LRRK2 and MET are two proteins that are overexpressed in Papillary Renal Cell Carcinoma. LRRK2 has also been found to be overexpressed in other solid tumor formations as well as implicated in many genetic cases of Parkinson’s disease. Studying these proteins will hopefully give insight in how to more effectively fight these cancers in the future.

Cell can bud off parts of their membranes to create endosomes or little compartments which transport things around the cell. LRRK2 is believed to be involved in the process of sorting where these endosomes travel within a cell. This is important in conjunction with the MET receptor because MET has been found to activate different signaling pathways within the cell depending on whether it is on the cell surface or on the surface of these endosomes. MET is a specific type of growth factor receptor that activates different pathways which help a cell prepare for growth and division. It activates the necessary machinery within the cell to allow it to obtain nutrients for growth and to make the necessary components to divide.

This summer, we worked primarily with one normal renal cell line and two different renal cancer cell lines. The first experiments we worked on determined the total amount of MET on the surface of these different cell lines. We used flow cytometry to measure the abundance of the MET receptor on the cancer cell lines compared to the amount on the normal kidney cells. Flow cytometry allowed us to look at live cells and get a real time read out of the amounts of MET receptor on cells as they flow through a machine one by one. The cancerous cell lines were found to have increased amounts of the MET receptor.

Experiments were also run using soft agar as a platform for cell growth in three dimensions. Three dimensional growth or tumor formation is characteristic of cancer cells. The results confirmed the two renal cancer cell lines could grow in three dimensions while the normal renal cell line could not. These experiments will be used in the future to determine what role, if any, MET has in allowing cells to grow in three dimensions.

Another experiment we performed was to look at the effects of changing the amount of MET a cell can produce. We looked at the chronic, or permanent changes of the amounts of a protein within a cell, and acute, short term changes in the amount or activity of a protein, of the receptor MET. We used shRNA methods to chronically knockdown the amount of MET in cell. The cells were then analyzed using flow cytometry and immunoblotting. Immunoblotting is a method for determining the total amount of protein and the amount of activated protein within a cell. With chronic knockdown of MET, the cells stopped proliferating and ultimately started to die off. To look at the results of acute knockdown of MET within cells, the amount of MET was decreased using siRNA. This method decreased the amount of MET on the cells but the effects of this change wear off over time as the cells divide. The acute knockdown of MET was also performed using a special drug called INCB028060 which inhibits the MET receptor from becoming activated. When the cells were analyzed using flow cytometry and immunoblotting, the activity of MET was shown to be almost completely inhibited using the siRNA and the drug INCB. However, surprisingly, the cell proliferation seemed to be unaffected by the acute knockdown of MET.

Researching under Dr. Looyenga has been an extremely rewarding experience. Dr. Looyenga brings a passion for teaching and research that is contagious and ultimately creates an exciting atmosphere which I had the pleasure of working in this summer. The professors and other students in the various labs have made this experience very enjoyable. I enjoyed being hands on in the lab and working to gain an understanding of how signaling pathways can go wrong within a cell. I hope to attend medical school after next year and this research experience has strengthened my desire to continue researching while in medical school. I am very thankful to Calvin College for providing an environment where students are able to thrive and learn from professors who are passionate and genuine in their interactions with students.