

Strategies to overcome the hurdles to treat fibrosis, a major unmet clinical need

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Fibroproliferative disorders are estimated to contribute to 45% of deaths in the USA (1). Whilst this huge number includes atherosclerosis, the biggest killer in the developed world, less common conditions such as idiopathic pulmonary fibrosis, fibrosis of the liver and kidney and systemic sclerosis are also associated with high mortality. Localized fibrotic conditions, which receive much less attention, including endometriosis, abdominal adhesions, frozen shoulder and Dupuytren's disease, also cause considerable morbidity and together affect more than 10% of the population. The magnitude of the unmet clinical need has resulted in intense efforts to develop novel therapeutic strategies. Despite this, success remains elusive for a multitude of reasons. (i) Detection: Fibrosis is the end stage of a process that develops over many years, often decades, and patients usually present late, once organ function has been significantly compromised. Early predictors and biomarkers are scarce. (ii) Reversibility: Elimination of the initiating insult such as hepatitis B viral infection results in regression of fibrosis and restoration of function in the liver (2). However, in the majority of organs and even other liver disorders such as non-alcoholic steatohepatitis, this process is irreversible. (iii) Matrix: Late stage fibrotic tissues are relatively acellular, leaving few cells to target and a densely cross-linked matrix that is barely susceptible to proteolysis. (iv) Tissue availability: The study of primary cells from diseased human tissues has proven to be highly successful in the identification of therapeutic targets, as exemplified by TNF in inflammatory arthritis (3). In fibrotic diseases human samples are available in very limited quantities and, even when they can be accessed, have failed to provide insights that have translated to successful clinical trials. (v) Cell and animal models: Consequently, investigators have turned to the study of cells in culture to identify and study potential new targets in fibrosis. The main effector cells in fibrosis are myofibroblasts derived from a heterogeneous pool of precursors to produce and remodel collagen matrix into scar tissue. However, it is difficult to emulate in cell culture the mechanical and biochemical conditions found in vivo. Animal models of fibrosis have provided useful information but also have significant weaknesses and invariably involve the administration of toxins or other insults that are rarely encountered in clinical practice (4). Consequently, many targets identified in cell culture and tested in animal models have failed to translate to the clinic (5). For instance, activation of cultured fibroblasts following exposure to relatively high doses of the pro-fibrotic cytokine TGF- β 1 has provided valuable insights into myofibroblast biology but all late phase clinical trials directly targeting this cytokine have

failed to reach their primary endpoints. A trial in patients with systemic sclerosis (SSc) reported dose-dependent increase in morbidity and mortality (6).

In this issue of PNAS, Elhai et al. address some of these limitations and propose inhibition of the OX40-OX40 ligand (OX40L) axis as a novel approach to treat systemic sclerosis (7). SSc is relatively uncommon and affects ~100,000 individuals in the USA. However, there is an urgent need to develop new effective therapeutics as the diffuse form, which affects extensive areas of the skin as well as visceral organs such as the lungs, and has a 10 year survival of ~55% (8). The authors present OX40L as a possible biomarker and predictor of SSc, showing increased expression in the skin of SSc patients, especially those with diffuse cutaneous SSc. They also analysed the serum from 177 patients compared to 100 healthy controls and found that circulating levels of soluble OX40L were higher in the SSc group, particularly in those with the diffuse form, and an elevated level at baseline was highly predictive of deterioration in pulmonary disease. This is an important as a therapeutic is most cost effective if it is directed at the sub-population most likely to benefit. SSc is characterised by widespread vascular damage and T cells have been shown to play a prominent role in the pathogenesis of the disorder; in particular the Th2 phenotype contributes to elevated levels of the pro-fibrotic cytokines IL-4 and IL-13 (9). However, the interplay between the Th1/Th2/Th17 and Treg subtypes of T cells in fibrosis is complex and indiscriminate abrogation of T cell activity may lead to unwanted effects such as immune suppression. By targeting OX40L, Elhai et al. (7) elegantly avoid some of these issues. OX40, a member of the TNF receptor superfamily, promotes T cell expansion and survival, blocks natural Treg activity, antagonises generation of inducible Tregs, promotes generation of memory CD4⁺ and CD8⁺ T cells and mice deficient in OX40 or OX40L demonstrate attenuated Th2 responses (10). Crucially, OX40/OX40L are present at low levels in normal tissues, are only transiently expressed after engagement of the T cell receptor and are upregulated on the most recently activated T cells within inflammatory lesions, making it an attractive target for immune modulation, and an OX40 agonist has been used in clinical trials for patients with cancer (11).

In addition, Elhai et al. show that OX40L deficient mice are protected against bleomycin-induced skin fibrosis and have reduced local immune cell infiltration accompanied by lower

local levels of proinflammatory cytokines. Neutralizing OX40L antibody was protective and additionally led to regression of established fibrotic lesions in mice. The latter finding may not necessarily translate to clinical practice as the potential for resolution of late stage fibrosis in patients is limited. Mice that over-express OX40 and OX40L in the skin and lung also demonstrated improvement when treated with the OX40L neutralising antibody. The path to therapeutic translation is open, with clinical trials using neutralizing OX40L antibody (MEDI6469 – AstraZeneca) ongoing in cancer. Interestingly Elhai et al. (7) found that OX40L blockade affected both hematopoietic and non-hematopoietic cells, consistent with their finding that OX40L was expressed at the immunohistochemical level in B cells, endothelial cells, fibroblasts, myofibroblasts as well as T cells. Indeed, a multitude of cells have been shown by others to express OX40-OX40L (12). This raises the possibility that OX40L blockade may also be effective in other forms of fibrosis less dependent on T cell activity. However, diffuse SSc is a systemic disorder affecting blood vessels in a variety of tissues and other fibrotic diseases are less dependent on T cell activity. For example, following myocardial infarction, Th1 and Th17 T cells are rarely seen and in the early stages and CD4⁺ T cells, particularly the Tregs, contribute to wound healing, although the role of the CD4⁺ Th1 cells that predominate in the first few days remains unclear (13).

Nonetheless, the concept of potential unifying mechanisms and therapeutic targets across multiple systems is very enticing. Mehal et al. proposed identification of core pathways across a multitude of fibrotic diseases by studying several animal models combined with cell-based systems (14). A core element in fibrotic lesions is the myofibroblast. Targeting myofibroblasts is advantageous as, unlike many other pathological processes, the myofibroblast is key in all forms of fibrosis and absent from most normal tissues (15, 16). Alternatively, Wick et al. make a persuasive case for *all* fibrosis, irrespective of the organ affected or the underlying etiological process, to be considered as representing the final common pathway of the body's response to persistent inflammation (17). A multitude of innate and adaptive immune cells are involved in fibrotic diseases. The initiating event may lead to an influx of neutrophils through to macrophages. Whilst macrophages with the inflammatory phenotype may predominate initially, those typically associated with a wound healing phenotype appear later, persist and secrete the Th2-type cytokines that in turn promote the fibrotic response (18). Mast cells are a rich source of secreted cytokines and

also play a key role, with mast cell-deficient mice being relatively resistant to bleomycin-induced fibrosis (17). The adaptive immune response also appears to be skewed towards the Th2 type response, although the role of Tregs in fibrosis remains controversial (15).

An alternative approach is to consider is targeting cytokines and growth factors that regulate activation and activity of myofibroblasts. The challenge is identifying factors specific for myofibroblasts without affecting other cell types. Inhibiting the obvious candidate TGF- β 1 has not translated to the clinic partly due to the pleiotropic nature of the cytokine, although new strategies are being developed to block TGF- β 1 activation in immunity and fibrosis (19, 20). Study of primary cultures of rheumatoid synovium (21) as well as diseased and appropriate control human tissues led to the identification of TNF as another therapeutic target. This approach has considerable merit. Neutralisation of secreted factors is easier than intracellular pathways or particular cell types. Study of diseased and appropriate control human tissues resulted in the identification of TNF as a therapeutic target for Dupuytren's disease (22), and a phase II clinical trial is in progress (23).

We would support the core pathway approach (14) but would suggest that progress to identify valid therapeutic targets would be optimally achieved by studying human tissues at a relatively early stage. The potential target can then be validated using appropriate animal models, as elegantly demonstrated by Elhai et al. (7) in this issue of PNAS.

Fig 1. A persistent insult leads to low but chronic levels of inflammation typified by the activity of macrophages with a wound healing phenotype, mast cells and a Th2-type adaptive immune response. Soluble mediators from these immune cells lead to the differentiation of myofibroblasts from local or circulating precursors. Myofibroblasts produce and contract extracellular matrix. Increasing matrix stiffness and persistence of the soluble mediators leads to continuing myofibroblast activity. Identification of pivotal soluble mediators may form the basis of successful therapeutic interventions.

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