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Loss of Contact Inhibition in Papillary Renal Cell Carcinoma

Papillary Renal Cell Carcinoma (pRCC) arises from mutated kidney cells that continuously grow with loss of contact inhibition and have and increased sensitivity growth signal. Contact inhibition means that once there are enough cells to form a single layer they signal to each other to stop growing. The cells attach to adjacent cells through a protein called E-cadherin. E-cadherin binds to the cytoskeleton of one cell, extends out through the membrane, and attaches to the same protein in another cell. In pRCC cancer cells this protein is lost and the cells continue to grow inappropriately. This is analogous to a car being unable to stop because it lost its brakes. Another mechanism cells use to stop growing is by shutting down responses to growth signals. MET is a protein that sits on a cell membrane and triggers the cell to grow when it receives and external signal. Normal cells realize when to stop growing and deactivate MET so the cell stops growing. The pRCC cancer cells don't deactivate MET and continue to grow. This is similar to a car with the gas pedal stuck down.

One of the main methods I used was immunoblotting. This process involves collecting a sample of cells, splitting them open, and analyzing the proteins inside. The proteins are separated by size on a gel before antibodies are used to detect the presence of particular proteins. A second antibody is added that binds to the first antibody and gives off light that can be measured. This method allows to determine if MET is activated in cells and if E-cadherin is present. One immunoblot I did shows that as normal cells grow closer together, MET is deactivated and the amount E-cadherin increases. This means normal cells bind together and stop growing. In the cancer cells, MET was always active and E-cadherin was never present.

I am working to create a system to test the relationship between MET and E-cadherin. Earlier data from the Looyenga lab suggests that E-cadherin might deactivate MET and stop cells from responding to growth signals. In order to test this hypothesis, I am working to alter cancer cells so that they express E-cadherin. DNA for two different genes is inserted into the cancer cells: E-cadherin with green fluorescent protein and antibiotic resistance. Green fluorescent protein is attached to E-cadherin as a visible marker to tell if E-cadherin is present in the cells. The antibiotic resistance gene allows me to treat the cells with antibiotic and only the ones with the altered genetics will survive. The cells with the altered genetics will only produce E-cadherin when treated with a drug which enables me to control how much is expressed in the cells. Most of the summer was spent on creating the tools to set up this system and I am still waiting for cells to grow up. Once I have enough of these altered cancer cells, I can force them to express E-cadherin and see what affect it had on MET activation and cell growth.

I am also working to stop E-cadherin in the normal cells to see if it will cause them to behave like the cancer cells. To do this, I use an enzyme to cut the E-cadherin gene out of the DNA and replace it with an antibiotic resistance gene. This allows me to select for only the cells without E-cadherin when I treat them with antibiotics. Since I've spent a lot of time setting up this system, I have only been able to try and alter cells once. This attempt was unsuccessful but I look forward to trying again soon.

Research this summer was a great experience and taught me more in ten weeks than I could ever learn in a classroom. I learned a lot about cellular genetics and cancer from Professor Looyenga and the many academic journal articles I read. This information will be beneficial to know as I hope to attend medical school after graduating from Calvin. On top of the biochemistry, I also learned how to respond to failure. Many of the steps in my experiments took several tries to achieve and some projects simply didn't work. Instead of giving up and becoming frustrated, I learned how to remain hopeful about future projects. Whether doing research or any other job, it's important to celebrate all the little victories along the way in order to stay optimistic when things don't work out.