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Genetic Analysis of Planarian Protonephridia using Fluorescent In Situ Hybridization and TUNEL

Liz Maastricht

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Liz Maastricht and Ryan King

Introduction

Polycystic Kidney Disease is one of the most common inherited genetic conditions and is often caused by dysfunction of cellular appendages called cilia. Loss of ciliary function leads to over-proliferation of kidney cells causing renal cysts that impair kidney function as well as a wide range of other disorders called ciliopathies.

The planarian (*Schmidtea mediterranea)* excretory system has cellular and molecular similarities (Figure 1) to the human kidney that could serve as a model for understanding the pathways affected in cystic kidneys[1]. They also contain cilia which are cellular appendages involved in fluid movement, sensation, and signal transduction (Figure 2A and 2B).

Previous research in the lab examined knockdown of core cilia genes, such as rootletin, which were hypothesized to negatively impact cilia structure. These knockdowns result in protonephridia dysfunction, but cilia were still present with motility and at approximately the correct length, suggesting that cilia may be required for more than generating a fluid flow within the system.

We hypothesized that, like the human kidney, cilia in the planarian protonephridia may also have a sensory function. To explore this possibility, we cloned various genes related to ciliary structure and function (Table 1) with the goal of examining their requirements in maintenance of protonephridia cell differentiation.

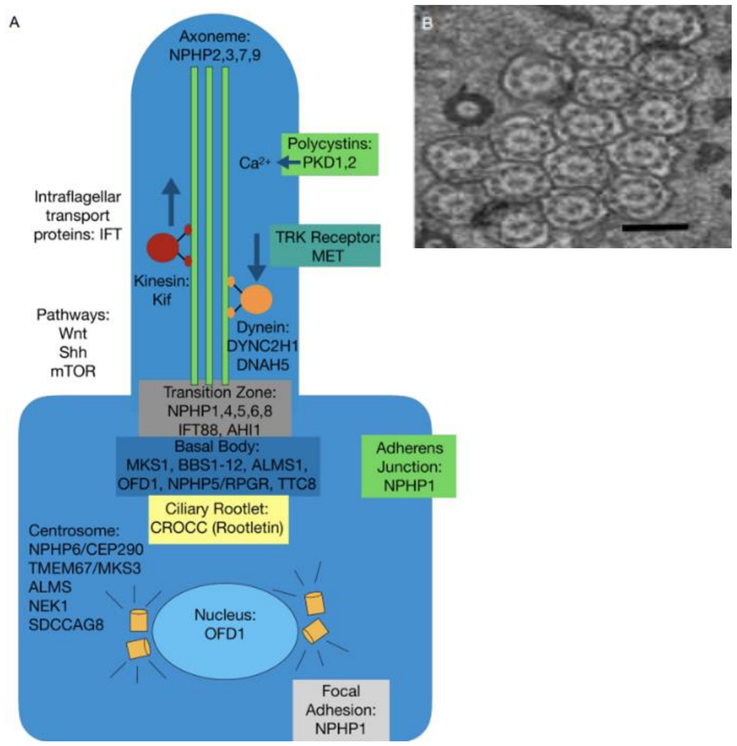

Figure 2: (A) Genes important in various aspects of cilia anatomy. (B) TEM cross-section of the 9+2 motile cilia structure, basal bodies, and cilia roots found in protonephridia.

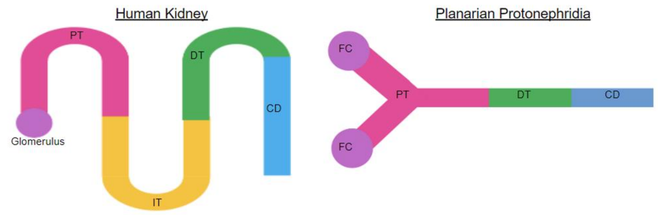

Figure 1: Homologies between Excretory Systems. Schematic representation of human kidney and planarian protonephridia. **Key:** PT-Proximal Tubule, IT-Intermediate Tubule, DT-Distal Tubule, CD-Collecting Duct, FC- Flame Cell.

Approach

- Identified planarian homologs of core cilia genes and genes related to protonephridia and kidney disease (Table 1)
- Designed primers for each gene to clone them from cDNA
- Used the cDNA clones to make dsRNA for use in RNA interference (RNAi) and knockdown of gene functions (Figure 3)
- Worked out the TUNEL protocol which marks apoptotic cells[2] (Figure 4)
- Optimized the combination of the FISH protocol[3] with TUNEL (Figure 5)

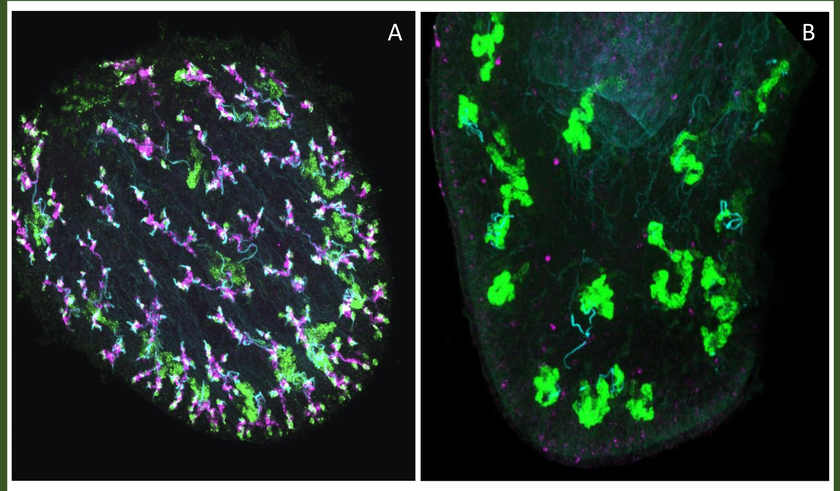

Figure 3: Fluorescent In Situ Hybridization (FISH) of a RNAi planarian. In both images, the light blue stain shows Flame Cells, green is Proximal Tubules, and pink is Proximal and Distal Tubules. (A) A negative control (53.2) which shows proper staining of the protonephridia. (B) A positive control (26) which resulted in reduced protonephridia staining.

Results

Disruption of genes that may function downstream of cilia using RNAi led to the degradation of protonephridia cells, which can be seen using FISH (Figure 3). To determine whether the protonephridia cell loss is due to the initiation of programmed cell death, we had to develop a combination of FISH and whole-mount TUNEL. We started by optimizing a whole-mount TUNEL staining protocol[2] (Figure 4). Once we had this working, we adapted it to our Fluorescent In Situ Hybridization protocol to simultaneously mark protonephridia cells and apoptotic cells (Figure 5A-D).

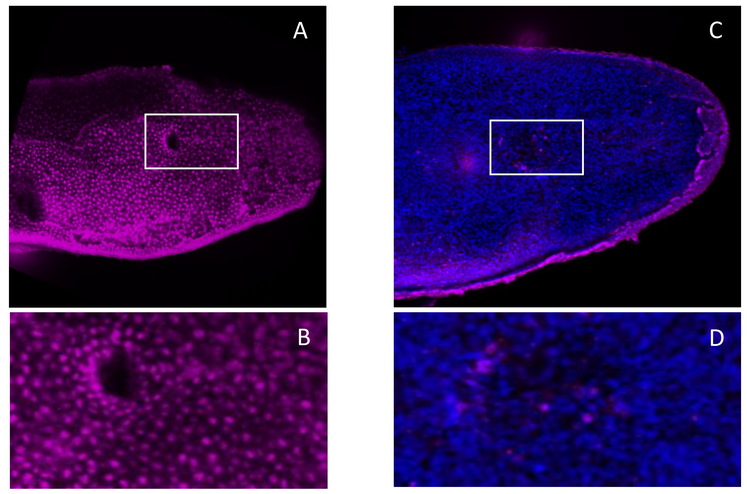

Figure 4: (A) TUNEL positive control using HCl. (B) A zoomed in portion of A. (C) TUNEL regeneration model using a poked planarian. The pink stain is showing the apoptotic cells. DAPI, the blue stain, is used as a control to stain the nuclei of cells. (D) A zoomed in portion of C.

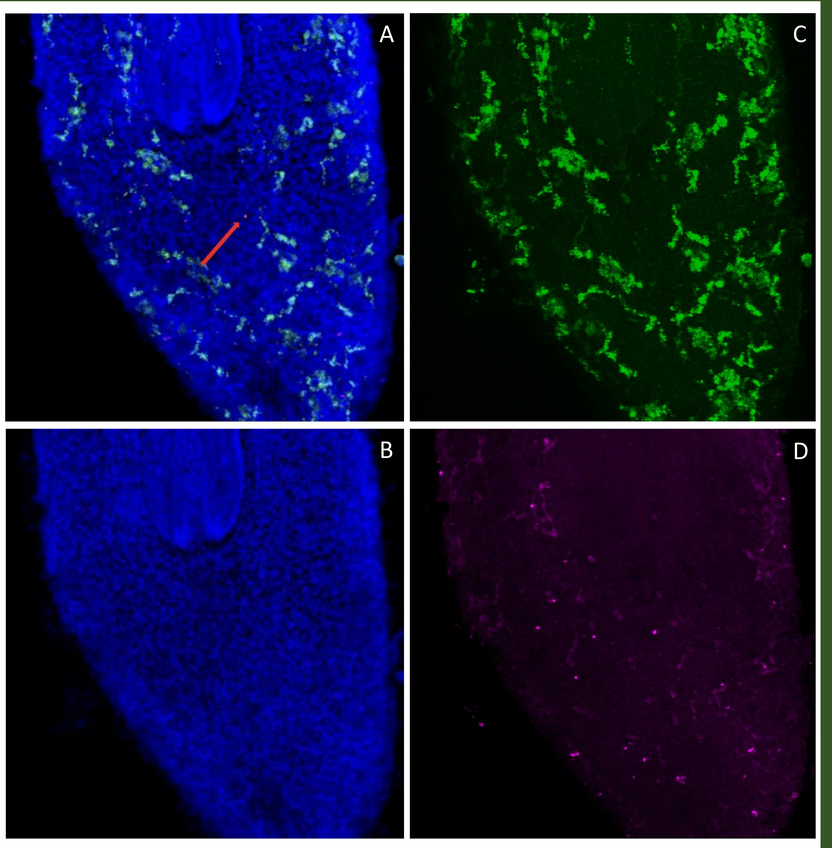

Figure 5: (A) A 26 RNAi (positive control) planarian fixed and stained using the Fluorescent In Situ Hybridization and TUNEL protocols. An example of one of the apoptotic cells are marked by a red arrow. (B) DAPI was used as a control to mark the nuclei of cells. (C) The FISH protocol used FITC probes to mark the protonephridia development of the worm. (D) The TUNEL protocol used DIG antibody to mark apoptotic cells within the planarian.

Human Genes	Planarian Homolog	Function of Gene	Association with Kidney Disease
foxJ1	foxJ1	Key transcription factor	Controls classical motile ciliogenesis
STK or MST	Hippo	Cytoplasmic kinase necessary in epidermal differentiation[4]	Causes cysts and improper kidney development[5]
YAP	Yorkie (Yki)	Key restrictive regulator for scaling in a regenerative response[6]	Loss leads to loss of kidney cells[5]
NPHS	SMED-NPHS 1-6	Adhesion molecules in the glomerular filtration barrier	Congenital Nephrotic Syndrome
KIRREL3	SMED-NEPH-3	Transmembrane protein and cell adhesion molecules	Thin Basement Membrane Nephropathy
DNAAF1	SMED-LRRC50	Stability of ciliary architecture	Primary Ciliary Dyskinesia
DNAH17	SMED-DNAHb-1	Produces a microtubule associated motor protein	Chronic Kidney Disease
RPGRIP1L	SMED-NPHP8	Negatively regulate signaling in primary cilia and ciliated cells	Nephronophthisis
IQCB1	SMED-NPHP5	Nephrocystin protein which plays a role in ciliary function	Senior-Loken Syndrome & Nephronophthisis
IFT172	SMED-ift172	Intraflagellar transport subcomplex used in ciliary maintenance	Joubert Syndrome and related disorders

Future Directions

Now that the combined FISH and TUNEL staining protocol is working, an injury timeline needs to be established in knockdowns of both core cilia genes and genes found in Table 1 to determine the best time to perform staining to identify apoptotic protonephridia cells.

References

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