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Cytoskeletal characteristics of myofibroblasts in benign neoplastic and reactive fibroblastic lesions

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Summary. The characteristics of the cytoskeleton of myofibroblasts were examined immunohistochemically in 10 extra-abdominal desmoid tumours, 3 palmar and 2 plantar fibromatoses and 5 nodular fasciites; in the cultured cells of one desmoid tumour, and also ultrastructurally in 3 desmoid tumours. Polyclonal anti-desmin antibody reacted with the cells in 7 extra-abdominal desmoid tumours, 1 palmar fibromatosis, 1 plantar fibromatosis and 3 nodular fasciites. Monoclonal anti-desmin antibody reacted with cells in only 2 desmoid tumours. Desmin-positive spindle cells were scattered throughout these lesions. There were no marked ultrastructural differences between desmin-positive and desmin-negative desmoids. All specimens except one specimen of nodular fasciitis showed immunoreactivity for alpha-smooth muscle actin and vimentin. Muscle actin-positive cells were observed in all specimens. Cultured cells gave positive reactions with polyclonal desmin antibody as well as to vimentin antibodies and two preparations of actin antibodies, whereas the original tumour did not react with desmin antibody. The present studies suggested that the cytoskeleton of some myofibroblasts in both neoplastic and reactive lesions resembles that of smooth muscle cells.

Key words: Myofibroblast – Cytoskeleton – Desmoid tumour – Desmin – Immunohistochemistry

Introduction

The term 'myofibroblast' was first used by Majno et al. (1971) to define the spindle cells of connective tissue that exhibit ultrastructural features of both smooth muscle cells and fibroblasts. Myofibroblasts have been observed in the following tissues: injured or repair tissue (Gabbiani et al. 1971; Majno et al. 1971; Adler et al. 1981; Ballardini et al. 1988) stroma of malignant tumours (Ghosh et al. 1980; Nakanishi et al. 1981; Schürch et al. 1984) fibroblastic neoplasms, such as desmoid tumours (Stiller and Katenkamp 1975; Goellner and Soule 1980), palmar and plantar fibromatoses (Gabbiani and Majno 1972; Meister et al. 1979; Schum and McFarlane 1988), malignant fibrous histiocytomas (Churg and Kahn 1977; Nakanishi et al. 1981; Hirose et al. 1989), myofibroblastomas (Hashimoto et al. 1982; Fletcher et al. 1987; Wargotz et al. 1987), and infantile digital fibroma (Bhawan et al. 1979); and tumour-like lesions of fibroblasts, such as nodular fasciitis (Wirman 1976), and proliferative fasciitis (Craver and McDivitt 1981). However, it is still uncertain whether myofibroblasts are of fibroblastic or smooth muscle nature. In the present study, using antibodies to cytoskeletal proteins we examined the cytoskeletal properties of myofibroblasts immunohistochemically in myofibroblastic tumours (extra-abdominal desmoid and palmar and plantar fibromatoses) and reactive lesions containing numerous myofibroblasts (nodular fasciitis). In addition, we examined cultured cells of one desmoid tumour immunohistochemically and 3 extra-abdominal desmoid tumours ultrastructurally.

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Materials and methods

Ten extra-abdominal desmoids, 3 palmar fibromatoses, 2 plantar fibromatoses and 5 nodular fasciites were studied.

Tumour samples obtained from an extra-abdominal desmoid were cultured by the method of Sethi et al. (1977). Cells were grown in RPMI 1640 medium (Nissui) with 10% heat-

inactivated fetal calf serum at 37° C in an atmosphere of 5% CO_2 in air. For passage, cells were treated for 2 to 3 min with 0.05% trypsin and EDTA. After several passages, cells were fed and subdivided at 1:2 dilution.

Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections of 4 μm thickness were prepared for staining with haematoxylin-eosin, phosphotungstic acid-haematoxylin (PTAH) and silver impregnation for reticulin, and for immunohistochemical examination. For cytological examination, cultured cells were grown in chamber slides (Lab-Tek, Miles Lab.). Cells fixed in 10% neutral buffered formalin were stained with haematoxylin-eosin. For immunocytochemistry, cells were fixed in cold methanol for 5 min and air dried. Immunohistochemical and immunocytochemical stainings were carried out by the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. (1981) with an ABC kit (Vector Lab., USA). The primary antibodies used are shown in Table 1.

For electron-microscopy, material from 3 extra-abdominal desmoid tumours were cut into 1-mm cubes and fixed in 3% glutaraldehyde in phosphate buffer. The specimens were then washed with the buffer solution, post-fixed in 1% OsO_4 in phosphate buffer, dehydrated, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H-300 electron microscope.

Table 1. Antibodies used in immunohistochemical analyses

Primary antibody	Source	Dilution
Vimentin	DAKO, m	× 10
Cytokeratin		
keratin	DAKO, p	× 500
PKK1	Labsystems, m	× 100
Desmin		
DP-1	our laboratory[a], p	×1000
DP-2	DAKO, p	× 500
DM	DAKO, m	× 50
Alpha-smooth muscle actin (ASMA)	Biomakor, m	×1000
Muscle actin (MA)	Enzo, m	×1000

m: monoclonal, p: polyclonal, [a] Hirose et al. (1988)

Results

The extra-abdominal desmoid tumours were poorly circumscribed and invaded surrounding skeletal muscle. The tumours consisted of a moderately cellular proliferation of ill-defined fascicles of spindle cells. Dense collagen fibers between individual tumour cells were clearly identified in preparations stained for reticulin. The nuclei were vesicular and had one to three minute nucleoli (Fig. 1a). Palmar and plantar fibromatoses showed similar histological characteristics. In some cases, spindle cells were smaller and thinner than those of extra-abdominal desmoids. All five lesions of nodular fasciites were round or oval nodules in the subcutis, consisting mainly of plump fibroblasts arranged in short fascicles and irregular bundles, with a few scattered chronic inflammatory cells. The fibroblasts with weakly staining, oval nuclei with prominent nucleoli contained delicate fibrils demonstrated by PTAH staining. Mitotic

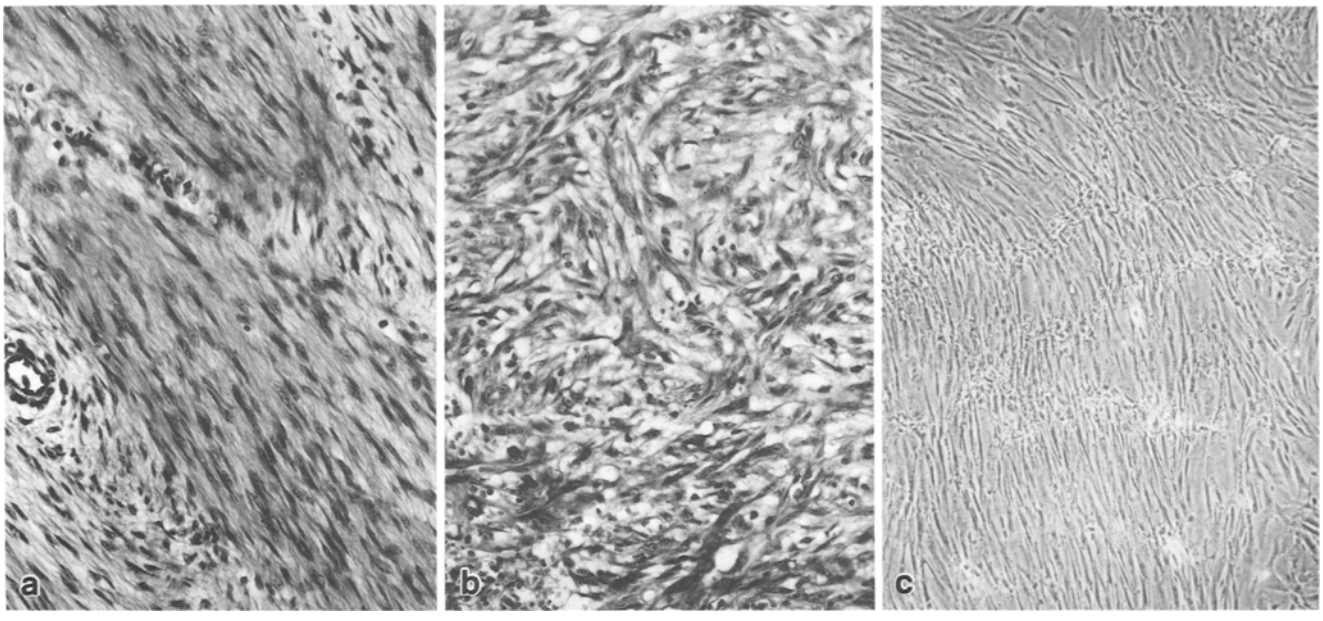


Fig. 1. a Extra-abdominal desmoid showing spindle-shaped tumour cells and abundant collagen fibers arranged in sweeping bundles. H.E. ×200. **b** Nodular fasciitis showing short irregular bundles and fascicles of plump fibroblasts and scattered chronic inflammatory cells. H.E. ×200. **c** Cultured desmoid tumour cells forming a confluent monolayer of spindle cells. Phase micrograph, ×10

Table 2a. Immunohistochemical reactivities of myofibroblastic lesions for desmin, vimentin, ASMA, and MA

Case	Diagnosis	Percentage of positive cells					
		Desmin			Vimentin	ASMA	MA
		DP-1	DP-2	DM			
1.	Extra-abdominal desmoid	10	30	0	90	10	60
2.	Extra-abdominal desmoid	10	10	0	80	40	70
3.	Extra-abdominal desmoid	10	10	0	90	70	60
4.	Extra-abdominal desmoid	30	20	0	90	80	80
5.	Extra-abdominal desmoid	30	10	0	70	60	60
6.	Extra-abdominal desmoid	20	20	10	80	60	50
7.	Extra-abdominal desmoid	0	0	0	10	10	50
8.	Extra-abdominal desmoid	0	0	0	20	10	10
9.	Extra-abdominal desmoid	0	0	0	70	50	50
10.	Extra-abdominal desmoid	40	10	10	80	70	50
Positive cases/tested cases		7/10	7/10	2/10	10/10	10/10	10/10

Table 2b. Immunohistochemical reactivities of myofibroblastic lesions for desmin, vimentin, ASMA, and MA

Case	Diagnosis	Percentage of positive cells					
		Desmin			Vimentin	ASMA	MA
		DP-1	DP-2	DM			
11.	Palmar fibromatosis	0	0	0	40	40	90
12.	Palmar fibromatosis	0	0	0	50	20	60
13.	Palmar fibromatosis	40	30	0	90	60	30
14.	Plantar fibromatosis	30	0	0	80	50	70
15.	Plantar fibromatosis	0	0	0	90	80	70
16.	Nodular fasciitis	30	0	0	90	60	70
17.	Nodular fasciitis	30	0	0	90	60	70
18.	Nodular fasciitis	10	0	0	80	40	50
19.	Nodular fasciitis	0	0	0	0	0	30
20.	Nodular fasciitis	0	0	0	80	80	80
Positive cases/tested cases		5/10	1/10	0/10	9/10	9/10	10/10

figures were common, but no atypical mitoses were observed (Fig. 1b). Cell cultures in flasks consisted of closely packed, confluent monolayers with bundles of aligned cells converging at various angles (Fig. 1c). Stained monolayer cells on glass slides were long, thin cells arranged in clusters. They contained plump nuclei and a few minute nucleoli. These cytological features were similar of those of myofibroblasts in the original desmoid tumour.

Immunohistochemical results are shown in Table 2. None of the specimens showed immunoreactivity for cytokeratin. Polyclonal anti-desmin antibodies, DP-1, DP-2, reacted with some cells in 7 extra-abdominal desmoids and in one palmar fibromatosis. DP-1-positive cells were also present in one plantar fibromatosis and 3 lesions of nodular fasciitis, but DP-2 was not detected in these 4 lesions. Monoclonal anti-desmin antibody, DM, reacted with only 2 desmoid tumours, which also showed reactivity with polyclonal antibodies. Desmin-positive spindle cells were seen scattered throughout sections of these lesions. Immunoreactivity with DP-1 antibody was the most intense and was seen in many cells (Fig. 2a).

All specimens except one of nodular fasciitis showed immunoreactivities for alpha-smooth muscle actin (ASMA) and vimentin. MA antibody reacted with cells in all 20 lesions. Vimentin-positive cells were more numerous than desmin-positive cells and were distributed throughout the lesions (Fig. 2b). Immunoreaction products for ASMA and MA were present in numerous spindle-shaped cells in addition to pericytes and smooth muscle cells of the vessels (Fig. 2c, d).

Cultured cells in Case 8 showed uniformly weak reactions with the two anti-desmin anti-

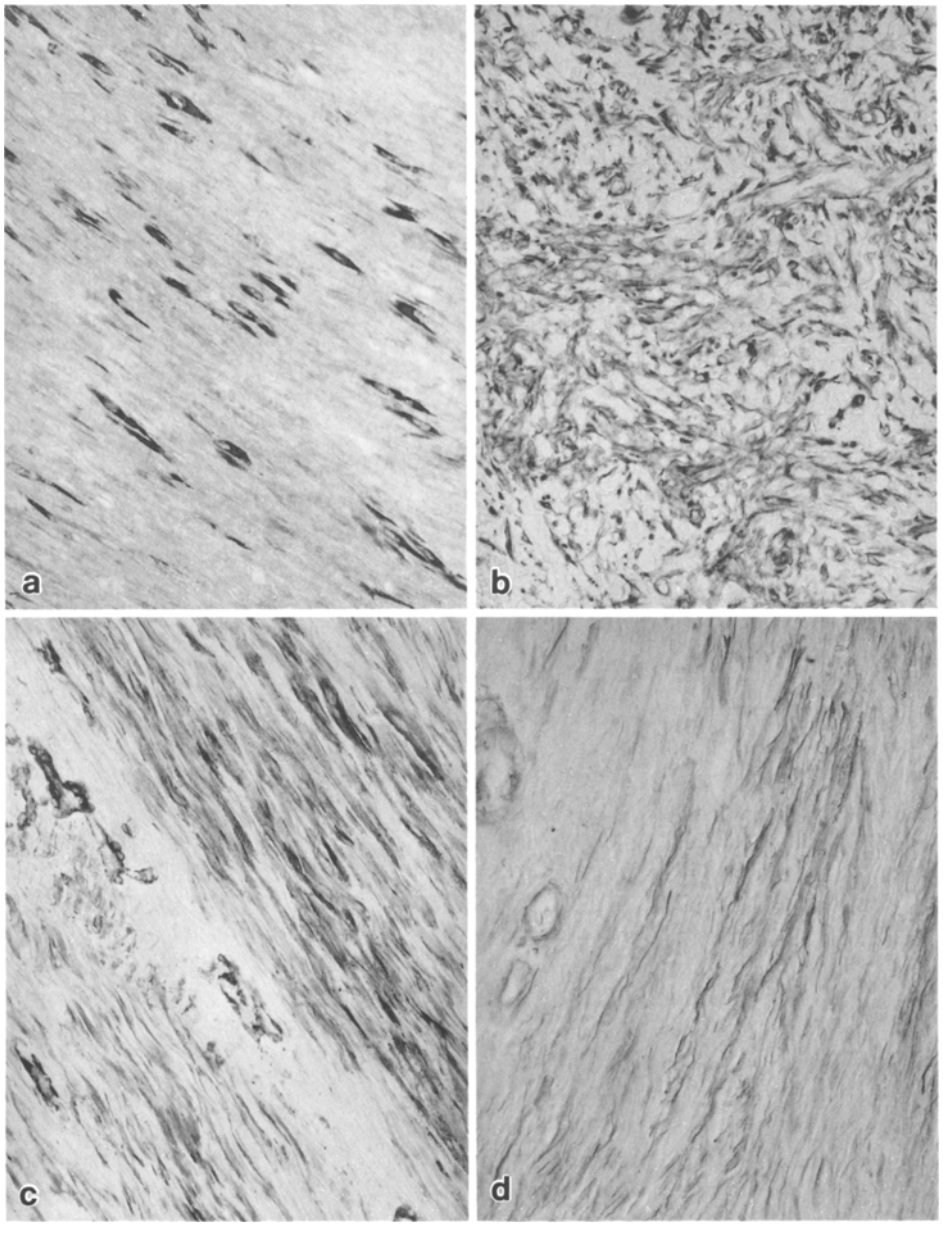


Fig. 2. a Polyclonal desmin (DP-1)-immunoreactivity in some tumour cells in a desmoid. ×200. **b** Vimentin-reactivity in numerous cells in nodular fasciitis showing irregular fascicles. ×200. **c** ASMA-immunoreactivity in many spindle cells of a desmoid. Pericytes of the vessels are also positive for ASMA. ×200. **d** Fibrillary MA-immunoreactivity observed in plantar fibromatosis. ×400

bodies, DP-1 and DP-2, whereas the original tumour did not show any reactions with these antibodies (Fig. 3a). The cultured cells also gave positive reactions for vimentin, ASMA, and MA. ASMA- and MA-immunostainings revealed some intracellular filamentous structures, stress fibers, running parallel to the long axis of the cells (Fig. 3b).

Ultrastructurally in all three desmoid tumours, 2 desmin-positive (Cases 6 and 10) and one desmin-negative (Case 7), spindle or stellate tumour cells had round or oval nuclei with occasional indentations and small nucleoli. Their cytoplasm contained a well-developed rough endoplasmic reticulum (RER), prominent Golgi apparatus, and some mitochondria. Many tumour cells had bundles of microfilaments with dense bodies, running parallel to the long axis of the cell body, numerous pinocytotic vesicles on the cell surface and occasional incomplete external laminae (Fig. 4). These ultrastructural features of tumour cells were consistent with those of myofibroblasts. There were no remarkable ultrastructural differences in the quantity of microfilaments or the development of pinocytotic vesicles and external laminas between the desmin-positive and desmin-negative tumors, although some tumour cells of the desmin-negative tumour contained microtubules (Fig. 5).

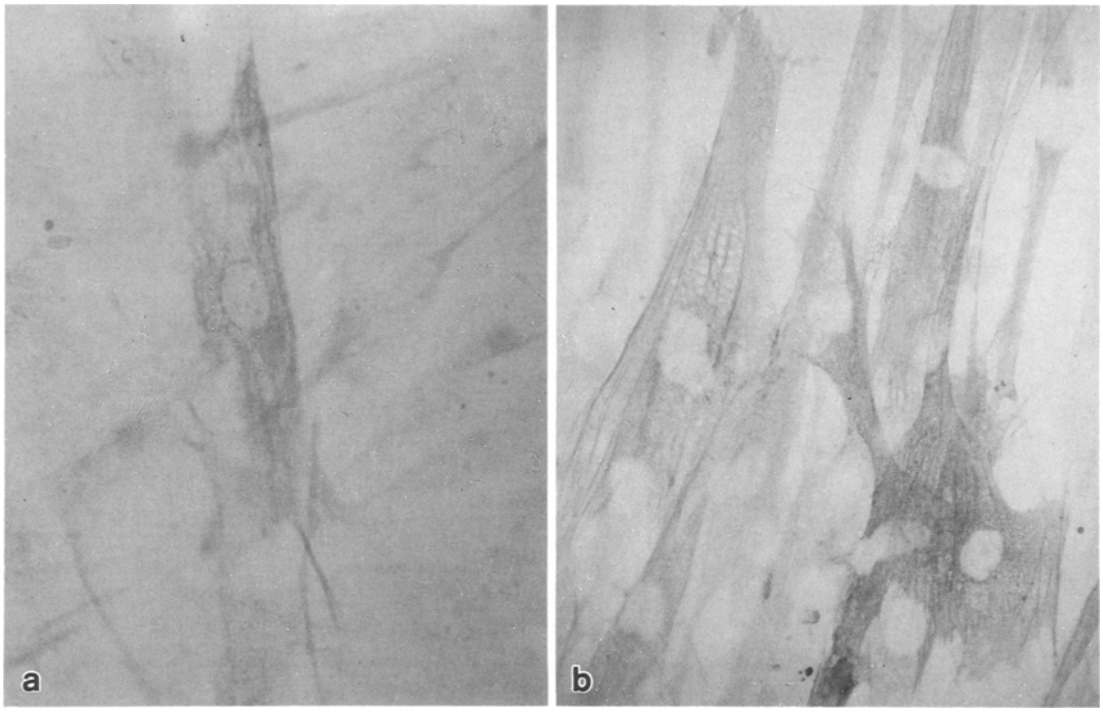


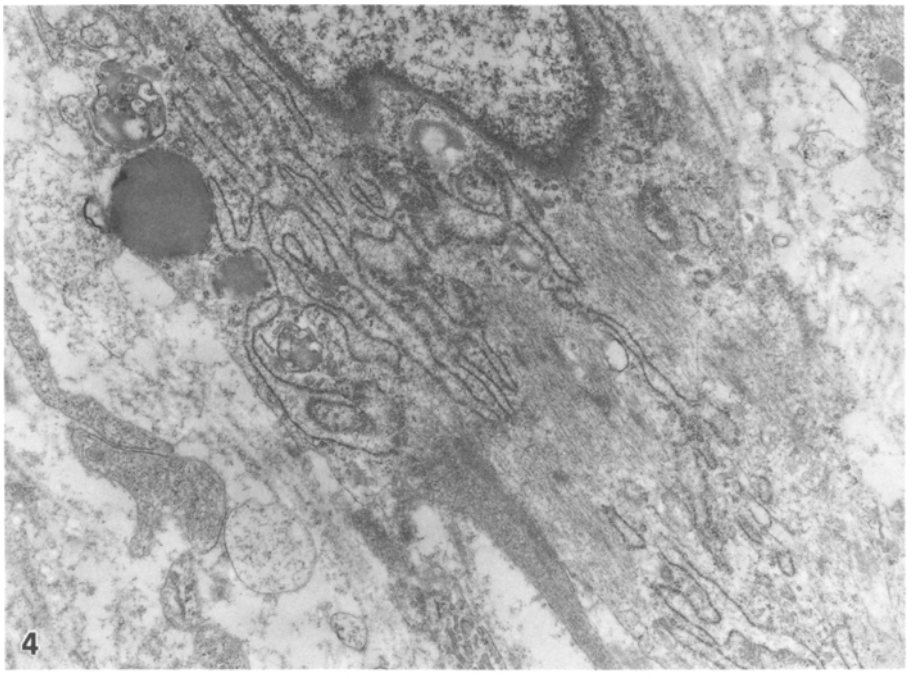
Fig. 3. **a** Filamentous immunoreactivity for desmin (DP-1) antibody in some cultured cells of a desmoid tumour. ×480. **b** Stress fibers stained with ASMA observed in numerous cultured cells. ×400

Discussion

Since their initial recognition by Gabbiani et al. (1971), myofibroblasts have been studied extensively, using a variety of techniques, such as electron microscopy, immunohistochemistry, and in vitro physiological studies. It is still uncertain whether myofibroblasts are modified fibroblasts or modified smooth muscle cells, or are derived from other mesenchymal cells including stem cells. In this study we found that the ultrastructural features of myofibroblasts were intermediate between those of fibroblasts and smooth muscle cells and the origin and nature of these cells is thus difficult to deduce from their morphological features alone. Recently, the characteristic cytoskeletal properties of myofibroblasts have been reported. In general, cells and tissues are considered to have specific types of intermediate filament (Gabbiani et al. 1981a; Osborn and Weber 1983). Schürch et al. (1984) and Iwasaki et al. (1987) reported that the only detectable intermediate filament protein of myofibroblasts in some myofibroblastic lesions and in vitro is vimentin. Therefore, they postulated that myofibroblasts originate from fibroblasts, not from smooth muscle cells.

Conversely, some investigators have recently demonstrated desmin-immunoreactivities in some myofibroblastic lesions, such as myofibroblastomas of the breast (Wargotz et al. 1987), infantile myofibromatosis (Fletcher et al. 1987), and experimental liver fibrosis (Ballardini et al. 1988). Leader et al. (1987), Lawson et al. (1987), and Hirose et al. (1989) interpreted desmin-positive reactions in malignant fibrous histiocytomas as being due to myofibroblastic differentiation of some tumour cells. In addition, Miettinen (1988) and Skalli et al. (1989) demonstrated desmin-positive cells in desmoid tumours and suggested the existence of a desmin-positive subset of myofibroblasts.

In the present immunohistochemical study, 12 of 20 myofibroblastic lesions contained desmin-positive cells. Desmin-positive cells were more numerous in desmoid tumours than in palmar and plantar fibromatoses and nodular fasciites. Similar quantitative differences in different pathological conditions were also reported by Skalli et al. (1989). Furthermore, cultured cells obtained from a desmoid showed diffuse desmin-immunoreactivity, although the original tumour was desmin-negative. Thus, myofibroblasts could be induced to express desmin protein even in vitro. Hence, different types of intermediate filaments, vimentin and desmin, are concurrently expressed in these myofibroblastic lesions. This heterogenous expression of intermediate filaments is not unexpected, because



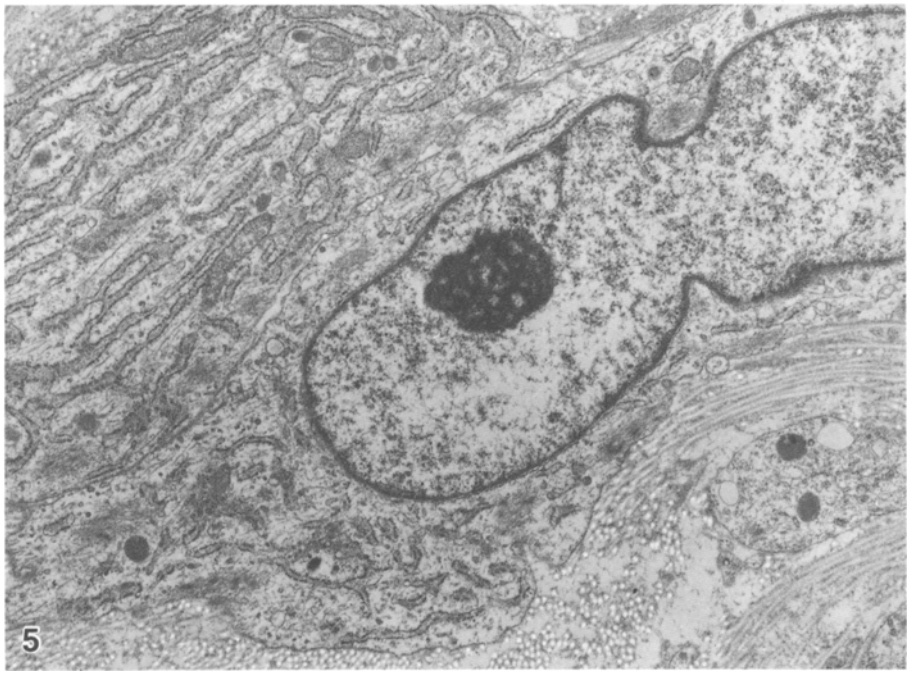


Fig. 4. Bundles of microfilaments with dense bodies running parallel to the long axis of the cell observed in tumour cells of a desmoid. Case 10, ×10000

Fig. 5. Cytoplasm of a desmoid cell containing well developed rough endoplasmic reticulum, Golgi apparatus, and microfilaments. Note some microtubules in deeper regions within the cell. Case 7, ×5000

it has also been observed in some smooth muscle cells: parenchymal smooth muscle cells of the respiratory, gastrointestinal, and urogenital tracts contain numerous desmin-positive cells (Schürch et al. 1987), whereas some vascular smooth muscle cells contain only vimentin (Gabbiani et al. 1981 b; Osborn et al. 1981). In the present ultrastructural study no marked morphological differences were found between myofibroblasts in desmin-positive and desmin-negative tumours.

Actin is a ubiquitous cytoskeletal protein of microfilaments and is also present in myofibroblasts (Schürch et al. 1984). The recent development of monoclonal antibodies that are specific for actin isoforms has facilitated studies on the distribution and expression of muscle-type actins in normal tissues and neoplasms. Antialpha-smooth muscle antin (ASMA) antibody has been reported to react mainly with smooth muscle cells and well-differentiated smooth muscle cell tu-

mours, but not with fibroblastic tissues (Skalli et al. 1986, 1987; Schürch et al. 1987). Antimuscle actin (MA) antibody recognizes alpha-actin isoforms of smooth, skeletal and cardiac muscle cells and gamma-actin of smooth muscle cells (Tsukada et al. 1987a). Therefore, both antibodies have been considered to be useful for the detection of smooth muscle cell differentiation in normal and pathological conditions (Schürch et al. 1987; Tsukada et al. 1987a).

There have been only a few reports on the actin isoforms expressed in myofibroblastic lesions. MA-positive myofibroblasts were detected in palmar fibromatosis, desmoid tumours and the stroma of breast and ovarian carcinomas (Tsukada et al. 1987b; Miettinen 1988). Skalli et al. (1989) also reported that myofibroblasts in hypertrophic scars and fibromatoses showed immunohistochemical features of smooth muscle differentiation with distinct expression of desmin and ASMA besides vimentin. These features were, however, not found in normally healing granulation tissue (Skalli et al. 1989). In the present immunohistochemical study, positive reactions for both ASMA and MA were observed in almost all cases of fibromatoses and nodular fasciites. Cultured cells showed linear distributions of ASMA- and MA-immunoreactivities, suggesting the presence of stress fibers. Similar immunoreactions were reported in cultured aortic smooth muscle cells by Tsukada et al. (1987a) and Skalli et al. (1986).

The immunoreactivities for desmin, ASMA and MA demonstrated in the present studies indicated that the cytoskeletal properties of some myofibroblasts, at least in neoplastic and reactive proliferative lesions, are analogous to those of smooth muscle cells. However, the myosin of myofibroblasts in granulation tissue and hypertrophic scars is reported not to be of the muscle type (Eddy et al. 1988), although we could not study the type of this protein. Furthermore, the natures of the intermediate filaments in given cell type are now known not to be so constant as previously thought (Gould 1985). The origin of myofibroblasts cannot thus be determined by studies on their cytoskeletal expression alone. Nevertheless, the findings show that cytoskeletal findings are important in considering the nature of these cells and are practically useful in the diagnoses of some soft tissue tumours.

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