STRUCTURAL CHARACTERIZATION OF SELECTIVE INHIBITORS OF TGFβ1 ACTIVATION WITH DIFFERING MECHANISMS OF ACTION AND CLINICAL POTENTIAL

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Abstract

Background: Transforming growth factor beta-1 (TGFβ1) is an important cytokine that regulates a variety of cellular functions, with notable roles in regulation of the extracellular matrix and the immune response. TGFβ1 signaling has been implicated in the development of pathogenic fibrosis and immune cell exclusion in tumorigenesis (Meng 2016, Derynck 2020, Budi 2016). As such, therapeutic blockade of the TGFβ1 pathway has been sought for treatment of fibrosis and cancer progression.

Previous efforts to inhibit TGFβ1 signaling, either by targeting the mature growth factor itself or the membrane receptor were unsuccessful due to toxicities (Anderton 2011, Gueorguieva 2014, Morris 2014, Stauber 2014, Brandes 2016, Ruike 2017, Melisi 2018 & Mitra 2020). However, these previous approaches were not specific for TGFβ1; rather, they also inhibited signaling from the related paralogs TGFβ2 and TGFβ3, and this broad-spectrum inhibition has been hypothesized as the source of the observed toxicities in patients (Martin 2020 & Welsh 2021).

TGFβ1 is produced by cells as a pro-protein that requires further processing by the Furin protease that cleaves the pro-domain or latency associated peptide (LAP) from the growth factor (GF) (Dubois 1995). The homodimeric complex, containing two TGFβ1 LAP domains noncovalently bound to two GF domains, forms the small latent complex (Shi 2011). The small latent complex (SLC) can form covalent linkages to one of several potential presenting molecules (LTBP1, LTBP3, GARP, and LRRC33) to form the various large latent complexes (LLC), which bear latent TGFβ1 in different biological roles, poised for activation and signaling within these different molecular contexts (Robertson 2015, Stockis 2009, & Qin 2018). Activation of TGFβ1 can occur via integrins, which can apply a tensile force to the latent complex and release the GF, or via proteases that can cleave the LAP and release the GF for signaling (Jenkins 2008, Aluwihare 2009, Kojima 2010 & Dong 2017).

Aims: We sought to characterize the mechanism of action for our antibodies capable of selectively inhibiting the activation of latent TGFβ1.

Methods: We have employed structural biology to characterize the epitope: paratope interaction between our antibodies and latent TGFβ1 and further understand the mechanism by which antibody binding mediates inhibition of activation. Further biochemical and cell-based assays of latent TGFβ1 activity help to characterize this inhibitory activity.

Results: Presented here are data showing the inhibitory activity for three TGFβ1 context-independent antibodies, TGFb1-36993, TGFb1-37021, and TGFb1-36956 with two epitope regions on TGFβ1 and two unique mechanisms of action. Further data show the inhibitory activity of a LTBP1/3 context-specific inhibitor of TGFβ1 activation, LTBP-49247 (Manuscript accepted). Structural data characterizing the epitopes for each of these antibodies illustrates the mechanism of action for latent TGFβ1 inhibition. Additional data shows how some TGFβ1 inhibitory epitopes, where the antibody contacts both the LAP and GF, are capable of blocking integrin and protease mediated activation. An alternative epitope that lies entirely within the LAP can compete with integrin binding (and therefore inhibits integrin-mediated activation) but does not inhibit protease-mediated activation. Treatment of TGFβ1 with oxidizing compounds (Ascorbate/Iron (III) Chloride) was shown to make TGFβ1 susceptible to activation within a cell-based assay of TGFβ1 signaling as seen previously (Barcellos-Hoff 1996, Pociask 2004 & Jobling 2006), however this treatment did not cause the growth factor to dissociate from the latent complex when analyzed biochemically. Like the protease mediated activation, our inhibitory antibodies that contact both the LAP and the GF blocked this oxidation-mediated activation, whereas the integrin competitive epitope did not.

Conclusion: We have discovered context-independent TGFβ1 inhibitory antibodies capable of binding and preventing the activation of latent TGFβ1, irrespective of presenting molecule context. One of these antibodies is being developed further and has demonstrated proof of mechanism in the clinic for checkpoint (anti-PD(L)1)-resistant cancer patients (NCT04291079, SITC 2023 #726, ASCO 2024 #2507). We have also discovered an LTBP1 and LTBP3 context-dependent antibody that selectively binds and inhibits activation of LTBP1- or LTBP3-presented TGFβ1, which we expect to have clinical potential for the treatment of TGFβ1-driven fibrosis progression.

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

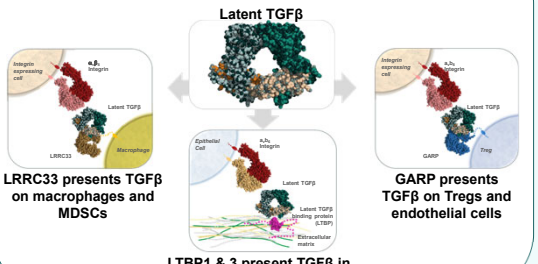
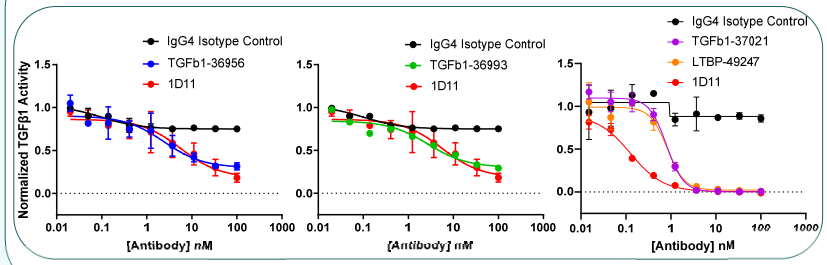
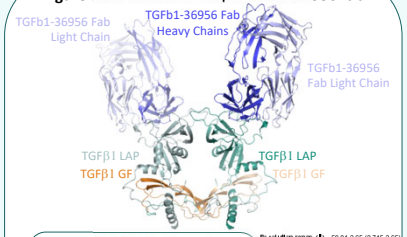


Figure 2. Several antibodies were identified as inhibitors of integrin-mediated activation of latent TGFβ1 in cell-based screens



- LN229 cells (human glioblastoma) are transiently transfected with murine LTBP1 and proTGFβ1
- LN229 cells endogenously express $\alpha_v\beta_8$ integrin that activates TGFβ1
- Cells are treated with test antibodies and co-cultured with a TGFβ reporter cell line

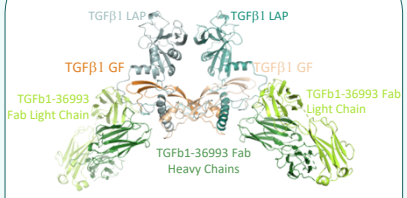
Figure 3. *Structure of TGFβ1:TGFb1-36956 fab*



TGFb1-36956 binds on the LAP, stabilizing the RGD loop, which is an integrin binding competitive epitope

Resolution range (Å)	59.04–2.65 (2.745–2.65)
Space group	C 1 2 1
Completeness (%)	99.0 (99.0)
R-work	0.217 (0.409)
R-free	0.272 (0.435)
Average B-factor	89.33
macromolecules	89.40
solvent	63.46

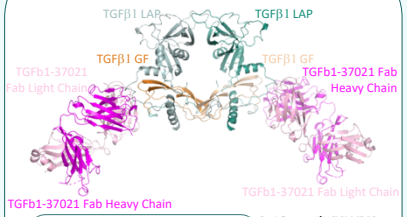
Figure 4. Structure of TGFβ1:TGFb1-36993 fab



TGFb1-36993 contacts the LAP latency lasso and the GF finger domains, stabilizing the complex together

Resolution range (Å)	113-3.50 (3.59-3.50)
Space group	C 2 2 2
Completeness (%)	99.6 (99.1)
R-work	0.326 (0.301)
R-free	0.350 (0.353)
Average B-factor	114.19
macromolecules	114.19
solvent	No Waters

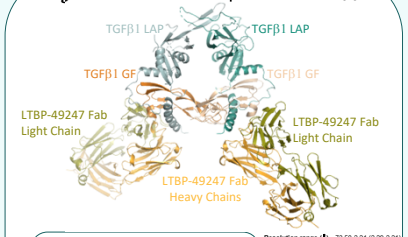
Figure 5. Structure of TGFβ1:TGFb1-37021 fab



TGFb1-37021 also contacts the LAP latency lasso and the GF finger domains, stabilizing the complex together

Resolution range (Å)	59.04–2.65 (2.745–2.65)
Space group	C 1 2 1
Completeness (%)	99.0 (99.0)
R-work	0.217 (0.409)
R-free	0.272 (0.435)
Average B-factor	89.33
macromolecules	89.40
solvent	63.46

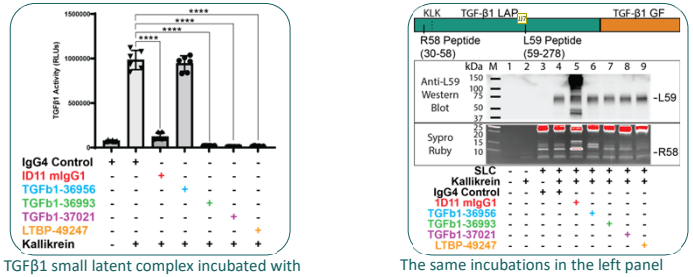
Figure 6. Structure of TGFβ1:LTBP-49247 fab



LTBP-49247 contacts the LAP α-helix 1 and the GF finger domains, stabilizing the complex together

Resolution range (Å)	73.50-2.21 (2.29-2.21)
Space group	P 2 21 21
Completeness (%)	99.5 (94.8)
R-work	0.232 (0.369)
R-free	0.264 (0.423)
Average B-factor	82.15
macromolecules	82.47
solvent	60.93

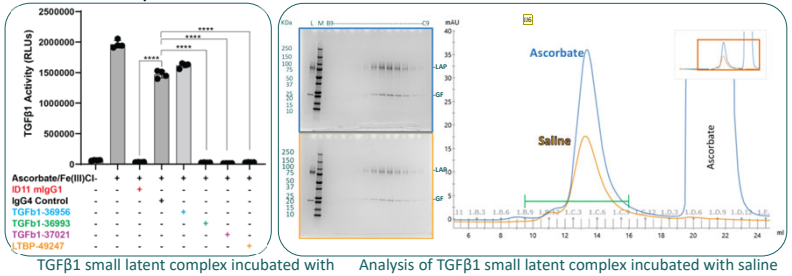
Figure 7. Antibodies with stabilizing epitopes can inhibit Kallikrein mediated activation of TGFβ1



TGFβ1 small latent complex incubated with Kallikrein and inhibitory antibodies prior to addition to the TGFβ1 reporter cells

The same incubations in the left panel analyzed with SDS-PAGE followed by Sypro Ruby staining or western blot

Figure 8. Antibodies with stabilizing epitopes can inhibit Reactive Oxygen Species (ROS)-mediated activation of TGFβ1



TGFβ1 small latent complex incubated with ascorbate/Fe(III)Cl and inhibitory antibodies prior to addition to the TGFβ1 reporter cells

Analysis of TGFβ1 small latent complex incubated with saline or ascorbate/Fe(III)Cl (first 2 samples on left) and further analyzed by size exclusion chromatography and SDS-PAGE

Conclusions

- We have discovered context-independent TGFβ1 inhibitory antibodies capable of binding and preventing the activation of latent TGFβ1, irrespective of presenting molecule context. One of these antibodies is being developed further and has demonstrated proof of mechanism in the clinic for checkpoint (anti-PD(L)1)-resistant cancer patients (NCT04291079, SITC 2023 #726, ASCO 2024 #2507). We have also discovered an LTBP1 and LTBP3 context-dependent antibody that selectively binds and inhibits activation of LTBP1- or LTBP3-presented TGFβ1, which we expect to have clinical potential for the treatment of TGFβ1-driven fibrosis progression.

Acknowledgements

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Slide 1

SN0 I got rid of the bullet point before these
Samantha Nicholls, 2024-06-24T13:20:56.490

SN1 Paper ref?
Samantha Nicholls, 2024-06-24T13:25:41.346

SN2 Did any of it come from IALS and/or the Harvard med core for the 021?
Samantha Nicholls, 2024-06-24T13:26:33.258

SN3 Don't leave this red...
Samantha Nicholls, 2024-06-24T13:29:41.425

MM4 this conclusion makes some good points on application and gets at my comment on your conclusion slide in the short talk
Molly MacLeod, 2024-06-27T13:27:27.232

MM5 this looks great!
Molly MacLeod, 2024-06-27T13:31:35.349

JJ6 I think you could delete "wholly within the LAP"
Justin Jackson, 2024-06-30T23:44:17.830

JJ7 As above
Justin Jackson, 2024-06-30T23:44:49.808