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

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Abstract

Transforming growth factor-β1 (TGFβ1) is a cytokine with crucial and diverse biological functions, including regulation of immune responses and tissue homeostasis. TGFβs are expressed as pro-proteins that are proteolytically cleaved into an N-terminal prodomain and a C-terminal growth factor. The secreted 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. Dysregulated TGFβ1 activation has been associated with a number of pathologies, including fibrotic diseases, where chronic TGFβ1 activation drives myofibroblast transdifferentiation and overexpression of extracellular matrix proteins. The role of TGFβ1 in driving fibrosis has led to the development of multiple therapeutics to inhibit its activity. However, inhibition with potent anti-pan-TGFβ antibodies was found to cause dose-limiting heart valvulopathies, leading to concerns about toxicity of this therapeutic approach. The alternative strategy 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 prodomains of TGFβ2 and TGFβ3, and have identified SR-AB1, a fully human monoclonal antibody that specifically binds to and inhibits activation of latent TGFβ1 with no detectable binding to latent TGFβ2 or TGFβ3. This novel mechanism allows isoform specificity unachieved by biologics that bind and block the TGFβ1 growth factor/receptor interaction and prevents latent TGFβ1 activation by both αVβ6 and αVβ8 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 myofibroblasts and hepatic stellate cells, and Treg activity *in vitro* and *in vivo*. In addition, we tested the *in vivo* efficacy of TGFβ1 inhibition 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

TGFβ structure

- The three TGFβ 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 TGFβ 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.

TGFβ 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 TGFβ Binding Proteins 1 & 3 (LTBP1 and LTBP3) - fibrillin-like proteins that link latent TGFβ1 to the ECM.
  - Glycoprotein-A Repetitions Predominant (GARP) & Leucine-Rich Repeat-Containing Protein 33 (LRRC33) - transmembrane proteins that present latent TGFβ1 on the surface of activated regulatory T cells (Tregs) and myeloid cells, respectively.

Biological functions of TGFβ

- As demonstrated by the distinct phenotypes of the three TGFβ 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 myofibroblast transdifferentiation and overexpression of extracellular matrix proteins.

Therapeutic TGFβ inhibition

- Approaches include antibodies or soluble ligand traps that bind and block the TGFβ growth factors, or small molecular inhibitors of the downstream TGFβ receptor kinase ALK5.
- pan-TGFβ inhibition was found to cause dose-limiting heart valvulopathies, 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 TGFβ growth factors.

Hypothesis

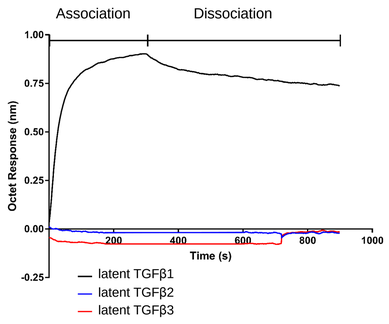
We hypothesize that inhibitors targeting the much less conserved TGFβ1 prodomain would achieve TGFβ1 isoform specificity, potentially providing a superior safety profile compared to pan-TGFβ inhibition.

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.

Results

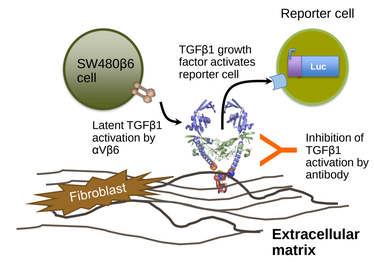
Figure 1: SR-AB1 specifically binds prodomain of latent TGFβ1



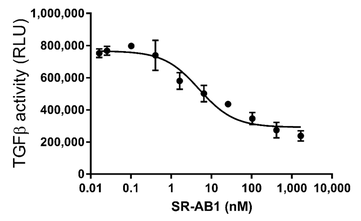
- Human monoclonal antibody SR-AB1 discovered by yeast display
- Specifically binds latent TGFβ1, without detectable binding to latent TGFβ2 or TGFβ3 (Fig. 1)
  - No binding of TGFβ1 growth factor
  - Antibody epitope maps to straitjacket of TGFβ1 prodomain
  - Crossreacts with mouse, rat, cynomolgus monkey proteins

Figure 2: SR-AB1 inhibits activation of human fibroblast-expressed latent TGFβ1

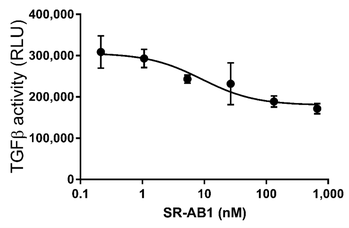
A *Schematic of fibroblast co-culture assays*



B *TGFβ1 inhibition in human dermal fibroblasts*



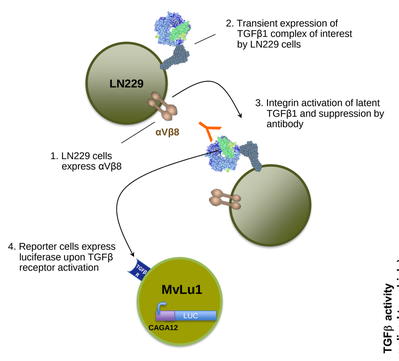
C *TGFβ1 inhibition in human hepatic stellate cells*



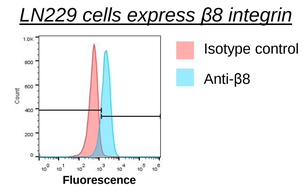
- Co-culture of human fibroblasts with αVβ6-expressing cells activates endogenous fibroblast TGFβ (Fig. 2A)
- SR-AB1 blocks αVβ6-dependent activation of latent TGFβ1 produced by dermal fibroblasts or activated hepatic stellate cells (Fig. 2B,C)

Figure 3: SR-AB1 is an inhibitor of latent TGFβ1 activation irrespective of presenting molecule

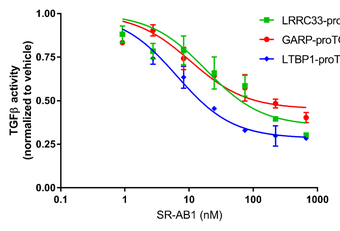
A *Schematic of LN229 assay*



B *LN229 cells express β8 integrin*



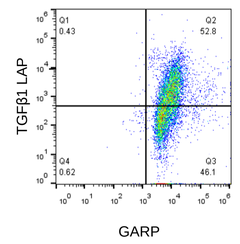
C *SR-AB1 inhibits activation of TGFβ1 irrespective of presenting molecule*



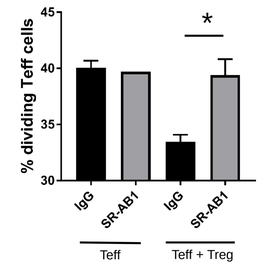
- Inhibitory activity of SR-AB1 was tested on overexpressed TGFβ1 complexes (Fig. 3A)
  - LN229 glioblastoma cells express αVβ8 (Fig. 3B)
  - Transfected LN229 cells express latent LTBP1-proTGFβ1, GARP-proTGFβ1, or LRRC33-proTGFβ1
  - Latent TGFβ1 complexes activated through αVβ8
  - TGFβ1 activity detected by reporter cells
- SR-AB1 inhibits all latent TGFβ1 complexes tested (Fig. 3C)
  - SR-AB1 is a context-independent inhibitor of TGFβ1 activation

Figure 4: SR-AB1 blocks regulatory T cell (Treg) function *in vitro* and *in vivo*

A *CD4+CD25+FoxP3+ Tregs express GARP and TGFβ1*

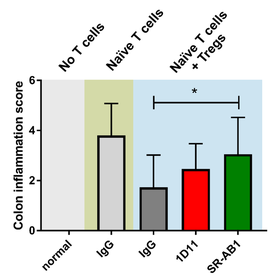


B *In vitro Treg activity is suppressed by SR-AB1*



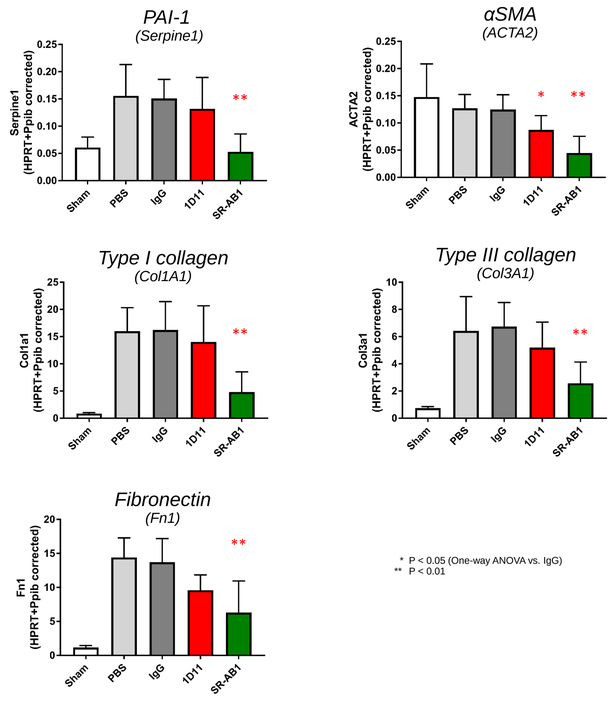
* P < 0.05 (two-tailed T test)

C *In vivo Treg activity is suppressed by SR-AB1*



- SR-AB1 suppresses Treg function *in vitro*
  - Activated human Tregs express GARP and TGFβ1 prodomain (LAP) on their surface (Fig. 4A)
  - Division of activated human effector T cells (Teff) is suppressed by autologous Tregs (Fig. 4B)
  - SR-AB1 inhibits suppression by Tregs, but not division of Teff cells alone (Fig. 4B)
- SR-AB1 suppresses Treg function *in vivo* (Fig. 4C)
  - Transfer of CD45Rb^{hi} naïve T cells into *scid* mice induces severe colitis, as determined by histopathology 45 days after T cell transfer
  - Pathology is ameliorated by co-transfer of CD45Rb^{low} CD25+ Tregs
  - Suppressive Treg activity is blocked by SR-AB1 (30 mg/kg) or pan-TGFβ-inhibitory antibody 1D11

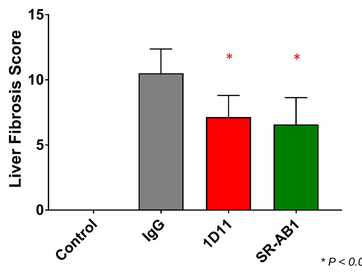
Figure 5: SR-AB1 suppresses profibrotic gene expression in UUO model of kidney fibrosis



* P < 0.05 (One-way ANOVA vs. IgG)
** P < 0.01

- SR-AB1 (30 mg/kg) inhibits activation of latent TGFβ1 in the kidney
  - Male CD-1 mice underwent sham or unilateral ureteral occlusion (UUO) surgery
  - Injured kidneys were collected 5 days post surgery and mRNA levels analyzed by multiplexed Quantigene RNA assays
  - Dosing of mice with SR-AB1 suppressed expression of TGFβ-responsive and profibrotic genes
- Specific inhibition of TGFβ1 was at least as efficacious as pan-TGFβ inhibition with 1D11

Figure 6: TGFβ1 inhibition with SR-AB1 ameliorates CCl4-induced liver fibrosis

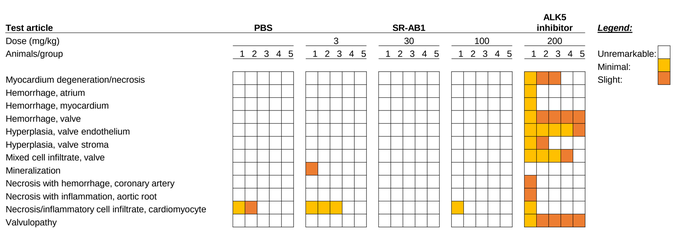


- Treatment of mice with SR-AB1 (30 mg/kg) reduced fibrosis, confirming suppressive activity of SR-AB1 in liver.
  - Liver fibrosis was induced in BALB/c mice with CCl4.
  - Therapeutic dosing with antibodies was initiated after two weeks and continued for four weeks.
  - Liver pathology was assessed by histology on picrosirius red-stained liver slices, and extent of fibrosis was scored.
- Similar to kidney, specific inhibition of TGFβ1 isoform was as effective as pan-TGFβ inhibition with 1D11

* P < 0.0001 (One-way ANOVA vs. IgG)

Figure 7: No cardiac toxicity observed with SR-AB1 up to 100 mg/kg in 4-week rat study

Cardiac pathology (microscopic findings)



- TGFβ1-specific inhibitor SR-AB1 tested at three dose levels in 4-week rat toxicology study
- No treatment-related cardiac toxicity of SR-AB1 up to 100 mg/kg per week, the highest dose tested
- No toxicology findings with SR-AB1 in comprehensive list of tissues and organs
- Dosing of ALK5 inhibitor for 5 days recapitulated published cardiac valve toxicity of pan-TGFβ inhibition
- Efforts underway to determine minimum efficacious dose of SR-AB1 *in vivo*