Myofibroblasts. I. Paracrine cells important in health and disease

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Powell, D. W., R. C. Mifflin, J. D. Valentich, S. E. Crowe, J. I. Saada, and A. B. West. Myofibroblasts. I. Paracrine cells important in health and disease. Am. J. Physiol. 277 (Cell Physiol. 46): C1–C19, 1999.—Myofibroblasts are a unique group of smooth-muscle-like fibroblasts that have a similar appearance and function regardless of their tissue of residence. Through the secretion of inflammatory and anti-inflammatory cytokines, chemokines, growth factors, both lipid and gaseous inflammatory mediators, as well as extracellular matrix proteins and proteases, they play an important role in organogenesis and oncogenesis, inflammation, repair, and fibrosis in most organs and tissues. Platelet-derived growth factor (PDGF) and stem cell factor are two secreted proteins responsible for differentiating myofibroblasts from embryological stem cells. These and other growth factors cause proliferation of myofibroblasts, and myofibroblast secretion of extracellular matrix (ECM) molecules and various cytokines and growth factors causes mobility, proliferation, and differentiation of epithelial or parenchymal cells. Repeated cycles of injury and repair lead to organ or tissue fibrosis through secretion of ECM by the myofibroblasts. Transforming growth factor-β and the PDGF family of growth factors are the key factors in the fibrotic response. Because of their ubiquitous presence in all tissues, myofibroblasts play important roles in various organ diseases and perhaps in multisystem diseases as well.

platelet-derived growth factor; stem cell factor; transforming growth factor-β; wound repair; fibrosis; inflammation; immunophysiology

A GROWING BODY OF LITERATURE over the last decade has made it evident that there is phenotypic heterogeneity among fibroblasts and that some express features of smooth muscle differentiation (73, 136, 214, 216). These smooth-muscle-like cells, or myofibroblasts as they were termed by Gabbiani (82, 83) who pioneered this field, take part in the growth, development, and repair of normal tissue as well as the diseases affecting many different organs. These cells belong to a unique class, and, even allowing for specific functions for those cells in a given organ or tissue, there is an amazing similarity in their morphology, function, and biochemical repertoire regardless of their location. Nonetheless, in a given tissue, they may express some specific appearances and functions, i.e., phenotypic and functional heterogeneity. Because of this propensity and their location next to epithelial or parenchymal cells, we have suggested they might be termed “juxtaparenchymal cells” (247).

In this review, we give an overview of myofibroblasts, illustrating similarities and differences in their biochemical/physiological/immunologic properties, and we indicate the role that these cells play in specific disease states. The major soluble factors secreted by these cells are discussed, and important receptors on myofibroblasts are listed. We have slanted the discussion toward the intestinal myofibroblasts (247): the interstitial cells of Cajal (ICC) and the subepithelial intestinal myofibroblast. This review is not meant to be entirely comprehensive of the field of myofibroblasts. It focuses on recently discovered information about the interactions of myofibroblasts with epithelial and parenchymal cells and the molecules that mediate these interactions. Furthermore, we have purposefully referenced review articles when possible to amplify the reference base.

ROLES IN HEALTH AND DISEASE

Table 1 lists various tissue myofibroblasts and what is thought to be their normal function. Some of these functions are well proven, and others can be inferred from the known properties of myofibroblasts in other
In general, there are several common normal activities of myofibroblasts. First, through mesenchymal-epithelial interactions, myofibroblasts are key components of organogenesis or morphogenesis, i.e., the growth and differentiation of the tissue or organ (227). They do so through the secretion of soluble mediators of inflammation and growth factors (Table 2) and expression of their receptors (Table 3) and through secretion and formation of interstitial matrix and/or basement membrane molecules (Table 4) (20, 73, 82, 247). Myofibroblasts also play a fundamental role in many disease states, either through activation and proliferation or through deletion (Table 5) (51, 214, 216). They play a central role in wound healing, presumably as an extension or accentuation of their role in normal growth and differentiation (82, 83, 99, 120, 136). They appear to be involved in the formation and repair of the extracellular matrix (ECM) and proliferation and differentiation of epithelial (or parenchymal), vascular and neurogenic elements (50, 215, 250, 262).

Healing is facilitated by the fact that the myofibroblasts are contractile, which aids in reducing the amount of denuded surface area of wounded tissue (163, 192, 242). An extension of this contractile capability allows these cells to participate in the ejection of fluid from the gastric glands (237) and in the motility of intestinal villi (120). Their relationship to myoepithelial cells, which have this function in the breast lobule (204) and the seminiferous tubules of the testis (106), is unclear. The contractile property of pericytes and spec-
Receptors expressed by myofibroblasts

Table 3. Receptors expressed by myofibroblasts

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Growth Factors</th>
<th>Inflammatory Mediators</th>
<th>Neurotransmitters and Paracrine Mediators</th>
<th>Adhesion Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 (139, 178)</td>
<td>TGF-α/EGF (138, 214)</td>
<td>Prostaglandins (19)</td>
<td>Acetylcholine (102)</td>
<td>ICAM-1 (100, 178)</td>
</tr>
<tr>
<td>IL-1Ra (102)</td>
<td>TGF-β RI and RII (25, 58, 159, 203)</td>
<td>HETEs (210)</td>
<td>Histamine (19)</td>
<td>VCAM-1 (178)</td>
</tr>
<tr>
<td>TNF-α (100, 101)</td>
<td>PDGF-α (26, 111, 263)</td>
<td></td>
<td>Serotonin (19)</td>
<td>NCAM (133)</td>
</tr>
<tr>
<td>IL-6R (179)</td>
<td>PDGF-β (111, 141, 234)</td>
<td></td>
<td>Bradykinin (19)</td>
<td>MCP-1 (151, 235)</td>
</tr>
<tr>
<td>IL-8R (31)</td>
<td>c-kit (18, 109, 174)</td>
<td>Endothelin (80, 142, 246)</td>
<td></td>
<td>αβ1 integrin (192)</td>
</tr>
<tr>
<td>IL-4R (128, 156)</td>
<td>aFGF and bFGF R (111)</td>
<td>Atrial natriuretic factor (246)</td>
<td></td>
<td>CD18 (31)</td>
</tr>
<tr>
<td>IL-11Ra (143)</td>
<td>IGF-IR (144)</td>
<td>Aldosterone or ANG II (34, 258)</td>
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</tbody>
</table>

EGFR, epidermal growth factor receptor; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NCAM-1, neural cell adhesion molecule-1; RII and RII, types I and II receptors.
DEFINITION OF A MYOFIBROBLAST

Myofibroblasts may be defined morphologically and immunologically through identification of expressed cytoskeletal proteins (214, 216). The simplest definition of a myofibroblast is that they are smooth-muscle-like fibroblasts. Some investigators choose to call them smooth-muscle-like cells or activated smooth muscle cells (159, 163, 237). Others refer to them as lipocytes smooth-muscle-like cells or activated smooth muscle cells (159, 163, 237). Myofibroblasts possess several distinguishing morphological characteristics, some of which are present in fibroblasts or smooth muscle cells (Fig. 1). They display prominent cytoplasmic actin microfilaments (stress fibers), and they are connected to each other by adherens and gap junctions (51, 239). These cells are also in contact with the ECM by focal contacts once known as the fibronexus, a transmembrane complex made up of intracellular contractile microfilaments and the ECM protein fibronectin (65). Both fibronexus formation and stress fiber assembly are regulated by Rho, a newly described member of the RAS superfamily of small guanosine triphosphatases (GTPases) (94), specifically in mammalian cells by RhoA. These small, monomeric GTP-binding proteins also regulate myofibroblast morphology (191, 265). Often, an incomplete basal lamina surrounds the myofibroblasts. Gap junctions couple some myofibroblasts to the tissue smooth muscle, and the cells are commonly in close apposition to varicosities of nerve fibers (134, 212, 243).

In some tissues, e.g., the liver (Ito cells) (88), intestine (both the ICC (243) and the subepithelial myofibroblasts) (78, 246), the orbital myofibroblast (195, 254), the synoviocyte of the joint space (11), and brain (astrocyte) (15, 166, 193), the myofibroblasts exist in two distinct morphological states (Fig. 2): 1) the "activated" myofibroblast, as described above, and 2) the stellate-transformed myofibroblast, which is considered to be a transiently differentiated myofibroblast. This generalization, correlating appearance and function, has not been verified in every tissue where such morphological heterogeneity has been seen. Agents (e.g., prostaglandins, cholesterin, vasoactive intestinal polypeptide) that increase the cAMP content of the cells are commonly in close apposition to varicosities of nerve fibers (134, 212, 243).

Immunohistochemical characterization of myofibroblasts is based on antibody reactions to two of the three filament systems of eukaryotic cells (75, 116). These three systems are composed of 1) actin, a component of the microfilaments; 2) vimentin, desmin, lamin, or glial fibrillary acidic protein (GFAP), members of the intermediate filament system; and 3) the tubulins of the microtubules. Myofibroblasts have not been characterized with regard to tubulins. The β and γ actins are expressed by all cells, including myofibroblasts, which may also express α-smooth muscle (α-SM) actin (214, 216). Myofibroblasts stain negatively for α-cardiac and α-skeletal actin (216). Myofibroblasts are not well characterized with regard to the newly defined myosin isoforms (75, 161, 217). In some tissues, such as the intestine and reticular cells of lymph nodes and spleen,
myofibroblasts stain positive for smooth muscle heavy chain myosin or tropomyosin (old terminology) (216, 243).

Vimentin, desmin, and α-SM actin are the three filaments most often used to classify myofibroblasts (161). Expression of these proteins may vary with the tissue studied within species and is subject to environmental factors, e.g., whether the cells are studied in situ or in culture and, even within a given tissue, whether the cells are activated by hormonal or cytokine treatment or by disease (191). Based on immunohistochemical staining of these filaments in a given tissue, a classification system has been proposed (134, 216). Myofibroblasts that express only vimentin are termed V-type myofibroblasts, those that express vimentin and desmin are called VD-type, those that express vimentin, α-SM actin, and desmin are called VAD-type, those that express vimentin and myosin are called VM-type.

<table>
<thead>
<tr>
<th>Tissue or Organ</th>
<th>Activation/ Proliferation</th>
<th>Deletion or Damage</th>
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</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Scleroderma; keloid; Dupuytren's contracture (73, 213, 224); psoriasis (63)</td>
<td>Microaneurysms, edema, and hemorrhage (26, 239)</td>
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<tr>
<td>Pericyte</td>
<td>Atherosclerosis and restenosis (149, 159); hypertension (208)</td>
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<tr>
<td>Mouth</td>
<td>Periodontal disease (136, 214)</td>
<td></td>
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<tr>
<td>Periodontal ligament</td>
<td>Gingival hypertrophy secondary to drugs (cyclosporin and Dilantin) (135, 136, 212, 214, 216)</td>
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<tr>
<td>Gingival myofibroblasts</td>
<td>Gingival hypertrophy secondary to drugs (cyclosporin and Dilantin) (135, 136, 212, 214, 216)</td>
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<tr>
<td>Heart and pericardium</td>
<td>Myocardial fibrosis, atherosclerosis, and coronary artery restenosis (35, 149, 159, 258)</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>Exophthalmos (proptosis) of Grave's disease (9, 221, 254)</td>
<td></td>
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<tr>
<td>Orbital fibroblast</td>
<td></td>
<td>Diabetic microaneurysm (26, 142, 239)</td>
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<tr>
<td>Retinal myofibroblast</td>
<td>Proliferative vitreoretinopathy (253)</td>
<td></td>
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<tr>
<td>Anterior capsule of lens</td>
<td>Anterior capsular cataract (172, 217)</td>
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<tr>
<td>Corneal myofibroblast</td>
<td>Corneal scarring (184)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Proliferative and sclerosing glomerulonephritis (108, 184, 239)</td>
<td>Absence of glomerular structure (141, 234)</td>
</tr>
<tr>
<td>Mesangial cell</td>
<td>Renal tubulointerstitial fibrosis (171, 177, 198, 239)</td>
<td></td>
</tr>
<tr>
<td>Interstitial cell</td>
<td>Renal tubulointerstitial fibrosis (171, 177, 198, 239)</td>
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<tr>
<td>Liver</td>
<td>Fibrosis and cirrhosis (72, 88, 150)</td>
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<tr>
<td>Perisinusoidal stellate (Ito cell)</td>
<td>Ischemia-reperfusion injury of hepatic transplantation (206)</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreatic fibrosis (4, 8)</td>
<td>Emphysema (25)</td>
</tr>
<tr>
<td>Periacinar stellate cell</td>
<td></td>
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<tr>
<td>Lung</td>
<td>Pulmonary interstitial fibrosis, idiopathic and drug-induced; sarcoidosis (105, 209, 214)</td>
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<tr>
<td>Interstitial contractile cell</td>
<td>Diffuse alveolar damage disease (176)</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pulmonary hypertension (123)</td>
<td></td>
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<tr>
<td>Stomach and intestine</td>
<td>Abnormal intestinal motility; hypertrophic pyloric stenosis; Hirschsprung's disease; megacolon of piebaldism; idiopathic pseudo-obstruction (33, 52, 115, 183, 212, 243, 248, 249)</td>
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<tr>
<td>Subepithelial myofibroblast</td>
<td>Collagenous colitis; villous atrophy and crypt hyperplasia; polyp formation; psoriasis (2, 86, 114, 131, 153)</td>
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<tr>
<td>Brain</td>
<td>Healing gastric ulcer (170)</td>
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<tr>
<td>Astrocyte</td>
<td>Produce glial scar tissue (166)</td>
<td>Human immunodeficiency virus-associated cognitive motor disease; spongiform encephalopathy (166)</td>
</tr>
<tr>
<td>Breast</td>
<td>Fibrocytic disease: desmoplastic reaction to breast cancer (73, 214)</td>
<td>Aplastic anemia (182, 218)</td>
</tr>
<tr>
<td>Stromal myofibroblast</td>
<td></td>
<td></td>
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<tr>
<td>Bone marrow</td>
<td>Fibrosis in myelodysplasia and neoplastic diseases (182, 218)</td>
<td></td>
</tr>
<tr>
<td>Stromal cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint</td>
<td>Rheumatoid pannus formation (11)</td>
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</table>
In the intestine, the ICC express immunoreactive vimentin, γ-SM actin, and smooth muscle tropomyosin, suggesting that they are members of the V or VM (243) class of myofibroblasts. However, we have not been able to find references indicating that ICC have been explored with antibodies to α-SM actin. The intestinal subepithelial myofibroblasts (ISEMFs) stain positive for vimentin and α-SM actin and negative (or weakly)

Fig. 1. A: transmission electron micrograph of a cultured lumen intestinal subepithelial myofibroblast (18Co). The cell membrane displays numerous caveolae. Stress fibers (bundles of actin microfilaments) are prominent. The cytoplasm is rich in rough endoplasmic reticulum, Golgi apparatus, and mitochondria. B: nucleus of an activated myofibroblast shows multiple indentations. Adherens (C) and gap junctions (D) are present between myofibroblasts. [From Valentich et al. (246).]

Fig. 2. Phase-contrast micrographs (A and B) and scanning electron micrographs (C and D) of stellate 18Co cells. The stellate myofibroblast displays a highly refractile cell body on phase-contrast microscopy and possesses a highly arborized array of cell processes with several orders of bifurcation. The cell processes are devoid of microvilli, whereas the cell body shows a dense array of long microvilli, giving it a shaggy appearance. [From Valentich et al. (246).]
for desmin (VA-type). They also express smooth muscle
myosin (thus may be called VAM-type myofibroblasts),
although expression of myosin is less than that seen in
the corresponding smooth muscle cells in the same tissue
(120, 153, 246). It is possible that both intestinal
myofibroblasts, the ICC and ISEMF, could be the
VA(M)-type.

Specific monoclonal antibodies have been developed to
identify myofibroblasts in certain tissues. For ex-
ample, the monoclonal antibody Gb42 recognizes placen-
tal myofibroblasts (134). The 8E1 monoclonal antibody
reacts with many of the stellate-shaped myofibroblasts,
such as GFAP-positive astrocytes, and both intestinal
myofibroblasts, the ICC and ISEMF (79). Anti-GFAP
antibody stains astrocytes, pancreatic periacinar stellate
cells, and hepatic stellate (Ito) cells (30). The PR2D3
antibody stains subepithelial myofibroblasts in the
stomach and intestine, lung myofibroblasts, periductu-
lar 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 (199). Antibodies against the protooncogene
C-kit, the receptor for stem cell factor (SCF or steel
factor), react with ICC (109, 132, 244) and possibly with
pulmonary alveolar myofibroblasts (61). No systematic
studies have been reported concerning the reactivity of
all the other myofibroblasts to C-kit antibodies.

In many of the studies quoted above, only a subset of
the (myo)fibroblasts stain with α-SM actin antibodies
in vivo or in vitro (culture). In vivo, not all fibroblastic-
appearing cells are myofibroblasts. In culture, treat-
ment with transforming growth factor-β (TGF-β) may
induce uniform α-SM actin staining of all the cells,
providing the cells are of a single done (226). Further-
more, activation to an α-SM actin-expressing pheno-
type may require both TGF-β and a specific cell-matrix
interaction (118, 222) (see ACTIVATION, PROLIFERATION,
AND MIGRATION OF MYOFIBROBLASTS).

ORIGIN OF MYOFIBROBLASTS AND ROLE IN GROWTH
AND DEVELOPMENT

It is unclear whether myofibroblasts originate from
progenitor stem cells (possibly neuroepithelial stem
cells) (24, 157) from the neural crest (117) or simply
transdifferentiate from resident tissue fibroblasts (81)
or from tissue (e.g., vascular, intestinal, or uterine)
smooth muscle cells (204). The close anatomic
relationship of pericytes to vascular smooth muscle and
of intestinal myofibroblasts to intestinal smooth muscle
suggests a bidirectional route of transdifferentiation.
These various possibilities are depicted in Fig. 3. It has
recently been suggested that renal tubular cells (a cell
of endoderm origin) might differentiate into myofibro-
blasts (mesenchymal cells) under noxious stimuli (171,
177, 198). However, it is equally likely that the peri-
tubular interstitial myofibroblasts are proliferating un-
der these circumstances and simply replacing apoptotic
tubular cells in these disease states.

Two soluble factors have been shown to promote
differentiation from embryonic stem cells: PDGF and
SCF. PDGF has two chains, A and B, and exists as a
homodimer (PDGF-AA or PDGF-BB) or as a het-
 erotdimer (PDGF-AB). Each form acts on separate
receptors: α receptors that are nondiscriminatory and
can bind AA, BB, and AB trimers or β receptors that are
specific for the B chain (126). After ligand binding,
there are two separate intercellular signaling path-
ways for the PDGF receptor: a mitogen-activated pro-
kine (MAPK) path and one involving phosphati-
dylinositol 3-kinase (PI3K). Depending on the cell
types, one pathway may be required for cell activation
and/or proliferation and the other pathway for cell
motility (migration) (3, 72, 150). For example, smooth
muscle cells proliferate in response to the MAPK
cascade and migrate in response to the PI3K path,
whereas hepatic stellate cells and endothelial cells
respond with both proliferation and migration via the
PI3K (72).

Disruption of the PDGF-AA gene in mice is lethal in
50% of affected animals (26). The surviving animals are
almost completely devoid of lung alveolar myofibro-
blasts (also called pulmonary contractile interstitial
cells) and develop emphysema due to failure of lung
septation. In contrast, animals born with disruption of
the PDGF-BB gene have a virtual absence of renal
mesangial cells and failure of the formation of the
complex structure of the glomerulus (141, 234). The
PDGF-BB-deficient animals also lack pericytes and
diusplosippere onosacnernaryiee, reminiscen of those
seen in the diabetic retina, and leaky vessels that cause
tissue edema and hemorrhage (142). Intestinal subepi-
thelial myofibroblasts (119) and hepatic stellate cells
(147) proliferate in response to low concentrations of
PDGF-BB, suggesting that this growth factor is impor-
tant in the growth and development of these cells.

The protooncogene C-kit is the transmembrane glyco-
protein tyrosine kinase (III) receptor (160 kDa molecu-
lar mass) for SCF, a growth factor secreted by epithelial
cells, white blood cells, and (myo)fibroblasts. SCF is
also a member of the PDGF family, and the tyrosine
kinase type III family also includes receptors for granu-
locyte/macrophage colony-stimulating factor (GM-
CSF). Intestinal ICC (in situ and in culture) express
c-kit as detected by rat anti-kit (ACK2) monoclonal
antibodies (18, 109, 132, 174, 244). Mutations in the
c-kit locus, the W mutants, result in abnormalities in
the number, structure, and function of the ICC (18, 52,
174, 212, 249). Furthermore, mutants of the ligand
SCF, steel (Sl) mutants, also show morphological and
functional abnormalities of the ICC (257). Thus the
PDGF family of growth factors seems crucial for the
embryological development of myofibroblasts. Unfortu-
nately, no systematic study of the various different
tissue myofibroblasts has been reported in PDGF or
SCF knockout mice or in mutants of their respective
receptors.

TGF-β, PDGF, insulin-like growth factor II (IGF-II),
and interleukin-4 (IL-4) appear to be the most impor-
tant growth factors for the transdifferentiation of fibro-
blasts to myofibroblasts or of stellate-transformed myo-
fibroblasts into activated myofibroblasts (45, 61, 145,
211, 239). When myofibroblasts from the intestine (74),
breast (203), skin (17, 111, 263), liver (88), lung (214), prostate (181), nose (256), and joint synovium (214) are treated with TGF-β in serum-containing media, they express α-SM actin, reduce the number of vitamin A lipid droplets, and expand the rough endoplasmic reticulum, i.e., they take on the morphology of an activated myofibroblast. Conversely, interferon (IFN)-α and IFN-γ (56, 90) decrease the expression of α-SM actin in myofibroblasts. It is not clear whether they do so by transdifferentiating myofibroblasts back to the fibroblast state, inducing them to undergo stellate transformation, or simply downregulating the amount of α-SM actin in the cell.

**ACTIVATION, PROLIFERATION, AND MIGRATION OF MYOFIBROBLASTS**

Fibroblasts or stellate-transformed myofibroblasts become activated and proliferate when cultured on plastic in serum-containing growth culture media, especially when seeded at low cell density (74, 155). In vivo activation, as signified by the development of α-SM actin positivity, may be separable from proliferation. Whereas many fibrogenic cytokines [IL-1, tumor necrosis factor (TNF)-α, PDGF, fibroblast growth factor (FGF), and TGF-β] have been incriminated in this process (138), TGF-β appears to be the most important cytokine causing the development of α-SM actin staining and an activated phenotype (25, 45, 88, 97, 155, 165, 223, 238) capable of collagen secretion (25, 45, 165). The source of TGF-β in damaged tissue may be from white blood cells, parenchymal or epithelial cells, or from the myofibroblast itself in an autocrine fashion (22, 25, 45, 88). Recently, it has been determined that the activation of the myofibroblast requires the presence of matrix molecules, specifically, the ED-A (EIIIA) domain of fibronectin (118, 222). Tissue injury gives rise to this specific ED-A domain splice variant of fibronectin. ED-A is the binding site for cell membranes and for other matrix molecules. It has been shown in both skin granulation tissue (222) and hepatic (118) models that this fibronectin ED-A domain 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 (29), appears to be the factor primarily responsible for myofibroblast proliferation (71, 72, 89, 119, 147). TGF-β was once considered the prime factor (88, 214, 216), but it is now thought that TGF-β acts predominantly through the induction of PDGF receptors on or synthesis of CTGF by the myofibroblasts (72, 89, 111, 112, 263). Thus TGF-β is predominantly a cytodifferentiating rather than a proliferating growth factor.

The TGF-βs are a large superfamily of soluble factors important in growth, development, and fibrogenesis (149). TGF-β1, TGF-β2, and TGF-β3 are encoded from three separate genes. TGF-β1 is the isoform usually upregulated in the presence of tissue injury. It is secreted in a latent form after cleavage from a large promolecule and then noncovalently binds to another peptide on the cell membrane called the latency-associated peptide, which, in turn, is formed from the cleavage fragments of the TGF-β precursor. This latent TGF-β is stored on the surface of the cell or on the extracellular matrix, awaiting conversion by unknown mechanisms to active TGF-β. In contrast to their apparent (but probably indirect) proliferative effect on myofibroblasts (see above), TGF-βs cause G1 phase cell cycle arrest of epithelial and smooth muscle cells and may even induce apoptosis (25). TGF-β acts through a superfamilly of serine-threonine kinase cell surface receptors. All three TGF-β bind first to the type II (RII) receptor that assembles and phosphorylates the type I (RI) receptor, activating this serine-threonine kinase and transducing the signal (25). Microsatellite (genomic) instability due to defects in DNA mismatch repair systems of the TGF-β receptors has been incriminated in the unregulated growth of cancer and in vascular atherosclerosis/restenosis (159). This raises the question of a role for myofibroblasts in these two diseases [see part II of this review (191)].

TGF-α (138), a member of the epidermal growth factor (EGF) family, as well as EGF itself (138, 214, 216, 226), GM-CSF (25), both acidic and basic FGF (aFGF and bFGF, respectively) (25, 119, 185, 214, 216, 226), and IGF-I and IGF-II (25, 226) are candidate growth factors promoting myofibroblast proliferation (see more details in Growth Factors). Proinflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-8 may also cause activation and proliferation (119, 138, 177, 185, 246) as does IL-4, a protein generally thought of as an anti-inflammatory cytokine (61, 156, 211).

ANG II or aldosterone, thrombin, and endothelin are also important soluble factors reported to promote myofibroblast activation (15, 34, 35, 77, 246, 258). Endothelin is capable of rapidly transdifferentiating the stellate morphology of intestinal myofibroblasts to the activated phenotype within 30 min of addition to cell culture media (78, 80, 246). After it activates the myofibroblasts, endothelin may subsequently inhibit their proliferation (147, 148).

Cocultures of fibroblasts and myofibroblasts with cancer cells of several different types induce transdifferentiation of fibroblasts to myofibroblasts and activation and proliferation of myofibroblasts (13, 19, 46, 70, 136, 149, 153, 204, 246, 264). This property of neoplastic cells, perhaps via secretion of growth hormones such as TGF-β, may well be responsible for the desmoplastic reaction (excessive fibrosis) seen in many cancers.

ROLE IN WOUND REPAIR

The process of wound healing is a highly orchestrated event that entails the release of proinflammatory cytokines, eicosanoids of the cyclooxygenase, lipoxygenase, and cytochrome P-450 family, nitric oxide, and a host of growth factors; the secretion of collagen and other matrix proteins; the elaboration of angiogenic, angiosclerotic, and nerve growth factors; and, finally, if it is a deep or open wound, the formation of granulation tissue that then becomes a scar (fibrosis) (20, 57, 187, 208, 214, 247, 252). Myofibroblasts appear to be key cells in these various events. They become activated and proliferate in the early stages of wounding. They respond to proinflammatory cytokines with elaboration of matrix proteins and additional growth factors and then disappear by apoptosis following repair or scar formation (51, 54, 55, 113, 164, 266).

Repair Processes

Epithelial tissues such as the intestine or stomach, in contrast to organs such as the liver, kidney, or lung, do not commonly sustain widespread injury that leads to uniform fibrosis. However, gastrointestinal epithelial tissues are often superficially injured. In fact, exfoliation of the epithelium is viewed as a defense response to certain noxious insults such as toxins, microbiological invasion, or gut anaphylaxis (163). The process of repair of the epithelium occurs through two separate mechanisms (252). If the basement membrane underlying the sloughed epithelium is intact, residual epithelial cells at the edges of the wound become motile and move along the basement membrane until they meet advancing epithelial cells from the other side of the wound and form new tight junctions. This process is called restitution (189, 225). Prostaglandins from COX-1 or COX-2 activation are key factors promoting restitution (23) and preserving the epithelial cells from damage (47). Myofibroblast-secreted growth factors such as TGF-β, TGF-α, EGF, aFGF, and bFGF and inflammatory cytokines such as IL-1β and IFN-γ also promote restitution (58, 59, 189, 190, 200).

Conversely, if the wound is deep, the subepithelial tissues that contain interstitial substance, blood vessels, nerves, and fibroblasts must be reconstituted. If the basement membrane has been destroyed by the noxious stimulus, epithelial cells and mesenchymal elements form a new basement membrane (252). Epithelial stem cells then undergo mitosis and proliferate and migrate along the newly formed basement membrane. This latter process is a coordinated event involving secretion of matrix proteins and growth factors. Thus myofibroblasts appear to play roles both in the restitution and repair processes.

A key event in the process of wound repair by either restitution or proliferation is contraction of the underlying granulation tissue or gastrointestinal lamina pro-
pria to limit the exposed surface area of the wound (51, 120, 163, 192, 208, 242). Myofibroblasts contain smooth muscle myosin isoforms in addition to α-SM actin, the requisite machinery for contraction and/or motility. The ability of myofibroblasts to carry out these processes depends on changes in the cellular cytoskeleton as well as in the Rho-regulated fibronexus and on the expression of integrins that allow attachment of the myofibroblasts to the extracellular matrix (192, 242).

The fibronexus, discovered and characterized by Eyden (65) and Singer and colleagues (228–232) connects the myofibroblast to the extracellular matrix through a transmembrane αβ integrin complex that joins the actin stress fiber of the myofibroblast to ECM fibronectin (32, 192). The Rho family of small GTPases includes Rac 1–3, Cdc 42, and Rho A–H, which respond to PDGF, TNF-α or bradykinin, or lipopolysaccharide A, respectively. In mammals, Rho A acts on the actin cytoskeleton to cause myofibroblast shape change or motility (6, 265).

ECM

The ECM is a complex mixture of collagen, other glycoproteins, and proteoglycans distributed in each organ or tissue in unique proportions (220, 227). These matrix proteins have several general functions: they are the scaffold for tissue formation and growth; through binding to cell receptors (integrins), they initiate intercellular signaling events; and they bind to growth factors and thus supply sustained concentration of these factors for epithelial or parenchymal cell migration, proliferation, and differentiation (20, 219, 227). There are at least 19 different collagens in the collagen superfamily, with types I, III, IV, and VIII being secreted by myofibroblasts (154, 168). Proteoglycans are proteins with large sulfated polysaccharide side chains of several types (Table 4). The major glycoproteins secreted by the myofibroblasts are the various laminins, which include fibronectin (see ACTIVATION, PROLIFERATION, AND MIGRATION OF MYOFIBROBLASTS) and tenascin. Laminin is a constituent of the basement membrane along with type IV collagen, entactin, and chondroitin sulfate (all of mesenchymal origin) and perlecan, a large, low-density proteoglycan composed of heparan sulfate side chains of epithelial cell origin (20, 227). Basement membranes and matrix are degraded by a family of Zn2+-dependent matrix metalloproteinases (MMPs 1–3) also secreted by myofibroblasts (146, 251). They 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 cascin (16, 251). These MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs) (16). Growth factors may bind to heparan sulfate proteoglycans or collagen, thus controlling their availability both temporally and spatially, and so modify their biological activity (20, 219, 227, 242).

Growth Factors

The growth factors secreted by myofibroblasts have three general functions as follows: 1) they initiate or increase cell mobility, 2) they induce proliferation, i.e., they are paracrine mitogens for epithelial or parenchymal cells and perhaps autocrine mitogens for themselves, or 3) they induce terminal differentiation of these cells, even driving the cells to apoptosis. Some growth factors seem to have all three effects.

Individual growth factors may be produced by the epithelial cells alone (trefoil proteins), by mesenchymal cells such as myofibroblasts or inflammatory cells, particularly macrophages and lymphocytes, and some by both cell types (67). Furthermore, the various inflammatory cytokines, eicosanoids, and growth factors released during tissue damage may directly affect the epithelium or parenchymal cell of the injured tissue, or these agents may act more proximally on myofibroblasts to induce these cells to secrete additional cytokines, eicosanoids, or growth factors (67). Thus an in vivo epithelial proliferative response could be the result of a cytodifferentiating effect of mediators on the myofibroblasts, inducing them to express receptors for other factors or to secrete specific epithelial proliferating growth factors. Examples of this are TGF-β1, which induces the expression of PDGF receptors on or CTGF secretion by the myofibroblasts, causing them to proliferate in response to PDGF (137), or the secretion of hepatocyte growth factor (HGF) or keratinocyte growth factor (KGF) by myofibroblasts in response to IL-1 (37) or immune stimulation (10, 66).

IL-1 (60), IL-6 (261), IL-15 (196), and TNF-α (121, 139) have also been identified as being involved in tissue repair and have been shown to be mitogenic for several mesenchymal and epithelial cell lines. Furthermore, combinations of cytokines and growth factors may have offsetting effects on epithelial proliferation, so the ultimate consequence of these various factors in vivo can be quite complicated.

Factors secreted by myofibroblasts such as EGF and TGF-α (12, 188), IGF-I and IGF-II (144, 145), HGF (28, 84, 173), and members of the FGF family, including aFGF, bFGF (also known as FGF-2) (110), KGF (also known as FGF-7) (107, 207), and IL-11 (38, 175, 179), have been demonstrated to be the major paracrine growth factors for epithelial and parenchymal cells. The trefoil peptides, secreted by the epithelial cells themselves, have similar effects through autocrine stimulation (186). These factors may also have nonmitogenic effects on intestinal cells as well, e.g., they may regulate secretory and contractile processes as well as regulate blood flow (245).

The trefoil peptides are so named because of a distinctive pairing of six cysteine residues that results in three interchained loops, thus giving a “three leaf” trefoil shape (38, 186). There are three such trefoil proteins that are small, highly stable molecules secreted principally by the goblet (mucus)-secreting cells of the epithelium and not by myofibroblasts. The stomach secretes peptide pS2 in the fundus and spasmylytic
polypeptide (SP) in the antrum, whereas the breast epithelium secretes only pS2 and the pancreas secretes only pancreatic SP (pSP) (129). In contrast, the intestinal epithelium secretes only intestinal trefoil factor (ITF). Targeted gene disruption of ITF causes abnormal epithelial cells and increased susceptibility to various models of injury, resulting in a colitis-like picture (7, 130). Exogenous administration of ITF repairs the gastric mucosa against other injuries such as those induced by ethanol or chronic indomethacin administration (7). PS2 gene knockout mice have a different disease phenotype; they develop extensive neoplastic adenomas in the antrum of the stomach, which then progress to carcinoma in situ (140).

TGF-α, EGF, and the EGF human homologue urogastrone (EGF/URO) are members of the same family of polypeptides and act on a common cell membrane receptor (12, 188). The TGF-α/EGF receptor appears to be upregulated in the mucosa of injured intestine and other organs. TGF-α is expressed in epithelial cells, myofibroblasts, and monocytes/macrophages, whereas EGF seems to be produced primarily by the epithelial cells of the salivary gland and Brunner’s glands of the duodenum. TGF-α is synthesized as a 160-amino acid precursor molecule that spans the cell membrane. Proteases release the soluble 50-amino acid form. It is unclear whether the membrane-bound form is active as a growth factor for adjacent cells. The soluble factor is trophic (mitogenic) for a number of cell lines in vitro and intestinal epithelial cells in vivo. It may well have differentiating functions as well. Ulceration of the human gastrointestinal mucosa causes the development of a specific cell lineage from epithelial stem cells that bud from the crypts next to an ulcer and then ramify to form a small gland. These budding glands secrete EGF/URO, which stimulates epithelial proliferation and promotes ulcer healing (260).

The FGF family (aFGF and bFGF) are important mitogens for myofibroblasts and have powerful neurotrophic and angiogenic properties that are important for tissue healing (68, 110). Other angiogenic factors secreted by myofibroblasts include the CXC family of cytokines such as IL-8 and epithelial neutrophil-activating peptide (ENA-78) (5, 127, 178). Cell-to-cell contact such as that occurring in restitution or wound healing has its antiapoptotic action via the adhesion molecule N-cadherin. When the adjacent cells touch, there is homophilic binding of N-cadherin molecules, which activate the FGF family of receptors. In this way, cell contact mimics the antiapoptotic effect of bFGF (91).

IGF-I and IGF-II are structurally related polypeptides that have various metabolic, proliferative, and differentiating effects through endocrine, autocrine, and paracrine mechanisms (144, 145). The effects are mediated by IGF-I receptors and insulin receptors. There is an IGF-II receptor, but its role in signal transduction is unclear. IGF is present in the circulation (from liver) and is also secreted in a paracrine fashion by myofibroblasts adjacent to epithelial and parenchymal cells (144). The IGF actions are determined by the availability of free IGF, the form that interacts with its receptors. In turn, the amount of free IGF is modulated by the level of high-affinity IGF-binding proteins (IGFBPs), of which six have been identified (42–44). These IGFBPs not only regulate the bioavailability of IGF but also inhibit or enhance its action on target tissues. Although the IGFs are weakly mitogenic for epithelial and parenchymal cells, they seem to be powerfully mitogenic for myofibroblasts (226) and other smooth muscle cells (255).

A new member of the family of factors stimulating epithelial growth is IL-11, a multifunctional cytokine originally derived from bone marrow stromal cells (175, 194). It regulates the growth of hematopoietic and lymphoid cells by acting on the IL-6 family of cytokine receptors. It stimulates proliferation of small intestinal crypts and accelerates recovery of the intestinal mucosa from models of damage (143). It also has trophic effects on neurons, preadipocytes, and myofibroblasts of the lung (175). TGF-β and IL-1 are potent stimulants of IL-11 production. Paradoxically, IL-11 has been shown to inhibit epithelial cell proliferation in the lung by altering phosphorylation of the retinoblastoma protein (194). Thus it is possible that the proliferative effect of IL-11 on epithelia occurs via activation of myofibroblasts, with subsequent secretion of epithelial proliferating factors by these cells, rather than being a direct effect of IL-11 on the epithelium itself.

KGF is a member of the FGF family (FGF-7) (107, 207). This factor is unique because, unlike other members of the FGF family, it does not appear to have activity on fibroblasts, endothelial cells, or other nonepithelial targets. This is a consequence of the epithelial cell expression of the KGF receptor (KGFR), a transmembrane tyrosine kinase that binds KGF and aFGF with high affinity and binds bFGF much more poorly. The KGFR is nearly identical to the FGF receptor type II, except for alterations in a 49-amino acid residue in one of its extracellular loops. FGFR-II does not bind KGF but shows a high affinity for both aFGF and bFGF. KGFR expression is limited to epithelial cells, whereas FGFR-II is present in a variety of tissues including fibroblasts. KGF, initially isolated from lung fibroblasts, appears to be a myofibroblast-secreted epithelial growth factor with specific roles in epithelial growth and differentiation. The KGFR is expressed on the epithelial cells, and KGF has been shown to induce proliferation and differentiation of a host of epithelial and parenchymal cells, including intestinal epithelial cells, type II pneumocytes, hepatocytes, and keratinocytes of the skin. Its expression and secretion are regulated by IL-1 (37). Its synthesis is significantly upregulated in the lamina propria of inflamed intestine (66). Thus KGF represents a prime example of a mediator causing a specific mesenchymal-epithelial interaction.

HGF, also known as scatter factor because it induces cell migration as well as proliferation, is synthesized and secreted by fibroblasts and myofibroblasts (28, 84). HGF is a glycoprotein heparin-binding heterodimer
related to plasminogen, consisting of a heavy α chain and a light β chain held together by disulfide bonds (84). It is produced as a single-chain precursor protein and proteolytically cleaved to form HGF. The HGF receptor, prominently expressed by epithelial cells, is encoded by the protooncogene c-met. This receptor is a heterodimeric glycoprotein of 190 kDa linked with two disulfide bonds; the α chain is extracellular, while the membrane-spanning β chain has the cytoplasmic domain of a tyrosine kinase. C-met also is regulated by proteases that cleave both chains from a 178-kDa common precursor. Thus HGF is only active if the correct proteases are present. Like TGF-β, HGF has effects on cell division, motility, and apoptosis and appears to have angiogenic activity (28). Its synthesis is stimulated by IL-1 (28). Not only does it cause proliferation of epithelial cells but it also affects parenchymal cells such as liver and bone (28). Thus HGF, like KGF, is a major mediator of epithelial-mesenchymal interactions and epithelial morphogenesis (207).

The process of repair is completed by the terminal differentiation of epithelial and parenchymal cells and by apoptosis of the α-SM actin myofibroblasts (51, 81, 216). The factors that terminate the repair process are poorly understood. The role of IL-10, INF-γ, and INF-α in either the downregulation (90, 197) of myofibroblasts or induction of their apoptosis (95, 128) needs further investigation.

ROLE IN FIBROSIS

With repeated cycles of injury and repair or if, for unclear reasons, there is loss of the signals that discontinue the healing process, organ fibrosis occurs. The important functions of the myofibroblasts in the fibrosis of tissues such as the skin, lung, pancreas, and kidney are well described (see references in Table 4). The effects of PDGF, TGF-β, and other growth factors in the fibrotic process have been studied in detail (72, 93, 167, 214, 239) and are beyond the scope of this review (see Refs. 29 and 96 for detailed reviews of fibrosis).

Factors that act on myofibroblasts are important in tissue fibrosis. Recently, the key role of TGF-β in fibrosis has been accentuated by the finding of fibrosis of multiple organs, including the liver, kidney (both renal interstitium and glomerulus), and adipose tissue in a transgenic mouse overexpressing TGF-β (45). PDGF-BB causes fibrosis in the kidney (239) and, given the propensity of TGF-β to upregulate PDGF receptors, an equally important role for PDGF cannot be ruled out. IGFl has been shown also to induce collagen mRNA and IGF binding protein-5 mRNA in rat intestinal smooth muscle (269), raising the question of an important role for this growth factor in organ fibrogenesis (268). IL-1, IL-6, INF-γ, TNF-α, and bFGF have also been incriminated as fibrogenic cytokines (101, 205). Potential abnormalities in matrix secretion, degradation of matrix by MMPs, and inhibition of MMPs by TIMPs (see above) that might result in fibrosis are under investigation (21, 96, 154). An understanding of these processes and the development of effective pharmacological or biological inhibitors would be important advances in the treatment of disease.

CONCLUSIONS AND SPECULATION

Myofibroblasts are ubiquitous cells with similar properties and functions that play important roles in growth and development, wound repair, and disease. Either their absence or their activation and proliferation in a given tissue or organ can lead to specific diseases as outlined in Table 3. However, because they are present in virtually every tissue, it is possible that they may play a role in multimystem diseases as well. For example, do abnormalities in pericytes account for some of the multifocal effects of chronic hypertension (208)? Are the multiple abnormalities of diabetes mellitus due to stimulation of or damage to vascular, renal, intestinal, and skin myofibroblasts? It is intriguing that high glucose concentrations induce TGF-β1 production by the glomerular mesangial cell (135), and PDGF and bFGF improve wound healing in genetically diabetic animals (87). What is the role of myofibroblasts in aging, a condition in which myofibroblasts are reported to be morphologically abnormal (169)? These are but a few of the intriguing questions raised by this unique family of pleiotropic cells.

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