Dnr GU 2021/2597

Study plan stipend Fall 2021-2022 (1 year)

To thrive and proliferate, cancer cells must counteract the oxidative stress they are constantly subjected to. In order to do so, they must either obtain antioxidants from their surrounding environment (exogenous) or upregulate their synthesis (endogenous). KEAP1 is a negative regulator of NRF2, the master transcriptional regulator of the endogenous antioxidant response.

Our lab is interested in studying the impact of the KEAP1-NRF2 pathway in cancer, with a major focus on lung cancer. Treating KRAS mutant lung adenocarcinoma (LUAD) remains a major clinical challenge given the difficulties associated with directly inhibiting the KRAS oncoprotein. Approximately 20% of KRAS mutant LUAD carry loss-of-function (LOF) mutations in KEAP1, a negative regulator of NRF2. NRF2 enables cells to maintain oxidative homeostasis by scavenging reactive oxygen species (ROS). Higher metabolic activity makes the cellular and organelle membranes, due to their high content of polyunsaturated fatty acids (PUFAs), susceptible to reactive oxygen species (ROS) damage, a process known as lipid peroxidation. Ferroptosis, a nonapoptotic, iron-dependent form of cell death is mainly activated in cells which involve genetic changes in iron homeostasis and lipid peroxidation metabolism. NRF2 has been a shown to positively regulate several genes that are relevant to ferroptosis, including those involved in GSH biosynthesis and iron homeostasis.

This research project will focus on identifying genes and pathways that modulate cell survival in response to ferroptosis modulators and understand the distinct mechanism behind it.

To answer this question, the student will, together with a doctoral student, perform, and analyze a genome wide functional CRISPR (Clustered Regularly Interspaced Short Palindrome Repeats) - associated nuclease Cas9 screen using lentiviral libraries on human A549 LUAD cells, which have KRAS and KEAP1 mutations. This will lead to the identification of genes whose loss (depleted gRNAs in the final population) would render cells more sensitive to sublethal doses of ferroptosis inducers. Top hits will be validated *in vitro* and mechanism(s) of action dissected. We will define the growth and signaling properties of cells depleted for the gene(s) of interest, determine the cells metabolic profile, antioxidant and ROS levels, and lipid peroxidation assays levels. These investigations will be performed by a variety of methods including cell culture, cytotoxicity and viability assays, effects on gene and protein expression by qPCR and western blot, and other molecular biology based methods.

At the end of the study period the student should know how to work with different *in vitro* models of lung cancer, as well as to assess cell growth, viability (by luminescent and colorimetric methods), proliferation, and to measure ROS levels, The student should also be able to set up western blot and genetic expression assays.

At the end of the study period the student should know how to plan and set up simple experiments (number of replicates, controls, biological vs technical replicates, etc.), how to report and present results (image quantification, graphical interpretation, simple statistical analysis), and how to analyze and interpret his/her own results.

Months 1-2

1. Study the effects of ferroptosis inducers on mutant cell pool cell through viability and proliferation assays
2. Optimizing the different drug concentrations for subsequent CRISPR screening

Months 2-4

1. Starting the CRISPR screen with optimized drug concentrations
2. Genomic DNA isolation from cells and library preparation to send sample for sequencing

Months 4-7

1. Bioinformatic analysis of the data generated form sequencing to identify the top genes and pathway.

Months 7-12

1. Setting up experiments and performing experiments to validate the screen hits *in vitro* using different assays.