**Summer 2014 Research project**

**Using the Glo-Max 96 well plate reader**

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The purpose of this project was two-fold. First, being new to research on cancer cells, I had to learn cell culture techniques. In the Krucher laboratory, several breast cancer (BC) cells are studied. They have to be grown in culture, and maintained under strict aseptic conditions. They each require a different media and growing conditions. Dr. Krucher started my training using MCF7 breast cancer cells. They were originally obtained from a BC patient who had early-stage disease. Dr. Krucher has used these cells for many years studying the proliferation and cell death (apoptosis) in these cells. Once I could maintain the cells, I learned how to count the cells and seed them in special 96 well dishes we required for the experiments. Counting cells require they be carefully mixed and spread on a hemocytometer slide and careful counting. Eventually, I expanded my cell culture knowledge to the use of Hs578T BC cells, which are metastatic and grow more quickly than the MCF7 cells.

The second goal of my research project this summer was to troubleshoot the use of the new Promega Glo-Max fluorescent/bioluminescent 96 well plate reader. It arrived in our laboratory in May, and the details of its use needed to be worked out. So I was given the project of determining the optimal assay and assay conditions for the BC cell lines to determine cell death (apoptosis) in the cells. This project has several parameters, and each had to be worked out. I had to determine the exact number of cells needed at seeding to have an appropriate number for drug dosing 24 hours later. We tried 6000, 8000, 10,000 and 12,000 cells per well. To induce cell death in the cells, we used several drugs that are known to cause apoptosis by acting as inhibitors of important growth regulating kinases (see figure legend below). In some experiments we utilized a RNA interference method (RNAi) to induce cell death (1). First we tried an assay called the CellTox Green Cytotoxicity assay which measures cell death at several intervals for ex: at 24, 48 and 72 hours by fluorescence. Unfortunately, the results from this assay were inconsistent with induction of cell death, and so we tried a bioluminescence assay called CellTiter-Glo 2.0 assay. This assay consistently gave a true reading of what we believed were the expected results. In Figure 1 we show representative results using the CellTiter-Glo assay. In both cell types, Staurosporine reduced cell number by the greatest amount. Also in both cell types, LY had little effect at the concentrations used. Differences between the cell types are interesting in that AKT inhibition does not affect Hs578T cells whereas it does by 30% in MCF7 cells. This difference may be due to the stage of BC each of these cell types are taken from: MCF7 (early) and Hs578T (metastatic). Overall, these results indicate this assay on this plate reader will be useful for future studies quantifying cell death. Dr. Krucher hopes to use this assay further to determine cell death in BC cells growing in 3D tumor models. Also, I gained valuable training in cell culture, experimental design and analysis and a view into what working in a research laboratory would be like. I hope to continue working in the research lab with Dr. Krucher through the Provost’s research program during the 2014-2015 academic year (if awarded!) or if not, as a Bio 480 Research in Biology student in Spring 2015. Thank you.

Figure 1: Effect of Apoptosis-inducing drugs on Cell Number

CT LY AKT ATM S(low) S (high) CT LY AKT ATM S (low) S (high)

Figure legend:

Cells (MCF7 or Hs578T) were plated at 12,000 cells per well and 24 hours later drugs (1mM) were administered. CT: control, LY: LY inhibitor, AKT: AKT inhibitor, ATM: ATM inhibitor, S low:Staurosporine (.25mM); S high:Staurosporine (.5mM). Cell number is shown on the vertical axis. Error bars represent the standard deviation of the mean of triplicate samples.

Reference:

(1) De Leon, G., Sherry, T and Krucher, NA, Reduced expression of PNUTS leads to activation of Rb-Phosphatase and Caspase-Mediated Apoptosis, *Cancer Biology and Therapy* 7:833-841 (2008).