CTNS mRNA as a potential treatment for nephropathic cystinosis

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Abstract Layman:

Cystinosis is a genetic lysosomal storage disease caused by mutations in the *CTNS* gene, with the kidneys as the most affected organs and the current therapy, cysteamine, not able to fully cure the disease. mRNA has revolutionized the world of molecular therapies and RNA-based therapeutics have started to emerge in the kidney field. In mRNA-based therapeutics, a synthetic mRNA is designed with a 5' cap structure and poly-A tail to protect the mRNA and improve the stability, while the untranslated regions can influence the amount of gene that is expressed. In order to reduce the risk of an immune response against the synthetic mRNA, it is further modified to include similar adaptations that the mRNA from the own cells also use to avoid the recognition by the immune receptors.

Our aim is to investigate mRNA-based gene replacement to treat cystinosis, where we introduce the healthy cystinosin protein in affected cells and tissues. For this study, we used both a cell model, the cystinotic proximal tubular cells, and a cystinotic zebrafish model. The mRNA was introduced in these models and resulted in protein expression in the cells and zebrafish. In the cells, we were able to show that cystine levels were successfully reduced for up to 2 weeks. Additionally, mRNA-based treatment resulted in the amelioration of the kidney dysfunction in the zebrafish, with both the renal Fanconi syndrome and proteinuria showing improvement.

In conclusion, our results show that mRNA-based therapy can be a viable treatment option for nephropathic cystinosis, resulting both in cystine reduction for a prolonged period of time after a single dose and amelioration of the kidney disease.

Abstract Technical:
Cystinosis is an autosomal recessive lysosomal storage disorder caused by mutations in the *CTNS* gene and the current standard treatment, cysteamine, is non curative. mRNA has revolutionized the world of molecular therapies and RNA-based therapeutics have started to emerge in the kidney field. In mRNA-based therapeutics, a synthetic mRNA is designed with a 5' cap structure and poly-A tail to protect the mRNA and improve the stability, while the untranslated regions can modulate gene expression levels. The immunogenicity of the synthetic mRNA is reduced by using sequence modifications that are also used by the host mRNA itself to avoid recognition by the immune receptors.

Our aim is to investigate mRNA-based gene replacement to treat cystinosis, where we introduce the healthy cystinosin protein in affected cells and tissues by transfection or injection of the *CTNS* mRNA. More specifically, patient derived proximal tubular epithelial cells (PTECs) and podocytes (PODOs) were transfected with synthetic 3HA-tagged *CTNS*-mRNA. Transfection efficiency and half-life were studied by means of immunofluorescence staining and cystine measurement was performed at specific timepoints. Cystinotic PTECs and PODOs showed detectable lysosomal cystinosin expression within 24h after transfection and for up to 4 days. Furthermore, transfection with 500ng/ml *CTNS-3HA* mRNA resulted in a significant reduction of cystine levels for up to 14 days in PTECs and 18 days in PODOs. Additionally, *ctns* $^{-/-}$ zebrafish embryos were injected with mCherry-tagged *CTNS*-mRNA and the kidney phenotype evaluated. Cystinosin protein expression led to significant decrease in cystine levels and reduced proteinuria. The expression of proximal tubular multi-ligand receptor megalin was increased by mRNA treatment, leading to improved proximal tubular reabsorption of a labelled low molecular weight dextran molecule (Fig.1).

In conclusion, our results show that mRNA-based therapy results in detectable cystinosin protein expression and a restoration of the cystinosis phenotype in cell and zebrafish models of cystinosis.