Lysosomal Enzymes in Extracellular Matrix Remodeling and Outflow Pathway Function

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**Purpose:** To investigate the role of lysosomal enzymes in the degradation of proteoglycans and structural ECM components, and their effect on outflow pathway function.

**Methods:** Degradation of proteoglycans was investigated in vivo in mice deficient in L-iduronidase (IDUA), a lysosomal enzyme responsible for the degradation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate. Degradation of structural ECM components was investigated in vitro in human trabecular meshwork cells. Intraocular pressure (IOP) was monitored using a TonoLab tonometer. Outflow facilities were evaluated by ex-vivo perfusion of mouse enucleated eyes. RGC count was performed in whole-retina flat mounts using a Brn3a antibody. Tissue morphology and ultrastructure was analyzed in plastic sections and electron micrographs, respectively. ECM degradation was visualized by in-situ zymography using DQ-labeled components, and quantified by flow cytometry. Protein expression levels were evaluated by WB and immunofluorescence. Cathepsin activities were monitored using Magic Red fluorescence substrates.

**Results:** Elevated IOP (12.34±2.6 and 15.05±3.6 mmHg), reduced outflow facility (0.0164 and 0.013 μL/min/mmHg), and decreased RGC count (1113±120 and 969±80 total RGC per 0.5mm²) was found in IDUA deficient mice (IDUA +/− and IDUA −/−, respectively) compared to age-matched C57BL/6 controls (10.85±0.5 mmHg, c= 0.023 μL/min/mmHg, 1283±148 RGC per 0.5mm²). Ultrastructural analysis showed the presence of extracellular and intracellular vacuoles in the outflow pathway region, as well as partial collapse of the Schlemm’s canal. In vitro experiments demonstrated the pericellular and intralysosomal degradation of structural ECM components associated with active cathepsin B (CTSB), which was significantly decreased in the presence of CTSB inhibitors (8.890±1.29 vs 13.596±2.7 RFU, p<0.05). Expression of IDUA and CTSB was found to be downregulated in glaucomatous TM cells compared to age-matched controls.

**Conclusions:** Our findings clearly indicate a role of the lysosomal enzymes IDUA and CTSB in ECM remodeling and outflow pathway physiology and pathophysiology, and support the use of these lysosomal enzymes as potential therapeutic targets for ocular hypertension and glaucoma. Our data additionally suggest IDUA deficient mice as a suitable animal model to study the role of GAGs in outflow pathway function and for the testing of therapeutic approaches.

**Layman Abstract (optional):** Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.