Primary cilia and aqueous humor outflow.

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Purpose

The Schlemm's canal (SC) inner wall endothelial cells (SCE) are the last barrier to the outflow of aqueous humor (AQH). SCE mechanosensor(s) detect IOP elevation-induced shear stress and stretch to increase the permeability of the SC inner wall, increasing AQH outflow. Primary cilia function as a mechanosensor in cells. Hence, we have investigated the role of primary cilia in controlling AQH outflow in SC using mice.

Methods

We immunostained mouse SC with ARL13B to identify cilia. We then quantified the morphological features of SC primary cilia. Next, we identified candidate proteins implicated in mechanotransduction in SC cilia using immunofluorescence. We also determined the physiological role of SC cilia by disrupting cilia in the adult mouse SC by knocking out the gene encoding the intraflagellar transport 88 protein (IFT88) using cre-loxP technology using mice bearing an endothelial-specific tamoxifen inducible cre (Scl-cre ERT) and the *ift88* floxed allele (*ift88* ECKO or *ift88* EChet). We also evaluated ift88 mice lacking cre as controls. We visualized cilia in *ift88* EChet and *ift88* ECKO mice. We then measured the facility to determine if aqueous humor outflow was affected in the homozygous knockout mice compared to the heterozygous mice and control mice.

Results

Our results show that the Schlemm’s canal inner wall cells have primary cilia. On average, the SC cilia were 1.19µm long, 4.7µm^3 in volume, and slightly bent with an average bending index of 1.15 (n=1358 cilia). The cilia expressed ARL13B, IFT88, and ADCY3. Tamoxifen treatment resulted in loss of cilia in *ift88* ECKO compared to *ift88* EChet. The outflow facility (C) measured in tamoxifen-treated ift88 eyes was 4.8±1.97 nl/min/mmHg (n=21, 95%CI mean: 3.63-5.75), *ift88* EChet 5.22±1.7 nl/min/mmHg (95%CImean: 4.42-6.17) and *ift88* ECKO 5.75±2.53 nl/min/mmHg (95%CImean: 4.16-6.88). The outflow facility in the *ift88* ECKO was weakly bimodal. The mean associated deviation for the *ift88* ECKO facility data was 1.9 compared to 1.5 for *ift88* and 1.03 for *ift88* EChet facility data. This increase in the spread of the outflow facility data in SC lacking cilia suggests a dysregulation of outflow.

Conclusions

Together, our results suggest that primary cilia play a role in regulating outflow. We are currently determining the effect of IOP elevation and age on the morphological characteristics of the cilia.