Looking at GluT1 in Osteosarcomas
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Osteosarcomas are pediatric bone cancers. They form very dense tumors, which makes it hard for oxygen to penetrate the core of the tumor. This lack of oxygen triggers stabilizes a protein called HIF1 that binds to DNA and upregulates enzymes and proteins that increase the amount of glucose in the cells, increases fermentation, and shuts down other metabolic pathways. Our area of interest is looking to see if GluT1, a glucose transporter, is expressed at higher levels in these tumors. This is interesting to find out because there is a clinical connection. There seems to be a correlation between increased levels of GluT1 in osteosarcoma and worse patient outcomes. This could be due to a variety of factors, and we are looking solely on GluT1.

To begin, I performed a variety of assays to characterize osteosarcoma and GluT1. I performed qRT-PCR (quantitative real time polymerase chain reaction) that allowed us to look at the expression of GluT1 in cells that we grew in spheres (to mimic a tumor environment), or cells grown on a flat monolayer. This works by amplifying our gene of interest (GluT1) and 'housekeeping genes" (genes that are kept at relatively constant levels in the cell), and we compare the expression of GluT1 to the genes that are kept at constant levels. From this, we found that there is an increased expression of GluT1 in the cells grown in spheres than in the single layer of cells, which is what we would expect. We reasoned that just because GluT1 is more expressed in the spheroids doesn't necessarily mean that the GluT1 is functional. To test functionality, we completed Celltiter Glo assays. For this, we add an inhibitor to GluT1 in decreasing concentrations (as to have a range), let them incubate for a set amount of time (usually 1-3 days), then add the Celltiter Glo reagent that breaks open the cells. When we read the pate of cells on a plate reader, the program looks at the amount of ATP (energy cells use), and from there, we can see how well cells are surviving under the set conditions. These results showed little difference between the monolayer of cells versus the spheroids, so we decided to run this experiment again, but this time, use glucose-only media instead of complete media. In complete media, cells have access to other nutrients to grow besides glucose, and in the glucose-only media, cells will be forced to transport and use glucose for growth. Under the glucose-only conditions, we saw in one cell line, MHOS, a dramatic decrease in cell survival, and in the other cell line, 143B, it didn't matter much. We suspect that MHOS rely much more on GluT1 to import glucose, and the 143B rely on other glucose transporters. Data from last summer agree with these findings. What we can conclude is that GluT1 expression varies between cell lines, and that there is an increase in the expression on GluT1 in spheroids than in a monolayer of cells, but this expression doesn't necessarily correlate with functionality.

This research experience has been greatly beneficial for me personally. I am interested in going into medicine as a career, and it has been amazing to conduct cancer research. I like that I get a new perspective on disease that isn't a clinical experience; it's very unique. I can use my knowledge that I have learned during classes and directly apply it to research.