Natural Asp-tRNA Synthetase Fragment Interacts with LTBP-1 on the ECM Promoting Myofibroblast Apoptosis and Reducing Fibrosis

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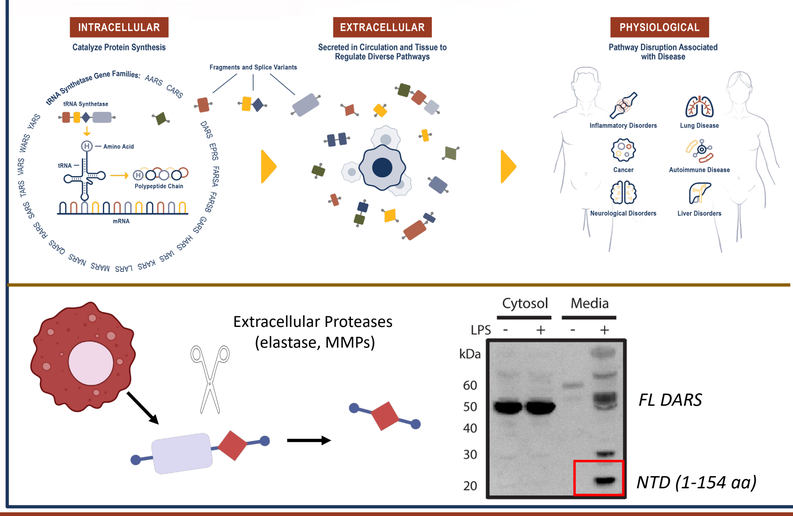
Introduction

Background: Evolutionarily conserved domains of aminoacyl-tRNA synthetases (aaRS) undergo proteolytic cleavage or alternative splicing to generate fragments with specialized extracellular signaling roles that regulate disease states. Previously, aTyr has developed one such splice variant of histidyl-tRNA synthetase into the therapeutic molecule, efzofitimod. This candidate effectively mitigates lung inflammation through targeting myeloid cells and is currently being evaluated for the treatment of pulmonary sarcoidosis and systemic sclerosis-ILD in clinical trials. Further research efforts have focused on identifying and characterizing the therapeutic potential of other aaRS family members. To this end, we have developed ATYR0101, a molecule derived from a unique proteolytic fragment of aspartyl-tRNA synthetase (DARS), which selectively induces myofibroblast apoptosis via binding latent-transforming growth factor beta binding protein 1 (LTBP-1) found in the extracellular matrix (ECM).

Aim: In this study, we aim to reveal a novel anti-fibrotic mechanism for ATYR0101 and examine its therapeutic potential using *in vivo* models of pulmonary and renal fibrosis.

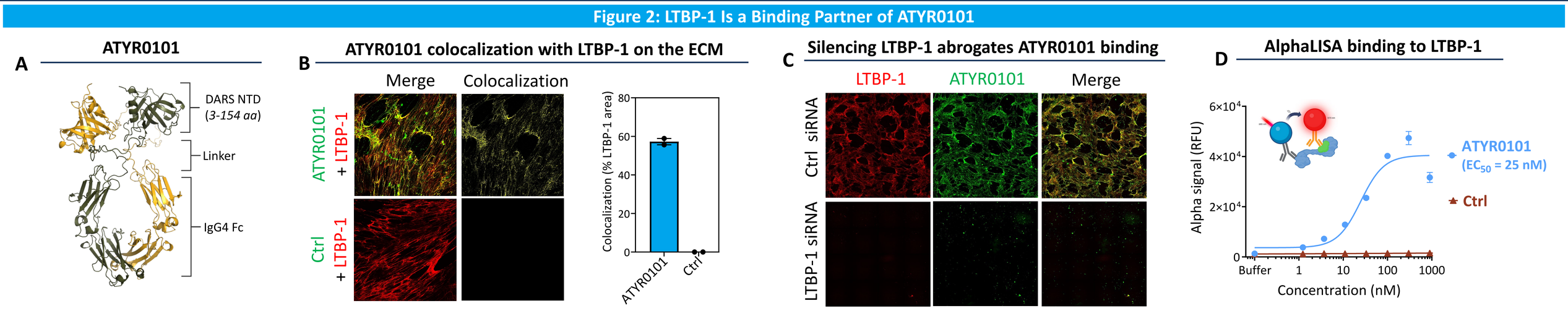
Figure 1: tRNA Synthetase Drug Discovery Platform



- 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 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 elucidated a novel anti-fibrotic mechanism of ATYR0101 via targeting LTBP-1 on the ECM. ATYR0101 demonstrated pharmacological activities in experimental models of lung and kidney fibrosis suggesting a potentially broad anti-fibrotic application across diverse organ systems.

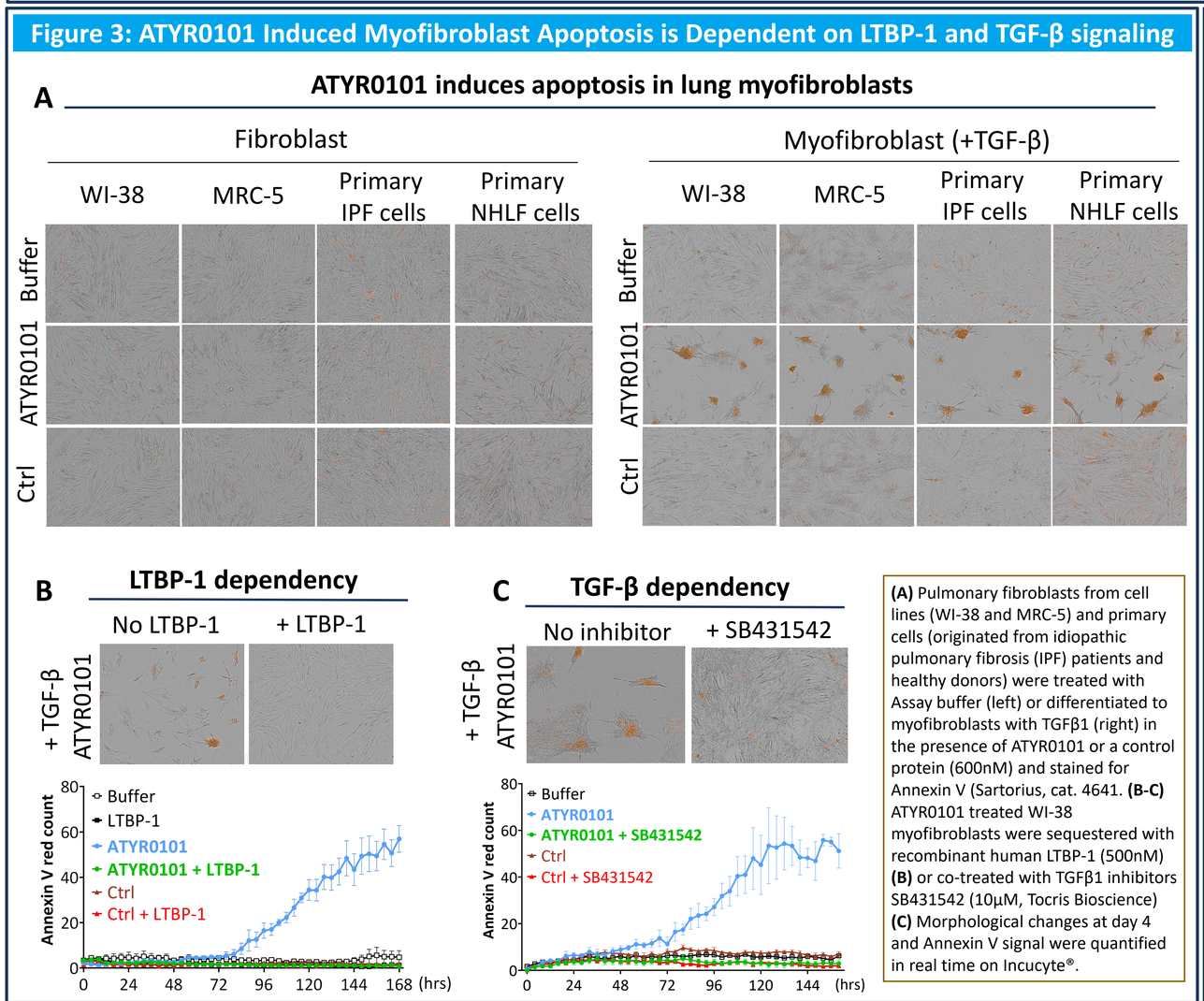
Results

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



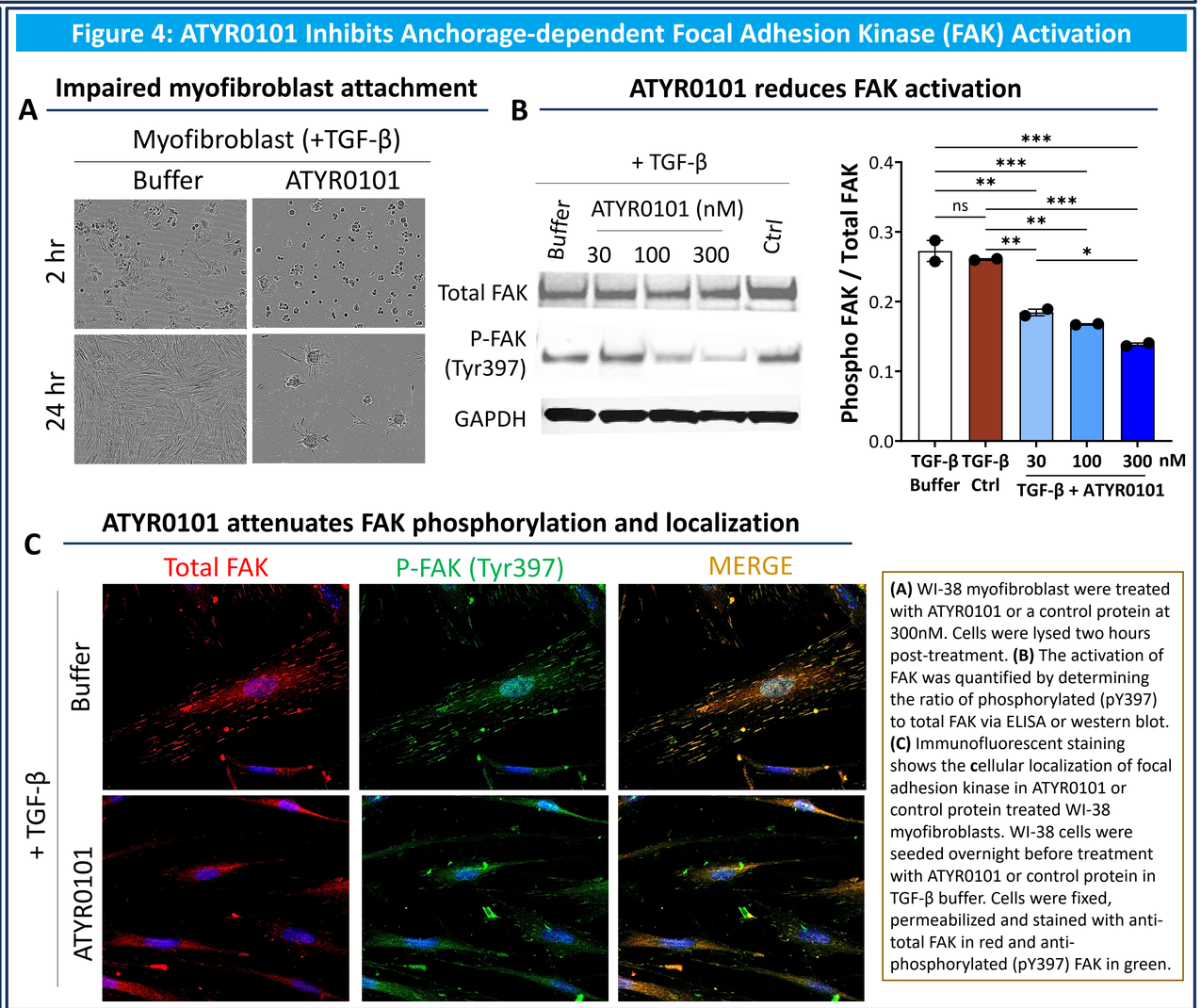
(A) Structure of ATYR0101. **(B)** Human embryonic lung fibroblasts (WI-38 cells) were treated with TGFβ1 for 6 days to deposit extracellular matrix (ECM). Cells were removed and ECM were stained with ATYR0101 or a control protein in green and LTBP-1 in red. ATYR0101 showed colocalization with LTBP-1 on the ECM deposited by TGFβ1 activated WI-38 cells. **(C)** ATYR0101 lost binding to the ECM deposited from LTBP-1 knockdown WI-38 myofibroblasts. TGFβ1 activated WI-38 cell were treated with control siRNA (top) or LTBP-1 siRNA (bottom panel) in a protocol as described in (B). **(D)** A direct interaction between ATYR0101 and recombinant human LTBP-1 was confirmed by AlphaLISA protein-protein interaction assay.

Figure 3: ATYR0101 Induced Myofibroblast Apoptosis is Dependent on LTBP-1 and TGF-β signaling



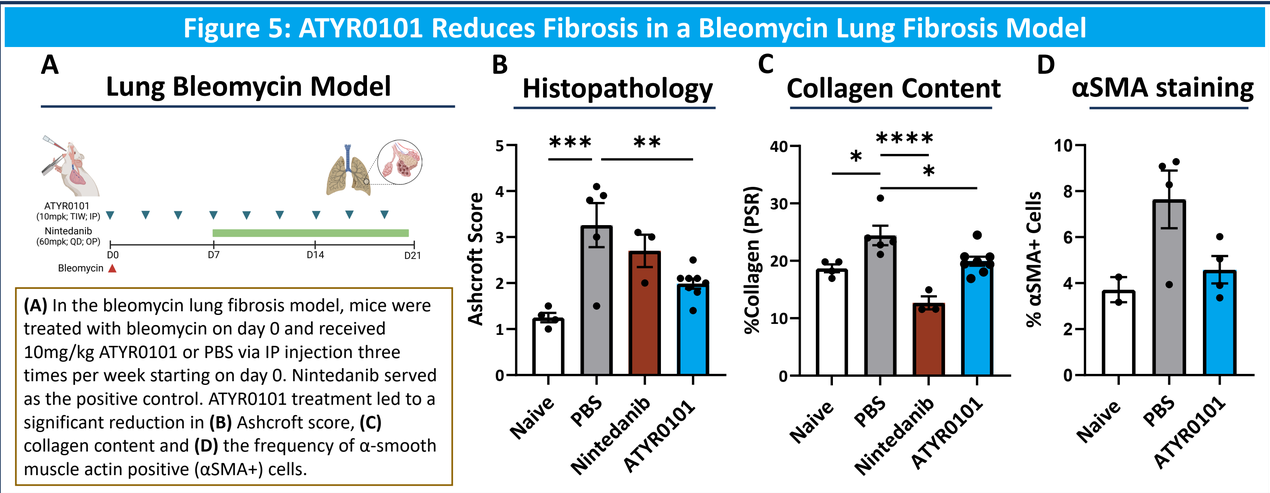
(A) Pulmonary fibroblasts from cell lines (WI-38 and MRC-5) and primary cells (originated from idiopathic pulmonary fibrosis (IPF) patients and healthy donors) were treated with Assay buffer (left) or differentiated to myofibroblasts with TGFβ1 (right) in the presence of ATYR0101 or a control protein (600nM) and stained for Annexin V (Sartorius, cat. 4641. **(B-C)** ATYR0101 treated WI-38 myofibroblasts were sequestered with recombinant human LTBP-1 (500nM) **(B)** or co-treated with TGFβ1 inhibitors SB431542 (10μM, Tocris Bioscience) **(C)** Morphological changes at day 4 and Annexin V signal were quantified in real time on Incucyte®.

Figure 4: ATYR0101 Inhibits Anchorage-dependent Focal Adhesion Kinase (FAK) Activation



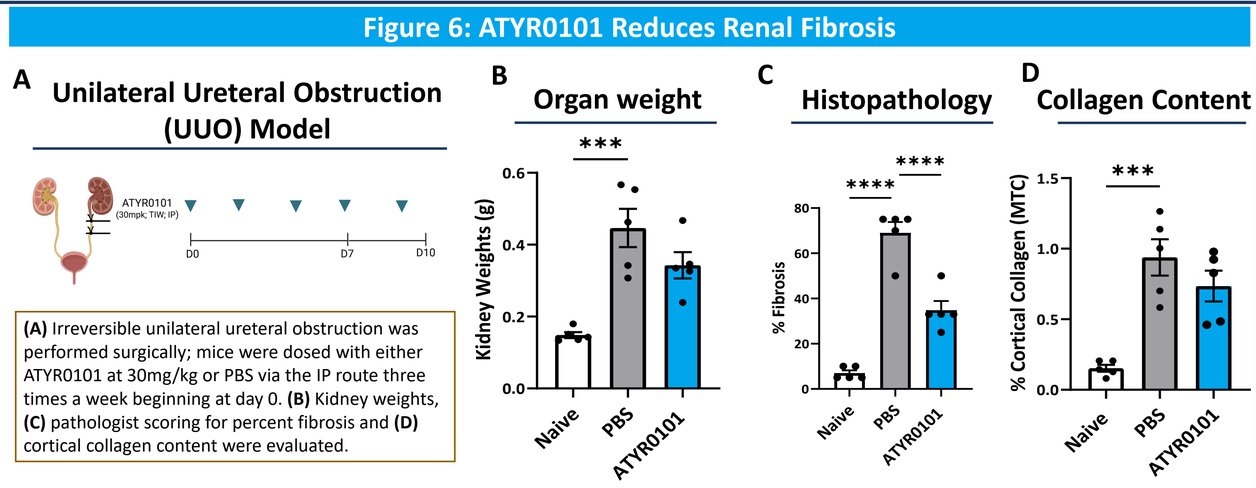
(A) WI-38 myofibroblast were treated with ATYR0101 or a control protein at 300nM. Cells were lysed two hours post-treatment. **(B)** The activation of FAK was quantified by determining the ratio of phosphorylated (pY397) to total FAK via ELISA or western blot. **(C)** Immunofluorescent staining shows the cellular localization of focal adhesion kinase in ATYR0101 or control protein treated WI-38 myofibroblasts. WI-38 cells were seeded overnight before treatment with ATYR0101 or control protein in TGF-β buffer. Cells were fixed, permeabilized and stained with anti-total FAK in red and anti-phosphorylated (pY397) FAK in green.

Figure 5: ATYR0101 Reduces Fibrosis in a Bleomycin Lung Fibrosis Model



(A) In the bleomycin lung fibrosis model, mice were treated with bleomycin on day 0 and received 10mg/kg ATYR0101 or PBS via IP injection three times per week starting on day 0. Nintedanib served as the positive control. ATYR0101 treatment led to a significant reduction in **(B)** Ashcroft score, **(C)** collagen content and **(D)** the frequency of α-smooth muscle actin positive (αSMA+) cells.

Figure 6: ATYR0101 Reduces Renal Fibrosis



(A) Irreversible unilateral ureteral obstruction was performed surgically; mice were dosed with either ATYR0101 at 30mg/kg or PBS via the IP route three times a week beginning at day 0. **(B)** Kidney weights, **(C)** pathologist scoring for percent fibrosis and **(D)** cortical collagen content were evaluated.

Conclusions

- ATYR0101 demonstrates potential as a novel anti-fibrotic therapeutic candidate with a unique mechanism of action.
- ATYR0101 induces myofibroblast apoptosis via a novel binding interaction with LTBP-1 resulting in a significant reduction of fibrosis in lung and kidney models.
- ATYR0101 has significant promise as an innovative pharmacological approach to restore tissue homeostasis through depletion of pathogenic myofibroblasts.

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