Mesenchymal cell proliferation and programmed cell death: key players in fibrogenesis and new targets for therapeutic intervention

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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 300: G703–G708, 2011. First published January 13, 2011; doi:10.1152/ajpgi.00504.2010.—An exquisite equilibrium between cell proliferation and programmed cell death is required to maintain physiological homeostasis in inflammatory bowel disease (IBD), and especially in Crohn’s disease (CD), enhanced proliferation along with defective apoptosis of immune cells are considered key pathogenic elements. This notion is supported by the histological observation that a large number of inflammatory cells infiltrate the bowel wall in patients with active IBD. Such leukocyte infiltration must result from either enhanced leukocyte recruitment from the bloodstream, an abnormal expansion of these cells within the bowel wall, or a combination of both events.

Several studies have demonstrated that T cells taken from CD patients have the ability to proliferate more than those from normal controls. Ina et al. (24) observed an increase in CD T cell proliferation upon IL-2 stimulation whereas T cells from ulcerative colitis (UC) multiplied less than control T cells. Sturm et al. (72) further expanded these observations by demonstrating that, compared with normal cells, CD T cells cycle faster, express increased phosphorylated retinoblastoma protein and decreased phosphorylated p53 levels, and undergo vigorous cellular expansion upon CD2 and CD3 stimulation. The contribution of immune cell proliferation to the development of IBD is further underlined by the efficacy shown by immunosuppressive agents such as azathioprine, 6-mercaptopurine, and methotrexate in the treatment of IBD (16, 20, 56, 59, 67, 67a).

Similarly, a growing body of evidence suggests that mucosal CD T cells display an increased resistance to undergoing apoptosis. In the above mentioned study by Ina et al. (24), less apoptosis occurred in CD than control T cells upon IL-2 deprivation, a difference that could be explained by the marked decrease in the proapoptotic protein bax and an increase in the antiapoptotic protein bcl-2 found in the CD T cell population. In keeping with these results, Sturm et al. (72) showed that CD T cells display less caspase activity, but more telomerase activity, resulting in a significantly decreased rate of programmed cell death. More recently, it has been demonstrated that FAS-mediated apoptosis was lower in CD than in UC and control T cells, whereas enhanced expression of both long and short Flip (a Flice inhibitor protein) isoforms was present in both biopsy specimens and purified mucosal T cells taken from CD patients. Moreover, the authors identified that inhibition of Flip by antisense oligonucleotides could reverse the resistance of CD mucosal T cells to FAS-induced apoptosis (48). Intrinsinc defects in the control of programmed cell death in mucosal T cells are strongly implicated in the pathogenesis of IBD. In addition, they may also be used to differentiate between the cellular and molecular mechanisms underlying the pathogenesis of UC and CD (57).

In the last decade several investigators have demonstrated that almost all drugs with clinical efficacy in IBD, including 5-aminosaliclates, steroids, azathioprine, methotrexate, and infliximab, are able to induce apoptosis of immune cells in vitro. This observation, combined with the fact that etanercept,
an anti-TNF-fusion protein that does not induce apoptosis of immune cells, was not efficacious in the treatment of CD (45, 67, 71), led to two conclusions: 1) induction of immune cells apoptosis is a key mechanism of action of many drugs useful to treat active CD patients, and 2) when searching for new CD therapies, induction of immune cells apoptosis seems to be a requirement to be fulfilled. These concepts were broadly accepted by the IBD community until quite recently when another anti-TNF-α agent, certolizumab pegol, lacking the antibody Fc fragment and therefore unable to induce cell apoptosis, was shown to be useful for the induction and maintenance of remission in active CD (69, 70).

Therefore, the presence of abnormal immune cell proliferation and programmed cell death do contribute to CD pathogenesis and most drugs used to treat CD patients have demonstrated the ability to reverse these abnormalities, underlining the pathogenic relevance of these processes.

**Contribution of Abnormal Mesenchymal Cell Proliferation to Fibrogenesis**

The development of fibrosis results from an imbalance in extracellular matrix (ECM) deposition and degradation. The balance can be tipped toward a net increase in collagen and ECM production when individual cells produce a greater amount of ECM, when there are a greater number of ECM producing cells, or a combination of the two. 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 programmed cell death.

Marked heterogeneity has been observed in the function of fibroblast-like cells between different tissues and even within the same tissue. Although it is agreed that fibrosis is almost invariably preceded by inflammation, it is unclear whether fibroblast-like cells require continuous exposure to the inflammatory microenvironment to induce fibrosis or whether a “fibrogenic” phenotype may emerge following prolonged exposure to inflammation. This was initially examined using fibroblasts isolated from idiopathic pulmonary fibrosis (IPF), a condition characterized by derangement of the alveolar wall secondary to collagen deposition. Fibroblasts from fibrosed lung tissue demonstrated markedly elevated proliferation rates in vitro when compared with those taken from normal pulmonary tissue suggesting the existence of fibroblast subgroups within pulmonary fibrosis (30). In the liver the retinoid-storing quiescent cells, in the presence of chronic inflammation, transdifferentiate into hepatic stellate cells (HSC) that display a myofibroblast phenotype and acquire contractile, proinflammatory, and fibrogenic properties (18). Similar findings are also observed in other fibrotic conditions such as scleroderma (17), urethral strictures (4), and the ECM changes associated with breast carcinoma (68).

In the intestine there also appears to be an inflammation-induced fibroblast phenotype with fibroblast-like cells isolated from IBD patients demonstrating significantly faster proliferation than those from control intestine. The proliferation rates did not differ significantly regardless of whether cells were derived from fibrosed CD, inflamed CD, and UC did not differ significantly. Similarly, following stimulation with basic fibroblast growth factor and insulin-like growth factor-I, the enhancement in proliferation was similar among the different IBD groups (32). It has also been observed that in IBD the development of intestinal fibrosis localizes to regions of active inflammation and does not differ between the type of IBD (33). This suggests that, as in the lung and liver, there is a functionally distinct subset of intestinal fibroblasts that proliferate more rapidly, is induced by chronic inflammation, and is independent of the disease type.

It seems obvious that the most effective way to prevent or limit the extent of fibrosis is to remove the underlying causal agent. This in many cases is not possible. However, if inhibition of fibroblast proliferation can be achieved, then potentially the level of ECM production and tissue fibrosis could also be reduced. Numerous agents have been suggested as potentially effective against fibrogenesis; however, data are extremely limited for most of them.

The nonsteroidal anti-inflammatory drugs (NSAID) are, as their name suggest, anti-inflammatory and block the synthesis of prostaglandins (PGs). PGE1 and 2 are known to inhibit smooth muscle cell proliferation (28) and inhibit both TNF-α and IL-1β-induced fibroblast proliferation (12). Reduced PGE2 levels are associated with the development of fibrosis in IPF (82) and the use of the NSAID indomethacin has been shown to markedly increase dermal fibrosis (43). Its use in the 2,4,6-trinitrobenzenesulfonic acid (TNBS) mouse model of intestinal fibrosis is also associated with markedly increased intestinal fibrosis (31). In IBD, PGE2 synthesis is increased in the inflamed mucosa of CD patients (2) and its levels are also increased by sulfasalazine treatment (55), but any therapeutic role that PGE2 may play in intestinal fibrosis requires further examination.

The polyunsaturated lecithin soybean extract, phosphatidylcholine (PC), has demonstrated an ability to prevent cirrhosis in a baboon model of alcohol-induced cirrhosis (35, 36), whereas it also decreased stricture formation in the rat TNBS intestinal fibrosis model (50). Benefit has also been observed with its use against tissue necrosis in immune-mediated chronic hepatitis (52) and chronic active hepatitis (9). Any role on fibroblast proliferation, however, has yet to be investigated, but polyunsaturated fatty acids like PC are precursors to PGE2, and their mechanism of action may, potentially, be through inhibition of cellular proliferation.

Another potential agent for the modification of fibrosis in IBD is the steroid hormone retinoic acid (RA). In mice its use inhibited both radiation and bleomycin-induced pulmonary fibrosis (73), whereas in humans it inhibited fibroblast proliferation in both IPF and neonatal lungs (75). In the liver it inhibits HSC proliferation (14) and dermal fibroblast proliferation both in vitro and in vivo (13). Investigation of its effect in the intestine is limited to the TNBS mouse model of intestinal fibrosis, where its use was associated with a reduction in intestinal fibrosis (31). In contrast to the above, a deficiency in RA has been associated with the development of hepatic fibrosis in the rat (82). Again, however, further work is required.

Angiotensin type 1 (AT1) receptor blockers are also potentially antifibrogenic and have been shown to attenuate liver fibrogenesis with reduction in both collagen deposition and the accumulation of myofibroblasts (49). Angiotensin II is known to induce HSC proliferation through its binding to AT1 receptors (5), whereas inhibition of angiotensin II is able to induce liver myofibroblast apoptosis (53) and reduced proliferation in...
the intestinal fibroblast (41). Heparin is also known to inhibit fibroblast proliferation, as well as collagen production in human intestinal smooth muscle cells in a reversible dose-dependent manner in vitro (19). But as with all the above potentially useful agents, there is a great need for further investigation before there is enough known to recommend their clinical use.

**Contribution of Abnormal Mesenchymal Programmed Cell Death to Fibrogenesis**

During the perpetuation of fibrosis, mesenchymal cells activation involves discrete changes in cell behavior: proliferation, chemotaxis, contractility, matrix production, and resistance to apoptosis. It has been demonstrated that apoptosis is responsible for mediating the reduction in HSC number during the resolution of hepatic fibrosis (26) and, conversely, that induction of HSC apoptosis has an antifibrotic effect (83).

Whereas previous work has 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. TIMP-1 suppresses HSC apoptosis both in vitro and in vivo (51), highlighting a potential role for HSC survival in liver fibrosis. So far, however, no similar work has been carried out in CD myofibroblasts.

In 1996, a susceptibility locus for CD located adjacent to the centromere on chromosome 16 was first identified (23). Further analysis of this region identified a strong association with the gene NOD2, also known as caspase-recruitment domain protein 15 (CARD15), which is involved in the recognition of bacteria with CD (80). This gene contains two amino-terminal effector domains, known as caspase-recruitment domains (CARDs), which induce the nuclear factor-$\kappa$B (NF-$\kappa$B) signaling cascade (10). The CARD domain, however, is also implicated in signal transduction that results in apoptosis via the caspasers.

**NOD2/CARD15** was originally shown to be expressed by monocyte/macrophage cells, but a more recent study has demonstrated expression also by intestinal myofibroblasts (54). Overexpression of NOD2/CARD15 enhances apoptosis through induction of caspase-9 expression. It is, therefore, attractive to speculate that mutations of this protein are implicated in the apoptotic pathway and may trigger an impaired proapoptotic response on activated cells resulting in continued activation. Indeed, a cohort study describing genotype/phenotype correlation in CD patients and NOD2 variants showed a correlation with fibrostenosing CD (1).

In 2007, two independent genome-wide association studies (GWAS) identified ATG16L1 as a susceptibility variant for CD (21, 63). The ATG16L1 gene product is part of a multimeric protein complex that is essential for autophagy, a biological process that mediates the bulk degradation of cytoplasmic components in lysosomes and vacuoles. In the Wellcome Trust Case Control Consortium GWAS, a second autophagic gene was also identified with multiple SNPs in the IRGM gene, and this was highly associated with CD (80). It is clear from these genetic studies that autophagic processes may be associated with the pathogenesis of CD, and other studies have also demonstrated that autophagy plays an important role in clearance of apoptotic bodies (61). Persistence of apoptotic bodies as a result of incomplete autophagy could be a potential contributor to the continual inflammatory process that characterizes CD.

Until now, it was thought that NOD2 and autophagy independently influenced the development of CD. Recently, however, studies provide a link between these two major pathways. Cooney et al. (11) identified that NOD2 engagement by pentagalactans induces autophagy and that this process is disturbed in individuals bearing risk alleles for either NOD2 or ATG16L1, suggesting that these two genes share a common pathway. Two other polymorphisms have been also implicated in the development of fibrostenotic lesions in CD. In 2006 an association was demonstrated between T280M polymorphism of CX3CR1 gene and fibrostenosing CD (7), whereas in 2008 V249I polymorphism of CX3CR1 gene were also associated with fibrostenotic disease behavior (65).

CX3CR1 is a highly selective chemokine receptor for fractalkine and surface marker of NK cells, T lymphocytes, and $\gamma$6 $\delta$ T cells, as well as monocytes (77). The two SNPs, 249I and 280M, associated with fibrostenosis in CD are functionally relevant since they influence the binding of fractalkine to CX3CR1 (44) and result in fewer receptor binding sites and decreased ligand affinity (15, 47).

CX3CR1 is expressed on activated HSCs and, importantly, fractalkine represses TIMP-1 gene expression in these cells. The binding of fractalkine to CX3CR1-V249I, however, is associated with elevated TIMP-1 mRNA expression in hepatitis C virus-infected liver compared with CX3CR1-V (78). This effect by itself could explain the association of this allele with fibrosis given that TIMP-1 suppresses scar matrix degradation and protects HSCs from apoptosis.

Reduction in fibrosis occurs when myofibroblasts undergo apoptosis or senescence, or revert to a more quiescent phenotype, and the regulation of the balance between myofibroblasts survival vs. death may impact on the development of tissue fibrosis (58). As an example, myofibroblast apoptosis becomes evident during resolution of fibrosis and reduction in ECM content in liver cirrhosis (25) and renal fibrosis (3), suggesting a role for myofibroblast apoptosis in the resolution of tissue fibrosis.

Previous studies have demonstrated that hepatocyte growth factor (HGF) reduces lung fibrosis in murine models (76, 84) and there is ample evidence that HGF plays an essential part in parenchymal repair and protection in other organs (6, 42). It has been suggested that HGF is a potent inducer of ECM-degrading enzymes such as the matrix metalloproteinases (MMPs) (42), which are overexpressed during myofibroblasts apoptosis (25). MMPs induce apoptosis in lung myofibroblasts through the extracellular degradation of fibronectin and that the antifibrotic effects of HGF observed in lung were due to upregulation of MMPs and MMP-dependent myofibroblast apoptosis (46).

In that regard, a variety of dietary components, including vitamin E, have attracted attention for their health benefit and harmless consumption profile. Specifically, tocotrienols, which have proven efficacy in inducing apoptosis and autophagy on activated rat pancreatic stellate cells (PSC) and human intestinal myofibroblasts (HIFs) (62, 40). Tocotrienols are able to induce apoptosis in activated fibroblasts by activating caspase 3, 8, and 9 and increasing DNA fragmentation Furthermore, upon treatment with tocotrienols, both PSCs and HIFs display an autophagic response by converting LC3I to LC3II. Impor-
tantly, tocotrienols have no such effects in quiescent PSCs and acinar cells from the pancreas, showing selectivity to activated cells. Interestingly, tocotrienols are also able to upregulate expression of MMPs on HIFs in vitro (39), which, besides inducing accumulated ECM degradation, could be responsible for the induction of fibroblast apoptosis observed upon tocotrienol treatment.

NF-κB signaling promotes survival of hepatic myofibroblasts (79). Angiotensin II, which is locally synthesized in the injured liver promotes HSC proliferation (5), myofibroblast survival and liver fibrosis through the activation of NF-κB (53). Losartan, an angiotensin II type I receptor, has some efficacy in attenuating liver fibrosis (49, 86) and triggers apoptotic cell death in human pancreatic cancer (60) and stellate cells (37). The angiotensin-converting enzyme inhibitor captopril can also prevent fibrosis development in experimental colitis in the rat (81) and attenuates the progression of rat hepatic fibrosis (29). No studies, however, have been carried out to assess its apoptotic effect on fibroblasts, but it does induce apoptosis in other cell types, including human vascular myocytes (8) and vascular smooth muscle cells (22).

Anti-inflammatory and antifibrotic effects of the widely used cholesterol level-lowering 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) have been also examined in several in vitro models. Statins may be effective antifibrotic agents through inhibition of the activation and proliferation of fibrogenic cells and ECM production (27, 34, 38, 64, 66). Lovastatin is able to induce lung fibroblasts apoptosis (74) and pravastatin induces apoptosis of HSC (85). Of the antifibrotic mechanisms of the statins, induction of activated fibroblasts apoptosis appears be the most important. Fibrosis has been considered traditionally as an irreversible process but experimental and clinical literature data published in the last decade have suggested that an effective therapy can result in significant regression of fibrosis. This is usually associated with induction of apoptosis of mesenchymal cells.

Conclusions

Despite the limited attention that has been given to research efforts devoted to intestinal fibrosis to date, there is evidence that suggests that enhanced proliferation along with defective programmed cell death of mesenchymal cells can significantly contribute to the development of abnormal fibrogenesis in many different tissues. In line with these findings, there are a few therapies that have demonstrated potential antifibrogenic efficacy through the regulation of mesenchymal cell proliferation and programmed cell death. Further understanding of the pathways involved in the regulation of mesenchymal cell proliferation and apoptosis, as well as further evaluation of the potentially antifibrogenic agent, is required before there is going to be effective therapy directed against intestinal fibrosis.

REFERENCES


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