Histol Histopathol (2001) 16: 57-70

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

The fibronexus in reactive and tumoral myofibroblasts: further characterisation by electron microscopy

B. Eyden

Department of Histopathology, Christie Hospital NHS Trust, Manchester, United Kingdom

Summary. Forty two surgical specimens containing myofibroblasts were studied to clarify the criteria for identifying the fibronexus, an ultrastructural feature regarded as a marker for myofibroblastic differentiation. Granulation tissue, tumour stroma, fibro-proliferative lesions (nodular fasciitis, myofibromatosis, inflammatory myofibroblastic tumour) and malignancies (myofibrosarcoma and fibrosarcoma) were studied. Comparable results were found throughout these specimens, although fibronexus junctions were better developed in reactive compared with tumoral myofibroblasts. By electron microscopy, myofibroblasts were identified by abundant rough endoplasmic reticulum, peripheral smooth-muscle myofilaments with focal densities, and fibronexus junctions. The latter were recognised as the points of convergence on the myofibroblast surfaces of intracellular myofilaments and extracellular fibronectin fibrils. The fibronectin fibrils were often co-linear with myofilaments. Also, fibronectin fibrils were dark-staining, straight and rigid-looking, and had a longitudinal filamentous substructure. A striking feature was the tendency of fibronectin fibrils to project into the surrounding extracellular space, away from the myofibroblast surface: in these respects, they differed significantly from lamina ("basement membrane"). The presence of fibronectin fibrils correlated positively with fibronectin immunostaining by light and electron microscopy. Laminin and collagen IV showed variable and weak staining in the intercellular spaces in a minority of cases and never strongly stained myofibroblast surfaces. The data emphasise that the fibronexus has a number of distinctive features permitting identification, and constitute a reference-point for pathologists wishing to use electron microscopy to refine light microscopy diagnoses of putative myofibroblastic lesions. The role of the fibronexus in the definition of the myofibroblast is discussed.

Offprint requests to: Brian Eyden PhD, Department of Histopathology, Christie Hospital NHS Trust, Manchester M20 4BX, United Kingdom. Fax: +44 161 446 3300

Key words: Fibronexus, Myofibroblast, Electron-microscopy, Fibronectin , Basement-membrane

Introduction

The myofibroblast is a mesenchymal cell with a predominantly spindled morphology in histological and ultrathin sections, described initially in granulation tissue, and regarded as having a role in wound healing (Gabbiani et al., 1971; Majno et al., 1971). The cell was subsequently identified in reactive tumour stroma, in a variety of fibro-proliferative lesions such as nodular fasciitis, the fibromatoses and certain pseudo-tumours (Schürch et al., 1998), and as neoplastic elements in malignancies (Eyden et al., 1991, 1992; Eyden and Christensen, 1993; Smith et al., 1995; Taccagni et al., 1997; Mentzel et al., 1998; Gocht et al., 1999). The myofibroblast was originally defined by electron microscopy: it was found to have prominent rough endoplasmic reticulum (rER), modestly developed peripheral myofilaments of smooth-muscle-type, and a surface material first described as *basement membrane-like material*. The subsequent demonstration of a filamentous substructure and fibronectin content indicated that this was a structure differing significantly from lamina ("basement membrane"[1]), and it has, therefore, been referred to as the *fibronectin fibril*

[1]: Basement membrane is the classical light microscopy term for the proteinaceous interface between epithelium and endothelium on the one hand, and matrix on the other. Electron microscopy has shown that one of the principal constituents of this zone is a dense plate of protein called the basal lamina. The preference for most electron microscopists, therefore, is to use basal lamina rather than basement membrane. The term basal lamina is particularly appropriate where a cell shows an unambiguous apical-basal polarity. A nearly identical structure completely encircles individual muscle cells, adipocytes and nerve-sheath cells: here there is no apical-basal polarity and the term basal – although often used - is, therefore, inappropriate. For this reason, external lamina is often used, but since both basal and external laminae are outside the cell, the simple term lamina has been preferred (Eyden, 1996) and is used here.

(Eyden, 1993). Its other main characteristic is that, from its point of contact with the cell surface, it projects into the extracellular space, and has been regarded as a means of transmitting intracellular contractile force to matrix to achieve overall tissue contraction. The point of convergence on the cell surface of intracellular myofilaments and the extracellular fibronectin fibril is known as the *fibronexus* or *fibronexus junction*: this - in the context of other features (rER and myofilaments by electron microscopy, and smooth-muscle actin by immunostaining) - has been argued as an important marker for defining myofibroblastic differentiation (Eyden et al., 1991,1992; Eyden and Christensen, 1993; Eyden, 1993, 1996; Smith et al., 1995; Taccagni et al., 1997; Gocht et al., 1999).

In contrast to the perceived usefulness of the fibronexus in these papers, some authors have found the myofibroblast difficult to define (Mentzel and Fletcher, 1997; Mentzel et al., 1998) and the fibronexus difficult to identify (Mentzel et al., 1998; Fletcher, 1999). Given the varied experience in which some pathologists can and some can not identify the fibronexus, this paper has the objective of giving a detailed description of the fibronexus in reactive and tumoral myofibroblasts, which can act as a reference-point to help those working with soft-tissue lesions reach more precise, differentiation-based diagnoses.

Materials and methods

Clinical material

The study consisted of 42 specimens, which were either surgical accessions of the Christie Hospital, Manchester, or referrals from nearby district general hospitals. Diagnostic and clinical data are given in table 1. Fifteen cases were primary or metastatic squamous cell carcinomas, for which fresh tissue was available for electron microscopy and glutaraldehyde fixation. A further 10 carcinomas with varying diagnoses and 4 non-carcinoma malignancies were sampled opportunistically for myofibroblastic stroma during the course of ultrastructural examination for routine diagnostic purposes (these included two pseudo-angiosarcomatous variants of squamous carcinoma). Here, tissue for electron microscopy was retrieved from histological formalin. Three samples of human granulation tissue presenting clinically as scar tissue following surgery for neoplastic disease, and 10 tumours or tumour-like lesions were also studied, with tissue for electron microscopy again being retrieved from histological formalin.

Light and electron microscopy

All specimens were fixed in phosphate-buffered histological formalin, embedded in paraffin wax and sectioned at 4 μm for subsequent staining in haematoxylin-and-eosin (H&E) for morphology and for immunohistochemistry. For light microscope immunostaining, the following antibodies were obtained from Europath (Vector Labs), UK: anti-fibronectin (mouse monoclonal, code MFIB, dilution 1:5); anti-collagen IV (rabbit polyclonal, code PCO, 1:50), and anti-laminin (rabbit polyclonal, code PLA, 1:25). They were used in a 3-step biotin-streptavidin-horseradish peroxidase complex method. For electron microscopy, all specimens were processed and sectioned as already described (Eyden et al., 1998), and ultrathin sections were examined in a JEOL JEM-1220 electron microscope. For immuno-electronmicroscopy, formalin-fixed tissue without osmication was embedded in LRWhite hydrophilic resin (Agar Scientific, UK) polymerised at 50 °C. Ultrathin sections were collected on nickel grids, and exposed first to a primary unlabelled antibody, then to a secondary 15nm gold labelled antibody (British Biocell, UK).

Results

All myofibroblasts had a plump, spindle-cell appearance in H&E sections with cytoplasm lacking obvious myofibrillar striations (Fig. 1A). By electron microscopy, they were identified by the presence of abundant rER cisternae, modestly developed bundles of smooth-muscle myofilaments with focal densities, and fibronexus junctions. The latter were recognised as the points of convergence on the myofibroblast surfaces of intracellular myofilaments and extracellular fibronectin fibrils.

Fibronexus junctions were observed on myofibroblasts from all samples of tumour stroma (Figs. 1, 3) and granulation tissue (Fig. 2), although they showed variation in the degree of development from cell to cell and case to case. They were also seen in nodular fasciitis (2/2 cases) (Fig. 5A-C), myofibromatosis (2/2 cases) (Fig. 5D), and single cases of inflammatory myofibroblastic tumour (Fig. 5E), post-operative spindle cell nodule and paratesticular benign pseudo-tumour. A single example of fibroma of tendon sheath showed structures which were suggestive of fibronectin fibrils but which were less well developed and therefore less convincing as components of a fibronexus than the other specimens. Fibronexus junctions were also observed in 3 soft-tissue malignancies not hitherto documented (two

Fig. 1. Myofibroblasts from tumour stroma (case 1). **A.** Light micrograph of H&E-stained section to show plump, spindled myofibroblasts in a collagenised matrix between islands of tumour. x 400. **B.** Myofibroblasts and myofibroblast processes showing abundant rER, smooth-muscle myofilaments (mainly peripheral) (arrow-heads), and fibronectin fibrils (small arrows). x 5,000. **C.** Fibronexus characterised by intracellular myofilaments (mf) in contact with an area of cell membrane (arrow-heads), and fibronectin fibril (small arrow) arising from the same area, and projecting out into the extracellular matrix. x 16,000


A
mf
C
B

myofibrosarcomas (Fig. 6A, B) and a fibrosarcoma (Fig. 6C).

Fibronexus junctions in reactive[2] myofibroblasts

In tumour stroma and granulation tissue, the fibronexus was identified by intracellular smooth-muscle myofilaments interacting at a localised area of the cell membrane, usually a submembranous plaque, with an

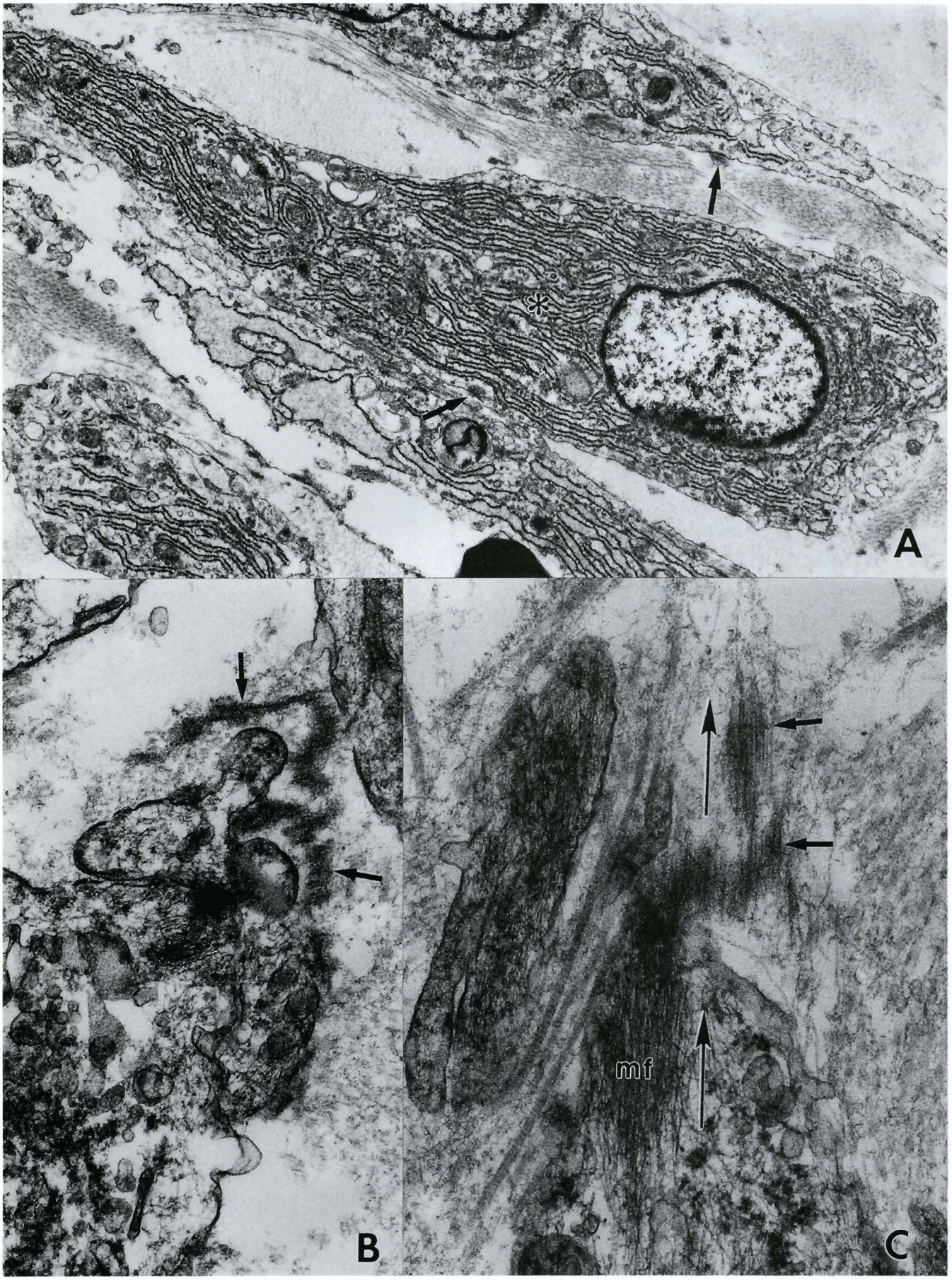
[2]: myofibroblasts from tumour stroma and from granulation tissue will be referred to collectively as reactive myofibroblasts.

Table 1. Diagnostic and clinical information on specimens containing myofibroblasts.

CASE	REACTIVE TUMOUR STROMA
1	primary keratinising SCC[1], cervical skin, M[2] 85 yr[3]
2	poorly differentiated SCC metastatic to supraclavicular lymph node, F[2] 41 yr
3	well differentiated SCC metastatic to inguinal lymph node, F 85 yr
4	keratinising SCC metastatic to cervical lymph node, F 65 yr
5	poorly differentiated SCC metastatic to axillary node, F 35 yr
6	primary well differentiated SCC, inguinal fibro-fatty tissue, F 48 yr
7	recurrent SCC, tongue, M 48 yr
8	poorly differentiated SCC metastatic to inguinal lymph node, F 56 yr[4]
9	extra-nodal deposit of moderately differentiated SCC (block neck dissection), M 52 yr
10	primary invasive moderately differentiated SCC, neck, M 89 yr
11	primary invasive, well differentiated SCC, nose, F 88 yr
12	poorly differentiated SCC metastatic to cervical node, M 59 yr
13	primary invasive moderately differentiated SCC, temple, M 66 yr
14	well differentiated SCC, leg, F 83 yr
15	primary moderately differentiated non-keratinising SCC, pinna, M 79 yr
16	primary pseudo-angiosarcomatous SCC of pinna of ear, M 78 yr
17	primary pseudo-angiosarcomatous SCC of breast, F 73 yr
18	adenocarcinoma of unknown primary site metastatic to cervical lymph node, M 44 yr
19	primary invasive poorly differentiated adenocarcinoma, colon, F 88 yr
20	carcinoma with chondroid metaplasia of unknown primary site metastatic to small bowel, F 42 yr
21	primary secretory carcinoma of breast, F 44 yr
22	primary undifferentiated large-cell carcinoma, skin of neck, M 55 yr[4]
23	primary pleomorphic lobular carcinoma, breast, F age not known
24	primary ductal carcinoma, breast, F age not known
25	primary ductal carcinoma, breast, F age not known
26	primary malignant myoepithelioma, submandibular gland, F 57 yr
27	primary desmoplastic malignant melanoma, naso-labial fold, F 79 yr
28	extraskeletal myxoid chondrosarcoma, thigh, M 14 yr
29	malignant fibrous histiocytoma, arm, M 65 yr
CASE	GRANULATION TISSUE
30	granulation tissue, knee, M 39 yr
31	granulation tissue, dermis (skin of calf), F 32 yr
32	granulation tissue, nose, F 65 yr[4]
CASE	TUMOURS/TUMOUR-LIKE LESIONS
33	nodular fasciitis, supra-pubic mass, F 66 yr
34	nodular fasciitis, calf, M 30 yr
35	musculo-aponeurotic fibromatosis, buttock, F 52 yr
36	inflammatory myofibroblastic tumour/pseudo-tumour, larynx, M 60 yr
37	post-operative spindle cell nodule, vulva, F age not known
38	fibroma of tendon sheath, M age not known
39	benign fibrous proliferation (fibrous pseudo-tumour), paratesticular mass, M 42 yr
40	small intestinal myofibrosarcoma metastatic to liver, F 17 yr
41	fibrosarcoma, biceps muscle, F 23 yr
42	myofibrosarcoma, skin of ear, M 64 yr

[1]: SCC, squamous cell carcinoma; [2]: M, male; F, female; [3]: yr, years; [4]: studied by immuno-electronmicroscopy.

Fig. 2. Myofibroblasts from granulation tissue (case 31). **A.** Low-magnification view showing abundant rER (*). Fibronexus junctions are inconspicuous (arrows). x 10,000. **B.** Medium-power view of "basement-membrane-like material" (fibronectin fibril) (arrows). The material looks amorphous owing to orientation within the section. x 30,000.**C.** High-magnification view of the fibronexus showing the end of a cytoplasmic process containing actin myofilaments (mf) in a co-linear orientation (long arrows) with respect to the extracellular fibronectin fibril (short arrows) which has a clearly defined filamentous substructure. x 60,000


A
B
C
mf


mf
A
C
*
mf
B

Fig. 3. Myofibroblasts from tumour stroma (A and C, case 1; B, case 3). **A.** Myofibroblast showing myofilaments (mf) and a fibronectin fibril (arrow), a part of which has sheet-like or ribbon-like area (arrow-heads). The distance between the arrow-heads is about 600 nm. x 22,000. **B.** Myofibroblast with comparatively modestly developed rER, but well developed peripheral myofilaments (mf) and fibronectin fibrils (arrows). The more slender fibril (asterisked arrow) has a superficially lamina-like appearance. x 20,000. **C.** Myofibroblast surface adorned with a large number of fibronectin fibrils (arrows). x 7,500

extracellular fibril, the *fibronectin fibril*. Generally at low magnifications, the fibronectin fibril was conspicuous and acted as a marker for the fibronexus. At low power particularly, fibronectin fibrils often appeared dense and somewhat irregularly organised (Fig. 1B). Very often, as revealed under higher magnification, they were associated with myofilaments in a *co-linear* fashion, i.e., the myofilaments and the fibronectin fibril had the same directional arrangement (Fig. 1C): this applied even to poorly developed fibronexus junctions (Fig. 2C). Typically, fibronectin fibrils were dark-staining, straight and rigid-looking, and had a longitudinal filamentous substructure (Figs. 1C, 3A). One of the most striking and characteristic features of the fibronectin fibril was its tendency to project out into the surrounding extracellular space, away from the myofibroblast surface (Figs. 1C, 3A). In this respect, the fibronectin fibril differed significantly from lamina, which normally follows the contour of the cell surface. On occasion, however, a lamina-like profile was seen (Fig. 3B). This was invariably restricted to a localised region of the cell surface and, given that many fibronectin fibrils appeared to have a broad ribbon-like profile (Fig. 3A), these lamina-like profiles (in the context of immuno-electronmicroscopy results – see below) were interpreted as being cross-sections of broad fibronectin ribbons. Appropriately sectioned myofibroblasts revealed large numbers of fibronectin fibrils at the cell surface (Fig. 3C).

Matrix protein staining in tumour stroma and granulation tissue

Staining for fibronectin was observed in all cases (Fig. 4A), although staining intensity varied. Often myofibroblast surfaces and extracellular matrix some distance from myofibroblasts were emphasised in fibril-like patterns of staining. Laminin and collagen IV showed variable staining in the intercellular spaces in a minority of cases and myofibroblast surfaces were never strongly stained.

Immuno-electronmicroscopy for fibronectin revealed highly specific staining for fibronectin fibrils (Fig. 4B) corresponding with the staining pattern by light microscopy (Fig. 4A). Staining with anti-collagen IV and anti-laminin antibodies gave sparse, widely dispersed and individual gold particles on the surfaces and over the cytoplasm of myofibroblasts (possibly representing a degree of non-specific staining) but consistently failed to show significant label on myofibroblast surfaces (Fig. 4C, D).

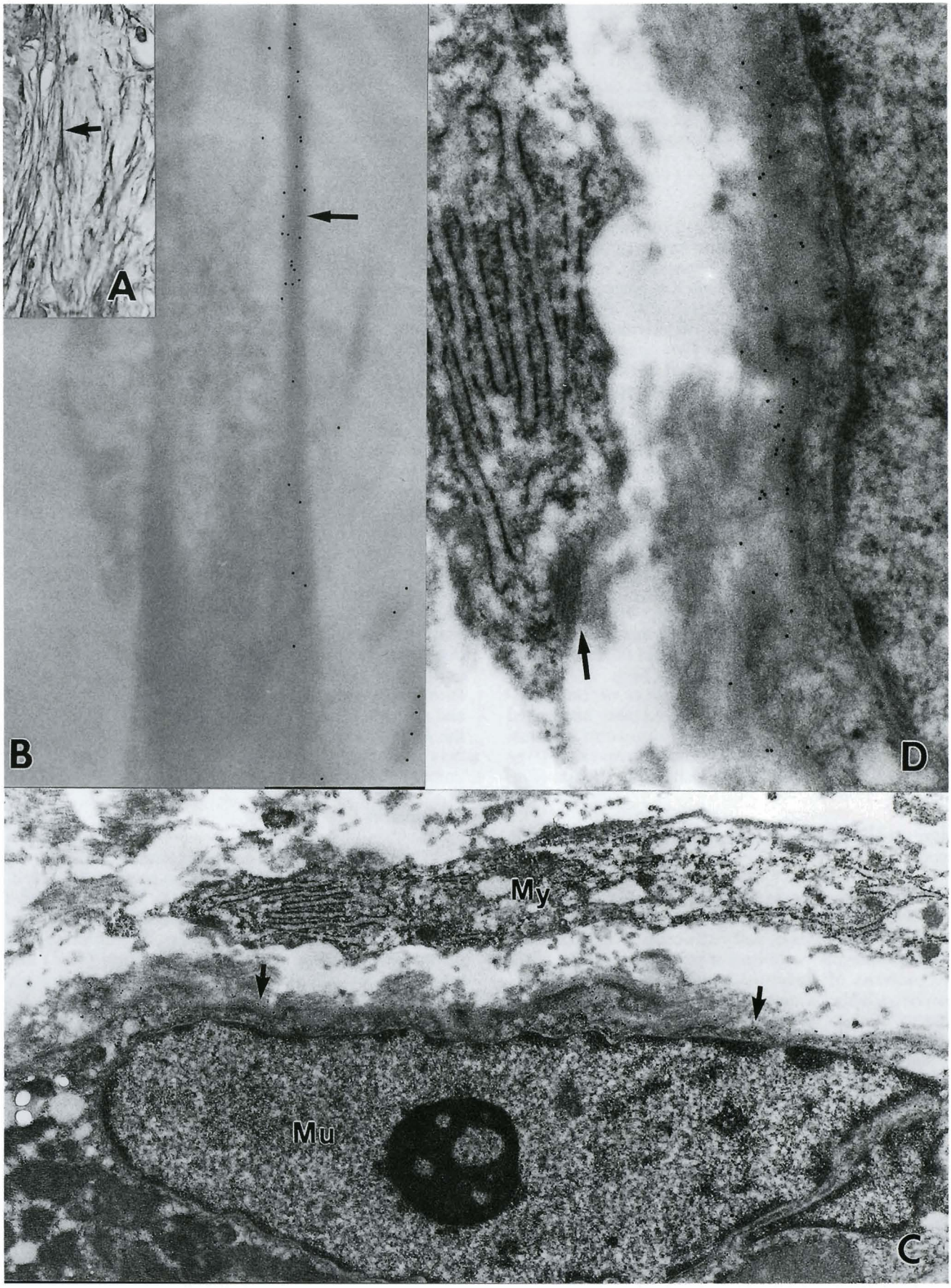
Tumoral fibronexus junctions

In nodular fasciitis, myofibromatosis, inflammatory myofibroblastic tumour (Fig. 5), post-operative spindle-cell nodule, paratesticular benign pseudo-tumour, myofibrosarcoma and fibrosarcoma (Fig. 6), fibronexus junctions were identified with the same architecture as in the reactive myofibroblasts. The degree of development varied, however, with exuberant expression of myofilaments and fibronectin fibrils in some of the benign lesions, and generally, less well developed or imperfecty formed fibronexus junctions in the malignancies. However, even among the latter the distinctive features of the fibronexus were identifiable: co-linearity of myofilaments and fibronectin fibrils, the latter being straight, having a filamentous substructure and diverging away from the cell surface (Fig. 6A,B). In the fibrosarcoma, an imperfectly organised fibronexus was observed, lacking the myofilaments in the immediate vicinity of a shelf of cell surface (Fig. 6C).

Discussion

This paper presents both novel and confirmatory findings on the myofibroblast, and in particular, the cell-to-matrix specialisation known as the fibronexus. Most authorities recognise that myofibroblasts from tumour stroma and granulation tissue are characterised by prominent rough endoplasmic reticulum (rER) and modestly developed bundles of smooth-muscle myofilaments with focal densities, consistent with the matrix production and contractility characteristic of healing wounds (Majno et al., 1971), and with the retraction phenomenon commonly observed, for example, in carcinomas (Schürch et al., 1982). A number of authors have recognised an additional feature of the

Fig. 4. Matrix protein imunostaining. A and B case 8 (tumour stroma); C and D case 32 (granulation tissue). **A.** Light micrograph showing a line of fibronectin immunoreactivity (arrow) consistent with a fibronectin fibril. x 800. **B.** Immuno-electron microscopy of gold-labelled anti-fibronectin antibody showing specific localisation over a fibronectin fibril. x 13,000. **C and D.** Absence of collagen IV staining over a myofibroblast. C shows a myofibroblast (My) close to an entrapped striated muscle cell (Mu) (the dense bodies lower left are obliquely sectioned sarcomeric fibrils). Collagen IV staining is observed over the muscle cell lamina (arrows). x 6,000. D is a detail from C. The myofibroblast has prominent rER, and, despite imperfect structural preservation due to the lack of osmium tetroxide in the processing schedule, a small bundle of myofilaments as part of a small fibronexus is evident (arrow). Note the absence of collagen IV staining over the myofibroblast compared with the lamina of the adjacent muscle cell. x 25,000


A
B
D
My
Mu
C

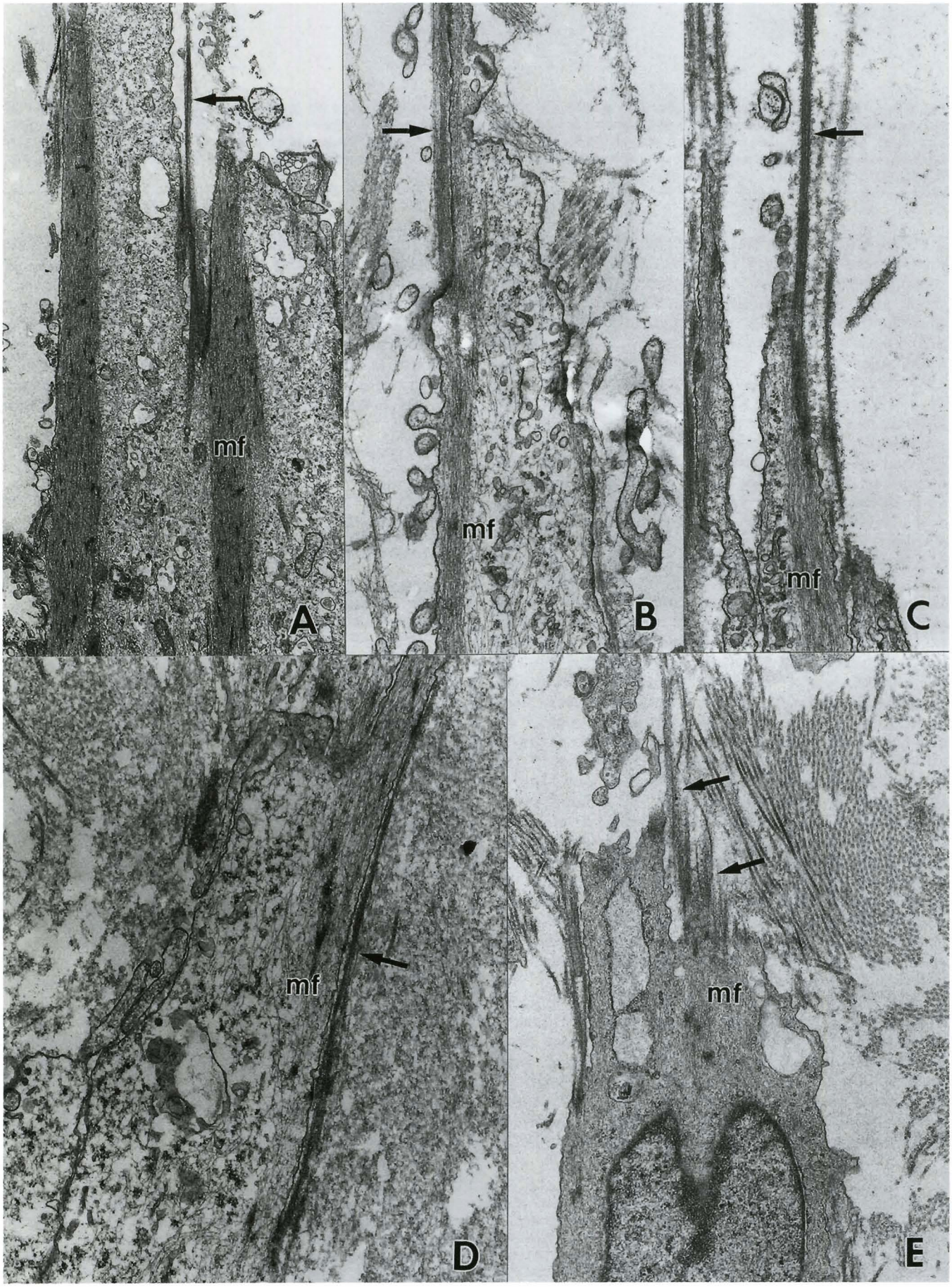

mf
A
mf
B
mf
C
mf
D
mf
E

Fig. 5. Fibronexus junctions in benign lesions. **A-C.** Nodular fasciitis (A and B, case 33; C, case 34). **D.** Musculo-aponeurotic fibromatosis, case 35. **E.** Inflammatory myofibroblastic tumour, larynx, case 36. In all figures, densely staining and relatively straight fibronectin fibrils are shown (arrows), associated with smooth-muscle myofilaments (mf) with focal densities. Throughout, the myofilaments and the fibronectin fibril follow the same direction. Note also in B how the plasma membrane overlying the myofilaments is ruffled, suggesting contraction and a folding of "excess" membrane. A, x 8,000; B, x 13,000; C, x 20,000; D, x 18,000; E, x 15,000

myofibroblast, originally described as basement membrane-like material (Gabbiani et al., 1971) and more recently referred to as the fibronectin fibril (Eyden, 1993), as an important ultrastructural feature for defining the myofibroblast and identifying myofibroblastic differentiation in tumours (Eyden et al., 1991, 1992; Eyden and Christensen, 1993; Eyden 1993; Smith et al., 1995; Taccagni et al., 1997; Gocht et al., 1999).

For others, however, the fibronexus is less important. One reason is that over the past 20 years electron microscopy as a diagnostic technique in general has lost ground in perceived importance to immunohistochemistry. Consequently, many use α-smooth-muscle actin immunostaining in an appropriate morphological and clinical setting for defining the myofibroblast (Darby et al., 1990; Nouchi et al., 1991; Foo et al., 1992; Ma et al., 1992; Pedagogos et al., 1997; Morgan and Pitha, 1998; Bisceglia and Magro, 1999). Another reason is the simple inability on the part of some service pathologists to recognise this cell structure (Mentzel et al., 1998; Fletcher, 1999). This might seem surprising given that, as indicated in table 2, there are at least 28 publications in the literature containing informative illustrations of the fibronexus. Clearly, however, these papers have not made an appropriate impact on diagnostic pathologists in terms of providing data to allow them to make confident interpretations of the fibronexus. Possibly, this is due to the fact that many of these papers have appeared in the scientific or specialised clinical literature and not the "mainstream" diagnostic pathology journals which are the required reading for practising service pathologists. The data in effect are relatively inaccessible and also dispersed: the importance of the present paper is that it assembles a comprehensive range of images of the fibronexus for the purpose of providing a reference-point for pathologists who wish to refine their diagnoses based on differentiation characteristics.

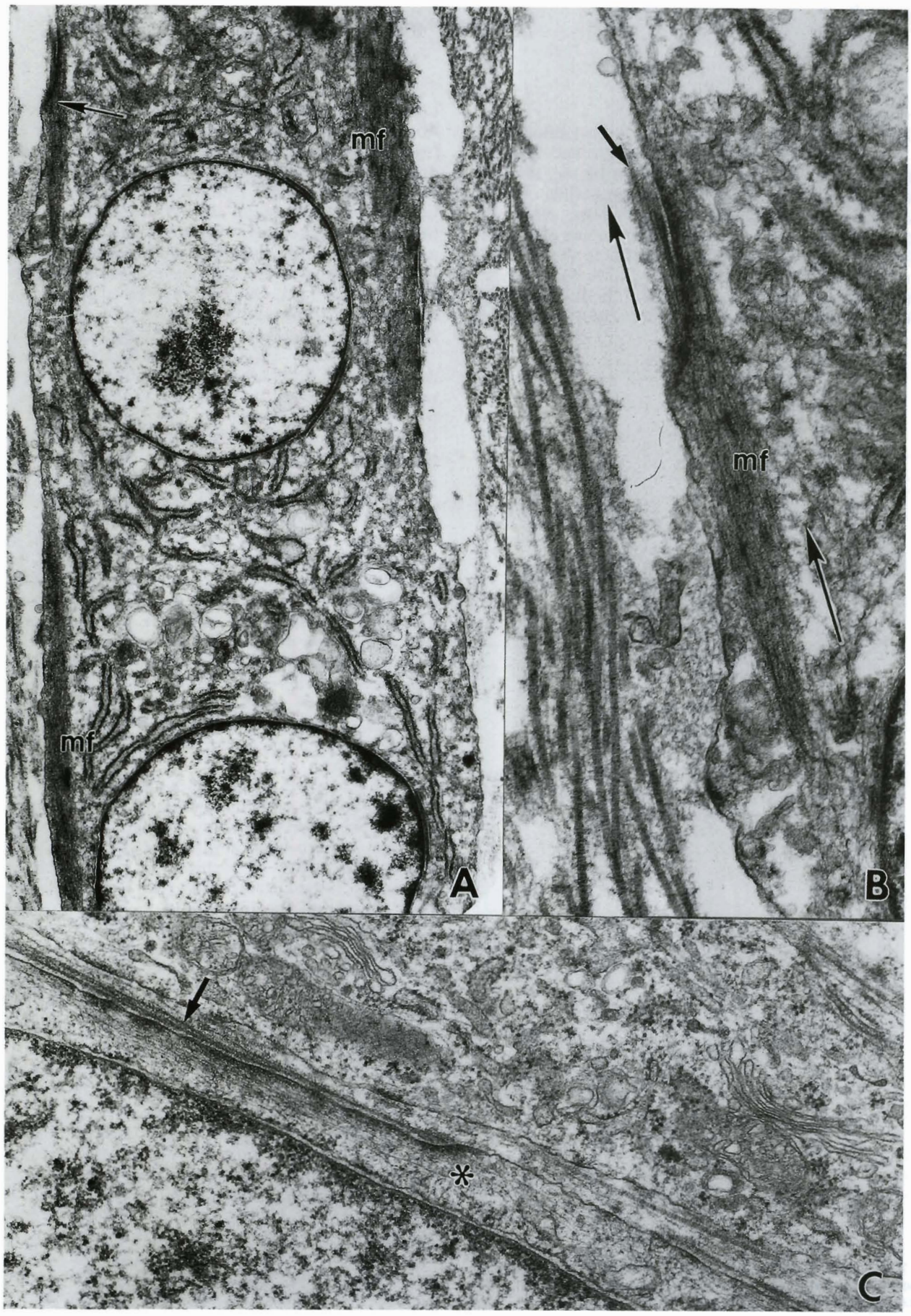
The main characteristics which these images illustrate are: the composite nature of the fibronexus (intracellular myofilaments and an extracellular fibril converging at a point or points on the cell surface, often marked by a submembranous plaque); co-linearity of myofilaments and fibronectin fibril; a finely filamentous substructure, relatively dense staining, and an often straight and rigid-looking profile to the fibronectin fibril; and finally, the divergence from the cell surface of the fibronectin fibril into the matrix (Fig. 7). In none of these characteristics does the fibronexus or fibronectin fibril resemble lamina ("basement membrane") (Reale, 1984; Eyden, 1996). The latter is often a more lightly staining structure, but more importantly, it typically adheres faithfully to cell surface contours, and it has a relatively homogeneous substructure. These images should therefore help to identify the fibronexus junction

Table 2. Publications illustrating the fibronexus/fibronectin fibrils.

REFERENCE	FIGURE	TISSUE/LESION
Bhawan et al., 1979	4	infantile digital fibroma
Baur et al., 1978	19	scar contracture
Baur and Parks, 1983	11a	granulation tissue and immature scars
Dominguez-Malagon, 1993	2	granulation tissue[1]
Ehrlich et al., 1994	3B	hypertrophic scar
Eyden et al., 1991	3	myofibrosarcoma
Eyden et al., 1992	9-11	myofibrosarcoma
Eyden, 1993	1	tumour stroma
Eyden, 1996	69A,B	tumour stroma
Eyden and Christensen, 1992	2-3	myofibrosarcoma
Flint and Poole, 1990	10.1	Dupuytren's disease
Gocht et al., 1999	4C	myofibrosarcoma
Holstein et al., 1996	6	seminiferous tubule[2]
Ishizaki et al., 1994	10A	traumatised cornea[3]
Kuhn and McDonald, 1991	34	idiopathic pulmonary fibrosis[5]
Lipper et al., 1980	10	desmoid[4]
Meek, 1994	4,5	malignant fibrous histiocytoma[6]
Ryan et al., 1974	8	granulation tissue
Santucci et al., 1989	12	multicystic peritoneal mesothelioma[7]
Schürch et al., 1998	1f	granulation tissue
Seemayer et al., 1980	20	tumour stroma
Singer, 1989	1A	in vitro fibroblasts
Singer et al., 1985	2a	granulation tissue
Skalli and Gabbiani, 1988	4	rat endothelial cell[6]
Smith et al., 1995	8	myofibrosarcoma
Taccagni et al., 1997	6	myofibrosarcoma
Tomasek et al., 1987	2,4,7,8	Dupuytren's disease
Vracko and Thorning, 1991	5B	myocardial scar[8]

[1]: also referred to as anchoring fibers/filaments; [2]: not well developed and not referred to as fibronexus; [3]: shows a fibronectin fibril and uses the term fibronexis; [4]: shows fibronectin fibril rather than entire fibronexus; [5]: referred to as microtendon; [6]: the specificity of the fibronexus is dealt with in a separate paper; [7]: associated with "mesenchymal-like mesothelial cells"; [8]: myofilaments referred to as nonsarcomeric myocytoskeletal arrangement.

Fig. 6. Fibronexus junctions in malignancies. A and B, myofibrosarcoma (case 40); C, fibrosarcoma (case 41). **A.** Spindled cell showing nuclear irregularity and disorganised rER cisternae, peripheral myofilaments (mf) and (arrow) a fibronexus junction. **B.** Detail showing the fibronectin fibril (arrow) projecting into the extracellular space, and co-linear (indicated by arrows) with a bundle of myofilaments (mf). **C.** Fibronectin fibril (arrow) contacting a shelf-like area of cell membrane at what can regarded as an incomplete fibronexus (*) given that myofilaments do not appear to be present. Another fibronectin fibril is overlying an area of cell surface at bottom right. A, x 12,000; B, x 30,000; C, x 20,000


mf
mf
A
mf
B
*
C

and distinguish it from lamina.

Not surprisingly, the fibronexus is best illustrated and best developed in granulation tissue and tumour stroma myofibroblasts, cells which can be regarded as close to normal in terms of their differentiation (in the sense of being non-neoplastic). Nonetheless, cells from fibro-proliferative lesions and pseudo-tumours also have well-defined fibronexus junctions which, as shown here, are readily identifiable. This paper shows for the first time the full complement of features of the fibronexus in nodular fasciitis and inflammatory myofibroblastic tumour, hinted at in earlier papers. Well developed fibronectin fibrils but without the demonstration of co-linearity with myofilaments have been illustrated in nodular fasciitis (figure 6 in Wirman, 1976), while in plasma cell granuloma (inflammatory pseudo-tumour) a well defined fibronectin fibril was illustrated with an observable longitudinal filamentous substructure close to a bundle of myofilaments (figure 3 in Chou et al., 1988). In both instances, the term *basement membrane-like material* was used. Our observations also confirm the presence of fibronectin fibrils already illustrated in fibromatoses (Bhawan et al., 1979; Lipper et al., 1980).

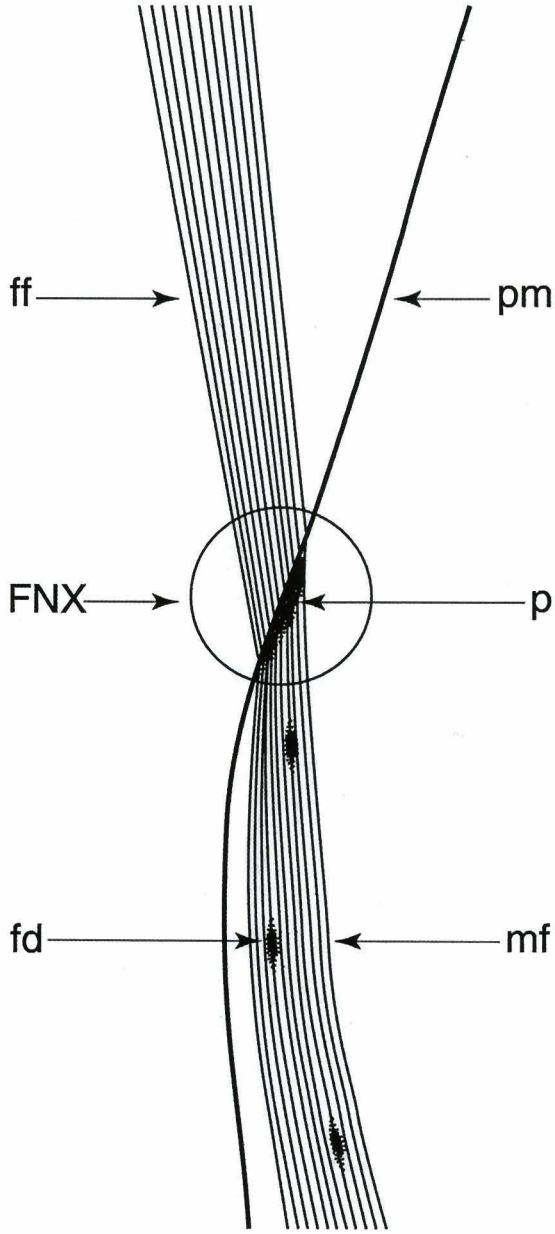


Fig. 7. Representation of the fibronexus. FNX: fibronexus; mf: smooth-muscle myofilaments; fd: focal density; pm: plasmalemma; p: sub-membranous plaque; ff: fibronectin fibril.

Preliminary immuno-ultrastructural results, demonstrating fibronectin but not laminin or collagen IV over the surfaces of myofibroblasts, are consistent with the idea that the fibonectin fibrils are distinct from lamina. Lamina-like profiles, however, can be seen on the surfaces of myofibroblasts (Fig. 3B) and lamina has sometimes been reported as a feature of myofibroblasts (Schürch et al., 1998). Given the immuno-ultrastructural lack of lamina proteins in this study, however, these lamina-like profiles may represent cross-sectional views of sheet-like fibronectin fibrils (Fig. 3A). This does not exclude the possibility that fibronectin fibrils and foci of true lamina co-exist on the myofibroblast surface, although at present the evidence for true lamina is tentative. Given the presence of lamina proteins in the matrix of some myofibroblast proliferations as shown here and elsewhere (Betz et al., 1992; Berndt et al., 1994; Kosmehl et al., 1995; Magro et al., 1997), however, the protein composition of the myofibroblast surface and surrounding matrix deserves further investigation.

In the cases of malignancies, one can expect a more pronounced loss of development of the fibronexus, but even in these tumours, unambiguous examples of the fibronexus have been published (Eyden et al., 1992; Smith et al., 1995; Taccagni et al., 1997; Gocht et al., 1999), and further examples are provided here, where all the features of the fibronexus as defined above in reactive myofibroblasts can be seen. Naturally again, in some instances, the fibronexus can be expected to be imperfectly assembled (as in the fibrosarcoma, Fig. 6C): in this respect the fibronexus is no different from other diagnostically important organelles such as melanosomes and desmosomes which can also show reduced or imperfect development (Eyden, 1996).

Having characterised the fibronexus, it is appropriate to comment on its significance for defining the myofibroblast. This is a complex subject in which widely different views have been expressed, ranging, on the other hand, from the use of light microscopy and especially α-smooth-muscle actin immunostaining (Darby et al., 1990; Nouchi et al., 1991; Foo et al., 1992; Ma et al., 1992; Pedagogos et al., 1997; Morgan and Pitha, 1998; Bisceglia and Magro, 1999) to, on the other, a combination of light microscopical and ultrastructural criteria including the fibronexus and other organelles (Eyden, 1993; Schürch et al., 1998, Lagacé et al., 1999).

It is beyond the scope of this discussion to go into the detail required to deal adequately with this question, but the following brief points will be made.

The fibronexus can be regarded as an important criterion for defining the myofibroblast or myofibroblastic differentiation in tumour cells because it is a well developed and consistently observed feature of tumour stromal and granulation tissue myofibroblasts, cells which can reasonably be regarded as the nearest approach to the normal counterparts of a myofibroblastic tumour. It is, however, only *one* feature among a number which collectively define myofibroblastic differentiation, the others being rER, modestly developed smooth-muscle myofilaments and α-smooth-muscle actin immunostaining. Observing all of these features gives maximum confidence of identifying myofibroblastic differentiation in tumours. These features were useful in enabling us to identify a spindle-cell tumour positive for α-smooth-muscle actin and diagnosed as a leiomyosarcoma by light microscopy as a myofibrosarcoma (Eyden et al., 1992). Incidentally, desmin has also been observed in certain myofibroblastic lesions (Skalli et al., 1989) and has sometimes been described in terms of a marker for myofibroblasts (van der Ven and Fürst, 1998). Given that desmin is also found in smooth muscle cells and leiomyosarcoma, it must be regarded as having limited value in the distinction, for example, of leiomyosarcoma and myofibrosaroma (Mentzel et al., 1998).

The absence of fibronexuses in an appropriate context can suggest smooth-muscle rather than myofibroblastic differentiation, and an example with implications for understanding differentiation is myofibroblastoma. These are widely regarded as being myofibroblastic. In our hands, however, a number of myofibroblastomas were found to lack fibronexus junctions and possess instead the lamina and attachment plaques alternating with plasmalemmal caveolae (Eyden et al., 1996, 1999) characteristic of smooth-muscle cells (Faussone-Pelegrini et al., 1989; Motta, 1990). They were, therefore, interpreted as kinds of smooth-muscle tumour. This may be a further reason why the fibronexus has been regarded as difficult to identify – that it has been sought but not found in tumours expected to harbour them because of an erroneous belief that they are myofibroblastic.

Acknowledgements. I would like to thank consultant pathologists Professor M. Harris, Dr S.S. Banerjee and Dr J.H. Shanks for providing histopathological diagnoses of the cases studied. Mrs Elizabeth Beckett (Biomedical Scientist in the Department of Histopathology, Christie Hospital) for carrying out the immunostaining, Mr Paul Chantry of the Department of Medical Illustration (Christie Hospital) for drawing figure 7, and Dr George Marshall (University of Glasgow, Scotland) for kindly providing a detailed protocol for immuno-electronmicroscopy.

References

Baur P.S. Jr and Parks D.H. (1983). The myofibroblast anchoring strand - the fibronectin connection in wound healing and the possible loci of collagen fibril assembly. J. Trauma 23, 853-862.

Baur P.S., Barratt G., Linares H.A., Dobrkovsky M., de la Houssaye A.J. and Larson D.L. (1978). Wound contractions, scar contractures and myofibroblasts: a classical case study. J. Trauma 18, 8-22.

Berndt A., Kosmehl H., Katenkamp D. and Tauchmann V. (1994). Appearance of the myofibroblastic phenotype in Dupuytren's disease is associated with a fibronectin, laminin, collagen type IV and tenascin extracellular matrix. Pathobiology 62, 55-58.

Betz P., Nerlich A., Wilske J., Tubel J., Wiest I., Penning R. and Eisenmenger W. (1992). Time-dependent pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts. Int. J. Legal Med. 105, 169-172.

Bhawan J., Bacchetta C., Joris I. and Majno G. (1979). A myofibroblastic tumor. Infantile digital fibroma (recurrent digital fibrous tumor of childhood). Am. J. Pathol. 94, 19-36.

Bisceglia M. and Magro G. (1999). Low-grade myofibrosarcoma of the salivary gland. Am. J. Surg. Pathol. 23, 1435-1436.

Chou P., Gonzalez-Crussi F., Cole R. and Reddy V.B. (1988). Plasma cell granuloma of the heart. Cancer 62, 1409-1413.

Darby I., Skalli O. and Gabbiani G. (1990). Alpha-smooth muscle actin is transiently expressed by myofibroblasts during wound healing. Lab. Invest. 63, 21-29.

Dominguez-Malagon H. (1993). Proliferative disorders of myofibroblasts. Ultrastruct. Pathol. 17, 211-220.

Ehrlich H.P., Desmouliere A., Diegelmann R.F., Cohen I.K., Compton C.C., Garner W.L., Kapanci Y. and Gabbiani G. (1994). Morphological and immunochemical differences between keloid and hypertrophic scar. Am. J. Pathol. 145, 105-113.

Eyden B.P. (1993). Brief review of the fibronexus and its significance for myofibroblastic differentiation and tumor diagnosis. Ultrastruct. Pathol. 17, 611-622.

Eyden B. (1996). Organelles in human tumor diagnosis: an ultrastructural atlas. Igaku-Shoin, New York and Tokyo: pp146-148 [lamina]; pp 53-63 [melanosomes]; pp 138-141 [desmosomes].

Eyden B.P. and Christensen L. (1993). Leiomyosarcoma versus myofibrosarcoma: observations and terminology. Ultrastruct. Pathol. 17, 231-239.

Eyden B.P., Banerjee S.S., Harris M. and Mene A. (1991). A study of spindle cell sarcomas showing myofibroblastic differentiation. Ultrastruct. Pathol. 15, 367-378.

Eyden B.P., Christensen L., Tagore V. and Harris M. (1992). Myofibrosarcoma of subcutaneous soft tissue of the cheek. J. Submicrosc. Cytol. Pathol. 24, 307-313.

Eyden B.P., Harris M., Greywoode G.I., Christensen L. and Banerjee S.S. (1996). Intranodal myofibroblastoma: report of a case. Ultrastruct. Pathol. 20, 79-88.

Eyden B.P., Manson C., Banerjee S.S., Roberts I.S. and Harris M. (1998). Sclerosing epithelioid fibrosarcoma: a study of five cases emphasizing diagnostic criteria. Histopathology 33, 354-360.

Eyden B.P., Shanks J.H., Ioachim E., Ali H.H., Christensen L. and Howat A.J. (1999). Myofibroblastoma of breast: evidence favoring smooth-muscle rather than myofibroblastic differentiation. Ultrastruct. Pathol. 23, 249-257.

Faussone-Pelegrini M.S., Pantalone D. and Cortesini C. (1989). An ultrastructural study of the smooth muscle cells and nerve endings of the human stomach. J. Submicrosc. Cytol. Pathol. 21, 421-437.

Fletcher C.D.M. (1999). Myofibroblastic sarcoma. Author's reply. Am J. Surg. Pathol. 23, 11433-1435.

Flint M.H. and Poole C.A. (1990). Contraction and contracture. In: Dupytren's disease. McFarlane R.M., McGrouther D.A. and Flint M.H. (eds). Churchill Livingstone. Edinburgh. pp 104-116.

Foo I.T., Naylor I.L., Timmons M.J. and Trejdosiewicz L.K. (1992). Intracellular actin as a marker for myofibroblasts in vitro. Lab. Invest. 67, 727-733.

Gabbiani G., Ryan G.B. and Majno G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 27, 549-550.

Gocht A., Bosmuller H.C., Bassler R., Tavassoli F.A., Moinfar F., Katenkamp D., Schirrmacher K., Luders P. and Saeger W. (1999). Breast tumors with myofibroblastic differentiation: clinico-pathological observations in myofibroblastoma and myofibro-sarcoma. Pathol. Res. Pract. 195, 1-10.

Holstein A.F., Maekawa M., Nagano T. and Davidoff M.S. (1996). Myofibroblasts in the lamina propria of human seminiferous tubules are dynamic structures of heterogeneous phenotyp. Arch. Histol. Cytol. 59, 109-125.

Ishizaki M., Wakamatsu K., Matsunami T., Yamanaka N., Saiga T., Shimizu Y., Zhu G. and Kao W.W. (1994). Dynamics of the expression of cytoskeleton components and adherens molecules by fibroblastic cells in alkali-burned and lacerated corneas. Exp. Eye Res. 59, 537-549.

Kosmehl H., Berndt A., Katenkamp D., Mandel U., Bohle R., Gabler U. and Celeda D. (1995). Differential expression of fibronectin splice variants, oncofetal glycosylated fibronectin and laminin isoforms in nodular palmar fibromatosis. Pathol. Res. Pract. 191, 1105-1113.

Kuhn C. and McDonald J.A. (1991). The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohisto-chemical features of sites of active extracellular matrix synthesis. Am. J. Pathol. 138, 1257-1265.

Lagacé R., Seemayer T.A., Gabbiani G. and Schürch W. (1999). Myofibroblastic sarcoma. Am J. Surg. Pathol. 23, 1432-1433 (Letter to the editor).

Lipper S., Kahn L.B. and Reddick R.L. (1980). The myofibroblast. Pathol. Annu. 15, 409-441.

Ma C.K., Zarbo R.J. and Gown A.M. (1992). Immunohistochemical characterization of atypical fibroxanthoma and dermatofibrosarcoma protuberans. Am. J. Clin. Pathol. 97, 478-483.

Magro G., Fraggetta F., Colombatti A. and Lanzafame S. (1997). Myofibroblasts and extracellular matrix glycoproteins in palmar fibromatosis. Gen. Diagn. Pathol. 142, 185-190.

Majno G., Gabbiani G., Hirschel B.J., Ryan G.B. and Statkov P.R. (1971). Contraction of granulation tissue in vitro: similarity to smooth muscle. Science 173, 548-550.

Meek W.D. (1994). Fibronexus in a case of malignant fibrous histiocytoma. Ultrastruct. Pathol. 18, 365-370.

Mentzel T. and Fletcher C.D.M. (1997). The emerging role of myofibroblasts in soft-tissue neoplasia. Am. J. Clin. Pathol. 107, 2-5.

Mentzel T., Dry S., Katenkamp D. and Fletcher C.D. (1998). Low-grade myofibroblastic sarcoma: analysis of 18 cases in the spectrum of myofibroblastic tumors. Am. J. Surg. Pathol. 22, 1228-1238.

Morgan M.B. and Pitha J.V. (1998). Myofibroblastoma of the breast revisited: an etiologic association with androgens? Hum. Pathol. 29, 347-351.

Motta P. (1990). Ultrastructure of smooth muscle. Kluwer Academic Publishers. Boston. pp 8-27.

Nouchi T., Tanaka Y., Tsukada T., Sato C. and Marumo F. (1991). Appearance of alpha-smooth-muscle-actin-positive cells in hepatic fibrosis. Liver 11, 100-105.

Pedagogos E., Hewitson T., Fraser I., Nicholls K. and Becker G. (1997). Myofibroblasts and arteriolar sclerosis in human diabetic nephropathy. Am. J. Kidney Dis. 29, 912-918.

Reale E. (1984). Electron microscopy of the basement membranes. In: Ultrastructure of the connective tissue matrix. Ruggeri A. and Motta P.M. (eds). Martinus Nijhoff Publishers. Boston. pp 192-211.

Ryan G.B., Cliff W.J., Gabbiani G., Irle C., Montandon D., Statkov P.R. and Majno G. (1974). Myofibroblasts in human granulation tissue. Hum. Pathol. 5, 55-67.

Santucci M., Biancalani M. and Dini S. (1989). Multicystic peritoneal mesothelioma. A fine structure study with special reference to the spectrum of phenotypic differentiation exhibited by mesothelial cells. J. Submicrosc. Cytol. Pathol. 21, 749-764.

Schürch W., Lagacé R. and Seemayer T.A. (1982). Myofibroblastic stromal reaction in retracted scirrhous carcinoma of the breast. Surg. Gynecol. Obstet. 154, 351-358.

Schürch W., Seemayer T.A. and Gabbiani G. (1998). The myofibroblast: a quarter century after its discovery. Am. J. Surg. Pathol. 22, 141-147 (Editorial).

Seemayer T.A., Lagacé R., Schürch W. and Thelmo W.L. (1980). The myofibroblast: biologic, pathologic, and theoretical considerations. Pathol. Annu. 15, 443-470.

Singer I.I. (1989). Fibronectin-cytoskeleton relationships. In: Fibronectin. Mosher D.F. (ed). Academic Press, San Diego, pp 139-161.

Singer I.I., Kazazis D.M. and Kawka D.W. (1985). Localization of the fibronexus at the surface of granulation tissue myofibroblasts using double-label immunogold electron microscopy on ultrathin frozen sections. Eur. J. Cell Biol. 38, 94-101.

Skalli O. and Gabbiani G. (1988). The biology of the myofibroblast. Relationship to wound contraction and fibrocontractive diseases. In: The molecular and cellular biology of wound repair. Clark R.A.F. and Henson P.M. (eds). Plenum Press. New York. pp 373-402.

Skalli O., Schürch W., Seemayer T., Lagacé R., Montandon D., Pittet B. and Gabbiani G. (1989). Myofibroblasts from diverse pathologic settings are heterogeneous in their content of actin isoforms and intermediate filament proteins. Lab. Invest. 60, 275-285.

Smith D.M., Mahmoud H.H., Jenkins J.J.3rd, Rao B., Hopkins K.P. and Parham D.M. (1995). Myofibrosarcoma of the head and neck in children. Pediatr. Pathol. Lab. Med. 15, 403-418.

Taccagni G., Rovere E., Masullo M., Christensen L. and Eyden B. (1997). Myofibrosarcoma of the breast: review of the literature on myofibroblastic tumors and criteria for defining myofibroblastic differentiation. Am. J. Surg. Pathol. 21, 489-496.

Tomasek J.J., Schultz R.J. and Haaksma C.J. (1987). Extracellular matrix-cytoskeletal connections at the surface of the specialized contractile fibroblast (myofibroblast) in Dupuytren disease. J. Bone Joint Surg. Am. 69, 1400-1407.

van der Ven P.F.M. and Fürst D.O. (1998). Expression of sarcomeric proteins and assembly of myofibrils in the putative myofibroblast cell line BHK-21/C13. J. Muscle Res. Cell Motil.19, 767-775.

Vracko R. and Thorning D. (1991). Contractile cells in rat myocardial scar tissue. Lab. Invest. 65, 214-227.

Wirman J.A. (1976). Nodular fasciitis, a lesion of myofibroblasts: an ultrastructural study. Cancer 38, 2378-2389.

Accepted July 14, 2000