World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.4291/wjgp.v4.i4.100

World J Gastrointest Pathophysiol 2013 November 15; 4(4): 100-107
ISSN 2150-5330 (online)

MINIREVIEWS

Fibrogenesis and fibrosis in inflammatory bowel diseases: Good and bad side of same coin?

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Received: June 28, 2013 Revised: September 9, 2013
Accepted: October 16, 2013
Published online: November 15, 2013

Abstract

Fibrogenesis in inflammatory bowel diseases is a complex phenomenon aimed at mucosal repair. However, it may provoke intestinal fibrosis with the development of strictures which require surgery. Therefore, fibrogenesis may be considered as a "two-faced" process when related to chronic intestinal inflammation. Many types of cells may be converted into the fibrogenic phenotype at different levels of the intestinal wall. A complex interaction of cytokines, adhesion molecules and growth factors is involved in the process. We report an overview of recent advances in molecular mechanisms of stricturizing Crohn's disease (CD) including the potential role of trasforming growth factor beta, protein kinase C and Ras, Raf and ERK proteins. Fibrotic growth factors such as vascular endothelial growth factor and platelet-derived growth factor, as well as the Endothelial-to-Mesenchymal Transition induced by transforming growth factor-β, are considered. Finally, our experience, focused on tumor necrosis factor α (the main cytokine of inflammatory bowel diseases) and the link between syndecan 1 (a heparan sulphate adhesion molecule) and basic fibroblast growth factor (a strong stimulator of collagen synthesis) is described. We hypothesize a possible molecular pattern for mucosal healing as well as how its deregulation could be involved in fibrotic complications of CD. A final clinical point is the importance of performing an accurate evaluation of the presence of fibrotic strictures before starting anti-tumor necrosis α treatment, which could worsen the lesions.

Key words: Fibrogenesis; Fibrosis; Tumor necrosis factor -α; Syndecan 1; Basic fibroblast growth factor; Cellular fibrogenic phenotype; Inflammatory bowel diseases

Core tip: The present minireview reports an outline of the mechanisms of fibrogenesis in inflammatory bowel diseases. Potential fibrogenetic cells and their characterization are detailed. Recent advances in possible molecular mechanisms are highlighted. Our experience, suggesting the hypothesis of a possible molecular mechanism of mucosal healing, is described. The modalities whereby a deregulation of this molecular pattern may lead to fibrotic strictures in Crohn's disease are also illustrated. Finally, possible clinical implications are outlined.

Principi M, Giorgio F, Losurdo G, Neve V, Contaldo A, Di Leo A, Ierardi E. Fibrogenesis and fibrosis in inflammatory bowel diseases: Good and bad side of same coin? *World J Gastrointest Pathophysiol* 2013; 4(4): 100-107 Available from: URL: http://www.wjgnet.com/2150-5330/full/v4/i4/100.htm DOI: http://dx.doi.org/10.4291/wjgp.v4.i4.100

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are immunologically mediated disorders of the gastrointestinal tract in which inflammation and damage are the main findings. Fibrosis may be a complication of both processes, occurring more frequently in CD and often requiring surgical treatment due to the development of stenosis and hence bowel occlusion. However, fibrogenesis is a phenomenon that is intimately involved in mucosal repair[1] and, therefore, fibrotic complications of the disorders are paradoxically closely linked to a physiological phenomenon aimed at restoring damaged mucosa.

On these bases, a better understanding of the modalities of the evolution of fibrogenesis into fibrosis is essential, and the issues of how fibrosis differs from normal tissue repair, as well as the identification of cellular mediators for testing possible therapies, are intriguing emerging research concepts.

CELLULAR BASIS OF FIBROSIS

Fibrosis in CD can be viewed as an extreme healing response to injury. This model predicts that injury causes an initial activation of normal intestinal mesenchymal cells, with a shift to a "fibrogenic" phenotype[2,3]. These cells are characterized by an enhanced ability to trigger extracellular matrix (ECM) synthesis. Following acute injury, however, the normal intestinal architecture is restored because post-transcriptional and post-translational mechanisms prevent the accumulation of ECM, while fibrogenic cells are eliminated. By contrast, in fibrosis the mechanisms serving to degrade ECM are not operative at appropriate levels and fibrogenic cells are not only maintained but increase in number. The mechanisms regulating these effects are unknown but may include factors associated with CD, such as cytokines or transmural inflammation.

The normal intestine has a large, heterogeneous population of mesenchymal cells, some of which synthesize significant amounts of collagen. These cells could be considered to have a fibrogenic phenotype and are mainly constituted by fibroblasts and smooth muscle cells or myofibroblasts, as shown by their immunostaining properties with antibodies to vimentin (V) and α-smooth muscle actin (α-SMA)[2,3].

Fibroblasts are V+/A-, while smooth muscle cells are V-/A+ and predominate in the normal muscularis mucosa and muscularis propria. Subepithelial myofibroblasts (SEMF) with a V+/A+ phenotype are found adjacent to epithelial cells. However, some of these, that share common features with V+/A+ myofibroblasts, do not express α but γ-SMA[2,3].

Interstitial cells of Cajal (ICC) are a myofibroblast-related subtype specific to the intestine. They are located between enteric smooth muscle layers and serve to regulate gut motility. The c-kit receptor, which binds the protooncogene stem cell or steel factor, is a marker of cells of Cajal. Recent studies suggest that in normal human intestine these elements also express vimentin, but not α-SMA. It has been suggested that ICC could transform into a collagen-expressing fibroblast or myofibroblast phenotype. Another possibility is that ICC are destroyed during fibrosis and replaced by cells with a fibroblast phenotype[2,3].

Inflammatory cells that infiltrate the gut in UC and CD include macrophages, lymphocytes, and plasma cells. These may have important interactions with mesenchymal cells and thereby impact fibrosis[4,5].

In normal intestine, SEMF and fibroblasts found in submucosa, serosa, and intermuscular connective tissue are the primary sites of expression of collagen mRNA and protein. In UC, collagen mRNA expression is upregulated in SEMF, suggesting that chronic inflammation further increases the activity of fibrogenic cells.

Recent studies in fibrotic intestine from CD patients indicate that V+/A- or V+/A+ fibroblasts and myofibroblasts are the major sites of increased collagen mRNA expression and collagen deposition[6]. An overgrowth of the muscularis mucosa and muscularis propria occurs in CD but not UC, and this contributes to the development of stenosis, strictures, and obstruction[7]. Muscularis overgrowth also occurs in some animal models of chronic intestinal inflammation and these data support the concept that in muscularis overgrowth in CD, but not in UC, a change in enteric smooth muscle cells towards a fibroblast or myofibroblast phenotype[8] is implicated.

MOLECULAR MECHANISMS OF FIBROGENESIS

Cytokines are a heterogeneous class of secretory proteins produced by several types of cells. For some of them, they act as growth factors, for others as regulators of cellular division and finally, cytokines may paradoxically trigger mechanisms which mediate cell death. All these effects occur *via* regulation of the immune system and inflammation, so cytokines are currently subdivided into pro and anti-inflammatory types. The main cytokine involved in the pathogenesis of both UC and CD is tumor necrosis factor-α (TNF-α)[9,10]. The sites of TNF-α production are the mononuclear phagocytes, antigen activated T-lymphocytes, activated mast cells and natural killer cells. Conventional stimulators of TNF-α production are the lipopolysaccharides of the Gram negative bacteria cellular wall, since they are the main mediators of the host response to these organisms. However, TNF-α may be seen as two-faced, since it is able to trigger a closed circle in which, starting from tissue damage, it generates an inflammatory response that exacerbates the damage itself. Moreover, increasing doses of TNF-α may have a lethal effect. Nevertheless, TNF-α plays a key role in the maintenance of tuberculous granuloma, allowing Koch's bacilli to "be walled alive" and thus pre-

venting their spread in the body of an infected person (miliary tuberculosis)[11].

Cell adhesion molecules (CAMs) are proteins located on the cell surface involved in binding with other cells or with the ECM. Essentially, cell adhesion molecules help cells to join together and to their surroundings. These proteins are typically transmembrane receptors and are composed of three domains: an intracellular domain that interacts with the cytoskeleton, a transmembrane domain, and an extracellular domain that interacts either with other CAMs of the same kind (homophilic binding) or with other CAMs or the extracellular matrix (heterophilic binding)[12]. A subtype of adhesion molecules containing heparan sulphate (syndecan family) is chemically a proteo-glycan and plays a significant role in tissue repair[13]. At intestinal level, syndecan 1 is located in the basolateral region of the columnar epithelium[14] and is a relevant factor in the reversal of inflammatory bowel disease (IBD) damage[15,16]. Indeed, in inflamed mucosa, these molecules are mainly located in the cells of the stroma and apical epithelium, where the repair of ulcerative lesions will presumably occur.

Basic fibroblast growth factor (bFGF) is a member of the fibroblast growth factor family[17], comprising at least 22 factors with pleiotropic functions[18]. This peptide is able to repair ulcerative lesions because of its capacity to bind epithelial and stromal cells. In normal tissue, basic fibroblast growth factor is present in basement membranes and in the subendothelial extracellular matrix of blood vessels. It stays membrane-bound as long as there is no signal peptide, when it acts as a potent angiogenic factor in patho-physiological processes that include wound healing and tissue repair[19-22]. The bFGF has been shown to promote proliferation of endothelial cells, to increase the number of fibroblasts and myofibroblasts and to activate these fibroblasts. The induction of collagen secretion from CD and UC fibroblasts by bFGF may be one of the mechanisms inherent to the stromal processes of the disease, including transmural fibrosis and stricture formation, as well as tissue repair and healing.

Klagsbrun *et al*[23] suggested that heparan sulphate proteoglycans (and, therefore, syndecan 1) change the bFGF morphology and modulate the structure of its receptors, allowing it to bind to the cells dedicated to the repair process, such as those located at the margins of an ulcerative lesion[24,25]. bFGF, when not activated by syndecan 1, is destroyed by luminal and circulating proteases, which may be activated by TNF-α, thus impeding the tissue restoration process.

Molecular mechanisms: recent advances

A relevant actor in the pathogenesis of fibrotic complications of CD is the cytokine transforming growth factor beta (TGF-β)[26]. TGF-β is secreted by many cell types, including macrophages, and has a controlling role in cellular proliferation, differentiation and apoptosis, immunoregulation, supervision of the inflammatory response, as well as fibrosis and other functions including tissue healing. It is known that TGF-β promotes collagen expression by intestinal fibroblasts and smooth muscle cells[27,28]. This process is mediated by an intracellular signaling pathway in which a cascade constituted by protein kinase C and Ras, Raf and ERK proteins plays a key role[26,29,30]. Moreover, it has been hypothesized that TGF-β promotes the overexpression of adhesion molecules (*e.g.*, intracellular adhesion molecule-1)[31] by fibroblasts and other pro-fibrotic growth factors such as vascular endothelial growth factor[32] and platelet-derived growth factor[33]. Finally, a recently revealed mechanism of fibrogenesis in CD, is the Endothelial-to-Mesenchymal Transition induced by TGF-β[34]. This cytokine is able to induce a protein expression pattern in endothelium that leads to a de-differentiation of these cells and to a transformation to a fibrogenetic phenotype, similar to fibroblasts.

In conclusion, the overexpression of TGF-β and its receptors in both the intestinal wall, and in fibroblast cultures taken from sites of intestinal stricture in patients with CD, suggests a potential regulatory role for this cytokine in intestinal fibrogenesis[35].

Adipokines are cell-to-cell signaling proteins produced by adipose tissue. The best known adipokines are leptin, adiponectin and resistin. They play a key role in regulating energy intake and expenditure, including appetite and hunger, metabolism, and behavior: indeed, their role has beenwidely studied in diseases like metabolic syndrome and type 2 diabetes. Recently, however, further functions of these molecules have been discovered, as mediators of systemic inflammation. It has been shown that obesity per se, and in particular visceral adiposity, is associated with systemic microinflammation, and disturbed circulating adipokines levels[36,37]. This is why numerous studies have been focused on the role of adipokines in the pathogenesis of IBD[38].

The outer intestinal wall in patients affected by IBD is enveloped by fat deposits called "wrapping" or "creeping" fat that seem to play an important role in the pathogenesis of IBD. An overexpression of leptin mRNA in mesenteric visceral adipose tissue (mWAT) has also been found in IBD subjects[39], and is correlated with local macrophages infiltration, which drives a high expression of interleukin (IL)-10, IL-6 and TNF-α[40]. On the other hand, adiponectin seems to have a protective function against inflammation in IBD[41,42]. Rodrigues *et al*[43] evaluated serum adiponectin and leptin by enzyme-linked immunosorbent assay in patients with active CD (ACD group), CD in remission (RCD group) and in six healthy controls, and found that serum adiponectin was lower in the ACD group as compared to controls, whereas there were no differences between the ACD and RCD groups.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is involved in the control of the expression of DNA sequences that affect basic cell functions, like cell growth, differentiation and death[44]. This pathway is triggered by the binding

of a ligand (usually a pro-inflammatory cytokine such as IL-6[45], IL-12, IL-1, TNF-α or interferon gamma[46]) to a tyrosine-kinase membrane receptor. Wu *et al*[47] evaluated whether intestinal myofibroblasts could produce nitric oxide (NO) in response to the IBD-associated cytokines IL-1b, TNF-α, and interferon gamma in a JAK-STAT dependant pathway, using intestinal myofibroblasts isolated from mice and cultured. The result was an increasing expression of inducible nitric oxide synthase (iNOS) mRNA (evaluated by real time polymerase chain reaction, RT-PCR), but not endothelial NOS or neuronal NOS. This mechanism was shown to be enhanced by a protein cascade constituted by JAK-STAT, Akt and NF-κB. The importance of NO in the pathogenesis of IBD has long been known and is widely discussed in literature[48,49]. Furthermore, genetic studies investigating polymorphisms in the *JAK* gene[50] revealed that same genetic variants (the G allele of rs744166 and the C allele of rs3816769) increase not only the risk of onset of CD, but even the risk of strictures requiring surgery, because of the interaction with the *CARD15* gene[51]. Considering the JAK2 rs10758669 polymorphism, the homozygous C/C or heterozygous A/C genotypes had a higher risk of CD as compared with the homozygous A/A (OR = 1.76, 95%CI: 1.26-2.45 and OR= 2.36, 95%CI: 1.44-3.86, respectively). On these bases, future therapy with JAK inhibitors for their anti-inflammatory effects appears promising[52].

A novel field of genetics which is attracting the attention of researchers is epigenetics, *i.e.*, the study of all the heritable modifications that vary gene expression while not altering the DNA sequence. Micro RNAs (miRNA)[53] are just one example. miRNAs are small non-coding RNA oligonucleotides that can regulate the expression of a large number of genes and have been implicated in different human diseases like cancer[54] and inflammatory diseases[55,56] including IBD[57]. A very recent study[58] performed on NCM460 human colonocytes incubated with interleukin-6 and on colon biopsies from pediatric and adult patients with UC revealed that a deregulation (low levels) of miRNA-124 can cause a hyper-phosphorylation of STAT3 (and consequently, hyper-activation), *via* a mechanism induced by IL-6, very likely resulting in a pathogenic system leading to IBD. miRNA-124 is only a second lead on the crowded stage of miRNAs: miRNA-192, which is normally expressed in colonic epithelial cells, is significantly reduced in tissues of patients with active UC[59]; miR-150 is up-regulated in mice with dextran sulfate sodium-induced colitis and in colon tissues from patients with UC[60]; an overexpression of miRNA-21, which promotes inflammation, has been reported in several studies of patients with active UC and CD colitis[61].

The process of DNA methylation is another form of epigenetic regulation of gene expression. It consists in the binding of a methyl group to cytosines that are part of cytosine-guanine dinucleotides (CpG), and has a gene silencing function. The main genes whose methylation is involved in the pathogenesis of IBD are the CDH1[62], BCL3, STAT3, OSM, STAT5[63] proteins involved in the IL-12 and IL-23 pathways.

Finally, histone modifications are an epigenetic process that may modify genomic expression. Histones are alkaline proteins that package and order the DNA into structural units called nucleosomes and chromatine[64]. They have N-terminal amino acid tails that protrude and can be modified by acetylation, methylation, ubiquitination, and phosphorylation. Acetylation, however, is the most closely studied phenomenon, because it improves gene transcription and recruitment of transcriptional factors. In the course of IBD, the main genes targeted by histone acetylation are *p53*, *STAT3*, and *HIF1a*[65]. Furthermore, an innate immune response to microbiota has been proposed to link histone modifications with inflammation: butyrate, an endogenous metabolite formed during fermentation of dietary fibers by the intestinal microbiota, is a histone deacetylase inhibitor. Butyrate increases the expression of NOD2 by increasing histone acetylation in its promoter region[66].

The importance of all these epigenetic mechanisms lies in possible future therapeutic applications: inhibitors of deacetylation, demethylating agents and miRNA produced by genetic engineering could be potential targets in IBD[67].

Molecular mechanisms in mucosal healing and strictures: our experience

Our previous investigations[68] demonstrated that a decrease of TNF-α induced by anti-TNF-α (infliximab) treatment is accompanied by a decrease in both syndecan 1 and bFGF when mucosal healing occurs. A possible explanation is that infliximab therapy may downregulate, *via* a marked reduction of TNF-α mucosal levels, the bFGF/syndecan 1 link. This molecular profile could represent a pathway of mucosal healing. However, the parallel trend of TNF-α, syndecan 1 and bFGF could be just a simultaneous consequence of the control of inflammation. To dispel this doubt, we analyzed the "timing" of the TNF-α decrease and bFGF/syndecan 1 reversal to normal levels and sites in cultured biopsy samples taken from patients with both CD and UC and incubated in a medium containing comparable amounts of infliximab similar to those reached in the serum of treated patients. After 24 h we assayed TNF-α, syndecan 1 and bFGF in tissue homogenates. TNF-α was found to be decreased, while syndecan 1 and bFGF levels were still high when evaluated by both a molecular method (reverse transcriptase real time polymerase chain reaction) and immunohistochemistry[69]. This last finding supports our primary hypothesis that a mucosal TNF-α reset, induced by biological drugs, is followed by a mucosal restoration in which syndecan 1 modulates the strong reparative bFGF aptitudes. Finally, in healed mucosa, cytokines, adhesion molecules and growth factors resume their normal pattern.

A report by Bousvaros *et al*[70] showed that in chil-

dren with CD there was a strong correlation between the bFGF level and disease activity. The relationship of bFGF with disease activity persisted even after adjusting for other covariates (including age, sex, hematocrit, albumin, and sedimentation rate) in a multivariate linear regression model. There was also a statistically significant, although less strong, correlation between the bFGF level and disease activity in UC. Although bFGF is not a specific marker for CD, its serum levels reflect disease activity. Therefore, bFGF release may be important in mediating the angiogenesis and wound healing seen in CD. This report, as well as our experience both *in vivo* and *in vitro*[68,69], suggest a similar molecular mechanism for mucosal healing both in CD and UC.

In a further study, we investigated the pattern of TNF-α, syndecan 1 and bFGF in patients with CD complicated by fibrotic stenosis undergoing surgical resection[71]. We observed that TNF-α mucosal levels were not significantly increased. A possible explanation for this finding may be that an overgrowth of fibrotic tissue may be a successive stage after inflammation, where the increase in TNF-α is the peculiar aspect. Therefore, at the stage of fibrotic stenosis requiring surgery, inflammatory mucosal changes may be an irrelevant phenomenon in most patients. Syndecan 1 levels were increased, showing a pattern similar to the one observed in damaged tissues. It may be presumed that the molecule location, albeit limited to the mucosal layer, reflects an attempt at bFGF modulation. However, this function cannot be effectively carried out due to the bFGF overexpression and location all along the intestinal wall, *i.e.*, outside the district where syndecan 1 could operate[14]. Indeed, bFGF overexpression affects all intestinal wall layers, being expressed in epithelium, stroma, muscularis propria and endothelium. It is possible that: (1) the low levels of TNF-α may provoke a failure in cytokine induced bFGF proteolysis; (2) the presence of syndecan 1 is limited to the mucosal layer with a consequent very partial regulation of bFGF binding to specific receptors dedicated to tissue repair; and (3) an irreversible transformation of different type of cells to the fibrogenetic phenotype occurs, thus provoking the prevalence of fibrotic over inflammatory stenotic lesions[72].

In strictures, it is possible that the excess extracellular matrix cannot be inhibited by the regulatory mechanisms of the phenomenon, in accordance with the hypothesis proposed by Pucilowska *et al*[72]. On these bases, we may conclude that the different molecular patterns in repair dedicated fibrogenesis and stricturizing fibrosis in CD could be the consequence of different mucosal levels of TNF-α. These are very high in the active disorder, but undergo a progressive depletion in the long term, and this event may trigger a polymorphic regulation of the syndecan 1/bFGF system.

FINAL REMARKS

Fibrogenesis in inflammatory bowel diseases is a phenomenon aimed at tissue repair. Many cellular types are involved in this process and cytokines, adhesion molecules and growth factors interact to achieve mucosal repair. However, a deregulation of the healing molecular pathway can progress towards fibrosis and stenotic complications often requiring surgical therapy[73,74]. Therefore, fibrogenesis and fibrosis may represent the good and bad sides of the same coin, the former allowing lesion healing but the latter leading to severe complications. The main tool for discriminating between them is, in our experience, the presence/absence of inflammation (and, consequently, the level of TNF-α expression).

A final clinical consideration is the importance of making an accurate evaluation[75] in cases of stenosis in the course of CD using all available diagnostic tools (histology, ultrasonography[76] with Doppler evaluation of the resistance index, magnetic resonance[77], computed tomo-enterography[78], biochemical indices of inflammation[79,80]) in order to distinguish inflammatory from fibrotic stenosis. This could orient anti-TNF-α therapy[81], that should be limited to the first case, avoiding the risk that the cytokine decrease could support fibrotic complications rather than mucosal healing.

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