Discovery and Antifibrotic Activity of an Anti-LTBP-TGFβ1 Inhibitory Antibody

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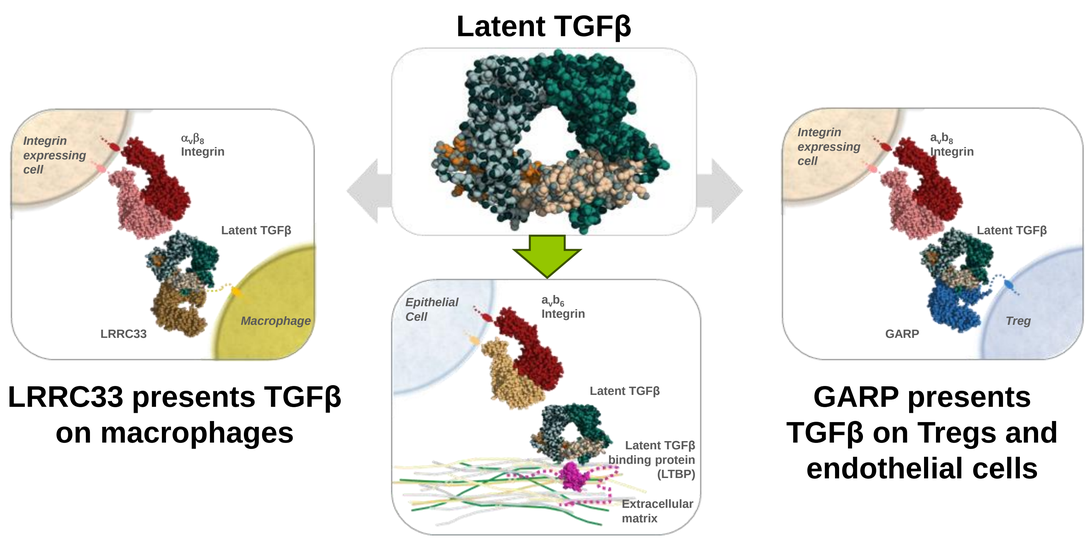
Abstract

Transforming growth factor beta 1, or TGFβ1, is involved in numerous cell processes including proliferation, differentiation, and apoptosis. Dysregulated signaling of TGFβ1 has also been implicated in numerous diseases including cancer, autoimmunity, and fibrosis of the liver, lung, and kidney. TGFβ1 is expressed as an inactive precursor, called proTGFβ1, which is posttranslationally processed by furin proteases that cleave proTGFβ1 into an N-terminal prodomain (known as latency associated peptide, or LAP) and a C-terminal mature growth factor. The prodomain remains noncovalently associated with the TGFβ1 growth factor to form the small latent complex (SLC), in which the prodomain sterically prevents growth factor signaling. The prodomain of TGFβ1 is linked via disulfide bonds to transmembrane proteins or proteins in the extracellular matrix (ECM), forming so called large latent complexes (LLCs). These LLC-forming proteins include the latent TGFβ binding proteins LTBP1 and LTBP3, both ECM proteins, glycoprotein A repetitions predominant (GARP), and leucine-rich repeat protein LRRC33. GARP and LRRC33 are complexed with TGFβ1 on the surface of cells of the immune system and are involved in immune system regulation. Scholar Rock generated large latent complexes of TGFβ1 and performed iterative antibody display campaigns to discover/engineer LTBP-49247, an antibody that selectively binds to LTBP complexes of TGFβ1 and inhibits the integrin-mediated activation of ECM-associated TGFβ1 without inhibiting TGFβ1 of the immune system. Importantly, inhibition of LTBP-complexed, ECM-associated TGFβ1 is sufficient to significantly decrease TGFβ signaling and reduce the progression of fibrosis in a mouse model of Alport Syndrome, a model of progressive fibrotic kidney disease. This highly-targeted approach may avoid immune cell activation and dose limiting toxicities historically associated with TGFβ inhibition and offer a superior therapeutic index compared to other strategies targeting the TGFβ pathway.

Figure 1. Latent TGFβ1 is covalently bound to distinct presenting molecules which are found in distinct cellular milieus

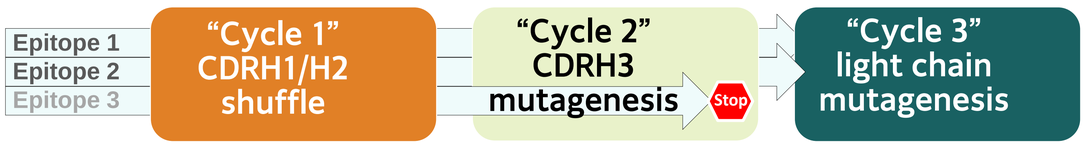


LRRC33 presents TGFβ on macrophages

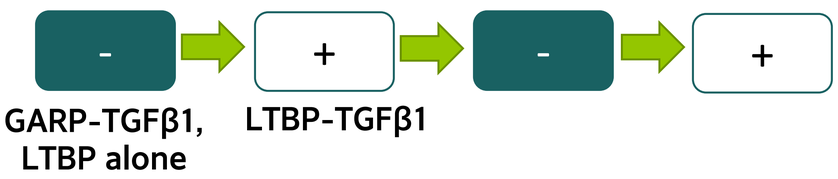
LTBP1 & 3 present TGFβ in connective tissue, the site of fibrosis

GARP presents TGFβ on Tregs and endothelial cells

Figure 2. Three cycles of antibody engineering were performed to identify high affinity antibodies to LTBP-TGFβ1

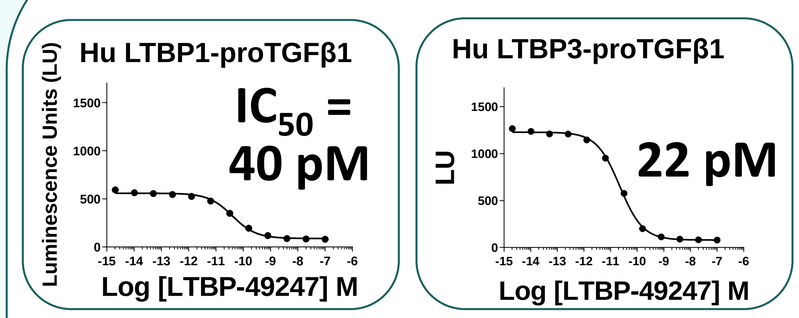


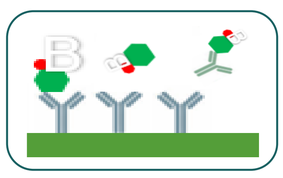
each cycle consisted of multiple rounds of positive and negative selections



- 3 low-affinity (K_D~100 nM) antibodies against TGFβ1 targeting non-crossblocking epitopes were selected for affinity maturation
- A yeast display campaign utilizing multiple rounds of positive selections with LTBP-TGFβ1 and negative selections with GARP-TGFβ1 and LTBP presenting molecule alone were performed
- Sub-nM affinity LTBP-complex-selective TGFβ1 antibodies were identified

Figure 3. LTBP-49247 targets LTBP-complexed TGFβ1 (MSD-SET)



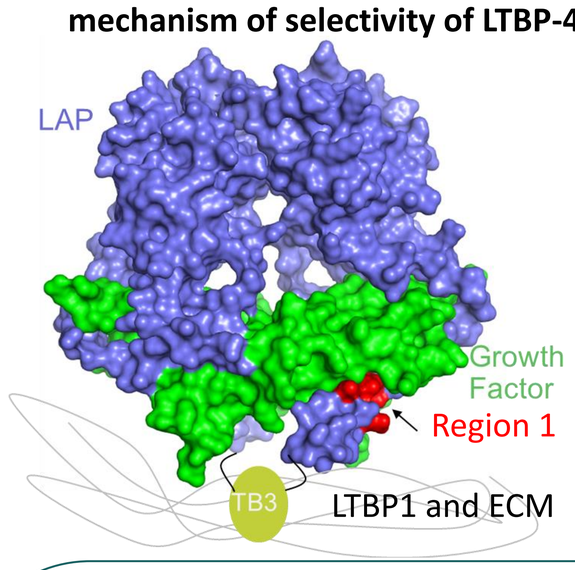


- LTBP-49247 is immobilized to a plate surface and used to capture biotinylated large latent complexes (LLCs) of TGFβ1 with a 150-second capture step
- Affinity measured by titrating LTBP-49247 in solution against a set concentration of LLCs
- No binding detected to LTBP alone, TGFβ2, or TGFβ3
- Similar binding profile to mouse, rat, and cyno TGFβ1 complexes

Conclusion

Multiple rounds of affinity maturation enabled the discovery of LTBP-49247, a highly selective antibody that potently inhibits the integrin-mediated activation of TGFβ1 from LTBP complexes, but not from GARP or LRRC33 complexes. Targeting TGFβ1 bound to LTBPs and deposited in the extracellular matrix was sufficient to reduce TGFβ signaling and fibrotic disease progression in a mouse model of Alport syndrome and this strategy may offer a superior therapeutic index compared to other strategies for targeting the TGFβ pathway, which is a well-established driver of fibrosis. Avoiding immune stimulation may offer superior antifibrotic efficacy, as fibrosis is characterized by chronic inflammation and immune cell activation.

Figure 4. Hydrogen deuterium exchange elucidates the epitope and potential mechanism of selectivity of LTBP-49247



- Region 1 is the most protected region of LTBP1-TGFβ1 when LTBP-49247 fab is bound
- Region 1 is immediately adjacent to the presenting molecule and modeling indicates that this epitope would be sterically occluded by GARP or LRRC33

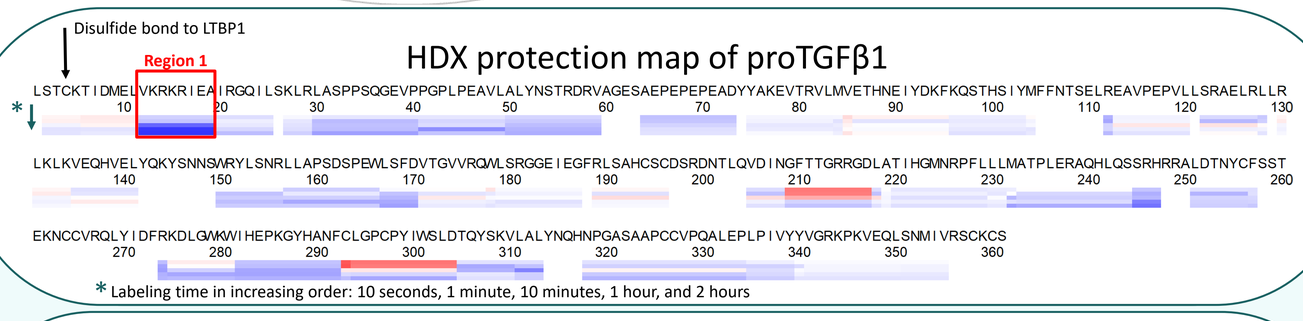
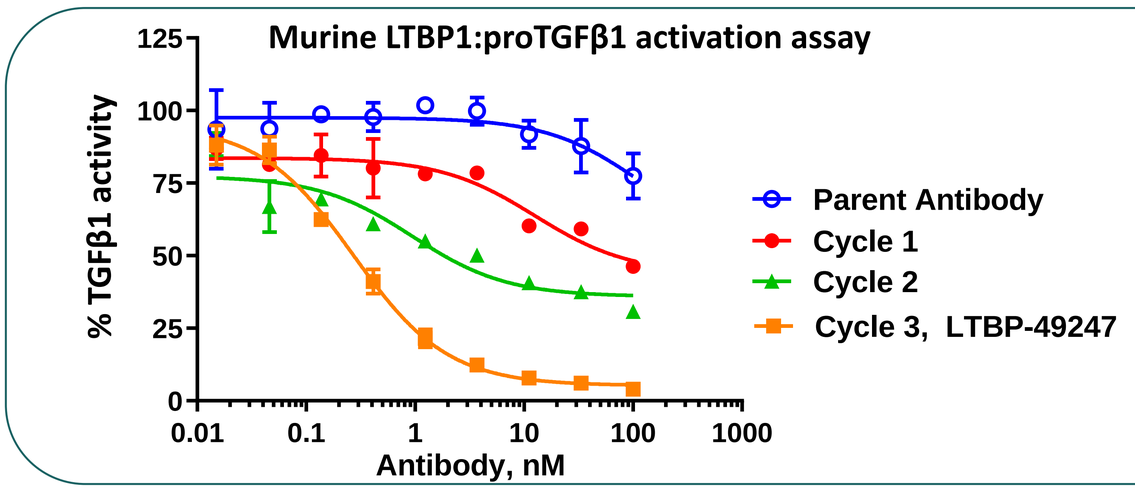
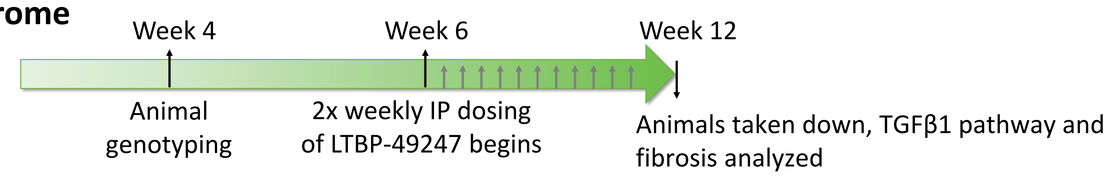


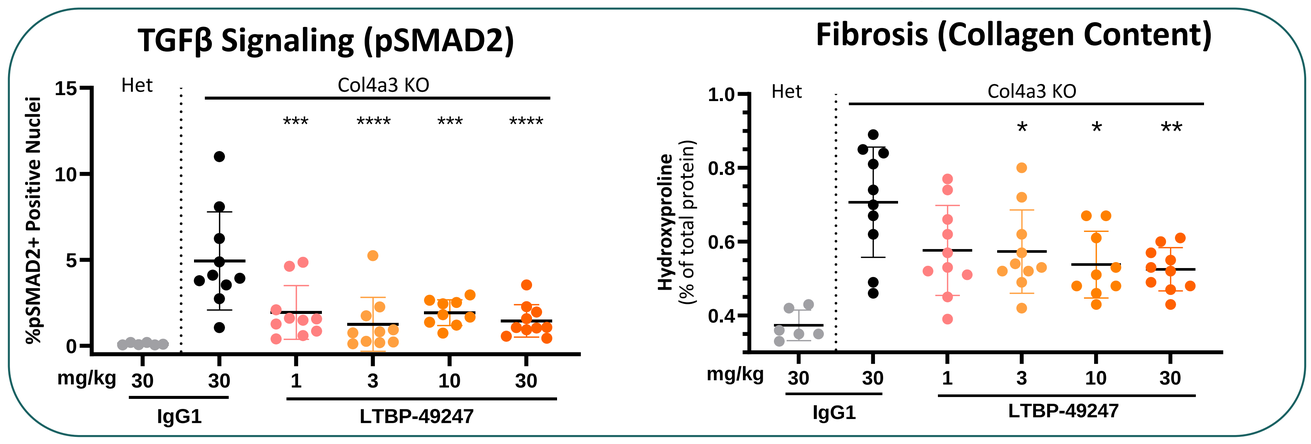
Figure 5. LTBP-49247 inhibits the integrin-mediated activation of TGFβ1



- LN229 cells (human glioblastoma) are transiently transfected with murine LTBP1 and proTGFβ1
- LN229 cells endogenously express αvβ8 integrin that activates TGFβ1
- Cells are treated with test antibodies and co-cultured with a TGFβ reporter cell line
- Inhibitory potency increased with each cycle of affinity maturation, culminating in LTBP-49247, a sub-nM IC50 inhibitor of the integrin-mediated activation of LTBP complexed TGFβ1

Figure 6. LTBP-49247 Reduces pSmad2 and Fibrosis in a Mouse Model of Alport Syndrome





- Alport syndrome is caused by genetic deficit in type IV collagen and affects the basement membrane in the ears, eyes, and kidneys, resulting in progressive kidney inflammation and fibrosis
- COL4A3 knockout mice on an F1 cross of S129 and C57Bl/6 background were utilized in this study

Acknowledgements

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