PROJECT SUMMARY/ABSTRACT

Heterozygous mutations in *Uromodulin* (*UMOD*) cause autosomal-dominant tubulo-interstitial kidney disease (ADTKD-UMOD) which results in chronic and end-stage renal disease, and so far no specific treatment is available for these patients. In different murine and cell culture models, it has been found that *UMOD* mutations impair UMOD protein trafficking. Misfolded UMOD accumulates within the endoplasmic reticulum, resulting in apoptosis of the thick ascending limb (TAL) cells and renal fibrosis. We have established a cell culture-based assay which monitors secretion dynamics of wild-type (WT) and human mutation C150S UMOD in culture medium of stable cell lines expressing luciferase-tagged UMOD plasmids, thereby measuring a disease-relevant endpoint. Our *objective* for this proposal is to identify and chemically optimize compounds that enhance the secretion of retained mutant UMOD protein as a new therapeutic modality for treating ADTKD-UMOD. Our *hypothesis* is that increasing secretion of retained, mutant UMOD protein will reduce cellular apoptosis, and will mitigate chronic kidney disease in ADTKD-UMOD. In *preliminary data*, we screened 8,000 compounds of a subset chemical library for enhancing the secretion of C150S UMOD. We identified five hits that increased C150S UMOD secretion to at least 75% compared to WT UMOD, providing proof-of-concept for our approach. The *rationale* of this project is to identify potent compounds accelerating secretion of different UMOD mutations using a human kidney cell line to ameliorate ADTKD-UMOD. After screening our established canine C150S UMOD expressing stable cell line against the optimized over 150,000 compounds containing UTSW chemical library, we will counter-screen identified candidates against stably transfected human kidney cell lines expressing the C150S and five other human *UMOD* mutations. All of these mutations affect one of 24 disulfide bridges, which are involved in up to 60% of *UMOD* mutations. With this approach, we attempt to treat a maximum number of patients. In aim 1, we will first optimize all plate-based secondary assays including a UMOD ELISA, an assay testing for non-specific upregulation of the secretory pathway, a caspase 3/7 assay, and an assay for testing different UMOD mutations. In aim 2, we will conduct a large phenotypic HTS of the over 150,000 compounds containing UTSW chemical library and will confirm hits in triplicates. A subsequent cell toxicity assay will exclude compounds resulting in false positive hits due to UMOD release caused by significant toxicity. Secondary assays optimized in aim 1 will be performed to decrease the number of promising hits. In aim 3, the best candidates will be tested in a primary cell culture model of the TAL from WT and a mutant Umod mouse model. We will test the stimulatory effect of compounds on mutant UMOD secretion by studying current density of the calcium channel TRPV5. SAR and chemical optimization of the best hits will be studied and tested in the assays outlined in aims 1 and 3. Finally, baseline pharmacology characteristics will be evaluated. Results from these experiments will be *significant* as they may provide novel and *innovative* therapeutic options for ADTKD-UMOD patients.