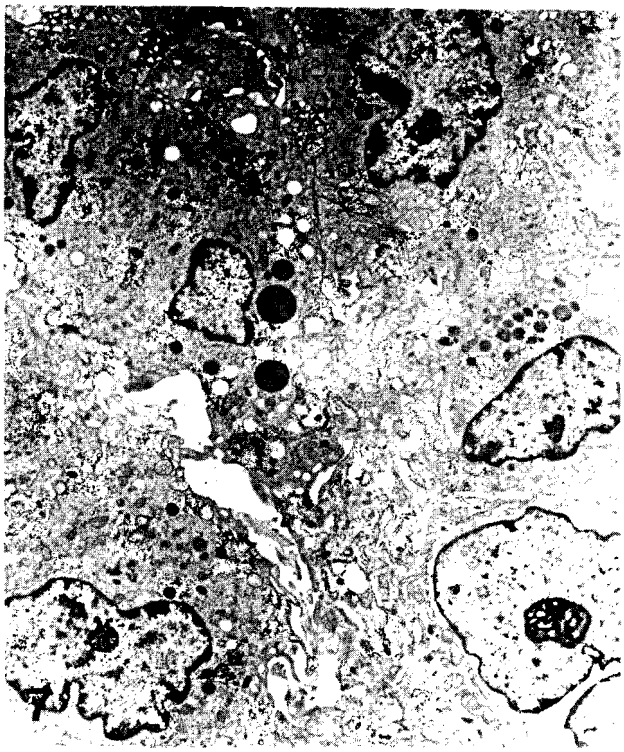
* Polyclonal monospecific and monoclonal antibodies to vimentin and desmin were used (see Materials and Methods).
** vascular smooth muscle cells are positive.

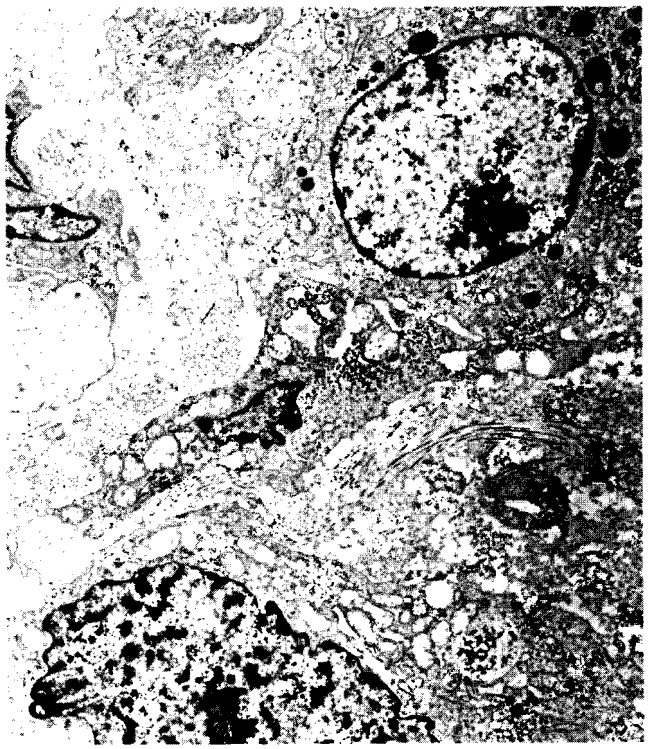


Figs. 1, 2 and 3 illustrate the light microscopical features of case 1, 2 and 3 respectively. Paraffin sections stained with H & E. Note the presence of multinucleated giant cells in figure 2. × 210



Figs. 4, 5 and 6 illustrate immunohistochemical staining for desmin in case 1, 2 and 3 respectively. Desmin could be detected on paraffin sections of case 1 and 2 and on frozen sections of case 3. Histiocyte-like and fibroblast-like cells are positive. Note the absence of staining for desmin in the multinucleated giant cells in figure 5b. × 320





Figs. 7, 8 and 9 represent electron microscopic illustrations of the cases 1, 2 and 3 respectively. Figure 7 shows fibrohistiocytes with irregular indented nuclei and prominent nucleoli. A developed rough endoplasmic reticulum is present. Figure 8 shows an oval-shaped fibrohistiocyte and an enlogated fibroblast-like cell. Lysosomes and long, sometimes branched strands of rough endoplasmic reticulum are present. Figure 9 also shows fibrohistiocytes. Rounded and indented nuclei, phagocytotic material, strands, sometimes dilated, of endoplasmic reticulum and long plasma membrane extensions can be seen. Many collagen-bundles are present in the extra-cellular matrix of cases 2 and 3. No pinocytotic vesicles, filamentous condensations or remnants of basement membranes were seen. × 3,750

Electron microscopy

Electron microscopy was extensively done on these three cases and samples, derived from different locations of the tumours were studied. In all three cases the population of tumour cells consisted of undifferentiated and fibroblast-like cells, fibrohistiocytes and multinucleated giant cells. Fibroblast-like cells and fibrohistiocytes are shown in Figs. 7, 8 and 9.

The majority of the tumour cells had a slender appearance with an irregular cell surface. Nuclei were pleomorphic with indentations. A coarse chromatin pattern and a dense nucleolus were usually seen. Intermediate-sized filaments were sometimes arranged around the nuclei, but more often they showed a random arrangement. Strands of rough endoplasmatic reticulum and occasional lipid droplets were noticed. Varying numbers of lysosomal structures were present, mostly in the fibrohistiocytes and mono- and multinucleated histiocyte-like giant cells. Plasma membranes regularly formed long extensions, without interdigitating processes and without basement membranes. Intercellular junctions were not found and micropinocytosis was not noticed. Well-developed collagen-bundles were seen in the intercellular space, together with some amorphous material.

Discussion

EM-studies have been recommended as important diagnostic tools for pleomorphic sarcomas (Weiss and Warhol, 1984) but immunoperoxidase techniques can also be used successfully for the differential diagnosis of this type of sarcoma (Molenaar et al., 1985; De Jong et al., 1987). This study shows that immunolabelling can distinguish between MFH and leiomyosarcomas whereas electronmicroscopic studies often cannot. One of the reasons for this may be the paucity of ultrastructural features specific for smooth muscle cells (Churg and Kahn, 1977; Weiss and MacKay, 1981; King et al., 1982), and the ultrastructural heterogeneity of MFH (Jabi et al., 1987). It is not surprising therefore that on the basis of electronmicroscopic observations a distinction between MFH and leiomyosarcoma is frequently difficult or even imposible (Churg and Kahn, 1977). For ultrastructural interpretations of smooth muscle differentiation rather mature cellforms are needed (for example cells with myofibrils, pinocytotic activity or basement membrane), as it is not possible to distinguish between the different isoforms of actin or between vimentin and desmin ultrastructurally.

The immunolocalization of desmin in cells of sarcomas, diagnosed as MFH on the basis of light microscopic observations, has been described by Miettinen et al. (1982) and Leader et al. (1987). The light microscopic patterns of leiomyogenic sarcomas can also, however be suggestive for MFH (Enzinger and Weiss, 1983). In our opinion the presence of desmin in the former tumours should be interpreted as a feature of myogenic differentiation (De Jong et al., 1987). This is supported by the fact that myofibroblasts, a cell type frequently observed in sarcomas (Lagacé et al., 1980), lack desmin (Schürch et al., 1984; Iwasaki et al., 1987) and a clear immunohistochemical distinction can therefore be made between fibroblasts and myofibroblasts on the one hand and leiomyoblasts on the other. An ultrastructural distinction between these cell types is often difficult (Churg et al., 1977). Consequently we give priority to the immunomarkers and have classified the three tumours as (poorly differentiated) leiomyosarcomas.

Recently, studies describing the immunolocalization of desmin in smooth muscle tumours, have been published. In some of these neoplasms desmin was found (Leader et al., 1987; Schürch et al., 1987), whereas desmin could not be found in any of these types of neoplasm, located in the small intestine (Ricci et al., 1987). The study of Schürch et al. (1987) demonstrated that absence of immunoreactivity for desmin should be interpreted continously, as in their tumours studied ultrastructural evidence of smooth muscle differentiation was present. The immunohistological demonstration of desmin, however, can be absent, when formation fixed tissues are used (Table 1, case 3). A better immunopreservation of desmin is obtained when alcohol-fixed (Altmansberger et al., 1981) or frozen tissues are used (Table 1).

So desmin is not an absolute marker for leiomyosarcomas. Other immunomarkers for these sarcomas are the isoforms of actin and of myosin specific for smooth muscle cells. The alpha isoform of smooth muscle actin, however, is also not absolute, as its is apparently lost in leiomyosarcomas (Schürch et al., 1987).

It appears that MFH neoplasms cannot be diagnosed on the basis of specific microscopic features and that other types of soft tissue tumours are able to express histological characteristics similar to those described for MFH tumours. This was observed for, among others, neoplasms derived from patients known to have Recklinghausen's disease (Herrera and Pinto de Moraes, 1984), and also for dedifferentiated liposarcomas (Enzinger and Weiss, 1983). Another conclusion from these and other studied should be that although MFH tumours appear undifferentiated from a histopathological point of view, some of them are able to express markers which permit a possible differentiation. This conclusion fits into the hypothetical model of Brooks (1986). This model describes that MFH neoplasms are derived from primitive fibrohistioblasts, which have the capacity to differentiate into various differentiations. Indeed we found this in an experimental model (Roholl et al., 1988).

A great deal has been published concerning MFH and leiomyosarcomas, describing their histopathological differences (Fu et al., 1975; Churg and Kahn, 1977; King et al., 1982; Enzinger and Weiss, 1983; Hoffman and Dickersin, 1983). From these data it is clear that when light and electronmicroscopic features are used, often only a faint difference between these neoplasms can be detected. The three cases described in this study perfectly fulfilled the criteria for the diagnosis of MFH of the storiform-pleomorphic subtype. The presence of desmin did, however, not fit with this diagnosis and the absence of specific skeletal muscle proteins argued in favour of (poorly differentiated) leiomyosarcoma. It is therefore concluded that the tumour cells in fact belong to the smooth muscle cell lineage, without showing its ultrastructural characteristics. In other words, they lacked myofilaments, groups of pinocytotic vesicles, a basement membrane, and glycogen, but they did possess vimentin and desmin as intermediate filaments.

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