Cystinosis Research Foundation Progress Report

Title: Role of nutrient sensing and mTORC1 signaling in cystinosis

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Our previous research indicates that the loss of CTNS and the accumulation of cystine lead to abnormal activation of mTORC1 in the epithelial cells of kidney tubules in mice, rats, and zebrafish, highlighting the evolutionary conservation of this signaling pathway (Berquez et al., Nature Communications 14:3994, 2023). To understand how CTNS influences mTORC1 in response to lysosomal cystine levels, we conducted co-immunoprecipitation (co-IP) assays to assess the activation state of Rag GTPases by measuring their interaction with Ragulator. We immunoprecipitated the Ragulator subunits, p18/Lamtor1 and/or p14/Lamtor2, from CtnsKO mPTCs and analysed their interaction with RagA/B and RagC/D GTPases using immunoblotting with validated antibodies. Additionally, we knocked down components of the Ragulator-Rag GTPase lysosomal scaffold (e.g., RagC/D GTPases or p14/Lamtor2) using adenovirus-mediated RNA interference (RNAi) to establish a causal link between increased Ragulator-Rag GTPase complex assembly and mTORC1 hyperactivation in CTNS-deficient mPTCs affected by cystinosis. Our studies in *Ctns*^{KO} cells suggest that increased levels of cystine enable the recruitment of components of V-ATPase and Ragulator-Rag GTPase scaffold complex that enables mTORC1 at the surface of the lysosome. Furthermore, we investigated whether modulating mTORC1 signaling pathways could rescue lysosomal defects and proximal tubule dysfunction in cystinosis cells. Short-term treatment with rapamycin, an mTORC1 inhibitor, effectively reduced mTORC1 activity, restored lysosome-directed processing of ultrafiltered low molecular weight proteins (LMWPs), and improved proximal tubule function in *ctns*^{KO} zebrafish pronephros. Similar functional improvements were observed in mouse and rat *Ctns*^{KO} cells treated with low concentrations of rapamycin.

Taken together, these proof-of-concept studies indicate that CTNS-mTORC1 couples cystine sensing to the maintenance of PT homeostasis and physiological functions, and is a targetable pathway for cystinosis.

Future work: Considering the limitations of mTORC1 inhibitors in terms of availability, specificity, toxicity, and side effects, particularly on kidney function, we will explore alternative approaches to selectively target hyperactive mTORC1 signaling in cystinosis cells and in our *ctns*^{KO} zebrafish model, which recapitulates the cystine accumulation, lysosomal/autophagy defects, and progressive proximal tubule dysfunction observed in patients. These approaches may include intermittent fasting, dietary adaptations such as caloric restriction (CR) and time-restricted feeding (TRF), low-protein diets, reduction of essential and branched-chain amino acids (including cystine), or the use of CR mimetics (e.g., spermidine, resveratrol, epigallocatechin-3-gallate, and gallic acid). The positive effects of the dietary approaches will then be validated in more advanced systems including our *Ctns* rat model.