Cystinosis Research Foundation Progress Report

Title: Role of nutrient sensing and mTORC1 signaling in cystinosis

Principal investigator: Prof. Dr. med. Olivier Devuyst

PostDoc Fellow: Dr. Marine Berquez

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Overview: This proposal aims to explore the role of nutrient sensing and mTORC1 signaling in cystinosis by utilizing disease model organisms, physiologically relevant proximal tubule (PT) cellular systems, advanced cell biology tools, and lysosome-based functional assays. *We hypothesize that the loss of cystinosin (CTNS) and cystine accumulation disrupts metabolic homeostasis and cellular differentiation by persistently activating lysosomal mTORC1 signaling*. We plan to examine how CTNS loss and cystine storage impact mTORC1 signaling in kidney tubule epithelial cells affected by cystinosis. Additionally, we will investigate whether targeting the mTORC1 pathway through dietary, genetic, and pharmacological interventions can rescue lysosomal storage-related phenotypes and proximal tubulopathy in preclinical cystinosis models (rat and zebrafish).

Final Report: Our studies using preclinical disease models (mouse, rat, zebrafish) and PT cellular systems, along with organelle-based assays, revealed that cystine-accumulating lysosomes in CTNS-deficient cells show severe proteolytic defects. This results in the accumulation of intracellular components, such as misfolded proteins and damaged mitochondria, which are typically degraded via macroautophagy. These catabolic impairments are accompanied by upregulated anabolic processes that alter PT cell differentiation and homeostasis, ultimately leading to PT dysfunction as evidenced by low-molecular-weight (LMW) proteinuria — a consistent, early sign of cystinosis.

To identify drivers of the metabolic switch induced by CTNS deficiency and cystine storage, we conducted proteomic and metabolomic profiling of cystinosis-affected PT cells. Integrated biological network analysis of differentially expressed proteins and metabolites revealed significant enrichment of pathways regulating the mTORC1 signalosome, including glucose and amino acid metabolism, mTORC1 signaling, and downstream anabolic (protein, fatty acid, and nucleotide biosynthesis) and catabolic (autophagy) processes in CTNS knockout (KO) cells. These findings were further supported by the application of an AI-based platform (PandaOmics) that ranked disease-target associations, identifying mTORC1 as a key target for restoring homeostasis and correcting tubular dysfunction downstream of CTNS loss.

Inspired by both experimental and computational evidence, we hypothesized that lysosomal cystine recycling via CTNS modulates mTORC1 signaling in PT cells. Supporting this, CTNS deficiency resulted in constitutive mTORC1 activation, even under nutrient-deprived conditions, as evidenced by persistent phosphorylation of RPS6 (p-RPS6 [Ser235/236]) and EIF4EBP1 (p-EIF4EBP1 [Ser65]). Increased mTORC1 activity was observed in PT segments of CTNS KO mouse and rat kidneys, as well as in CTNS-deficient zebrafish, demonstrating the evolutionary conservation of this pathway.

Next, we tested whether cystine storage itself maintains mTORC1 localization at the lysosomal surface. Treatment of CTNS-deficient PT cells with cysteamine, which promotes cystine export via SLC66A1/PQLC2, or exposure of wild-type cells to cystine methyl esters, which independently accumulate within lysosomes, modulated mTORC1 activation. Additionally, reintroducing wild-type CTNS, but not the transport-deficient $CTNS^{G339R}$ mutant, restored nutrient-dependent mTORC1 regulation in CTNS KO cells. These results suggest that early lysosomal and mTORC1 pathway defects caused by CTNS deficiency impair autophagy-mediated turnover of damaged mitochondria, thereby disrupting PT metabolism and epithelial cell differentiation programs.

A key question is how CTNS regulates mTORC1 activation in response to lysosomal cystine levels. In nutrient-rich conditions, a protein complex consisting of heterodimeric RRAG GTPases, Ragulator, and the V-ATPase anchors mTORC1 to the lysosomal surface, where it is activated by RHEB. Remarkably, lysosomes from CTNS-deficient cells show elevated levels of V-ATPase subunits (ATP6V0D1 and ATP6V1B2), Ragulator (LAMTOR2), and RRAGC proteins, which are normalized by reintroducing wild-type CTNS but not the G339R mutant. Co-immunoprecipitation experiments

revealed that cystine accumulation enhances CTNS interaction with V-ATPase and the Ragulator-RRAG complex, recruiting mTORC1 to the lysosomal surface.

Finally, we examined whether inhibiting hyperactive mTORC1 could restore PT cell homeostasis in cystinosis. In PT cells, *Ctns* rat kidneys, and *ctns* zebrafish with a tubular proteinuria biosensor, low-dose rapamycin treatment recovered lysosomal function and autophagy, promoting PT cell differentiation and alleviating LMW proteinuria. These findings demonstrate the potential therapeutic value of mTORC1 modulators in treating cystinosis.

These studies provide critical insights into the biological pathways underlying proximal tubulopathy and kidney disease in cystinosis. They highlight potential therapeutic targets not only for cystinosis but also for other lysosome-related disorders.

The results of this project supported by the CRF have been published in *Nature Communications** and have gained global recognition through commentaries in peer-reviewed journals and widespread press coverage. Additionally, they have been presented at prominent conferences, including the Gordon Research Conference on Lysosome and Endocytosis (2023, USA), the Gordon Conference on Organelle Channels and Transporters (2023, Spain), the LysoFor2625 Symposium (2024, Germany), the CNE International Cystinosis Conference (2024, Manchester), and the Swiss Autophagy Symposium (2024, Lausanne).

The post-doc fellow, Dr. Marine Berquez, is now at the Perera laboratory of at the University of California, San Francisco (UCSF), where she investigates the mechanisms of autophagy-lysosome activation and how this organellar system contributes to metabolic reprogramming in cancer.

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