THE ROLE OF CILIA IN REGULATION OF APICAL ION CHANNELS ACTIVITIES IN COLLECTING DUCT CELLS

Student Researcher: Carlin D. Nelson, Burke High School
Mentor: Zhiren Zhang, PhD, Medical University, Bethesda, Maryland

Polycystic kidney disease (PKD) is a genetic disease, caused by the loss of structure/function of cilia, that results in cyst growth in the kidneys. PKD cysts can replace much of the mass of the kidneys, thereby reducing kidney function and leading to kidney failure. In the United States, about 500,000 people have PKD, and it is the fourth leading cause of kidney failure. The two main types of PKD are autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD is more common and can be inherited from either parent. On the other hand, ARPKD is a rare disorder in which both the parents must have the recessive disease gene to pass it on to their child. Since the parents each have only one copy of the disease gene, the disease is not manifested and they are referred to as carriers. For carrier parents, there is a 25% chance in each pregnancy that both copies of the disease gene will be transmitted to the baby. In ARPKD, the abnormality always involves both kidneys.

PKD not only affects humans; mice with a $Tg737^{orpk}$ gene have been found to have PKD. In these mice, the cilia (hair-like particles on the outside of a cell that allow it to move) were abnormally shorter or longer than normal cilia. Cilia move by creating a ''beating'' motion in one direction. On the return motion, the cilia are pulled back toward the cell's surface, which lets the cell move. The cilia then straighten out again and continue the motion. Each cilium is stimulated by the one in front of it, and they create ''a rowing motion'' and force movement on the cell or outside substances. It has been suggested that cilia are responsible for the fluid flow-induced increases in calcium ($[Ca^{2+}]i$) and that this increase in calcium may be mediated by members of the transient receptor potential (TRP) channels. My hypothesis was: Does a loss of cilia lead to an increase in intracellular calcium in orpk cilia(−) And, is this increase in ($[Ca^{2+}]i$) mediated by a TRP channel?

Calcium ($[Ca^{2+}]i$) ions play a central role in many cellular processes including muscle contraction, transmitter release, cell proliferation, gene transcription and cell death. TRP channels are a family of loosely related ion channels that are non-selectively permeable to cations, including calcium and magnesium. TRP channels contribute to changes in the cytosolic free Ca^{2+} concentration either by acting as calcium entry pathways in the plasma membrane or in membrane polarization. TRP Channels play an important role as multifunctional cellular sensors.

Cells were cultured on permeable supports for 6–10 days. Inside-out membrane patches were excised from the apical membrane of either orpk cilia (+) or cilia (−) cells into a calcium-containing solution. To coherently scrutinize the activity of these channels between orpk cilia (+) or cilia (−) duct cell lines originating from the $Tg737^{orpk}$ ARPKD mouse, we performed single-channel patch clamp recordings in these cells. The voltage clamp operates by negative feedback. The membrane potential amplifier measures membrane voltage and sends output to the feedback amplifier; this subtracts the membrane voltage from the command voltage, which it receives from the signal generator. This signal is amplified and output is sent into the axon via the current electrode.

Our results suggest increased activity of a non-selective cation channel at

the apical membrane in cilia (−) cells and that the activity of this channel is enhanced in these two cell types. This condition may contribute to the pathogenesis of ARPKD, such as unregulated increase in ([Ca^{2+}]i) in cilia (−) cells; these currents may be mediated by a non-selective channel, probably by an isoform of TRP family. We observed these channels in both cilium −/+ cells; however, the rate of occurrence of the channels are different between the two cell lines (64% in cilia [−] cells vs. 23% in cilia [+] cells). The rate of occurrence of the channels was significantly different between two cell types, where the channels appeared more frequently in cilia (−) cells upon application of negative pressure (78% in cilia (−) cells vs. 20% in cilia (+) cells. Moreover, the channel activities are much higher in cilium (−) cells. In conclusion, activation of these channels could lead to high intake of Calcium (Ca^{2+}) which could lead to cell proliferation and cyst formation in the kidneys.