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A Newly Evolved Domain of Asp-tRNA Synthetase Interacts with Latent Transforming Growth Factor Beta Binding Protein 1 (LTBP-1) to Induce Myofibroblast Apoptosis

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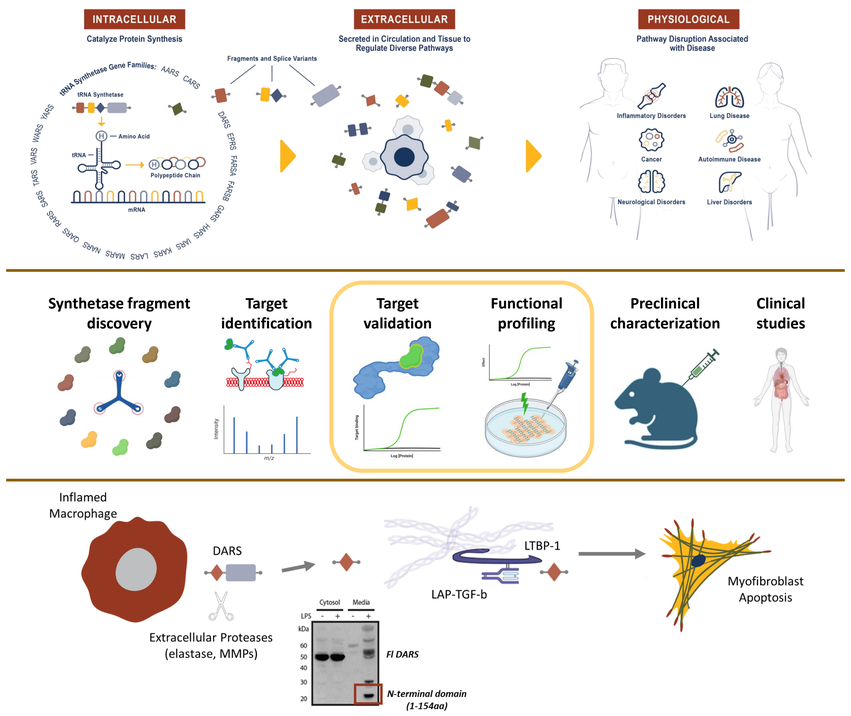
Introduction

Background: Throughout the course of evolution, aminoacyl-tRNA synthetases (aaRS) have established their indispensable function for protein synthesis. However, what is less widely known is that these aaRS have systematically acquired novel domains which are liberated through splicing and/or proteolytic cleavage to effect extracellular signaling. Upon release from cells, these novel aaRS domains interact with extracellular or membrane bound targets to impact a variety of biological activities which may have therapeutic utility. One such candidate, derived from a his-tRNA synthetase splice variant, has resulted in a therapeutic molecule, termed efzofitimod, which is currently in a Phase 3 clinical trial for pulmonary sarcoidosis. A second synthetase, asp-tRNA synthetase, has been shown to liberate an N-terminal fragment in inflamed macrophages which may provide opportunities for additional therapeutic development.

Aim: In this study, we aimed to examine the therapeutic potential of a novel asp-tRNA synthetase fragment (ATYR0101) by identifying its binding partner, latent transforming growth factor beta binding protein 1 (LTBP-1) and further revealing a novel anti-fibrotic mechanism that results from ATYR0101-induced signaling to induce myofibroblast apoptosis.

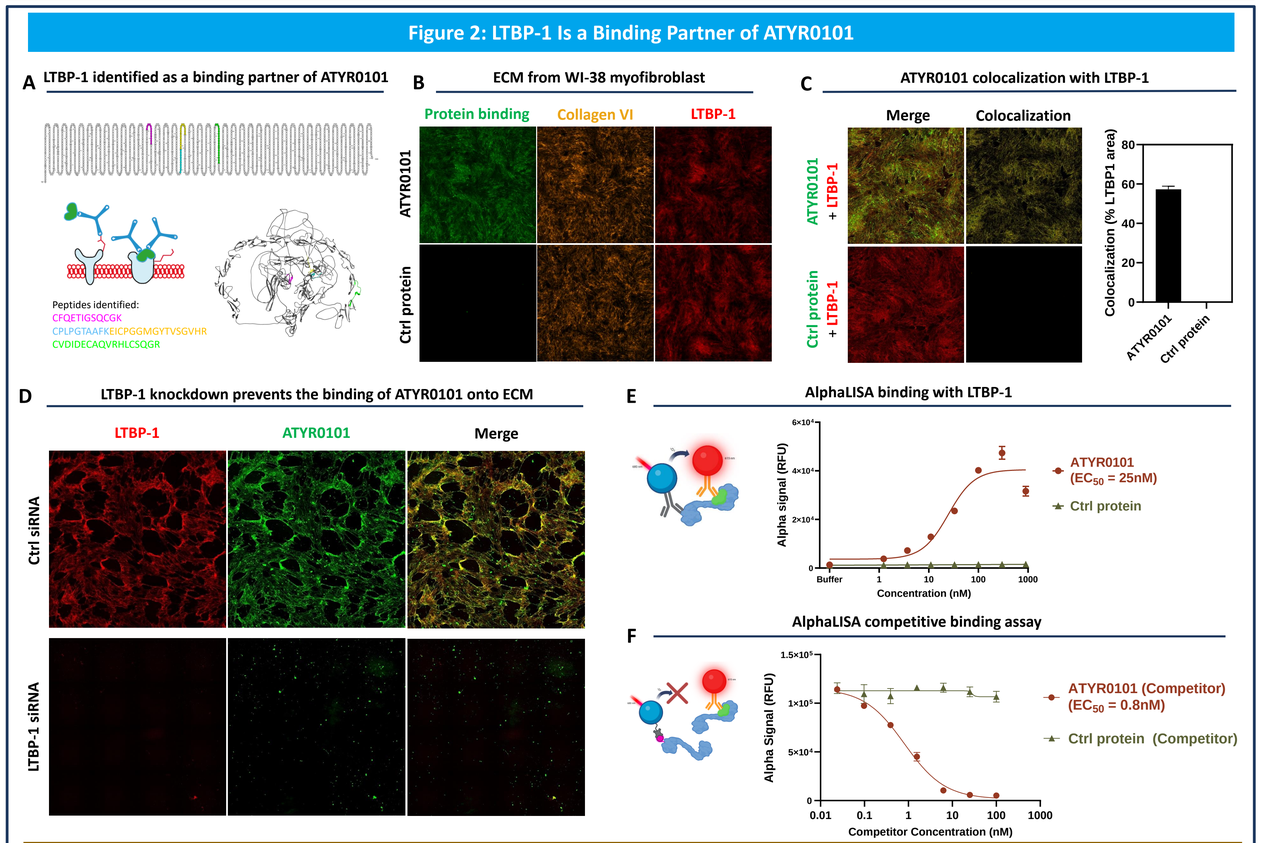
Figure 1: tRNA Synthetase Drug Discovery Platform



- Unique protein domains found in tRNA synthetases can be liberated via splicing or proteolysis and have been identified in extracellular spaces, where they have functions separate from their essential role in protein synthesis.
- aTyr has built an intellectual property portfolio covering >300 protein compositions derived from all 20 tRNA synthetase genes and is engaged in the discovery and development of potential first-in-class medicines based on newly discovered pathways effected by extracellular tRNA synthetases.
- An N-terminal fragment from asp-tRNA synthetase was identified in macrophages stimulated with LPS. This fragment was subsequently found to induce myofibroblast apoptosis.
- In this work, we build upon the discovery of LTBP-1 as a novel binding partner of an asp-tRNA synthetase fragment (ATYR0101) and further reveal a novel anti-fibrotic mechanism of ATYR0101.

Results

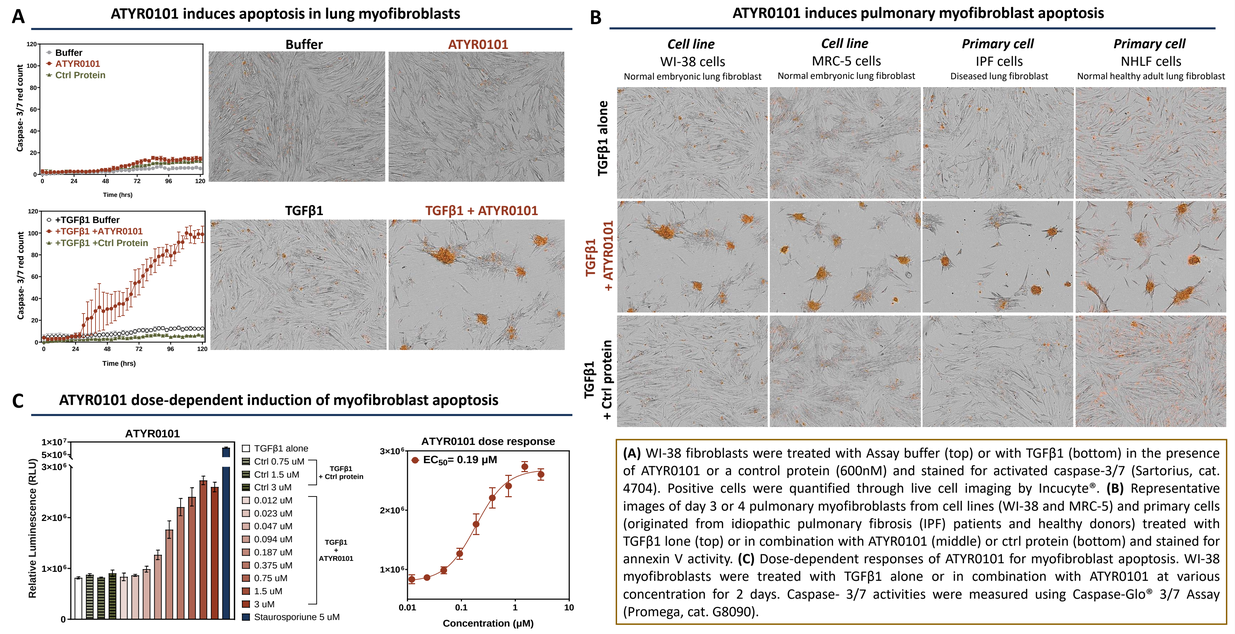
Figure 2: LTBP-1 Is a Binding Partner of ATYR0101



(A) Latent transforming growth factor beta-binding protein 1 (LTBP-1) was identified as a binding partner of ATYR0101 using a mass spectrometry-based receptor screening platform from Dualsystems' ligand-receptor capture technology. LTBP-1 peptide sequences identified are highlighted in LTBP-1 AlphaFold structure (entry Q14766). **(B)** WI-38 cells (human embryonic lung fibroblasts) were treated with TGFβ1 for 6 days to allow for extracellular matrix (ECM) deposition. Cells were removed and ECM were stained with ATYR0101 or a control protein (green), collagen VI (orange), and LTBP-1 (red). ATYR0101 showed binding to the ECM deposited by WI-38 cells. **(C)** Immunofluorescence staining showed ATYR0101 colocalized with LTBP-1 on the ECM deposited by TGFβ1 activated human fibroblasts. ECM deposited from TGFβ1 treated WI-38 cells were stained with ATYR0101 or a control protein (green) and LTBP-1 (red). **(D)** ATYR0101 lost binding to ECM from LTBP-1 knockdown WI-38 myofibroblasts. TGFβ1 activated WI-38 cell were treated with ctrl siRNA (top) or LTBP-1 siRNA (bottom panel) in a protocol as described in (B). **(E)** A direct interaction between ATYR0101 and recombinant human LTBP-1 was confirmed by AlphaLISA protein-protein interaction assay. **(F)** ATYR0101 competitive binding assay to measure the Alpha signal upon exogenous competitor, ATYR0101 or ctrl protein, in competing with the binding of ATYR0101 (donor beads) to LTBP-1 (acceptor beads).

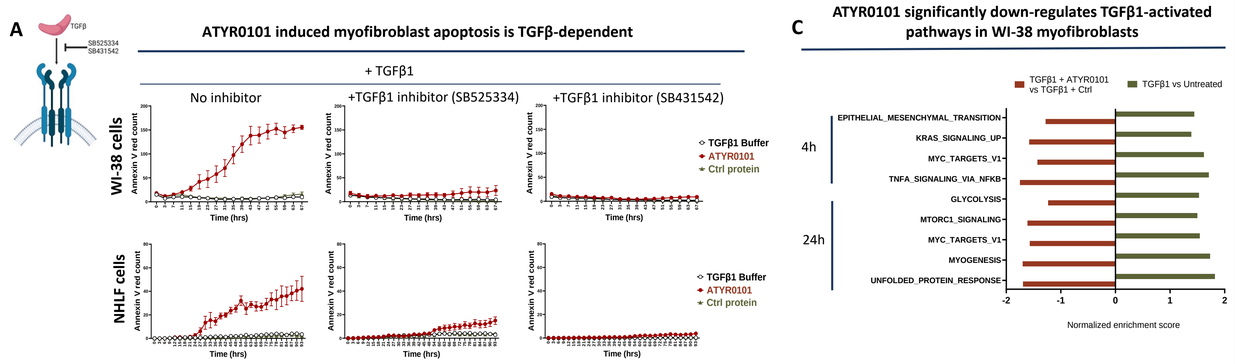
Results

Figure 3: ATYR0101 Induces Myofibroblast Apoptosis



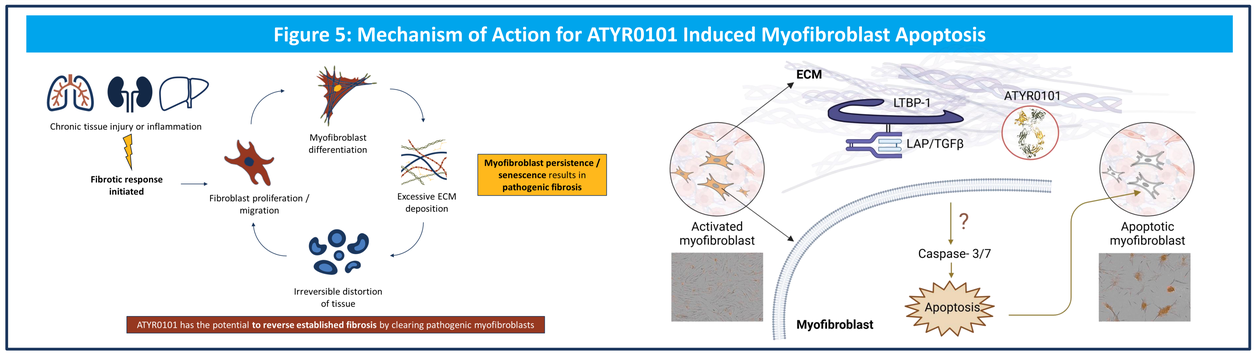
(A) WI-38 fibroblasts were treated with Assay buffer (top) or with TGFβ1 (bottom) in the presence of ATYR0101 or a control protein (600nM) and stained for activated caspase-3/7 (Sartorius, cat. 4704). Positive cells were quantified through live cell imaging by Incucyte®. **(B)** Representative images of day 3 or 4 pulmonary myofibroblasts from cell lines (WI-38 and MRC-5) and primary cells (originated from idiopathic pulmonary fibrosis (IPF) patients and healthy donors) treated with TGFβ1 lone (top) or in combination with ATYR0101 (middle) or ctrl protein (bottom) and stained for annexin V activity. **(C)** Dose-dependent responses of ATYR0101 for myofibroblast apoptosis. WI-38 myofibroblasts were treated with TGFβ1 alone or in combination with ATYR0101 at various concentration for 2 days. Caspase- 3/7 activities were measured using Caspase-Glo® 3/7 Assay (Promega, cat. G8090).

Figure 4: ATYR0101 Induced Myofibroblast Apoptosis Is TGFβ-dependent and Relied Upon Signaling Through LTBP-1 Interaction



(A) Changes in apoptosis signals in ATYR0101-treated-TGFβ1-differentiated myofibroblast with or without TGFβ1 inhibitors SB525334 (10 μM) or SB431542 (10 μM) (Tocris Bioscience). WI-38 myofibroblast were treated with ATYR0101 or ctrl protein (600nM) in a protocol as described previously. Annexin V activities were measured and quantified on the Incucyte® instrument over a time course of incubation. **(B)** ATYR0101 treated WI-38 myofibroblasts were sequestered with recombinant human LTBP-1 (500nM). Morphological changes at day 4 (left) and annexin V signal changes (right) were measured in real time on Incucyte®. **(C)** Gene set enrichment analysis (GSEA) of 50 hallmark pathways demonstrated that ATYR0101 significantly down-regulates multiple TGFβ1-activated pathways (e.g. epithelial-mesenchymal-transition, glycolysis, mTOR) that are critically involved in fibrosis development, suggesting ATYR0101 as a novel modulator of TGFβ1-signaling against fibrosis.

Figure 5: Mechanism of Action for ATYR0101 Induced Myofibroblast Apoptosis



Conclusions

- ATYR0101 binds directly to LTBP-1 resulting in caspase-3/7 mediated apoptosis in TGFβ1-differentiated myofibroblasts while having no effect on undifferentiated fibroblasts. This apoptotic activity can be observed in multiple cell types demonstrating the therapeutic potential in several organ systems.
- Analysis of the ATYR0101-induced myofibroblast apoptosis activity was confirmed to be dependent upon LTBP-1, TGFβ activation and downstream gene expression changes.
- ATYR0101 has promise as a novel and transformative anti-fibrotic therapeutic with a unique mechanism of action.

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