"The role of the primary cilium in the cellular response to a hyperosmolal microenvironment"

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Introduction: Due to its location on the luminal surface of renal tubular epithelial cells, the primary cilium is poised to assay the osmolality of the luminal environment including its osmolality. Transient receptor potential vanilloid (TRPV) channels localize to the primary cilium of olfactory neurons in *C. elegans* and mammalian cholangiocytes, and participate in osmosensation. It is unknown whether TRPV channels participate in ciliary osmosensation of the renal microenvironment, and we sought to investigate this role in cultured renal epithelial cells.

Methods: The 176-5 renal epithelial cell line, containing a floxed Kif3a gene and a tamoxifen-inducible Cre recombinase, was cultured both in the absence of tamoxifen, allowing expression of the primary cilium, and in the presence of tamoxifen conditionally deleting Kif3a and rendering it unable to hoist primary cilia (176-5Δ cells). Expression of TRPV channels TRPV1 and TRPV4 was assayed by RT-PCR in both 176-5 and 176-5Δ cells, and cellular localization was assessed in these cells by immunofluorescence. To determine the role of TRPV channels in ciliary osmosensation, both 176-5 and 176-5Δ cells were exposed to either isoosmolal or hyperosmolal culture conditions in the presence and absence of a TRPV4 antagonist. Cell proliferation under these conditions was assessed through cell cycle analysis performed by flow cytometry, and expression of osmotic response proteins SMIT and aldose reductase under these conditions was determined by western blot analysis.

Results: Both 176-5 and 176-5Δ cells were found to express TRPV4, but not TRPV1, by RT-PCR. TRPV4 appeared to localize primarily to the cell membrane, although cilial localization could not be definitively excluded. Exposure of ciliated 176-5 cells to hyperosmolal NaCl conditions in the presence of TRPV4 antagonist led to a small increase of cells in S phase, although this was not statistically significant. Decreased expression of SMIT and aldose reductase was also noted in 176-5 cells exposed to hyperosmolal conditions in the presence of TRPV4 antagonist.

Conclusion: This data collectively suggests that TRPV4 participates in ciliary osmosensation. Further work is underway to better characterize the role of TRPV4 in the osmosensory function of the primary cilium, and how this role affects the cellular response to hyperosmolar conditions.