Identification of LTBP Complex-Specific Inhibitors of Latent TGFβ1 Activation

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Abstract

Transforming growth factor beta 1 (TGFβ1) is expressed as a pro-protein that is proteolytically cleaved into a C-terminal growth factor and an N-terminal prodomain. After cleavage, the prodomain remains noncovalently associated with the growth factor, preventing receptor binding. This latent TGFβ1 forms a large latent complex (LLC) through disulfide bonds that link the prodomain to presenting molecules, and these large latent complexes are then deposited into the extracellular matrix (ECM) or brought to the cell surface. These presenting molecules provide an anchor for specific αVβ integrins to exert traction force on latent TGFβ1, thus releasing the growth factor from the complex to allow signaling. Four TGFβ1 presenting proteins have been identified: Latent TGFβ Binding Protein-1 (LTBP1) and LTBP3 are deposited in the extracellular matrix, while Glycoprotein-A Repetitions Predominant (GARP/LRRC32) and Leucine-Rich Repeat-Containing Protein 33 (LRRC33) present latent TGFβ1 on the surface of immune cells. TGFβ1 is involved in tissue homeostasis processes and regulation of immune responses, and dysregulation of its activation is a key driver of organ fibrosis, cancer, and autoimmunity. However, non-selective targeting of TGFβ activity for therapeutic purposes has been challenging due to dose-limiting toxicities reported for pan-TGFβ pathway inhibitors, as well as immune system activation through chronic TGFβ suppression. In an effort to address this therapeutic need for both isoform- and context-selectivity for TGFβ1 targeting, we have identified isoform-specific monoclonal antibodies that bind the latent TGFβ1 prodomain, with no detectable binding to latent TGFβ2 or TGFβ3, and that inhibit integrin-mediated activation of latent TGFβ1 in vitro with context-selectivity. In order to facilitate our antibody discovery and characterization efforts, we developed context-dependent cell-based assays of TGFβ1 activation. Antibodies discovered by screening in these assays revealed two novel classes of antibodies: one group that binds and suppresses the activation of latent TGFβ1 irrespective of its presentation molecule, and a second that binds and inhibits TGFβ1 only when presented by LTBP. Because members of this latter class of LTBP-specific antibodies do not inhibit TGFβ1 in the context of the immune-associated TGFβ1 presenters, GARP and LRRC33, such antibodies may be optimal candidates for the treatment of fibrotic indications, and could allow chronic dosing that would avoid TGFβ-related immune system activation.

Figure 1. Targeting the latent form of TGFβ1 provides isoform and context specificity

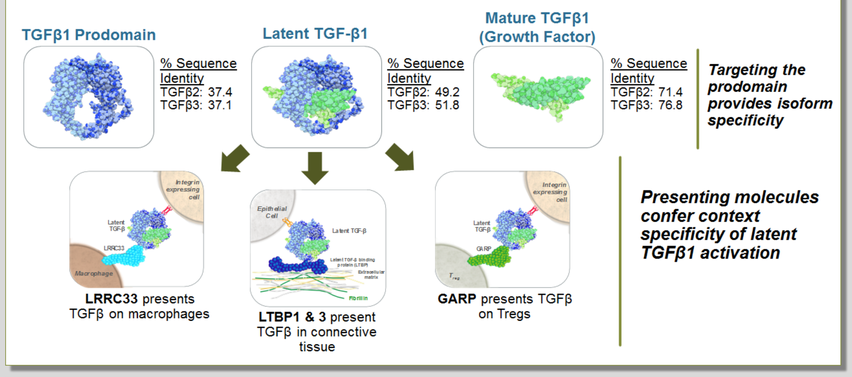
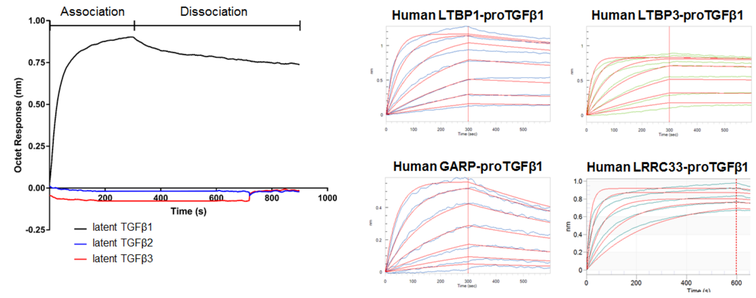
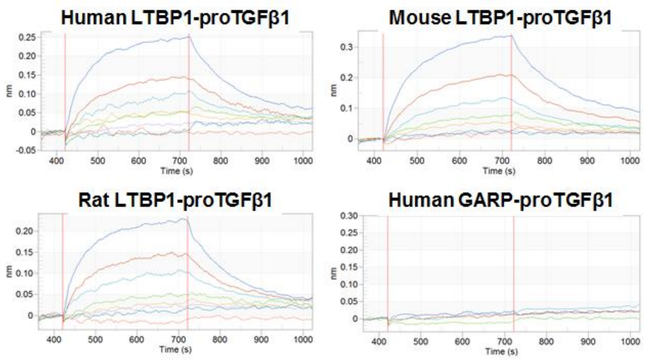


Figure 2. Identification of isoform-specific and LTBP-specific binders of TGFβ1 large latent complexes

A. SR-AB1 binds TGFβ1 LLCs independent of presenting molecule



B. SR-AB2 binds LTBP-presented TGFβ1 LLCs

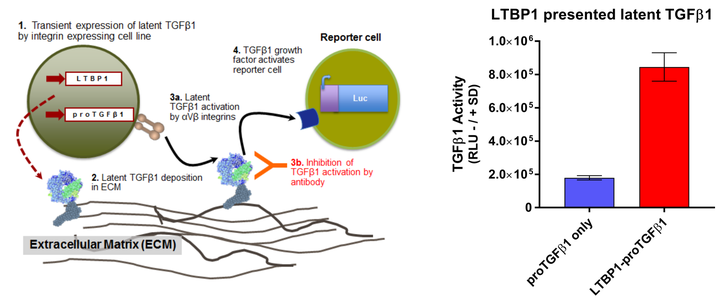


A. SR-AB1 is a human monoclonal antibody that specifically binds latent TGFβ1
- No detectable binding to latent TGFβ2 or TGFβ3, or to TGFβ1 growth factor
- Cross-reacts with mouse, rat and cynomolgus monkey proteins
- Binds to all 4 latent TGFβ1 complexes

B. SR-AB2 is a human monoclonal antibody that specifically binds LTBP-presented latent TGFβ1
- No detectable binding to GARP-proTGFβ1 complex
- Cross-reacts to rodent LTBP1-proTGFβ1

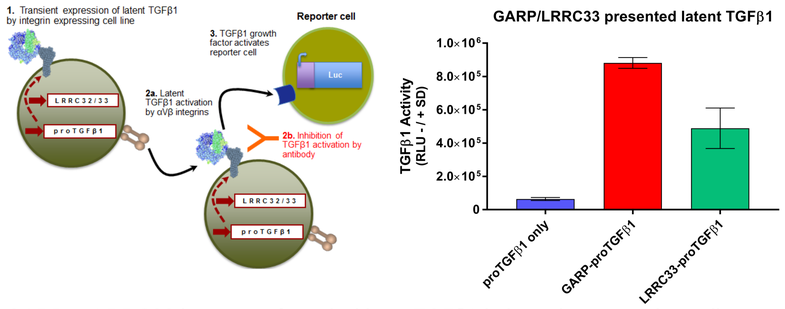
Figure 3. Functional assay to detect the inhibition of TGFβ1 large latent complex activation by integrin

A. Activation of LTBP1 presented latent TGFβ1 deposited in ECM



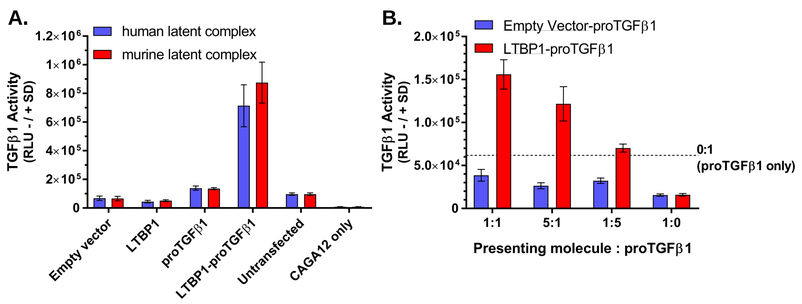
- LTBP1 co-transfected with proTGFβ1 in integrin-expressing cells
- Transiently transfected cells are seeded in assay plates in the presence of inhibitors. Latent LTBP1-proTGFβ1 complex is embedded in ECM.
- TGFβ reporter cells are then added to system; free growth factor (released by integrin) signals and is detected as luciferase activity

B. Activation of latent TGFβ1 presented on cell surface



- GARP or LRRC33 is co-transfected with proTGFβ1 in integrin-expressing cells. Latent TGFβ1 is expressed on the cell surface by the presenting molecule
- TGFβ reporter cells and inhibitors are then added to system; free growth factor (released by integrin) signals and is detected as luciferase activity

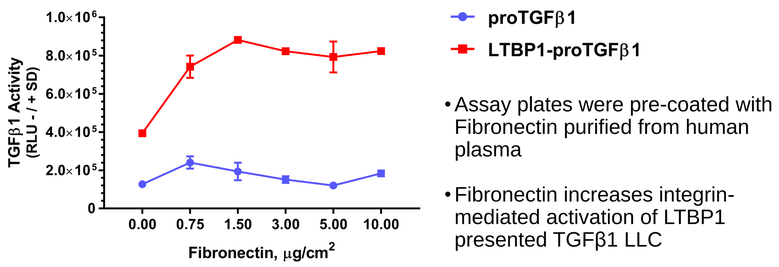
Figure 4. Optimization of recombinant functional assay



A. Relative contribution of presenting molecule and/or proTGFβ1 to latent TGFβ1 activation
- Significant increase in latent TGFβ1 activation upon co-transfection of presenting molecule and proTGFβ1

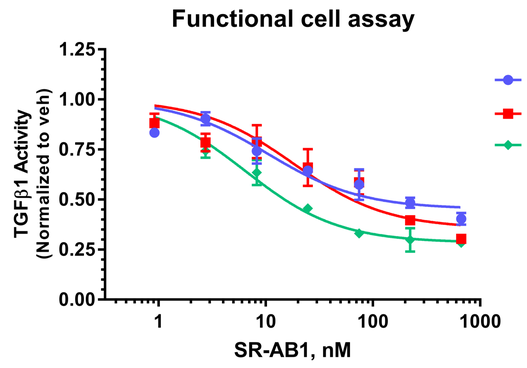
B. Optimization of co-transfection: Ratio of plasmid DNAs for presenting molecule and proTGFβ1
- Equivalent amounts of each plasmid is optimal for co-transfection

Figure 5. Fibronectin promotes integrin activation of LTBP1 presented TGFβ1 large latent complex



- Assay plates were pre-coated with Fibronectin purified from human plasma
- Fibronectin increases integrin-mediated activation of LTBP1 presented TGFβ1 LLC

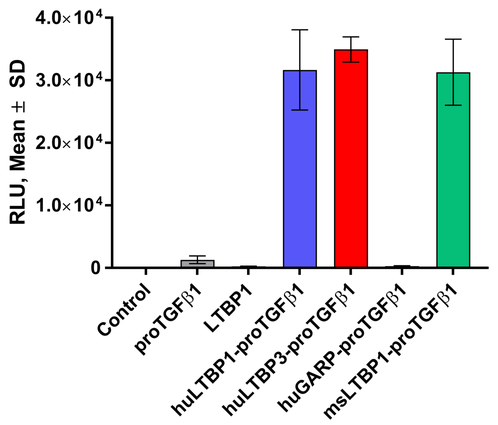
Figure 6. SR-AB1 is a context-independent inhibitor of TGFβ1 large latent complex activation by integrin



- SR-AB1 inhibits the integrin activation of TGFβ1 LLC independent of the presenting molecule

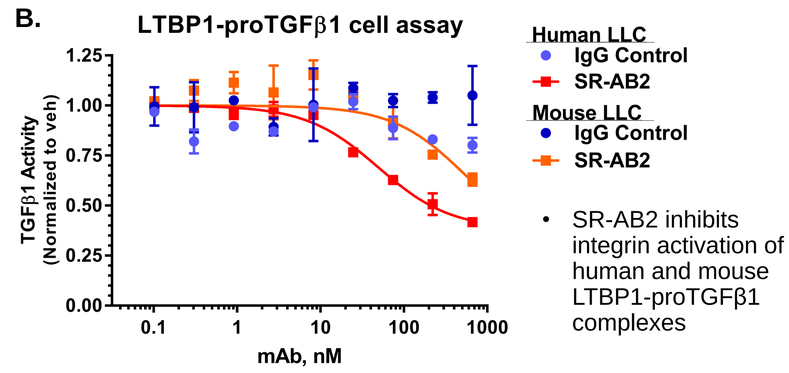
Figure 7. Validation of a LTBP-specific inhibitor of TGFβ1 large latent complex activation by integrin

A.



- SR-AB2 only binds LTBP-proTGFβ1 complex; it does not bind proTGFβ1 or LTBP1 alone, and it does not bind GARP-proTGFβ1 complex

B.



- SR-AB2 inhibits integrin activation of human and mouse LTBP1-proTGFβ1 complexes

Conclusions

We discovered anti-TGFβ1 large latent complex (LLC) antibodies that can be divided into two classes: 1.) "context independent" antibodies that bind all TGFβ1 LLCs, regardless of presenting molecule and 2.) "context-selective" antibodies that bind only LTBP-presented TGFβ1 LLCs. To determine whether these binders were functional, we developed cell-based assays to measure LLC activation in the context of each known TGFβ1 presenting molecule (LTBP1, LTBP3, GARP, and LRRC33). Using these assays, we identified antibodies that are capable of inhibiting integrin-mediated TGFβ1 LLC activation in the context of all presenting molecules (SR-AB1) or specifically in LLCs containing LTBP1 (SR-AB2). Thus, we demonstrate that prodomain-targeted antibodies can inhibit TGFβ1 activation, and can be used to tailor TGFβ1 inhibition to specific LLC complexes. This strategy for generation of antibodies with different TGFβ1 LLC selectivity profiles may enable a significantly more targeted approach to therapeutic inhibition of TGFβ signaling, thus mitigating some of the risks associated with broad inhibition of this important pathway.