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REVIEW ARTICLE

Intestinal fibrosis in inflammatory bowel disease — Current knowledge and future perspectives

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Abstract

Background and aims: Intestinal fibrosis is a common complication of IBD that can become seriously symptomatic and may require surgical intervention if stricture formation ensues. This review discusses existing and developing knowledge of intestinal fibrosis and its implications for therapy.
Methods: Review of the literature, personal communications, unpublished observations.
Results: Known mechanisms of intestinal fibrosis include fibroblast proliferation and migration, activation of stellate cells, and extraintestinal fibroblast recruitment. However, novel mechanisms are being uncovered, including epithelial-to-mesenchymal transition, endothelial-to-mesenchymal transition, pericyte differentiation, and fibrocyte recruitment. Most of the traditional and novel mechanisms underlying intestinal fibrosis are associated to the presence of chronic inflammation, but is also possible that fibrosis develops independently of persistent immune activation in the gut. At the moment, the development of preventive, non-interventional, and more effective management of intestinal fibrosis is hampered by the lack of a greater knowledge of its basic pathophysiology and predisposing factors.
Conclusions: It is reasonable to expect that therapy of IBD-associated fibrosis will radically improve once the underlying mechanisms are better understood, and therapeutic modalities will emerge that prevent or reverse this complication of IBD.

Contents

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1. Intestinal fibrosis — an overview of the clinical problem

Intestinal fibrosis, commonly defined as an excessive deposition of extracellular matrix (ECM) resulting from chronic inflammation and impairment of intestinal wound healing, represents a serious complication of IBD and has important clinical implications. This is true for both ulcerative colitis (UC) and Crohn's disease (CD). In UC the involvement of the mucosal and submucosal layers causes a thickening of the muscularis mucosae with accumulation of ECM that may contribute to shortening or stiffening of the colon, whereas in CD the transmural nature of the inflammatory process is followed by bowel wall thickening, and eventually formation of stricture and stenosis.[1]

More than one-third of the patients with CD develop a distinct fibrostenosing phenotype, manifested by progressive narrowing of the intestinal lumen and potential obstruction.[2] Together with fistulae, intestinal stenosis represents the main indication for surgery in CD, whereas in UC indication of surgery because of bowel stenosis is a far more sporadic event .[3,4] Up to 80% of all patients suffering from CD undergo surgery at least once during the course of their disease.[5] In approximately half of these patients stricture formation and obstruction secondary to bowel wall fibrosis are the main reason for surgery, denoting that excessive scar tissue formation is underlying the need for an operation in approximately one-third of all CD patients.[6,7] Recurrence of disease at the site of anastomosis is common, and recurrent stricture formation may also occur.[8,9] It is well established that CD is a dynamic disorder whose phenotype may evolve with time. While location of inflammation is a relatively stable clinical feature, changes in disease behavior occur in approximately one-third of patients who progressively switch from a pure inflammatory to a stricturing or penetrating phenotype over a period of 10 years or longer.[10] The time-dependent phenotypic change of the disease suggests that, as long as intestinal inflammation endures, fibrosis may follow, although this is not always the case, as patients may display a chronic inflammatory pattern without ever developing significant intestinal fibrosis or stricture. Despite substantial advances in its management, IBD still displays a chronic inflammatory course, and the incidence of stricture formation and stenosis secondary to inflammation has not significantly changed during the last 25 years.[11]

In contrast to the remarkable success of new pathophysiology-based anti-inflammatory therapies in IBD,[12] relatively minor progress has occurred with respect to the therapeutic approach to intestinal fibrosis.[13] Bowel resection and strictureplasty remain the basic interventions for complications secondary to intestinal fibrosis.[14] Less invasive procedures for treatment of strictures are increasingly used, such as balloon dilatation,[15,16] polyvinyl over-the-guidewire dilatation[17] and injection of glucocorticoids into the strictures after dilatation.[18] However, the long-term efficacy of these measures is limited by the frequent recurrence of the problem. In order to develop better therapeutic approaches a much greater understanding of the mechanism of intestinal fibrosis is needed, which underscores the need of more studies of the cellular and molecular events underlying its pathophysiology.

Currently fibrosis is seen as the irreversible end stage result of chronic inflammation. Applying this view to the gut, recurrent inflammation is regarded to be an absolutely necessary process for the development of intestinal fibrosis.[7] However, novel concepts emerging from *in vivo* and *in vitro* experimental models suggest that fibrogenic mechanisms can be distinct and, to some degree, independent of those regulating inflammation.[19] In the case of IBD related fibrosis, however, it is practically impossible to separate the inflammatory from the fibrotic response as the cells responsible for each type of response are intimately associated and influencing each other in the mucosa microenvironment. Of note, the cells that are primarily responsible for ECM deposition, such as myofibroblasts, do so under the influence of signals derived from surrounding inflammatory cells.[13] Myofibroblasts are defined as an activated or differentiated form of fibroblasts.[20] In reality, at any site of inflammation, local mesenchymal cells are in a constant state of de- and transdifferentiation among fibroblast, myofibroblast and smooth muscle cell phenotypes.[21] For sake of simplicity and the specific goals of this review only the term "fibroblast" will be used when referring to these interrelated cell types (Fig. 1).

Fibroblasts are found in the interstitium of all normal tissues and organs where they are crucial contributors to local homeostasis.[22] Morphologic, phenotypic, molecular and functional differences among fibroblast from different locations have been described.[20,23] In case of persistent stimulus, injury or inflammation, fibroblasts become activated and express receptors for pro-inflammatory cytokines, such as TNF-α, becoming primary targets of the immune

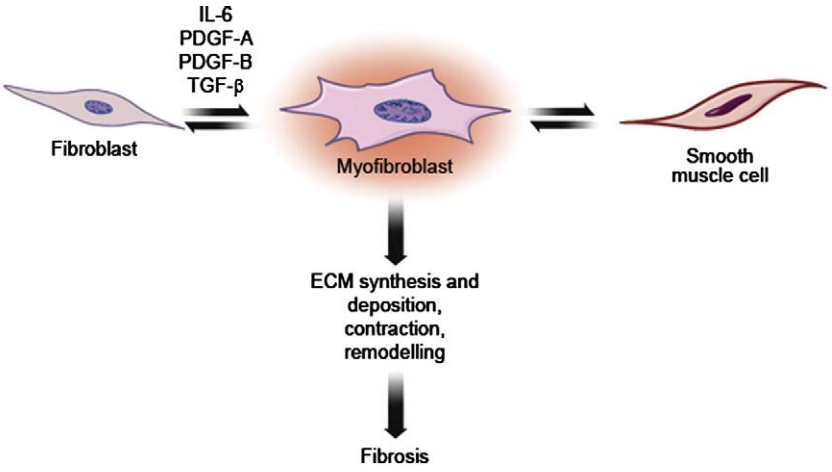

Figure 1 Transdifferentiation among mesenchymal cells. Intestinal mesenchymal cells are in a constant state of trans- and de-differentiation among fibroblast, myofibroblast and smooth muscle cell phenotypes. This process is driven by a variety of mediators present under both physiological and pathophysiological conditions of the intestinal mucosa. Therefore, all mesenchymal cell types can directly or indirectly contribute to intestinal fibrosis.

response.[24] They expand in number and secrete increased amounts of a large variety of molecules, including mediators that foster local inflammation and ECM proteins that contribute to local tissue remodeling and fibrosis.[13,25] This review will present some of the recent progress made in the understanding of intestinal fibroblast origin, differentiation, and function, and discuss the relevance of these processes to the pathophysiology of and potential new therapeutic approaches to intestinal fibrosis.

2. Known mechanisms of intestinal fibrosis

2.1. Fibroblast proliferation

To date the core mechanism responsible for the development of intestinal fibrosis is believed to be the growth and numerical increase of the resident fibroblast population. In support of this concept there are reports showing that fibroblasts isolated from IBD mucosa spontaneously display a faster rate of proliferation compared to that of fibroblast derived from non-IBD normal mucosa.[26,27] This difference was observed regardless of the type of IBD, increased proliferation being observed with fibroblasts from inflamed or fibrosed CD tissue, as well as inflamed UC mucosa. In addition to spontaneous proliferation, intestinal fibroblasts can increase their growth rate when exposed to various *in vitro* conditions, like those found in the inflamed gut. These include activation by several growth factors such as insulin-like growth factor I (IGF-I), basic fibroblast growth factor (bFGF), epithelial growth factor (EGF), connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), but also pro-inflammatory cytokines like interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α.[7,26,28–30] Although multiple mediators stimulate intestinal fibroblast proliferation, most reports show no differences in regard to *in vitro* growth rates of IBD and normal mucosa-derived cells.

Transforming growth factor (TGF)-β1 is generally considered as the chief mediator of fibrosis in essentially all organs, including the gut. Surprisingly, this factor has not been shown to have a definitive role in promoting proliferation of intestinal fibroblasts, despite doing so for fibroblasts from other tissues and organs.[26,31,32] However, TGF-β1 may indirectly impact on intestinal fibroblast proliferation through its capacity to upregulate the PDGF receptor, increase synthesis of CTGF, and promote expression of IGF-1, all of which could directly affect proliferation.[20,28] Thus, the function of TGF-β1 may be more directed at modulating differentiation and secretion rather than proliferation, in addition to playing critical role in pro-fibrotic pathways as discussed below.

In addition to soluble factors, other mechanisms and events may induce growth of fibroblasts. Direct cell-to-cell contact with inflammatory cells, such as mast cells or eosinophils, which are present in increased numbers in active IBD mucosa, can stimulate proliferation *in vitro*.[33–35] Most likely, T cells can also induce fibroblast proliferation through a direct cell-to-cell contact mechanism[36,37] (Fig. 2).

2.2. Fibroblast migration

Migration, defined as the active movement of fibroblasts into and through the surrounding ECM, likely represents another component of intestinal fibrosis.[7] During inflammation a chemotactic gradient is created due to the secretion of various molecules that induce cell migration into the affected area. Depending on the location of the inflammatory focus in the bowel wall, migration can probably arise from all surrounding tissue layers, including the mucosa, submucosa or muscle. As inflammation abates, the chemotactic gradient subsides and eventually disappears, resulting the cessation of fibroblast migration. How much fibrosis results as a consequence of migration largely depends on the intensity and duration of inflammation. A large number of soluble molecules with the potential for triggering fibroblast migration are found in essentially all tissues.[38,39] In the gut, fibroblasts can stimulate their own migration through autocrine or paracrine processes.[40,41] Fibronectin, which is synthesized by fibroblasts in large quantities, is considered

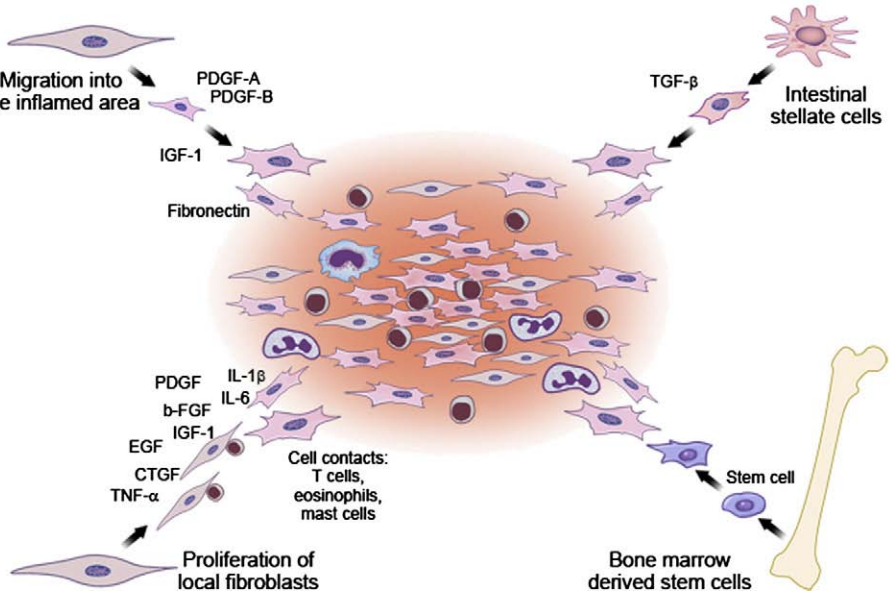

Figure 2 Different cellular sources in intestinal fibrosis. Fibroblasts contributing to intestinal fibrosis can derive from migration into the inflamed area, proliferation of local fibroblasts, differentiation from intestinal stellate cells, and influx from bone marrow-derived fibroblast precursors.

one of the most potent inducers of autocrine migration.[40] PDGF-A, PDGF-B, IGF-I and EGF also enhance migration, but their effect appears to be fibronectin-dependent.[40] The migratory response of fibroblasts has two components: an increase in chemokinesis (random movement) and chemotaxis (gradient-directed movement).

Once fibroblasts have been recruited to the inflammatory focus they must be locally retained, an action mediated by additional pro-inflammatory mediators, such as TNF-α and IFN-γ, both of which can reduce intestinal fibroblast migration *in vitro*.[42] This reduction persists as long as the cells are maintained in culture, and is more pronounced when fibroblasts isolated from CD mucosa are used, under spontaneous as well as cytokine-mediated conditions.[42] This behavior makes the intestinal fibroblast a cell highly reactive to the surrounding inflammatory milieu. This reactivity, however, may not to be generalizable to all fibroblasts, as studies show inconsistent results when fibroblasts isolated from different organs are tested.[43–45] How much a reduced migratory capacity contributes to fibrosis in IBD *in vivo* is still unclear. In fact, this mechanism remains speculative because growth factor-induced fibroblast migration occurs in the context of other biological responses and complex interactions with local immune and epithelial cells, and there are still not enough experimental data to meaningfully integrate these intricate responses (Fig. 2).

2.3. Intestinal stellate cells

It is well established that stellate cells are major contributors to fibrosis, a notion primarily based on a vast literature of studies of liver (where they are also termed fat- or vitamin A-storing cell, or Ito cell)[46] and pancreatic fibrosis.[47] Stellate cells are mesenchymal cells precursors that display low mitogenic activity and contribute to retinoic acid metabolism.[48] Upon activation, stellate cells differentiate into fibroblasts at sites of inflammation and become responsible for ECM accumulation by secreting a variety of matrix components and influencing their turnover.[49]

In contrast to the wealth of data on the role of stellate cells in liver and pancreatic fibrosis, very limited information is available on intestinal stellate cells. Cells with cytoplasmic projections compatible with stellate morphology and containing retinoid-rich lipid droplets have been described in the intestinal submucosa,[50] but their paucity and lack of specific markers make the definitive identification only tentative. Recently, our laboratory has started the functionally characterization of primary stellate cells directly isolated from the human intestine (Leite A., unpublished observations). Particularly interesting is the observation that stellate cells from CD and UC mucosa differentiate into fibroblasts at a much faster pace than those from normal non-IBD mucosa, as demonstrated by the quick acquisition of α-smooth muscle actin, a typical marker of mature mesenchymal cells. In addition, IBD stellate cells show an increased proliferation rate and produce collagen earlier in the differentiation process and at higher amounts compared to control cells. The differential behavior of IBD *vs* control cells suggest that stellate cells can be conditioned *in vivo* to acquire a pro-fibrotic behavior by their exposure to the chronic inflammatory milieu of the IBD mucosa (Fig. 2).

2.4. Extraintestinal fibroblast recruitment

In recent years it has become evident that adult bone marrow stem cells are not restricted to generation of cells of hematopoietic lineage as previously thought. Stem cells actually show a remarkable degree of plasticity, can engraft non-hematopoietic tissues, and can differentiate into an assortment of adult lineages found in those tissues, including fibroblasts, hepatocytes, endothelial cells, myocytes and epithelial cells.[51–54] The capacity to engraft is intensified in

damaged or diseased tissues. In both humans and animals stem cells have been shown to differentiate into intestinal pericryptal fibroblasts.[55] In addition, transplanted bone marrow cells contribute to intestinal tissue repair by generating activated fibroblasts. For instance, in TNBS-induced colitis transplanted bone marrow cells give rise to intestinal fibroblasts whose number increases with worsening disease severity.[56] In the IL-10$^{-/-}$ model of colitis a dramatic number (of up to 45%) of colonic subepithelial myofibroblasts can be of bone marrow origin.[57]

Fibroblasts derived from the bone marrow are as functional as native resident fibroblasts.[58] As an example, systemic administration of CD34-negative cells derived from the bone marrow or peripheral blood can enhance tissue repair in IBD even without ablation of the recipient original immune system.[59] In addition, in various conditions including tumor angiogenesis, tissue ischemia and corneal neovascularization, pericytes and mesenchymal cells enveloping small vessels can be bone marrow-derived. This is relevant to fibrosis because pericytes represent another cell type with the capacity to transdifferentiate into activated fibroblasts[60] (Fig. 2).

3. New mechanisms of intestinal fibrosis

In addition to the above cells and means contributing to intestinal fibrosis, evidence has recently emerged indicating that fibrosis can also result from entirely different mechanisms involving previously unknown cell differentiation, transformation and recruitment processes. It is also abundantly clear now that a number of mature non-mesenchymal cells are far more plastic than traditionally thought, and that mature fibroblasts are not necessarily directly derived from cells of mesenchymal origin.

3.1. Epithelial-to-mesenchymal transition

Throughout the body a sizeable amount of fibroblasts is generated through a process called epithelial-to-mesenchymal transition (EMT), a process that contributes to tissue fibrosis. EMT occurs in a variety of physiological and pathological situations, being initiated under the influence of embryonal, inflammatory or neoplastic events and characterized by dramatic changes in epithelial cell phenotype and function.[61,62] Epithelial cells lose classical epithelial markers like E-cadherin, catenins and cytokeratins, and acquire a spindle shape morphology, fibroblast proteins like fibroblast-specific protein (FSP)-1, α-SMA, and vimentin, and the capacity to produce interstitial collagens and fibronectin. In addition, changes in migratory and infiltrating ability also occur. Finally, cells that underwent EMT are more resistant to apoptosis and show a reduced rate of mitosis.[61,62] Among several molecules involved in EMT, TGF-β1 is the best established inducer. Various cytokines and growth factors may foster or accelerate transition, including IGF-1 and -2, EGF, FGF-2 and TNF-α, but also ECM molecules may promote EMT, like fibronectin and fibrin, as well as disruption of the basement membrane.[61,63,64] Interestingly, reactive oxygen species have also been shown to induce EMT[65] (Fig. 3).

There is convincing evidence that EMT occurs in multiple organs. The strongest evidence derives from studies of renal

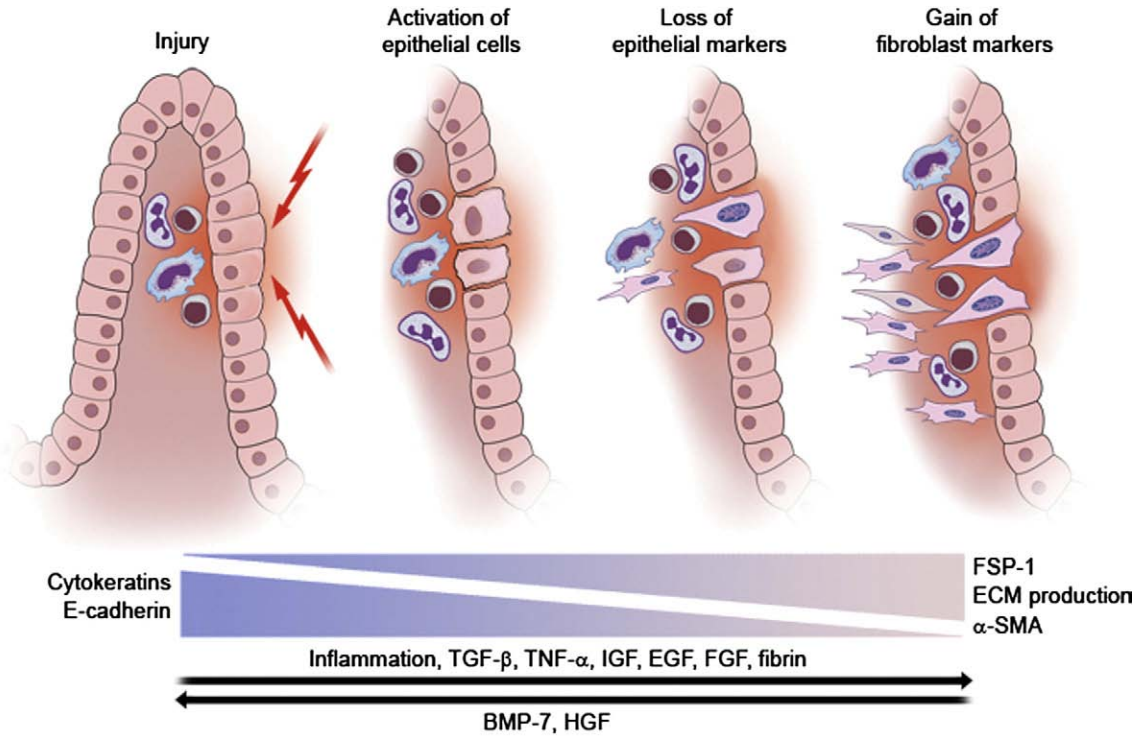

Figure 3 Epithelial-to-mesenchymal transition. Epithelial cells can transdifferentiate into fibroblasts under the influence of several factors produced under intestinal inflammatory conditions. This transition is accompanied by the progressive loss of typical epithelial cell markers (cytokeratins, E-cadherin) and the acquisition of typical mesenchymal cell markers (FSP-1, α-SMA, ECM production). This process can be reverted by the administration of BMP-7 or HGF.

fibrosis. Some studies indicate that, under chronic injury condition, more than 30% of renal fibroblasts arise from transformation of tubular epithelial cells.[66] EMT also contributes to pulmonary and liver fibrosis.[67,68] There is preliminary evidence that EMT occurs in the gut in the setting of IBD, suggesting a possible role for EMT in the process of fistula formation in patients with CD (Bataille F., unpublished observations). These reports open the door to the development of specific antifibrotic therapy. Bone-morphogenetic protein-7 (BMP-7) and hepatocyte growth factor (HGF) are able to antagonize EMT not only *in vitro*, but also *in vivo*. In animal models of kidney and liver fibrosis BMP-7 shows not only preventive but also therapeutic efficacy in reversing EMT,[66,67] and HGF overexpression also prevents fibrosis in these organs.[69–71]

3.2. Endothelial-to-mesenchymal transition

Endothelial-to-mesenchymal transition (EndoMT) is another form of cellular transformation relevant to fibrosis. In a murine system it can be shown that endothelial cells can derive from a common embryonic stem cell precursor which also gives rise to smooth muscle cells (SMC).[72] Of particular interest is the observation that during their differentiation such endothelial cells can switch their phenotype to a mesenchymal lineage, demonstrating a high degree of plasticity before they reach a final stage of differentiation. However, even after reaching their "final" differentiation stage, endothelial cells still retain the capacity to transdifferentiate, as they have been shown to transform into mesenchymal cells. Frid et al.[73] demonstrated that adult endothelial cells of bovine aortic or pulmonary artery origin can differentiate into SMCs *in vitro* . Transdifferentiation of endothelial cells into mesenchymal cells is also supported by findings in experimental wound repair systems where capillary endothelial cells converted into connective granulation tissue cells, and by the transition of microvascular endothelial cells into spindle-shaped mesenchymal cells under the influence of chronic inflammatory stimuli.[74–76] In a mouse model for cardiac fibrosis it has been calculated that endothelial cells contribute up to one-third of the total pool of tissue-infiltrating fibroblasts.[77]

Far less is known of the factors and events involved in the process of EndoMT when compared to existing knowledge on EMT. However, there are mechanistic similarities between the two processes, as TGF-β1also plays a central role as an inducer of EndoMT.[77,78] Insulin-like-growth factor-II, which is considered essential to embryonic development, can also induce EndoMT,[79] and a pro-inflammatory environment (IL-1β or TNF-α) can induce cutaneous endothelial cells to undergo EndoMT *in vitro*.[76] As for EMT, BMP-7 has the capability to not only prevent but also reverse EndoMT[77] (Fig. 4). Information on EndoMT in the gut microvasculature has yet to be reported. In this regard, it is intriguing that the cardiac and intestinal vascular systems bear some striking developmental, functional and morphological similarities.[80] This observation and the presence of key inducers of EndoMT in gut chronic inflammation make it likely that EndoMT also contributes to the pool of fibroblasts in chronic intestinal inflammatory processes like IBD.

3.3. Pericyte differentiation

In the mature vascular system arteries and veins are surrounded by single or multiple layers of vascular smooth muscle cells (vSMC), whereas capillaries are partially lined by single cells called pericytes.[81] Both cell types derive from Flk1-positive angioblasts, and share common cytoskeletal components such as α-SMA and desmin.[72] Several additional pericyte markers have been described: high molecular weight melanoma-associated antigen (HMW-MMA), platelet-

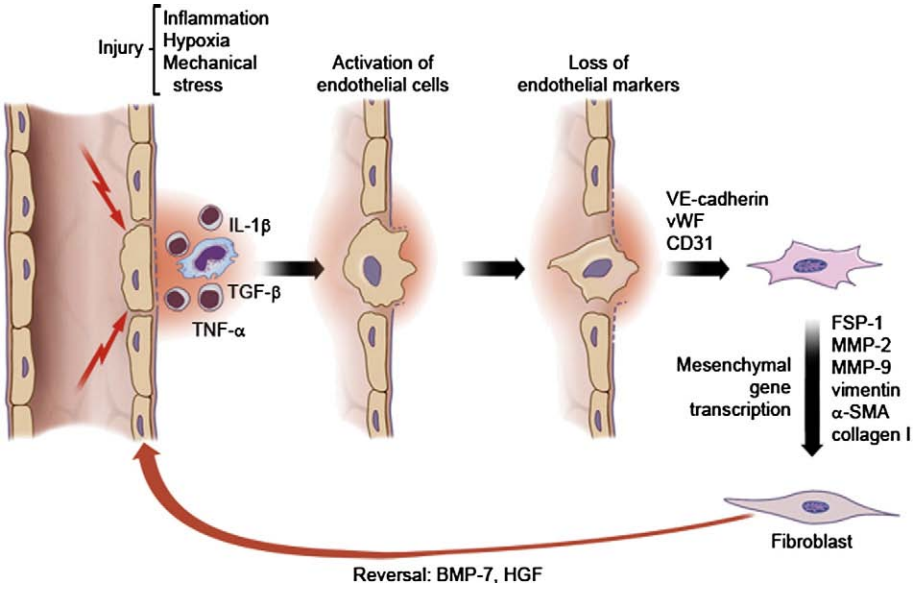

Figure 4 Endothelial-to-mesenchymal transition. Endothelial cells can transdifferentiate into fibroblasts under the influence of several factors produced under intestinal inflammatory conditions. This transition is accompanied by the progressive loss of typical endothelial cell markers (VE-cadherin, vWF, CD31) and the transcription of typical mesenchymal cell markers (FSP-1, MMP-2, MMP-9, vimentin, α-SMA, collagen I). This process can be reverted by the administration of BMP-7 or HGF.

derived growth factor β-receptor (PDGFR-β), aminopeptidase N, the promotor trap transgene XlacZ4, the regulator of G-protein signaling-5 (RGS5) and 3G5.[60,82,83] Their expression level is variable and none of these markers detects all types of pericytes.

Pericytes reside at the interface between the endothelium and the interstitium and, because of this peculiar location, exert multiple functions during inflammation, including sensing of endothelial signals, contributing to angiogenesis, controlling endothelial cell differentiation, and mediating of ECM degradation.[84] In addition, pericytes display an intermediate phenotype between vSMC and fibroblasts, and represent a cellular reservoir for fibroblasts during tissue repair.[84] Thus, pericytes also contribute to inflammation-associated tissue fibrosis. It has been proposed that, in cutaneous wound healing, pericytes detach from vessels and differentiate into a collagen type-I-producing fibroblast-like cell.[85] This may explain why in the initial phase of organ fibrosis there is marked ECM deposition in close proximity to the blood vessels, whereas in later stages fibrosis is more diffuse[86,87] (Fig. 5).

Little is known so far about the role of pericytes in intestinal inflammation and fibrosis, and investigation into this field is limited by the lack of adequate *in vitro* culture systems.[88] Using a mouse model of intestinal inflammation, Brittan et al. nicely demonstrated that both vSMCs and pericytes can be recruited from the bone marrow. Their contribution to intestinal fibrosis is still uncertain,[56] but because of their well defined involvement in both inflammation and fibrosis, pericytes may also be considered as potential new targets for controlling intestinal fibrosis.[89,90]

3.4. Fibrocyte recruitment

Fibrocytes are bone marrow-derived circulating mesenchymal progenitors that co-express hematopoietic and mesenchymal markers, including the stem cell antigen CD34, the leukocyte common antigen CD45, the monocytic cell marker CD14, and produce typical fibroblast proteins like collagens and α-SMA.[91,92] It is estimated that fibrocytes comprise up to 0.5% of all non-erythrocytic circulating cells.[93] They constitutively express ECM components as well as ECM-modifying enzymes, and differentiate into fibroblasts both *in vitro* and *in vivo*. Under normal conditions these cells likely contribute to the tissue-resident macrophage and dendritic cell population through a maturation process that takes place in the blood stream *before* entering the tissue.[94] In contrast, during inflammatory conditions, fibrocytes are released in high numbers from the bone marrow and migrate directly to inflamed tissue sites through a CCR2-mediated pathway. Once localized, in addition to macrophages and dendritic cells, they may differentiate into several other cell types, including epithelial, endothelial, neuronal cells and mesenchymal cells.[94–96] Fibrocytes can be distinguished from circulating or tissue-resident mesenchymal stem cells because these are CD90-positive and fail to express CD34, CD45, and monocyte markers. The combination of expression of CD34, CD45 or myeloid antigens, like CD11b and CD13, and collagen production, is considered a sufficient criterion to discriminate fibrocytes from resident leukocytes, dendritic cells, endothelial cells and tissue-resident fibroblasts.[91] When fibrocytes mature into fibroblasts at the site of tissue injury the expression of CD14 and CD34 is downregulated while that of α-SMA and collagen increase[91,97] (Fig. 6). TGF-β1, PDGF, IL-4, IL-13 and co-culture with T cells promote the differentiation of CD14-positive percursors into fibrocytes,[91,98] while activation of CD32 or CD64, or exposure to IFN-γ, IL-12 or serum amyloid P (SAP) inhibits their differentiation.[99] Interestingly, SAP is upregulated in the early stages of inflammation,[100] as IL-12 and IFN-γ also are, and this could in part explain the lack of fibrosis in acute inflammation.

Evidence of a causal link between the accumulation of fibrocytes at sites of injury and ensuing tissue fibrosis has been demonstrated in animal models of pulmonary, cardiac, renal and vascular diseases.[97,101–103] In these models, inhibition of fibrocyte accumulation results in reduced collagen deposition and decreased number of myofibroblasts. In humans fibrocytes have been detected

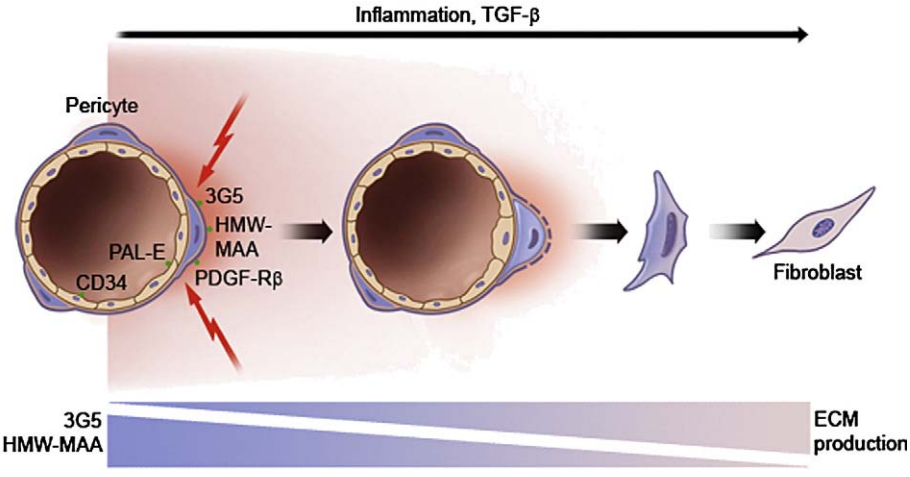

Figure 5 Pericyte-to-mesenchymal cell transition. Pericytes represent an additional cellular reservoir for fibroblasts in states of inflammation, tissue repair and fibrosis. They are attached to capillaries (indicated by the blood-vessel endothelial cell specific markers Pal-E and CD34) and differentiate into fibroblasts by losing pericyte markers (3G5, HMW-MAA, PDGF-Rβ) and acquiring typical fibroblast markers and functions.

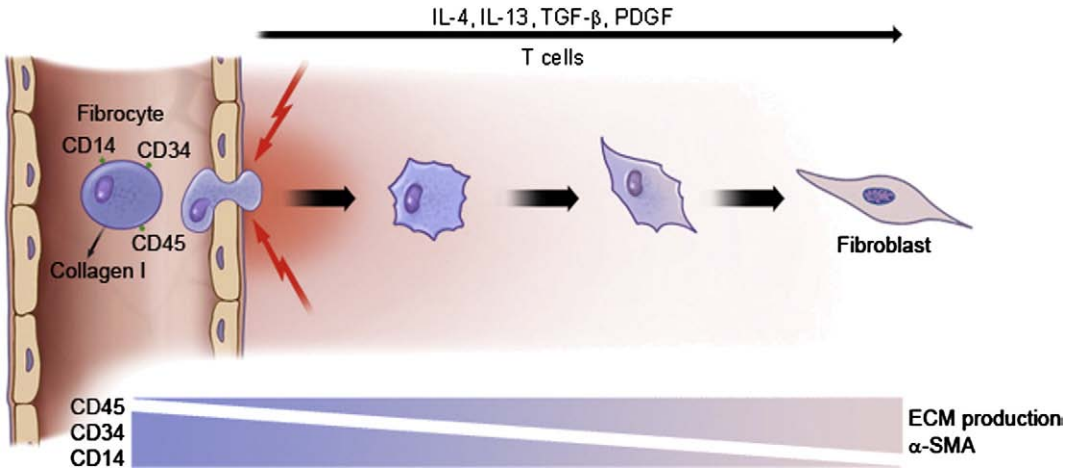

Figure 6 Fibrocyte recruitment. Fibrocytes are circulating mesenchymal precursor cells that are recruited to sites of inflammation, tissue repair and fibrosis. They differentiate into fibroblasts by losing fibrocyte markers (CD14, CD34, CD45) and acquiring typical fibroblast markers and functions (ECM production, α-SMA).

in tissues affected by post-burn hypertrophic scars and keloids, asthma, nephrogenic fibrosis, systemic sclerosis, atherosclerosis, chronic pancreatitis, chronic cystitis, and tumor-associated stromal reaction.[97,104–109] In all these conditions there are persistent inflammatory infiltrates concurrently with recruitment of inflammatory cells and fibrocytes. Similar events do occur in IBD and, therefore, a contribution of fibrocytes to the development of intestinal fibrosis is likely.

4. Conclusions and implications for therapy

4.1. Intestinal fibrosis: is it all inflammation-dependent?

Due to the characteristically chronic nature of the disease process, development of intestinal fibrosis is probably a fairly common event in IBD, at least at the tissue level. On the other hand, only a relative minority of patients will seek medical attention because of complaints primarily related to intestinal fibrosis, and the vast majority of them will do so because of symptoms secondary to difficulties created by the existence of a narrowed intestinal segment. By the time this series of events has fully unfolded most of the pathophysiological processes described in the preceding sections, including fibroblast proliferation, migration and recruitment, activation and differentiation of stellate cells, EMT and EndoMT, pericyte differentiation and fibrocyte recruitment, have already taken place. This obviously implies that whatever anti-inflammatory measures have been adopted at the bedside they have failed to prevent, block or reverse inflammation-driven intestinal fibrosis. In addition, concerns have been raised that some anti-inflammatory therapies, notably the use of infliximab, might even induce or worsen intestinal strictures due the scarring accompanying the healing process. In reality, this assumption is not supported by recent reports describing the safe administration of infliximab to CD patients with known fibrotic strictures,[110,111] and a lack of association between infliximab use and the development of strictures.[112] Even the injection of infliximab directly into a CD stricture appears safe and effective and not followed by stricture formation.[113] Some triggers, signals or patient-intrinsic predisposition may result in fibrosis regardless of whether anti-inflammatory measures are effective, and it has been proposed that fibrosis may develop independently of inflammation.[19] Current management of symptoms due to intestinal fibrosis is primarily invasive, more so as in the case of segmental resections or strictureplasty, or less so, as for balloon dilatation or local injections.[114]

4.2. Managing intestinal fibrosis in IBD: future perspectives

Taking all this evidence into consideration, it is clear that more effective management of intestinal fibrosis in IBD is badly needed. To this end, it may be worthwhile to establish a parallel between gut inflammation and fibrosis as far as the progress achieved in these two areas based on knowledge of the underlying mechanisms. In doing so a striking contrast becomes apparent: the impressive advances in IBD medical therapy experienced in the last decade can be ascribed to the development of new drugs – most of them biologicals – that are directly derived from knowledge acquired from investigation of the cellular and molecular mechanisms of mucosal immunity and inflammation; on the other hand, during the same period of time, progress in the management of intestinal fibrosis has been trivial, and this is so because negligible progress has been achieved in trying to understand why and how fibrosis develops in the setting of gut inflammation. Thus, the answer to how to better handle the problem of fibrosis in IBD obviously depends on a better understanding of its predisposing and pathogenic factors, and this can be done at different levels.

At a clinical level, tools should be developed to screen for individuals particularly susceptible to the development intestinal fibrosis. It could be argued that is the case when genetic testing detects *NOD2/CARD15* mutations in young

CD patients that go on developing ileal strictures associated with an increased risk of surgery.[115] A family history may help, but the identification of mutations in genes specifically encoding molecules involved in stimulating or modulating mesenchymal cell function or ECM protein production may help even more in screening for individuals at risk. Still at a clinical level, measurement of markers for mesenchymal cell or ECM turnover products in the circulation could also be valuable, such as anti-glycan antibodies (Rieder F., unpublished observations). Detection of fibrosis with new imaging modalities, some of which are currently under investigation, such as magnetization transfer MRI, MR elastography, US elastography, PET-MRI and PET-CT may help in identifying the very early stages of fibrosis and intervening accordingly.

At a more basic research level, studies should be carried out to understand whether gut fibrosis is developing as an event inherently linked to mucosal inflammation, or whether fibrosis can develop independently, totally or in part, from inflammation based on an entirely separate set of triggering and signaling pathways. This, of course, would help in deciding if a therapeutic anti-inflammatory intervention targeting the immune system would be the best choice, or whether cells and products of the mesenchymal lineage would be a better alternative target. In regard to the latter possibility, although we do not fully understand the mechanisms that regulate activation of fibroblasts and their accumulation during tissue fibrosis, it is reasonable to believe that the fibroblasts themselves might serve as a novel target in intestinal fibrosis. Similarly, targeting stellate cells, fibrocytes, pericytes, and EMT and EndoMT, as done in some *in vivo* models,[66,77,90,116] represents novel approaches to the prevention and therapy of IBD-associated fibrosis and its complications.

How to specifically target cells or events directly linked to development of intestinal fibrosis is at the moment rather challenging given the multiplicity of cells, factors and mechanisms involved in this process. Trying to block TGF-ββ1 makes theoretical sense based on current knowledge of IBD pathophysiology, but the potential dangers of blocking this critical immunosuppressive factor may overshadow its benefits. The administration of BMP-7 also makes sense considering its ability to antagonize EMT and EndoMT,[66,77] but safety and clinical efficacy would have to be very carefully evaluated. Trying to block recruitment and migration of fibrocytes, stellate cells and fibroblasts with antibodies to cell surface receptors would represent an alternative approach, with the potential risk of reducing the ability of repairing and healing an injured mucosa. *N*-(3′,4′-dimethoxycinnamoyl) anthranilic acid (Tranilast), a substance that inhibits TGF-β1-related functions, decreases fibrosis in experimental models,[117,118] and a report claims that its administration to CD patients with asymptomatic stenosis increases the symptom-free time and the diameter of the stricture lumen compared to placebo.[119] The significance of this observation is unclear at the moment. Thus, it is evident that additional studies and more progress must be accomplished before we can transfer a greater knowledge on the pathogenesis of intestinal fibrosis to the bedside.

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