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Novel Mechanisms and Clinical Trial Endpoints in Intestinal Fibrosis

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Summary

The incidence of inflammatory bowel diseases (IBD) worldwide has resulted in a global public health challenge. Intestinal fibrosis leading to stricture formation and bowel obstruction is a frequent complication in Crohn's disease (CD), and the lack of anti-fibrotic therapies makes elucidation of fibrosis mechanisms a priority. Progress has shown that mesenchymal cells, cytokines, microbial products and mesenteric adipocytes are jointly implicated in the pathogenesis of intestinal fibrosis. This recent information puts prevention or reversal of intestinal strictures within reach through innovative therapies validated by reliable clinical trial endpoints. Here, we review the role of immune and non-immune components of the pathogenesis of intestinal fibrosis, including new cell clusters, cytokine networks, host-microbiome interactions, creeping fat, and their translation for endpoint development in anti-fibrotic clinical trials.

Keywords

Intestinal fibrosis; inflammatory bowel disease; myofibroblasts; single cell RNA (scRNA) sequencing; cytokine; creeping fat

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Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a relapsing inflammatory condition of the gastrointestinal tract. It has become a healthcare burden with the highest prevalence in Europe and North America, and accelerating incidence in Asia, Africa and South America ¹. While UC is restricted to the colon with continuous superficial inflammation of the mucosa layer, CD can occur in both small and large intestine with patchy, transmural inflammation ². Intestinal fibrosis which results in obstruction and need for surgical interventions is a serious and common complication of CD ³. Deposition of extracellular matrix (ECM) is part of physiologic tissue repair and restitution after injury. However, in states of chronic inflammation such as CD, that are associated with persistent inflammation lasting for months or years, progressive fibrosis with excessive accumulation of ECM, hardening and scarring of intestinal tissues ensues, leading to impaired function and organ damage ⁴. Fibroblasts and myofibroblasts are the primary ECM producers, but emerging evidence points towards additional mechanisms of gut obstruction. In fact, thickening of the intestinal muscularis propria appears to be the major contributor to luminal narrowing and development of clinical symptoms ⁵. In their lifetime more than half of CD patients develop clinically apparent strictures, which are characterized on cross sectional imaging or histopathology by the presence of intestinal luminal narrowing, induced by bowel wall thickening ⁶. However, in these population-based studies, diagnosis of strictures is mostly based on symptoms, and likely underestimates the real incidence of fibrosis.

Despite awareness of its clinical importance in CD, fibrogenesis and stricture formation, have been largely overlooked in UC. Colonic fibrosis in UC has significant clinical implications, including motility abnormalities, anorectal dysfunction, rectal urgency, and incontinence ⁷. Stricture formation is an infrequent event in UC [1–10%] as compared with small bowel CD, but interestingly, rates of colonic strictures are comparable in CD and UC. UC is generally acknowledged to be an inflammatory process confined to the mucosal/submucosal layers. However, in a study with 706 tissue cross-sections derived along the length of 89 consecutive UC colectomy specimens and compared to CD, diverticular disease and normal areas from colorectal cancer specimens, submucosal fibrosis was detected in 100% of UC specimens ⁸. Submucosal fibrosis and thickening of the muscularis mucosae were associated with severe and chronic injury, but not active inflammation, suggesting fibrosis and muscularis mucosae thickening are common complications of chronic progressive UC ⁸. Another recent investigation showed persistent abnormalities even in endoscopically healed UC mucosa, including ECM remodeling, profibrotic cytokines production, and activated TGF- β signaling pathways, again suggesting that while inflammation is necessary to initiate fibrogenesis, suppression of inflammation is not sufficient to prevent intestinal fibrosis ⁹. The fact that most experimental models of intestinal fibrosis have a colonic location ¹⁰ and most preclinical target validation occurs in these models, supports the notion that intestinal fibrosis occurs in areas of inflammation irrespective of disease location.

In this review, we highlight cutting-edge advances in the field of intestinal fibrosis, including single cell RNA (scRNA) sequencing, novel cytokines, and aspects unique to the intestine that have therapeutic potential, namely the microbiota and the interaction of intestinal muscle with mesenteric fat. We also highlight a pathway for translation of these findings into clinical practice.

Overview of IBD pathogenesis

Although the origin of IBD remains incompletely understood, several studies have demonstrated that complex interactions between genetic predisposition and environmental factors have a crucial role in the development of this chronic disease¹¹. Genome-wide association studies have identified more than 200 loci associated with the risk of CD or UC, including *NOD2*, *ATG16L1*, *CARD9* and *IL23R*¹²⁻¹⁴. It is worth noticing that many of these IBD risk loci are found in regions encoding molecules that regulate cytokine signaling, immune response to microbes and autophagy signaling¹⁵⁻¹⁷, indicating the essential role of cytokine and host-microbe interactions in IBD pathogenesis. Environmental risk factors (collectively grouped under the rubric “exposome”), including smoking, diet, food additives, the use of antibiotics, air and water pollution are likely also involved in the onset or progression of IBD¹⁸. These exposome factors interact and likely alter the composition of the gut microbiome, which plays a major role in human health and is a key factor in IBD pathogenesis¹⁹. The interplay between genetic and environmental factors leads to impaired epithelial barrier function, resulting in the translocation of microbial products into the bowel wall, where they first are recognized by innate immune cells such as dendritic cells and macrophages²⁰. Once activated these cells produce cytokines and chemokines, resulting in recruitment of additional immune cells and initiation of adaptive immune responses^{21,22}. The excessive and uncontrolled innate and adaptive responses trigger chronic intestinal inflammation, further impairment of barrier function, gut tissue damage, persistence of IBD, and fibrogenic events²³.

Mechanisms and cellular components of intestinal fibrogenesis

Fibrosis develops to preserve tissue architecture and functional integrity, and is an essential component of wound healing and tissue repair that usually follows tissue injury and associated inflammation^{24,25}. However, prolonged exposure to injurious stimuli may lead to progressive fibrosis characterized by excess deposition of ECM, scarring of various tissues, impaired function and organ damage^{26,27}. A majority of IBD patients, particularly those with CD, will develop intestinal fibrosis, a serious and common complication that ultimately results in obstruction and surgical interventions²⁸. Intestinal mesenchymal cells, including fibroblasts, myofibroblasts and smooth muscle cells are the main cell types responsible for ECM secretion, and the combined increase of mesenchymal cell numbers and excessive ECM secretion are considered the hallmark features of intestinal strictures⁴. Other than secretion, ECM accumulation is also regulated by matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). MMPs are proteolytic enzymes that break down ECM, while TIMPs counteract their degrading activity. An imbalance between MMPs and TIMPs may lead to ECM accumulation and subsequently fibrosis²⁹. Here we will discuss the main cell types and cell differentiation mechanisms involved in ECM secretion and fibrosis in the intestine (Figure 1).

Mesenchymal cells.—Mesenchymal cells are non-epithelial, non-endothelial, non-haematopoietic cells that differentiate from mesenchymal stem cells and act as a scaffold for tissue structure although they also exert other functions³⁰. In the intestine, mesenchymal cells are found in all anatomical compartments and most of them participate in the process of intestinal fibrosis, including intestinal subepithelial myofibroblasts, lamina propria fibroblasts, pericytes, bone marrow-derived mesenchymal stromal or stem cells, and smooth muscle cells (SMC), as well as the interstitial cells of Cajal and fibrocytes, the latter usually considered non-conventional mesenchymal cells³¹.

Fibroblasts, smooth muscle cells and myofibroblasts.—According to the current marker-based classification system, intestinal fibroblasts express the surface marker CD90 and the intermediate filament protein vimentin, but not desmin, whereas smooth muscle cells are CD90 (–), vimentin (low), α -SMA (+), and desmin (+)³². Myofibroblasts are CD90 (+), vimentin (+), α -SMA (+), and desmin (– or low), granting them an intermediate phenotype between fibroblasts and smooth muscle cells³¹. In the inflamed gut, resident mesenchymal cells actively differentiate and dedifferentiate between these three interrelated mesenchymal cell phenotypes³³. In case of acute injury or inflammation, fibroblasts are activated, migrate to and proliferate at the site of injury and contribute to ECM production. Upon stimulation with specific growth factors such as TGF- β 1, they start expressing α -SMA and differentiate into myofibroblast-like cells^{34,35}. Similarly, smooth muscle cells can also transdifferentiate into myofibroblasts³⁶. However, upon stimulation by pro-inflammatory cytokines, myofibroblasts can differentiate back into smooth muscle cells, induce SMC hyperplasia/hypertrophy, leading to thickened muscularis propria, stricture formation and intestinal obstruction³⁷. Accumulating evidence suggests that myofibroblasts are the primary producers of ECM³⁸. Myofibroblasts can be divided into two main types, interstitial cells of Cajal and intestinal subepithelial myofibroblasts. Interstitial cells of Cajal reside in the submucosa and the muscularis propria and provide signals that facilitate gastrointestinal motility, whereas intestinal subepithelial myofibroblasts are located within the lamina propria directly under only one layer of epithelial cells³⁸. Subepithelial myofibroblasts are dense in the crypt region and sparse at the colonic surface and the villi of the small bowel³⁸. In IBD chronic inflammation fosters production of inflammatory and profibrotic mediators that sustain the activation and proliferation of myofibroblasts, leading to ECM overproduction and fibrosis³⁹.

Fibrocytes.—Fibrocytes are bone marrow-derived mesenchymal stem cells⁴⁰. Under certain circumstances, fibrocytes circulating in the peripheral blood travel to affected tissues where they differentiate into fibroblasts/myofibroblasts upon stimulation by inflammatory and pro-fibrotic growth factors⁴¹. Fibrocytes may enhance inflammation by producing TNF- α and can directly augment fibrosis by producing ColI⁴². In CD patients, levels of circulating fibrocytes are increased compared to healthy controls^{42,43}, but circulating fibrocytes can also be elevated in UC patients⁴⁴. To date there is no evaluation of fibrocytes function in intestinal fibrosis.

Pericytes.—Another type of ECM-producing cells are pericytes, which are found surrounding blood vessels, adjacent to endothelial cells³⁸, and in histological sections they

display the shape of myofibroblasts⁴⁵. Pericytes are defined as α -SMA(-) and desmin-positive cells, exhibiting an intermediate phenotype between vascular smooth muscle cells and fibroblasts³¹. Under acute or chronic inflammatory conditions, pericytes differentiate into fibroblasts by losing their original markers, expressing typical fibroblast markers and, by producing large quantities of ECM components⁴⁶, may contribute to intestinal fibrosis. Increased numbers of activated pericytes are found in the inflamed mucosa of patients with long lasting UC⁴⁷. However, the exact role of pericytes in intestinal fibrosis remains unclear.

Epithelial-Mesenchymal Transition (EMT).—EMT represents a cellular trans-differentiation event where typical epithelial cell markers like adherens and tight junctions (E-cadherin, ZOs and claudins) and cytoskeletal proteins (cytokeratins) are lost in epithelial cells, which then acquire mesenchymal neural cadherin (N-cadherin) expression together with the upregulation of α -SMA, vimentin, fibronectin, collagens and the transcription factors Twist, Snail, Slug, Zeb1/2. Along with these phenotypic changes, these events help transitioning stationary epithelial cells to migratory and invasive cells^{48,49}. EMT was first described in organs such as the kidney, liver and lung, and contribute to the fibrogenic process by generating epithelial-derived fibroblasts that become responsible for ECM deposition and tissue scarring⁵⁰. Later, a role for EMT in the pathogenesis of IBD-associated intestinal fibrosis also emerged⁵⁰. A recent study demonstrated the occurrence of EMT in fibrotic intestinal CD tissue independent of the effect of inflammation⁵¹. Another report detected fibrosis and expression of EMT markers in samples from CD patients, irrespective of the clinical behavior [stricturing (Montreal classification: B2) or penetrating (Montreal classification: B3) behavior]⁵². The functional role of EMT in IBD still needs to be determined.

Endothelial-Mesenchymal Transition (EndoMT).—EndoMT can be viewed as EMT occurring in endothelial cells. Similarly to epithelial cells, during the process of EndoMT endothelial cells lose the expression of typical endothelial markers and acquire fibroblast-like morphology and gene expression profile⁵³. Primary human intestinal microvascular endothelial cells (HIMECs) undergo EndoMT when exposed to TGF β , IL-1 β and TNF α , or supernatants from lamina propria primary mononuclear cells⁵⁴. During the process of EndoMT, HIMECs acquire an elongated morphology and broad changes in the gene expression profile, including downregulation of genes typically expressed by endothelial cells (CD31, VE-cadherin, vWF), downregulation of ECM components normally produced by endothelial cells (Collagen IV, entactin) and, conversely, upregulation of genes encoding ECM components expressed during intestinal fibrosis (Collagen I, fibronectin)⁵⁴. Evidence of *in vivo* EndoMT can be found in human colonic tissues from both CD and UC patients⁵⁴. EndoMT is also involved in radiation-induced rectal fibrosis, and in mucosal and submucosal vessels is proportional to the radiation injury score of patients with radiation-induced proctitis⁵⁵.

Activated fibroblast clusters and intestinal fibrosis

While traditional mesenchymal cell phenotypes have strongly contributed to the field of intestinal fibrosis, recent single cell genomics approaches are transforming our knowledge

on fibrotic disease across various organ systems⁵⁶. Its unprecedented resolution allows the interrogation of cell types and functional states with unmatched accuracy, opening a new window into disease mechanisms. Little is known about the application of single cell sequencing (scRNA-seq) in intestinal fibrosis. However, scRNA-seq analysis from other organs, such as lung, liver, skin, kidney and heart, provide new clues about cell clusters related to fibrosis and will help to guide the establishment of scRNA-seq in intestinal fibrosis studies.

In the lung, using single-cell transcriptome analyses, a new mesenchymal cells subtype expressing high levels of *Pdgfrb* was identified, with increased *Pdgfrb* expression in fibrotic mesenchymal cells compared to normal mesenchymal cells⁵⁷. scRNA-seq of alveolar fibroblasts also revealed a subset expressing *Wnt5a* scattered throughout the alveolar region, most near an *Axin2*+ *AT2* cell⁵⁸. In liver fibrosis, hepatic stellate cells (HSC) are considered the major source of myofibroblasts^{59,60}, with several scRNA-seq studies describing the HSC/myofibroblasts and immune cell populations involved. In a study using isolated HSCs and activated myofibroblasts from the carbon tetrachloride (CCl₄)-induced liver fibrosis mouse model, S100 calcium binding protein A6 (S100A6) was found to be a universal marker of activated myofibroblasts, but heterogeneity was noticed in both HSCs and activated myofibroblasts, indicating the existence of functionally relevant subsets in hepatic fibrosis⁶¹. Pathogenic subpopulations of *TREM2*+*CD9*+ macrophages, *ACKR1*+ and *PLVAP*+ endothelial cells and *PDGFR α* + collagen-producing myofibroblasts were also identified, revealing intra-scar activity of several pro-fibrogenic pathways, including *TNFRSF12A*, *PDGFR* and *NOTCH* signaling⁶². Central vein-associated HSCs were described as the dominant collagen-producing cells in a mouse model of centrilobular fibrosis, and *LPAR1* was identified as a therapeutic target for collagen-production in a rodent liver fibrosis non-alcoholic steatohepatitis NASH model⁶³. scRNA-seq also revealed the contribution of monocytes to myofibroblasts in kidney fibrosis⁶⁴. Two distinct populations of myofibroblasts were identified, resident myofibroblasts (*PDGFR β* +*CD45*-) largely associated with ECM production, and circulating cells-derived myofibroblasts (*PDGFR β* +*CD45*+) which exhibit a monocyte-like phenotype associated with immune responses⁶⁴. Another study used both single-cell RNA sequencing and single-nucleus RNA sequencing to uncover novel cell types and cell states related to kidney fibrosis⁶⁵. In this study, two distinct α -SMA positive activated fibroblast populations were identified: mannose receptor 2-expressing renal myofibroblasts, which bind and internalize collagen and attenuate renal fibrosis, and tenascin C (an extracellular glycoprotein)-expressing renal myofibroblasts⁶⁵ that promote renal fibrosis⁶⁶.

scRNA-seq technologies have also been applied to IBD. A recent study of the colonic mesenchyme revealed four distinct subsets of fibroblasts in addition to pericytes and myofibroblasts⁶⁷. The mesenchymal niches are dysregulated in intestinal inflammation with appearance of an activated fibroblast population expressing interleukin (IL)-33, lysyl oxidases (LOX), *TNFRSF14* and fibroblastic reticular cell-associated genes, leading to impaired epithelial function. This highlights novel types of fibroblasts and how intestinal fibroblasts drive inflammation and barrier dysfunction in IBD⁶⁷. In the context of ileal CD cellular heterogeneity may contribute to resistance to treatment with anti-TNF therapy. 82,417 lamina propria cells from 22 samples were sequenced in one study and interestingly

the tissue in the anti-TNF resistant group contained two subsets of fibroblasts, one of which was characterized by an activation program including strong expression of THY1 (CD90), PDPN (podoplanin), CTHRC1 (collagen triple-helix repeat-containing 1), and CHI3L1⁶⁸. CTHRC1+ fibroblasts are of particular interest, since this cell population expresses the highest amount of collagen, can be found in fibroblast foci in idiopathic pulmonary fibrosis (IPF), and show high migration and invasion capacity *in vitro*⁶⁹. However, the role of these activated fibroblasts or other mesenchymal cell populations in the pathogenesis of intestinal fibrosis is not yet clear. Discovery of spatially discrete, functionally unique fibroblast subsets with non-overlapping functions will have a critical impact in the rational design of therapies aimed at precisely modulating inflammation, fibrosis and tissue repair.

Cytokine networks and immune cells in intestinal fibrosis

In IBD mucosal fibroblasts and myofibroblasts in the mucosa are exposed to a highly complex microenvironment consisting of multiple cytokines released by several different cell types, with immune cells in the lamina propria being the most important sources. Investigation in both humans and animal models suggests that the release of cytokines during chronic inflammation could trigger the onset and directly promote intestinal fibrosis⁷⁰. However, it remains unclear whether the neutralization of a single cytokine or a combination of several cytokines will prevent or even reverse intestinal fibrosis. Hence, it is worth discussing the cytokines related to intestinal fibrosis and the main cell types releasing these cytokines, particularly newly described pro-fibrotic factors such as IL-11, IL-33, IL-34 and IL-36 (Figure 2).

TGF- β .—TGF- β is considered the key driver of intestinal fibrosis in IBD. In 1990 it was reported that TGF- β 1 could selectively augment collagen production by human intestinal smooth muscle cells *in vitro*⁷¹. Following studies elucidated the overexpression of TGF- β s and their signaling receptors in tissue samples from intestinal CD resections, indicating a potential role of these regulatory molecules in the pathophysiology of CD⁷². Furthermore, the expression levels of TGF- β transcripts and phosphorylated Smad2/3 were elevated in the mucosa overlying strictures but not non-strictured areas in CD tissues⁷³. Consistently, myofibroblasts from mucosa overlying strictured gut also showed higher expression of TGF- β transcripts than myofibroblasts from mucosa overlying non-strictured gut⁷³.

After binding to its receptor, TGF- β activates intracellular signaling through both the Smad-dependent and Smad-independent pathways. In the Smad-dependent pathway, the TGF- β receptor complex directly phosphorylates Smad2 and Smad3, which then forms a complex with Smad4 and enters the nucleus to regulate the transcription of target genes⁷⁴. Smad6 and Smad7, which compete with Smad2 and Smad3 for TGF- β receptor I kinase and promote the degradation of the TGF- β receptor, negatively regulate the TGF- β /Smad2–3 signaling pathway⁷⁵. Additionally, Smad7 was reported to have a lower expression level in the mucosa overlying strictured than non-strictured areas⁷³. Smad-independent TGF- β signaling pathways, including mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3-kinase (PI3K) cascade, can also be activated by TGF- β directly⁷⁶. Activated TGF- β promotes the differentiation from fibroblasts to myofibroblasts, resulting in elevated expression of α -SMA, increased proliferation and decreased apoptosis³⁸. In

addition, TGF- β treatment enhances the migration and collagen production of myofibroblasts^{77,78}. In human intestinal organoids which contain both epithelial and mesenchymal cells, including myofibroblasts, TGF- β stimulation increases the production of a series of pro-fibrotic factors, such as collagen I, α -SMA and fibronectin⁷⁹. In animal models, TGF- β 1 peptide-based vaccine suppresses excessive TGF- β 1 bioactivity and ameliorates fibrosis in a mouse model of chronic colitis⁸⁰. Knockdown of Smad7 with a specific antisense oligonucleotide attenuated colitis and colitis-driven colonic fibrosis in a 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) mice model⁸¹. These results may result from the many different effects of TGF- β in inflammation and fibrosis, which poses a challenge in generating anti-fibrotic treatments targeting TGF- β . Recently, several new proteins and factors have been reported to have a role in intestinal fibrosis through regulation of the TGF- β signaling pathway, such as ER stress protein glucose-regulated protein (GRP)78⁸², fibrin⁸³, Cadherin-11⁸⁴, anaxekto (AXL)⁸⁵, nuclear factor erythroid 2-related factor 2 (Nrf2)⁸⁶ and others.

IL-1 family members.—There are 11 members of the IL-1 family of cytokines, which can be divided into 3 subfamilies: the IL-1 subfamily (containing IL-1 α , IL-1 β , IL-33, and IL-1Ra), IL-18 subfamily (containing IL-18 and IL-37), and IL-36 subfamily (containing IL-36Ra, IL-36 α , β , γ and IL-38)⁸⁷. Besides the well-established inflammatory properties of IL-1 family members, some of them, such as IL-1, IL-33 and IL-36, also have an emerging role in intestinal fibrosis.

IL-1 α and IL-1 β : IL-1 α can function via its cell surface receptor IL-1R1 or regulate gene expression directly⁸⁸. IL-1 α is constitutively expressed in epithelial cells, but also can be expressed by other cell types upon their activation, such as macrophages, monocytes, endothelial cells and others⁸⁸. IL-1 α released from damaged intestinal epithelial cells acts as a damage-associated molecular pattern (DAMP), contributing to the initiation and development of chronic intestinal inflammation³⁸. In a DSS-induced colitis mouse model, IL-1 α -deficient mice exhibited mild disease symptoms with improved recovery, and neutralization of IL-1 α in control mice during acute colitis led to alleviation of clinical and histological manifestations. IL-1 β deficiency correlated with disease exacerbation and treatment with rIL-1Ra or anti-IL-1 β antibodies was not effective in DSS-induced acute colitis mice model⁸⁹. Intestinal epithelial cell lysate-induced activation of human intestinal fibroblasts (HIFs) is mediated by IL-1R and IL-1 α but not IL-1 β ⁹⁰. Furthermore, intestinal epithelial cell-derived IL-1 α induces cytokine production by HIFs⁹⁰. IL-1 α and TNF- α also increased TGF- β 1 and TIMP-1 production by colonic epithelial cells⁷⁷. In other organs, IL-1 α also acts as a profibrotic cytokine, as IL-1 α -deficient mice exhibit reduced collagen deposition in response to bleomycin treatment in lung fibroblasts⁹¹.

IL-1 β is mainly produced by mononuclear phagocytes, acting as a pro-inflammatory effector in intestinal inflammation⁷⁰. IL-1 β enhances TGF- β 1-induced EMT in human bronchial epithelial cells⁹². IL-1 β over-expression induces acute lung injury and chronic repair leading to pulmonary fibrosis in a rat model and the IL-1R1/MyD88 axis is considered essential in pulmonary inflammation and fibrosis in mice^{93–95}. In liver fibrosis, although IL-1 β promoted HSC proliferation and was associated with the degree of liver fibrosis in

Abcb4^{-/-} mice, IL-1 antagonism showed anti-fibrotic effects in vitro but not in Abcb4^{-/-} mice⁹⁶. IL-1 β induces a metabolic switch in platelet-derived growth factor receptor (PDGFR β) + kidney stromal cells, which promotes tubulointerstitial fibrosis⁹⁷. IL-1 β stimulates the secretion of collagens I and IV, IL-8, monocyte chemoattractant protein (MCP)-1 and MMP-1 by colonic subepithelial myofibroblasts⁹⁸. However, another report suggested IL-1 β inhibited collagen synthesis and induced collagenase and TIMP-1 production in intestinal smooth muscle cells^{99,100}. The role of IL-1 β in intestinal fibrosis remains unclear.

IL-36: IL-36 consists of three agonists, IL-36 α (IL-1F6), IL-36 β (IL-1F8), IL-36 γ (IL-1F9), and IL-36 receptor antagonist (IL-36Ra)^{101,102}. The IL-36 agonists have demonstrated pro-inflammatory effects and recent evidence suggests additional roles in intestinal fibrosis^{101,102}. In the intestinal tract, IL-36 is generated by many different cell types, including fibroblasts, myofibroblasts, epithelial cells, goblet cells, macrophages, and glial cells^{103–108}. In turn, many cell types can respond to IL-36, including immune cells, epithelial cell, endothelial cells, and mesenchymal cells, and IL-36 induces distinct gene expression and functional changes¹⁰⁹. IL-36 α and IL-36 γ were upregulated in endoscopic biopsies of patients with active colonic CD compared to unaffected biopsies from the same patients¹¹⁰. In addition, increased expression of IL-36 α and IL-36 γ , but not of IL-36 β , was observed in the intestinal mucosa in UC patients rather than control or CD patients^{107,108}. A pro-fibrotic role of IL-36 has been suggested in pulmonary fibrosis and renal fibrosis^{88,111}. In the intestine, increased IL36A expression was also found in tissues from patients with fibrostenotic CD, with high numbers of activated myofibroblasts¹¹². In both mouse and human fibroblasts, IL36R activation enhances the expression of genes that regulated fibrosis and tissue remodeling, with higher expression of collagen VI¹¹². IL-36R-deficient mice or mice injected with anti-IL-36R antibody develop less severe colitis and fibrosis in DSS- or TNBS-induced colitis¹¹², suggesting that blockade of IL36R signaling has the potential to be an efficient mechanism for prevention and treatment of intestinal fibrosis in IBD patients.

IL-33: IL-33 is expressed throughout various organs in the body and can be produced by both non-hematopoietic and hematopoietic cells, including fibroblasts, adipocytes, smooth muscle cells, endothelial cells, bronchial, intestinal epithelial cells, macrophages and dendritic cells¹¹³. IL-33 signals through its receptor ST2 and drives the production of pro-inflammatory and type 2 helper T cell (Th2)-associated immune responses, which lead to the development of fibrotic diseases in various organs, such as the lung, liver, kidney, heart, skin, and pancreas in an ST2- and macrophage-dependent manner, regulating EMT, or contributing to abnormal fibroblast proliferation, leukocyte infiltration and morphologic differentiation of human endothelial cells¹¹⁴. In the gut, IL-33 is mainly expressed by fibroblasts, smooth muscle cells, endothelial cells, and adipocytes, as well as intestinal epithelial cells and infiltrating lamina propria mononuclear cells in active UC¹¹³. Increased expression levels of IL-33 and its receptor, ST2, was found in IBD patients, particularly in UC^{115,116}. In adherent invasive E-coli (AIEC)-elicited experimental intestinal fibrosis, the bacterial protein flagellin potentiated the expression of the IL-33 receptor ST2 in the intestinal epithelium¹¹⁷. The IL-33-ST2 axis promoted intestinal fibrosis in a TLR5- and NOD-like receptor family CARD domain-containing protein (NLRC) 4-dependent manner.

Blockade of IL-33-ST2 signaling by an anti-ST2 blocking antibody suppresses fibrosis-related gene expression and the accumulation of collagen in the colon¹¹⁷. MyD88, which is known for its essential role in TLR signaling pathways as an adaptor protein in innate immune responses, was demonstrated to act downstream TLR5 and regulate intestinal fibrosis¹¹⁸. In a α -SMA-specific MyD88 deletion mouse model, MyD88 deletion prior to, but not after induction of, experimental colitis resulted in decreased intestinal fibrosis¹¹⁸. α -SMA(+) HIMFs selectively respond to flagellin with enhanced fibronectin or Coll1 production in an MyD88-dependent manner, mediated by eIF2 alpha and 4EBP1¹¹⁸. These results further indicate signaling pathways involved in flagellin induced intestinal fibrosis.

IL-6 family members.—The interleukin (IL)-6 family of cytokines includes IL-6, IL-11, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin 1 (CT-1), cardiotrophin-like cytokine (CLC), and IL-27¹¹⁹, all of which utilize a common signaling receptor subunit glycoprotein 130 kDa (gp130). Blockade of IL-6 family cytokines has been shown to be beneficial in autoimmune diseases, but bacterial infections and metabolic side effects have been observed¹¹⁹. Current studies suggest IL-6 and IL-11 may have a role in intestinal fibrosis.

IL-6: IL-6 can be expressed by intestinal mononuclear cells, epithelial cells and mesenchymal cells, especially upon challenge with inflammatory cytokines⁷⁰, and the role of IL-6 is well established in IBD¹²⁰. Besides its known pro-inflammatory activity, IL-6 also contributes to fibrosis. High levels of IL-6 characterize early idiopathic pulmonary fibrosis acute exacerbations and an increase in the levels of IL-6 associates with worse outcome in idiopathic pulmonary fibrosis patients¹²¹. The HLF/IL-6/STAT3 circuit drives HSC activation in liver fibrosis and IL-6 and p-STAT3 levels were increased in patients with fibrotic livers¹²². Upon TGF- β activation, intestinal mesenchymal cells are activated and increase their autocrine production of a number cytokines including IL-6, as well as excess ECM production including collagen I¹²³. In the strictured intestine, immunoreactive IL-6 is increased in α -SMA(+) muscle cells compared with the normal resection margin in structuring (Montreal classification: B2) CD patients. Treatment of normal muscle cells with IL-6 phenocopied muscle cells from strictured intestine whereas neutralization of autocrine IL-6 reversed STAT3 phosphorylation and normalized expression of TGF- β 1 in strictured intestinal muscle¹²⁴. The pro-fibrotic role of IL-6 *in vivo* needs further clarification.

IL-11: IL-11 was characterized as a profibrotic cytokine secreted by myofibroblasts and damaged epithelial cells as well as smooth muscle cells^{125,126}. Its receptor, IL-11RA, is highly expressed on stromal cells, including fibroblasts, smooth muscle cells, adipocytes, and hepatic/pancreatic stellate cells or pericytes, and also on epithelial/polarized cells, such as hepatocytes, alveolar epithelial cells, and kidney tubular epithelial cells^{127,128}. IL-11 signaling drives myofibroblast activation, parenchymal cell dysfunction, inflammation and promotes fibrosis in several organs, including lung, liver, heart and skin^{127–129}. In UC, an inflammation-associated fibroblast subset was identified with elevated expression of IL-11, IL-24, and IL-13RA2¹³⁰. Transgenic mice (III1^{SMC}) with smooth muscle cell-specific conditional overexpression of murine IL-11 develop loose stools, progressive bleeding and rectal prolapse, with inflamed, fibrotic and a thickened bowel wall, accompanied by

activation of ERK and STAT3¹²⁵. A similar phenotype was observed in a second model with fibroblast-specific expression of IL-11, indicating that both mesenchymal cell types are mechanistically important in driving fibro-inflammation and an inflammatory bowel phenotype in mice¹²⁵. However, additional studies using a loss-of-function approach for IL-11 are needed, such as using IL-11 deficient mice or IL-11 neutralizing antibodies, to further determine the impact of IL-11 on intestinal fibrosis.

TNF- α family members—Two TNF- α family members, TNF- α and TL1A, have an established role in the pathogenesis of IBD^{70,131}. Accumulating evidence suggests they may also have direct effects on myofibroblasts or EMT, and thus be related to fibrosis in the intestine and other organs^{70,131}.

TNF- α : Anti-TNF blocking monoclonal antibodies have a well-established role in IBD therapy^{132,133}. TNF- α induces an increase in IL-8, MCP-1 and MMP-1, as well as a change in type I and IV collagen secretion in subepithelial myofibroblasts, but does not affect their proliferation⁹⁸. TNF- α upregulates TGF- β and TIMP-1 in colonic epithelial cells and conditioned medium from epithelial cells pretreated with TNF- α induces MMP-9 in colonic subepithelial myofibroblasts⁷⁷. The anti-TNF antibody Infliximab induces a significant dose-dependent increase in TIMP-1 production in CD myofibroblast with enhanced myofibroblast migration and decreased collagen production¹³⁴. In a peptidoglycan-polysaccharide (PG-PS) induced enterocolitis model with granulomatous inflammation that leads to prominent fibrosis mimicking CD, rat-specific anti-TNF- α treatment prevents inflammation and fibrosis¹³⁵. In a study evaluating the possible effect of anti-TNF- α antibodies on small bowel stenosis, no progression of pre-existing stenoses, no appearance of new ones, and complete regression of 1/22 (4.5%) small bowel stenoses was observed after the induction phase¹³⁶. However, in another study with pediatric CD patients, those who received early anti-TNF α therapy were less likely to have penetrating but not stricturing complications than those who did not receive early anti-TNF α therapy¹³⁷. It is generally considered that anti-TNF antibodies do not prevent strictures in CD¹³¹.

TL1A: TL1A, a protein encoded by *TNFSF15*, binds to death domain receptor 3 (DR3) and is expressed by a variety of cell types including immune cells, epithelial cells, and fibroblasts. TL1A was originally described as a T cell-costimulatory cytokine, but later found to affect multiple cell lineages with cell-type specific effects, such as proliferation, cytokine secretion or cell differentiation among others^{138,139}. TL1A mRNA and protein expression is upregulated in IBD, particularly in areas involved by CD¹⁴⁰. TL1A may contribute to IBD pathogenesis via local but not systemic induction of IL-17A, but not IL-4, IL-13 or IFN- γ ¹⁴¹. In human translational studies, CD patients with higher peripheral TL1A expression also exhibited intestinal strictures and worsened ileocecal inflammation with relative sparing of rectosigmoid inflammation¹⁴². Constitutive expression of TL1A in either lymphoid or myeloid cells in transgenic mice leads to enhanced intestinal and colonic fibrosis^{143,144}. In the DSS and adoptive T cell transfer models there is proximal migration of colonic inflammation, worsen patchy intestinal inflammation and long gross intestinal strictures in TL1A transgenic mice compared to wild-type littermates, with increased T-cell activation markers and interleukin-17 expression¹⁴². Furthermore, TL1A-mediated

intestinal fibrosis and fibroblast activation are dependent on specific microbial populations¹³⁹. Anti-TL1A antibody can reduce intestinal inflammation and fibrosis by inhibiting the activation of intestinal fibroblasts and reducing the collagen synthesis in the T cell transfer model of chronic colitis¹⁴⁵. In established murine colonic fibrosis, treatment with neutralizing TL1A antibodies results in lowered expression of TGF- β 1, with reduced number of fibroblasts and myofibroblasts¹⁴⁶. Primary intestinal myofibroblasts express DR3 and functionally respond to direct TL1A signaling by increasing collagen¹⁴⁶. In a recent study, TL1A overexpressing naïve T cells were transferred into Rag-/-, Rag-/- mice lacking DR3 in all cell types (Rag-/-Dr3-/-), or Rag-/- mice lacking DR3 only on fibroblasts (Rag-/-Dr3-/-Coll1a2) to induce colitis and fibrosis, and the results showed that Rag-/-Dr3-/- developed decreased inflammation and fibrosis, and despite similar clinical disease and inflammation as Rag-/-, Rag-/-Dr3-/-Coll1a2 exhibited reduced intestinal fibrosis and attenuated fibroblast activation and migration¹⁴⁷.

IL-17 and Th17 cells. —It is generally believed that Th1-cell-associated cytokines drive inflammation, whereas uncontrolled type 2 and type 17 cell responses might drive tissue fibrosis through the excessive deposition of ECM⁷⁰. This implies a switch in immunophenotype over time, possibly driven by chronic inflammation itself.

It is becoming increasingly clear that T-cell phenotypes are more heterogenous than previously anticipated. There is evidence demonstrating the regulatory role of IL-17 in the development of fibrosis in multiple organs, such as the liver, skin, lung and heart^{38,131}, but its contribution to IBD and intestinal fibrosis remains controversial¹⁴⁸. IL-17 cytokines, primarily produced by Th17 cells, consists of six related proteins: IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F, which signal through five receptor subunits IL-17RA-IL-17RE to¹⁴⁹. In humans, IL-6, IL-21, IL-23, IL-1 β , and TGF- β are critical for Th17 development and, in addition to IL-17A-F, Th17 cells also produce IL-21, IL-22, IL-9, IL-26, TNF- α , and chemokine (C-C motif) ligand 20 (CCL20)¹⁴⁸. Th17 cell numbers and Th17-related cytokines, including IL-17, IL-21 and IL-22, are significantly increased in active IBD¹⁵⁰. IL-17A is significantly overexpressed in CD strictured areas compared with non-strictured areas¹⁵¹. In addition, IL-17A significantly inhibits myofibroblast migration and promotes the production of MMP-3, MMP-12, TIMP-1 and collagen by myofibroblasts from strictured CD tissues¹⁵¹. In subepithelial myofibroblasts, IL-17A enhances the production of collagen I and heat shock protein 47 (HSP47), which is significantly elevated in the intestinal tissues of patients with active CD and contributes to IL-17A-induced collagen I expression¹⁵². Occurrence of EMT and high levels of IL-17A are observed in the intestinal mucosa of CD patients and IL-17A may induce EMT in intestinal epithelial cells¹⁵³. Anti-IL-17A treatment alleviated intestinal fibrosis by reducing EMT in mouse intestines¹⁵³. Consistent with these data, in a TNBS-induced intestinal fibrosis mouse model, treatment with anti-IL-17 antibody significantly alleviated intestinal fibrosis and reduced both mRNA and protein levels of collagen 3, TNF- α , TIMP-1, and MMP-2, as well as the decreased levels of profibrogenic cytokines IL-1 β , TGF- β 1, and TNF- α ¹⁵⁴. However, secukinumab, a human anti-IL-17A monoclonal antibody, failed to show efficacy in CD and in fact led to worsening of intestinal

inflammation in some patients, suggesting that baseline levels of IL-17 are necessary for intestinal homeostasis ¹⁵⁵.

IL-4, IL-13 and the Th2 response.—IL-4 and IL-13 are prototypical Th2 cytokines. Although it was initially believed that Th1 cells are involved in CD whereas Th2 or Th2-like cells are associated with UC ¹⁵⁶, Th2 immune responses have also been detected in late stages of experimental and human CD ^{157,158}. Previous studies in several fibroblastic synovial cell lines indicated that IL-4 might be a pro-fibrogenic cytokine promoting collagen and fibronectin synthesis in normal healing and pathological fibrosis ¹⁵⁹. In addition, the treatment of human hepatic fibroblasts with IL-4 increased collagen production ¹⁶⁰. However, in IBD the role of IL-4 is still controversial. Although IL4 mRNA expression in the intestinal mucosa in both CD and UC patients is almost undetectable ¹⁶¹, anti-IL-4 administration leads to a striking amelioration of oxazolone colitis ¹⁶². IL-13 signals through IL-13R α 2 to activate the TGF- β 1 promoter and the prevention of IL-13R α 2 expression reduced production of TGF- β 1 in oxazolone- or TNBS-induced colitis ^{163,164}. Additionally, IL-13 and its receptor were found to be overexpressed in areas of fibrosis in CD patients ¹⁶⁵. However, anrukinzumab, an anti-interleukin 13 monoclonal antibody, showed no therapeutic effect in patients with active UC in a phase IIa randomized multicenter study ¹⁶⁶. The effect of another IL-13-neutralizing antibody, tralokinumab, was evaluated in a randomized, double-blind, placebo-controlled, phase IIa study as add-on therapy in adults with moderate-to-severe UC despite standard treatments and the results demonstrated that did not significantly improve clinical response, although a higher than placebo clinical remission rate suggested that tralokinumab might benefit some UC patients ¹⁶⁷.

IL-34.—IL-34 is a novel cytokine identified as a tissue-specific ligand of CSF-1 receptor (CSF-1R) ¹³⁰. Although IL-34 shares the receptor CSF-1R with CSF1 and M-CSF, IL-34 shows very little homology with them and IL-34 has also two distinct receptors (PTP- ζ) and CD138 (syndecan-1) ¹⁶⁸. It belongs to the 4-helical cytokine family, which usually functionally mimics the PDGF and VEGF cystine knot dimers ¹³⁰. In humans, IL-34 has a wide distribution in various tissues, including the heart, brain, lung, liver, kidney, spleen, thymus, testis, ovary, small intestine, prostate, colon, and spleen, and it can be expressed by synovial fibroblasts, immune, epithelial, endothelial, cancer cells and adipocytes ¹⁶⁹. IL-34 induces lymphocyte differentiation, proliferation, and regulates the synthesis of inflammatory components and aberrant expression of IL-34 in several autoimmune disorders, such as lupus, arthritis, systemic sclerosis ¹³⁰. IL-34 is overexpressed in chronic hepatitis C liver fibrosis and induce profibrotic macrophages, and serum interleukin-34 level can be an indicator of liver fibrosis in patients with chronic hepatitis B, as well as a marker of liver fibrosis in patients with non-alcoholic fatty liver disease ¹⁷⁰⁻¹⁷². IL-34 deficient mice exhibit less renal fibrosis during the chronic phase after ischemia/reperfusion injury ¹⁷³. In the gut IL-34 is constitutively expressed in human small intestine and colon and shows a higher expression level in stricturing CDs compared to controls ¹⁷⁴. It functions as a direct activator of intestinal fibroblasts, who respond with increased collagen expression, including COL1A1 and COL3A1, in a p38MAPK dependent manner ¹⁷⁴. IL-34 knockdown results in decreased collagen production in CD fibroblasts isolated from CD strictures without affecting cell survival ¹⁷⁴.

Host-microbiome interactions and intestinal fibrosis

The gut microbiota, a highly complex and dynamic population of microorganisms, has become the subject of intense investigation in recent years and plays a major role in human health and disease¹⁷⁵, and compositional and metabolic alterations of the gut microbiota (dysbiosis) are key factors involved in IBD pathogenesis¹⁹. In individuals with a genetic susceptibility to IBD, microbial antigens and products induce functional changes of the intestinal mucosa and immune response which are linked to IBD onset and progression¹⁷⁶.

Innate immune response is the first line of defense against invading pathogens, and initiation of this response depends on the recognition of pathogen-associated molecular patterns (PAMPs) by a series of pathogen recognition receptors (PRRs)¹⁷⁷. PRRs are expressed by both intestinal immune (dendritic cells, macrophages, lymphocytes, etc.) and non-immune cells (epithelial cells, fibroblasts, endothelial cells, etc.)¹⁷⁸. Primary human intestinal myofibroblasts express multiple PRRs, including toll-like receptor (TLR) 1–9, as well as nucleotide-binding oligomerization domain-containing protein (NOD) 1 and NOD2¹⁷⁹. Lipopolysaccharide (LPS), predominantly derived from gram-negative bacteria and recognized by TLR4, promotes profibrotic activation of intestinal fibroblasts, with enhanced NF- κ B promoter activity and increased collagen production¹⁸⁰. DSS-induced intestinal fibrosis is decreased in TLR4-deficient mice, with reduced TGF- β production by peritoneal macrophages and less collagen production¹⁸¹. Flagellin, which potentiates the expression of the IL-33 receptor ST2 in the intestinal epithelium, promotes intestinal fibrosis in a TLR5- and NOD-like receptor family CARD domain-containing protein (NLRC) 4-dependent manner¹¹⁷. Supporting the notion of microbial driven fibrogenesis, CD patients carrying NOD2 mutations display a fibrostenotic phenotype¹⁸².

Bacteria-driven IBD animal models, microbes or microbial components are used to study intestinal fibrosis, such as AIEC infection and *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) infection mouse models^{182,183}. On one hand, research in these animal models provides evidence of the relationship between gut microbiome and intestinal fibrosis and, on the other hand, uncovers new molecules and mechanisms involved in intestinal fibrogenesis.

AIEC is a pathotype of *E. coli* which adheres to the gut epithelium and causes chronic intestinal inflammation in genetically susceptible hosts¹⁸⁴. AIEC strains are found more frequently in ileal specimens from CD patients and are suspected to be involved in the initiation and/or progression of local inflammation¹⁸⁵. In mice, chronic AIEC infection leads to tissue pathology in the small and large bowel of mice, especially in the cecum, with elevated Th1 and Th17 responses¹⁸⁶. In addition, cecum from AIEC-infected mice exhibits extensive ECM deposition, higher expression levels of collagen type I and III, and enhanced expression of profibrotic mediators, such as TGF- β 1, connective-tissue growth factor (CTGF) and insulin-like growth factor I (IGF-I), all of which are also observed in CD patients¹⁸⁶. However, intestinal strictures do not occur in this model, and inflammation and fibrosis is mouse strain-dependent.

S. Typhimurium is a common gastrointestinal bacterial pathogen that causes food poisoning and gastroenteritis in millions of people each year. *S. Typhimurium* infection induces

chronic *Salmonella* colonization of the murine cecum and colon, with severe transmural inflammation¹¹⁷. *S. Typhimurium* infection also increases the susceptibility of DSS-treated and IL-10^{-/-} mice to intestinal inflammation¹⁸⁷. Although in humans *S. Typhimurium* infection has not been implicated in the pathogenesis of CD or intestinal fibrosis, *S. Typhimurium*-infected mice exhibit enhanced production of TGF- β and IGF-I, along with extensive collagen I deposition in the cecal mucosa, submucosa, and muscularis mucosa¹⁸⁷ and large numbers of fibroblasts and myofibroblasts in fibrotic areas¹⁸⁸. A modified *Salmonella*-induced intestinal fibrosis model has been reported and was used to detect the role of flagellin and IL-33 in intestinal fibrosis¹¹⁷. In this model, AIEC and an attenuated strain of *S. Typhimurium* were co-colonized and induced intestinal fibrosis in a flagellin-dependent manner with the activation of IL-33-ST2 signaling¹¹⁷. No animal model can fully mimic human IBD fibrosis, but deeper investigation of the mechanism of IBD and further characterization of novel animal models will support the interrogation of fibrotic mechanisms in vivo.

Creeping fat and smooth muscle hyperplasia in intestinal fibrosis

Fat wrapping, also called creeping fat, a pathologically altered mesenteric fat tissue adjacent to CD-involved intestinal segments and was described almost 100 years ago, but its functional implications have only recently received attention¹⁸⁹. Creeping fat is highly linked to stricturing disease and its extent correlates closely with the degree of transmural inflammation¹⁹⁰, a clinically relevant observation. When the mesentery is removed extensively for ileocolic resections (as opposed to minimal mesenteric resection) CD recurrence is reduced, and fewer reoperations are required⁴³. The mechanism underlying the formation of creeping fat remains unclear. One factor essential to the formation of mesenteric adipose tissue in CD is adipocyte hyperplasia, which occurs by recruitment and differentiation of adipose tissue-derived stem cells (ASCs). CD ASCs have elevated proliferative, invasive and phagocytic capacities, which may contribute to the creeping fat development¹⁹¹. In CD ASCs exhibit a unique DNA methylation and gene expression profile¹⁹² and CD creeping and mesenteric fat shows a microbiome signature that is absent in subcutaneous fat¹⁹³. Immune-non-immune cell interactions are critical to the function of creeping fat. Compared with tissue from control subjects, both T and B memory cells are increased within the creeping fat of CD patients¹⁹⁴, and CD mesenteric tissue contains greater amounts of adipocyte-derived chemokines which may actively recruit T and B lymphocytes¹⁹⁴. Of critical relevance, CD creeping fat is associated with muscularis propria hyperplasia, which is now believed to be the major factor contributing to the luminal narrowing in gut stenosis (Figure 2)^{189,195}. Our own preliminary observations suggest a novel role of creeping fat derived fatty acids on human intestinal muscle cells (HIMCs) hyperplasia, which contributes to the intestinal stricture formation^{196,197}. Exposure of HIMC to whole creeping fat tissue and fat-conditioned medium dramatically upregulates HIMC proliferation compared with UC and normal mesenteric fat¹⁹⁷. Creeping fat-derived mediators such as free fatty acids, but not adipokines, increase a differential and selective proliferative response by HIMC, which is dependent on p38MAPK, PKC, and PI3K^{196,197}. In addition, creeping fat may be linked with gut wall fibrosis. The adipocyte-dependent microenvironment within the creeping fat of patients with CD has been reported to promote

an M2 macrophage subtype with secretion of large amounts of pro-fibrotic factors such as TGF- β , leading to intestinal fibrosis¹⁹⁸.

Pathway to develop anti-fibrotic trials in IBD

Despite the significant advances in the field of fibrosis, including features unique to the intestine such as creeping fat and microbial influence, trials testing drugs with an anti-fibrotic mechanism have yet to be started in IBD. This is largely due to the major obstacle of missing consensus on definitions and the absence of clinical endpoints. While multiple biomarkers have been proposed to stratify patients without any complications at diagnosis into at-risk populations for future strictures, none of the current markers are able to discriminate future stricturing from penetrating behavior with high enough accuracy to allow enrolling CD patients into fibrosis prevention trials^{199,200}. In addition, at present no accurate biomarkers or imaging technique can accurately quantify fibrosis in a stricture, which make studies relying solely on ECM changes as endpoints challenging. Cross-sectional imaging techniques, including ultrasound, computed tomography enterography and magnetic resonance enterography can detect intestinal strictures or assess inflammation with high accuracy but cannot determine fibrosis degree²⁰¹, making it practically impossible to assess responses to anti-fibrotic therapy. One possible starting point is to choose a population with existing strictures, in which resection studies have provided an assessment of the amount of present fibrosis, muscle thickening and inflammation²⁰². In these patients a background therapy with anti-inflammatory drugs can lead to a mitigation in obstructive symptoms that then allows the randomization of patients to an anti-fibrotic therapy versus placebo. Clinically meaningful endpoints could be absence of obstructive symptoms, stricture morphology changes on cross sectional imaging or reduced number of endoscopic or surgical interventions.

To accomplish translation of novel anti-fibrotics the field of stricturing IBD is in dire need of reliable definitions. Systematic reviews reveal tremendous heterogeneity in the definitions for stricturing IBD on endoscopy and cross-sectional imaging²⁰¹. Recently, the global consortium Stenosis Therapy and Anti-Fibrotic Therapy (STAR) created clear definitions for what defines a stricture and what constitutes improvement²⁰³. Multiple projects are now underway to build the monitoring tools and endpoints for clinical trials in fibrostenosing IBD, including a patient reported outcome tool, a stricture radiology index and dynamic fibrosis turnover markers.

Finally, successful development of markers to predict or quantify fibrosis in the future will be based on development of a solid histopathologic gold standard. Typically, candidate markers are evaluated in patients with symptomatic strictures, who then undergo imaging, followed by bowel resection with grading of the intestinal tissue for inflammation and fibrosis^{204–211}. Next, construct validity is determined by correlating histologic evaluations of the degree of fibrosis and/or inflammation on the resected tissue to imaging results. Multiple histopathology indices have been proposed, but none has been validated following modern methodological standards²¹², and their evaluation is highly variable across indices²¹³. A reliable, validated and responsive histopathologic standard would deliver the needed discriminatory ability as a basis to optimize and compare cross sectional imaging

techniques. An international working group comprised of IBD pathologists and gastroenterologists under the umbrella of the STAR consortium is working on building a validated histopathology index for stricturing IBD. Ultimate progress will be fueled by combining mechanistic preclinical studies with the newly created endpoints in state-of-the-art drug development programs (Figure 3).

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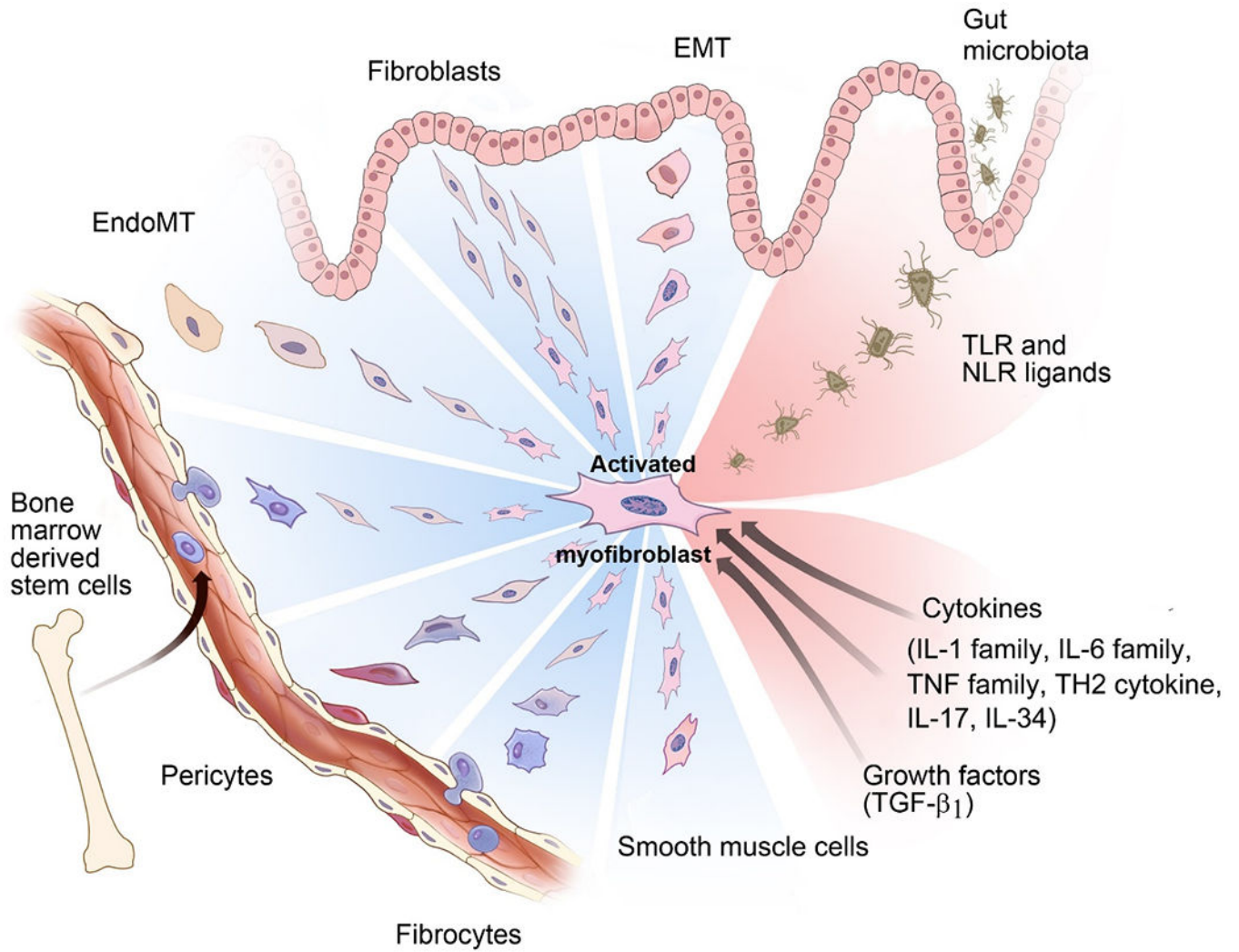


Figure 1. The main cell types and cell differentiation mechanisms involved in ECM secretion and fibrosis in intestine. EMT, epithelial-mesenchymal transition; EndoMT, endothelial-to-mesenchymal transition; TLR, Toll-like receptor; NLR, Nod-like receptor; IL, interleukin; TNF, tumor necrosis factor; TGF- β 1, transforming growth factor beta 1.

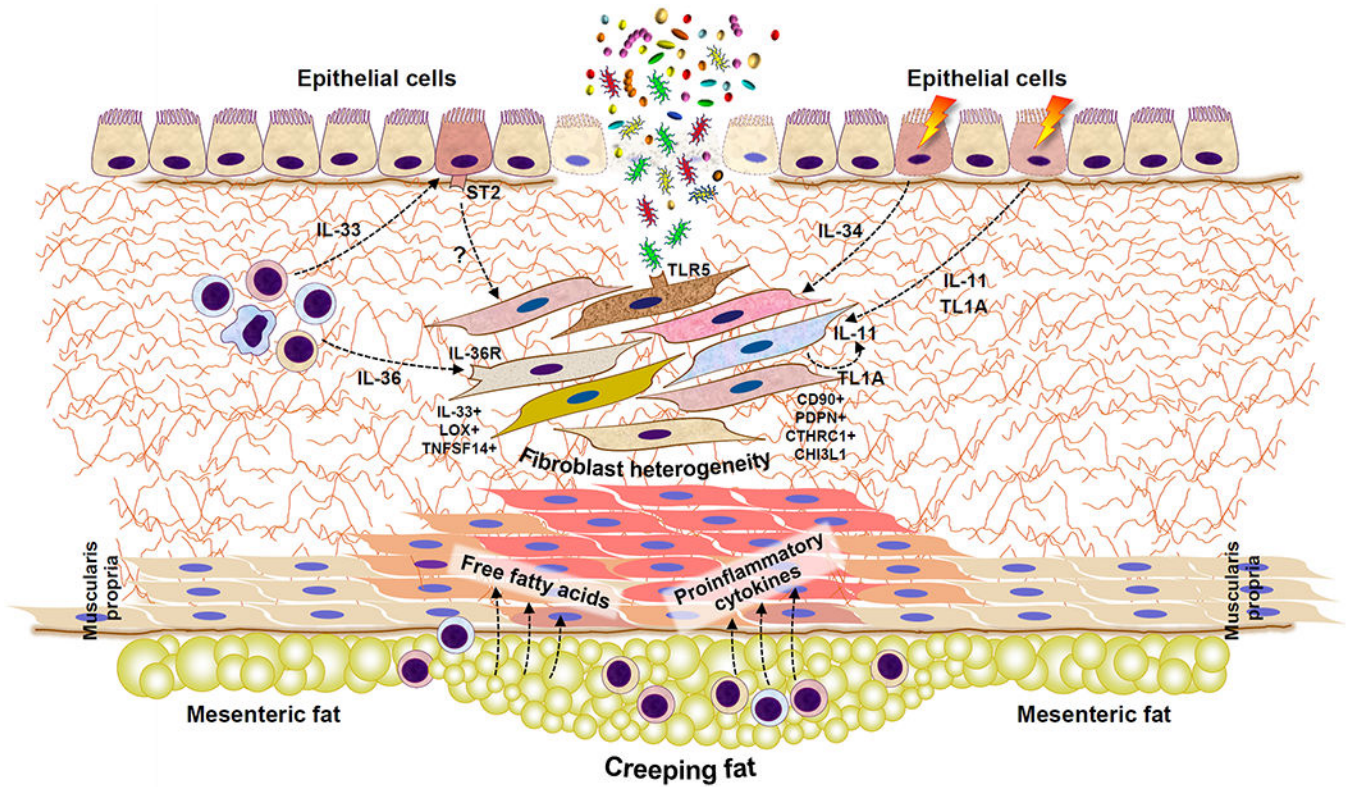


Figure 2.

Novel mechanisms underlying intestinal fibrosis. IL, interleukin; TLR, Toll-like receptor; LOX, lysyl oxidases; TNFSF14, tumor necrosis factor superfamily member 14; PDPN, podoplanin; CTHRC1, collagen triple-helix repeat-containing 1; CHI3L1, chitinase-3-like 1.

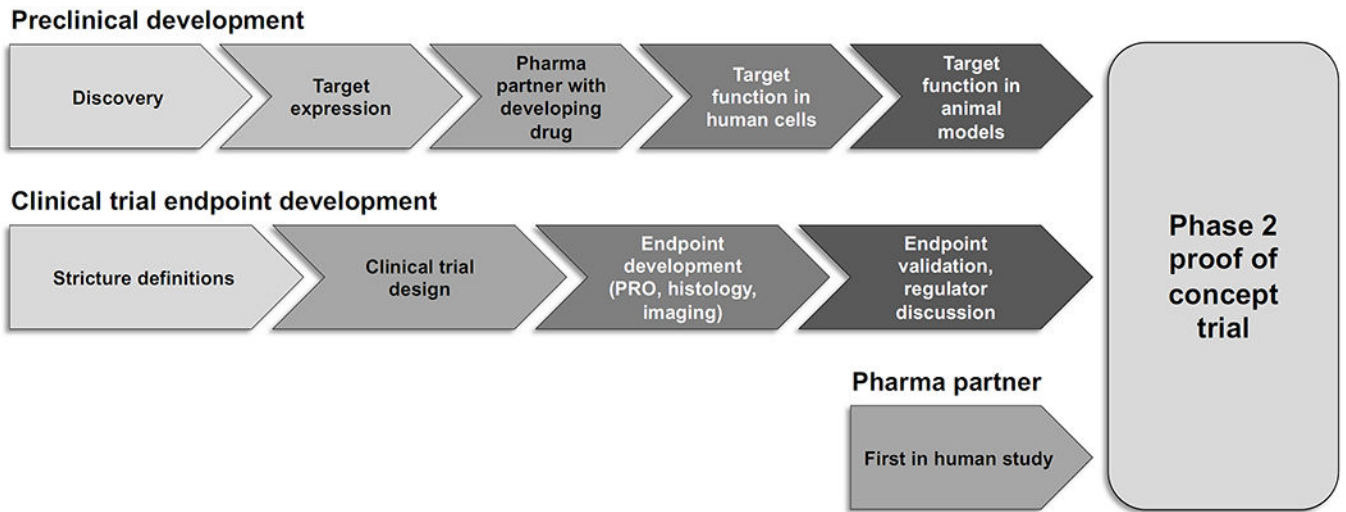


Figure 3.
Proposed pathway to develop anti-fibrotic medications in IBD

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