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Review Article

Review Article

Cellular and Molecular Mediators of Intestinal Fibrosis

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Abstract

Intestinal fibrosis is a major complication of the inflammatory bowel diseases (IBD) and although inflammation is necessary for its development, it would appear that it plays a minor role in its progression as anti-inflammatory treatments in IBD do not prevent fibrosis once it has started. The processes that regulate fibrosis would thus appear to be distinct from those regulating inflammation and, therefore, a detailed understanding of these pathways is vital to the development of anti-fibrogenic strategies. There have been several recent reviews exploring what is known, and what remains unknown, about the development of intestinal fibrosis. This review is designed to add to this literature but with a focus on the cellular components that are involved in the development of fibrogenesis and the major molecular mediators that impact on these cells. The aim is to heighten the understanding of the factors involved in intestinal fibrogenesis so that detailed research can be encouraged in order to advance the processes that could lead to effective treatments.

Key Words: inflammatory bowel disease; intestinal fibrosis; myofibroblast; fibroblast; extracellular matrix; growth factor; interleukin; chemokine

1. Introduction

Intestinal fibrosis is a common and serious complication of the inflammatory bowel disease (IBDs). It is a consequence of the chronic, recurrent, or unresolved, intestinal inflammation that leads to ongoing tissue damage without reconstitution of the tissue structure resulting in excessive extracellular matrix (ECM) deposition and loss of normal function.[1] In ulcerative colitis (UC) the inflammation is localized to the submucosa and thus ongoing inflammation can result in colonic shortening and a loss of normal colonic structure leading to the 'lead pipe' appearance of the colon.

In Crohn's disease (CD), however, the inflammation is frequently transmural and while most patients initially present with purely

inflammatory disease, within 10 years of diagnosis more than 70% of patients will develop a stricturing or perforating complication.[2] As a consequence, over a third of CD patients will have intestinal narrowing and obstruction necessitating surgery.[3,4] Surgery, however, does not prevent disease recurrence with up to 70% of patients suffering recurrent endoscopic disease at 1 year, nor does it prevent fibrotic changes and need for recurrent surgery. CD is a life-long, incurable, disabling inflammatory disorder frequently diagnosed between 15 and 35 years that is continuing to increase in frequency worldwide. In developed countries, the prevalence of IBD is 100–220/100,000 population,[5,6] it is associated with high morbidity, reduced employment opportunities and earning capacity as well as school and work absenteeism and increased use of disability services. In Canada, the direct cost for patient medical management was $1.2 billion in 2012 while the indirect societal and patient cost, such as long-term work loss, was an additional $1.6 billion.[7]

Despite great progress in the understanding and management of IBD inflammation, almost no progress has been made in the development of anti-fibrotic therapies in IBD. This lack is due in part to a lack of understanding of the principle, and more specific cellular and molecular, pathways that can lead to fibrosis. It is also notable that despite the advances in inflammation control in IBD, the development of intestinal fibrosis has not significantly altered and can still present many years after control of the inflammation.[8,9] Although inflammation is needed for the initiation of the events that may result in fibrosis, observations suggest that the mechanisms promoting fibrosis are different to those that impact inflammation.[10,11] The need for investigation into the mechanisms that underlie the development of intestinal fibrosis is thus vital to prevent long-term sequelae of disease and the resultant social and financial burdens.

There have been several recent reviews focusing on what is known, and highlighting what still needs to be investigated, into the mechanisms impacting intestinal fibrosis formation. This review aims to add to this literature with a more detailed discussion of the cellular and molecular mediations of fibrogenesis that have not been covered in the recent papers.

2. Cellular mediators of fibrosis

Recurrent, or persistent, epithelial injury is crucial for initiating and sustaining intestinal inflammation and fibrogenesis. Epithelial and endothelial damage release chemotactic factors promoting recruitment and activation of innate and adaptive inflammatory cells, as well as mesenchymal cell precursors of the activated myofibroblasts (Fig. 1).[11,12] Myofibroblasts secrete ECM proteins and promote an altered cytokine milieu that supports the fibrotic process. Under normal conditions the fibrotic matrix is degraded by matrix metalloproteinases (MMPs), myofibroblasts apoptosis, or reverts to a non-activated state, while the epithelium undergoes repair. Thus intestinal fibrosis is characterized by abnormal ECM deposition by activated myofibroblasts[10,13–17] and constitutive activation of collagen secreting myofibroblasts is ultimately responsible for increased tissue stiffness and progressive organ dysfunction.[18] This then is enhanced by the innate and adaptive immune systems, which promote fibrogenesis through the differentiation, recruitment, proliferation and activation of ECM-producing myofibroblast progenitors.[11,12]

2.1. Innate and adaptive immune cells

The innate immune cells, monocytes, neutrophils, mast cells, eosinophils and basophils, produce pro-inflammatory and pro-fibrotic molecules like the interleukins (ILs), tumor necrosis factor-α (TNFα), transforming growth factor (TGF)-β1 and platelet-derived growth factor (PDGF). Innate immune signaling pathways, by provoking cellular activation and fibrosis, are also important drivers of myofibroblast transdifferentiation.

Monocyte-derived cells, including macrophages and dendritic cells, also impact both inflammation and fibrosis. These cells regulate the activated myofibroblast and their progenitors by direct effects on the matrix.[12,19,20] The recruitment of distinct functional subsets of macrophages, and their relative concentrations during injury, can also determine whether the inflammatory response leads to tissue repair or fibrosis. Classically activated M1 pro-inflammatory macrophages are induced by interferon (IFN)-γ, TNFα or bacterial products, which activate MyD88 and NF-kB. M1 macrophages activate myofibroblasts and fibrosis by both cytokine-dependent and independent mechanisms, and reactive oxygen species (ROS), by cause additional tissue injury and promoting myofibroblast resistance to apoptosis.

M2a macrophages are produced following exposure to IL-4 or -13, which signal through the common IL-4 receptor α, and through STAT6 activation. M2a macrophages produce crucial pro-fibrotic factors including TGF-β1, connective tissue growth factor (CTGF), PDGF, fibroblast growth factor (FGF) and insulin-like growth factor (IGF). M2c/reg (regulatory) phenotype macrophages occur following IL-10 exposure, are anti-fibrotic and act, in part, via STAT1 and NF-kB inhibition. Regulatory macrophages (M2c/reg) inactivate myofibroblasts and inhibit M1- and M2a-type macrophages through the local production of IL-10 and/or Arginase-1 (Fig. 2). Macrophages can also change phenotype and function as tissue repair, or fibrosis, progresses, although the precise factors regulating these transitions *in vivo* remain poorly defined.

Neutrophils are quickly recruited after epithelial injury and remove tissue debris and kill invading microbes. If inflammatory neutrophils are not eliminated, they can exacerbate tissue-damage and promote ECM-myofibroblast activation through the release of pro-fibrotic cytokines, chemokines and reactive oxygen and nitrogen species. Mast cells and eosinophils promote fibrosis by recruiting inflammatory leukocytes and by producing pro-fibrotic mediators like TGF-β1 and IL-13. Basophils have a less clear role in the development of fibrosis, although may be a source of type 2 cytokines.

Adaptive cells include T helper cell subsets (Th1, Th2, Th17), regulatory T cells (Tregs) and B cells. The Th17-type immune response is pro-inflammatory and pro-fibrotic. Th2-type immunity, defined by the production of IL-4, -5 and -13, is also potently fibrogenic with IL-13 considered the dominant pro-fibrogenic mediator. By contrast, Th1-type immunity expressing IFN-γ may have anti-fibrotic activity (Fig. 2). The role of Tregs in fibrogenesis is less clear, although could suppress Th17- and Th2-driven fibrosis.[11]

2.2. Myofibroblasts and their mesenchymal cell precursors

Myofibroblasts are derived from a variety of sources, not only resident mesenchymal cells, like fibroblasts (Føs), sub-epithelial myofibroblasts and smooth muscle cells (SMCs), but also from dedifferentiation of epithelial cells by epithelial–mesenchymal transition (EMT),[21] and endothelial cells via epithelial/endothelial–mesenchymal transition, stellate cells, pericytes, and bone marrow stem cells.[10,14–17] Bone marrow-derived circulating fibrocytes in particular, enter tissues following injury and contribute to healing and scarring.[22,23] (Fig. 3)

The relative contribution that each cellular source has on the myofibroblast population in inflamed and fibrotic tissue, however,

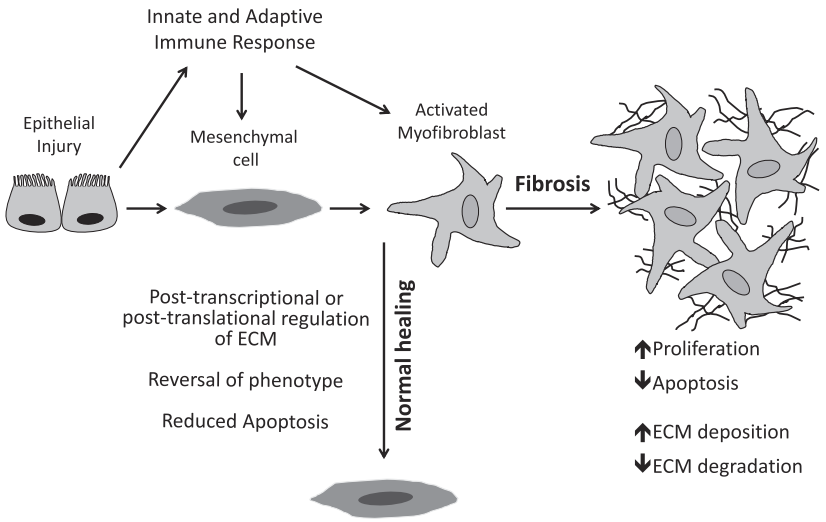

Figure 1. Process of tissue repair and fibrosis. Epithelial and endothelial damage promotes the release of chemotactic factors that promote the recruitment and activation of innate and adaptive inflammatory cells, as well as mesenchymal cell precursors. The activated myofibroblasts then secrete ECM proteins and promote an altered cytokine milieu that further supports the fibrotic process instead of normal healing.

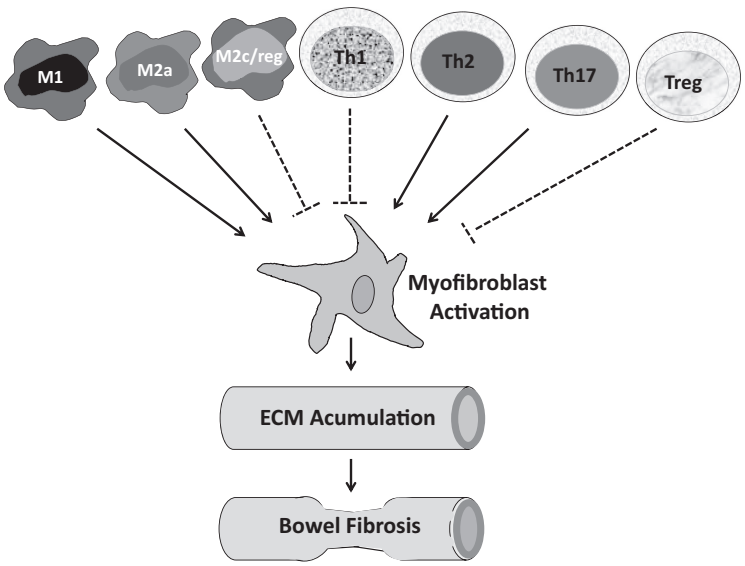

Figure 2. Intersection of immune response and fibrosis. Activated M1 pro-inflammatory macrophages activate myofibroblasts and fibrosis by both cytokine-dependent and independent mechanisms. M2a macrophages signal through STAT6 and produce crucial pro-fibrotic factors. M2c/reg (regulatory) phenotype macrophages are anti-fibrotic and inactivate myofibroblasts as well as inhibiting M1- and M2a-type macrophages. Th2-type T cells are potently fibrogenic as is the Th17-type immune response. By contrast, Th1-type immunity may have anti-fibrotic activity. Treg's role in fibrogenesis is less clear, but may suppress Th17- and Th2-driven fibrosis.

is not known. Also unknown are the major triggers that promote myofibroblast activation, the markers that identify the activated myofibroblast and if once activated the myofibroblast can be 'deactivated,' or if once activated this a 'point of no return' in intestinal fibrosis development.

2.2.1. Fibroblasts (Føs)

Føs (vimentin$^+$, α-smooth muscle actin [α-SMA]$^-$, desmin$^-$),[21] located in the interstitium of normal tissue, are central in maintaining structural integrity, healing and regeneration, while an increase in resident Fø populations is pivotal to fibrosis development.[10,14–17] Føs isolated from IBD mucosa proliferate faster than normal and this increase also occurs after exposure to growth factors, pro-inflammatory cytokines and after direct cell-to-cell contact with inflammatory cells.[13,24,25] Fø migration patterns through the ECM also represent another aspect that contributes to the development of intestinal fibrosis. Fibronectin, which is synthesized in large quantities by Føs, is one of the most potent inducers of autocrine migration. Paracrine processes, driven by PDGF-A and B, IGF-I, epidermal-like growth factor (EGF), can also stimulate Fø migration in a fibronectin-dependent way[26] and it is noted that different fibronectin isoforms are able to impact on the rate of Fø migration.[27]

Føs produce cytokines and chemokines that modify the quality, quantity and duration of inflammation.[28] Føs, however, also contribute to inflammation resolution by withdrawing several survival signals and normalizing chemokine gradients, allowing for the

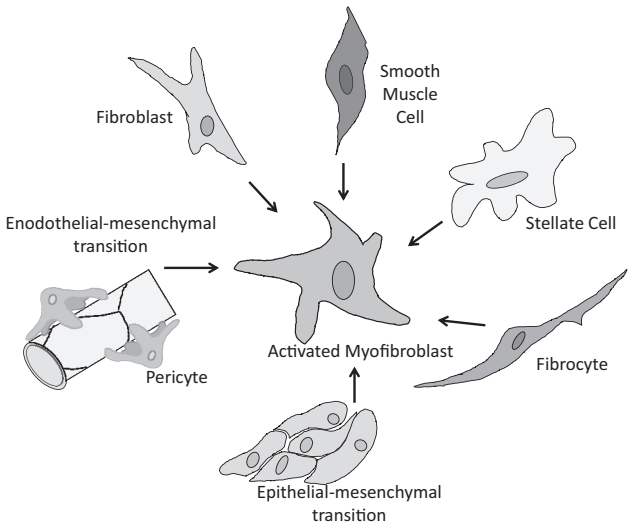

Figure 3. Progenitors of intestinal activated myofibroblasts. The activated mesenchymal cells are central to fibrogenesis. These cells are derived from resident mesenchymal fibroblasts and smooth muscle cells as well as from dedifferentiation of epithelial cells by epithelial–mesenchymal transition (EMT), and endothelial cells via epithelial/endothelial–mesenchymal transition, stellate cells, pericytes, and bone marrow stem cells.

infiltrating leukocytes to apoptose (programmed cell death) or leave the mucosa.[29] Their ability to produce, and respond to growth factors, also allows for paracrine interactions to occur and these maintain the homeostasis of adjacent cell types such as epithelial and endothelial cells. Cytoskeletal proteins, in association with cell surface integrins and the ECM, also facilitate cell motility and the generation of contractile forces is important in tissue homeostasis and wound healing[21]

The differentiation of Føs into ECM-producing myofibroblasts is controlled by the combined actions of IL-1β, TGF-β1 and mechanical tension,[30–33] and this is associated with a distinct change in mRNA expression profiles.[31] Increased tissue stiffness and decreased elasticity, result in mechanical stress that exacerbates tissue injury and promotes local α-SMA expression by Føs. Mechanical stress also induces EMT via TGF-β1 driven mechanisms, Wnt-β-catenin and hyaluronan.[32,33] Intestinal fibroblasts are thus main effector cells of gastrointestinal fibrosis and tissue repair.[34,35]

2.2.2. Myofibroblasts

Myofibroblasts (vimentin$^+$, α-SMA$^+$, desmin$^-$) are highly contractile cells that exhibit a "hybrid" phenotype between Føs and SMCs and, when activated, synthesize high levels of ECM.[10,11,14,16,17,34] Besides roles in tissue growth and differentiation, myofibroblasts are central to wound healing and fibrosis. Two types of myofibroblasts occur within the intestine, the intestinal sub-epithelial myofibroblasts (SEMFs) and the interstitial cells of Cajal (ICC).[10,14,17,36,37] SEMFs are located primarily at the intestinal crypt base in the lamina propria, while the ICC are located in the submucosa and muscularis propria in association with the smooth-muscle layer.[36,37] ICC are pacemaker cells, which regulate smooth muscle motility. Mediators which promote myofibroblast proliferation and ECM production are numerous including PDGF, EGF, IGF-1&2, CTGF, IL-1, IL-13, stem cell factor (SCF), endothelins (ET-1, -2, -3), angiotensin II (ANG II), TGF-α, TGF-β, bFGF and peroxisome proliferator activator receptor-γ (PPAR-γ).[10,11,15,16,34] It is these activated myofibroblasts that are central to fibrosis.

While in other organs the source of ECM-producing myofibroblasts is restricted to a few cell types, in the intestine multiple cell types may become activated ECM-producing myofibroblasts.[10,15,16] These ECM-producing cells are activated by paracrine signals derived from immune and non-immune cells, autocrine factors, and pathogen-associated molecular patterns (PAMPs) derived from microorganisms that interact with pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[10,15,16] Myofibroblasts are also activated by products derived from injured cells, the 'so-called' damage-associated molecular patterns (DAMPs) including DNA, RNA, ATP, HMGB, microvesicles, and fragments of ECM molecules.[16]

2.2.3. Smooth muscle cells (SMCs)

SMCs (vimentin$^-$, α-SMA$^+$, desmin$^+$) are one of the three cell phenotypes into which intestinal mesenchymal cells can differentiate, and in chronic inflammation can trans-differentiate into myofibroblasts.[10,15] A dynamic equilibrium thus exists between the myofibroblast and SMC phenotype and in pathological conditions the myofibroblast can express desmin and α-SMA indicating a myofibroblast phenotype more closely resembling the SMC. The SMC can also acquire the ultrastructural characteristics of the myofibroblast,[38] supporting the concept that SMCs are progenitors of the myofibroblast. It is suggested that the myofibroblast and SMC are cellular isoforms with a common ancestor cell and that the Fø, together with the SMC, are embryologically derived from the same primitive mesenchymal cell. Within the intestine, the SMC is confined primarily to the muscularis mucosa and secretes collagen types I, III and V with a greater proportion of type III being secreted compared to the Fø.[39]

In UC, SMCs lead to considerable thickening of the muscularis mucosae and in CD to bowel wall thickening with potential stricture formation. These cells contribute to fibrogenesis in IBD by inducing collagen and MMP production in response to mediators, like TGF-β and IL-1β. Activated SMCs also release significant amounts of IL-6.[40]

2.2.4. Stellate cells

Stellate cells are mesenchymal cell precursors that contribute to retinoic acid metabolism, which impacts fibrosis and, when activated, may differentiate into myofibroblasts.[10,41,42] Stellate cells are major contributors to fibrosis in the liver and pancreas.[42,43] Only limited information, however, is available on intestinal stellate cells, but in IBD mucosa they differentiate into Føs faster than those from normal mucosa and proliferate faster, and produce collagen earlier and at higher levels.[43]

2.2.5. Pericytes

Pericytes are derived from undifferentiated mesenchymal cells and they surround capillary and small blood vessel endothelial cells.[44] They reside at the interface between the endothelium and interstitium. Pericytes display an intermediate phenotype between vascular SMCs and Føs, and are defined as α-SMA$^+$ and desmin$^+$-vascular SMCs. Pericytes control endothelial cell differentiation, endothelial signalling, angiogenesis and ECM deposition.[10,15,45] They represent a useful reserve of Føs during tissue repair and inflammation-associated fibrosis. Pericytes increase ECM deposition near blood vessels during the initial phase of fibrosis.[46] In a transplant model of trinitrobenzene sulfonic acid (TNBS) colitis, vascular SMCs and pericytes are recruited from the bone marrow,[47] however, due to a lack of good *in vitro* culture systems, only limited information about the role of pericytes in intestinal fibrosis is available.[10,15]

2.2.6. Epithelial and endothelial cell transformation

The main intestinal fibrogenic cells may also be derived from non-mesenchymal cells, including epithelial and endothelial cells via transformation. Epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndoMT) are characterized by dramatic changes in cell phenotype and function.[48,49] Epithelial/endothelial cells assume a spindle-shaped morphology, lose classical cell markers and gain Fø, or myofibroblast, markers. In animal models, and human primary cells cultures, EMT and EndoMT contribute to intestinal fibrogenesis, and TGF-β1 is clearly involved in EMT and EndoMT.[48,49] Various other cytokines and growth factors may foster, or accelerate, this transition, including IGF-1 and 2, EGF, FGF-2, IL-1β and TNFα. ECM molecules like fibronectin and fibrin may promote cellular transition as can disruption of the basement membrane.[50] Interestingly, ROS also induces EMT, while hepatocyte growth factor (HGF) antagonize the transformation *in vitro* and *in vivo*, while bone-morphogenetic protein-7 (BMP-7) not only prevents, but also therapeutically can reversing EMT, an HGF overexpression prevents fibrosis.[51–53]

2.2.7. Bone marrow stem cells

The bone marrow contains hematopoietic and mesenchymal stem cells. Hematopoietic stem cells (HSCs) give rise to three classes of blood cells, whereas mesenchymal stem cells (MSCs) can differentiate into other cell types, including myofibroblasts.[54,55] The peripheral blood fibrocyte is a bone marrow-derived progenitor for mesenchymal cells.[10,15,56] Fibrocytes co-express hematopoietic and mesenchymal markers and produce typical Fø proteins like CD34, CD45, CD14, collagens and α-SMA. In normal conditions, they mature before entering the tissue, where they differentiate into tissue-resident macrophages and dendritic cells.[57] In inflammation, fibrocytes migrate into the inflamed tissue in a CCR2-mediated way where they then differentiate into epithelial, endothelial, neuronal and mesenchymal cells.[58]

Fibrocytes are distinguished from MSCs, as they are CD90 positive and fail to express CD34, CD45, and monocyte markers. In animal models, a causal link between the accumulation of fibrocytes and fibrosis has been demonstrated and this appears to be involved in intestinal repair and fibrosis in IBD.[10,17,56] IL-1, TGF-β and Serum Amyloid P (SAP) modulate fibrocyte function leading to fibrosis. Fibrocytes themselves also produce growth factors, inflammatory cytokines and chemokines that in turn promote resident Fø proliferation and their differentiation into myofibroblasts.[17,56]

3. Cellular proliferation, apoptosis and autophagy

An exquisite equilibrium exists between cell proliferation and apoptosis in order to maintain physiological homeostasis within the intestine. In fibrosis, however, there are greater numbers of ECM-producing cells secondary to an increase in proliferation and a decrease in apoptosis.[10,15,16] As apoptosis mediates the reduction in myofibroblast numbers during fibrosis resolution, the induction of myofibroblasts apoptosis could be profoundly anti-fibrotic.[35]

The main regulators of apoptosis include the caspases, Bcl-2, Bax, p53 and focal adhesion kinase (FAK). Caspases are a family of cysteine-dependent aspartate-directed proteases integral to apoptosis. Caspases are either initiators, or effectors, of apoptosis, depending on where they enter the cell death process. Bcl-2 is the prototype anti-apoptotic protein as it blocks the recruitment, and activation, of pro-apoptotic proteins in the mitochondria, such as Bax. FAK inhibits the activity of p53 with the transcriptional targets p21, while Bax and Mdm-2 work through protein–protein interactions. NOD2 and ATG16L1 (an autophagy gene) are also expressed by myofibroblasts and enhance apoptosis through the induction of caspase activation.[35] In CD, variants of these genes increase the risk of small bowel fibrostenosis.[59] Tissue inhibitors of metalloproteinases (TIMPs) are also important in fibrosis as they inhibit matrix degradation. Individual TIMPs, however, may regulate cell division and apoptosis independent of this activity.[35] TIMP-1 is also overexpressed in CD fibrostenosis where it reduces matrix degradation and suppresses myofibroblast apoptosis.

HGF reduces fibrosis by increasing apoptosis. HGF is a potent inducer of ECM-degrading enzymes, which are overexpressed during myofibroblast apoptosis[353535]. MMPs induce apoptosis in myofibroblasts through the degradation of fibronectin and the anti-fibrotic effect of HGF is due to up-regulation of MMPs and MMP-dependent myofibroblast apoptosis. Proliferation and apoptosis of ECM-producing cells are important steps in intestinal fibrogenesis and possible new targets for therapeutic intervention[35] with some therapies demonstrating potential anti-fibrogenic efficacy through the regulation of mesenchymal cell proliferation and apoptosis.

In addition to myofibroblast proliferation and apoptosis, understanding of the physiological function of autophagy suggests that defective autophagy of the Fø and myofibroblast may also be important in IBD fibrosis[60] as autophagy is important both in ECM secretion and degradation. TGF-β impairs autophagic processes. CD patients with ATG16L1 mutations also have increased risk of small bowel fibrostenosis,[61] while rapamycin, a powerful autophagy inducer, improves patient outcomes with severe fibrotic disease.[62] Learning to manipulate autophagy could thus provide new therapies in the management of IBD fibrosis.

3.1. Summary

- Injury and inflammation lead to changes within critical intestinal cells that can trigger, maintain and perpetuate fibrogenesis by regulating ECM-producing myofibroblast activation.
- ECM-producing myofibroblasts derive from resident mesenchymal, epithelial, endothelial, and stellate cells, pericytes, and bone marrow stem cells.

3.2. Questions that need to be addressed

- What are the cellular triggers leading to intestinal fibrosis?
- What is the main source of ECM-producing myofibroblasts in intestinal fibrosis?
- What are the main mediators of myofibroblast activation?
- What are the specific molecular markers of activated myofibroblasts?
- Is there a 'point of no return' in intestinal fibrosis and stricture formation?

4. Major molecular mediator of fibrosis

A concert of paracrine signals derived from immune and non-immune cells, autocrine factors secreted by myofibroblasts, pro- and anti-fibrotic mediators can modulate myofibroblasts and enhance intestinal wall ECM production and degradation (Fig. 3). The most important of these include TGF-β, activins, CTGF, PDGF, IGF-1&2, EGF, ET-1, -2, -3, and various cytokines and products of oxidative stress (Fig. 4 and Table 1). These, factors, however, are not alone as there are other more novel factors like the renin–angiotensin system, integrins, TLRs and others that can impact fibrogenesis. These more novel factors, however, have been discussed in detail in a recent review and will not be covered in this section.[63]

4.1. Growth factors

4.1.1. TGF-β

TGF-β is the prototypical fibrogenic molecule and in mammals there are three isoforms. TGF-β1 is primarily produced by macrophages and Føs and it up-regulates collagen, fibronectin, tenascin, laminin and entactin production. TGF-β regulates tissue TIMP expression and is the most potent inductor of α-SMA (Fig. 5). It induces the myofibroblast phenotype promoting both EMT and endo-MT. TGF-β1 is increased in IBD mucosa[64] and its overexpression in experimental chronic colitis leads to fibrosis.[65,66]

TGF-β is stored as a disulphide-bonded homodimer, non-covalently bound to a latency-associated protein (LAP), which keeps it inactive. TGF-β binding to its receptors requires dissociation of LAP, a process catalyzed by plasmin, urokinase-type and plasmin activators, tissue-type plasminogen activators, MMPs, cathepsins and integrins.[11,34] TGF-β plays a critical role in intestinal mesenchymal cell activation and ECM production.[10,14–17,65,66] The canonical TGF-β intracellular signal transduction pathway is mediated by Smad proteins as TGF-β receptor activation phosphorylates Smad2 and Smad3 and induces binding with Smad4.[67] The Smad2/3–Smad4 complex translocates into the nucleus where it regulates TGF-β target genes. Smad7 inhibits TGF-β signaling. TGF-β also modulates, in a Smad-independent manner, other signal transduction pathways including ERK/cJUN/p38 MAP kinases. Importantly, the Smad-dependent pro-fibrotic effects of TGF-β include myofibroblast activation, collagen, CTGF and TIMP stimulation, and MMP inhibition.

The TGF-β/Smad pathway is crucial in intestinal fibrosis as in other organs.[10,15,16,65,66] Both TGF-β, and its receptors, are over-expressed particularly in fibrostenotic CD and in animal models of intestinal fibrosis.[64–66,68] Adenovirus-mediated overexpression of TGF-β in the murine colon leads to colonic fibrosis,[69] conversely, the loss of Smad3 confers resistance to TNBS-induced colorectal fibrosis.[70,71] Disruption of the TGF-β/Smad signaling pathway, either by the loss of Smad3, or increase of Smad7, expression, can reduce fibrosis in several organs including the intestine.[71–80] Decreased Smad7, and increased pSmad2/3, expression in intestinal CD strictures also supports the pro-fibrogenic role of the TGF-β/Smad pathway.[81]

TGF-β induces intestinal myofibroblast/Fø secretion of the major parenchymal collagens, COLI and COLIII. The amount and relative proportions of COLI and COLIII secreted, however, varies greatly on the tissues' physiological and pathological state. During the early regeneration phase following tissue injury, the COLI/III ratio is low and as healing progresses towards maturation the COLI/III ratio becomes high and then progresses to equilibrium upon reconstitution of the tissue.[82,83] A persistent shift towards a high COLI/III ratio is usually associated with dysregulation of the regenerative phase that frequently results in fibrosis.[1]

The MMPs are central to ECM as well as collagen digestion. MMP activity is tightly controlled by the TIMPs,[84] thus the balance between MMPs and TIMPs is crucial for normal tissue reconstitution, or progression to fibrosis. TGF-β1 and 2 stimulate collagen synthesis and up-regulate the TIMPs thus reducing collagen digestion.[85] By contrast, exogenous TGF-β3 reduces collagen deposition.[86] Higher TGF-β1 levels occur in inflamed/fibrosed IBD tissue and in combination with enhanced TGF-β2, and reduced TGF-β3 expression by intestinal myofibroblasts, fibrosis may occur.[1,86,87] Control of the delicate balance between deposition and degradation is thus central for tissue reconstitution, and TGF-β1:β3 ratio regulation could potentially impact on intestinal scarring.

Blockade of TGF-β signaling is also a potential strategy for the prevention of fibrosis[10,88] as the TGF-β/Smad pathway drives fibrosis. TGF-β, however, is also involved in several vital cellular functions so fully blocking its function, and that of Smad2 and Smad4, is lethal in animal models.[89–91] Selective blockage of individual intracellular mediators of the TGF-β/Smad pathway such as disrupting Smad3, however, could be of benefit as this results in mice that survive to adulthood[92–94] and are resistant to fibrogenesis.[70,71,77,78,80] In addition, the natural inhibitors of the TGF-β/Smad pathway HGF, BMP-7 and decorin are anti-fibrogenic supporting a therapeutic role in disease.[95–99]

4.1.2. Activins

Activins are members of the TGF-β superfamily; they activate Smad transcription factors and the MAP kinase signalling pathways. Important functions for the activins, particularly activin A, occur in tissue inflammation, repair and fibrosis.[100,101] Activin levels are increased in IBD, and many other inflammatory diseases, suggesting that they play a significant role in inflammation/fibrosis.[100]

4.1.3. CTGF

CTGF is a downstream mediator of TGF-β where it stimulates cell proliferation and ECM synthesis. CTGF is co-expressed with TGF-β

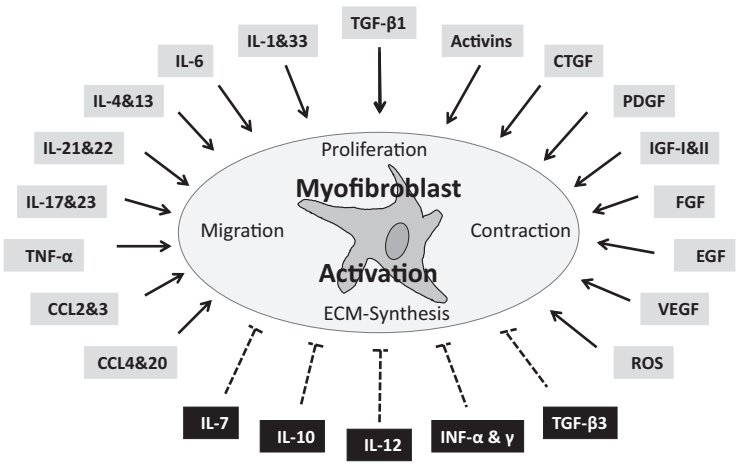

Figure 4. Promoting effects of multiple growth factors and cytokines on myofibroblasts. Numerous factors impact on fibrosis through their effect on the activated myofibroblast. The effect of numerous of these are demonstrated here with either their pro or anti-fibrogenic effect.

in every fibrotic disorder and is considered a key determinant of fibrosis (Fig. 5).[102] CTGF expression is controlled by TGF-β in a Smad-dependent manner. In addition to TGF-β, other regulators of CTGF expression include VEGF, TNFα and ROS.[103] CTGF is, therefore, an interesting molecule for future anti-fibrotic therapies as its inhibition might block the pro-fibrotic TGF-β effects, without affecting TGF-β's immunosuppressive and anti-inflammatory roles.[102] CTGF is also more specific than the growth factors for fibrosis inhibition and various proposed treatments targeting CTGF have demonstrated favorable anti-fibrotic effects.[104,105]

4.1.4. PDGF

PDGF is increased in inflamed IBD mucosa, especially CD and collagenous colitis.[106,107] Intestinal Føs, SEMFs and ICC are activated, and proliferate, in response to PDGF. PDGF also enhances fibronectin-dependent Fø and myofibroblast migration.[108] PDGF induces α-SMA expression and increased PDGF activity promotes ECM deposition. Recent preclinical studies suggest that selective tyrosine kinase inhibitors that target c-Abl, PDGF receptor or Src kinases are anti-fibrotic[109] as dual inhibition of c-Abl and the PDGF receptor by imatinib and nilotinib, and inhibition of Src kinases, either selectively by SU6656 or in combination with c-Abl and PDGF by dasatinib, demonstrate potent anti-fibrotic effects.

Table 1. Summary of the pro and anti-fibrogenic effects of the major molecular mediators of fibrosis.

	Pro or anti-fibrogenic	Actions
IL-1	Pro	Induces myofibroblast activation Induces chemokine secretion Induces MMP secretion Enhances EMT
IL-4	Pro	Myofibroblast differentiation Induces collagen production
IL-5	Pro	Induces IL-13 production Induces TGF-β production
IL-6	Pro	Induces myofibroblast activation Induces fibroblast proliferation Induces TGF-β expression With IL-21 promotes TH17 cell development
IL-7	Anti	Increases Smas7 Inhibits TGF-β production
IL-10	Anti	Inhibits collagen deposition
IL-12	Anti	Stimulates IFN-γ production
IL-13	Pro	Myofibroblast differentiation Induces collagen production Induces TGF-β expression
IL-17	Pro	Pro inflammatory Involved in intestinal homeostasis Indices chemokine production Induces collagen production Induces EMT
IL-21	Pro	Promotes macrophage migration Promotes macrophage survival Simulates IL-4 and IL-13 receptor expression With IL-6 promotes TH17 cell development
IL-22	Anti	Blocks collagen deposition
IL-23	Undetermined	Pro inflammatory Involved in intestinal homeostasis Induces TNFα
IL-33	Pro	A member of the IL-1 family Induces angiogenesis and fibrosis
TNFα	Pro	Induces myofibroblast proliferation Induces collagen production Inhibits MMP activity
IFN-α	Anti	Inhibits TGF-β activity Inhibits fibroblast proliferation Inhibits collagen production

4.1.5. IGF

IGF-I and II and their respective receptors are expressed in the intestine and interact principally with Føs, epithelial and endothelial cells and regulate collagen deposition.[110] IGF-1 enhances myofibroblast migration and increases intestinal SMC and myofibroblasts.[108,111,112] It is up-regulated in CD and animal models of intestinal fibrosis.[1,111,112]

4.1.6. FGF

The FGFs are heparin-binding proteins and interactions with cell-surface-associated heparan sulfate proteoglycans are essential for FGF signal transduction.[113] In vertebrates, FGFs have intracrine (FGF11/12/13/14 subfamily), paracrine (FGF/1/2/5, FGF3/4/6, FGF7/10/22, FGF8/17/18 and FGF9/16/20 subfamilies) and endocrine (FGF15/19/21/23 subfamily) functions.[113] Paracrine and endocrine FGFs act via cell-surface FGF receptors (FGFR1-4), while intracrine FGFs act independent of FGFRs. FGF1 and FGF2, also named acidic FGF (aFGF) and basic FGF (bFGF) are released from damaged cells or by an exocytotic mechanism.

bFGF is a potent mitogen for SMCs, myofibroblasts and Føs (Fig. 5).[10] It stimulates collagen production and CTGF cooperates with bFGF.[113] Serum bFGF levels are raised in CD intestinal strictures while serum bFGF levels correlate with bowel wall thickness suggesting a role for bFGF in transmural CD fibrogenesis.[114] Conversely, bFGF accelerates acute and chronic wound healing, suggesting a potential anti-fibrogenic role.[115] Different FGF subtypes also have differing functions as FGF2 and FGF23 promote cardiac hypertrophy and fibrosis, while FGF16 and 21 prevent these by competing with FGF2 for the binding site of FGFR1c.[116] FGF23, however, is involved in the development of renal fibrosis.[117]

4.1.7. EGF

EGF is the prototype member of a family comprising different peptides with a similar primary structure that bind to a family of EGF receptors.[118] EGF induces intracellular protein phosphorylation regulating transcription, translation, cell architecture, cell proliferation, and production of inflammatory mediators. EGF can be isolated from the intestine and its receptors are located on monocytes and myofibroblasts.

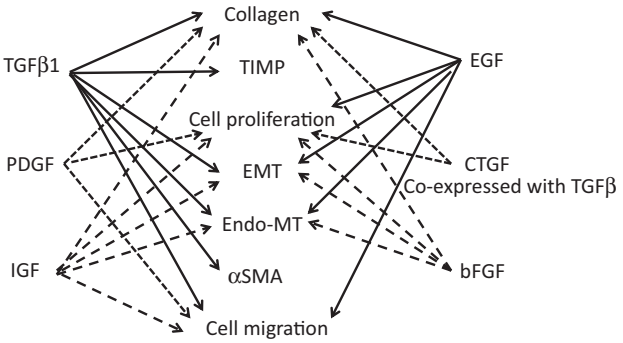

Figure 5. Effects of the key pro-fibrogenic growth factors. The pro-fibrogenic effects that several key growth factors have on collagen, TIMP levels, cell proliferation, EMT, etc., are demonstrated.

EGF is involved in lung fibrosis as it stimulates Fø proliferation and ECM production in idiopathic pulmonary fibrosis.[119] The role of EGF in IBD, however, is unclear, since its receptor (EGFR) is up-regulated in TNBS-induced colitis, while EGF administration reduces TNBS-induced colitis severity.[120] EGF also regulates human colonic Fø and myofibroblast migration.[121,122] Further studies, however, are needed to fully elucidate the role of EGF and EGFR in IBD fibrosis.

4.1.8 Cytokines

4.1.8.1. Interleukins (IL)

IL-1 contributes to fibrosis during chronic intestinal inflammation through myofibroblast activation, and chemokine and MMP secretion.[123] IL-1, in combination with TNF and IFN-γ also increases TGF-β-induced EMT.[124] IL-5, however, acts as an amplifier rather than direct mediator of fibrosis and thus it may facilitate pro-fibrotic cytokine IL-13 and/or TGF-β production.[11,34]

IL-6 is increased in CD where it stimulates fibrogenetic mesenchymal cells, TGF-β and TGF-βR2 expression and Fø proliferation,[125–127] and IL-6 neutralization decreases fibrosis. By contrast, IL-7 inhibits fibrosis by modulating the Smad and EMT pathway and inhibiting both TGF-β production and signaling by Føs.[128–130] This, however, requires an intact JAK1/STAT1 signal transduction pathway. IL-7 increases Smad7, which suppresses TGF-β signalling. In the presence of IL-7, Smad7 dominant negative Føs restore TGF-β-induced collagen synthesis showing that reduced TGF-β signaling occurs with increased Smad7.[129]

IL-4 and IL-13 are immunoregulatory cytokines secreted by activated Th2 cells. IL-13 signals through IL-4Rα/IL-13Rα1 and IL-13Rα2 and is the dominant pro-fibrotic mediator in numerous models of fibrosis.[65,66,131–133] IL-13 acts on Føs, but also indirectly induces TGF-β1 release from monocytes and macrophages.[134] Furthermore, IL-13 induces several CC-chemokines.[135] IL-4 and IL-13 are overexpressed in fibrosis and induce differentiation of Føs to myofibroblasts and stimulate collagen production.[11,34] IL-13 signaling through IL-13Rα induces TGF-β production, while IL-13 inhibition reduces TGF-β and fibrosis.[65,66] Soluble IL-13Rα2-Fc is a highly effective decoy receptor of IL-13 that can reduce the progression of established fibrosis.

IL-10 inhibits fibrosis in numerous experimental models.[11,34] The IL-13 decoy receptor and IL-10 suppress collagen deposition and act as endogenous factors that slow fibrosis progression.

IL-21 and IL-22 are related to IBD-associated intestinal fibrosis.[10] IL-21 promotes fibrosis by enhancing the development, survival and migration of Th2 cells. Moreover, it stimulates IL-4 and IL-13 receptor expression in macrophages, induces their activation, stimulates Føs ECM-degrading enzyme secretion and the secretion of T-cell chemoattractants by epithelial cells.[136,137] IL-21 is produced in excess in CD. IL-21, together with IL-6, is critical for the development of Th17 cells. By contrast, blockade of IL-22 can enhance collagen deposition in the lung suggesting a protective role for IL-22 in lung fibrosis.[138]

The IL-23/IL-17 axis plays an important role in intestinal homeostasis, nevertheless the role of these cytokines in fibrogenesis remains to be elucidated.[10] In normal intestine, constitutive production of IL-23 and IL-17 protect the epithelial layer, fortify epithelial cell tight junctions and inhibit bacterial colonization. In inflammation, activated dendritic cells produce large amounts of IL-23, which activate innate immune cells to produce pro-inflammatory and pro-fibrotic cytokines. IL-23 also induces the production of IL-17 and TNFα. Targeting IL-23 by employing a p40 peptide-based vaccine improves TNBS-induced acute, and chronic, murine colitis with a significant decrease in collagen deposition.[139]

The main action of IL-17 is to promote chemokine production that recruits and activates granulocytes.[140] IL-17 is also a potent activator of mesenchymal cells.[141] IL-17A increases the synthesis, and secretion, of collagen and induces EMT in a TGF-β1-dependent manner.[142] IL-1β-mediated pulmonary fibrosis is IL-17A-dependent and blocking IL-17A attenuates myocarditis-induced cardiac fibrosis confirming a role of IL-17A in fibrogenesis.[143,144]

IL-33, a novel member of the IL-1 family, induces mucosal pathology *in vivo* and may lead to the development of fibrosis and angiogenesis.[145] TLR-3 is one of the strongest promoters inducing IL-33, which activates myofibroblasts and pericytes.

4.1.8.2. TNFα

TNFα is abundantly expressed in IBD and is central to fibrosis by inducing myofibroblast proliferation and collagen accumulation.[146] TNFR2 is essential for these processes.[147] Furthermore, TNFα induces TIMP-1 expression, reduces MMP-2 activity and collagen degradation while, in combination with IGF-1, synergistically stimulates intestinal myofibroblast proliferation and collagen production.[112]

TNF-like cytokine 1A (TL1A)/TNF superfamily member 15 (TNFSF15) is pro-inflammatory and the TNFα superfamily member that is linked to IBD pathogenesis. TL1A is induced in innate immune cells and signals via death domain receptor 3 (DR3) that modulates the adaptive immune response. Constitutive TL1A expression in mice leads to spontaneous ileitis with increased collagen deposition, elevated levels of small intestinal IL-13 and goblet cell hyperplasia while its neutralization ameliorates inflammation.[148–150] Constitutive TL1A expression in T-cells and myeloid cells also leads to severe fibrostenotic intestinal disease with more Føs and collagen deposition.[150] TL1A is elevated in IBD mucosa and *TNFSF15* gene variants are associated with IBD.[151]

4.1.8.3. IFN-γ

IFN-γ and IL-12 suppress fibrosis.[10,11,34] IFN-γ antagonizes TGF-β-induced phosphorylation of Smad3.[152] IFN-γ acts through a Janus kinase (Jak1)-dependent pathway, the transcription factor STAT1 and by the induction Smad7 expression. IFN-γ also directly inhibits Fø proliferation, TGF-β1-induced expression of genes encoding procollagens I and III, and collagen synthesis in activated myofibroblasts.[153]

IFN-γ affects the cellular distribution of fibronectin and the cytoskeleton that interferes with Fø migration.[154] By virtue of its ability to stimulate IFN-γ production in Th1 and natural killer cells, IL-12 also has anti-fibrotic activity.[11,34,155] Despite evidence for an anti-fibrotic role for IFN-γ, however, clinical studies investigating its therapeutic potential have been disappointing.[156]

4.1.8.4. Chemokines

Chemokines are leukocyte chemoattractants that cooperate with profibrotic cytokines in fibrogenesis by recruiting myofibroblasts, macrophages and other key effector cells to sites of tissue injury.[10,11,34] Although a large number of chemokine signaling pathways are involved in fibrogenesis, the CC- and CXC-chemokine receptor families have important regulatory roles. Specifically, CCL2 (monocyte chemoattractant protein-1 [MCP1]), CCL3 (macrophage inflammatory protein-1 [MIP1]), CCL4 (MIP-1β), and CCL20 (MIP-3α) are pro-fibrotic mediators and are elevated in IBD.[10,11,34] Interrupting specific chemokine signalling pathways could thus impact on fibrosis. Blockade of CC- and CXC chemokine receptors decreases fibrosis in association with decreased IL-4 and IL-13. A direct link between CC-chemokine activity and IL-13 has also been suggested.[34]

4.1.8.5. ROS

ROS are involved in acute and chronic inflammatory processes, and are key mediators in collagen gene regulation.[157] Antioxidants can protect against experimental pulmonary and hepatic fibrosis[158,159] and ROS is involved in intestinal fibrosis with their inhibition improving murine colitis.[160]

4.1.8.6 Microvascular changes

Microvascular changes are common in fibrosis.[11,34] Control of angiogenesis and lymphangiongenesis, might represent an alternative approach to fibrosis treatment, particularly due to the connection between vascular remodeling and fibrogenesis in chronic intestinal inflammation.[161–164] Evidence suggests that the microvasculature plays an integral role in IBD pathophysiology and it contributes to chronic inflammation through altered leukocyte recruitment, impaired perfusion, and angiogenesis leading to tissue remodeling.[162–164] VEGF expression is increased in IBD and its blockage can reduce fibrosis in animal models.[165] Because various members of the CXC-chemokine family exhibit potent angiogenic, or angiostatic activity, targeting the CXC-chemokines might control angiogenesis and lymphangiongenesis and act as a novel therapy in IBD to prevent, or reverse, fibrosis.[11,34]

4.2. Key points

- ECM-producing cells act synergistically and are under the control of numerous biological mediators.
- Blockage of selective signalling pathways can prevent, or reverse, intestinal fibrosis.

4.3. Questions to be addressed in the future

- Which factors determine the switch from inflammatory to fibrosing disease?
- What molecular mechanisms lead to the auto-propagation of intestinal fibrosis?
- What are the main mediators of myofibroblast activation?
- Can timing, concentration and the source of the main pro-fibrotic mediators affect their contribution to tissue remodelling and fibrosis?
- Is the simultaneous action of pro-fibrotic mediators relevant to fibrogenesis?
- Which factors represent the driving force ("core pathway") of intestinal fibrosis?
- Which factors with anti-fibrotic properties play a critical role in intestinal fibrosis?
- What is the role of the *"new generation"* of fibrogenic molecules in intestinal fibrosis?

5. Conclusion

Intestinal fibrosis is common and results in high morbidity and surgical rates in IBD patients. It occurs as a consequence of chronic intestinal inflammation, but this is not the whole story as better control of the inflammation has not reduced the rate of fibrotic obstructions, and fibrosis can present many years after control of the inflammation. As multiple intestinal cell types may become activated ECM-producing myofibroblasts, and all contribute to ECM production, it is vital to understand the interactions and ways that these cells can be modified. Understanding the impact that specific growth factors, cytokines and chemokines have on the ECM-producing cells is also fundamental in unraveling the fibrotic processes with the aim of developing effective therapeutic strategies.

Conflict of interest

There are no conflicts of interest for any author.

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