Identification of key extracellular binding proteins implicate role in inflammation and fibrosis for alanyl- and aspartyl-tRNA synthetases

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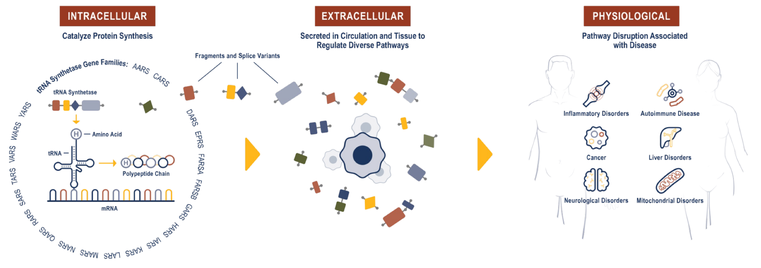
Introduction

Background: Full length and fragments of aminoacyl tRNA synthetases reach the extracellular space where they have functions that are distinct from their essential role in protein synthesis.[1,2] tRNA synthetases therefore, represent a novel set of physiologic modulators and therapeutic targets. The therapeutic potential of extracellular tRNA synthetases has recently been demonstrated with efzofitimod, a novel biologic immunomodulator composed of an Fc-fragment fused to a histidyl-tRNA synthetase (HARS) splice variant, which binds to Neuropilin-2. Efzofitimod was safe, well tolerated, and demonstrated dose related improvements on multiple measures of efficacy in a phase 1b/2a trial in pulmonary sarcoidosis, a rare fibrotic lung disease.[3]

Aim: Alanyl-tRNA synthetase (AARS) and aspartyl-tRNA synthetase (DARS) also reach the extracellular space; however, their receptor targets and biological functions remain unknown. The goal of this project was to identify and characterize the interactions of alanyl and aspartyl tRNA synthetase fragments (AARS-1 and DARS-1) with novel binding partners. Utilizing aTyr's tRNA synthetase drug discovery platform, we were able to show that AARS-1 and DARS-1 bind to the surface of human cells. Moreover, we have identified fibroblast growth factor receptor 4 (FGFR4) as a target receptor of AARS-1. This indicates that AARS may have therapeutic potential in fibrosis, inflammation or cancer.

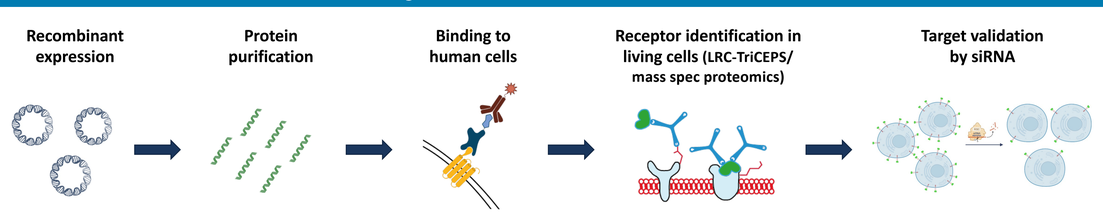
tRNA synthetase drug discovery platform



	SYNTHETASE FRAGMENT DISCOVERY	TARGET ID, VALIDATION, AND FUNCTIONAL PROFILING	PRECLINICAL CHARACTERIZATION	CLINICAL PROOF OF CONCEPT
Efzofitimod				
AARS-1				
DARS-1				
Other tRNA synthetases				

- 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.
- Recent research has further reinforced the idea that tRNA synthetases may play important roles in cellular responses to certain disease states, in particular, cellular stress and imbalances in tissue homeostasis.
- 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.
- Our tRNA synthetase platform has produced efzofitimod, an Fc-fused fragment of HARS, which recently demonstrated clinical proof of concept in fibrotic lung disease.
- In this work, we present our progress on the study and development of fragments derived from AARS and DARS.

Target identification and validation methods

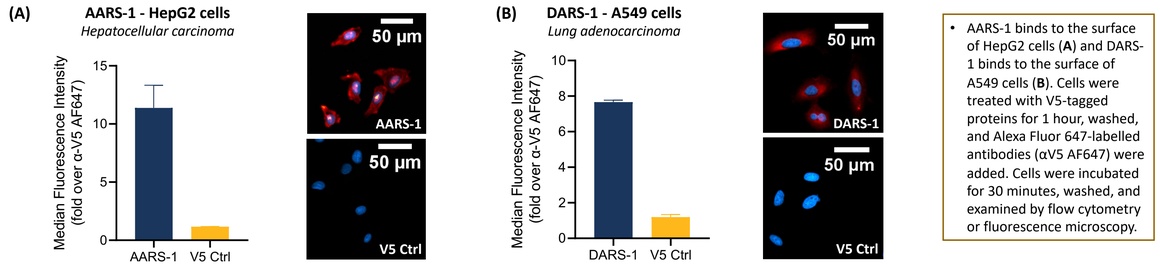


- AARS-1 and DARS-1 were expressed and purified, and binding to human cells was confirmed. Ligand receptor capture was used to identify the target receptor of AARS-1.[4] The interaction between AARS-1 and its target was confirmed by siRNA knockdown.

REFERENCES:
1. Kwon, N.H., Fox, P.L., Kim, S. Aminoacyl-tRNA synthetases as therapeutic targets. Nat Rev Drug Discov 2019.
2. Adams RA, Fernandes-Cerqueira C, Notarnicola A, Mertsching E, Xu Z, Lo WS, Ogilvie K, Chiang KP, Ampudia J, Rosengren S, Cubitt A, King DJ, Mendlein JD, Yang XL, Nangle LA, Lundberg IE, Jakobsson PJ, Schimmel P. Serum-circulating His-tRNA synthetase inhibits organ-targeted immune responses. Cell Mol Immunol 2019.
3. Sporn PHS, 2022 Safety and Efficacy ATYR1923, a Novel Immunomodulator for Pulmonary Sarcoidosis: Results of a Phase 1b/2a Randomized Placebo Controlled Trial. Am J Respir Crit Care Med 2022.
4. Dualsystems Biotech AG.

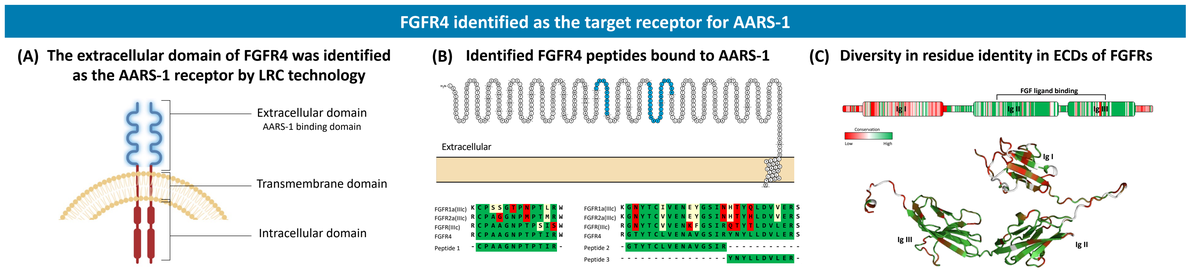
Results

AARS-1 and DARS-1 bind to human cells



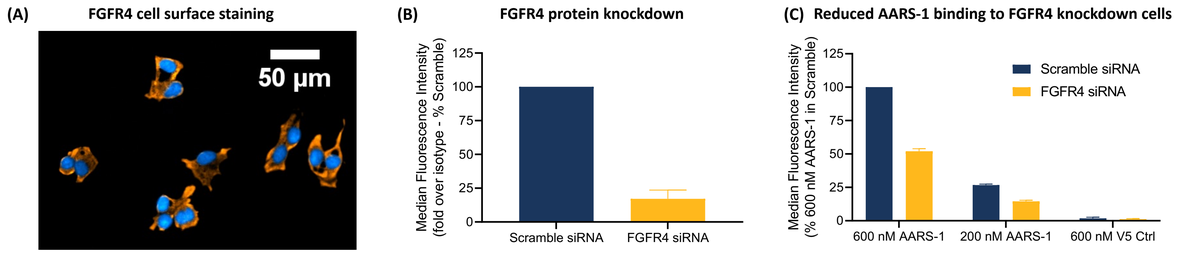
- AARS-1 binds to the surface of HepG2 cells (**A**) and DARS-1 binds to the surface of A549 cells (**B**). Cells were treated with V5-tagged proteins for 1 hour, washed, and Alexa Fluor 647-labelled antibodies (αV5 AF647) were added. Cells were incubated for 30 minutes, washed, and examined by flow cytometry or fluorescence microscopy.

FGFR4 identified as the target receptor for AARS-1



- A mass spectrometry-based receptor screen employing Dualsystems' ligand-receptor capture technology LRC-TriCEPS 3.0 reagent identified the extracellular domain (ECD) of fibroblast growth factor receptor 4 (FGFR4) as the target receptor for AARS-1 in A549 and THP-1 cells (**A**). The specific FGFR peptides identified by the screen in both cell lines are highlighted in blue (**B** - top) and are specifically derived from FGFR4's sequence versus other FGFR isoforms (**B** - bottom).
- The amino acid conservation of the FGFR ECDs are shown in (**C**), with green indicating higher and red indicating lower sequence conservation. Pairwise identity of the ECD of FGFR4 compared to that of other FGFRs range from 47-53%. The 3D structure of FGFR4 ECD shown here is predicted by AlphaFold.

siRNA knockdown of FGFR4 inhibits AARS-1 cell binding



- Immunocytochemistry assays show that FGFR4 is highly expressed on the surface of HepG2 cells (**A**).
- RNA interference experiments were performed using Integrated DNA Technologies' DsiRNA Kit and RNAiMAX Lipofectamine for transfections of HepG2 cells. Flow cytometry experiments showed a marked decrease in FGFR4 expression (**B**), which was accompanied by a decrease in AARS-1 cell binding (**C**).

Conclusions

- AARS-1 and DARS-1 bind specifically to the surface of select human cells.
- AARS-1 is found in complex with FGFR4 in A549 and THP-1 cells using ligand-receptor capture technology (LRC-TriCEPS) and mass spectrometry. Several unique peptides were identified in the extracellular domain of FGFR4.
- siRNA-induced knockdown of FGFR4 in HepG2 cells reduced the binding of AARS-1 to the surface of these cells by flow cytometry.
- The methods utilized in this study can be further employed to identify and validate new molecular targets from aTyr's tRNA synthetase platform.

Acknowledgements: This work was supported by aTyr Pharma, Inc. The authors would like to acknowledge Dualsystems Biotech for assistance with the receptor screening experiments.