Epithelial Cells and Their Neighbors
I. Role of intestinal myofibroblasts in development, repair, and cancer

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Powell, D. W., P. A. Adegboyega, J. F. Di Mari, and R. C. Mifflin. Epithelial Cells and Their Neighbors. I. Role of intestinal myofibroblasts in development, repair, and cancer. Am J Physiol Gastrointest Liver Physiol 289: G2–G7, 2005; doi:10.1152/ajpgi.00075.2005.—Intestinal myofibroblasts are α-smooth muscle actin-positive stromal cells that exist as a syncytium with fibroblasts and mural cells in the lamina propria of the gut. Through expression and secretion of cytokines, chemokines, growth factors, prostaglandins, and basal lamina/extracellular matrix molecules, as well as expression of adhesion molecules and receptors for many of the same soluble factors and matrix, myofibroblasts mediate information flow between the epithelium and the mesenchymal elements of the lamina propria. With the use of these factors and receptors, they play a fundamental role in intestinal organogenesis and in the repair of wounding or disease. Intestinal neoplasms enlist and conscript myofibroblast factors and matrix molecules to promote neoplastic growth, carcinoma invasion, and distant metastases.

Intestinal myofibroblasts (IMFs), also called pericryptal fibroblasts, are a syncytium of α-smooth muscle actin (α-SMA)-positive (+) stromal (mesenchymal) cells, which reside subjacent to the basement membrane of the small and large intestines (22, 23). IMFs belong to a family of α-SMA(+) fibroblast-like cells, such as the cells of the peridental ligament and the hepatic stellate (Ito) renal mesangial/tubulointerstitial, the lung interstitial contractile cell, and the pancreatic stellate cells, to name a few (22). In various tissues and organs, these myofibroblasts (MFs) express and secrete an extensive repertoir e of cytokines, growth factors, chemokines, hormones, neurotransmitters, inflammatory mediators, and adhesion proteins, as well as express receptors for many of these ligands (22). This allows these cells to act in a paracrine fashion to mediate information flow in both directions to and from the intestinal epithelium and the immune and the other mesenchymal and neural elements of the lamina propria. IMFs also secrete collagen and various matrix proteins, as well as families of matrix-modifying proteins such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). These latter molecules are important in tissue remodeling and tissue repair (22).

This Themes article focuses mostly on new knowledge about IMFs that has been published since our 1999 reviews concerning the definition and organization of this syncytium, the origin of these stromal cells, and new information about their role in organogenesis, repair, and tumorigenesis. To maximize the readers’ access to references, we have often quoted timely reviews rather than primary studies. In addition to interacting with the gastrointestinal (GI) epithelium and regulating many of its functions, IMFs also have important roles in mucosal immunity and in intestinal fibrosis; areas not covered in this brief review.

DEFINITION AND STRUCTURAL ORGANIZATION

Simply defined, IMFs share phenotypic characteristics of both fibroblasts and smooth muscle cells but do not express smooth muscle markers such as smoothelin, caldesmon, or (in the normal colon and small intestine) desmin. Instead, they express α-SMA, smooth muscle heavy chain myosin, vimentin, and certain fibroblast markers such as prolyl 4-hydroxylase and Thy-1 (CD90) (22, 23). Intestinal fibroblasts can be transdifferentiated into IMFs by treatment with transforming growth factor (TGF)-β (22), but it is uncertain whether they can be made to differentiate into intestinal smooth muscle cells and what factors might govern such transdifferentiation. α-SMA(+) colonic MFs (CMFs) are found just under the epithelium of normal mucosa and are connected to the smooth muscle cells that make up the muscularis mucosae (Fig 1) (1). Furthermore, the IMFs, which were originally believed to be a two-dimensional network that encompasses the lamina propria, much like a hair net (discussed in Ref. 23), are really a three-dimensional syncytium in regions of the lamina propria where the network is more than one cell layer thick. Thus the network ramifies throughout the lamina propria, extending from the subepithelial MFs into the core of the lamina propria, where it interdigititates through gap junctions and tight junctions with typical α-SMA(−) fibroblasts and α-SMA(+) pericytes. These pericytes, also called mural cells, surround the capillaries of the lamina propria (see Fig. 2) (23).

In both intestinal inflammation (27) and neoplasia (adenomatous polyps) (1), there is an increased number of α-SMA(+) MFs in the lamina propria (Fig. 2). The syncytial nature of the MFs also exists in the polyps, although it is not clear that this formal structure remains in areas of intense intestinal inflammation (Fig. 2). This increase in the number of MFs in injury and neoplasia may derive from TGF-β-mediated transdifferentiation of existing fibroblasts to MFs or to the recruitment and expansion of MFs by chemotactic and proliferative agents (e.g., PDGF).

NICHE PROPERTIES AND ORIGIN OF IMFs

Specific properties of gut MFs, which are retained ex vivo after passage in culture, have been demonstrated in IMFs cultured from a specific niche. These MFs retain their unique characteristics through multiple passages. As reviewed in Ref. 23, Kedinger’s laboratory has shown that IMFs isolated from jejenum, ileum, and colon secrete different growth factors [hepatocyte growth factor (HGF), TGF-β1, and epimorphin]
profiles, and these specific profiles are reproducible after multiple passage. Furthermore α-SMA(−) fibroblast-like cells isolated from the gut lamina propria will drive the rat fetal endoderm to form proliferative crypts, whereas primary isolates of α-SMA(+) IMFs will drive the endoderm to form well-developed villi containing differentiated enterocytes, goblet cells, and enteroendocrine cells. Similarly, preliminary studies in our laboratory using microarray technology on CMFs cultured from normal colonic lamina propria, adenomatous polyps, or colonic adenocarcinoma have revealed 395 differentially expressed genes, as defined by one-way ANOVA at a confidence level of 99% (R. Mifflin, et al., unpublished observations). These studies raise the important question as to how do IMFs in different regions or in different pathological conditions in the GI tract develop specific gene-expression profiles that exist through replicated culture passage, i.e., how do these IMFs become genetically “hardwired”?

α-SMA(+) IMFs can be found in the lamina propria of the human colon as early as the 13th week, and certainly, the 21st week of gestation (P. A. Adegboyega, and D. W. Powell, unpublished observations and Ref. 23). From recent studies in several laboratories, we now understand that these IMFs are replaced by bone marrow mesenchymal stem cells (MSCs), and this replacement is increased by wounding, inflammation, and perhaps by signals elaborated from neoplastic epithelia. Thus it seems likely that IMFs become genetically hardwired by entering a specific niche as undifferentiated bone marrow MSCs, and there, in response to specific signals, they differentiate into IMFs with specific characteristics.

Brittan and Wright (6) showed that bone marrow stem cells derived from male mice (muMSC) or humans could be detected via the Y chromosome in the intestinal MFs when transplanted into female recipients. In the mouse studies, the bone marrow-derived MFs were first seen at 7 days, and by 6 wk, they essentially populated the entire subepithelial sheath. These findings were confirmed in additional studies in mice using both gender-mismatched donors and recipients and also using lentivirus-transfected, purified MSCs. It was further demonstrated that these transfused MSCs could also be found not only in the IMFs, but also as donor-derived hepatocytes, lung epithelial cells, renal tubular cells, and MFs in the stomach, esophagus, and adrenal capsule (4, 6).

It is unclear what exactly is the chemotactic signal for these vascularly transfused, or native stem cells, but signals arising from proliferating cells and/or inflammation are certainly possibilities. When muMSC from β-galactosidase transgenic/recombination-activating gene 1-deficient mice were injected into SCID mice simultaneously with human pancreatic cancer cells, α-SMA(+) stromal MFs expressing the β-galactosidase marker were found extensively in the stroma surrounding the tumor implants (16). Tissue wounding increases the number of engrafted IMFs (6), and muMSCs can be shown to repopulate the stromal MFs in a murine TNBS experimental colitis model (6). A recent report demonstrated that muMSCs are recruited to the gastric epithelium under conditions of epithelial injury, repair, and hyperproliferation (a murine model of Helicobacter infection) (14). This latter study raises the important question as to what are, in fact, the factors that direct circulating stem cells into an epithelial versus mesenchymal stromal niche and whether hematopoietic stem cells usually populate a different niche (e.g., the epithelium) than do mesenchymal stem cells (stromal compartment).

DEVELOPMENT, REPAIR, AND CANCER

As pointed out by Weinberg and colleagues (8, 13), cancer is better viewed as a complex tissue in which transformed epithelial cells have enlisted and conscripted cells of the microenvironment (immune cells, endothelial cells, and, most importantly, fibroblasts/MFs) to sustain growth and to foster local spread and distant metastasis. Thus cancer is no longer considered to be simply a nidus of genetically transformed cells that by themselves accomplish devastating growth and spread. The two major functions of IMFs are to drive embryonic development and to orchestrate tissue repair after injury or disease. By coopting these mesenchymal cells of the stromal microenvironment and using both soluble factors and extracellular matrix (ECM) laid down by MFs in proximity to the cancer cells, the neoplasm develops a set of “acquired capabilities”: 1) self-sufficiency in growth signals, 2) insensitivity to antigrowth signals, 3) ability to evade apoptosis, 4) limitless self-renewal potential, and 5) genetic instability.

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replicative potential, 5) sustained angiogenesis, and 6) tissue invasion and metastasis (13). Thus any discussion of the role of IMFs in development and repair also applies to their roles in tumorigenesis and carcinogenesis.

BASEMENT MEMBRANE AND ECM

The matrix molecules secreted by IMFs are composed of a diverse group of complex macromolecules that impart unique characteristics to basement membranes, cell surfaces, and the ECM. Via their ability to bind growth factors, act as coreceptors for growth factors, antagonize or agonize growth factor receptors, alter transcellular adhesion and migration, and reorganize the extracellular environment, matrix molecules (particularly, proteoglycans) can either inhibit or promote cellular activities associated with development, repair, or carcinogenesis.

The basement membrane or basal lamina that separates epithelial cells from IMFs is composed mainly of collagen IV, laminins, and heparan sulfate proteoglycans (nidogen, perlecan). Both cell types contribute to basement membrane synthesis: collagen IV and nidogen are primarily synthesized by IMFs; ECM in which cells of the lamina propria are embedded is primarily synthesized by IMFs and interstitial fibroblasts. The ECM contains collagens, proteoglycans, glycoproteins, and hyaluronate (reviewed in Refs. 22 and 23).

Proteoglycans are proteins that are glycanated with at least one glycosaminoglycan chain of either chondroitin, dermatan, keratan, or heparan sulfate. Proteoglycans play important roles in epithelial-mesenchymal interactions through their ability to sequester growth factors and chemokines, act as coreceptors for various growth factors, act as ligands for growth factor receptors, and modulate cellular processes such as adhesion, proliferation, and migration (15). For example, heparan sulfate proteoglycans play important roles in embryonic development, serving as growth factor coreceptors for the TGF-β family members and other heparin-binding growth factors such VEGF, HGF, IGF-2, Wnts, Shh, FGF-1, and FGF-2 and may also be important for the establishment of gradients of components of Wnt and hedgehog signaling pathways (15). Decorin, a secreted chondroitin sulfate proteoglycan, also binds members of the TGF-β family with high affinity and, depending on the environment, can either inhibit or augment TGF-β bioactivity. Decorin also interacts with the EGF receptor leading to transient activation followed by a rapid down-regulation of its activity. Enforced decorin expression, using recombinant adenovirus, dramatically reduces the growth of colon cancer xenografts and injection of the decorin core protein suppresses breast cancer metastasis in animal models (24). Mesenchymal expression of syndecan family members, under the control of the FoxL1 transcription factor, affects the

![Image](http://ajpgi.physiology.org/)

Fig. 2. A: high-power micrograph of normal colonic mucosa with α-SMA-positive pericryptal (subepithelial) myofibroblasts (white arrows) and pericytes (mural cells, red arrows). The pericytes are located immediately subjacent to α-SMA-negative endothelial cells (black arrow heads). Note that the nonpericytal fibroblasts and other cells in the lamina propria of the normal colonic mucosa are α-SMA negative. B: diffuse expression of α-SMA (stained brown) by pericytal and nonpericyctal cells in the lamina propria of colorectal adenoma, in contrast to what it obtains in normal colonic mucosa (A). Also, note the processes of pericyctal myofibroblasts, nonpericyctal myofibroblasts, and mural cells (red arrows) connect in a syncytium. C: expression of cyclooxygenase-2 (COX-2) in the stromal cells (myofibroblasts, macrophages, and endothelial cells) of adenomatous polyp. Myofibroblasts have been shown to constitute the majority of these COX-2-expressing cells. D: hyperplasia of α-SMA-positive myofibroblasts (short arrows) in the base of a colonic mucosa ulcer. The crypts (long arrows) are sectioned tangentially and so their luminal connections to the ulcer bed (*) are not shown in this micrograph. Original magnifications: A, ×600; B, ×720; C, ×100; D, ×100.
growth and survival of the epithelium by modifying Wnt signaling (21). IMF also secrete enzymes responsible for matrix remodeling. MMPs are enzymes that are part of a 24-or-more-member family of neutral proteinases with various substrate specificities: collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -11), elastases (MMP-12), membrane types (MMP-14, -15, -16, -17, -24, -25), and others (MMP-19, -20, -23, -26, -27, -28) (20). Activation or inhibition of MMP activity can lead to release and activation of seques-
tered growth factors, shedding of surface-bound receptors, generation of anti- or proangiogenic peptides, and promotion of cellular migration. These MMPs and their small molecule inhibitors, TIMPs, play a role in several GI diseases: inflammatory bowel disease, necrotizing enterocolitis, celiac disease, collagenous colitis, diverticulitis, peptic ulcer disease, and colorectal cancer (20).

In summary, the basement membrane and ECM of the GI tract are not the static, solely structural elements they were once considered to be. Instead they represent dynamic sites of cellular regulation that are largely influenced by IMFs.

DEVELOPMENT

Epithelial-mesenchymal interactions are necessary for proper gut morphogenesis. These interactions are not only required for enterocyte differentiation from endoderm, but they also specify regional identity to the differentiating epithelium. The mesenchyme differentiates into the connective tissue components of the GI tract that include IMFs, fibroblasts, and smooth muscle cells. Given their subepithelial location, IMFs are particularly important for the process of epithelial differentiation and gut morphogenesis. A number of mesenchymally expressed genes has been identified that modulate gut morphogenesis. These include mesenchyme-specific transcription factors (FoxL1, Nkx2.3, HOX family), cell- and matrix-associated factors (tenascin, syndecans and other proteoglycans), and secreted factors such as FGFs, HGF, KGF, TGF-β, Bmps, and Wnts. Recent studies have highlighted the importance of Bmp-2 and -4 as secreted morphogens that regulate epithelial differentiation. For example, Bmp-4 expression is regulated by epimorphin in IMFs (10), and Bmp-2 and Bmp-4 are downstream targets of the paracrine signaling of endoderm-derived Hedgehog factors (Shh, Ihh) on patch-expressing mesodermal cells (25). Bmp-2 and Bmp-4 have also been identified as downstream targets of the mesenchymal FoxL1 winged helix transcription factor and the homeobox factor Nkx2.3 (25). FoxL1 also regulates aspects of Wnt signaling to the epithelium by controlling expression levels of extracellular proteoglycans that function as coreceptors for Wnt ligands (21).

Little is known about how IMFs are derived during development. Endoderm-derived Shh signaling, in combination with other yet to be identified factors, regulates the radial differentiation of the mesoderm by induction of lamina propria and submucosa and suppression of smooth muscle and enteric neuron differentiation (29). During development, PDGF signaling is important for mesenchymal recruitment, because stromal depletion occurs in mice deficient for PDGFA or PDGFRα, resulting in abnormal villus architecture (17). Kruppel-like transcription factor 5 (KLF5) is an important upstream mediator of this process. KLF5 expression upregulates PDGF A chain and α-SMA expression (3, 18), and KLF5 knockout mice have a phenotype very similar to that observed in the PDGFA and PDGFRα knockouts mentioned above (discussed in Ref. 3).

Much is known about factors that regulate MF proliferation, migration, and activation in adults. PDGF family members (PDGF AA, AB, BB, CC, and DD) and their receptors (PDGF-Rα, β, αβ) are critical players in IMF motility and prolif-
eration. In the intestine, the most important PDGF agonists appear to be PDGF BB acting through receptors PDGF-Rβ and αβ (22, 23). However, EGF, bFGF, IGF-I, and IGF-II, TGF-β, and CTGF (a downstream mediator of TGF-β action) also play a role (11, 27). IMFs also contract and become motile in response to endothelin and are relaxed by atrial natriuretic peptide (7). TGF-β is the important soluble factor initiating IMF differentiation and activation, although activation of P311 (PTZ17) might represent a TGF-β-independent pathway (19). Activation of CMFs is suppressed by agents that increase intracellular cyclic AMP levels, which also drives a phenotypic change in the cells to become more stellate or dendritic in appearance (22).

REPAIR

MFs play important roles in the healing of intestinal wounds and influence the processes of epithelial restitution and subsequent proliferation (23). Factors contributing to the restitution phase include IMF-derived HGFs, KGFs (FGF-7, FGF-10), prostaglandins, and CXCL12 (stromal-derived factor 1α) (23, 28). HGF and keratinocyte growth factor (KGF) represent factors that act solely in a paracrine fashion. These factors are secreted by mesenchymal cells, MFs in particular, whereas their receptors (c-met for HGF and FGFR2IIIb for KGFs) are expressed in epithelial cells exclusively. KGF is overexpressed in IMF inflamatory bowel disease (9) and in celiac disease (26) and is capable of enhancing small intestinal ulcer healing in rats (12).

TGF-β and intestinal trefoil factors, which are also products of epithelial cells, also facilitate epithelial migration. TGF-β plays an important role in the MF activation observed during wound healing in which IMFs increase αSMA expression (enhancing contractibility) and increase synthesis of MMPs and matrix components necessary for restoration of the intact tissue (20, 22).

CANCER

As much as 60–90% of the mass of colonic neoplasms are made up of stromal cells [fibroblasts, MFs, white blood cells, blood vessels (endothelium + mural cells)] that are all embedded in ECM. It is not surprising, then, that there is a clear role for the stromal microenvironment in tumorigenesis and carcino-
genesis. This phenomenon is variously termed mesenchy-
mal-epithelial interactions, tumor-stromal interactions, tumor microenvironment, or the “landscaper effect.” Space does not permit a detailed description of the various specific growth factors and matrix molecules that are involved in tumor growth, local invasion, or distant metastasis. Suffice it to say that the various cytokines, chemokines, growth factors, ECM, and matrix modifying molecules mentioned in previous sections have been discovered as playing roles in cancer progres-
sion as well. Thus the tumor has usurped the normal developmental and repair mechanisms to survive and enhance tumor growth. A number of excellent review articles has been published recently that cover this topic in more detail (5, 8, 13).

Most of the individual studies reviewed deal with the role of stroma in the progression or metastasis of frank carcinoma. In our laboratory, we have attempted to study the role of IMFs and their products at an earlier stage of this process: the transition from normal colonic tissue to adenomatous polyps. We have found global MF activation in precancerous tubular adenomas. Although TGF-β expression is increased in colonic neoplasms; however, IGF and PDGF support of the transdifferentiation hypothesis, TGF-β is important in cancer progression (30), our studies of polyps that COX-2 (both stromal and epithelial) is undoubtedly important in cancer progression, and then in <50% of the cancers (2). Although it is clear that COX-2 (both stromal and epithelial) is undoubtedly important in cancer progression (30), our studies of polyps change the target of nonsteroidal anti-inflammatory drugs and specific COX-2 antagonists in their role of cancer chemoprevention from the epithelium to the stromal MFs.

We have cultured IMFs from normal, adenomatous, and adenocarcinomatous human colonic tissues and compared their mRNA expression profiles using microarray analyses. Interestingly, clusters of genes were identified that distinguish each class of cells from the others. Because TGF-β is capable of mediating MF activation and COX-2 expression, we hypothesized that it would play a role in modulating the MF phenotype we observed in premalignant colorectal polyps and malignant adenocarcinomas. Although TGF-β certainly plays a role in these changes, other unidentified factors are also involved. Future studies need to identify the other factors critical for the observed phenotypic changes.

Future important areas of research also include identification of the sources of polyp- and cancer-associated MFs, i.e., do these activated populations arise from transdifferentiation of resident interstitial fibroblasts, proliferation of pericryptal MFs, or colonization by circulating mesenchymal stem cells? In support of the transdifferentiation hypothesis, TGF-β synthesis is increased in colonic neoplasms; however, IGF and PDGF levels are also elevated, supporting the proliferation hypothesis. Furthermore, the tumor microenvironment preferentially promotes mesenchymal stem cell engraftment, making the latter hypothesis a viable possibility as well (6). The participation of TGF-β in modulation of the tumor microenvironment also needs to be revisited, because it has been shown recently that abrogation of TGF-β signaling in FSP1-expressing stromal cells can drive epithelial transformation in the forestomach and prostate (5). This latter study also raises again the interesting question of whether genetic defects within stromal cells drive epithelial carcinogenesis in some instances. Epigenetic modifications have been observed within the stroma of preneoplastic colonic lesions, and juvenile polypody syndrome is characterized by the loss of function of BMPR1A or SMAD4 primarily within interstitial fibroblasts (5). Finally, the observations of Campisi and colleagues that senescent fibroblasts can drive the progression of premalignant epithelium to cancer may be the link between aging and the increased incidence of cancer (discussed in Ref. 5).

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