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Can we prevent, reduce or reverse intestinal fibrosis in IBD?

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Abstract. – Intestinal fibrosis is a common complication of in inflammatory bowel disease (IBD) and can occur in both ulcerative colitis (UC) and Crohn's disease (CD), but is much more prevalent in CD. Fibrosis is a consequence of local chronic inflammation and is characterized by abnormal deposition of extracellular matrix (ECM) proteins producted by activated myofibroblasts. Current anti-inflammatory therapies used in IBD do not prevent nor they reverse established fibrosis and strictures. Despite the therapeutic advance in the treatment of IBD in the last two decades, the incidence of intestinal strictures in CD has not significantly changed. This implies that control of intestinal inflammation does not necessarily affect the associated fibrotic process. The conventional view that intestinal fibrosis is an inevitable and irreversible process in patients with IBD is progressively changing in light of improved understanding of the cellular and molecular mechanisms that underline the pathogenesis of fibrosis. Comprehension of the mechanisms of intestinal fibrosis may pave the way for the developments of anti-fibrotic agents and of new possible therapeutic approches in IBD. Nevertheless, there are important clinical issues that need further investigations, in particular the identification of factors relevant for the development of the intestinal fibrosis in IBD and the need of accurate and effective monitoring of the fibrotic progression and of effectiveness of the new proposed treatments.

Key Words:
Inflammatory bowel disease, Ulcerative colitis, Crohn's disease, Intestinal fibrosis, Therapy, Anti-fibrotic agents.

Abbreviations

IBD = Inflammatory bowel disease; UC = Ulcerative colitis; CD = Crohn's disease; ECM = Extracellular matrix; PAMPs = Pathogen-associated molecular patterns; PRRs = Pattern recognition receptors; TLRs = Toll-like receptors; DAMPs = Damage-associated molecular patterns; NOD = Nucleotide oligomerization domain; CARD = Caspase recruitment domain; ILs = Interleukins; INF-α = Interferon-α; INF-γ = Interferon-γ; TGF-α = Transforming Growth Factor-α; TGF-β = Transforming Growth Factor-β; TNF-α = Tumor necrosis factor-α; CTGF = Connective Tissue Growth Factor; PDGF = Platelet-Derived Growth Factor; bFGF = Basic fibroblast growth factor; IGFs = Insulin-like Growth Factors; EGF = Epidermal Growth factor; RAS = Renin Angiotensin System; NO = Nitric oxide; ROS = Reactive Oxygen Species; PPAR-γ = Peroxisome Proliferator Activated Receptor-γ; RXR = Retinoid X receptor; mTOR = Mammalian Target Of Rapamycin; VEGF = Vascular endothelial growt factor; MMPs = Matrix metalloproteinases; TIMPs = Tissue inhibitors of metalloproteinases; IGFBP = Insulin-like growth factor binding protein; MCP-1 = Monocyte chemotactic protein-1; MIP-1 = Macrophage inflammatory protein-1; ICC = Interstitial cells of Cajal; SEMFs = Intestinal subepithelial myofibroblasts; SMCs = Smooth muscle cells; α-SMA = α-Smooth-muscle actin; EMT = Epithelial-to-mesenchymal transition; EndoMT = Endothelial-to-mesenchimal transition; ETs = Endothelins; ACE = Angiotensin converting enzyme; ANG II = Angiotenin II; HSCs = Hematopoietic stem cells; MSCs = Mesenchymal stem cells; SAP = Serum Amyloid P; SCF = Stem cell factor; HGF = Hepatic growth factor; BMP-7 = Bone morphogenetic protein-7; MAPK = Mitogen-activated protein kinase; ERK = Extracellular signal-regulated kinase; JNK = c-Jun N terminal kinase; PIKK = Phosphatidylinositol 3-kinase-related kinase

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, progressive and relapsing inflammatory disorders of unknown etiology that may cause disability over time. Genetic, environmental and intestinal microbial factors have been reported to play a role in the etiology, pathogenesis and outcomes of IBD[1,2]. IBD represents a life-long disorder that may occur at any time from early childhood to late adulthood, although over 80% of cases are currently diagnosed in the second or third decade of life. UC is character-

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ized by inflammation in the large bowel mucosa, whereas CD is a trans-mural inflammation that may involve various sites of gastrointestinal (GI) tract, in 40-70% the terminal ileum[3]. Approximately 50% of the patients with IBD present a slightly evolutive disease with a low prevalence of relapses, hospitalizations, and complications[3]. Other patients have a more severe course and may develop complications that require surgery.

Progression of intestinal lesions may range from weeks to decades; however, it can be slowed down, stopped, or reversed spontaneously or by means of medical therapy[3,4]. Superficial mucosal lesions are most prone to heal, whereas deep ulcers or transmural fissures may heal with more difficulty and may be followed by the development of fibrotic strictures. IBD becomes symptomatic when lesions are extensive or distal, associated with a systemic inflammatory response, or when associated with local complications such as dilatation (toxic megacolon), massive hemorrhage, strictures, perforation (abscesses and fistulas) and cancer. Fibrosis and stricture formation is the result of an uncontrolled and an excessive process of intestinal healing, while perforation and fistulas a defective process of the tissue repair[5,6]. In CD, intestinal strictures, internal or perianal fistulas or abscesses are frequent, being reported in approximately one-third of patients. Colorectal lesions usually present more and early symptoms, whereas small bowel lesions may remain latent for several years[3,4]. The disease course is generally characterized by a sequence of flares and remission of varying duration, while approximately one fifth of these patients undergo a chronic, active, continuous disease course. Abdominal pain, abnormal bowel functions and rectal bleeding are the patients' main complaints that significantly alter their quality of life.

Acute intestinal inflammation is usually followed by physiologic healing of the damaged tissue and restoration of the normal structure and function of the intestine[5]. If this does not occur, chronic inflammation can develop and characterized by continuous events of injury and repair that may lead to the development of fibrosis[6]. In IBD, it is still unclear which factor triggers the road to chronicity. In addition, once intestinal inflammation is chronic, it is not yet understood what sets the stage for the later development of intestinal strictures.

Several lines of evidence suggest that inflammation is necessary to trigger the onset of the fibrotic process, but subsequently plays a minor role in progression of the disease[6]. Anti-inflammatory treatment in IBD and in other chronic inflammation-associated fibrotic conditions in various organs (lung, liver, kidney) does not prevent evolution of fibrosis once the process of excessive extracellular matrix (ECM) deposition has started. Mechanisms that regulate fibrosis, therefore, appear to be distinct from those regulating inflammation.

Fibrosis represents a common complication of IBD and follows the distribution and location of inflammation[7-9]. In UC, the deposition of ECM is restricted to the mucosal and submucosal layers of the large bowel and can induce structural changes (haustral loss, colonic shortening), and motility disorders of the colon. In CD, fibrosis can involve the entire bowel wall of the GI tract including the mucosa, submucosa, muscularis mucosa, muscularis propria and serosa layers and can result in critical narrowing of the lumen and strictures or stenosis, commonly leading to obstruction that requires surgery. The higher prevalence of fibrosis in CD is probably a consequence of transmural bowel inflammation which exposes all the mesenchimal cells producing ECM to inflammatory mediators released by the activated immune and non-immune cells[7]. Of note, course and extent of intestinal fibrosis in IBD display significant variability among individual patients. This suggests that the susceptibility to intestinal fibrosis may have a genetic component. Host genetic factors are likely to play key roles in the modulation of intestinal fibrosis and to contribute to the overall variability in disease progression. In recent years, different genetic polymorphisms that may influence the progression of intestinal fibrosis have been identified in animal models and human case-control studies. These findings indicate that variants of genes encoding immunoregulatory proteins, pro- and anti-inflammatory cytokines, and fibrogenic factors may determine the development of intestinal fibrosis in patients with IBD. In particular, patients with CD associated with mutation of NOD2/CARD15 genes, either alone or in combination with mutation of toll-like receptors (TLRs) (especially TLR4) or ATG16L1 (an autophagy gene) have an increased risk of fibrostenosis of the small bowel[10,11]. Furthermore, an association between T280M and V249I polymorphisms of CX3CR1 gene and fibrostenosing CD was reported. In addition, CD patients with a stronger immune response to microbial peptides are more likely to develop earlier fibrostenotic

disease[12,13]. Genetic association studies have a great potential for identification of fibrogenic genes, but large-scale, well-designed studies are required to clarify their actual relevance in IBD and to provide a solid framework for future therapeutic strategies.

Current anti-inflammatory therapies used in IBD do not prevent nor they reverse established strictures, which may present years after remission of active inflammation. Despite the therapeutic advance in the treatment of IBD in the last two decades, the incidence of intestinal strictures in CD has not significantly changed[14-17]. This implies that control of intestinal inflammation does not necessarily affect the associated fibrotic process. In IBD, in contrast to the intensive investigation of the immunological mechanisms of intestinal inflammation, the pathophysiology of fibrosis is remained largely unexplored. The lack of efficient and well-tolerated anti-fibrotic drugs is partly due to the fact that the main and specific cellular and molecular pathways leading to fibrosis remain to be identified[18]. An other major obstacle in the development of anti-fibrotic drugs is the slow evolution of the intestinal fibrosis in IBD. A clinical benefit may only be observed after a prolonged period of treatment: clinical trials could be long and expensive. Therefore, there is a urgent need of noninvasive methods, such as serum markers (growth factors, ECM turnover products) or imaging techniques (magnetization transfer MRI, MR elastography, US elastography, PET-MRI, PET-CT), to quickly quantify changes in the natural history of a disease and in particular in the response to specific medical treatments.

Cellular and Molecular Mediators of Intestinal Fibrosis

The conventional view that intestinal fibrosis is inevitable and irreversible process in patients with IBD is progressively changing in light of improved understanding of the cellular and molecular mechanisms that underline the pathogenesis of fibrosis[6-9,18].

Fibrosis is a chronic and progressive process acting through complex cell/matrix/cytokine and growth factors interactions but it may be a reversible event[6-9].

Intestinal fibrosis results from an abnormal response to a chronic local injury and is characterized by abnormal production and deposition of ECM proteins by activated myofibroblasts, which are also called ECM-producing cells[6-9,18-21] (Figure 1). Multiple ECM component contribute to the excessive net deposition ECM that typify intestinal fibrosis in IBD. These include type I, II and IV collagen, fibronectins and laminins. ECM-produc-

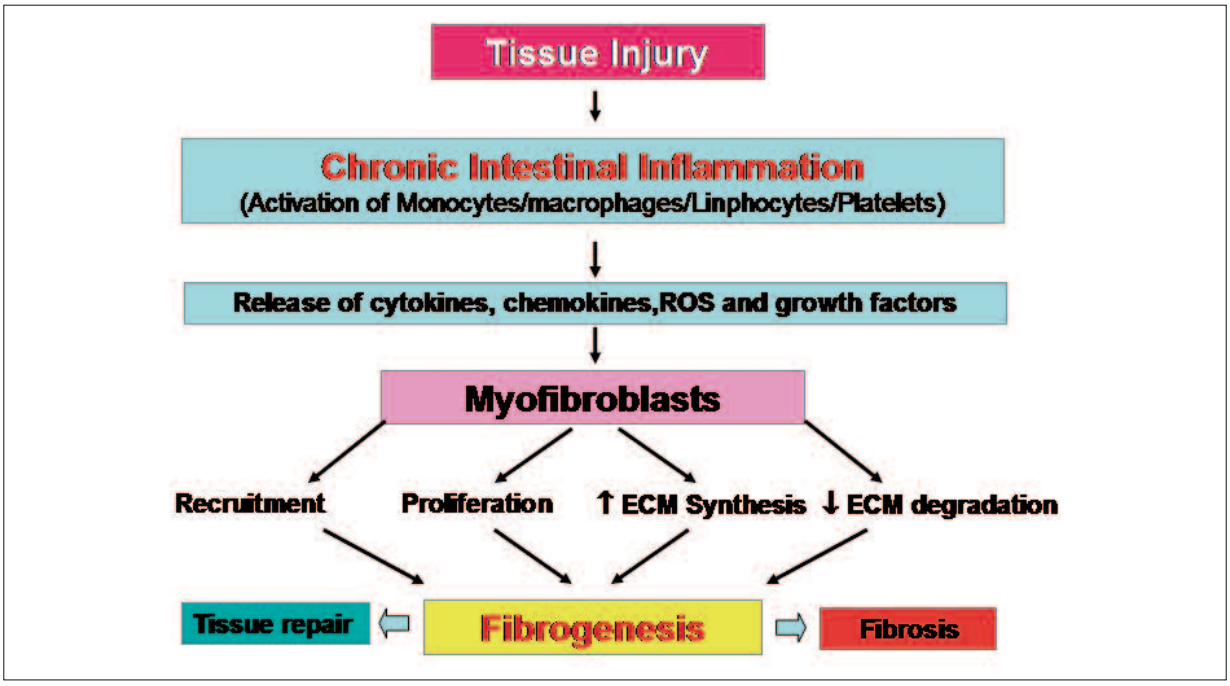

Figure 1. Sequence of events occurring from tissue injury to fibrosis. ROS = Reactive Oxygen Species; ECM = Extracellular Matrix.

ing cells are derived not only from resident mesenchymal cells (fibroblasts, sub-epithelial myofibroblasts and smooth muscle cells) but also from epithelial and endothelial cells (by a process known as epithelial/endothelial-mesenchymal transition), stellate cells, pericytes, and intestinal or bone marrow stem cells[6-9,18] (Figure 2).

Fibroblasts, located in the interstitium of normal tissue, play a central role in maintaining structural integrity and taking part in healing and regenerative processes. The increase in the resident fibroblast population is a pivotal mechanism for the development of intestinal fibrosis[6-9]. Several growth factors found in the inflammed gut, such as insulin-like growth factors (IGF-I), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF), platelet derived growth factor (PDGF), and pro-inflammatory cytokines, such as interleukins (IL-1β, IL-6) and tumor necrosis factor-α (TNF-a), increase their proliferation rate[6-9].

Myofibroblasts represent a highly contractile cell type that exhibit a "hybrid" phenotype between fibroblasts and smooth muscle cells and, when activated, synthesize high levels of ECM[18-23]. Besides their normal activities in growth and differentiation of tissues, the myofibroblasts play a central role both in wound healing and fibrosis. Two types of myofibroblasts are present in the intestine: the intestinal sub-epithelial myofibroblasts (SEMFs) and the interstitial cells of Cajal (ICC)[22-25]. SEMFs are mainly located at the base of the intestinal crypts in the lamina propria and form a three-dimensional network in connection with epithelial cells. ICC are located in the submucosa and muscularis propria in association with the smooth-muscle layer of the gut[24,25]. ICC are pacemaker cells which regulate gastrointestinal smooth muscle motility. It is unknown whether SEMFs and ICC differentiate from a common precursor. Mediators which promote myofibroblasts proliferation and ECM production are numerous including PDGF, EGF, IGF-1 and 2, CTGF, IL-1, IL-13, stem cell factor (SCF), endothelins (ET-1, -2, -3), angiotensin II (ANG II), transforming growt factor-α (TGF-α), transforming growth factor-β (TGF-β), bFGF and peroxisome proliferator activator receptor-γ (PPAR-γ)[6-9,21,26].

Smooth muscle cells (SMCs) are one of the three cell phenotypes into which intestinal mesenchymal cells can differentiate and in the chronic inflammation can trans-differentiate into myofibroblasts[8,9]. SMCs act in different way in IBD: in UC lead to a considerable thickening of the muscolaris mucosa and, in CD, to a remarkable thickening of the bowel wall, with subsequent stricture formation and obstruction. These cells actively contribute to the development of fibrosis

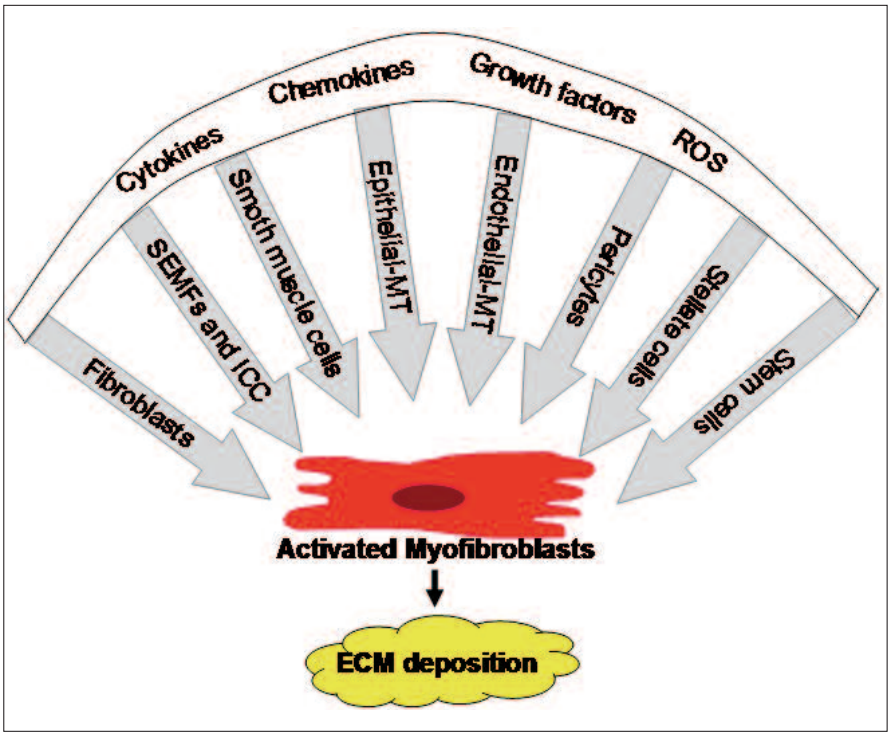

Figure 2. Cellular mediators of intestinal fibrogenesis. SEMFs = subepithelial myofibroblasts; ICC = interstitial cells of Cajal; Epithelial-MT = epithelial-to-mesenchymal transition; Endothelial-MT = endothelial-to-mesenchymal transition; ECM = Extracellual Matrix.

in IBD by inducing collagen and matrix metalloproteinases (MMPs) production in response to several inflammatory mediators, such as TGF-β and IL-1β. SMC are also able to release significant amounts of IL-6 contribuiting to the inflammatory process[27].

Stellate cells has been detected in several organs, such as the liver, pancreas, lung, uterus, kidney and gut and their important contribution to the development of fibrosis has been shown[28,29]. These cells have been isolated and cultured from the human intestinal mucosa[30]. In IBD, intestinal stellate cells show a higher proliferation rate, faster differentiation into myofibroblasts, and an earlier and higher collagen production than those from the normal mucosa[30].

Pericytes, derived from non-differentiated mesenchymal cells, surround the endothelial cells of capillaries and small blood vessels[31]. Pericytes control endothelial cell differentiation, endothelial signalling and angiogenesis and ECM deposition. They represent a useful reserve of fibroblasts during tissue repair and inflammation-associated fibrosis[30]. Pericytes increase the deposit of ECM proteins in proximity to the blood vessels during the initial phase of fibrosis.

The main intestinal fibrogenic cells (fibroblasts, myofibroblasts) may also derive from non-mesenchymal cells, including epithelial and endothelial cells via transformation. Epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndoMT) are characterized by dramatic changes in cell phenotype and function and play a key role in fibrosis[32,33]. By this process, epithelial or endothelial cells assume a spindle-shape morphology, lose classical cell markers and gain typical fibroblast or myofibroblast markers. It has been shown in animal models and in human primary cells that EMT and EndoMT can contribute to intestinal fibrogenesis[32,33].

Myofibroblasts may derive from stem cells, a non-differentiated cell type capable of producing all the specialized cell types of the tissue[34,35]. Bone marrow contains hematopoietic and mesenchymal stem cells, which are able to migrate to the majority of organs and differentiate into various cell types. Hematopoietic stem cells (HSCs) give rise to 3 classes of blood cells (leukocytes, erythrocytes and thrombocytes), whereas mesenchymal stem cells (MSCs) can differentiate into several cell types, including myofibroblasts. A class of bone marrow-derived cells that become progenitors for mesenchymal cells is represented by fibrocytes that circulate in the peripheral blood[8,9,19,36]. These cells appear to be involved in the intestinal repair and fibrosis in IBD[19,36]. Fibrocyte functions that lead to tissue fibrosis are modulated by IL-1, TGF-β and Serum Amyloid P (SAP). Fibrocytes themselves produce growth factors (TGF-β, CTGF), inflammatory cytokines and chemokines that in turn promote the proliferation of resident fibroblasts and their differentiation into myofibroblasts[19,36].

Fibrogenesis is a "physiological process" triggered by the onset of inflammation that may lead either to tissue repair or fibrosis depending on the balance between production of ECM proteins and enzymatic degradation[6-9] (Figure 1). Increased production of ECM in intestinal fibrosis is related to the abnormal function of activated intestinal myofibroblasts (proliferation, migration, contraction, ECM production, and resistance to apoptosis). Myofibroblasts are activated by a variety of mechanisms including paracrine signals derived from immune and non-immune cells, autocrine factors secreted by myofibroblasts, and pathogen-associated molecular patterns (PAMPs) derived from micro-organisms that interact with pattern recognition receptors (PRRs) such as TLRs[9,18]. Myofibroblsts can also be activated by products derived from injiured cells, the so-called damage-associated molecular patterns (DAMPs). These include several products such as DNA, RNA, ATP, HMGB, microvesicles, fragments of ECM molecules.

It is well known that an exquisite equilibrium between cell proliferation and programmed cell death (apoptosis) is required to maintain physiological homeostasis in any tissue. The main regulators of apoptosis include caspases, Bcl-2, Bax, p53 and focal adhesion kinase (FAK). Caspases are a family of cysteine-dependent aspartate-directed proteases that play an integral role in the cascade that leads to apoptosis. Caspases are grouped as either initiators or effectors of apoptosis, depending on where they enter the cell death process. Bcl-2 is the prototype anti-apoptotic protein that localizes to the mitochondria and blocks the recruitment and activation of pro-apoptotic proteins, such as Bax, to the mitochondria. FAK inhibited activity of p53 with the transcriptional targets: p21, Bax and Mdm-2 through protein-protein interactions. In the presence of tissue fibrosis, there uniformly are a greater number of ECM-producing cells, which is secondary to an increase in their proliferation and a decrease in their apoptosis[7-9]. It has been demonstrated that

apoptosis is responsible for mediating the reduction in myofibroblasts number during the resolution of fibrosis of various organs and, conversely, that induction of myofibroblasts apoptosis has an antifibrotic effect[14]. NOD2/CARD15 and ATG16L1 genes, also expressed by myofibroblasts, enhances apoptosis through induction of caspases expression[14]. In CD, mutations of these genes are associated with an increased risk of the small bowel fibrostenosis[10-14]. Whereas several studies have emphasized the potential importance of tissue inhibitor of metalloproteinases (TIMPs) to fibrosis via the inhibition of matrix degradation, individual TIMPs may regulate cell division and apoptosis independently of this activity[14]. TIMP-1, overexpressed in CD fibrostenosis, suppresses myofibroblasts apoptosis both *in vitro* and *in vivo*, highlighting a potential role for myofibroblasts survival in fibrosis. Hepatocyte growth factor (HGF) reduces fibrosis by increasing apoptosis. HGF is a potent inducer of ECM-degrading enzymes such as the matrix metalloproteinases (MMPs), which are overexpressed during myofibroblasts apoptosis[14]. MMPs induce apoptosis in myofibroblasts through the extracellular degradation of fibronectin and that the antifibrotic effects of HGF is due to upregulation of MMPs and MMP-dependent myofibroblast apoptosis. Proliferation and apoptosis of ECM-producing cells can represent key players in intestinal fibrogenesis and new targets for therapeutic intervention[14]. Some therapies have demonstrated potential antifibrogenic efficacy through the regulation of mesenchymal cell proliferation and apoptosis.

ECM degradation is mediated by MMPs and TIMPs. The fine balance between MMPs and TIMPs appears to be disturbed in chronically impaired wound healing in IBD[37-39]. It is unclear which specific MMPs and TIMPs are involved and how they are regulated in this process. Accumulating data indicate that an imbalance of tissue-degrading enzymes and their inhibitors may cause intestinal fibrosis. Nevertheless, effective pharmacological modulation of the MMP/TIMP-system could be helpful in the reversal of already established tissue fibrosis.

ECM is not an inactive structure, but directly regulates the inflammatory response and the process of healing and fibrosis by focal adhesions with immune and non-immune cells, such as myofibroblasts[5]. Intestinal ECM can act as a binding partner or reservoir for profibrotic tissue factors. ECM can anchor, store and release cytokines and chemokine[9]. TNF-α, TGF-β and bFGF interact with various ECM moieties. ECM fragments can bind to and activates TLR2 and TLR4 and trigger an innate immune response, as well as can modulate migration and proliferation of immune ad non-immune cells, including myofibroblasts.

All intestinal cell types that produce ECM proteins act synergistically and are under the control of various biological mediators, such as growth factors, cytokines, chemokines, proteolytic enzymes, complement components, vasoactive amines and peptides[6-9,21,26] (Table I). The most important of these molecules include, TGF-β, activins, CTGF, PDGF, IGF-1 & 2, EGF, ET-1, -2, -3, various cytokines such as IL-1, -4, -6, -13, -17, -21, -22, -23, TNF-α, components of the renin-angiotensin system (RAS), angiogenic factors (e.g., vascular endothelial growth factor – VEGF), PPARs, mammalian target of rapamycin (mTOR), and products of oxidative stress[6-9,21,26]. Other molecules, such as MMPs and TIMPs, are also involved in regulating ECM turnover. All these molecules are being investigated as potential targets of anti-fibrotic drugs. Pharmacological modulation of tissue ECM deposition by reducing activated ECM-producing cells and their profibrogenic effects (proliferation, motility, contraction, ECM production) could be useful in the prevention and treatment of intestinal fibrosis[6-9,21,26]. Soluble factors with anti-inflammatory properties have been identified including Interferon-α (INF-α), INF-γ, IL-7, IL-10, IL-12, Smad7 protein, adiponectin, nitric oxide (NO), as well as MMPs.

Timing, concentration and sources of the main pro-fibrotic mediators might affect their individual contribution to tissue remodelling and fibrosis. Furthermore, a simultaneous action of some pro-fibrotic mediators appear relevant in the development of fibrosis.

Of all the pro-fibrotic molecules, TGF-β and its extra- and intracellular pathways appear to play a critical role in regulating the development, proliferation, and differentiation, as well as in activation of intestinal mesenchymal cells and in stimulation of ECM proteins synthesis leading to fibrosis[6,8,9,18-21]. The canonical TGF-β intracellular signal transduction pathway is mediated by Smad proteins[40,41]. The activation of TGF-β receptors phosphorylates Smad2 and Smad3 binding with the common mediated Smad4. The Smad2/3-Smad4 complex translocates into the nucleus where it regulates specific TGF-β target

Table I. Molecules involved in fibrogenesis and fibrosis.

Fibrogenic	Anti-fibrogenic
• Transforming growt factor-β (TGF-β) • Smad2/3 proteins • Activin A • Connective tissue growth factor (CTGF) • Platelet derived growth factor (PDGF) • Insulin-like growth factors (IGF-I and II) • Epidermal growth factor (EGF) • Basic fibroblast growth factor (bFGF) • Cytokines (IL-1β, IL-4, IL-6, IL-13, IL-17, IL-21, IL-22, IL-23, IL-33, TNF-α) • CC- and CXC-chemokines (CCL2, CCL3, CCL4, CCL20) • Reactive Oxygen Species (ROS) • Mammalian Target Of Rapamycin (mTOR) • TLR2&4 ligands • Vascular endothelial growth factor (VEGF) • Endothelins (ET-1) • Angiotensin Converting Enzyme (ACE) • Angiotensin-II (AT-II) • Norepinephrine • Thrombospondin-1,2 • Leptin • Tissue inhibitor of Metalloproteinases (TIMPs)	• Peroxisome Proliferator Activator Receptor-γ (PPAR-γ) • Interferon-α (INF-α) • Interferon-γ (INF-γ) • IL-7, IL-10, IL-12 • Smad7 protein • PGE2 • Hepatocyte growth factor (HGF) • Adiponectin • Nitric Oxide (NO) • Relaxin • Matrix Metalloproteinases (MMPs)

genes. TGF-β signaling is negatively regulated by inhibitory Smad7. Besides Smads downstream patways, TGF-β can also modulate, in a Smad/independent manner, other signal transduction pathways such ERK/cJUN/p38 MAP kinases. Important Smad-dependent profibrotic effects of TGF-β include activation of myofibroblasts, stimulation of collagens, CTGF and TIMPs, as well as the inhibition of MMPs.

The TGF-β/Smad pathway plays a crucial role in promoting intestinal fibrosis[6,21]. Both TGF-β and its receptors are over-expressed in intestinal cell of patients with IBD, and in particular in fibrostenotic CD, as well as in in animal model of experimental intestinal fibrosis[41-43]. Adenovirus-mediated overexpression of TGF-β in the murine colon leads to colonic fibrosis and obstruction[44]. On the other hand, Smad3 loss confers resistance to the development of TNBS experimental colorectal fibrosis as demonstrated in our previous studies in which the colon from Smad3 wild-type mice showed a marked increase in submucosa and serosa layer thickness compared to Null mice[45,46] (Figure 3). Several studies have demonstrated that disruption of the TGF-β/Smads signalling pathway, either by the loss of Smad3 or the increase of Smad7 expression, confers resistance to tissue fibrosis in several organs including skin, kidney, lung, liver and intestine[46-55]. The findings of a decreased Smad7 and an increased pSmad2/3 expression observed in intestinal strictures in CD support the profibrogenic role of the TGF-β/Smad pathway in this disease[56].

Although TGF-β/Smad pathway represents the driving force of fibrotic process ("core pathway"), several pro-fibrogenic molecules, such as activins, CTGF, PDGF, bFGF, IGF-1, interleukins (IL-1, IL-6, IL-13), TNF-α, angiotensin converting enzyme, angiotensin II, αvβ6 integrin and mTOR, as well as anti-fibrogenic molecules (PPAR-γ, Smad7, IL-7, IL-10, IL-12, INF-α and γ, HGF) seem to interact directly with TGFβ/Smad pathway (Figure 4).

Activins, members of the TGF-β superfamily, activate Smad transcription factors and the MAP kinase signaling pathways. Important functions of activins, in particular of activin A, in tissue inflammation, repair and fibrosis of several organs, including the intestine, have been reported[57,58]. Activin levels are increased in IBD and in many other inflammatory diseases, thus giving rise to the hypothesis that it plays a significant role in the inflammatory response, as well as in fibrosis[57].

CTGF acts as a downstream mediator of TGF-β action on connective tissue cells, where it stimulates cell proliferation and ECM synthesis. CTGF is co-expressed with TGF-β in principally every fibrotic disorder and is considered a possible key determinant of progressive fibrosis[59]. CTGF expression is controlled by TGF-β in a Smad-de-

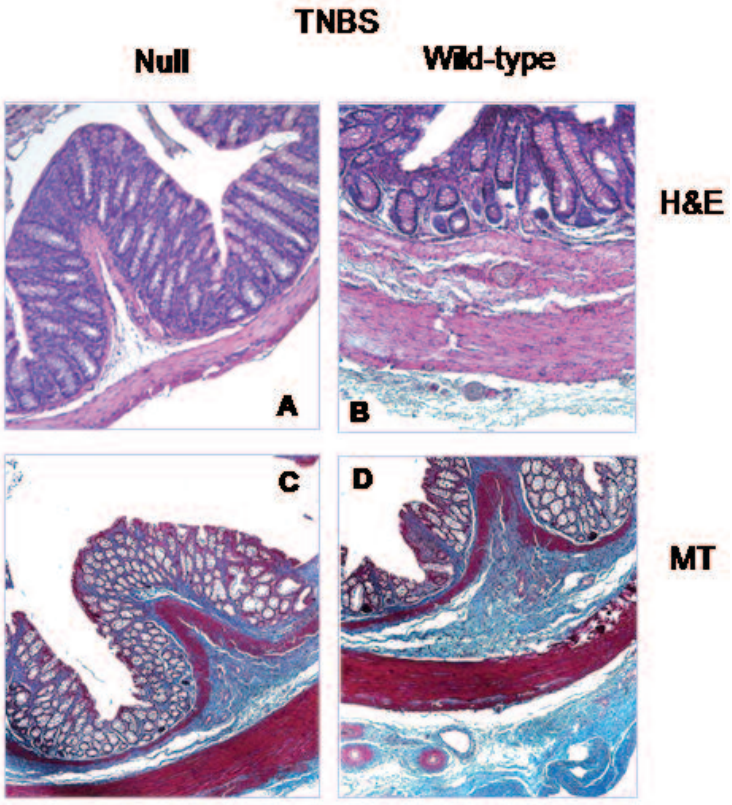

Figure 3. Haematoxilyn-Eosin (H&E) and Masson's Trichrome (MT) staining (O.M. 4X). Following TNBS treatment, the colon from Smad3 wild-type mice (B/D) showed a marked increase in submucosa and serosa layer thickness (mainly due to abnormal deposition of connective tissue) compared to Smad3 Null mice (A/C).

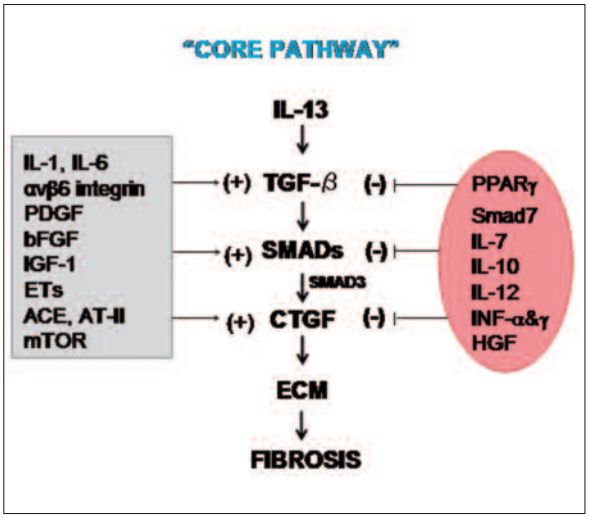

Figure 4. Relationship among several pro-fibrotic and anti-fibrotic mediators in the development of fibrosis. IL-13 = Interleukin-13; TGF-β = transforming growt factor-β; CTGF = connective tissue growth factor; PDGF = platelet derived growth factor; IGF-I = insulin-like growth factors I, EGF = epidermal growth factor; bFGF = basic fibroblast growth factor; ETs = endothelins; ACE = angiotensin convertingenzyme; AT-II = angiotensin-II; mTOR = mammalian target of rapamycin; PPAR-γ = peroxisome proliferator activator receptor-γ; INF-α and β = interferon-α and β; HGF = hepatic growt factor.

pendent manner. In addition to TGF-β, a number of other regulators of CTGF expression have been identified, including VEGF, TNF-α and reactive oxygen species (ROS)[60]. Due to the multiple biological actions of TGF-β, CTGF may serve as a more specific target for selective intervention in processes involving connective tissue formation during fibrotic disorders[59]. CTGF is an interesting molecule for future anti-fibrotic therapies as it is possible that inhibition of CTGF might block the pro-fibrotic effects of TGF-β, without affecting TGF-β's immunosuppressive and anti-inflammatory effects.

PDGF expression is significantly increased in the inflamed intestine of patients with IBD, especially in CD, and collagenous colitis[61,62]. Intestinal fibroblasts, intestinal SEMFs and ICC are activated and proliferate in response to the PDGF family. PDGF also enhances migration of fibroblasts, and its effects seem to be fibronectin-dependent. Increased activity of PDGF is also responsible for an excessive deposition of ECM in fibrotic processes of the intestine.

IGF-I and II and their respective receptors are expressed in the intestine and interact principally with fibroblasts and epithelial and endothelial

cells. IGF-I plays a relevant role in the deposition of collagen and fibrosis. It has been shown to be up-regulated in the bowel of animals with experimental intestinal fibrosis and of patients with CD[63]. IGF-I, through insulin-like growth factor binding proteins (mainly IGFBP-5), appear to be able to modulate proliferation of fibroblasts/myofibroblasts and collagen synthesis[64].

IL-1 contributes to the development of fibrosis during chronic intestinal inflammation through the induction of myofibroblast activation and induction of chemokines and MMPs secretion[65]. Furthermore, IL-1, in combination with TNF-α and INF-γ, is able to increase the TGF-β-induced epithelial-mesenchymal transition (EMT), an important cellular process of fibrogenesis[66]. Recently, it has been reported that IL-33, a novel member of the IL-1 family, may lead to the development of fibrosis[67].

IL-6 is markedly increased in CD where it appears to stimulate fibrogenetic mesenchymal cells[68]. IL-6 modulates TGF-β and TGF-βR2 expression and stimulates fibroblasts proliferation[69,70]. IL-6 neutralization improve fibrosis.

IL-4 and IL-13 are overexpressed in fibrotic processes and induce activation and differentiation of fibroblasts to myofibroblasts and production of collagens[21,26]. IL-13 signalling through the corresponding receptor IL-13Ra induces production of TGF-β[42,71]. Intestinal fibrosis development in TNBS-induced chronic colitis depends upon IL-13 binding to the IL-13 receptor to induce TGF-β[42,71]. IL-13 signalling inhibition leads to a reduced production of TGF-β and a lower amounts and fibrosis[42,71]. Soluble IL-13Ra2-Fc is a highly effective decoy receptor of IL-13, which can reduce the progression of established fibrotic disease. IL-10 has also been shown to inhibit fibrosis in numerous experimental models[21,26]. The IL-13 decoy receptor and IL-10, by suppressing collagen deposition, act as endogenous factors that slow the progression of fibrosis.

TNF-α, abundantly expressed in the intestine of patients with CD and UC, is an other central mediator of the fibrotic process[72]. TNF-α is able to induce intestinal fibrosis by stimulating myofibroblasts proliferation and up-regulating collagen accumulation. TNFR2 is essential for TNF-α-induced intestinal myofibroblasts proliferation and collagen synthesis[73]. Furthermore, TNF-α induces TIMP-1 expression and reduces MMP-2 activity and collagen degradation. TNF-α also appears to have additional effects on collagen synthesis when combined with IGF-I[64]. IGF-I and TNF-α synergistically stimulate intestinal myofibroblast proliferation and collagen production[64].

Chemokines are leukocyte chemoattractant that cooperate with profibrotic cytokines in the development of fibrosis by recruiting myofibroblasts, macrophages and other key effector cells to sites of tissue injury[21,26]. Although a large number of chemokine signaling pathways are involved in the fibrogenesis, the CC- and CXC-chemokine receptor families have exhibited important regulatory role. Specifically, CCL3 (macrofage inflammatory protein-1 – MIP1) and CCL2 (monocyte chemoattractant protein-1 – MCP1) were identified as profibrotic mediators. Interrupting specific chemokine signaling pathway could have a significant impact on the treatment of fibrosis. Blockade of CC- and CXC chemokine receptors decreases the progression of fibrosis in association with decreased IL-4 and IL-13. Among these chemokines, CCL2 (MCP-1), CCL3 (MIP-1a), CCL4 (MIP-1b) and CCL20 (MIP-3a) are increaded in IBD tissue[9].

Local renin-angiotensin system (RAS) has novel functions including the regulation of cell growth, differentiation, proliferation and apoptosis, generation of ROS, expression of cytokines (such as TGF-β_1, IL-6, INF-α), activation of endothelial cells, as well as tissue inflammation, ECM production and fibrosis[74]. It has been reported that all components of the RAS exist in the human large bowel[75]. Angiotensin II (ANGII), the principal effector of RAS, participates in the pathogenesis of chronic fibrogenetic diseases of several organs, including kidney, hearth, blood vessels, lung, pancreas, liver and intestine, through the regulation of both inflammatory and fibrotic processes[76,77]. ANGII is increased in the colonic mucosa of CD patients[78]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin types 1 (AT1) receptor antagonists have been shown to diminish inflammatory markers and inflammatory cell infiltration[79]. ANGII through AT1 receptor promotes ECM accumulation and fibrosis by increasing the local production of profibrotic molecules such as TGF-β, Smad2/3 and CTGF. Intestinal fibrosis is significantly improved or even reversed by ACE inhibitors and AT1 receptor antagonists, findings that are closely correlated to the reduction of TGF-β_1 and CTGF expression[80,81]. These observations suggested that the neutralization of the fibrogenic ACE and ANGII could be a beneficial therapeutic target in intestinal fibrosis.

Other potent fibrogenetic molecules, such as αvβ6 integrin, mTOR, and PPAR-γ seem to interact directly or indirectly with TGF-β/Smad pathway.

Integrins are involved in regulating a variety of cellular processes, including proliferation, differentiation and apoptosis, as well as development of fibrosis[82]. The αvβ6 integrin is upregulated on tissue with inflammatory-fibrotic pathology. αvβ6 ligands include fibronectin, tenascin and LAP (latency-associated peptides of TGF-β_1). Interaction with LAP activates latent TGF-β and promotes fibrosis. αvβ6 is not expressed in normal condition but it is up-regulated in many human fibrosis diseases of various organs (kidney, liver and lung)[83].

mTOR, a phosphatidylinositol 3-kinase-related kinase (PIKK), forms at least two distinct complexes[84]. The mTOR complex 1 (mTORC1) which is composed of mTOR, GβL and Raptor and controls protein synthesis and cell growth and proliferation, as well as autophagy, angiogenesis and fibrosis. The mTOR complex 2 (mTORC2) which consists of mTOR, GβL and Rictor and is involved in the cell proliferation and survival, metabolic regulation and actin cytosckeleton organization. mTOR signaling is activated by hormones, growth factors, amino acid levels, stress and alterations in cellular energy status[84]. mTOR inhibitors (mTORis) exerts direct antifibrotic activities both by reducing the number of fibroblast and myofibroblasts and by down-regulating the production of fibrogenic cytokines, such as IL-4, IL-6, IL-13, IL-17, and TGF-β_1, and the synthesis of type I and III collagen[85-88]. Their antifibrotic effectivines have been reported in fibrotic diseases of various organs including skin, lung, kidney, liver and intestine.

PPARs are nuclear receptors, which regulate gene transcription by binding to retinoid X receptors (RXR)[89]. PPAR-γ isoform, identified mainly in the colorectal mucosa, but also in adipocytes, liver, vascular tissue and several inflammatory cells (monocytes and macrophages, dendritic cells, B and T cells) seems to be involved in several physiological processes, such as differentiation of adipocytes, glucose homeostasis, lipid metabolism, inflammatory and immune processes, as well as fibrosis[90]. PPAR-γ activation seems to be strongly related to the TGF-β/Smads pathway. The stimulation of PPAR-γ with specific ligands interferes with the Smad3 pathway by directly antagonizing Smad3 or downregulating CTGF expression (a downstream effector of TGF-β/Smad3-induced ECM proteins)[91,92].

There are evidences, therefore, that the above mentioned molecules form with TGFb/Smad3 pathway a complex signaling network with extensive crosstalk and strong effects on fibrosis development.

In addition to the above factors know to be relevant to intestinal fibrosis, new inducing and modulators factor are emerging including PAMPS, DAMPS, fragments of ECM, the Indian Hedgehog (Ihh) and the Wnt/β-catenin pathways, epigenetic modifications, non coding RNAs of which microRNAs are the most extensively studied, as well as gut microbiota[18]. All these factors must undergo intense scrutiny and investigation in order to obtain new insights in the complex and dynamic fibrogenic process.

Fibrotic responses are modulated by transcriptional activators and cofactors, epigenetic modifications, and microRNAs that can amplify or inhibit cellular signalings regulating generation and apoptosis of of myofibroblasts, as well as ECM deposition. Epigenetics is the study of all heritable and potentially reversible changes in genome function that do not alter the nucleotide sequence within the DNA, but might be considered in simpler terms as the regulation of gene expression.Various epigenetic procedures, including DNA methylation, histone modifications (histone methylation, acetylation, phosphorylation, sumoylation/ubiquination) and formation of particular chromatin structure, play crucial roles in the gene transcriptional expression in ECM-producing cells, regulating various phases of wound healing and fibrogenic processes. Degradation of extracellular matrix is also regulated through epigenetic modulation on matrix associated enzymes. Epigenetic marks may be the missing link that connects both the internal microenvironmental and external macroenvironmental exposure in genetically predisposed individuals to transcriptome changes associated with the development of intestinal fibrosis.

Evidence of the contribution of the gut microbiota to intestinal fibrogenesis can be found *in vivo* in various animal models as well as *in vitro* models[6,18,43]. Products of the abundant and diversified gut microbiota can induce a profibrogenic response by activating mesenchymal cells through Toll-like and NOD-like receptors. Interestingly, not all bacterial products are profibrogenic, and some of them may protect against intestinal fibrosis[18]. Thus, whether intestinal fibrosis develops or not may depend not only on the type of local inflammatory process but also on the specific com-

position of the luminal or mucosa adherent microbiota in the affected segment, a notion with potential therapeutic implications.

Whereas the microbiota is part of the internal microenvironment of the gut, this organ is also subject to the influence of the external macroenvironment. The concept that all elements present in the environment can potentially influence disease development is established, and several environmental factors (diet, contaminants) can influence the immune response and lead to fibrosis in various organs including the intestine. Given the enormity and diversity of the external environment, new tools and approaches are needed to evaluate the impact of what has been recently named the "exposome" on intestinal disease in general and intestinal fibrosis in particular[18].

Therapies for Fibrosis in IBD

The main end point in the treatment of any chronic inflammatory disease is to induce the clinical remission and the healing of lesions and to prevent the development of fibrosis[17,18,93-95]. Reduction or reversal of fibrosis is also an important goal to achieve, but this represent a very challenge. Anti-fibrotic therapies may be complicated by the fact that a wound-healing response is essential to preserve bowel wall integrity and functions during inflammation[5]. At present, there are no approved or effective medical therapies aimed specifically at intestinal fibrosis in IBD. Therefore, intestinal fibrosis and associated complications, still remain the major causes of surgical intervention[17,96]. Surgical correction, by means of intestinal resection or stricturoplasty, is necessary in up to 75% of CD patients during the course of their disease[3,4]. However, surgical resection is associated with a high rate of recurrent stricturing disease and the need for repeated surgery is high, therefore, exploration of new therapeutic approaches has now become mandatory.

Theoretically, for the treatment of intestinal fibrosis in IBD we can act at three different levels: 1. To eliminate the primary cause of intestinal damage, 2. To use various anti-inflammatory and immunomodulatory drugs, 3. To use anti-fibrotic agents (Figure 5).

The best antifibrotic treatment would be represented by any strategy able to eliminate the primary cause of intestinal damage, but it is not the case for IBD since we do not know their etiology. In animal models in which IBD has been induced by the administration of exogenous agents, the tissue changes associated with both early and late stage of fibrosis disappear after removal of the irritant, which demonstrate the reversibility of intestinal fibrosis[43].

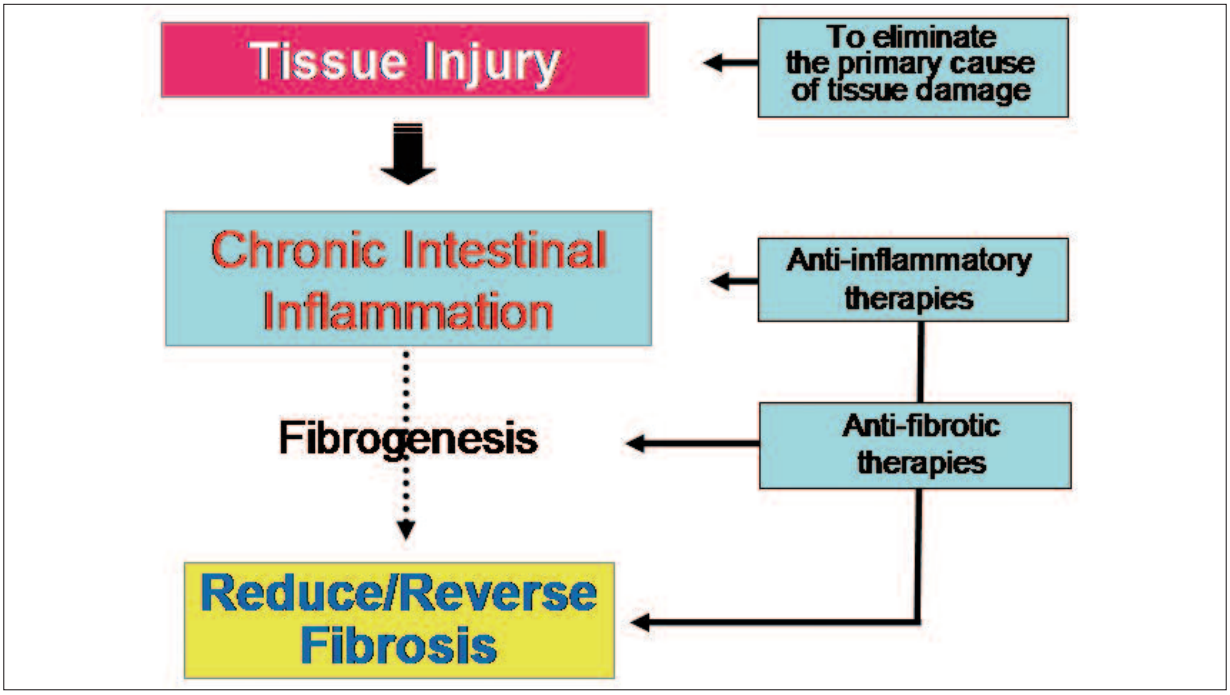

Figure 5. Steps and approaches for the treatment of intestinal fibrosis in IBD.

The agents currently used for the treatment of IBD (salicylates, antibiotics, steroids, immunosuppressive drugs, biological therapies) may relieve the inflammatory symptoms, but do not improve fibrostenotic obstruction[95-98]. The results of medical treatment aimed at stricturing or penetrating CD are poor, since 64% of these patients ultimately require surgery within one year[99]. There is no doubt that these agents work best when introduced early in the course of the disease, when inflammation predominate and the fibrosis is still at a reversible phase. A crucial point is the timing of commencement of early treatment. In clinical practice, early CD is usually considered as a newly diagnosed case, and this does not always correspond to the early purely inflammatory form of the disease. Approximately 50% of the patients already present a stricturing or penetrating disease, at the time of diagnosis[100], thus indicating a late disease which is more resistant to treatment both with immunosuppressants and biological agents.

Some evidence suggests that particular anti-inflamatory therapies may exacerbate fibrosis. Nonetheless, some of these agents may have indirect anti-fibrotic effects[101].

Several anti-inflammatory mechanisms of 5-aminosalycilic acid (5-ASA) have been described, but no data are available for a possible antifibrotic effects of this drug.

Corticosteroids are effective for controlling inflammation in the majority of inflammatory diseases. Anti-fibrotic effects of corticosteroids have been observed in several fibrotic diseases such systemic sclerosis, idiopatic pulmonary fibrosis and retroperitoneal fibrosis, but with discordant results in the intestinal fibrosis[102-106]. The antifibrotic effects of steroids may cause deficiency in general wound healing. Furthermore, long-term systemic use of steroids is not recommended due to several significant adverse events.

Immunosuppressive agents, such as azatioprine, 6-mercaptopurine, cyclosporine and methotrexate, are largely used for the treatment of several chronic inflammatory disease including IBD. Although some of these drugs were effective for the treatment of pulmunary and retroperitoneal fibrosis[107,108], no significant effects were observed in the treatment of intestinal fibrosis in IBD[3,4]. Improvement in medical treatment consisting of an earlier and more frequent use of immunosuppressive drugs have not resulted in a decreased risk of development of intestinal complications (strictures and/or fistulas), as well as of need of intestinal resection[15-17,96]. The successful use of azathioprine in preveting post-operative recurrence of CD supports the hypothesis that azathioprine can prevent, or at least slows down the development of intestinal fibrosis in CD[109].

The antifibrotic effects of anti-TNF-α (infliximab, adalimumab, certolizumab) agents on intestinal fibrosis is not clear. In early reports, obstructive complications were observed in some patients treated with anti anti-TNF-α antibodies, but this unfavourable effect remains to be confirmed[110-112]. *In vitro* studies, anti-TNF-α antibodies modulated myofibroblasts migration, as well as collagen and TIMP-1 production[113]. *In vivo* studies, serum levels of bFGF and VEGF, both involved in intestinal fibrosis, were decreased by these agents[114].

Although data from RCTs and observational studies suggest that biological use may reduce the need for surgery in the short term, the real impact of biologics on the lifetime risk of surgery remains to be established[115-117]. Recent data from population-based cohorts have shown that in the pre-biologic era, the rate of surgery ranged between 27% and 61% within 5 years after diagnosis, and, in the era of anti-TFN-α, ranged between 25% and 33% thus suggesting that the need for surgery remains high also in the era of biologics[118]. Although biological therapies have shown disease-modifying characteristics in other diseases, more data are required before it can be known whether they can influence the long term natural history of CD[119-120].

IBD is driven by the trafficking of lymphocytes from the circulation into the gut tissue that is mediated by adhesive interactions between the lymphocytes and endothelial cells[121,122]. The adhesion molecules involved represent attractive targets for the development of new therapeutics which should aid in the resolution of existing inflammation, prevent recurrence of inflammation, and may potentially lead to long-term control of disease. Natalizumab, a humanized monoclonal antibody against adhesion molecule α_4 integrin, showed significant anti-inflammatory effects in moderately-to-severely active CD, but not anti-fibrotic properties. On the contrary, it presents severe adverse events including a form of progressive multifocal leukoencephalopathy[123].

From all these important observations one has to infer that controlling intestinal inflammation alone is not sufficient to prevent or eliminate the associated fibrotic response. Thus it follows that fibrosis-specific therapies must be developed, and perhaps therapies specific for intestinal fibro-

sis, since the underlying pathophysilogy may be different from other organs, particularly with regard to the presence of luminal microbiota[18].

Putative anti-fibrotic drugs can include: (1) Agents able to reduce the activation of ECM-producing cells and their pro-fibrogenic properties (proliferation, motility, ECM deposition, contraction); (2) Agent with pro-apoptotic effect for ECM-producing cells; (3) Agents able to increase ECM degradation (Table II). There is a growing list of novel mediators and pathways that could be developed as anti-fibrotic treatments[21,26]. These include TGF-beta signaling modifiers, pro-fibrotic cytokines and cytokines receptors antagonists, profibrotic chemokines and chemokines receptors antagonists, anti-fibrotic cytokines and chemokines, TLR antagonists, angiogenesis antagonists, antihypertensive drugs, vasoactive substances, integrin/adhesion molecule antagonists, TIMP inhibitors, pro-apoptotic drugs that target myofibroblasts, gene silencing strategies, stem cell transplantation technologies (Table III). It should be stressed that most of the evidence indicating a beneficial effect of these drugs have been derived from studies performed *in vitro* or in animal models of fibrogenesis[18,43,124]. Therefore, the real effectiveness of these agents remains to be defined.

Table II. Mechanisms of action of antifibrogenic agents (Chemical & biological).

- Reduce inflammation
- Reduce activated ECM-producing cells and their profibrogenic actions:
  - Proliferation
  - Motility
  - Contraction
  - ECM deposition
- Promote ECM-producing cells apoptosis
- Promote ECM degradation

Blockade of the TGF-β signaling, either at extracellular (ligand, receptors) or intracellular level (signal transduction pathways), may offer a potential molecular strategy to prevent and/or treat fibrosis[6]. However, since TGF-β is not only the driving force of fibrotic process, but is also involved in other important cellular functions

Table III. Classes of antifibrogenic agents.

TGF-β signaling modifiers: relaxin, HGF, BMP7, Smad7, inhibitors of TGF-β_1, TGF-βR1, TGF-βRII, Smad3
Growth factors antagonists: inhibitors of CTGF, PDGFs, IGFs, EGF, bFGF
Cytokine and cytokine receptor antagonists: inhibitors of IL-1β, IL-6, IL-13, IL-17, IL-21, IL-22, IL-33, IL-4R, IL-13Rα1, TNFα
Chemokine and chemokine receptor antagonists: inhibitors of CCL2, CCL3, CCL6, CCL18, CXCL1, CXCL2, CXCL12, CCR2, CCR3, CCR5, CCR7, CXCR2, CXCR4
Cytokines and chemokines: Interferon-α & γ, IL-7, IL-10, IL-12, CXCL10, CXCL11
TLR antagonists: inhibitors of TLR2, TLR3, TLR4, TLR9
Angiogenesis antagonists: VEGF-specific antibodies
Vasoactive substance antagonists: inhibitors of ET-1 receptors
Renin-angiotensin system antagonists: inhibitors of ACE and Angiotensin II receptors
Integrin/adhesion molecule antagonists: inhibitors of α_1 β_1 and αvβ6 integrins, integrin linked kinase, ICAM-1, VCAM-1
Peroxisome proliferator activator receptors modulators: 15-D-PGJ2, Tiazolinediones.
mTOR inhibitors: rapamycin, sirolimus, everolimus
Proapoptotic drugs that target myofibroblasts
MMP antagonists: inhibitors of MMP2, MMP9, MMP12
TIMP antagonists: TIMP-1 specific antibodies
Gene silencing strategies and gene therapy: shRNA for TGF-β_1, TGF-βR1, TGFβRII
Stem/progenitors cell transplantation technologies
Other targets and approaches
Antioxidants
Nitric oxide donors (nitroglycerin), Relaxin
HMG-CoA reductase inhibitors (Statins): lovastatin, simvastatin, atorvastatin, provastatin
Prostaglandins (PGE2, 15-D-PGJ2), COX2-inhibitors
Leptin receptors antagonist, Adiponectin
Cannabinoid receptor 1 antagonists, Cannabinoid receptor 2 agonists
Pentoxifylline, Gliotoxin, Halofuginone
Probiotics/prebiotics, microflora methabolites (Butyrate, Propionate)
Herbal medicines: salvia miltiorrhiza, Scutellaria baicalensis or curcumine with putative antinflammatory and antifibrotic effects

(differentiation, proliferation, transformation, immuneregulation), the total blockade of this growth factor will be likely be problematic. Targeting of individual intracellular mediators could lead to selective blockade of pathological TGF-β fibrotic responses such as fibrosis without involving physiologically important, and even vital, TGF-β responses. In fact, the targeted disruptions of TGF-β, Smad2 and Smad4 are lethal[125-127], whereas the disruption of Smad3[128-130] results in the birth of mice which survive to adulthood. Loss of Smad3 confers resistance to tissue fibrosis in several organs including skin, kidney, lung, liver and intestine[45-50].

Inhibitors of the TGF-β receptor kinases, neutralizing antibodies that interfere with ligand and receptor interactions, antisense oligonucleotides reducing TGF-β expression, and soluble receptor ectodomains that sequester TGF-β have been developed and are currently being investigated for the treatment of several fibroproliferative disorders of various organs including lung, kidney, skin and liver[131,132].

Membrane αvβ6 integrin catalyzes the activation of latent TGF-β on epithelial cells. It is upregulated in many fibrosis diseases of various organs (kidney, liver and lung) and its inhibition prevents the development of fibrosis[133-136].

Hepatic growth factor (HGF), Bone morphogenetic protein 7 (BMP-7) and decorin are three natural inhibitors of TGF-β/Smad pathway showing antifibrotic effects in liver, renal and pulmunary fibrosis[137-140].

There is also considerable cross-talk between TGF-beta$_1$ and MAP kinase signaling pathways in the synthesis and turnover of extracellular matrix. Thus, blockade of p38 and JNK pathways may have therapeutic potential for the treatment of fibrosis[141].

Recent pre-clinical studies suggest that selective tyrosine kinase inhibitors that target c-Abl, PDGF receptor or Src kinases might be promising targets for anti-fibrotic approaches[142]. Dual inhibition of c-Abl and PDGF receptor by imatinib and nilotinib, and inhibition of Src kinases either selectively by SU6656 or in combination with c-Abl and PDGF by dasatinib exerted potent anti-fibrotic effects. Imatinib, nilotinib, dasatinib and SU6656 reduced dose-dependently the synthesis of extracellular matrix proteins. Clinical data from patients with chronic myelogenous leukaemia suggest that imatinib, nilotinib and dasatinib are well tolerated. Based on the promising pre-clinical data, imatinib is currently evaluated in clinical trials for the treatment of fibrosis in systemic sclerosis, pulmonary fibrosis, hepatic fibrosis and chronic kidney disease[142,143].

As far as the growth factors are concerned, CTGF appear to be a more specific target for selective intervention in processes involving connective tissue formation during fibrotic disorders. Various forms of treatment targeting CTGF effects have been proposed whith favourable effects in fibrosis of several organs, including the intestine[144,145].

Of all components of renin-angiotensin system, ACE and ANG-II appears to be the dominant molecules responsible for fibrosis and their inhibition results to slow the progression of fibrosis in cardiovascular, renal and hepatic chronic diseases, as well as in experimentally induced chronic intestinal inflammation[21,26,80,81,101]. Daily administration of the ACE inhibitor captopril in rats with chronic TNBS-induced colitis significantly reduced the macroscopic and microscopic pattern of both colonic inflammation and fibrosis, decreased the colon collagen content, and reduced TGF-β_1 mRNA levels by about 60%[80]. The antifibrotic mechanism of captopril could be related to the inhibition of ANG II-mediated TGF-β_1 overexpression, and/or to a direct down-regulation of TGF-β_1 transcripts. Likewise, the use of losartan, a specific AT1 receptor antagonist, significantly improved the macro- and microscopic scores of experimentally induced colorectal fibrosis and reduced TGF-β_1 concentration, thus suggesting that this drug has a preventive effect on colorectal fibrosis complicating the TNBS-induced chronic colitis by a downregulation of the TGF-β_1 expression[81]. In view of these data, RAS could be considered a future target for new antifibrotics in IBD.

Of the profibrotic interleukines, IL-13 could be the most important target of antifibrotic therapy[21,26]. IL-13 signaling via the IL-13R alpha2 is a key initiation point for a complex fibrotic program in the intestine consisting of TGF-β_1 activation, IGF-I and Egr-1 expression, myofibroblast activation, and myofibroblast production of collagen[146]. IL-13 production results in the induction of an IL-13R formerly thought to function only as a decoy receptor, IL-13Ralpha2, and this receptor is critical to the production of TGF-β_1 and the onset of fibrosis. Thus, if IL-13 signaling through this receptor is blocked by administration of soluble IL-13Ralpha2-Fc, or by administration of IL-13Ralpha2-specific small interfering RNA, TGF-β_1 is not produced and colorectal fibrosis does not occur[42,71,147].

ROS are involved in acute and chronic inflammatory processes, as well as in fibrosis. ROS appear to be a key mediator in collagen gene regulation[148]. Antioxidants protect against experimental pulmonary and hepatic fibrosis[149,150]. ROS is also involved in intestinal fibrosis. Inhibition of oxygen radical secretion improves experimental colitis in mice[151].

mTOR signalling is considered an attractive target for antifibrotic intervention. mTOR inhibitors (mTORis) constitute a relatively new category of immunosuppressive and antineoplastic drugs[152]. Their clinical applications have recently expanded significantly to cover a wide spectrum of immune and non-immune-mediated disorders, various solid organ and haematological malignancies, metabolic problems such as diabetes mellitus and obesity, and even fibrotic diseases, including skin, pulmonary, renal, hepatic and intestinal fibrosis[153-157]. Combined immunosuppressive and anti-fibrotic action of rapamycin and its analogues may result in a promising treatment approach of fibrotic chronic enteropathies such as CD. This has been confirmed by two case reports in which two patients with severe refractory CD were successfully treated with two different analogues of rapamycin, respectively sirolimus and everolimus[158,159]. In addition, a recent clinical trial demonstrated the effectiveness of everolimus to maintain steroid-induced remission in patients with moderate-to-severe active CD[160].

Overexpression of PPAR-γ prevents the development of tissue fibrosis, whereas its loss increases susceptibility to fibrosis[161,162]. Therefore, PPAR-γ should be regarded as an innate protection from excessive fibrogenesis and a potential new target for the development of novel anti-fibrotic agents[163]. Experimental studies have shown that PPAR-γ agonists attenuate fibrosis in various organs including lung, kidney, pancreas, liver and intestine, antifibrotic effects that are abolished by the use of a PPAR-γ selective antagonists[164-167].

Accumulating data demonstrate that some of MMPs are constitutively expressed and regulate physiologic processes such as barrier function and mucosal defense, while others are undetectable in normal intestine but their dysregulated expression during inflammation may play a role in cell adhesion, immune and non-immune cell migration, and impaired wound healing and fibrosis[168,169]. The final outcome of inflammatory and fibrogenetic responses depends on the balance between MMPs and TIMPs. The beneficial effects of the use of MMPs or TIMPs inhibitors in the treatment of intestinal fibrosis in IBD remain to be defined.

Although HMG-CoA reductase inhibitors (statins) reduce expression of TGF-β and CTGF and the production of ECM components, the specific antifibrotic effect is based on the inhibition of Smad3 phosphorylation[170]. Simvastatin attenuates intestinal fibrosis independent of the anti-inflammatory effect by promoting fibroblast/myofibroblast apoptosis in the regeneration/healing process from TNBS-induced colitis[171,172].

Control of events that are secondary to IBD, susch as angiogenesis and lymphangiongenesis, might represent an alternative approach to treatment, particularly in view of the connection between vascular remodeling and fibrogenesis in chronic intestinal inflammation[9,121,122,173,174]. Growing evidence suggests that the microvasculature plays an integral role in the pathophysiology of IBD. The microvasculature contributes to chronic inflammation through altered leukocyte recruitment, impaired perfusion, and angiogenesis leading to tissue remodeling[121,122,173,174]. Increased expression of VEGF in IBD and it blockade were therapeutically effective in preclinical animal models[175]. The control of angiogenesis and lymphangiongenesis has been proposed as a novel therapeutic approach to IBD, and also has the potential to prevent or reverse fibrosis in affected patients.

Furthermore, correcting intestinal mucosal barrier leakiness may decrease or eliminate the excessive absorption of bacterial products that stimulate immune and mesenchimal cells involved in fibrotic process, and could be an useful approach to prevention or treatment of fibrosis in IBD[176-178]. Detailed characterization of barrier defects offers the opportunity to consider and test therapeutic interventions. Beside cytokine antagonists, different plant compounds and probiotics have been shown to stabilize the barrier function by affecting epithelial tight junction proteins expression and distribution.

Recently, a role of MicroRNAs in pulmonary, renal and intestinal fibrosis has been reported[179-181]. MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene and protein expression. miRNAs are critical to a normal immune response and have altered expression in multiple immune-mediated disorders including IBD[182]. Specific microRNAs downregulate smad-3 activity and the expression of matrix proteins and prevente TGF-β-dependent EMT. The changes in microRNA expression in IBD and the evidence for their role in the fibrosis suggest that microRNAs should be evaluated as therapeutic targets in intestinal fibrosis complicating IBD.

Conclusions

The available current therapies do not affect intestinal fibrosis. The only treatment option for fibrosis in patients with IBD is still surgery (resection or strictureplasty) or repeated mechanical palliation (endoscopic balloon dilatation, metallic stents).

The concept of intestinal fibrosis has changed from being a static and irreversible entity to a dynamic and reversible disease, as also occur in other organs. Novel therapeutic strategies are under investigation to target specific steps in the process of fibrogenesis with the aim of reducing or reversing advanced intestinal fibrosis in IBD. One hope that researchers, funding agencies and pharmaceutical industries accelerate their efforts to identify and develop safe and effective antifibrotic therapies.

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Conflicts of Interest and Financial Disclosures

None to declare.

References

1) Schirbel A, Fiocchi C. Inflammatory bowel disease: Established and evolving considerations on its etiopathogenesis and therapy. J Dig Dis 2010; 11: 266-276.
2) Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med 2009; 361: 2066-2078.
3) Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology 2011; 140: 1785-1794.
4) Latella G, Papi C. Crucial steps in the natural history of inflammatory bowel disease. World J Gastroenterol 2012; 18: 3790-3799.
5) Rieder F, Karrasch T, Ben-Horin S, Schirbel A, Ehehalt R, Wehkamp J, de Haar C, Velin D, Latella G, Scaldaferri F, Rogler G, Higgins P, Sans M. Results of the 2nd scientific workshop of the ECCO (III): basic mechanisms of intestinal healing. J Crohns Colitis 2012; 6: 373-385.
6) Speca S, Giusti I, Rieder F, Latella G. Cellular and molecular mechanisms of intestinal fibrosis. World J Gastroenterol 2012; 18: 3635-3661.
7) Burke JP, Mulsow JJ, O'Keane C, Docherty NG, Watson RW, O'Connell PR. Fibrogenesis in Crohn's disease. Am J Gastroenterol 2007; 102: 439-448.
8) Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease–Current knowledge and future perspectives. J Crohns Colitis 2008; 2: 279-290.
9) Rieder F, Fiocchi C. Intestinal fibrosis in IBD-a dynamic, multifactorial process. Nat Rev Gastroenterol Hepatol 2009; 6: 228-235.
10) Rieder F, Schirbel A, Ouygan Z, West G, Rho H, de la Motte C, Fiocchi C. Pro-fibrogenic activity of Toll-like receptor (TLR) and NOD-Like Receptor (NLR) ligands on Human Intestinal Myofibroblasts (HIF)–linking bacterial innate immunity to intestinal fibrosis. Gastroenterology 2010; 138: S-35.
11) Gazouli M, Pachoula I, Panayotou I, Mantzaris G, Chrousos G, Anagnou NP, Roma-Giannikou E. NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease. World J Gastroenterol 2010; 16: 1753-1758.
12) Dubinsky MC, Kugathasan S, Mei L, Picornell Y, Nebel J, Wrobel I, Quiros A, Silber G, Wahbeh G, Katzir L, Vasiliauskas E, Bahar R, Otley A, Mack D, Evans J, Rosh J, Hemker MO, Leleiko N, Crandall W, Langton C, Landers C, Taylor KD, Targan SR, Rotter JI, Markowitz J, Hyams J; Western Regional Pediatric IBD Research Alliance; Pediatric IBD Collaborative Research Group; Wisconsin Pediatric IBD Alliance. Increased immune reactivity predicts aggressive complicating Crohn's disease in children. Clin Gastroenterol Hepatol 2008; 6: 1105-1111.
13) Rieder F, Lawrance IC, Leite A, Sans M. Predictors of fibrostenotic Crohn's disease. Inflamm Bowel Dis 2011; 17: 2000-2007.
14) Luna J, Masamunt MC, Lawrance IC, Sans M. Mesenchymal cell proliferation and programmed cell death: key players in fibrogenesis and new targets for therapeutic intervention. Am J Physiol Gastrointest Liver Physiol 2011; 300: G703-G708.
15) Angelucci E, Cesarini M, Gentile P, Necozione S, Fieri G, Caprilli R, Latella G. Available medical therapies do not affect development of major complications and need for surgery in Crohn's isease: Long-term prospective study on a population of 193 consecutive patients. J Crohns Colitis 2011; 5: S120-S121.
16) Cosnes J, Nion-Larmurier I, Beaugerie L, Afchain P, Tiret E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. Gut 2005; 54: 237-241.
17) Spinelli A, Correale C, Szabo H, Montorsi M. Intestinal fibrosis in Crohn's disease: medical treatment or surgery? Curr Drug Targets 2010; 11: 242-248.
18) Fiocchi C, Lund PK. Themes in fibrosis and gastrointestinal inflammation. Am J Physiol Gastrointest Liver Physiol 2011; 300: G677-G683.
19) Mifflin RC, Pinchuk IV, Saada JI, Powell DW. Intestinal myofibroblasts: targets for stem cell therapy. Am J Physiol Gastrointest Liver Physiol 2011; 300: G684-G696.
20) Pucilowska JB, Williams KL, Lund PK. Fibrogenesis IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models. Am J Physiol Gastroinest Liver Physiol 2000; 279: G653-G659.

21) Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008; 214: 199-210.

22) Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts I. Paracrine cells important in health and disease. Am J Physiol Cell Physiol 1999; 277: C1-C9.

23) Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts II. Intestinal subepithelial myofibroblasts. Am J Physiol Cell Physiol 1999; 277: C183-C201.

24) Sanders KM, Ordög T, Ward SM. Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 2002; 282: G747-G756.

25) Vetuschi A, Sferra R, Latella G, D'Angelo A, Catitti V, Zanninelli G, Continenza MA, Gaudio E. Smad3-null mice lack interstitial cells of Cajal in the colonic wall. Eur J Clin Invest 2006; 36: 41-48.

26) Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007; 117: 524-529.

27) Ng EK, Panesar N, Longo WE, Shapiro MJ, Kaminski DL, Tolman KC, Mazuski JE. Human intestinal epithelial and smooth muscle cells are potent producers of IL-6. Mediators Inflamm 2003; 12: 3-8.

28) Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, Ramadori G. Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 1999; 117: 1205-1221.

29) Apte, M. Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut 1999; 44: 534-541.

30) Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease: progress in basic and clinical science. Curr Opin Gastroenterol 2008; 24: 462-468.

31) Gerhardt H, Betsholtz C. Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res 2003; 314:15-23.

32) Flier SN, Tanjore H, Kokkotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem 2010; 285: 20202-20212.

33) Rieder F, Kessler SP, West GA, Bhilocha S, de la Motte C, Sadler TM, Gopalan B, Stylianou E, Fiocchi C. Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of intestinal fibrosis. Am J Pathol 2011; 179: 2660-2673.

34) Umar S. Intestinal stem cells. Curr Gastroenterol Rep 2010; 12: 340-348.

35) Ishii G, Sangai T, Sugiyama K, Ito T, Hasebe T, Endoh Y, Magae J, Ochiai A. *In vivo* characterization of bone marrow-derived fibroblasts recruited into fibrotic lesions. Stem Cells 2005; 23: 699-706.

36) Quan TE, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. Curr Rheumatol Rep 2006; 8: 145-150.

37) Von Lampe B, Barthel B, Coupland SE, Riecken EO, Rosewicz S. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. Gut 2000; 47: 63-73.

38) Ravi A, Garg P, Sitaraman SV. Matrix metalloproteinases in inflammatory bowel disease: boon or a bane? Inflamm Bowel Dis 2007; 13: 97-107.

39) Meijer MJ, Mieremet-Ooms MA, van Hogezand RA, Lamers CB, Hommes DW, Verspaget HW. Role of matrix metalloproteinase, tissue inhibitor of metalloproteinase and tumor necrosis factor-alpha single nucleotide gene polymorphisms in inflammatory bowel disease. World J Gastroenterol 2007; 13: 2960-2966.

40) Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 2003; 425: 577-584.

41) Roberts AB, Russo A, Felici A, Flanders KC. Smad3: a key player in pathogenetic mechanisms dependent on TGF-beta. Ann NY Acad Sci 2003; 995: 1-10.

41) Babyatsky MW, Rossiter G, Podolsky DK. Expression of transforming growth factor alpha and beta in colonic mucosa in inflammatory bowel disease. Gastroenterology 1996; 110: 975-984.

42) Fichtner-Fiegl S, Strober W, Geissler EK, Schlitt HJ. Cytokines mediating the induction of chronic colitis and colitis-associated fibrosis. Mucosal Immunol 2008; 1: S24-S27.

43) Rieder F, Kessler S, Sans M, Fiocchi C. Animal models of intestinal fibrosis: new tools for the understanding of pathogenesis and therapy of human disease. Am J Physiol Gastrointest Liver Physiol 2012; 303: G786-G801.

44) Vallance BA, Gunawan MI, Hewlett B, Bercik P, Van Kampen C, Galeazzi F, Sime PJ, Gauldie J, Collins SM. TGF-beta$_1$ gene transfer to the mouse colon leads to intestinal fibrosis. Am J Physiol Gastrointest Liver Physiol 2005; 289: G116-G128.

45) Zanninelli G, Vetuschi A, Sferra R, D'Angelo A, Fratticci A, Continenza MA, Chiaramonte M, Gaudio E, Caprilli R, Latella G. Smad3 knock-out mice as a useful model to study intestinal fibrogenesis. World J Gastroenterol 2006; 12: 1211-1218.

46) Latella G, Vetuschi A, Sferra R, Zanninelli G, D'Angelo A, Catitti V, Caprilli R, Flanders KC, Gaudio E. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colorectal fibrosis. Eur J Clin Invest 2009; 39: 145-156.

47) Lakos G, Takagawa S, Chen SJ, Ferreira AM, Han G, Masuda K, Wang XJ, DiPietro LA, Varga J. Targeted disruption of TGF-beta/Smad3 signaling modulates skin fibrosis in a mouse model of scleroderma. Am J Pathol 2004; 165: 203-217.

48) Zhao J, Shi W, Wang YL, Chen H, Bringas P Jr, Datto MB, Frederick JP, Wang XF, Warburton D.

Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. Am J Physiol Lung Cell Mol Physiol 2002; 282: L585-L593.

49) Inazaki K, Kanamaru Y, Kojima Y, Sueyoshi N, Okumura K, Kaneko K, Yamashiro Y, Ogawa H, Nakao A. Smad3 deficiency attenuates renal fibrosis, inflammation, and apoptosis after unilateral ureteral obstruction. Kidney Int 2004; 66: 597-604.

50) Latella G, Vetuschi A, Sferra R, Catitti V, D'Angelo A, Zanninelli G, Flanders KC, Gaudio E. Targeted disruption of Smad3 confers resistance to the development of DMN-induced hepatic fibrosis in mice. Liver Int 2009, 29: 997-1009.

51) Fukasawa H, Yamamoto T, Togawa A, Ohashi N, Fujigaki Y, Oda T, Uchida C, Kitagawa K, Hattori T, Suzuki S, Kitagawa M, Hishida A. Down-regulation of Smad7 expression by ubiquitin-dependent degradation contributes to renal fibrosis in obstructive nephropathy in mice. Proc Natl Acad Sci USA 2004; 101: 8687-8692.

52) Dooley S, Hamzavi J, Breitkopf K, Wiercinska E, Said HM, Lorenzen J, Ten Dijke P, Gressner AM. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rats. Gastroenterology 2003; 125: 178-191.

53) Dong C, Zhu S, Wang T, Yoon W, Li Z, Alvarez RJ, ten Dijke P, White B, Wigley FM, Goldschmidt-Clermont PJ. Deficient Smad7 expression: a putative molecular defect in scleroderma. Proc Natl Acad Sci USA 2002; 99: 3908-3913.

54) Nakao A, Fujii M, Matsumura R, Kumano K, Saito Y, Miyazono K, Iwamoto I. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. J Clin Invest 1999; 104: 5-11.

55) Latella G, Zanninelli G. Prevention and treatment of intestinal fibrosis: up-regulate smad7 or inhibit smad3 expression. Eur J Clin Invest 2008; 38: 878-880.

56) Di Sabatino A, Jackson CL, Pickard KM, Buckley M, Rovedatti L, Leakey NA, Picariello L, Cazzola P, Monteleone G, Tonelli F, Corazza GR, MacDonald TT, Pender SL. Transforming growth factor beta signalling and matrix metalloproteinases in the mucosa overlying Crohn's disease strictures. Gut 2009; 58: 777-789.

57) Munz B, Hübner G, Tretter Y, Alzheimer C, Werner S. A novel role of activin in inflammation and repair. J Endocrinol 1999; 161: 187-193.

58) Werner S, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev 2006; 17: 157-171.

59) Grotendorst GR. Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. Cytokine Growth Factor Rev 1997; 8: 171-179.

60) Blom IE, Goldschmeding R, Leask A. Gene regulation of connective tissue growth factor: new targets for antifibrotic therapy? Matrix Biol 2002; 21: 473-482.

61) Kumagai S, Ohtani H, Nagai T, Funa K, Hiwatashi NO, Shimosegawa, Nagura H. Platelet-derived growth factor and its receptors are expressed in areas of both active inflammation and active fibrosis in inflammatory bowel disease. Tohoku J Exp Med 2001; 195: 21-33.

62) Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. Cytokine Growth Factor Reviews 2004; 15: 255-273.

63) Simmons JG, Ling Y, Wilkins H, Fuller CR, D'Ercole AJ, Fagin J, Lund PK. Cell-specific effects of insulin receptor substrate-1 deficiency on normal and IGF-I-mediated colon growth. Am J Physiol Gastrointest Liver Physiol 2007; 293: G995-G1003.

64) Zimmermann EM, Li L, Hou YT, Mohapatra NK, Pucilowska JB. Insulin-like growth factor I and insulin-like growth factor binding protein 5 in Crohn's disease. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1022-G1029.

65) Gieling RG, Wallace K, Han YP. Interleukin-1 participates in the progression from liver injury to fibrosis. Am J Physiol Gastrointest Liver Physiol 2009; 296: G1324-G1331.

66) Liu X. Inflammatory cytokines augments TGF-beta$_1$-induced epithelial-mesenchymal transition in A549 cells by up-regulating TbetaR-I. Cell Motil Cytoskeleton 2008; 65: 935-944.

67) Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, Kasprzycka M, Sørensen DR, Nilsen HR, Küchler AM, Vatn MH, Haraldsen G. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. Am J Pathol 2010; 177: 2804-2815.

68) Ito H. IL-6 and Crohn's disease. Curr Drug Targets Inflamm Allergy 2003; 2: 125-130.

69) JA, Booth AJ, Lu G, Wood SC, Pinsky DJ, Bishop DK. Critical role of IL-6 in hypertrophy and fibrosis in chronic cardiac allograft rejection. Am J Transplant 2009; 9: 1773-1783.

70) Luckett-Chastain LR, Gallucci RM. Interleukin (IL)-6 modulates transforming growth factor –beta expression in skin and dermal fibroblasts from IL-6-deficient mice. Br J Dermatol 2009; 161: 237-248.

71) Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta$_1$ production and fibrosis. Nat Med 2006; 12: 99-106.

72) Sanchez-Muñoz F, Dominguez-Lopez A, Yamamoto-Furusho JK. Role of cytokines in inflammatory bowel disease. World J Gastroenterol 2008; 14: 4280-4288.

73) Theiss AL, Simmons JG, Jobin C, Lund PK. Tumor Necrosis Factor (TNF) a Increases Collagen Accumulation and Proliferation in Intestinal Myofibroblasts via TNF Receptor 2. J Biol Chem 2005; 280: 36099-36109.

74) Paul M, Poyan Mehr A, Kreutz R. 139 Physiology of local renin-angiotensin systems. Physiol Rev 2006; 86:747-803.

75) Hirasawa K, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Immunohistochemical localization of an-

giotensin II receptor and local renin-angiotensin system in human colonic mucosa. J Histochem Cytochem 2002; 50: 275-282.

76) Bader M. Tissue renin-angiotensin-aldosterone systems: Targets for pharmacological therapy. Annu Rev Pharmacol Toxicol 2010; 50: 439-465.

77) Ruiz-Ortega M, Ruperez M, Esteban V, Rodríguez-Vita J, Sánchez-López E, Carvajal G, Egido J. Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. Nephrol Dial Transpl 2006; 21: 16-20.

78) Jaszewski R, Tolia V, Ehrinpreis MN, Bodzin JH, Peleman RR, Korlipara R, Weinstock JV. Increased colonic mucosal angiotensin I and II concentration in Crohn's colitis. Gastroenterology 1990; 98: 1543-1548.

79) Inokuchi Y, Morohashi T, Kawana I, Nagashima Y, Kihara M, Umemura S. Amelioration of 2,4,6-trinitrobenzene sulphonic acid induced colitis in angiotensinogen gene knockout mice. Gut 2005; 54: 349-356.

80) D Wengrower, G Zanninelli, O Pappo, G Latella, M Sestieri, A. Villanova, Y Faitelson, M Pines, E Goldin. Prevention of fibrosis in experimental colitis by captopril: the role of TGFβ1. Inflamm Bowel Dis 2004; 10: 536-545.

81) Wengrower D, Zanninelli G, Latella G, Necozione S, Metanes I, Israeli E, Lysy J, Pines M, Papo O, Goldin E. Losartan reduces trinitrobenzene sulphonic acid-induced colorectal fibrosis in rats. Can J Gastroenterol 2012; 26: 33-39.

82) van der Flier A, Sonnenberg A. Function and interactions of integrins. Cell Tissue Res 2001; 305: 285-298.

83) Margadant C, Sonnenberg A. Integrin-TGF-beta crosstalk in fibrosis, cancer and wound healing. EMBO Rep 2010; 11: 97-105.

84) Tsang CK, Qi H, Liu LF, Zheng XF. Targeting mammalian target of rapamycin (mTOR) for health and diseases. Drug Discov Today 2007; 12: 112-124.

85) Wang S, Wilkes MC, Leof EB, Hirschberg R. Noncanonical TGF-beta pathways, mTORC1 and Abl, in renal interstitial fibrogenesis. Am J Physiol Renal Physiol 2010; 298: F142-F149.

86) Poulalhon N, Farge D, Roos N, Tacheau C, Neuzillet C, Michel L, Mauviel A, Verrecchia F. Modulation of collagen and MMP-1 gene expression in fibroblasts by the immunosuppressive drug rapamycin. A direct role as an antifibrotic agent? J Biol Chem 2006; 281: 33045-33052.

87) Osman B, Akool el-S, Doller A, Müller R, Pfeilschifter J, Eberhardt W. Differential modulation of the cytokine-induced MMP-9/TIMP-1 protease-antiprotease system by the mTOR inhibitor rapamycin. Biochem Pharmacol 2011; 81: 134-143.

88) Lieberthal W, Levine JS. The role of the mammalian target of rapamycin (mTOR) in renal disease. J Am Soc Nephrol 2009; 20: 2493-2502.

89) Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, Oplinger JA, Kliewer SA, Gampe RT, McKee DD, Moore JT, Willson TM. Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proc Natl Acad Sci USA 2001; 98: 13919-13924.

90) Rousseaux C, Desreumaux P. The peroxisome-proliferator-activated gamma receptor and chronic inflammatory bowel disease (PPARgamma and IBD). J Soc Biol 2006; 200: 121-131.

91) Zhao C, Chen W, Yang L, Chen L, Stimpson SA, Diehl AM. PPARgamma agonists prevent TGFbeta$_1$/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 2006; 350: 385-391.

92) Zhang GY, Cheng T, Zheng MH, Yi CG, Pan H, Li ZJ, Chen XL, Yu Q, Jiang LF, Zhou FY, Li XY, Yang JQ, Chu TG, Gao WY. Activation of peroxisome proliferator-activated receptor-gamma inhibits transforming growth factor-beta$_1$ induction of connective tissue growth factor andextracellular matrix in hypertrophic scar fibroblasts *in vitro*. Arch Dermatol Res 2009; 301: 515-522.

93) Lund PK, Riby RJ. What are the mechanisms of fibrosis in IBD? Inflamm Bowel Dis 2008; 14: S127-S128.

94) Latella G, Fiocchi C, Caprili R. News from the "5th International Meeting on Inflammatory Bowel Diseases" CAPRI 2010. J Crohns Colitis 2010; 4: 690-702.

95) Caprilli R, Latella G, Frieri G. Treatment of inflammatory bowel diseases: to heal the wound or to heal the sick? J Crohns Colitis 2012; 6: 621-625.

96) Latella G, Caprilli R, Travis S. In favour of early surgery in Crohn's disease: a hypothesis to be tested. J Crohn Colitis 2011: 5; 1-4.

97) Van Assche G, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J; European Crohn's and Colitis Organisation (ECCO). The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. J Crohn Colitis 2010; 4: 63-101.

98) Dignass A, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP; European Crohn's and Colitis Organisation (ECCO). The second European evidence-based consensus on the diagnosis and management of Crohn's disease: Current management. J Crohn Colitis 2010: 4: 28-62.

99) Samimi R, Flasar MH, Kavic S, Tracy K, Cross RK. Outcome of medical treatment of stricturing and penetrating Crohn's disease: a retrospective study. Inflamm Bowel Dis 2010; 16: 1187-1194.

100) Papi C, Festa V, Fagnani C, Stazi A, Antonelli G, Moretti A, Koch M, Capurso L. Evolution of clinical behaviour in Crohn's disease: predictive factors of penetrating complications. Digest Liver Dis 2005; 37: 247-253.

101) Szabò H, Fiorino G, Spinelli A, Rovida S, Repici A, Malesci AC, Danese S. Review article: anti-fibrotic agents for the treatment of Crohn's disease–lessons learnt from other diseases. Aliment Pharmacol Ther 2010; 31: 189-201.

102) Vaglio A, Palmisano A, Corradi D, Salvarani C, Buzio C. Retroperitoneal fibrosis: evolving concepts. Rheum Dis Clin North Am 2007; 33: 803-817.

103) Badea I, Taylor M, Rosenberg A, Foldvari M. Pathogenesis and therapeutic approaches for improved topical treatment in localized scleroderma and systemic sclerosis. Rheumatology 2009; 48: 213-221.

104) Rogliani P, Mura M, Assunta Porretta M, Saltini C. New perspectives in the treatment of idiopathic pulmonary fibrosis. Ther Adv Respir Dis 2008; 2: 75-93.

105) Graham MF, Willey A, Adams J, Diegelmann RF. Corticosteroids increase procollagen gene expression, synthesis, and secretion by human intestinal smooth muscle cells. Gastroenterology 1995; 109: 1454-1461.

106) Videla S, Vilaseca J, Medina C, Mourelle M, Guarner F, Salas A, Malagelada JR. Selective inhibition of phosphodiesterase-4 ameliorates chronic colitis and prevents intestinal fibrosis. J Pharmacol Exp Ther 2006; 316: 940-945.

107) Warnatz K, Keskin AG, Uhl M, Scholz C, Katzenwadel A, Vaith P, Peter HH, Walker UA. Immunosuppressive treatment of chronic periaortitis: a retrospective study of 20 patients with chronic periaortitis and a review of the literature. Ann Rheum Dis 2005; 64: 828-833.

108) Dheda K, Lalloo UG, Cassim B, Mody GM. Experience with azathioprine in systemic sclerosis associated with interstitial lung disease. Clin Rheumatol 2004; 23: 306-309.

109) Peyrin-Biroulet L, Deltenre P, Ardizzone S, D'Haens G, Hanauer SB, Herfarth H, Lémann M, Colombel JF. Azathioprine and 6-mercaptopurine for the prevention of postoperative recurrence in Crohn's disease: a meta-analysis .Am J Gastroenterol 2009; 104: 2089-2096.

110) Vasilopoulos S, Kugathasan S, Saeian K, Emmons JE, Hogan WJ, Otterson MF, et al. Intestinal strictures complicating initially successful infliximab treatment for luminal Crohn's disease. Am J Gastroenterol 2000; 95: 2503 (A315).

111) Toy LS, Scherl EJ, Kornbluth A, Kornbluth A, Marion JF, Greenstein AJ, et al. Complete bowel obstruction following initial response to infliximab therapy for Crohn's disease: A series of a newly described complication. Gastroenterology 2000; 118(Suppl 2, Part 1): A569 (2974).

112) Lichtenstein GR, Olson A, Travers S, Diamond RH, Chen DM, Pritchard ML, Feagan BG, Cohen RD, Salzberg BA, Hanauer SB, Sandborn WJ. Factors associated with the development of intestinal strictures or obstructions in patients with Crohn's disease. Am J Gastroenterol 2006; 101: 1030-1038.

113) Di Sabatino A, Pender SL, Jackson CL, Prothero JD, Gordon JN, Picariello L, Rovedatti L, Docena G, Monteleone G, Rampton DS, Tonelli F, Corazza GR, MacDonald TT. Functional modulation of Crohn's disease myofibroblasts by anti-tumor necrosis factor antibodies. Gastroenterology 2007; 133: 137-149.

114) Di Sabatino A, Ciccocioppo R, Benazzato L, Sturniolo GC, Corazza GR. Infliximab downregulates basic fibroblast growth factor and vascular endothelial growth factor in Crohn's disease patients. Aliment Pharmacol Ther 2004; 19: 1019-1024.

115) Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P; ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359: 1541-1549.

116) Lichtenstein GR, Yan S, Bala M, Blank M, Sands BE. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. Gastroenterology 2005; 128: 862-869.

117) Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, Hoffman I, Van Steen K, Vermeire S, Rutgeerts P. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut 2009; 58: 492-500.

118) Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? Gut 2011; 60: 1178-1181.

119) Sorrentino D. Role of biologics and other therapies in stricturing Crohn's disease: what have we learnt so far? Digestion 2008; 77: 38-47.

120) Van Assche G, Vermeire S, Rutgeerts P. The potential for disease modification in Crohn's disease. Nat Rev Gastroenterol Hepatol 2010; 7: 79-85.

121) Danese S. Inflammation and the mucosal microcirculation in inflammatory bowel disease: the ebb and flow. Curr Opin Gastroenterol 2007; 23: 384-389.

122) Danese S. Nonimmune cells in inflammatory bowel disease: from victim to villain", Trends Immunol 2008; 29: 555-564.

123) Ghosh S, Panaccione R. Anti-adhesion olecule therapy for inflammatory bowel disease. Therap Adv Gastroenterol 2010; 3: 239-258.

124) Koboziev I, Karlsson F, Zhang S, Grisham MB. Pharmacological intervention studies using mouse models of the inflammatory bowel diseases: translating preclinical data into new drug therapies. Inflamm Bowel Dis 2011; 17: 1229-1245.

125) Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 1993; 90: 770-774.

126) Nomura M, Li E. Smad2 role in mesoderm formation, left-right patterning and craniofacial development. Nature 1998; 393: 786-790.

127) Yang X, Li C, Hu X, Deng C. The tumor suppressor Smad4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. Proc Natl Acad Sci USA 1998; 95: 3667-3672.

128) Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of Smad3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J 1999; 18: 1280-1291.

129) Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol 1999; 19:2 495-2504.

130) Zhu Y, Richardson JA, Parada LF, Graff JM. Smad3 mutant mice develop metastatic colorectal cancer. Cell 1998; 94: 703-714.

131) Hawinkels LJ, Ten Dijke P. Exploring anti-TGF-β therapies in cancer and fibrosis. Growth Factors 2011; 29: 140-152.

132) Varga J, Pasche B. Antitransforming growth factor-beta therapy in fibrosis: recent progress and implications for systemic sclerosis. Curr Opin Rheumatol 2008; 20: 720-728.

133) Hahm K, Lukashev ME, Luo Y, Yang WJ, Dolinski BM, Weinreb PH, Simon KJ, Chun Wang L, Leone DR, Lobb RR, McCrann DJ, Allaire NE, Horan GS, Fogo A, Kalluri R, Shield CF 3rd, Sheppard D, Gardner HA, Violette SM. Alphav beta6 integrin regulates renal fibrosis and inflammation in Alport mouse. Am J Pathol 2007; 170: 110-125.

134) Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, Simon KJ, Hahm K, Allaire NE, Rinaldi NJ, Goyal J, Feghali-Bostwick CA, Matteson EL, O'Hara C, Lafyatis R, Davis GS, Huang X, Sheppard D, Violette SM. Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. Am J Respir Crit Care Med 2008; 177: 56-65.

135) Sullivan BP, Weinreb PH, Violette SM, Luyendyk JP. The coagulation system contributes to alphaVbeta6 integrin expression and liver fibrosis induced by cholestasis. Am J Pathol. 2010; 177: 2837-2849.

136) Katsumoto TR, Violette SM, Sheppard D. Blocking TGFβ via Inhibition of the αvβ6 Integrin: A Possible Therapy for Systemic Sclerosis Interstitial Lung Disease. Int J Rheumatol 2011; 2011: 208-219.

137) Panganiban RA, Day RM. Hepatocyte growth factor in lung repair and pulmonary fibrosis. Acta Pharmacol Sin 2011; 32: 12-20

138) Liu Y, Yang J. Hepatocyte growth factor: new arsenal in the fights against renal fibrosis? Kidney Int 2006; 70: 238-240.

139) Weiskirchen R, Meurer SK, Gressner OA, Herrmann J, Borkham-Kamphorst E, Gressner AM. BMP-7 as antagonist of organ fibrosis. Front Biosci 2009; 14: 4992-5012.

140) Baghy K, Iozzo RV, Kovalszky I. Decorin-TGFβ axis in hepatic fibrosis and cirrhosis. J Histochem Cytochem 2012; 60: 262-268.

141) Ma FY, Sachchithananthan M, Flanc RS, Nikolic-Paterson DJ. Mitogen activated protein kinases in renal fibrosis. Front Biosci 2009;1:171-187.

142) Distler JH, Distler O. Intracellular tyrosine kinases as novel targets for anti-fibrotic therapy in systemic sclerosis. Rheumatology 2008; 47(Suppl 5): v10-1.

143) Gordon J, Spiera R. Imatinib and the treatment of fibrosis: recent trials and tribulations. Curr Rheumatol Rep 2011; 13: 51-58.

144) Phanish MK, Winn SK, Dockrell ME. Connective tissue growth factor-(CTGF, CCN2)--a marker, mediator and therapeutic target for renal fibrosis. Nephron Exp Nephrol 2010; 114: e83-92.

145) Huang G, Brigstock DR. Regulation of hepatic stellate cells by connective tissue growth factor. Front Biosci 2012; 17: 2495-2507.

146) Fichtner-Feigl S, Young CA, Kitani A, Geissler EK, Schlitt HJ, Strober W. IL-13 signaling via IL-13R alpha2 induces major downstream fibrogenic factors mediating fibrosis in chronic TNBS colitis. Gastroenterology 2008; 135: 2003-2013.

147) Fichtner-Feigl S, Fuss IJ, Young CA, Watanabe T, Geissler EK, Schlitt HJ, Kitani A, Strober W. Induction of IL-13 triggers TGF-beta$_1$-dependent tissue fibrosis in chronic 2,4,6-trinitrobenzene sulfonic acid colitis. J Immunol 2007; 178: 5859-5870.

148) Urtasun R, Conde de la Rosa L, Nieto N. Oxidative and nitrosative stress and fibrogenic response. Clin Liver Dis 2008; 12: 769-790.

149) Serrano-Mollar A, Closa D, Prats N, Blesa S, Martinez-Losa M, Cortijo J, Estrela JM, Morcillo EJ, Bulbena O. *In vivo* antioxidant treatment protects against bleomycin-induced lung damage in rats. Br J Pharmacol 2003, 138: 1037-1048.

150) Svegliati-Baroni G, Saccomanno S, van Goor H, Jansen P, Benedetti A, Moshage H. Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells. Liver 2001; 21: 1-12.

151) Rachmilewitz D, Karmeli F, Okon E, Bursztyn M. Experimental colitis is ameliorated by inhibition of nitric oxide synthase activity. Gut 1995; 37: 247-255.

152) Sofroniadou S, Goldsmith D. Mammalian target of rapamycin (mTOR) inhibitors: potential uses and a review of haematological adverse effects. Drug Saf 2011; 34: 97-115.

153) Ong CT, Khoo YT, Mukhopadhyay A, Do DV, Lim IJ, Aalami O, Phan TT. mTOR as a potential therapeutic target for treatment of keloids and excessive scars. Exp Dermatol 2007; 16: 394-404.

154) Korfhagen TR, Le Cras TD, Davidson CR, Schmidt SM, Ikegami M, Whitsett JA, Hardie WD. Rapamycin prevents transforming growth factor-alpha-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2009; 41: 562-572.

155) Geissler EK, Schlitt HJ. The potential benefits of rapamycin on renal function, tolerance, fibrosis, and malignancy following transplantation. Kidney Int 2010; 78: 1075-1079.

156) Patsenker E, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. J Hepatol 2011; 55: 388-398.

157) Neef M, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. J Hepatol 2006; 45: 786-796.

158) Massey DC, Bredin F, Parkes M. Case report Use of sirolimus (rapamycin) to treat refractory Crohn's disease Gut 2008; 57: 1294-1296.

159) Dumortier J, Lapalus MG, Guillaud O, Poncet G, Gagnieu MC, Partensky C, Scoazec JY. Everolimus for refractory Crohn's disease: a case report. Inflamm Bowel Dis 2008; 14: 874-877.

160) Reinisch W, Panés J, Lémann M, Schreiber S, Feagan B, Schmidt S, Sturniolo GC, Mikhailova T, Alexeeva O, Sanna L, Haas T, Korom S, Mayer H. A multicenter, randomized, double-blind trial of everolimus versus azathioprine and placebo to maintain steroid-induced remission in patients with moderate-to-severe active Crohn's disease. Am J Gastroenterol 2008; 103: 2284-2292.

161) Nan YM, Han F, Kong LB, Zhao SX, Wang RQ, Wu WJ, Yu J. Adenovirus-mediated peroxisome proliferator activated receptor gamma overexpression prevents nutritional fibrotic steatohepatitis in mice. Scand J Gastroenterol 2011; 46: 358-369.

162) Kapoor M, McCann M, Liu S, Huh K, Denton CP, Abraham DJ, Leask A. Loss of peroxisome proliferator-activated receptor gamma in mouse fibroblasts results in increased susceptibility to bleomycin-induced skin fibrosis. Arthritis Rheum 2009; 60: 2822-2829.

163) Wei J, Ghosh AK, Sargent JL, Komura K, Wu M, Huang QQ, Jain M, Whitfield ML, Feghali-Bostwick C, Varga J. PPARγ downregulation by TGFß in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. PLoS One 2010; 5: e13778.

164) Wu M, Melichian DS, Chang E, Warner-Blankenship M, Ghosh AK, Varga J. Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. Am J Pathol 2009; 174: 519-533.

165) Kawai T, Masaki T, Doi S, Arakawa T, Yokoyama Y, Doi T, Kohno N, Yorioka N. PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. Lab Invest 2009; 89: 47-58.

166) Aoki Y, Maeno T, Aoyagi K, Ueno M, Aoki F, Aoki N, Nakagawa J, Sando Y, Shimizu Y, Suga T, Arai M, Kurabayashi M. Pioglitazone, a peroxisome proliferator-activated receptor gamma ligand,suppresses bleomycin-induced acute lung injury and fibrosis. Respiration 2009; 77: 311-319.

167) Yang L, Stimpson SA, Chen L, Wallace Harrington W, Rockey DC. Effectiveness of the PPARγ agonist, GW570, in liver fibrosis. Inflamm Res 2010; 59: 1061-1071.

168) Ravi A, Garg P, Sitaraman SV. Matrix metalloproteinases in inflammatory bowel disease: boon or a bane? Inflamm Bowel Dis 2007; 13: 97-107.

169) Clutterbuck AL, Asplin KE, Harris P, Allaway D, Mobasheri A. Targeting matrix metalloproteinases in inflammatory conditions. Curr Drug Targets 2009; 10: 1245-1254.

170) Shyu KG, Wang BW, Chen WJ, Kuan P, Hung CR. Mechanism of the inhibitory effect of atorvastatin on endoglin expression induced by transforming growth factor-beta$_1$ in cultured cardiac fibroblasts. Eur J Heart Fail 2010; 12: 219-226.

171) Burke JP, Watson RW, Murphy M, Docherty NG, Coffey JC, O'Connell PR. Simvastatin impairs smad-3 phosphorylation and modulates transforming growth factor beta$_1$-mediated activation of intestinal fibroblasts. Br J Surg 2009; 96: 541-551.

172) Abe Y, Murano M, Murano N, Morita E, Inoue T, Kawakami K, Ishida K, Kuramoto T, Kakimoto K, Okada T, Narabayashi K, Umegaki E, Higuchi K. Simvastatin attenuates intestinal fibrosis independent of the anti-inflammatory effect by promoting fibroblast/myofibroblast apoptosis in the regeneration/healing process from TNBS-induced colitis. Dig Dis Sci 2012; 57: 335-344.

173) Sakamoto I, Ito Y, Mizuno M, Suzuki Y, Sawai A, Tanaka A, Maruyama S, Takei Y, Yuzawa Y, Matsuo S. Lymphatic vessels develop during tubulointerstitial fibrosis. Kidney Int 2009; 75: 828-838.

174) Danese S. Role of the vascular and lymphatic endothelium in the pathogenesis of inflammatory bowel disease: 'brothers in arms'. Gut 2011; 60: 998-1008.

175) Scaldaferri F, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, Yla-Herttuala S, Fiocchi C, Danese S. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 2009; 136: 585-595.

176) Hering NA, Fromm M, Schulzke JD. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. J Physiol 2012; 590: 1035-1044.

177) Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. Gut 2012; 61: 1619-1635.

178) Hartmann P, Chen WC, Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. Front Physiol 2012; 3: 402.

179) Yang S, Banerjee S, de Freitas A, Sanders YY, Ding Q, Matalon S, Thannickal VJ, Abraham E, Liu G. Participation of miR-200 in pulmonary fibrosis. Am J Pathol 2012; 180: 484-493.

180) Wang B, Koh P, Winbanks C, Coughlan MT, McClelland A, Watson A, Jandeleit-Dahm K, Burns WC, Thomas MC, Cooper ME, Kantharidis P. miR-200a Prevents renal fibrogenesis through repression of TGF-β2 expression. Diabetes 2011; 60: 280-287.

181) Chen Y, Ge W, Xu L, Qu C, Zhu M, Zhang W, Xiao Y. miR-200b is involved in intestinal fibrosis of Crohn's disease. Int J Mol Med 2012; 29: 601-606.

182) Pekow JR, Kwon JH. MicroRNAs in inflammatory bowel disease. Inflamm Bowel Dis 2012; 18: 187-193.