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REVIEW ARTICLE

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# Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity

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**Abstract** Granulation tissue fibroblasts (myofibroblasts) develop several ultrastructural and biochemical features of smooth muscle (SM) cells, including the presence of microfilament bundles and the expression of $\alpha$-SM actin, the actin isoform present in SM cells and myoepithelial cells and particularly abundant in vascular SM cells. Myofibroblasts have been suggested to play a role in wound contraction and in retractile phenomena observed during fibrotic diseases. When contraction stops and the wound is fully epithelialized, myofibroblasts containing $\alpha$-SM actin disappear, probably as a result of apoptosis, and the scar classically becomes less cellular and composed of typical fibroblasts with well-developed rough endoplasmic reticulum but with no more microfilaments. In contrast, $\alpha$-SM actin expressing myofibroblasts persist in hypertrophic scars and in fibrotic lesions of many organs, including stroma reaction to epithelial tumours, where they are allegedly involved in retractile phenomena as well as in extracellular matrix accumulation. The mechanisms leading to the development of myofibroblastic features remain to be investigated. In vivo and in vitro investigations have shown that $\gamma$-interferon exerts an antifibrotic activity at least in part by decreasing $\alpha$-SM actin expression whereas heparin increases the proportion of $\alpha$-SM actin positive cells. Recently, we have observed that the subcutaneous administration of transforming growth factor-$\beta$1 to rats results in the formation of a granulation tissue in which $\alpha$-SM actin expressing myofibroblasts are particularly abundant. Other cytokines and growth factors, such as platelet-derived growth factor, basic fibroblast growth factor and tumour necrosis factor-$\alpha$, despite their profibrotic activity, do not induce $\alpha$-SM actin in myofibroblasts. In conclusion, fibroblastic cells are relatively undifferentiated and can assume a particular phenotype according to the physiological needs and/or the microenvironmental stimuli. Further studies on fibroblast adaptation phenomena appear to be useful for the understanding of the mechanisms of development and regression of pathological processes such as wound healing and fibrocontractive diseases.

**Key words** Cytoskeleton · Wound healing · Fibrosis
Extracellular matrix · Cytokine

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## Introduction

During the healing of an open wound, acute inflammation is followed by formation of granulation tissue, which is implicated in the synthesis of new connective tissue and in the active reduction of the wound space, as suggested by Carrel and Hartmann early in this century [48]. The process ends with the formation of a permanent scar. Granulation tissue consists of fibroblastic cells disposed in several layers separated by a collagenous matrix containing capillary buds and inflammatory cells. For many years, it was accepted that collagen is essential for wound contraction but in the mid-1950s, two reports contradicted this theory. It was found that in scorbotic guinea pigs wound contraction takes place normally despite altered collagen synthesis and organisation [1] and that fibroblasts could be induced to contract in vitro after permeabilisation [121]. These observations suggested that cells play a central role in tissue contraction. Further work showed that granulation tissue fibroblasts have structural and biological properties intermediate between those of resident fibroblasts and those of smooth muscle (SM) cells suggesting that they produce the force of wound contraction. These cells were first characterized morphologically [95] and called myofibroblasts; later

several biochemical features of myofibroblasts were defined [65, 238] and the observations of myofibroblasts were extended to fibrocontractive conditions (for review, see [227]) and normal tissues (for review, see [220]). This review will discuss the presence of myofibroblasts in normal tissues and pathological situations, the cellular origin of these cells and the biological mechanisms explaining the modifications of fibroblastic cell activities during physiological and pathological phenomena.

## Ultrastructural and biochemical features of the myofibroblast

According to the initial description [95], myofibroblasts are characterized by a well-developed cytoplasmic actin microfilament system which is not present in fibroblasts of normal tissues (Fig. 1a, b), but which is similar to the bundles of actin microfilaments found in SM cells [50] or in cultured fibroblasts [94]. Furthermore, myofibroblasts are interconnected by gap junctions [96] and are connected to the extracellular matrix by the fibronexus, a transmembrane complex involving intracellular microfilaments in apparent continuity with extracellular fibronectin fibres [233, 234]. The nucleus of myofibroblasts shows multiple indentations (Fig. 1b), an ultrastructural feature that has been correlated with cellular contraction in several systems [88, 169]. The presence of an incomplete layer of basal lamina on the cell surface is frequently observed.

In characterizing myofibroblastic phenotypic features, cytoskeletal proteins are of particular value since they display multiple variants, encoded by multigene families, or are the result of differential mRNA splicing and represent good differentiation markers. The cytoskeleton of eukaryotic cells is composed of three filamentous systems (for review, see [24]): microfilaments, made up mainly of actin and myosin; intermediate filaments formed by at least six distinct classes of proteins [2] (in mesenchymal cells, intermediate filaments are generally homopolymers of vimentin or desmin [198] and lamins are present, as in other cells of different embryological origin, in nuclear membranes); and microtubules consisting mainly of tubulin. By two-dimensional-polyacrylamide gel electrophoresis [101] and amino acid sequence analysis [254], six actin isoforms, produced by different genes [120, 149, 211], have been described in mammalian tissues; the $\beta$- and $\gamma$-cytoplasmic isoforms which are expressed by all cells, and the $\alpha$-cardiac, $\alpha$-skeletal and $\alpha$- and $\gamma$-SM isoforms, which are limited to specific cell types. Myosin is a polymeric molecule composed of two heavy chains and four light chains, each chain displaying multiple isoformic variations [11, 53, 179]. The analysis of cytoskeletal elements in fibroblastic and SM cells has been facilitated by the production of specific antibodies for the SM and non-muscle myosin heavy chain isoforms [23, 34, 76, 102], for the intermediate filament proteins vimentin and desmin [89, 100, 143, 189], and for actin isoforms [107, 236, 237, 249]. A monoclonal antibody against the $\alpha$-SM isoform of actin [236], which is predominant in vascular SM cells [97], has been particularly useful for the characterization of myofibroblast phenotypic features. The regulation of specific actin mRNA expression in fibroblasts and SM cells has been studied by means of specific probes [12, 29, 142]. Using cytoskeletal markers, the presence of four main myofibroblastic phenotypes has been described (for review, see [220]) which co-express in addition to cytoplasmic actin isoforms: vimentin (V-type), vimentin and desmin (VD-type), vimentin and $\alpha$-SM actin (VA-type); and vimentin, desmin and $\alpha$-SM actin (VAD-type). Among the SM cell markers expressed by myofibroblastic populations in vivo, $\alpha$-SM actin is the most common, followed by desmin and SM myosin; this last marker is more and more investigated in physiological and pathological situations (for review, see [220]). The use of recently described antibodies against non muscle and SM myosin isoforms [7, 44, 54] will be an essential adjunct in defining the different myofibroblastic phenotypes.

## Myofibroblasts in normal tissues

Cells with morphological features similar to those of myofibroblasts have been found in a variety of organs (Table 1) such as rat intestinal villi [113, 134], periodontal ligament [19, 266], developing human palatal mucosa [37], rat and mouse adrenal gland capsule [38], rat testicular capsule [106], bovine endometrial caruncle [243], pulmonary alveolar septa of rats, humans, lambs and monkeys [137], renal glomerulus (mesangial cells) of mouse, rat and human [18], external theca of ovarian follicles of rats [190], and liver perisinusoidal cells of fishes [92]. The presence of contractile proteins has been now demonstrated immunohistochemically in practically all these cells (Table 1; for review, see [220]) and has recently led to the recognition of a phenotypic heterogeneity of fibroblasts which has been related to different biological behaviours. All fibroblastic cells are generally thought to express vimentin, although defined subtypes of these cells have been found to express desmin, for example in the uterine mucosa [103], lymphatic organs, including the spleen [248], testicular stroma [236], hepatic perisinusoidal cells [225, 269] and lung septa [139]. Generally fibroblastic cells do not contain $\alpha$-SM actin but immunohistochemical studies have revealed that a category of reticular cells in lymph nodes and spleen [248], testicular [236] and bone marrow [223, 52] stromal cells, and cells of the theca externa of the ovary [64] express this protein (Table 1). Similarly, human intestinal pericryptal cells show SM differentiation features [218]. SM myosin is expressed in reticular cells of lymph nodes [195] and in testicular myoid and stromal cells [23]. These findings suggest that some normal stromal cells are equipped with SM structures and might participate in visceral contraction when specific functional needs are required. Thus it appears that fibroblasts of different tissues have distinct cytoskeletal features.

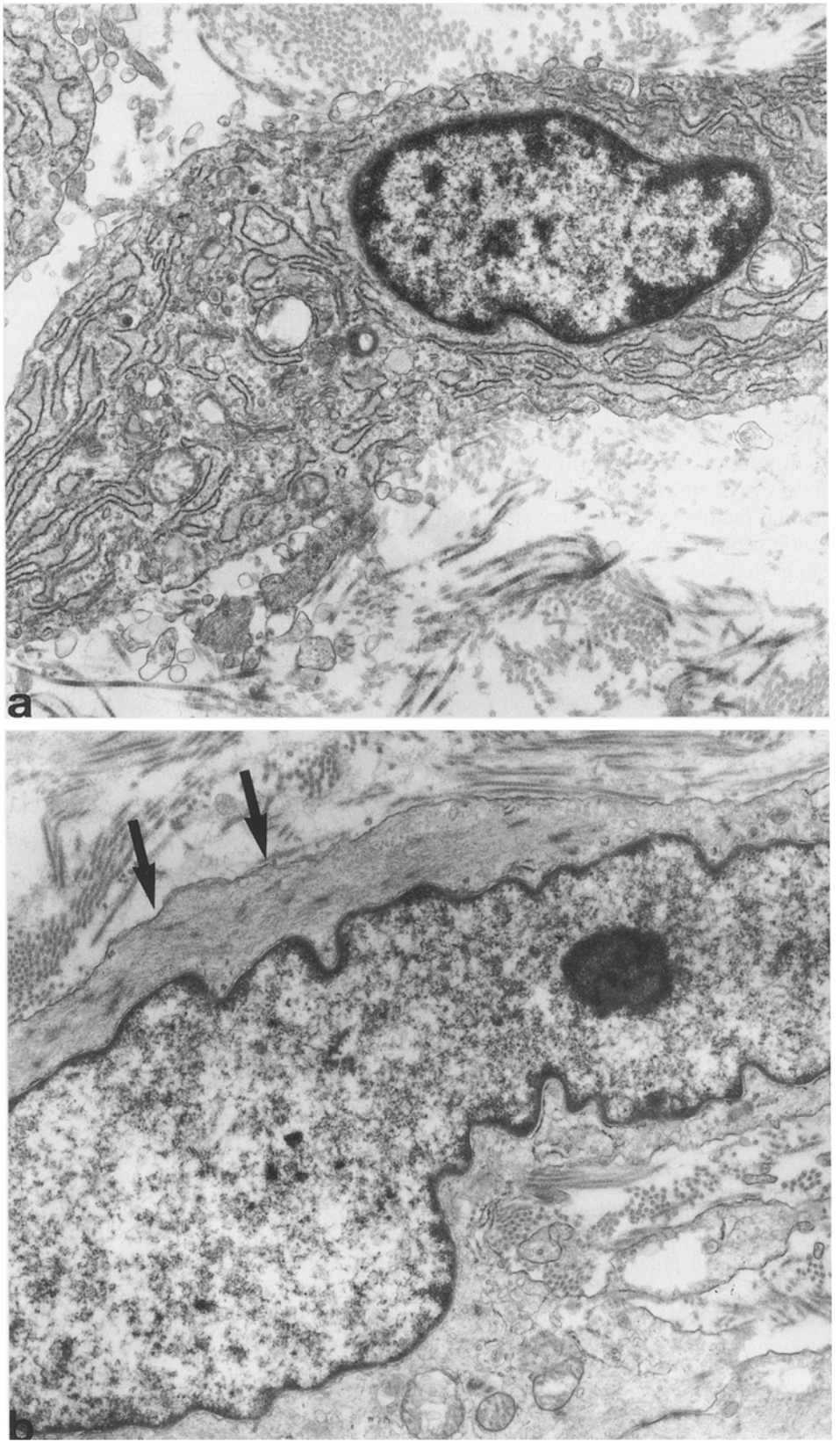


**Fig. 1** Transmission electron micrographs of fibroblastic cells observed in dermis (**a**) or in granulation tissue (**b**). Fibroblast (**a**) is characterized by a smooth nuclear outline and a cytoplasm rich in rough endoplasmic reticulum, Golgi apparatus, and mitochondria. In myofibroblast (**b**), stress fibres *(arrows)* are present often located beneath the plasmalemma and parallel to the main axis of the cell. ×10000

**Table 1** Fibroblastic cells of normal organs displaying ultrastructural and/or immunochemical features of smooth muscle (SM) differentiation

| Localization | Stress fibres | Desmin | $\alpha$-SM actin | SM myosin | Representative references |
|---|---|---|---|---|---|
| Uterine submucosa | – | + | – | – | [103] |
| Reticular cells of lymph nodes and spleen | + | + | + | + | [248] |
| Intestinal pericryptal cells | + | + | + | not known | [218] |
| Intestinal villous core | + | + | + | + | [140] |
| Testicular stroma | + | + | + | + | [236] |
| Theca externa of the ovary | + | not known | + | not known | [64] |
| Periodontal ligament | + | not known | not known | not known | [19] |
| Adrenal gland capsule | + | not known | not known | not known | [38] |
| Hepatic perisinusoidal cells | – | + | – | – | [269] |
| Lung septa | + | + | – | – | [139] |
| Bone marrow stroma | + | not known | + | not known | [52] |

The nature of extracellular matrix components secreted by differently localized fibroblasts also shows clearly the diversity of fibroblastic phenotypes [153]. Microenvironmental factors probably play an important role in the development of specific fibroblastic features. It has been suggested that each organ contains fibroblasts with specific features [220, 231]. Moreover, Fabra et al. [81] have shown that certain fibroblasts influence the invasive and metastatic potential of human colon carcinoma cells. Komuro [144] has proposed to categorize fibroblasts into subtypes depending on their main functions: in fibrogenesis, tissue skeleton or barrier; intercellular communication system; gentle contractile machinery; endocrine activity; and vitamin A-storing. Among these functions at least contractility and maintenance of tissue shape are directly related to cytoskeletal activities.

## Pathological situations related to myofibroblast appearance

### The myofibroblast in wound healing and hypertrophic scars

Myofibroblasts characterized by abundant cytoplasmic microfilaments, dense bodies and basal lamina-like material are a typical feature of granulation tissue (for review, see [227]). They are derived from gradual differentiation of local fibroblasts [65, 95]. Myofibroblasts are poorly developed in early granulation tissue, most numerous in the phase of wound contraction and progressively disappear in the late stage of cicatrization [65]. The myofibroblastic phenotype reverts to a quiescent form when the wound is closed or alternatively, myofibroblasts disappear selectively through apoptosis [65]. Microfilament bundles or stress fibres [235] composed of actin and associated proteins are probably the force resulting in wound retraction, while a well-developed rough endoplasmic reticulum indicates synthetic activities. Qualitative and quantitative differences in collagen types V, III and I synthesis have been observed in different granulation tissues [227]. The fibronexus is probably responsible for cell to matrix connections of myofibroblasts [233] and gap junctions account for the synchronization of retractile activity of these cells [96]. It has been proposed that wound contraction and scar formation may involve traction rather than contraction forces [117, 235]. Full-thickness skin autographs and cryosurgery are followed by weak wound contraction [212, 230]. This may result from a low degree of myofibroblast proliferation or a rapid disappearance of this cell type by apoptosis. Numerous physiological and pharmacological agents have been reported to influence the contraction of granulation tissue strips in vitro [93, 127, 215]. Among the substances most active in inducing contraction are serotonin, angiotensin, vasopressin, bradykinin, epinephrine and prostaglandin $F_1\alpha$, while among the most active in relaxing are papaverine and prostaglandins E1 and E2 [235]. Several studies suggest that reactivity of myofibroblasts from different organs to various stimulating agents are different.

The evaluation of cytoskeletal protein expression has been useful for the definition of phenotypic features of myofibroblasts during experimental and human wound healing: thus an heterogeneity in composition has been established during the evolution of this phenomenon. The V-type is present in early granulation tissue and is replaced by the VA-type during the period of active retraction [65]. In general, granulation tissue myofibroblasts are devoid of desmin and SM myosin, while these proteins are expressed more permanently in hypertrophic scars, fibromatosis and stroma reaction to tumors [238].

### Myofibroblasts in benign and malignant proliferative phenomena

#### *Fibromatosis*

Fibromatosis encompasses a spectrum of soft tissue proliferative lesions characterized by infiltrative growth pattern and tendency toward recurrence, but lack of metastatic potential, thus showing a quasi neoplastic biological behaviour. Examples include superficial or fascial fibromatosis such as Dupuytren's contracture, deep or

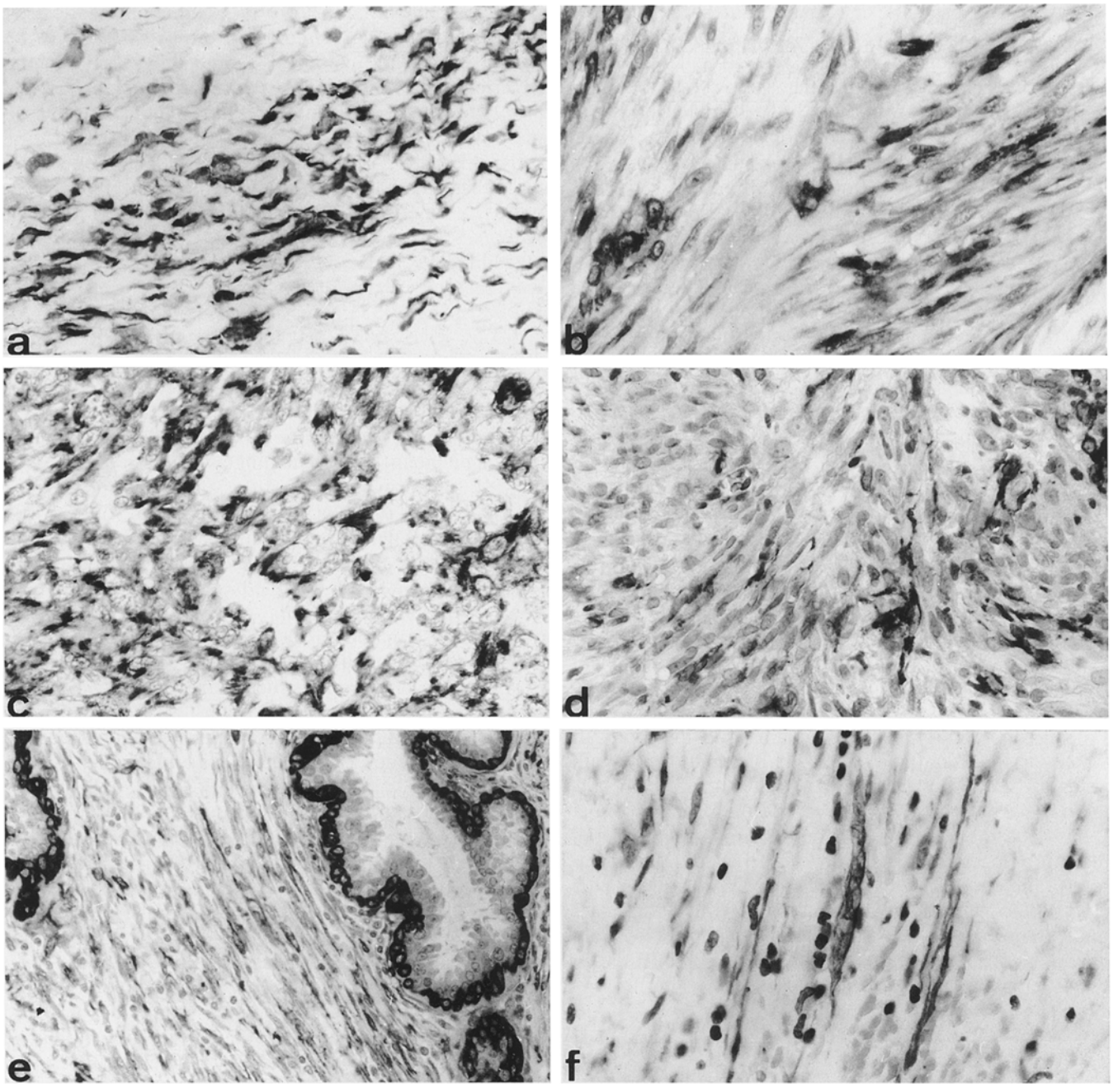


**Fig. 2** $\alpha$-SM actin expressing cells are demonstrated in: aggressive fibromatosis (**a**, ×180), a condition characterized by focal co-expression of desmin (**b**, ×400); malignant fibrous histiocytoma (**c**, ×90); AIDS-related Kaposi sarcoma (**d**, ×180); the hypercellular stroma of phylloides tumour of breast (**e**, ×90; **f**, ×420)

musculo-aponeurotic fibromatosis of abdominal, intra-abdominal or extra-abdominal types also referred to as desmoid tumours and other proliferations confined to infancy and childhood [79]. Gabbiani and Majno [93] described spindle-shaped cells with structural properties of myofibroblasts in Dupuytren's disease. Their ultrastructural observations were confirmed in similar lesions including desmoid tumours [82, 241] fibrous hamartoma of infancy and infantile myofibromatosis [79].

Characterization of cytoskeletal composition underlined the phenotypic heterogeneity of myofibroblasts during various fibromatoses [238]. In particular, the intermediate filament protein desmin, which is generally expressed in muscle cells, has been localized in an important proportion of fibromatotic myofibroblasts (VAD-type; Fig. 2a, b; [118, 238]). In Dupuytren's contracture, the proliferative stage coincides with the most important accumulation of VAD-type cells [227]. Apparently, a prominent myofibroblastic component correlates with an increased likelihood of recurrence after surgery [213].

During fibromatosis, cells are considered to generate tissue retraction and to synthesize variable amounts of interstitial collagens, especially types III and I [227]. To date the pathogenesis of fibromatosis is mysterious. Genetic predisposition, endocrine influences and tissue injury have all been implicated. However, despite genetic heterogeneity, these diseases show similar ultrastructural and biochemical composition of myofibroblastic cells, as described for familial and idiopathic gingival fibromatosis [244]. Cytokines released from bystander cells, such as macrophages or mast cells, may play a role in disease progression. It has been proposed that platelet-derived growth factor is a signal for myofibroblast proliferation in Dupuytren's disease [8]. The response of desmoid tumours to anti-oestrogen therapy suggests that fibrous proliferation may be affected by binding of steroid hormones to myofibroblasts [132, 260]. However, in mammary fibromatoses, absence of clinically important concentrations of oestrogen and progesterone receptors has been demonstrated [202]. Moreover, anti-oestrogen drugs such as tamoxifen have been shown to influence transforming growth factor-$\beta$ (TGF$\beta$) production and/or activation and this may furnish another interpretation for their mechanism of action. The mechanisms involved in spontaneous regression observed in occasional examples [22], such as infantile myofibromatosis, remain to be elucidated and may implicate apoptosis. Progression of fibromatosis to true fibrosarcoma has been reported in exceptional cases and may be related to radiotherapy. Further studies may help to explain why this group of soft tissue proliferations composed of spindle cells with at least a partial myoid differentiation lack metastatic potential despite their ability to invade the adjacent tissue. It has been speculated that a deregulation of $\alpha$-SM actin, desmin and SM myosin expression may play a crucial role in the development of these quasi neoplastic proliferative lesions. In bovine papillomavirus transgenic mice, transcription factors *jun* b and c-*jun* are selectively upregulated and seem to be functionally implicated in the development of aggressive fibromatoses and fibrosarcoma [35].

*Biphasic neoplasms*

Investigations based on cell typing using antibodies to cytoskeletal constituents have revealed that some tumours are composed of heterogeneous populations with cell types, each one expressing proteins found in different lineages. Evidence has accumulated indicating that mesenchymal or even epithelial neoplasms may contain a subset of tumour cells displaying a myofibroblastic phenotype. Such lesions appear distinct from fibromatoses or true SM tumours. Examples include fibrohistiocytic soft tissue tumours such as benign or malignant fibrous histiocytoma (Fig. 2c). They may contain subpopulations with ultrastructural characteristics of typical myofibroblasts [79] expressing $\alpha$-SM actin [200]. Invasive fibrous tumours of the tracheobronchial tree resembling both inflammatory pseudotumours and fibromatosis may be considered as a fibrous histiocytoma of low or borderline malignant potential [245]. From our experience, this locally invasive tumour contains a high proportion of $\alpha$-SM actin expressing spindle cells and of mast cells despite a general paucity of inflammatory cells. AIDS-Kaposi's sarcoma cells display some features that are characteristic of endothelial cells such as positivity for CD31. A subset of spindle-shaped cells express $\alpha$-SM actin in situ (Fig. 2d), while a higher degree of staining has been observed during long-term culture in the presence of activated CD4-positive T cells [259]. Studies using $\alpha$-SM actin antibody and/or electron microscopy have shown that biphasic tumours yielding a differentiation along epithelial and mesenchymal phenotypes may also contain myofibroblastic tumour cells as an intrinsic part of the lesion. Biphasic malignant mesotheliomas show a mixture of epithelial and sarcomatous components both containing cytokeratin and vimentin [28]. Fusiform elements of this and of the spindle cell type co-express $\alpha$-SM actin and cytokeratin. At the ultrastructural level these tumours disclose cells with myofibroblastic features such as bundles of microfilaments and focal contacts in relation to the basal lamina. The derivation of mesothelioma cells from a pluripotent submesothelial stem cell has been discussed. This stem cell may also differentiate into a stromal cell characterized by a myofibroblastic ultrastructure and co-expressing vimentin, cytokeratin and $\alpha$-SM actin [30]. This hypothesis is supported by the observation that proliferating subserosal spindle cells of the reactive pleura synthesize cytokeratins as well as $\alpha$-SM actin.

The histogenesis of cardiac myxoma still remains controversial. However, an origin from multipotent vasoformative cells with the potential of heterogeneous differentiation pathways is now generally favoured. Apparently, a subpopulation of neoplastic myxoma cells corresponds to myofibroblasts, while in the same tumour true epithelial structures may be encountered [221].

The pre-requisites for the diagnosis of phylloides tumour of the breast is the demonstration of both an epithelial and a mesenchymal component. Ultrastructural examination of both lesions has documented the presence of spindle cells with myofibroblastic traits. From our experience, overgrowth of stroma in relation to epithelium in phylloides tumour is characterized by spindle-shaped elements expressing $\alpha$-SM actin (Fig. 2e, f). Malignant tumours with intimately admixed epithelial and atypical mesenchymoid components have classically been designated as carcinoma, spindle cell carcinoma or metaplastic carcinoma. Recently, the concept of sarcomatoid carcinoma has been proposed including both purely sarcoma-like carcinomas and cases with divergent differentiation features such as fibrous and myoid elements, cartilage and bone. Many anatomical sites may give rise to sarcomatoid carcinomas such as nasopharynx, larynx, thyroid, lungs, oesophagus, breast, female and male urogenital tracts [60, 126, 262]. Different interpretations have been proposed to explain the biphasic tumours,

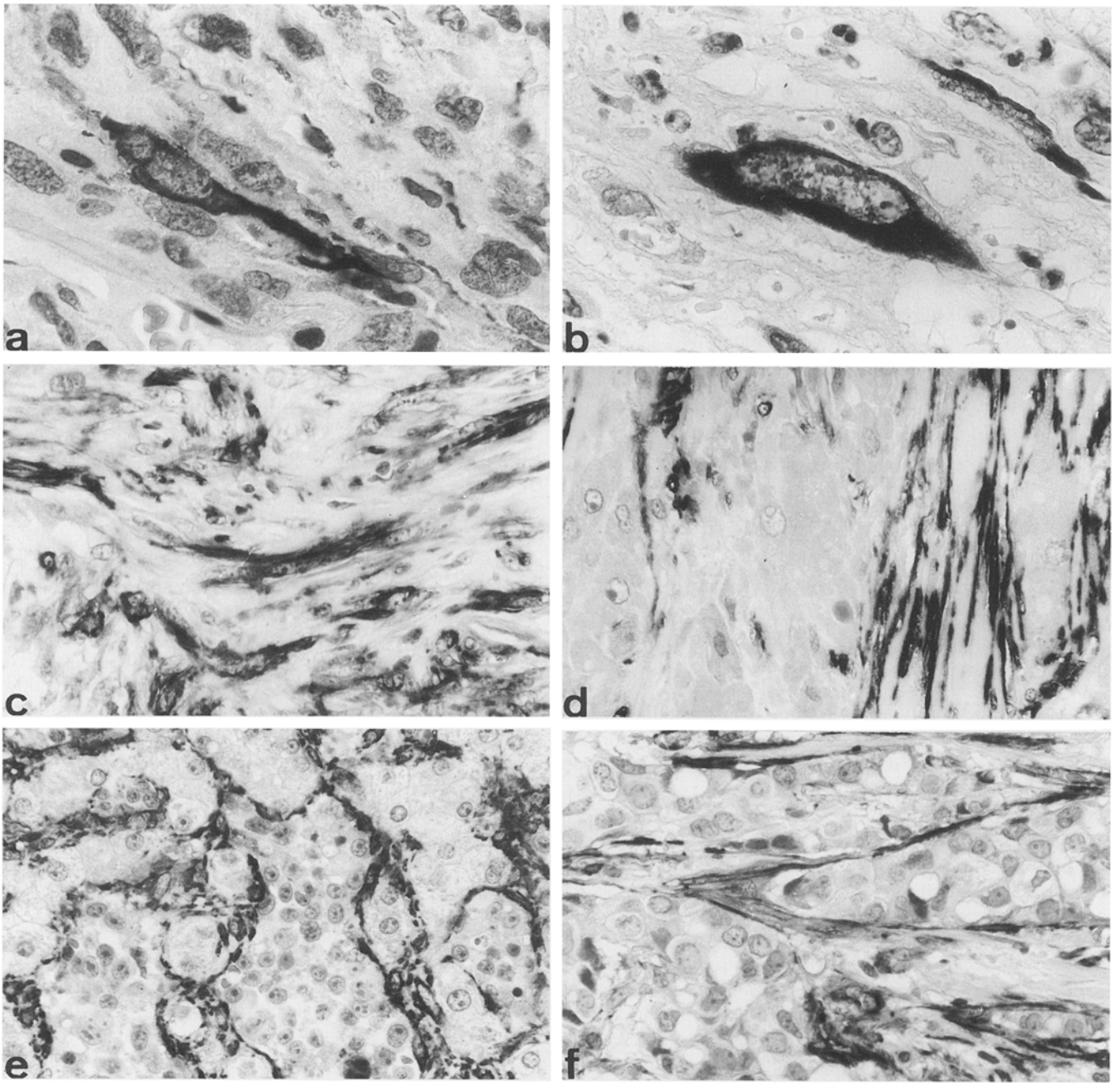


**Fig. 3** Tumour cell subsets staining for $\alpha$-SM actin may be seen focally in polypoid sarcomatoid carcinoma of the larynx (**a, b,** ×650), as a sarcomatoid component of homologous malignant mixed Müllerian tumour of the uterus (**c**, ×420), while myofibroblasts expressing $\alpha$-SM actin in fibrous strands separating columns of neoplastic hepatocytes in fibrolamellar carcinoma of the liver (**d**, ×420), in stroma of neuroendocrine carcinoma metastatic to liver (**e**, ×280) and in dense stroma of infiltrating ductal carcinoma (**f**, ×420) reflect a desmoplastic reaction

which may result from a pseudosarcomatous desmoplastic stromal response dominated by reactive myofibroblasts, a carcinosarcoma in which both elements are malignant or a pseudosarcomatous growth pattern of malignant epithelial cells corresponding to a so-called spindled squamous carcinoma. We had the opportunity to study a malignant polypoid tumour of the supraglottic area by means of electron microscopy and immunohistochemistry and observed pleomorphic proliferating cells co-expressing epithelial and myoid marker proteins (Fig. 3a, b). Thus, we may speculate that these peculiar neoplasms originate from pluripotent cells with the ability to acquire both an epithelial and a myofibroblastic phenotype.

In the ovary, $\alpha$-SM actin staining is evident in stromal cells which are an inherent component of biphasic neoplasms such as the Brenner tumour, and adenofibroma, and may also be seen in homologous malignant Müllerian tumour of the female genital tract (Fig. 3c; [64]).

These few examples support the concept that the presence of myofibroblasts within a tumour may not always reflect a stromal reaction, but may represent a true differentiation pathway of neoplastic cells. Now a broad consensus has emerged that the varying forms of biplasic tumours are carcinomas with a mesenchymoid evolution of the neoplastic clone but behavioral attributes of poorly-differentiated epithelial neoplasms. Recently, it has been shown that methylcholanthrene-induced pleomorphic sarcoma of mice mainly contain myofibroblast-like tumour cells and be classified as myofibrosarcoma [77]. However, the existence of tumours entirely composed of myofibroblasts referred to as myofibroblastoma when benign or myofibrosarcoma when malignant is poorly documented in human [80]. It is not yet clear whether intranodal myofibroblastoma, occurring in inguinal or submandibular lymph nodes and characterized by the proliferation of spindle cells positive for SM actin [86], is a true neoplasm or a reactive lesion. Moreover, there is still considerable debate as to the nature of human myofibrosarcoma which may represent a spectrum of soft tissue tumours such as malignant fibrous histiocytoma with a large proportion of cells showing a myofibroblastic differentiation.

## Myofibroblastic cells in desmoplasia

It is increasingly accepted that the co-ordinated activity of epithelial cells and their stroma is fundamental in controlling growth and differentiation in normal and pathological situations [72]. Examples include epithelial-mesenchymal cooperation in breast development [216]. Signalling may be accomplished by diffusible factors, extracellular matrix and/or direct cell to cell contacts [72]. Desmoplasia is considered as a response of host cells to inductive stimuli exerted by tumour cells [74]. In tumour formation, the stroma, essentially composed of inflammatory, fibroblastic and myofibroblastic cells and extracellular matrix components, reflects disturbed interactions between the neoplastic population and its surroundings [161, 251]. Studies of phenotypic features of stromal cells in normal, premalignant and malignant conditions have shown considerable diversity generated by regulatory influences which are, incompletely understood [222]. Stromal cells with myofibroblastic differentiation features are the predominant cell type surrounding primary (Fig. 3d) and metastatic (Fig. 3e) epithelial tumours and play a central role in the deposition of collagen as well as in retraction phenomena attributed to their contractile forces [227]. This situation is well-established in invasive ductal mammary carcinoma associated with skin retraction, umbilicated metastatic tumours in the liver and so-called scar carcinomas of the lungs which are now considered to be primary neoplasm with desmoplasia [167].

Myofibroblastic cells adjacent to neoplastic growth express large amounts of $\alpha$-SM actin, as seen in the stroma of breast carcinoma (Fig. 3f) [217], metastatic malignant melanoma [250], Hodgkin's disease (Fig. 4a) and myeloproliferative diseases (Fig. 4b) [223, 248]. Desmin and SM myosin have only been documented in a minority of such myofibroblasts [227, 238], a pattern supporting the notion that stromal myofibroblasts may correspond to modified fibroblasts rather than SM cells. Tumour derived cytokines are the best candidates to generate fibroblastic diversity with the emergence of myofibroblastic subsets. Granulocyte macrophage/colony stimulating factor and TGF$\beta$ have been shown (see below) to modulate the differentiation repertoire of mesenchymal cells characterized by induction of $\alpha$-SM actin expression [71, 210, 255]. The positive reaction to $\alpha$-SM actin observed in mesenchymal cells surrounding non-invasive epithelial proliferation such as cystic duct ectasia with epithelial hyperplasia (Fig. 4c), papillomatosis, sclerosing adenosis and ductal carcinoma in situ (Fig. 4d) of the breast [217] as well as cervical intraepithelial neoplasia [55] suggests that epithelial stromal signalling may be fundamental even in the absence of invasion. Although desmoplasia is generally considered to be a response of host cells to inductive stimuli exerted by tumour cells, recent findings argue that stromal cells have the ability to participate in tumour progression actively by secretion of proteolytic enzymes, thus allowing invasion and metastasis. Expression of stromelysin-3 gene has been demonstrated in stromal cells of invasive breast cancer [13]. Poulsom et al. [197] have shown that stromal cells of colorectal cancer have the ability to synthesize the metalloproteinase MMP-2 that degrades the basement membrane. These observations suggest that stromal cells may not only be implicated in desmoplastic tissue remodelling but may also contribute to tumour progression.

## Resident stromal cells acquiring a myofibroblastic phenotype in organ fibrosis

Much of our understanding of organ-specific subtypes of stromal cells acquiring partial SM phenotypes is derived from recent studies using antibodies to muscle specific proteins, especially to $\alpha$-SM actin. Here we briefly consider some well-documented examples of organs containing stromal cells which develop in particular situation myofibroblastic features demonstrated by immunohistochemistry and/or electron microscopy.

### *Kidney*

In the kidney, a modest basal expression of muscle-specific actins may be found in normal specimens suggestive of an ontogenetic relationship between mesangial and SM cells [3]. A markedly increased expression occurs in proliferative glomerulonephritis such as lupus nephritis [3]. A correlation between mesangial SM actin expression and proliferative activity of cells within the glomerulus has been suggested by simultaneous

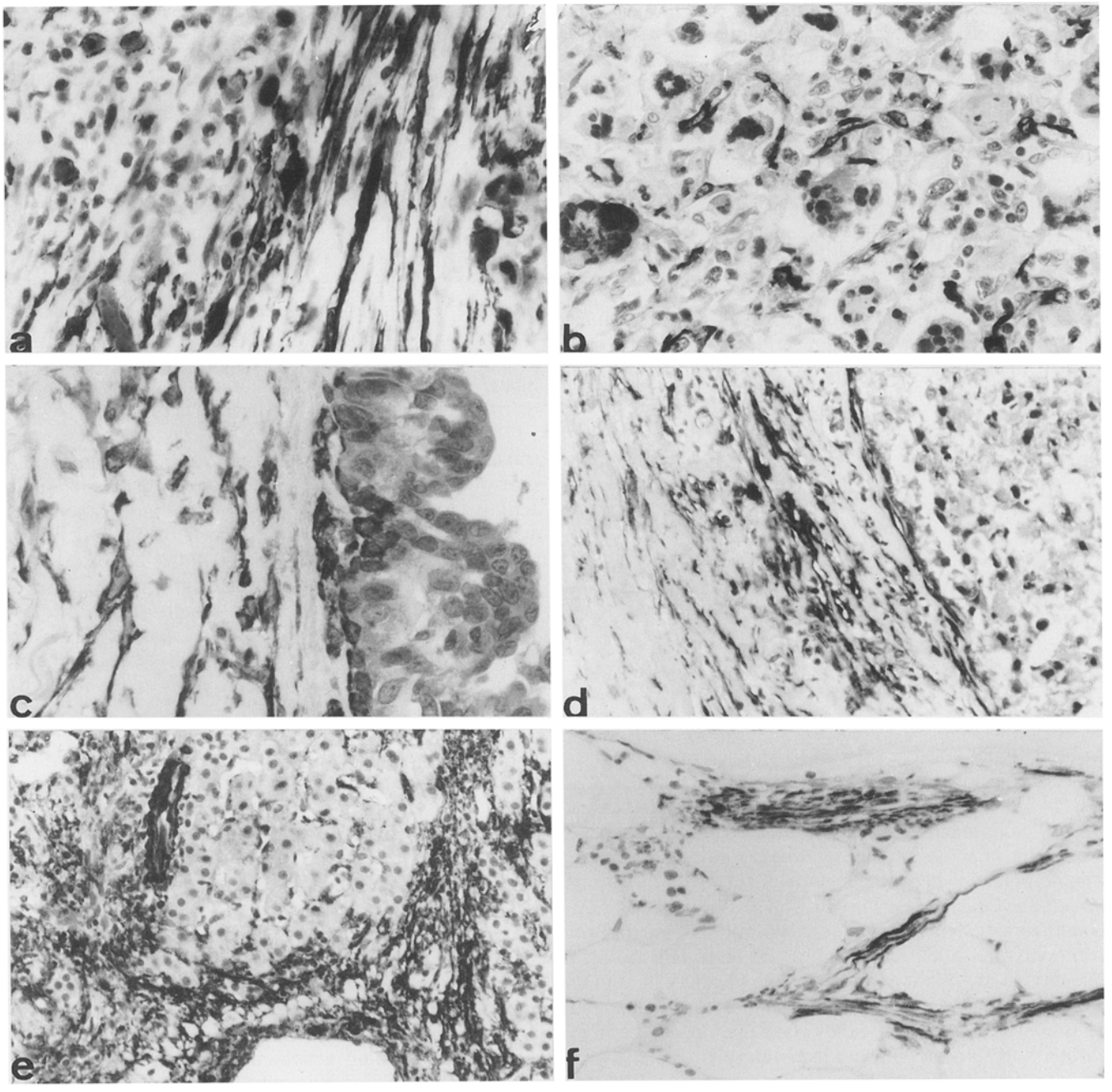


**Fig. 4** Myofibroblasts expressing $\alpha$-SM actin represent phenotypically altered stromal elements in: connective tissue bands subdividing the lymph node into distinct nodules in nodular sclerosis type of Hodgkin's disease (**a**, ×420); scanty extracellular matrix intimately intermingled with atypical megakaryocytes and megakaryoblasts in a lymph node showing extramedullary haematopoiesis in primary myelofibrosis (**b**, ×180); breast fibrous tissue adjacent to cystic duct ectasia with mild epithelial hyperplasia (**c**, ×420); periductal tissue in carcinoma in situ of comedo type (**d**, ×90); fibrous septa of cirrhotic liver (**e**, ×90); fibrotic bone marrow spaces of osteomyelofibrosis (**f**, ×190)

immunohistochemical analyses with antibodies recognizing the proliferating cell nuclear antigen and actin in human and experimental glomerulonephritis [130]. It has been proposed that a SM differentiation of mesangial cells during pathological conditions leads to an enhanced contractile potential of these cells and accounts for altered haemodynamics in glomerular injury [3]. Moreover, rodent models suggest that $\alpha$-SM actin expression may rise in parallel with active production of extracellular matrix in mesangial proliferative nephritis [87]. In this context it has been observed that the administration of decorin, a natural inhibitor of TGF$\beta$, may protect against scarring in experimental kidney disease [33]. Whether such treatment influences $\alpha$-SM actin expres-

sion by mesangial cells is unknown, but it has been shown that in vivo and in vitro treatment of mesangial cells with $\gamma$-interferon (which inhibits the expression of $\alpha$-SM actin in both SM cells [115] and fibroblasts [69]) results in a decrease of $\alpha$-SM actin expression.

*Lung*

Pulmonary myofibroblasts (also referred to as interstitial contractile cells) synthesize only cytoplasmic actin isoforms during normal conditions, while alveolar pericytes contain muscle specific actins [138]. In rat lungs, most interstitial myofibroblasts stain for desmin, while normal human interstitial cells are devoid of this intermediate-type filament protein. Thus, rat lung myofibroblasts show cytoskeletal features reminiscent of liver perisinusoidal cells and may also contain lipid droplets. During postcapillary hypertension and experimental lung fibrosis, myofibroblasts acquire the expression of $\alpha$-SM actin [138, 139]. In bleomycin-induced pulmonary fibrosis one sees an early phenotypic modulation of myofibroblasts which express de novo $\alpha$-SM actin. This change is followed by increased extracellular matrix deposition in the interstitium and scarring [256].

*Liver*

In human and experimental liver fibrosis, myofibroblastic cells are now generally considered to synthesize significant amounts of collagen, proteoglycans and other matrix components (for review, see [91]). It has been known for many years that myofibroblasts possess a contractile potential in cirrhotic rat liver produced by chronic carbon tetrachloride intoxication [127]. There is now convincing evidence that liver myofibroblasts are derived from perisinusoidal, fat storing or Ito cells undergoing a phenotypic modulation in response to a variety of stimuli (for review, see [199]). While normal rat perisinusoidal cells contain desmin only, they co-express $\alpha$-SM actin in carbon tetrachloride induced liver injury [185, 226]. Upon immunohistochemical examination at the electron microscopic level perisinusoidal cells from animals with toxic liver injury exhibit a reduced number of retinoid containing vesicles and acquire microfilament bundles decorated by $\alpha$-SM actin [226].

In contrast with the rat, normal human adult liver perisinusoidal cells are devoid of desmin; moreover they express $\alpha$-SM actin only exceptionally, while this actin isoform is frequently encountered in myofibroblasts occurring in a broad spectrum of pathological conditions [225]. Fibrous bands subdividing liver tissue in cirrhosis (Fig. 4e) and focal nodular hyperplasia as well as desmoplastic reactions adjacent to neoplastic growth yield myofibroblasts coexpressing $\alpha$-SM actin and desmin [225]. During murine liver *Schistosoma mansoni* infection mesenchymal cells of granuloma show a co-ordinate induction of desmin and collagen III synthesis suggestive of a myofibroblastic phenotype [31]. It has previously been shown that TGF$\beta$1 expression is markedly increased in murine schistosomiasis [63]. Thus, myofibroblastic transition of perisinusoidal cells may be modulated by numerous signals including cytokines, proteoglycans and other extracellular matrix constituents under in vivo and in vitro conditions [111, 173, 199].

*Bone marrow*

In the bone marrow microenvironment fibroblastic stromal cells are one of the components modulating the haematopoietic process through factors such as the c-*kit* ligand or stem cell factor. Cultured stromal cells of bone marrow synthesize muscle specific actin isoforms [51, 52, 159, 191]. In murine bone marrow derived stromal cells, Peled et al. [191] demonstrated a reversible expression of $\alpha$-SM actin inversely related to haematopoietic activity. A co-expression of $\alpha$-SM actin and vimentin is associated with a contractile morphology resembling myofibroblasts in granulation and fibrotic tissue [191]. In humans, bone marrow stromal cells may acquire a myofibroblastic phenotype in a spectrum of disorders usually associated with fibrosis (Fig. 4f). Examples include chronic myeloproliferative diseases such as agnogenic myeloid metaplasia, systemic mastocytosis and hairy cell laeukemia [223].

*Eye*

In the eye, myofibroblasts have first been documented by electron microscopy in a large series of epiretinal and vitreous membranes occurring in various ocular lesions including diabetic retinopathy [136]. This cell type is also evident at light microscopical level after decoration with $\alpha$-SM actin antibody. In human corneal lesions, myofibroblasts may be prominent in stages of early scarring in keratoconus. However, corneal fibroblasts assuming characteristics of myofibroblasts in experimental corneal wounds lacking neovascularisation were shown to contain non-muscle actins only [129] while this isoform has been observed in corneal wound myofibroblasts following radial keratomy [99]. With respect to the histogenesis of myofibroblasts, it is interesting that myofibroblastic features are not restricted to mesenchymal cells but may also be acquired by the ectodermally derived lens-forming cells in anterior capsular cataract [186, 224] and in cultured bovine lens cells [224]. It should be mentioned that the conditions characterized by the presence of myofibroblasts in the eye are more or less associated with scarring and retraction phenomena such as wrinkling of lens capsule and retinal detachment.

These examples support the concept of phenotypic plasticity of stromal cells which may be modulated into myofibroblasts during conditions often associated with active fibrotic processes.

## Cellular origin of myofibroblasts

Experimental evidence has suggested that granulation tissue fibroblasts arise locally from quiescent connective tissue cells. The local origin of granulation tissue fibroblasts was supported by autoradiographic and x-ray irradiation experiments [112, 163]. Ross et al. [209] described results in parabiotic rats showing that blood cells labelled with tritiated thymidine did not transform in granulation tissue fibroblasts. Myofibroblasts can theoretically derive from at least three different mesenchymal cell types, fibroblasts, pericytes, and SM cells. Shum and McFarlane [232] have proposed, on the basis of morphological observations, that myofibroblasts derived from vascular SM cells. It is clear that, as suggested by earlier observations [128, 154], an intimate relationship between myofibroblasts and blood vessel wall can be observed at the electron microscopic level, particularly during the initial steps of granulation tissue formation.

The analysis of cytoskeletal proteins does not allow a perfect definition of the origin of a mesenchymal cell. Fibroblasts, SM cells and pericytes can express $\alpha$-SM actin, SM myosin and desmin. Within experimental granulation tissue, myofibroblasts derived from local fibroblasts acquire temporarily markers of smooth muscle differentiation, such as $\alpha$-SM actin, which disappear when the wound is closed [65]. In this case, it seems clear that some local stimuli, probably distinct from those producing proliferation, induce SM differentiation markers in resident fibroblasts. These stimuli can be produced by neighbouring epithelial or mesenchymal cells, underlying the role of mesenchymal-epithelial interactions in these phenomena. Whether the distinct heterogeneity in the cytoskeletal phenotype of myofibroblasts is attributable to differentiation from a common cell type or from different cell types remains uncertain. It is conceivable that a common ancestor cell, the fibroblast or an undifferentiated mesenchymal cell, gives rise to myofibroblasts, pericytes, and SM cells, which would then represent examples of cellular isoforms [46]. Moreover, myofibroblasts during pathological situations may also originate from more specialized cells such as interstitial cells in the lung septa [138, 150, 178, 256], mesangial cells in the glomerulus [130], and perisinusoidal cells in the liver [10, 199, 225].

## Cultured fibroblasts and myofibroblasts

When grown in vitro, fibroblasts derived from normal tissues acquire several phenotypic features of myofibroblasts such as a system of microfilaments, called stress fibres. Microfilaments are mainly composed of actin, as shown by immunofluorescence or immunoelectron microscopy with specific antibodies [104, 264]. Several immunofluorescence studies have shown that stress fibres contain actin associated proteins such as myosin [258], tropomyosin [155], alpha-actinin [156], and filamin [257]. In addition to stress fibres, cultured fibroblasts also develop gap junctions [21]. The force generated by cultured fibroblasts can distort a sheet of silicon on which they are grown [36, 117]. Stress fibres are thought to be the force-generating element involved in wound contraction. When myofibroblasts from various sources (granulation tissue, Dupuytren's disease, and breast cancer stroma) are cultured, they maintain their morphology in vitro but grow significantly slower than fibroblasts [253]. We can assume that these cells have been induced to proliferate and differentiate in vivo; when placed in culture, these cells are less sensitive to factors contained in serum and their capacity to grow is reduced. Moreover, as suggested by Boswell et al. [36] and Streuli et al. [242], normal fibroblasts develop in vitro "differentiated" properties which resemble those exhibited in a "wounded" environment, presumably because cell culture conditions resemble basically those of an open wound.

The presence of $\alpha$-SM actin in primary and passaged fibroblastic populations has been reported by several laboratories [157, 236, 254]; it has also been shown that the expression of this protein is decreased after viral transformation [157, 187]. However, it has always been controversial whether these $\alpha$-SM actin expressing cells derive from SM cells and/or pericytes present in the tissue from which cultures have been produced or represent a true feature of fibroblastic cultures. We have observed that $\alpha$-SM actin is always present in a variable proportion of cells in rat, mouse, and human fibroblastic cultures [69]. The expression of desmin is generally low; in several populations, no desmin positive cells are found. The presence of SM myosin heavy chain containing cells has been evaluated in some fibroblastic populations [69]. Fibroblasts from rat fetuses or adult subcutaneous tissue and from normal human breast dermis or Dupuytren's nodules contain between 5 and 15% of SM myosin positive cells [69]. In human embryo lung fibroblasts and 3T3 cells, no SM myosin expression is detected.

Interestingly, fibroblasts derived from some pathological settings where $\alpha$-SM actin is not expressed (e.g. keloid), develop in culture higher proportion of $\alpha$-SM actin positive cells compared to fibroblasts isolated from $\alpha$-SM actin negative normal tissue (skin, unpublished observations).

In vivo, the concept of fibroblast heterogeneity is now well accepted [176, 192, 220]. Clonal heterogeneity in the response to mononuclear cell-derived mediators [147], in prostaglandin E2 [145], glycosaminoglycan [261], collagen [105, 125, 160], or collagenase [40] synthesis, and in the proliferative rate [133, 146, 206, 239, 252] has been reported in morphologically homogeneous fibroblastic populations. Cultured fibroblasts may also express different phenotypic features (for review, see [164]). A whole spectrum of differentiation steps has been described for fibroblastic cells in vitro [15, 16, 17].

To evaluate if a subpopulation of bona fide fibroblasts has the potential to express $\alpha$-SM actin, we have cloned and subcloned fibroblastic populations. Even after cloning and subcloning, a certain percentage of cells were

positive for $\alpha$-SM actin. It is noteworthy that $\alpha$-SM actin can be expressed by a proportion of cells in a population cultured from a single $\alpha$-SM actin positive or $\alpha$-SM actin negative cell. Moreover, when the expression of $\alpha$-SM actin increases during subculture, the proliferative activity decreases. We believe that $\alpha$-SM actin expression in cultured fibroblastic populations is a feature of fibroblastic cultures themselves, which may be related to functions exerted by fibroblasts under particular environmental conditions in vivo. This assumption is corroborated by the finding that $\alpha$-SM actin is expressed by fibroblasts cultured from organs where in situ fibroblasts have been verified not to contain this protein. This has been described in lens cells [224] and mammary gland stroma [207] and has been correlated with the observation that under pathological conditions in vivo both lens cells [224] and mammary gland fibroblasts [217] express $\alpha$-SM actin. However, presently, the genetic and environmental factors regulating $\alpha$-SM actin expression in fibroblasts are poorly known. The microenvironmental factors described above (cytokines and proteoglycans) may be important in producing the selection of $\alpha$-SM actin positive fibroblasts in a given population (see below).

Our results show that fibroblasts grown in vitro can be stimulated to divide and may also be stimulated to leave cell cycle and "differentiate" (i.e. expressing $\alpha$-SM actin) as fibroblast do in vivo in granulation tissue. Many factors can act at different levels inducing the proliferation or the differentiation of a particular fibroblastic subpopulation susceptible to specific stimuli. Fibroblastic cells repeatedly pressed to divide attain a "differentiated" state in which they are refractory to further mitotic stimulation and to subculturing under our culture conditions. We can assume, as suggested by previous work [20, 165, 170], that loss of division potential in vitro represents differentiation instead of aging. It seems clear that the loss of proliferation capacity observed in vivo during aging and in vitro after several passages represent different features induced by specific mechanisms which can not be correlated [43]. Furthermore, cloning and subcloning experiments show that features of the cultured parental population are representative of a subpopulation present in vivo and furnish evidence for selection events in mass culture [270].

## Factors implicated in the fibroblast phenotypic modulation

Little is known about the mechanisms leading to the development of fibroblastic cytoskeletal features similar to those of SM cells and to their persistence in some pathological conditions. As with SM cells, cytokines and extracellular matrix components are good candidates for modulating fibroblast phenotype and cytoskeletal protein expression. During the last years, the role of cytokines in cell differentiation during development and to maintain a complex equilibrium within cell-cell interactions has been well documented [27] and these mediators are implicated in pathological settings, particularly in the cascade of events observed during tissue repair [47, 148, 171, 204]. As suggested recently, during granulation tissue formation, we can distinguish among factors responsible for initiation, maturation, and termination and it is accepted that the repair process is a cascade in which many different stimuli are implicated and act on heterogeneous cell populations by means of autocrine and paracrine mechanisms. In this section, factors known to interact with fibroblastic phenotype pattern in vitro and/or in vivo will be described. Their role during wound repair process will be discussed.

### Extracellular matrix components

It is well accepted that the extracellular matrix represents a structural support for cellular constituents but evidence exists showing that the matrix plays a central role as a source of signals which are capable of influencing the growth and the differentiation of different cell types, including fibroblasts (for review, see [135]).

The fibroblast is the main cell type implicated in the production of the extracellular matrix during repair process. As for growth factors, we can assume that the action of extracellular matrix components can be autocrine or paracrine since many different fibroblastic subpopulations are implicated.

Among extracellular matrix components, different types of collagen, glycoproteins and proteoglycans are involved in fibroblastic differentiation. For instance, fibroblasts in floating collagen type I gels have been reported to decrease collagen- and to increase collagenase-synthesis compared to the same population cultured on a plastic dish. In vitro, adhesion, proliferation and migration of fibroblasts are modulated by extracellular matrix components. Recently, Streuli et al. [242] have shown that extracellular matrix regulates the expression of the TGF$\beta$1 gene. The authors propose that there is a feedback loop whereby TGF$\beta$1-induced synthesis of basement membrane is repressed once a functional basement membrane is present. This result illustrates the complex mechanisms by which growth factors and extracellular matrix components regulate cell activities. The components of the basal lamina, collagen type IV, laminin, and heparan sulphate, are known to maintain SM cells in a differentiated state. These components may also induce differentiation in undifferentiated or to partially differentiated cells such as fetal cells or quiescent fibroblasts.

It is well-known that heparin decreases the proliferation of SM cells in vivo [58] and in vitro [122]. Furthermore, heparin has also been shown to inhibit SM cell modulation from a contractile to a synthetic phenotype [49, 168], as well as the switch in actin isoform expression observed in SM cells after a balloon catheter-induced endothelial injury [59]. Heparin is able to induce $\alpha$-SM actin expression in cultured SM cells [68]. SM cell quiescence is probably actively maintained in the healthy arterial wall by heparan sulphates secreted by en-

**Table 2** Effect of heparin on tritiated-thymidine incorporation in synchronized cultured fibroblasts

| | Culture conditions* | |
|---|---|---|
| | 10% fetal calf serum (FCS) | 10% FCS +heparin |
| Percentage of $\alpha$-SM actin positive cells | 23.1 | 24.3 ($P>0.1$) |
| Percentage of tritiated-thymidine labelled cells | 31.9 | 28.2 ($P<0.01$) |
| Percentage of tritiated-thymidine labelled cells among $\alpha$-SM actin positive cells | 42.8 | 45.2 ($P<0.01$) |
| Percentage of tritiated-thymidine labelled cells among $\alpha$-SM actin negative cells | 26.0 | 19.7 ($P<0.001$) |
| Percentage of $\alpha$-SM actin positive cells among tritiated-thymidine labelled cells | 28.7 | 41.3 ($P<0.001$) |

*SEM were always lower than 5% of the values.

dothelial cells [263]. In cultured fibroblasts, heparin increases the expression of both $\alpha$-SM actin protein and mRNA [70]. This induction is observed in many different fibroblastic populations. Heparin in vitro accelerates the transformation of fibroblastic cells in myofibroblasts. We can assume (see below) that heparin facilitates the presentation to cell receptors of differentiation or maturation factors present in serum. The analysis of tritiated thymidine incorporation in synchronized cells suggests that heparin produces a selection of $\alpha$-SM actin expressing cells (Table 2). Growth-arrested rat subcutaneous fibroblasts were induced to enter the cell cycle by addition of 10% fetal calf serum (FCS) in the presence or absence of heparin. In heparin-treated cells, the percentage of tritiated-thymidine labelled cells was 45.2±0.6 among $\alpha$-SM actin positive cells ($P<0.01$, compared to cells in 10% FCS) but only 19.7±0.9 among $\alpha$-SM actin negative cells ($P<0.001$, compared to cells in 10% FCS). Moreover, among tritiated-thymidine labelled cells, the percentage of those positive for $\alpha$-SM actin was 28.7±0.9 in controls and 41.3±1.3 in heparin-treated cells ($P<0.001$). Thus, despite the fact that heparin induces a slight decrease of tritiated-thymidine incorporation in the whole fibroblastic population, it tends to produce a selection of $\alpha$-SM actin expressing cells since after heparin treatment the proportion of fibroblastic cells entering into the cell cycle is higher among the $\alpha$-SM actin positive cells and lower among the $\alpha$-SM actin negative cells compared to controls. For in vivo studies, osmotic minipumps filled with saline solution, non-anticoagulant heparin derivatives, recombinant-murine tumour necrosis factor-$\alpha$ (TNF$\alpha$) without or with non-anticoagulant heparin derivatives were implanted subcutaneously [70]. After 14 days, the newly formed connective tissue around the perfusion pumps was collected. In control animals and those treated with heparin derivatives, the capsule was thin and $\alpha$-SM actin staining was never detected. TNF$\alpha$ produced a significant fibroblast accumulation but the fibroblastic cells were positive for $\alpha$-SM actin only in the presence of heparin derivatives. Probably heparin derivatives induces $\alpha$-SM actin expression in a subpopulation of fibroblasts stimulated to proliferate by TNF$\alpha$. Thus proliferation and $\alpha$-SM actin synthesis by fibroblasts appear to be distinct phenomena during the formation and progression of granulation tissue. It is an old clinical observation that mast cell proliferation is accompanied by fibrotic changes [56]. Functional features of mast cells are regulated by fibroblasts [75, 98, 158]; we suggest that mast cells in turn exert a regulatory influence on fibroblast activities through their products, including heparin. Furthermore, heparin and proteoglycans are known to bind many growth factors [84, 214] such as basic fibroblast growth factor (FGF, [188], TNF$\alpha$ [152]) and transforming growth factor-$\beta$ (TGF$\beta$, [174, 175]) and these interactions may be essential to their activity. In conclusion, the effects of heparin are likely to participate in the complex modulation mechanisms of different connective tissue reactions.

## Platelet-derived growth factors (PDGFs)

PDGF is formed of two homologous peptides that are disulphide bonded, of approximately 16 and 14 kDa, termed the A and B chains. The molecule can take three forms consisting of an heterodimer of the two chains of homodimers of the individual A and B chains [208]. Furthermore, two distinct PDGF receptor subunits, named $\alpha$ and $\beta$, have been described. The $\alpha$ receptor subunit binds all three isoforms of PDGF, whereas the $\beta$ receptor subunit binds PDGF-BB with high affinity, PDGF-AB with lower affinity, and does not bind PDGF-AA [119, 228]. Activation requires the dimerization of receptor subunits. It has been shown that the regulation of the PDGF receptors plays a critical role in growth control in vivo. For example, in scleroderma fibroblasts which express SM markers such as $\alpha$-SM actin [219], a selective upregulation of PDGF $\alpha$ receptors by TGF$\beta$ is observed which may contribute to the expansion of these cells [265]. PDGF is a well-recognized mitogen for mesenchymal cells and is known to be a potent chemotactic agent for SM cells and fibroblasts. In the wound model designed by Mustoe et al. [181], a single application of PDGF-BB increased the volume of granulation tissue by 200% after 7 days. In these wounds glycosaminoglycan deposition consisting largely of hyaluronic acid was increased [194]. PDGF promotes the production of collagen [183] and upregulates fibronectin gene expression in human fibroblasts [26]; it also increases collagenase activity [14]. It is clear that PDGF is implicated in the modulation of fibroblast phenotype during repair process [5]. However, it seems that exogenous PDGF application

does not modify significantly the healing of a wound, although contradictory observations have been described in different models or situations [162, 193, 205]. As we will discuss below and as suggested by Lynch et al. [162], PDGF may have synergistic effects with other growth factors. PDGF appears to decrease the expression of $\alpha$-SM actin mRNA and protein in cultured SM cells [61] and fibroblasts (unpublished observations). Subcutaneous delivery of the heterodimeric form of PDGF induced the formation of an important granulation tissue albeit devoid of $\alpha$-SM actin positive myofibroblasts [210]. Further investigations are needed to evaluate the role of PGDF in combination with others factors on fibroblast phenotypic modulation and tissue repair.

### Fibroblast growth factors (FGFs)

The mitogenic activity for fibroblasts of substances contained in the brain was reported many years ago and two closely related peptides that can be distinguished by their different isoelectric point (acidic and basic FGFs) were characterized by their high affinity for heparin [203, 247]. The interaction of FGFs with different heparan sulphate proteoglycans plays a major role to modulate FGF activities [268]. Macrophages and endothelial cells have been recognized as the main producers of FGFs [9, 182, 201]. FGFs are potent mitogens for fibroblasts and endothelial cells and the influence of FGFs appears prominent on the connective tissue repair process, particularly through their potent angiogenic capacity. Basic FGF increases the accumulation of granulation tissue in subcutaneously implanted sponges by inducing fibroblast proliferation and collagen accumulation [66], but, as we have said for PDGFs, the results concerning the exogenous application of FGFs on wounds are depending on the model [83, 90, 110] and further studies are needed to evaluate the role of FGFs in combination with other growth factors or extracellular matrix components. We have seen that basic FGF decreases the expression of $\alpha$-SM actin in cultured fibroblasts but this action seems essentially due to its proliferative activity (unpublished observations).

### Granulocyte macrophage-colony stimulating factor (GM-CSF)

GM-CSF is mainly known for its haematopoietic effects [57] though some extra-haematopoietic activities have been described; GM-CSF stimulates the proliferation of different non-haematopoietic cells of mesenchymal origin such as endothelial cells [45] or bone marrow fibroblast precursors [67]. We have seen that the application of GM-CSF to the rat subcutaneous tissue induces the formation of an important granulation tissue rich in $\alpha$-SM actin positive myofibroblasts [210]. In vitro experiments have shown that GM-CSF does not directly stimulate $\alpha$-SM actin expression when added to the culture medium of rat or human fibroblasts. GM-CSF acts probably indirectly and to clarify this point, we have studied chronologically the formation of granulation tissue induced by GM-CSF treatment. After GM-CSF local treatment, the appearance of $\alpha$-SM actin rich myofibroblasts [210] is preceded by an accumulation of macrophage clusters [255] which could produce one or more $\alpha$-SM actin expression inducing factors. Moreover, in transgenic mice expressing GM-CSF, fibrotic nodules developed in areas where macrophages accumulate [151]. As suggested by Carrel [47] in 1922, we may assume that the utilisation of factors acting precociously in the cascade of events inducing granulation tissue formation and tissue repair are good candidates to induce the activation of local cells, particularly macrophages, in a way similar to that which occurs during wound healing.

### Transforming growth factor-$\beta$ (TGF$\beta$)

TGF$\beta$ is a 25 kDa protein consisting of two identical 12.5 kDa subunit chains joined covalently by disulphide bonds. Three isoforms (TGF$\beta$s 1, 2, and 3) are known in man. These different isoforms are synthesized in a defined pattern in specific cell populations in vivo. This well-defined and limited expression of TGF$\beta$1 in vivo is in contrast with the fact that all cells in culture can secrete TGF$\beta$1. This observation illustrates the complex regulation of TGF$\beta$ activity in which activation [85], receptor specific protein affinity [172] and extracellular matrix composition [242] play subtle roles.

Among factors secreted by activated macrophages and able to modulate the expression of $\alpha$-SM actin, TGF$\beta$1 is probably the most efficient. In human arterial SM cells, TGF$\beta$1 induced a growth inhibition and increased the expression of $\alpha$-SM actin [25]. Furthermore, Khalil et al. [141] have shown that, in pulmonary fibrosis induced by intratracheal instillation of bleomycin, an accumulation of $\alpha$-SM actin expressing myofibroblasts is observed around clustered macrophages with a high expression of TGF$\beta$.

Recently, we have shown that TGF$\beta$ is able to induce the expression of $\alpha$-SM actin in granulation tissue myofibroblasts [71]. As described above, other cytokines and growth factors, such as PDGF and TNF$\alpha$, despite their profibrotic activity do not induce $\alpha$-SM actin expression in myofibroblasts. In situ hybridization with an $\alpha$-SM actin probe showed a high level of $\alpha$-SM actin mRNA expression in TGF$\beta$1-induced granulation tissue myofibroblasts. Furthermore, the expression of $\alpha$-SM actin protein and mRNA (Fig. 5) by TGF$\beta$1 is induced in both growing and quiescent cultured fibroblastic populations. The expression of $\alpha$-SM actin observed in fibroblasts cultured in the presence of FCS is partly inhibited by the addition of antibodies against TGF$\beta$1. Thus TGF$\beta$1 could represent a regulator of $\alpha$-SM actin expression in fibroblasts. It is well-known that TGF$\beta$ increases the accumulation and the deposition of extracellular matrix compounds leading to the development of fibrosis [32,

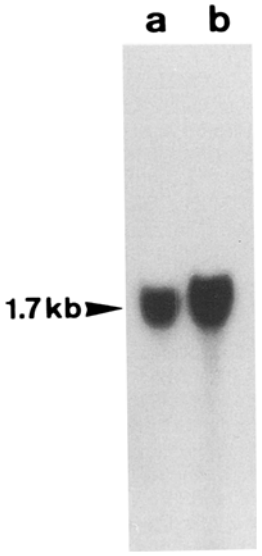


**Fig. 5** Effect of TGF$\beta$1 on $\alpha$-SM actin mRNA expression in cultured human subcutaneous fibroblasts. Hybridization of Northern blots with an oligonucleotide specific for $\alpha$-SM actin mRNA shows a unique band to 1.7-kb. In TGF$\beta$1-treated cells (**b**), $\alpha$-SM actin mRNA expression is increased compared to control cells (**a**). (From Desmoulière et al. 1993, with the kind permission of the publisher)

240]. TGF$\beta$ was found to accelerate healing of full-thickness, incisional wounds [180] and a single dose of TGF$\beta$, administered systemically to animals before wounding, enhances subsequent healing [4]. Furthermore, wounds treated with a neutralizing antibody to TGF$\beta$ presented a reduced inflammatory response and restitution of the dermal architecture without scarring, thus suggesting that TGF$\beta$ plays a role in wound retraction and scar formation in vivo [229]. Inhibition of TGF$\beta$ by decorin significantly reduced scarring in an experimental model of glomerulonephritis [32]. The action of TGF$\beta$1 on $\alpha$-SM actin expression confirms and extends the notion that TGF$\beta$ plays an important role in both fibroblast differentiation and fibrosis formation.

### Endothelin

Endothelin-1 (an acidic 21 amino acid peptide) has been isolated originally from the conditioned medium of cultured porcine endothelial cells and is the most potent vasopressor substance actually characterized [267]. Recently, Hahn et al. [114] have shown that quiescent vascular SM cells stimulated by growth factors such as PDGF-AA and TGF$\beta$ secrete endothelin-1. In a croton oil-induced granulation tissue model in rats, endothelin-1 induced a reversible concentration-dependent contraction of the granulation tissue [6]. The contractile activity is correlated with the development of myofibroblastic features. Furthermore, the level of synthesis of endothelin-1 by capillary endothelial cells displayed a time course compatible with a functional role in wound contraction. TGF$\beta$, which is expressed in granulation tissue [62], is a potent stimulator for endothelin-1 production [42]. Endothelin-1 has mitogenic activity on cultured rat fibroblasts [166] and acts synergistically with polypeptide growth factors such as TGF$\beta$ [41]. Endothelin-1 is able to induce the expression of $\alpha$-SM actin by cultured vascular SM cells [114]. It would be interesting to know if endothelin-1 is able to act directly on myofibroblastic differentiation by inducing $\alpha$-SM actin expression. These recent reports concerning the action of a vasopressor substance on myofibroblast differentiation confirm the role of this cell type in wound contraction [246]. Calcium antagonists modify the endothelin-1 effect in this tissue repair process; a possible therapeutic control in the treatment of fibrocontractive diseases can be considered.

### $\gamma$-Interferon ($\gamma$IFN)

In experimentally injured arteries and human atherosclerotic plaques, activated CD4 T lymphocytes secrete $\gamma$IFN which induces the expression of class II major histocompatibility antigens such as HLA-DR in SM cells [115, 131]. $\gamma$ IFN also inhibits both proliferation and expression of $\alpha$-SM actin in cultured SM cells [116].

We have shown that in cultured fibroblasts, $\gamma$ IFN decreases $\alpha$-SM actin protein and mRNA expression as well as proliferation [69]. $\gamma$ IFN has been shown, with some exceptions, to decrease proliferative activity and collagen production in fibroblastic cells [39, 73, 78, 108, 123, 124, 177, 184]. The properties of this cytokine make it a good candidate to exert anti-fibrotic activity in vivo as already suggested by Grandstein et al. [109]. Preliminary results [196] have shown that $\gamma$ IFN injection decreases the size of hypertrophic scars; in Dupuytren's disease, after $\gamma$ IFN treatment, nodules become smaller and the mobility is improved. In both cases, the expression of $\alpha$-SM actin in myofibroblasts is decreased. However, the development of a new appropriate way of $\gamma$IFN delivery is needed in order to perform larger clinical studies.

## Conclusions

It is increasingly recognized that the fibroblast appears to have a plastic phenotype and is capable of fulfilling distinct functions in normal and pathological situations as well as in different locations (for review, see [144, 220]. This suggests that most fibroblastic cells are relatively undifferentiated and can assume a particular phenotype according to the physiological needs and/or the microenvironmental stimuli. Moreover, during pathological situations myofibroblasts can develop from certain specialised cells typical of certain organs, such as the perisinusoidal cells in the liver [225] or mesangial cells in the glomerulus [130] which evidently do not exert myofibroblastic functions under normal conditions. In vitro and

cloning experiments do not support the notion of the presence of typical fibroblastic stem cells resulting, after replication, in a definite category of fibroblastic phenotypes, but rather support a plasticity of fibroblastic populations which evolve toward a given phenotype when submitted to the action of microenvironmental factors. This possibility is probably valid for other inflammatory cell such as the macrophage.

The modulation of fibroblasts into myofibroblasts, (as well as the appearance of a special macrophage phenotype upon stimulation by GM-CSF), represents an example of cellular adaptation resulting in the appearance of specific functional activities which may play an important role in the favorable evolution of a pathological phenomenon, such as in the case of wound contraction during wound healing or in the appearance of an inappropriate reaction, such in the case of connective tissue retraction during most fibrocontractive diseases. It is noteworthy that in general the modulation of fibroblastic cells involves several apparently independent biological activities including contractile activity and collagen synthesis; this suggests a co-ordinate activation of several genes.

We start now to understand the nature and the mechanisms of factors capable of modulating the fibroblastic phenotype. Further work in this direction will probably contribute to the understanding of the pathophysiology of wound healing and fibrocontractive diseases. It is already clear, however, that the activation and the suppression of myofibroblast differentiation involves the complex co-operation of several factors such as extracellular matrix components (e.g. heparin) and cytokines (e.g. TGF$\beta$ and $\gamma$IFN). Thus, we believe that the research of a single agent promoting myofibroblast development or disappearance is utopian. It is however possible that an appropriately stimulated cell, such as the macrophage, becomes capable of producing several products resulting in the appearance of typical myofibroblasts. The role of the macrophage in the evolution of granulation tissue is well-established [148]. It is noteworthy that GM-CSF induced macrophages are in turn capable to elicit the differentiation of typical $\alpha$-SM actin rich myofibroblastic cells [255]. This suggests that appropriately conditioned macrophages could be used in order to influence the evolution of experimental and clinical wound situations.

In conclusion, the study of fibroblast adaptation phenomena is important for the understanding of the mechanisms leading to the establishment of fibrotic reactions. It is conceivable that these studies will also lead to practical applications in the control of clinical situations in a not too distant future.

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