CellPress

Review

Antifibrotic Actions of Serelaxin – New Roles for an Old Player

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Fibrosis represents a failed wound healing response to tissue injury. It is characterized by the accumulation of excess connective tissue and is a significant cause of organ failure, morbidity, and mortality. Fibrotic disorders accompany a wide spectrum of conditions including both systemic and organ-specific diseases, for which there is currently no effective cure. Serelaxin, the recombinant form of the major stored and circulating form of human relaxin, has emerged as a pleiotropic drug with rapidly occurring antifibrotic actions. This review discusses the effectiveness of serelaxin as an antifibrotic, and how it augments the actions of several other therapeutics leading to its potential use not only as a monotherapy but also as an adjunct therapy with other antifibrotic agents.

Mechanisms and Functional Significance of Fibrosis

Fibrosis (see Glossary) is the universal pathological manifestation of chronic or severe injury, and consists of the accumulation of excess extracellular matrix (ECM) (Box 1). The process is indicative of organ failure both externally and internally (reviewed in [1]). Notwithstanding organ-specific differences, the pathogenesis of fibrosis in each organ resembles dysregulated wound healing. Normal wound healing involves consecutive but overlapping events in response to injury or stress: inflammation, ECM synthesis (fibrogenesis), and remodeling (Figure 1). An important and often forgotten fact is that ECM *per se* is not bad. It begins as a necessary and well-organized attempt to maintain basement membranes and structural integrity for repair and regeneration. The problem arises when ECM synthesis and deposition are ongoing and uncontrolled. Fibrosis is therefore an aberrant wound healing response, and is in effect a failure to repair and regenerate.

Fibrosis May Itself Be Pathological

Fibrosis itself may lead to further ongoing injury and ongoing ECM deposition (Figure 1). This has proven difficult to test experimentally, but we do know for instance that the acellular nature of fibrosis results in tissue hypoxia due to a loss of microvasculature, and that this exacerbates the progression of fibrosis [2]. Likewise, *in vitro* at least, the propagation of **fibroblasts** on matrix constituents that they are not normally exposed to stimulates their further proliferation [3].

Cellular Basis of Fibrosis

In vitro and *in vivo* studies have consistently shown that the fibroblasts, and other phenotypically similar mesenchymal cells, are the dominant source of ECM. Activation and differentiation of fibroblasts into **myofibroblasts** is a key event in this process [4]. Their differentiation, proliferation, collagen synthesis, and contraction are stimulated by a variety of cytokines and growth factors derived from adjacent stimulated epithelial cells, endothelial cells, leukocytes, or from fibroblasts themselves. Other influences include mechanical forces and surrounding ECM. A hierarchy exists among the profibrotic growth factors, with the most compelling evidence being

Trends

Progressive fibrosis is a universal pathological response to severe and ongoing injury and is a failure of wound repair, contributing to ~45% of deaths in the Western world.

Fibroblasts are the main cell that produces the excess extracellular matrix that constitutes fibrosis.

Relaxin is an endogenous inhibitor of fibrosis, while exogenous administration of recombinant relaxin (serelaxin) limits progression in diverse etiologies.

Serelaxin mediates signal transduction in fibroblasts through its cognate receptor RXFP1 and companion receptors, and involves ERK1/2, NO, and cGMP and longer-term changes in gene expression and function.

The antifibrotic efficacy of serelaxin is experimentally equivalent if not better than current standard care, and its effects are additive when combined with other therapies.

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Box 1. Key Features of Fibrosis

Definition: The overgrowth, hardening, and/or scarring of various tissues attributed to the excessive deposition of extracellular matrix components (mainly collagen), which results from a failed or even poorly controlled wound healing response to tissue injury.

Causes: Tissue injuries or diseases associated with:
- chronic inflammatory reactions,
- cell death,
- enhanced workload/stress (hypertrophy),
- metabolic disorders (hyperlipidemia, hyperglycemia), and
- the persistent stimulation of various cytokines, growth factors, and hormones.

Phases Involved
- Initiation of the response.
- Activation of effector cells.
- Influx of inflammatory cells that clear bacteria from the wound site and secrete factors that promote conversion of recruited matrix-producing fibroblasts into activated myofibroblasts.
- Interaction of activated myofibroblasts with the extracellular matrix (ECM) of connective tissues and deposition of excessive amounts of connective tissue components.
- Insufficient myofibroblast apoptosis and ECM absorption (once wound healing is complete).

Key Profibrotic Factors
- Transforming growth factor-β1.
- Angiotensin II.
- Connective tissue growth factor.
- Platelet-derived growth factor.
- Endothelin-1.

for **transforming growth factor-β1** (**TGF-β1**). Where injury is persistent or repeated, increasing numbers of injured epithelial cells stall between the G2 and M phases of the cell cycle, which results in an ongoing production of TGF-β1 [5]. It is also likely that fibroblasts are stimulated more directly in some diseases because high glucose concentrations [6] and angiotensin II (Ang II) [7] can stimulate *in vitro* fibroblast proliferation and ECM production.

As our understanding of fibrosis has increased, we have come to appreciate that the actions of profibrotic signals are counteracted by the activities of endogenous protective factors. Furthermore, ECM is broken down post-translationally by matrix metalloproteinases (MMPs), that are in turn regulated by tissue inhibitors of MMPs (TIMPs) [8]. Newly synthesized ECM, not yet crosslinked by tissue transglutaminase, is probably most susceptible to MMP degradation. Therefore, the balance of multiple opposing forces determines fibrosis progression.

Rational Therapeutic Strategies

Fibrosis is an attempt to maintain structure and therefore in itself is part of the repair process. In this respect, it is not necessary or even desirable to abrogate the process but rather to ameliorate it. The therapeutic aim should be to slow progression, which is a more achievable goal than outright prevention.

Preclinically, an enormous number of agents and approaches have been shown to reduce fibrosis experimentally. Successful clinical translation of these agents, however, is contingent on satisfying a number of criteria (Table 1). An ideal antifibrotic will have demonstrable direct antifibrotic effects, be efficacious in clinically relevant scenarios, be more effective than the current standard of care, and prevent progression of established fibrosis. Finally, we often forget that fibrosis can take a decade to develop, and potential treatment strategies will need to factor in long-term administration. Intriguingly, a growing body of evidence suggests that therapies based on the human gene-2 **relaxin** (or relaxin-2) hormone may satisfy many of these criteria (Table 1).

Glossary

Airway hyperresponsiveness: a feature of asthma consisting of a characteristic increased sensitivity of the airways to an inhaled constrictor agonist.
Airway remodeling: structural changes that occur in both small and large airways in airway diseases, such as asthma and chronic obstructive pulmonary disease.
Cardiomyopathy: chronic deterioration of the heart muscle.
Extracellular matrix: collection of extracellular molecules secreted by cells that provide structural and biochemical support to the surrounding cells; but which contribute to fibrosis when they accumulate in excess.
Fibroblast: a cell that produces collagen and other matrix components.
Fibrosis: the scarring and hardening of tissues characterized by the accumulation of excess connective tissue, representing a failed wound healing response to tissue injury.
Myofibroblast: an activated fibroblast that also has some properties of smooth muscle cells.
Relaxin: a two-chain peptide structurally related to insulin that possesses several actions that facilitate organ protection.
RXFP1: a family A G-protein-coupled receptor that is the cognate receptor stimulated by relaxin to activate pleiotropic signaling pathways.
Serelaxin: recombinantly produced form of the major stored and circulating peptide hormone human gene-2 relaxin.
Smads: intracellular proteins that translocate signals from TGF-β1 to the nucleus where they activate gene transcription.
Transforming growth factor β1 (TGF-β1): a secreted protein that promotes the differentiation of fibroblasts into activated myofibroblasts that synthesize large amounts of matrix proteins as part of the wound healing process, but which can contribute to fibrosis when persistently activated.