MYOFIBROBLASTS: PARACRINE CELLS IMPORTANT IN HEALTH AND DISEASE

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INTRODUCTION

It is now recognized that there is phenotypic heterogeneity among fibroblasts and that some express features of smooth muscle differentiation (1–5). These cells are known now as myofibroblasts (6,7). Although identified morphologically a century ago, we understand now that these specialized fibroblasts constitute a family of paracrine cells that play an important role in the regulation of fundamental processes such as cell motility, proliferation, differentiation, apoptosis, morphogenesis, tissue repair, inflammation and the immune response (3,4). They also take part in many disease states affecting many different organs. There are similarities in their morphology and function regardless of the tissue in which they reside, yet, in these tissues, they also express phenotypic and functional heterogeneity. In this article, we will portray some of the similarities and differences in their biologic functions, and indicate the role that these cells play in certain diseases.

DEFINITION OF A MYOFIBROBLAST

The simplest definition of myofibroblasts is that they are smooth muscle-like fibroblasts. Some investigators define them as activated smooth muscle cells (8,9); others call them lipocytes because of their propensity to store retinoids (Vitamin A) (10). They are also known as stellate cells due to a shape change when they are transiently differentiated.

In both cell culture and in native tissues, myofibroblasts possess several distinguishing morphologic characteristics (Figure 1). They display prominent cytoplasmic actin microfilaments (stress fibers) and are connected to each other by adherens and gap junctions (11). Thus,

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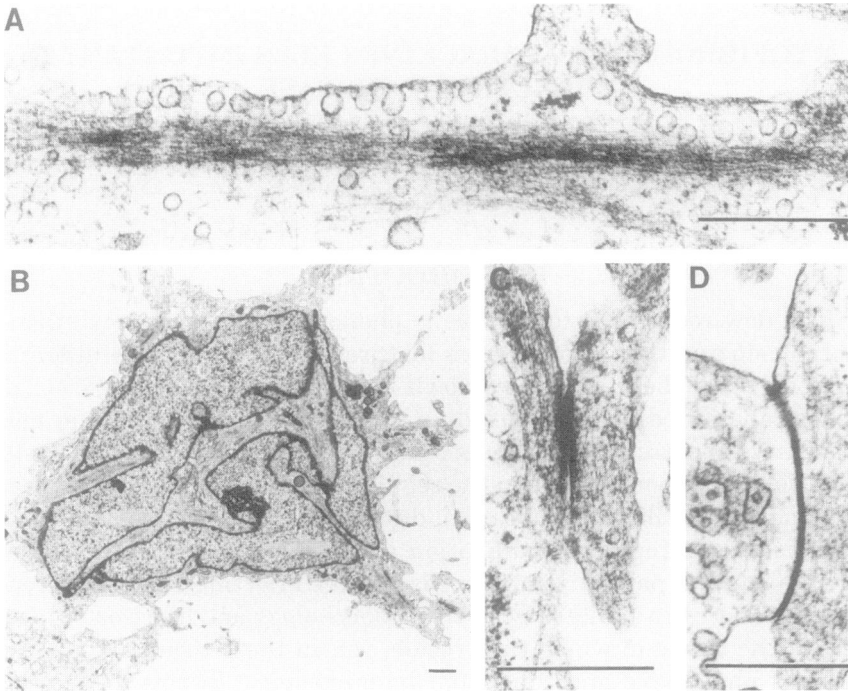

FIG. 1. (A) Transmission electron micrograph of an intestinal subepithelial myofibroblast (18Co). The cell membrane displays numerous caveolae, and the cytoplasm contains stress fibers (bundles of actin microfilaments). The cytoplasm is rich in rough endoplasmic reticulum, Golgi apparatus and mitochondria. (B) The nucleus of an activated myofibroblast shows multiple indentations. (C) Adherens and (D) gap junctions are present between myofibroblasts. (Reproduced by permission from ref. 11.)

these cells often exist in tissues as a syncytium (Figure 2) (12,13). They are also connected to the extracellular matrix (ECM) by focal contacts, which are transmembrane complexes made up of intracellular contractile microfilaments and the ECM protein fibronectin (14). Both the focal contacts (also called the fibronexus) and the stress fiber assembly are regulated by Rho, a newly described member of the RAS superfamily of small guanosine triphosphatases (GTPases) (15). These small, monomeric GTP-binding proteins also regulate myofibroblast morphology (16). The cells are often found in close apposition to varicosities of nerve fibers (17). Also, they may be connected to tissue smooth muscle by gap junctions (18).

In the eye (the orbital myofibroblast) (19), the joint (the synoviocyte) (20), the brain (astrocyte) (21), the liver (Ito cells) (22), and the intestine (both the interstitial cells of Cajal (23) and the subepithelial myofibroblasts (11)), the myofibroblasts exist in two distinct morpho-

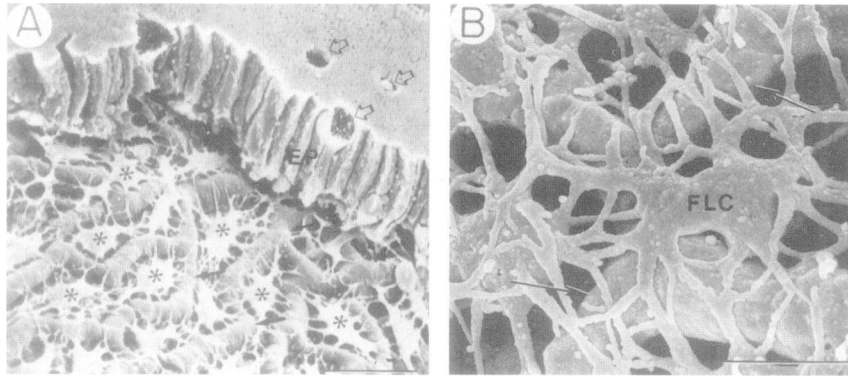

FIG. 2. (A) Intestinal subepithelial myofibroblasts, as shown here with scanning electron microscopy, are a syncytium of cells (*) underneath the epithelial cells (EP) which have been removed by freeze fracture. (Reproduced by permission from ref. 144) (B) Higher power view of the interconnecting fibroblast-like cells (FLC). (Reproduced by permission from ref. 145.)

logical states (Figure 3): 1) "activated" myofibroblast, as described above, and 2) stellate-transformed myofibroblast, a transiently differentiated myofibroblast. Agents that increase the cyclic adenosine 3′,5′ monophosphate (cyclic AMP) content of activated myofibroblasts (e.g., prostaglandins, cholera toxin, vasoactive intestinal polypeptide) induce stellate transformation in vitro within 24 hours (11) and stop myofibroblast proliferation (24).

Immunocytochemical characterization of myofibroblasts is based on antibody reactions to several antigens. As shown in Figure 4, neuroendocrine, endothelial and epithelial heritages are eliminated by negative reactions to chromogranins, Factor VIII and cytokeratin, respectively (25). Two of the three filament systems of eukaryotic cells (26,27), actin (a component of the microfilaments) and vimentin, desmin, lamin or glial fibrillary acidic protein (GFAP) (members of the intermediate filament system) differentiate myofibroblasts from smooth muscle cells. Myofibroblasts have not been characterized with regard to tubulins (proteins of the microtubules). Beta (β) and gamma (γ) actins are expressed by all cells, including myofibroblasts. Myofibroblasts stain negatively for α-cardiac and α-skeletal actin (3), but positively for α-smooth muscle (α-SM) actin (2). Myofibroblasts are not well characterized with regard to the newly defined myosin isoforms (28). In some tissues, such as the intestine and reticular cells of lymph nodes and spleen, myofibroblasts stain positively for smooth muscle heavy chain myosin or tropomyosin (3,23).

Expression of vimentin, desmin and α-SM actin, the three filaments

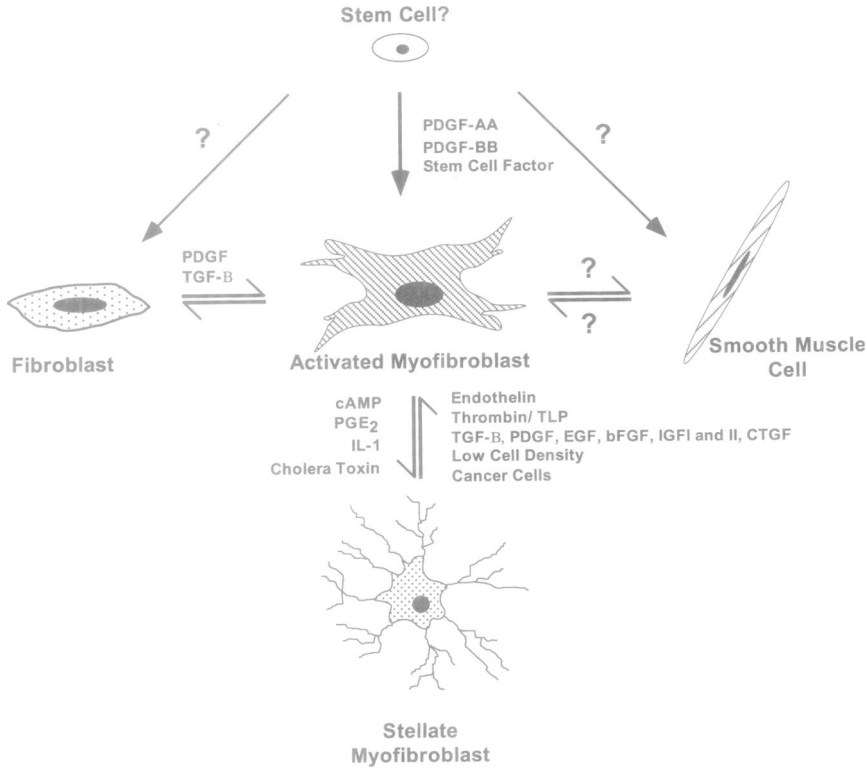

FIG. 3. Hypothesis for the origin, transdifferentiation, activation and stellate transformation of myofibroblasts. (Reproduced by permission from ref. 4.)

most often used to classify myofibroblasts, may vary in tissues. There is also variable expression depending on whether the cells are studied in situ or in culture and whether the cells are activated by hormones or cytokines, or by disease. A classification system has been proposed based on immunohistochemical staining of these filaments (3,29). Myofibroblasts that express only vimentin are termed V-type myofibroblasts; those that express vimentin and desmin are called VD-type; those expressing vimentin, α-SM actin and desmin are VAD-type and those that express only vimentin and α-SM actin are called VA-type. This classification suggests the possibility that myofibroblasts do not always express α-SM actin.

Monoclonal antibodies have been developed that identify myofibroblasts in certain tissues. For example, the monoclonal antibody Gb42 recognizes placental myofibroblasts (29), and 8E1 reacts with many of the stellate-shaped myofibroblasts such as astrocytes and both intes-

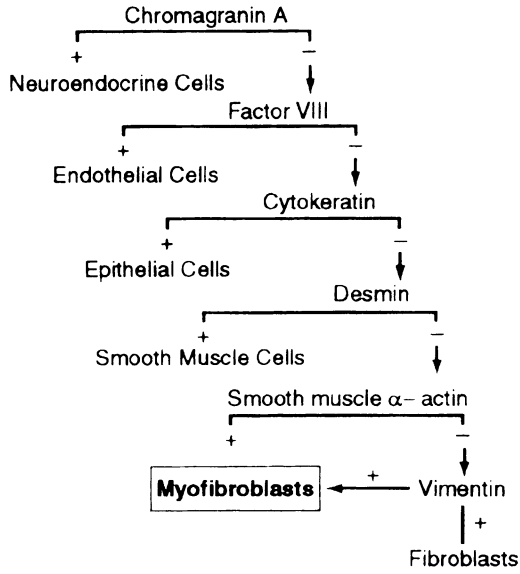

FIG. 4. Flow diagram illustrating the stepwise characterization of myofibroblasts. (Reproduced by permission from ref. 25.)

tinal myofibroblasts, the interstitial cells of Cajal (ICC) and intestinal subepithelial myofibroblasts (ISEMF) (30). Anti-GFAP antibodies will stain astrocytes, pancreatic periacinar stellate cells and hepatic stellate (Ito) cells (31). The PR2D3 antibody stains subepithelial myofibroblasts in the stomach and intestine; lung myofibroblasts; periductular myofibroblasts of the kidney, testes and breast; Ito cells of the liver; umbilical cord stellate myofibroblasts; and both vascular and tissue smooth muscle of most organs (32). Antibodies against the proto-oncogene *c-kit*, the receptor for stem-cell factor (SCF or steel factor), react with ICC (33).

ORIGIN OF MYOFIBROBLASTS

Myofibroblasts may originate from progenitor stem cells in the neural crest (34). Alternatively, they may simply transdifferentiate from resident fibroblasts (6) or from tissue (e.g., vascular, intestinal, or uterine) smooth muscle cells (35) (Figure 3). Transforming growth factor beta (TGF-β) seems to be the key in this transdifferentiating process. Activation to an α-SM actin-expressing phenotype may require both TGF-β and a specific cell-matrix interaction (36,37).

Platelet-derived growth factor (PDGF) and stem cell (Steel) factor (SCF) have been shown to promote myofibroblast differentiation from

embryonic stem cells. PDGF has two chains, A and B, and exists as either homodimers (PDGF-AA or PDGF-BB) or as a heterodimer (PDGF-AB). Each dimer acts on two different receptors: α which is nondiscriminatory and can bind AA, BB and AB dimers, or β receptors that are specific for the B chain (38). After ligand binding to the PDGF receptor, there are two separate intercellular signaling pathways: a mitogen-activated protein kinase (MAPK) path and a phosphatidylinositol 3 kinase (PI-3 kinase) path. In certain cell types, one pathway may be required for cell activation/proliferation and the other pathway for cell motility (migration) (39).

ROLE OF MYOFIBROBLASTS IN GROWTH AND DEVELOPMENT

Disruption of the PDGF-AA gene is lethal in 50% of affected mice (40). The surviving animals develop a lung devoid of alveolar myofibroblasts (also called the pulmonary contractile interstitial cells). These mice develop emphysema due to failure of lung septation. In contrast, animals born with disruption of the PDGF-BB gene develop a kidney devoid of renal mesangial cells, and the glomeruli in these mice lack the typical complex structure (41,42). The PDGF-BB-deficient animals also fail to develop pericytes, which leads to the formation of microaneurysms and leaky blood vessels, causing tissue edema and hemorrhage (43).

The proto-oncogene *c-kit* is the transmembrane glycoprotein tyrosine kinase (III) receptor for stem cell factor (SCF). SCF is a growth factor secreted by epithelial cells, white blood cells and (myo)fibroblasts, and it is also a member of the PDGF family. Intestinal interstitial cells of Cajal (ICC) express *c-kit* (33,44) and mutations in the *c-kit* receptor (the W-mutants) or in the ligand SCF (Steel mutants) results in abnormalities in the number, structure and the function of the ICC (18,45).

Thus, the PDGF family of growth factors seems crucial for the embryologic development of myofibroblasts. Unfortunately, no systematic study of all the different tissue myofibroblasts has been reported in the PDGF or SCF knockout mice or in mutants of their respective receptors.

ACTIVATION, PROLIFERATION AND MIGRATION OF MYOFIBROBLASTS

Some fibroblasts and all stellate-transformed myofibroblasts become activated and proliferate when cultured on plastic in serum-containing

growth culture media, especially when seeded at low cell density (46). When treated with TGF-β, myofibroblasts from the intestine (47), breast (48), skin (13), liver (22), lung (2), prostate (3), nose (49) and synovium (2) express α-SM actin, reduce the number of vitamin A lipid droplets, and expand the rough endoplasmic reticulum. These are the morphologic characteristics of an activated myofibroblast. Conversely, α and γ-IFN (50,51) decrease the expression of α-SM actin in myofibroblasts. This down-regulation occurs either by transdifferentiating myofibroblasts back to the fibroblast state, by inducing them to undergo stellate transformation, or simply by down-regulating the amount of α-SM actin in the cell.

TGF-β appears to be the most important cytokine causing the development of α-SM actin staining and an "activated" phenotype (52,53). The source of TGF-β in damaged tissue may be from white blood cells, parenchymal or epithelial cells, or from the myofibroblast itself (52,53). The activation of the myofibroblast requires the presence of matrix molecules, specifically, the ED-A (EIIIA) domain of fibronectin (36,37), as a binding site for cell membranes and other matrix molecules. This specific domain of a splice variant of fibronectin occurs during tissue injury and is necessary for TGF-β to trigger α-SM actin expression and collagen secretion by myofibroblasts.

Following activation of the myofibroblast, PDGF or connective tissue growth factor (CTGF), a member of the PDGF family, appear to be the principal factors responsible for myofibroblast proliferation (24,39,54,55). TGF-β was once considered the prime factor, but it is now thought that TGF-β acts predominantly through the induction of PDGF receptors on, or synthesis of CTGF, by the myofibroblasts (39,54). TGF-β appears to be predominantly a cytodifferentiating rather than a proliferating growth factor. Other growth factors, proinflammatory cytokines and regulatory lipids have been incriminated in the activation and proliferation of myofibroblasts, as well (Table 1).

ROLE IN WOUND REPAIR

The process of wound healing is a highly orchestrated sequence of events in which myofibroblasts appear to be key cells. During wound repair, there is the release of proinflammatory cytokines, eicosanoids of the cyclooxygenase, lipoxygenase and cytochrome P450 families, nitric oxide and a host of growth factors, as well as secretion of collagen and other matrix proteins. There is elaboration of angiogenic, angiostatic and nerve growth factors and, if it is a deep or open wound, the formation of granulation tissue which may become scar tissue (fibrosis)

TABLE 1
Cytokines, Growth Factors and Inflammatory Mediators That Induce Activation and Proliferation of Myofibroblasts (4,5)

Cytokines	Growth Factors	Steroids	Soluble Factors
IL-1	TGF-α	Aldosterone	Thrombin
IL-4	TGF-β		Angiotensin II
IL-6	EGF		Endothelin
IL-8	GM-CSF		
	PDGF-AA		
	PDGF-BB		
	aFGF		
	bFGF		
	IGF-I		
	IGF-II		
	SCF		

IL-1 or 6, Interleukin 1 or 6; TGF-α or β, transforming growth factor alpha or beta; EGF, epidermal growth factor; GM-CSF, granulocyte-macrophage colony stimulating factor; PDGF, platelet-derived growth factor; a or bFGF, acidic or basic fibroblast growth factor; IGF-I or II, insulin-like growth factor I or II; SCF, stem cell factor.

(56–58). Myofibroblasts become activated and proliferate in the early stages of wounding. In response to proinflammatory cytokines secreted by damaged epithelial cells and activated white blood cells, myofibroblasts then elaborate and secrete matrix proteins and additional growth factors. Then they disappear by apoptosis following completion of repair or scar formation (59–61).

Repair Processes. Epithelial tissues such as the intestine or stomach are often superficially injured. In fact, exfoliation of the epithelium is viewed as a defense response to certain noxious insults such as toxins, microbiologic invasion or gut anaphylaxis (62). The process of repair of the epithelium occurs through two separate mechanisms: restitution and reconstitution (58). If the basement membrane underlying the sloughed epithelium is intact, epithelial cells at the edges of the wound send out projections along the basement membrane until they meet advancing epithelial cells from the other side of the wound. This process is called *restitution* (63). Prostaglandins of myofibroblast origin from cyclooxygenase 1 or 2 (COX-1 or 2) activation are key factors promoting restitution (64) and in preserving the epithelial cells from damage (65). Myofibroblast-secreted growth factors such as TGF-β, TGF-α, EGF, aFGF and bFGF, and inflammatory cytokines such as IL-1β and interferon gamma (IFN-γ) also promote restitution (63,66–69).

If the wound is severe enough to destroy the subepithelial tissues with its interstitial substance, blood vessels, nerves, and fibroblasts,

healing must occur by reconstitution. If the basement membrane has been destroyed, epithelial cells and myofibroblasts form a new basement membrane (58). Epithelial stem cells then undergo mitosis, proliferate and migrate along the newly formed basement membrane in response to growth factors secreted by myofibroblasts. Thus, myofibroblasts appear to play roles in both the restitution and reconstitution repair processes.

An important event in the process of wound repair by either restitution or constitution is contraction of the underlying tissue to limit the surface area of the damaged tissue (12,62,70,71). This is accomplished because the myofibroblasts are connected to each other in a syncytium and because they contain α-smooth muscle actin and smooth muscle myosin isoforms. The ability of myofibroblasts to contract the tissue depends on changes in the cellular cytoskeleton (stress fiber formation), as well formation of the Rho-regulated focal contacts (fibronexus), and the expression of integrins which allow attachment of the myofibroblasts to the extracellular matrix (70,71).

Extracellular matrix (ECM). The ECM is a complex mixture of collagen, glycoproteins, and proteoglycans distributed in the tissue in unique proportions (72,73). These matrix proteins serve several functions. First, they are the scaffold for tissue formation. Through binding to cell receptors (integrins), they initiate intercellular signaling events. They bind to growth factors secreted into the damaged tissue. This creates a reservoir of concentrated factors which drives epithelial or parenchymal cell migration, proliferation and differentiation (56,74,75).

There are 19 different collagens in the collagen superfamily. Types I, III, IV, and VIII collagen are secreted by myofibroblasts (75,76). The major glycoproteins secreted by the myofibroblasts are the laminins such as fibronectin and tenascin. The basement membrane is composed of laminin, type IV collagen, entactin and chondroitin sulfate, all elaborated by (myo)fibroblasts, and perlecan, a large, low-density, heparin-rich proteoglycan of epithelial cell origin (56,73). Basement membranes and matrix are degraded by a family of Zn^{2+}-dependent matrix metalloproteinases (MMPs 1–3), also secreted by myofibroblasts (77,78). This promotes tissue remodeling following injury. MMPs are classified by the substrates they degrade: MMP 1 digests types I, II and III collagen; MMP 2 (gelatinase A) digests denatured collagens I and III and native collagen IV; and MMP 3 (stromelysin) degrades laminin, fibronectin, proteoglycans, type IV collagen and casein (78,79). MMPs are regulated, in turn, by tissue inhibitors of

metalloproteinases (TIMPs) that are also secreted by myofibroblasts (79).

Growth factors may bind to heparan sulfate proteoglycans or to collagen. This allows the matrix to control growth factor availability both temporally and spatially, and this regulates the biologic activity of these factors (56,71,73,74).

Growth Factors. The growth factors secreted by myofibroblasts have three actions: 1) they initiate or increase cell mobility, 2) they induce proliferation, and 3) they induce terminal differentiation of cells, even driving the cells to apoptosis. Some growth factors seem to have all three effects. These growth factors may act in an autocrine fashion on the myofibroblasts themselves or, via a paracrine fashion, on the epithelial or parenchymal cells in the tissue under repair.

Individual growth factors such as the trefoil proteins may be produced by the epithelial cells alone. Other growth factors are produced only by mesenchymal cells (myofibroblasts or inflammatory cells, particularly macrophages and lymphocytes), and some are produced by both mesenchymal and epithelial cells (17). Furthermore, the various inflammatory cytokines (IL-1, IL-6, IL-15, TNF-α), eicosanoids (PGE_2 and PGI_2) and growth factors (EGF, TGF-α, IGF-I and -II, HGF and KGF) released during tissue damage may directly affect the epithelium or parenchymal cell of the injured tissue, or these agents may act on the myofibroblasts to induce these cells to secrete additional cytokines, eicosanoids or growth factors which then act on the epithelial/parenchymal cells (17). Thus, in vivo, an epithelial proliferative response could be the result of a cytodifferentiating effect of mediators on the myofibroblasts, inducing them to express receptors or to secrete specific epithelial proliferating growth factors. For example, TGF-β1 induces the expression of PDGF or CTGF receptors on the myofibroblasts causing them to proliferate in response to PDGF (80). Another example is the secretion of HGF or KGF by myofibroblasts in response to IL-1 (81) or immune stimulation (82,83).

HGF and KGF have received special attention because they are secreted by the underlying (myo)fibroblasts and have major proliferative effects on epithelial and parenchymal cells. Keratinocyte growth factor (KGF) is a member of the fibroblast growth-factor family (FGF-7) (84). This factor is unique because, unlike other members of the FGF family, it does not appear to act on fibroblasts, endothelial cells or other nonepithelial targets because these cells do not express the KGF receptor (KGFR). KGFR expression is limited to epithelial cells. KGF induces proliferation and differentiation of epithelial and parenchymal cells, including intestinal epithelial cells, type II pneu-

mocytes, hepatocytes and keratinocytes of the skin. Its expression and secretion are regulated by IL-1 (81), and its synthesis is significantly up-regulated in inflamed tissues (83). Thus, KGF represents a prime example of a mediator released by mesenchymal cells and acting on epithelial cells.

Hepatocyte growth factor (HGF) is another factor synthesized and secreted by fibroblasts and myofibroblasts (85,86). The HGF receptor is encoded by the proto-oncogene *c-met* and is prominently expressed by epithelial cells. Like TGF-β, HGF has effects on cell division, motility, and apoptosis (85). Like KGF, its synthesis is also stimulated by IL-1 (85). In addition to proliferation effects on epithelial cells, it also affects liver and bone (85). Thus, HGF, like KGF, is a major mediator of epithelial-mesenchymal interactions and epithelial morphogenesis (84).

The process of repair is terminated by the differentiation of epithelial and parenchymal cells and by the eventual apoptosis of the α-SM actin myofibroblasts (59). The factors that terminate the repair process are poorly understood, but a role for IL-10, INF-γ and INF-α has been suggested.

ROLE OF MYOFIBROBLASTS IN INFLAMMATORY DISEASES

Myofibroblasts are incriminated in disease either because they are absent (see above) or because they are activated (inflammatory states). In endstage disease, these activated cells cause fibrosis (Table 2).

Myofibroblasts play a major role in the inflammatory response. These cells are avid producers of both chemokines and proinflammatory cytokines (4,5,87–89) and are capable of augmenting or down-regulating the inflammatory response by the secretion of these soluble mediators of inflammation. They also synthesize prostaglandins, expressing both the constitutive COX-1 (PHS-1) gene product and the inducible COX-2 (PHS-2) protein (90–93). In some tissues they make both NO and CO, important neurotransmitters and regulators of motility and inflammation (90,92,94–98). When activated, myofibroblasts express adhesion molecules such as ICAM-1, VCAM and NCAM (88,99,100). This allows lymphocytes, mast cells and neutrophils to associate with the myofibroblasts and promote immunological and inflammatory reactions (17,87,101,102). Through these or other properties, myofibroblasts participate in the formation of tissue granulomas (57), which secrete cytokines and other inflammatory mediators (103).

TABLE 2
Role of Myofibroblasts in Disease (4)

Myofibroblast	Disease
	Absent or Poorly Developed
Pericyte	Microaneurysm
Mesangial cell	Abnormal glomerulus
Pulmonary interstitial cell	Emphysema
Intestinal interstitial cell of Cajal	Hirschsprung's disease, hypertrophic pyloric stenosis
Bone marrow stromal cell	Aplastic anemia
	Activation and Proliferation
Coronary pericyte	Restenosis
Gingival myofibroblast	Gingival hypertrophy
Orbital myofibroblast	Proptosis of Graves' disease
Retinal myofibroblast	Proliferative vitreoretinopathy
Renal mesangial cell	Proliferative glomerulonephritis
Intestinal myofibroblast	Ischemic and radiation colitis, inflammatory bowel disease
Pulmonary interstitial cell	Diffuse alveolar damage
Joint synoviocyte	Rheumatoid arthritis
	Fibrosis
Skin	Scleroderma, keloid, Dupuytren's contracture
Corneal myofibroblast	Corneal scarring
Cardiac myofibroblast	Myocardial fibrosis
Renal mesangial cell	Sclerosing glomerulonephritis
Renal interstitial cell	Renal tubular interstitial fibrosis
Hepatic Ito cell	Cirrhosis
Pulmonary interstitial cell	Pulmonary interstitial fibrosis
Interstitial subepithelial myofibroblast	Collagenous colitis
Brain—atrophy	Glial scar
Bone marrow	Fibrosis of myelodysplasia

With repeated cycles of injury and repair or if there is loss of regulation of the healing process, organ fibrosis occurs. The role of the myofibroblasts in the fibrosis of the skin, lung, pancreas and kidney are well described. Factors that act on myofibroblasts are important in tissue fibrosis. The key role of TGF-β in fibrosis has been enforced by the finding of fibrosis of multiple organs including the liver, kidney (both renal interstitium and glomerulus) and in adipose tissue in a transgenic mouse over-expressing TGF-β (53). PDGF-BB causes fibrosis in the kidney (104). Given TGF-β's propensity to up regulate PDGF receptors, PDGF may be as important as TGF-β in organ fibrosis. IGF-I has been shown also to induce collagen mRNA and IGF-binding protein-5 mRNA in rat intestinal smooth muscle (105), raising the

question of an important role for this growth factor in organ fibrogenesis (106). The effects of PDGF, TGF-β and other growth factors in the fibrotic process have been studied in detail and are beyond the scope of this review (the reader is referred to refs. 56 and 107 for detailed reviews of the fibrotic process).

ROLE OF MYOFIBROBLASTS IN COLONIC POLYPS AND CANCER

Myofibroblasts are involved in the pathogenesis of intestinal inflammatory (hyperplastic) polyps, stromal tumors and hamartomatous tumors. They are also the primary mesenchymal element of colonic adenomas and cancer. Myofibroblasts undergo neoplastic transformation (108,109). The resulting mesenchymal tumors belong to the neoplasms previously called leiomyomas, leiomyosarcomas, fibrosarcomas, histiocytomas, spindle cell tumors and desmoid tumors. These neoplasms are now categorized under a single encompassing term of gastrointestinal stromal tumors (GISTs).

Myofibroblasts are the primary mesenchymal element in the lamina propria of sporadic, hyperplastic, hamartomatous and adenomatous polyps (3,110–112). They are also found in inherited neoplasms such as juvenile polyposis, Peutz-Jeghers syndrome and familial polyposis (110,111,113).

Intestinal pericryptal fibroblasts are believed to be important in local tumor growth patterns of colorectal neoplasms and in the metastatic process (114,115). Myofibroblasts appear to be responsible for the desmoplastic (fibrotic) reactions seen in many colon cancers (116,117). Intestinal myofibroblasts have been proposed to play an important role in tumor metastasis, in part by the secretion of MMPs which would promote detachment of tumor cells (67,118–126). There are several other proposed mechanisms whereby myofibroblasts might control the metastatic potential of cancer. Perhaps the most important possibility is that TGF-β secreted by tumor myofibroblasts may directly stimulate tumor cell motility (67,125,126).

The TGF-β Type II receptor (TGF-β RII) is mutated or absent in many colon carcinomas. Because TGF-β induces apoptotic cell death in many epithelial cells, such mutations might allow unregulated tumor development (127,128). Patients with colorectal cancer often have increased plasma levels of TGF-β, and this appears to be of lamina propria (? myofibroblast) origin (129). Because TGF-β production and receptors may be of either myofibroblast or epithelial origin, more

definition of this area is needed to sort out the role of this growth hormone and its receptors in colorectal cancer.

An important relationship between myofibroblasts and colorectal cancer is an understanding of the mechanisms whereby nonsteroidal anti-inflammatory drugs (NSAIDs) cause regression of polyps in familial adenomatous polyposis (FAP) (130–133) and the mechanism of NSAID chemoprevention of sporadic colon cancer (134–136). The mechanisms of this anti-tumorigenic and chemopreventive effect is unclear. The most recent hypothesis is that NSAIDs cause increased apoptosis of colonic epithelial cells (137–140) through inhibition of PG synthesis. This causes elevated arachidonic acid (AA) levels in the cell, and AA stimulates the conversion of sphingomyelin to ceramide, a known inducer of apoptosis (140). An alternative hypothesis is that NSAIDs may cause apoptosis of growth factor-secreting intestinal myofibroblasts, which leads secondarily to decreased epithelial proliferation or increased epithelial apoptosis.

COX-1 is detected in both normal and malignant colonic epithelial cells (65,141,142). COX-2 is rarely detectable in the normal colonic epithelia, but is found in over 90% of colon cancers. COX-2 expression is present in less than half of premalignant colonic polyps (141,142). In the adenomatous polyposis coli (APC) knockout mouse, specific COX-2 inhibitors have been shown to suppress the development of intestinal polyps (132). In these animals, the site of COX-2 expression is in the subepithelial myofibroblasts (132). Recent studies suggest that myofibroblasts in sporadic colon cancers are an important site of COX-2 expression (143).

CONCLUSIONS

Myofibroblasts are ubiquitous cells with similar properties and functions that play important roles in growth and development, wound repair and disease. Their absence or their activation and proliferation lead to specific diseases as outlined in Table 2. Their role in disease states will be the subject of much investigation in the coming decade.

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DISCUSSION

DAVIS, Charlottesville: We have heard a lot here about cancers and infectious disease. I'm thinking about some of the connective tissue diseases and I'm thinking here about scleroderma with all of the dysmotility, the sclerosis, of course, of the skin, and now the lungs that are such a major problem in those patients. I am wondering whether this dysregulation here may play a role in that spectrum of diseases and might be an object of treatment.

POWELL: Absolutely. Myofibroblasts have been identified as activated and present in both the skin and in the various tissues of patients with scleroderma, as well as in Dupuytren's contracture and some of the other fibrotic skin diseases. Also, it turn out that the synovial cells of rheumatoid arthritis are also activated myofibroblasts. These cells in many tissues and diseases are responding to immunologic challenge with an inflammatory response and eventually going on to cause a fibrotic state.

WINCHESTER, Washington: Following on from that question, I saw something about calcium channel blockers being used for Dupuytren's contracture. Do they have any effect on the myofibroblasts?

POWELL: Possibly. Intestinal myofibroblasts have cyclic changes in calcium content, presumably through calcium channels, and calcium levels regulate their ability to contract and to have a contractile function. It makes sense that they would, indeed,

perhaps be responsive to calcium channel blockers. I have not seen that paper. That is a very interesting idea.

WINCHESTER: Actually, it was on the television that I saw it.

STEVENSON, Stanford: Immature fetuses do not scar and the question is what is it about the cells in fetuses that make them such that they can be involved in wound healing and not scarring? Is there something we can do to ourselves now that would help us adults have wounds that heal without scarring?

POWELL: Well, it is pretty clear that these myofibroblasts have different stages of either activation or resting (stellate transformation) and, in these different states, are capable of varying amounts of extracellular matrix protein secretion. Depending on whether or not they are growing and proliferating, activated and secreting inflammatory mediators and collagen, or whether they are transiently differentiated and resting, may determine their capability to cause scar. A newborn child's myofibroblasts may be in a more stem-cell, proliferative state that is not secreting collagen or other extracellular matrix proteins. This is an interesting idea that needs to be studied.