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A NOVEL MOLECULAR MODEL OF INFLAMMATORY ACTIVATION IN GLAUCOMA

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Glaucoma is the leading cause of blindness, and the earliest treatable symptom of the disease is elevated intraocular pressure (IOP). In glaucoma, elevated intraocular pressure leads to retinal neuron death and blindness. Retinal ganglion cells (RGCs) receive visual information from photoreceptors and relay that to the brain via the optic nerve thus allowing you to see. RGC death is caused by an activation of a variety of pathways that causes the cells to be stressed and die and these cells do not regenerate. There seems to be some other mechanisms that have been activated that even after treating for IOP, still trigger RGC death. One of the proposed mechanisms by our lab is that a neuroinflammatory process is activated so there is inflammation going on in the eye. One of the candidate cells that our lab has identified is the retinal glial cells called Müller cells and they tend to activate before the onset of RGC death. By using an inflammatory marker called glial fibrillary acidic protein (GFAP), we can look at whether a cell is inflamed. From this we know that we can stimulate an inflammatory response by activating a channel inside Müller cells called TRPV4 using a drug called GSK101.

Specifically, my project looked at a channel called Piezo1 as although we know it is highly expressed in Müller cells, no one knows the function or if it is even functionally active in the retina. Piezo1 is a type of mechanosensitive channel found in Müller cells that open when responding to certain stimuli such as pressure. The structure of the channel looks like 3 propeller blades surrounding a central pore. To look at whether the channel is functionally active in Müller cells, we used a drug called Yoda1 (a synthetic compound that helps lower the threshold of activation of Piezo1 channels, so they are more likely to open) to open the channels. Piezo1 is a cation channel which means it lets cation in the cell like calcium and by using a technique called calcium imaging, we can look at whether the channel is functionally active when we stimulate it to open using Yoda1. Calcium imaging looks at the intracellular calcium in the cell and in this case, if we have an increase in intracellular calcium in the cell when we use Yoda1 to open the Piezo1 channel, it means that the channel is functionally active.

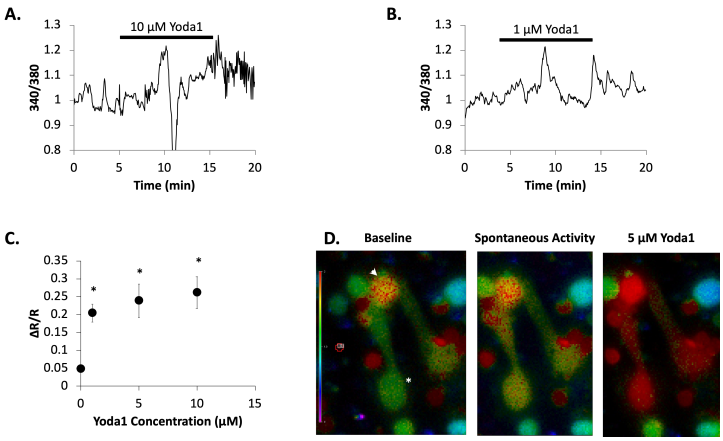
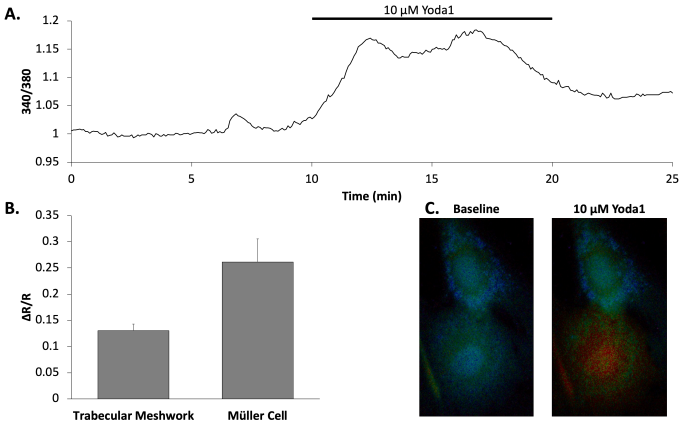


Figure 1 A-B shows a representative trace of the ratio of calcium levels in the Müller cell at different doses of the drug (10 µM and 1 µM). We can see from this that when we add the drug between 5-15 minutes, we can see a significant increase in the concentration of calcium indicating that these channels are actively functional. C shows a dose-dependent curve indicating that as we increase the concentration of the drug, we get a bigger response as more channels seem to be open. D shows what it looks like when I am calcium imaging as it shows what baseline (0-5 minutes) and 5 µM Yoda1 (5-15 minutes) look like as it is a heat map where red indicate an increase in calcium in the cells. During the experiment, there Is spontaneous activity going on in the cell even before we add the drug as channels just open and close but when we put the drug in, the response is much larger.

From the past, we knew that Piezo1 is actively functional in the trabecular meshwork (TM) cells, which is where IOP is controlled, and now we know it is also functional in Müller cells. We can compare Piezo 1 activity as they differ throughout the eye and by using the same dose of Yoda1 to activate these channels, our lab found that there is a smaller response in the TM than in Müller cells.



Overall, we found that Piezo1 channel are functionally active in Müller cells and at a range of different concentrations, in a dose dependent manner. We also knew that Piezo1 is functionally relevant in the anterior chamber but now we can say it is also functionally relevant in the retina. Some future works include looking at where on a Müller cell these channels are found and instead of activating Piezo1 in a Müller call, we can look at an intact retina instead to see if it affects other cells found in the retina. The goal is to find whether Piezo1 activation actually trigger inflammation that eventually cause RGC death.