Quantification of TRPP2 along Lengths of Primary Cilia

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is genetic disorder that leads to renal cysts and, ultimately, renal failure. ADPKD affects12.5 million persons worldwide. A decrease in steady-state intracellular Ca^{2+} in renal epithelial cells contributes to cystogenesis. Primary cilia and their associated proteins are likely key regulators of intracellular Ca^{2+}. For instance, the ciliary Ca^{2+}-conducting channel, Transient Receptor Potential Polycystin 2 (TRPP2) interacts with polycystin-1 (PC1) such that if one or both are mutated, cysts form. Clinically, both missense and nonsense mutations in human TRPP2 have been shown to lead to ADPKD.

Hypothesis

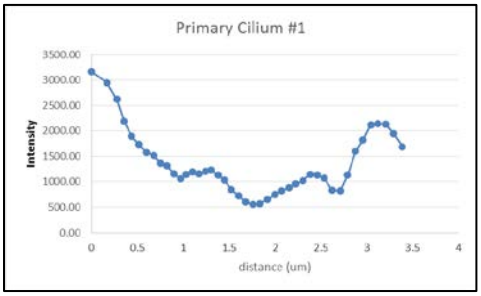
A discrepancy between the distribution and quantity of normal versus mutant TRPP2 on primary cilia is responsible for abnormal intracellular Ca^{2+} levels.

Methods

Murine renal epithelial cells were fluorescently immunostained for both acetylated tubulin, a primary cilium marker, and TRPP2. The fluorescent signals were oversampled with a confocal microscope (0.06 µm XY resolution, 0.1 µm Z step, 0.5 Airy unit pinhole, and 2x integration). The resulting 3D image stack was deconvolved (Lucy-Richardson algorithm in NIS Elements). Then, the intensity of TRPP2 staining along the lengths of the cilia were determined using 3D quantification software (Imaris).

Results

As seen in the graph, this methodology was capable of showing variation of TRPP2 staining along the length of a single primary cilium. For this particular example, we can see there is large proximal staining (at distance = 0.0 µm) and another local maximum at the distal end. This automated method will permit the quick processing of large numbers of cilia.



Conclusion

Through the development of our methodology, we have proven that immunostaining is a viable option to determine the localization of protein along a single primary cilium. This method will be used to determine how clinically relevant mutations in the TRPP2 protein contribute to deleterious alterations in intracellular Ca^{2+} levels.

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