IGFBP-5 induces epithelial and fibroblast responses consistent with the fibrotic response

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Abstract

Fibrosis involves activation of fibroblasts, increased production of collagen and fibronectin and transdifferentiation into contractile myofibroblasts. The process resembles aspects of wound-healing but remains unresolved and can be life-threatening when manifest in the kidneys, lungs and liver, in particular. The causes are largely unknown, but recent suggestions that repetitive micro-injury results in the eventual failure of epithelial cell repair due to replicative senescence are gaining favour. This is consistent with the onset of fibrotic diseases in middle age. Because epithelial injury often involves blood loss, inflammatory responses associated with the fibrotic response have been considered as therapeutic targets. However, this has proved largely unsuccessful and focus is now switching to earlier events in the process. These include EMT (epithelial–mesenchymal transition) and fibroblast activation in the absence of inflammation. TGFβ1 (transforming growth factor-β1) induces both EMT and fibroblast activation and is considered to be a major pro-fibrotic factor. Recently, IGFBP-5 [IGF (insulin-like growth factor)-binding protein-5] has also been shown to induce similar effects on TGFβ1, and is strongly implicated in the process of senescence. It also stimulates migration of peripheral blood mononuclear cells, implicating it in the inflammatory response. In this paper, we examine the evidence for a role of IGFBP-5 in fibrosis and highlight its structural relationship with other matrix proteins and growth factors also implicated in tissue remodelling.

Overview of the fibrotic response

Excessive fibroblast activation results in debilitating and fatal diseases including IPF (idiopathic pulmonary fibrosis) and SSc (systemic sclerosis). A lack of elasticity is fatal in IPF, which is a progressive lung disease characterized by lung scarring due to excessive collagen deposition [1], whereas SSc involves excessive fibrosis of the skin and internal organs leading to loss of function of the skin and major organs [2].

Although the classical hallmarks of fibrotic disease include activation of fibroblasts, with increased production of collagen and fibronectin, and transdifferentiation of fibroblasts into contractile myofibroblasts, EMT (epithelial–mesenchymal transition) is also a feature of the epithelial response to injury, which should be considered an integral part of this process. The fibrotic response also involves increased production of cytokines and growth factors, including TGFβ1 (transforming growth factor-β1) and CTGF (connective tissue growth factor). TGFβ1 has been shown to stimulate fibroblast proliferation and the production of ECM (extracellular matrix) components in vitro, making it a prime candidate as a causative factor [3,4].

Fibrosis also involves an inflammatory response including monocyte/macrophage activation, although attempts to control the progress of these diseases with anti-inflammatory therapies do little to affect disease progression. This has led to suggestions that inflammation is a secondary component of the condition and to a re-evaluation of the mechanism of fibrosis. Two new hypotheses are now under investigation. In the first hypothesis, cycles of micro-injury of the epithelium occur with such a frequency that epithelial cell proliferation and migration into the injury are too slow to maintain the barrier function and, as a consequence, a secondary response involving activation of fibroblasts occurs. Such a response resembles unresolved wound repair [5]. The second hypothesis argues for a role of senescence, where repetitive injury of the epithelium induces rounds of proliferation to effect repair but which leads to premature aging of the epithelium involving the process of replicative senescence. Cells typically exhibit a finite ability to replicate, termed Hayflick’s number [6]. It is thus proposed that repeated insults lead to replicative senescence of the epithelium and that a fibroblast response is evoked as a compensatory mechanism.

Although the major fibrotic diseases are considered to be IPF, SSc, renal fibrosis and cirrhosis of the liver, it is now evident that fibrosis is also a component of cardiovascular disease. Vascular inflammation has been considered to be an ‘inside-out’ response centred on the monocyte adhesion and lipid oxidation hypotheses but evidence is accumulating for a new ‘outside-in’ hypothesis, in which inflammation is initiated in the adventitia and travels inward to the intima [7].

Key words: epithelial–mesenchymal transition, fibrosis, insulin-like growth factor, senescence, tissue injury, transforming growth factor-β (TGFβ)."
is accumulating that fibroblasts located in the adventitia may drive hyperplasia in the intima in cardiovascular injury, after balloon angioplasty, for example. In addition IGFBP-5 [IGF (insulin-like growth factor)-binding protein-5] has been shown to be associated with calcification in plaques [8].

**TGFβ1 and fibrosis**

Considerable interest has focused on the major pro-fibrotic cytokine, TGFβ1, which induces many of the processes involved in fibrosis, including fibroblast to myofibroblast transdifferentiation, ECM deposition and EMT, although without inducing inflammation (reviewed in [9]). TGFβ1 bioavailability occurs by activation from a latent form involving removal of its LAP (latency-associated peptide). This can occur via cleavage by plasmin but, more recently, activation by αvβ6 integrin has also been shown to be important [10]. How αvβ6 integrin activates latent TGFβ1 is unclear but activation of the integrin also appears to be essential, since overexpression of the β6 integrin subunit, in the absence of injury, does not lead to TGFβ activation (see [10]).

**Evidence for a role of IGFBP-5 in fibrosis**

IGFBP-5 is one of a family of six IGFBPs, IGFBP-1–6, that bind to IGFs with high affinity [11]. Increased expression of IGFBP-3 and IGFBP-5 has been described in both SSc and IPF [12,13]. Skin fibroblasts from patients with SSc had elevated levels of IGFBP-5 mRNA and protein compared with skin from their healthy twins. IGFBP-5 induced collagen and fibronectin production from fibroblasts and fibroblast/myofibroblast transdifferentiation in vitro and in vivo [14,15]. Furthermore, adenoviral expression of IGFBP-3 and IGFBP-5 in primary human fibroblasts increased the expression of collagen and fibronectin [13] and in vivo expression of IGFBP-5 induced skin fibrosis in mice, including increased dermal thickness and collagen bundle diameter [14] and pulmonary fibrosis [15]. IGFBP-5-induced dermal fibrosis included an increase in the number of dermal fibroblasts expressing PCNA (proliferating-cell nuclear antigen), with increased vimentin and α-SMA (α-smooth-muscle actin) expression, suggesting that IGFBP-5 induces myofibroblast differentiation. These findings suggest that up-regulated expression of IGFBP-5 could be an initiating event in the overproduction of ECM components and the development of fibrosis. Is there also evidence for a role of IGFBP-5 in other aspects of fibrosis, including epithelial injury, EMT and senescence?

**IGFBP-5 and cell injury**

Dramatic increases in IGFBP-5 expression have been described in the involuting mammary gland and prostate during apoptosis [16,17]. When expressed as a transgene in the mammary gland, IGFBP-5 enhanced apoptotic events and impaired mammary development [18]. Evidence also exists for expression of IGFBP-5 during tissue injury. For example, IGFBP-5 is increased in the brain after ischaemia [19] and in atherosclerotic plaques [8]. This association of cellular injury/death with increased expression of IGFBP-5 may be a general phenomenon and is worthy of more extensive examination.

**IGFBP-5 and EMT**

IGFBP-5 induces a phenotypic EMT in a human breast cancer cell line, MCF-7, and activates tPA (tissue plasminogen activator) [20]. tPA is known to activate several MMPs (matrix metalloproteinases) involved in cellular migration/invasion and in activating TGFβ1. TGFβ1-induced EMT in epithelial cells has also been shown in cancer metastasis, chronic degenerative pulmonary and hepatic fibrosis [21,22]. IGFBP-5 has also been shown to increase the number of epithelial cells expressing α-SMA *in vivo* and to induce its expression in A549 lung epithelial cells *in vitro* [15].

**IGFBP-5 and senescence**

Cellular senescence is a process whereby cells lose the ability to proliferate. Normal somatic cells, when cultured *in vitro*, have a finite ability to divide, after which they enter a state of irreversible proliferative arrest, termed replicative senescence [6]. Interest in a role for senescence in pulmonary fibrosis has increased [23] and senescence in human dermal fibroblasts [24] and endothelial cells [25] is associated with increased IGFBP-5 expression. Senescence could be partially reversed by knockdown of IGFBP-5 in aged HUVEC (human umbilical-vein endothelial cells), whereas IGFBP-5 induced premature senescence in HUVEC *in vitro*. IGFBP-5-induced senescence was associated with induction of the tumour suppressor p53 [8].

There is, therefore, accumulating evidence for a role of IGFBP-5 in all aspects of the early responses present during the development of fibrosis: increased expression of IGFBP-5 after epithelial injury, induction of EMT, induction of epithelial senescence and activation of fibroblasts. Along with its expression in fibrotic tissues, these findings provide strong support for a role of IGFBP-5 in fibrosis. Most recently, IGFBP-5 has also been shown to induce migration of macrophages [26], an important component of the inflammatory response evident in fibrotic tissues and, intriguingly, an effect not induced by TGFβ1.

**Mechanisms of action of IGFBP-5**

Direct actions of IGFBPs, including IGFBP-5, have been reported and include a putative cell-surface receptor in osteoblasts [27], nuclear localization [28,29] and interactions with several proteins that are primarily expressed intracellularly [30,31], although some of these findings have been contested or difficult to reproduce [32]. For a more detailed discussion of these intracellular mechanisms, see [11,33].

Influencing the actions of IGFs is the most extensively studied effect of IGFBP-5 (see [11]). However, there is no evidence to date to suggest that the effects of IGFBP-5 on EMT, senescence or fibroblast activation involve inhibition of the actions of IGFs. It is, however, conceivable that the induction of epithelial apoptosis does involve this process, since IGFs are important survival factors for epithelial cells and we have shown that an IGF analogue that interacts very weakly with IGFBPs could overcome the
epithelial apoptosis induced by IGFBP-5 in transgenic mice overexpressing IGFBP-5.

IGFBP-5 is a secreted protein and evidence that it may act indirectly, extracellularly, in an IGF-independent fashion, comes from analogy with other proteins belonging to the secreted CCN (cysteine-rich) protein family, which includes CTGF, CYR61 (cysteine-rich heparin-binding protein 61) and Nov (CCN3). These proteins were temporarily renamed IGFBP8–10, because of their structural relationship with the IGFBP family. Perhaps it is more appropriate to consider IGFBP-5 as a member of the CCN family. This family is unusual in that only Nov has an identified cell-surface ‘receptor’, an integrin. These molecules are believed to exert their effects indirectly by interaction with growth factors. For example, CTGF binds to VEGF (vascular endothelial growth factor) and inhibits its actions [34] and binds to, and enhances the actions of, TGFβ1 [35]. IGFBP-5 may also act in a fashion analogous to that of CTGF, which is also a downstream mediator of the actions of TGFβ1 [36]. Further, albeit indirect, evidence to implicate IGFBP-5 in fibrosis comes from reports that IGFBP-5 interacts with several proteins from the matricellular family of proteins, which are expressed during wound-healing and metastasis and which play a role in cellular de-adhesion and migration. IGFBP-5 has been shown to bind to three members of this family: osteopontin, thrombospondin-1 [37] and tenascin-C [38].

Conclusions

Fibrosis encompasses a range of diseases of largely unknown aetiology. Downstream responses involved in the fibrotic process have thus, understandably, been the principal target for therapy. Anti-inflammatory approaches have, so far, failed to provide therapeutic benefit and, as a consequence, inhibition of TGFβ1 remains an active goal. Perhaps IGFBP-5, because of its involvement in the early response to injury and its ability to activate all aspects of the disease process, should also be given a similar consideration. IGFBP-5 is produced by epithelial cells during apoptosis and injury, induces EMT, activates fibroblasts and monocytes, activates tPA, generating plasmin, interacts with matricellular proteins and is associated with atherosclerotic plaques. Thus IGFBP-5 may be a central player in the initial response to epithelial injury, a role that may have developed out of its embryonic function as a molecule involved in epithelial instruction of the underlying mesoderm [39].

Funding

We thank the Biotechnology and Biological Sciences Research Council for its support via responsive mode grant [grant number BBF00205X1].

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Received 3 March 2009  
doi:10.1042/BST0370882