**Pathogenesis of cystinosis: Studies using transgenic zebrafish models**

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In order to study the pathogenesis of cystinosis and establish a model organism for drug screening, we generated a zebrafish knockout for *ctns* gene by transcription activator-like effector nucleases (TALEN) technology, which showed accumulation of cystine and impairment of lysosomal dynamics and autophagy at 5 days post-fertilization (dpf).1

**Lysosomal dysfunction and low-molecular-weight proteinuria in *ctns* knockout zebrafish**

We further analyzed the lysosomal function and morphology using transgenic reporter lines:

 *Tg(lfabp::½vdbp-mCherry)* line for assessment of low-molecular-weight (LMW) proteinuria and lysosomal processing in proximal tubule cells,2 and *Tg(PiT1::EGFP-hRAB7A)* line for analyzing the late endosome/ lysosome morphology in proximal tubule cells. Fluorescence imaging of 5 dpf-zebrafish larvae showed the accumulation of mCherry-positive vesicles in proximal tubule of *ctns*-deficient *Tg(lfabp::½vdbp-mCherry)* larvae and accumulation of EGFP-positive late endosome/lysosome in proximal tubule of *ctns*-deficient *Tg(PiT1::EGFP-hRAB7A)* larvae. These data confirm the lysosomal dysfunction in *ctns* knockout larvae at 5 days post-fertilization (dpf). Despite the lysosomal dysfunction, no ½vdbp-mCherry was detected in urine collected from *ctns*-deficient *Tg(lfabp::½vdbp-mCherry)* larvae at5 dpf, suggesting that the endocytic activity has not been affected by cystinosin-deletion at this early stage. Yet, the *ctns*-deficient *Tg(lfabp::½vdbp-mCherry)* larvae exhibited abnormal urinary excretion of ½vdbp-mCherry at 14 dpf, demonstrating that *ctns* knockout larvae developed proximal tubule dysfunction mimicking the situation observed in patients with nephropathic cystinosis.

**mTORC1 hyperactivation and dedifferentiation of proximal tubule cells in *ctns* knockout zebrafish**

Cystinosin interacts with components of vacuolar H+-ATPase/Ragulator complex, and the mTORC1 pathway was affected in immortalized proximal tubule cells derived from *Ctns-/-* mice.3 We performed phosphoproteome analysis of *ctns*wild type and knockout larvae at 14 dpf to better understand the molecular changes related to cystinosin deficiency. Two phosphoproteins of the mTORC1 signaling pathway (eef2b and rpS6) are among the top 10 proteins with most increased phosphorylation level, suggesting mTORC1 hyperactivity in *ctns* knockout larvae, confirming observations in *Ctns*-deficient mouse and rat.4 Analysis of mTORC1 signaling pathway confirmed the increased phosphorylation level of rpS6 protein in *ctns*knockout larvae at 5 dpf, persisting in *ctns*-deficient larvae at 14 dpf. Ultrastructural studies showed sporadic loss of brush border of the proximal tubule epithelial cells in *ctns* knockout larvae at 14 dpf, indicating the dedifferentiation of proximal tubule epithelial cells. Blocking of mTORC1 activity using mTORC1 inhibitors was able to rescue the LMW proteinuria in vivo. Altogether, these data demonstrate that proximal tubule dysfunction at 14 dpf is associated with mTORC1 hyperactivity and dedifferentiation of proximal tubule cells in *ctns*-deficient larvae.

The data of analyzing LMW proteinuria, mTORC1 signaling and pharmacological rescue in *ctns*-deficient larvae are included in a manuscript describing the mTORC1 hyperactivation in cystinosis – under advanced revision.4

**Rescue of LMW proteinuria by the genetic background in *ctns* knockout zebrafish**

The *ctns*-deficient *Tg(lfabp::½vdbp-mCherry)* larvae with *AB* genetic background progressively develop an abnormal urinary excretion of ½vdbp-mCherry (corresponding to LMW proteinuria), which was rescued by *TL* genetic background. A similar phenomenon was reported in the *Ctns* knockout mouse model, with the kidney phenotype being dependent upon genetic background 5. We performed studies to better understand the effect of the *AB* and *TL* background on the phenotypes related to *ctns*-deficiency. Cystine was accumulated at similar levels in *ctns* knockout larvae for both *AB* and *TL* background. The phosphorylation of rpS6 protein was also similarly increased in *ctns*-deficient larvae on the *AB* and *TL* backgrounds. These data demonstrate that the protective effect of *TL* background is not related to the difference in cystine level or in the activity of mTORC1. Comparative studies between *TL* and *AB* backgrounds are under way, with the aim to clarify the mechanism underlying the protective effect of *TL* background on kidney damage. These studies could provide novel insights into pathways relevant for the development of new therapies in cystinosis.

**References**

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