Highly specific inhibition of TGFβ1 activation with antibody SR-AB1 has antifibrotic activity

Thomas Schürpf, Abhishek Datta, Christopher Littlefield, Christopher D. Chapron, Kathy Morgan, Constance J. Martin, Ashish Kalra, Kimberly K. Long, Allan D. Capilli, Raleigh Pavlik, Justin W. Jackson, Gregory J. Carven, Stefan Wawersik, Alan Buckler

Scholar Rock Inc., 620 Memorial Drive, Cambridge, MA 02139, USA

Abstract

Transforming growth-factor β1 (TGFβ1) is a cytokine with crucial and diverse biological functions, including regulation of immune responses and tissue homeostasis. TGFβ1s are expressed as pro-enzymes that are proteolytically cleaved into an N-terminal prodomain and a C-terminal growth factor. The excreted growth factor remains noncovalently associated with the prodomain, preventing receptor binding and signaling. Latent TGFβ1 is covalently associated with presenting molecules through disulfide bonds that link latent TGFβ1 to the extracellular matrix or to the cell surface. These presenting molecules play a critical role in the activation of the latent complex, as they provide an anchor for integrins to exert traction force on latent TGFβ1, thus releasing the active growth factor. This activation mechanism has been associated with a number of pathologies, including fibrotic diseases, where chronic TGFβ1 activation drives pathological transdifferentiation and overexpression of extracellular matrix proteins. The role of TGFβ1 in driving fibrosis is critical to the development of novel therapeutic strategies to inhibit its pathological activity. For example, TGFβ1 is well-known to promote matrix deposition and organ fibrosis, and to suppress matrix turnover, ultimately leading to tissue damage and disease. Inhibiting TGFβ1 has been explored as a potential therapeutic strategy to reduce disease-limiting heart valveopathies, leading to concerns about toxicity of the therapeutic approach. The absence of toxicity of specifically targeting TGFβ1 is complicated by high homology between the TGFβ1 growth factor and its close relatives TGFβ2 and TGFβ3. We targeted the TGFβ1 prodomain, which has much lower homology to the prodomain of TGFβ2 and TGFβ3, and have identified SR-AB1, a fully human immunoglobulin antibody that specifically binds to and inhibits activation of latent TGFβ1 with no detectable binding of latent TGFβ2 or TGFβ3. This novel mechanism allows isoform specificity unachieved by biologics that bind to and block the TGFβ1 growth factor/receptor interaction and prevents latent TGFβ1 activation by both v8f1 and v8f2 integrins. SR-AB1 binds and inhibits latent TGFβ1 in complex with all four known TGFβ-presenting molecules, allowing targeting of latent TGFβ1 in multiple tissues. SR-AB1 inhibits endogenous TGFβ1 in a number of primary cells in vitro, including dermal fibroblasts and hepatic stellate cells, and Treg activity in vitro and in vivo. In addition, we tested the in vivo efficacy of TGFβ1 activation via this novel mechanism in multiple preclinical models of tissue fibrosis. We find that SR-AB1 suppresses the induction of profibrotic genes and tissue fibrosis to levels similar to those achieved in pan-TGFβ antibody-treated animals. Taken together, our data show that inhibition of latent TGFβ1 activation is efficacious in a preclinical fibrosis model and has a potentially superior safety profile as compared to pan-TGFβ inhibition.

Introduction

TGFβ1 structure

• The three TGFβ1 isoforms, TGFβ1, TGFβ2, and TGFβ3, are expressed as pro-proteins that are cleaved before secretion into an N-terminal prodomain and a C-terminal growth factor.

• The growth factor remains noncovalently associated with the prodomain, preventing receptor binding and signaling.

• The three TGFβ1 growth factors share a high degree of sequence identity across the three isoforms, which allows them to signal through the same receptor, but the prodomains are much less conserved.

TGFβ1 presenting molecules

• Latent TGFβ1 is covalently associated with presenting molecules through disulfide bonds.

• Presenting molecules provide an anchor for integrins to exert traction force on latent TGFβ1, releasing the active growth factor.

To date, four TGFβ1-presenting molecules have been identified:

- Latent TGFβ1 Binding Protein 1 (LTBP1) and LTBP2: (non)-ligand-like proteins that link TGFβ1 to the ECM
- Glypican-1: Receptor: Fibrillin (GAP) & Louise-Robin Repetitins Domain (LR), 3 (LR3D)− I-macrophages present latent TGFβ1 on the surface of activated regulatory T cells (Tregs) and myofibroblasts, respectively.

Biological functions of TGFβ1

• As demonstrated by the distinct phenotypes of the three TGFβ1 knockout mice, the isoforms have non-redundant biological functions.

- Dysregulated TGFβ1 activation has been associated with a number of pathologies, including fibrotic diseases.

- Chronic TGFβ1 activation drives pathological transdifferentiation and overexpression of extracellular matrix proteins.

Therapeutic TGFβ1 Inhibition

• Approaches include antibodies or soluble ligand traps that bind and block the TGFβ1 growth factors, or small molecular inhibitors of the downstream TGFβ1 receptor kinase ALK5.

- Pan-TGFβ1 inhibition was found to cause dose-limiting heart valveopathies, leading to concerns about toxicity of this therapeutic approach.

- Specifically targeting the TGFβ1 isoform has been challenging because of the high homology between the three TGFβ1 growth factors.

Hypothesis

We hypothesize that inhibitors targeting the much less conserved TGFβ1 prodomain would achieve TGFβ1 isoform specificity, potentially providing a superior safety profile compared to pan-TGFβ inhibition.

Conclusions

• Isoform-specific inhibition of TGFβ1 in vitro and in vivo can be achieved by targeting the prodomain of latent TGFβ1 with SR-AB1.

• Inhibition of TGFβ1 with SR-AB1 in preclinical models of kidney and liver fibrosis is at least as effective as pan-TGFβ inhibition.

• Specific inhibition of TGFβ1 avoids cardiac toxicity and valvulopathies associated with pan-TGFβ inhibition.