Investigating how UV exposure activates $K^+$ channels in corneal epithelial cells using electrophysiology
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I spent the summer looking at some of the mechanisms by which ultraviolet light (UV) can damage the cells on the surface of eyes. Corneal epithelial cells flatten along the surface of the eye, and, when covered in tear fluid, form a smooth optical surface. When these cells are exposed to UVB light apoptosis (programed cell death) can occur prematurely. In past years Professor Ubels (biology) and Professor Haarsma (physics) have demonstrated that high levels of potassium found in tear fluid can help prevent apoptosis, but the mechanism by which UV triggers apoptosis is still unknown.

Professor Haarsma and I used patch clamping techniques to monitor potassium movement across the membranes of HCLE cells. Patch clamping involves the placement of a very small pipet alongside a single cell. When brief suction is applied to the pipette a seal is formed between the pipet and the cell. The pipette contains a solution which nearly matches the ionic concentrations found inside the cell along with Amphotericin-B, a chemical which pokes holes in the cell membrane. This allows electrical access to the inside and outside of the cell. With electrical control across the membrane, voltage can be run across the membrane, and any currents present indicate the movement of $K^+$ ions through their channels. More current indicates lower resistance and more open potassium ion channels.

We can confirm that the increased currents were due to opening of $K^+$ ion channels by using a blocker for $K^+$ ion channels. When a cell had UVB activated $K^+$ currents, and BDS1 (a blocker of $K^+$ ion channels) was perfused across the cell, the currents would go away. When BDS1 was washed away from the cell, the currents would return.

The opening of these $K^+$ channels due to UV light is related to cell apoptosis. If more channels open, that indicates the cell is going into apoptosis and dying because the loss of $K^+$ through UV activated channels is a necessary step in apoptosis. We used siRNA knockdowns of proteins known to be involved in apoptosis to try and figure out which proteins are involved in UVB triggered apoptosis. By looking at cells with a single protein knocked down, we are able to determine whether potassium channels still open after exposure to UVB. If they still open, that protein is not involved in the UVB activated apoptosis pathway.

We looked at FAS and FADD as potential players in the UVB activated apoptosis pathway. FAS is a well-known activator of apoptosis pathways most often activated by FAS ligand. Some studies have shown it may be activated by UVB. FADD is often activated by FAS and activates caspase enzymes that chop up proteins to trigger apoptosis. However, in our experiments cells with FAS or FADD knockdown via siRNA still had UVB activated $K^+$ currents. These currents were reversibly blocked by BDS1 indicating that FAS and FADD are not required for the opening of $K^+$ channels in UVB triggered apoptosis.

Next summer it the goal will be to test other proteins involved in apoptosis pathways to determine if they are involved in the UV activation of apoptosis. Results of FAS and FADD knockdowns will also be confirmed using ion chromatography to look at $K^+$ levels inside and outside of cells before and after UVB exposure.

This project brings together aspects of biology, biochemistry, and physics. In this way it has helped me form concrete knowledge of how the three fit together. In class subjects can be taught in an abstract vacuum. This research has shown how things work together and give concrete example of biological, biochemical, and physical principles. It has been a lot of fun to learn new techniques and gain some understanding of a complicated pathway.