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Anti-Fibrotic Activity Observed Across Preclinical Models of Pulmonary and Renal Fibrosis for a Potential Therapeutic Based on Asp-tRNA Synthetase

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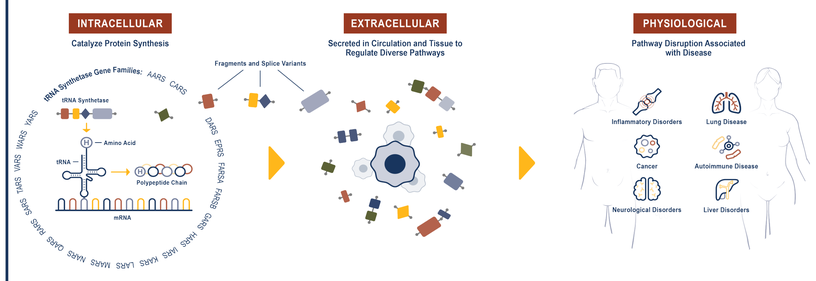
Abstract

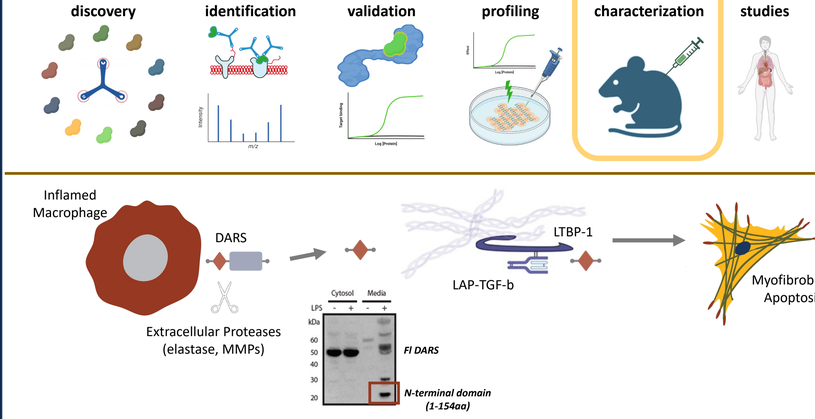
Introduction: Evolutionarily conserved domains of aminoacyl tRNA synthetases (aaRS) undergo proteolytic cleavage or alternative splicing to generate fragments with specialized extracellular signaling roles that regulate disease states. Previously, aTyr has developed one such splice variant of His-tRNA synthetase (HARS) into the drug candidate efzofitimod, which is currently in a Phase 3 clinical trial for the treatment of pulmonary sarcoidosis. 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 Asp-tRNA synthetase (DARS), which targets latent-transforming growth factor beta binding protein 1 (LTBP-1) and induces apoptosis in myofibroblasts *in vitro*. In this study we examine the therapeutic potential of ATYR0101 using *in vivo* models of pulmonary and renal fibrosis.

Results: We utilized the bleomycin (BLM) model of lung fibrosis and the unilateral ureteral obstruction (UUO) model of kidney fibrosis to examine the pharmacological activity of ATYR0101 in experimental models of fibrotic disease. LTBP-1 expression can be detected via immunohistochemistry both in naïve mouse lung and kidney tissues as well as in fibrotic foci of the lung and diseased kidney epithelia. In the lung BLM model, ATYR0101 treatment resulted in improved lung function as well as a significant reduction of Ashcroft score and collagen content, key measures of fibrosis. A pronounced reduction of myofibroblasts in ATYR0101-treated BLM lungs was also observed via immunofluorescence. In the UUO model, ATYR0101 treatment again resulted in reduced collagen content with a significant reduction of fibrosis observed by pathologist review.

Introduction

Figure 1: tRNA Synthetase Drug Discovery Platform

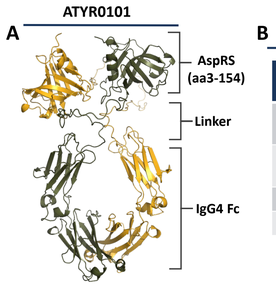




- Unique protein domains found in tRNA synthetases can be liberated via splicing or proteolysis and have been identified in extracellular spaces, where they have functions separate from their essential role in protein synthesis.
- aTyr has built an intellectual property portfolio covering >300 protein 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.
- DARS is released from inflamed macrophages and cleaved by extracellular proteases to produce a unique 154aa proteolytic fragment which can bind LTBP-1 and promote myofibroblast apoptosis.
- In this work, we show that ATYR0101 demonstrates pharmacological activity in experimental models of lung and kidney fibrosis suggesting that ATYR0101 may serve as a novel anti-fibrotic therapeutic agent with potentially broad applicability across diverse organ systems.

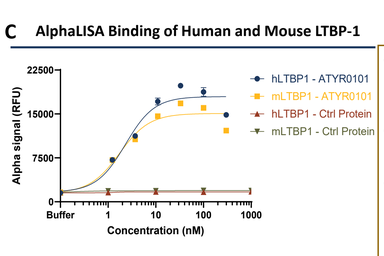
Results

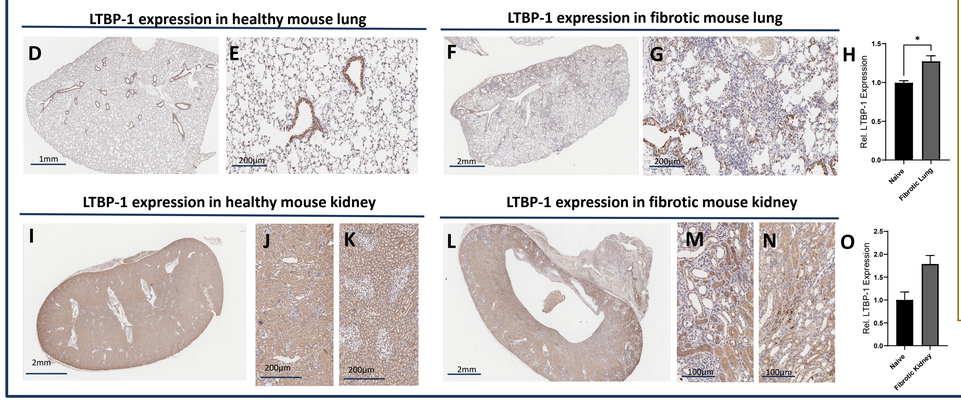
Figure 2: LTBP-1 is Expressed in Healthy and Diseased Mouse Lung and Kidney



B Human and Mouse LTBP-1 Sequence Similarity

Protein	Length (aa)	Cover (%)	Identity (%)
hDARS (aa1-154)	154	-	-
mDARS (aa1-154)	154	100	95
hLTBP1	1721	-	-
mLTBP1	1712	100	89

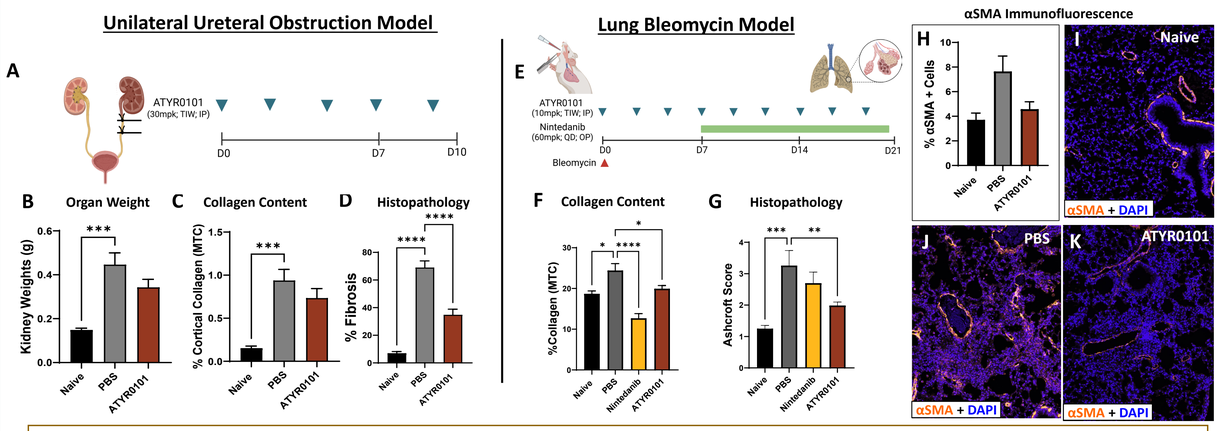




- ATYR0101 is comprised of a proteolytic fragment of Asp-tRNA synthetase (DARS), an Ala-Pro linker, and an IgG4 Fc (**A**).
- Both human and mouse DARS and LTBP-1 show high sequence similarity (**B**).
- AlphaLISA protein-protein interaction assay shows that ATYR0101 has a similar binding affinity for C-terminal fragments of human and mouse LTBP-1 (**C**).
- LTBP-1 expression can be detected in the bronchioles and alveoli of normal healthy mouse lung via IHC (**D-E**).
- LTBP-1 expression can also be detected and is elevated in fibrotic lungs from the bleomycin lung fibrosis model (**F-H**), including fibrotic foci within the lung (**G**).
- LTBP-1 is widely expressed in normal healthy mouse kidney (**I-K**), though it is absent from glomeruli in the cortex (**J**) as well as the interstitium (**K**).
- LTBP-1 is also widely expressed and elevated in fibrotic kidneys from the UUO model (**L-O**), dark granular staining can be detected in some tubules (**M**) as well as the interstitium (**N**).

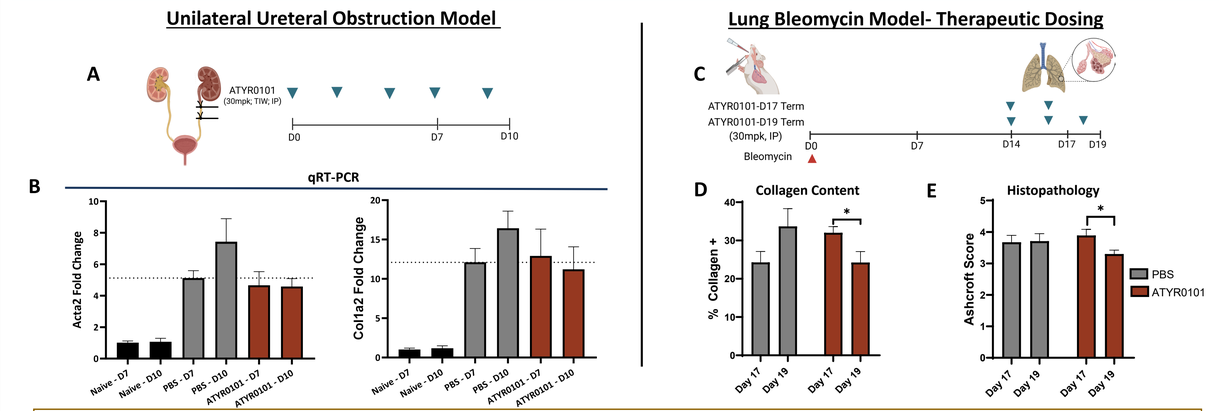
Results

Figure 3: ATYR0101 Exhibits Anti-fibrotic Activity in Models of Renal and Lung Fibrosis



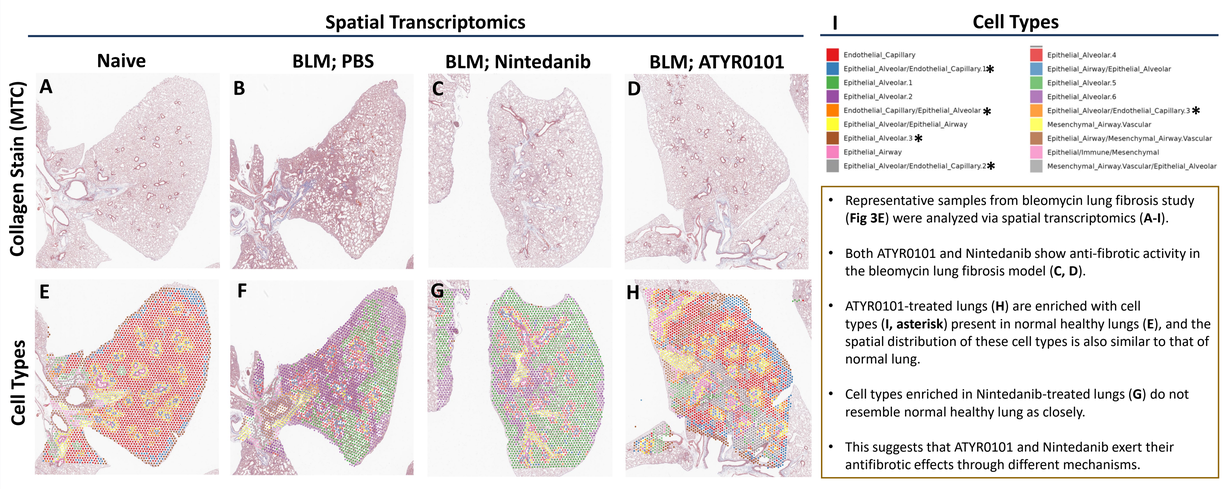
- In the mouse UUO kidney fibrosis model (**A**), ATYR0101 treatment leads to a reduction in terminal organ weight (**B**) and cortical collagen content (**C**), as well as a significant reduction in fibrosis as determined by pathologist review (**D**).
- In the mouse lung bleomycin model of fibrosis (**E**), ATYR0101 leads to a significant reduction in collagen content (**F**) and Ashcroft score (**G**).
- Immunofluorescence analysis shows that ATYR0101 treatment leads to a reduction of αSMA+ cells (**H**), this reduction is apparent in the fibrotic foci of animals treated with PBS (**J**) as compared to ATYR0101 (**K**).

Figure 4: Time Course Analysis Shows ATYR0101 Slows Progression of Fibrotic Disease



- In the mouse UUO kidney fibrosis model (**A**), ATYR0101 treatment maintains the expression of fibrosis-related genes between day 7 and day 10 whereas the PBS groups show an increase over time (**B**) indicating ATYR0101 can slow down disease progression in this model.
- When ATYR0101 is delivered therapeutically in the later myoproliferative phase of the mouse lung bleomycin model (**C**) a significant reduction of collagen content (**D**) and histopathological features (**E**) between day 17 and day 19 is observed. PBS control groups show the expected stable or progressive disease between day 17 and day 19.

Figure 5: ATYR0101 Treatment Reverts Cell Populations of the Lung Back to a Normal State



- Representative samples from bleomycin lung fibrosis study (**Fig 3E**) were analyzed via spatial transcriptomics (**A-I**).
- Both ATYR0101 and Nintedanib show anti-fibrotic activity in the bleomycin lung fibrosis model (**C, D**).
- ATYR0101-treated lungs (**H**) are enriched with cell types (**I, asterisk**) present in normal healthy lungs (**E**), and the spatial distribution of these cell types is also similar to that of normal lung.
- Cell types enriched in Nintedanib-treated lungs (**G**) do not resemble normal healthy lung as closely.
- This suggests that ATYR0101 and Nintedanib exert their antifibrotic effects through different mechanisms.

Conclusions

- ATYR0101 reduces fibrosis and fibrotic markers in models of kidney and lung fibrosis.
- The mechanism of action for ATYR0101 differs from the current standard of care in lung fibrosis.
- ATYR0101 has potential to be a novel anti-fibrotic therapeutic agent for renal and pulmonary fibrosis.

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