Study plan stipend June 2022 – May 2023 (1 year)

The survival of lung cancer patients has improved as a result of targeted cancer therapies that interfere with mutated proteins that are specific for the cancer cells. However, since the majority of lung cancer patients lack targetable mutations, there is an urgent need for new therapies broadening the repertoire of targetable mutations.

Recent studies show that oxidative stress is a barrier that lung tumors must overcome to progress. These studies indicate that lung cancer cells may therefore be susceptible to pro-oxidant therapies. Cancer cells maintain redox homeostasis by several interconnected systems. Vitamins and dietary antioxidants protect cells by scavenging oxygen and nitrogen radicals. Intermediate metabolism is rewired to maximize production of nicotinamide adenine dinucleotide phosphate (NADPH) – the main reducing power source – and minimize leakage of ROS from the electron transport chain. The antioxidants thioredoxin and glutathione reverse oxidation of cysteine thiols by transferring electrons to the thiol group. This prevents unwarranted formation of disulfide bonds and is essential for protein folding and redox signaling.

Glutathione is the most abundant intracellular antioxidant. Levels of glutathione or glutamyl-cysteine ligase – the rate-limiting enzyme in glutathione biosynthesis – correlate with tumor progression and patient mortality. The glutamyl-cysteine ligase inhibitor BSO has been used in combination with melphalan in phase I clinical trials with mixed results. In a synthetic lethality screen, we have shown that BSO in combination with cancer mutations that cause WNT pathway activation leads to increased death of human lung cancer cells (manuscript in preparation). This result raises the possibility that BSO treatment kills lung tumors carrying WNT mutations in a synthetic lethal manner.

In this current project, we will identify and validate mechanisms that modulate cell survival in response to glutathione biosynthesis inhibitors in combination with WNT pathway activation.

To achieve this, the student will, together with a senior supervisor, perform and analyze a genome wide CRISPR-Cas9[a] screen using lentiviral gRNA libraries on human A549 LUAD cells. Briefly, we will culture mutant cell pools in concentrations of BSO that are lethal in combination with recombinant WNT3A but not on its own, and use WNT3A to select for mutations (knockouts) that abolish or enhance WNT-induced synthetic lethality. 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, WNT signaling, and to measure ROS levels. The student should also be able to set up western blot and genetic expression assays.

In addition, 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.

[a] CRISPR: clustered regularly interspaced short palindromic repeat; CAS9: CRISPR associated protein 9

Months 1-2

1. Validate mechanisms behind increased BSO sensitivity after stimulation of WNT signaling
2. Optimize the different drug concentrations for subsequent CRISPR screening of BSO in combination with WNT signaling agonists

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 4-12

1. Bioinformatic analysis of the data generated form sequencing to identify the top genes and pathways.
2. Setting up experiments and performing experiments to validate the screen hits *in vitro* using different assays.

Months 8-12

1. Setting up and performing experiments to validate the screen hits in mouse cancer models and patient-derived organoids

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