"Mitochondrial Dysfunction in Cancer"

Targeting mitochondrial metabolism as a cancer therapeutic approach is a rapidly evolving research area, particularly with the realization that, despite the Warburg effect, tumors remain dependent on mitochondrial respiration [1-3]. This in turn has brought the use of respiratory chain inhibitors, such as the widely used antidiabetic drug and Complex I inhibitor Metformin, to the fore as possible anti-cancer treatments [4]. However, there remain controversies over the use of Metformin as an anti-cancer agent since the drug is only taken up by the liver, causing researchers to question the mechanism of its anti-tumor action [4]. Furthermore, the use of respiratory chain inhibitors can have undesirable side-effects on normal tissues. Nevertheless, the concept of targeting mitochondria as an anti-tumor strategy remains a compelling approach. Thus, we rationalized that a screen to identify novel drugs that modulate mitophagy or mitochondrial biogenesis may yield compounds with anti-tumor efficacy given the importance of both mitophagy and biogenesis to the tumor phenotype [5,6]. With this in mind and supported by the Fletcher Scholar award, we performed a high throughput screen for drugs in libraries of existing FDA-approved compounds looking for agents that modulated mitochondrial mass, either negatively or positively. The concept of drug repurposing has gained momentum in recent years with the cost of new drug development soaring as many drugs fail at advanced stages of clinical trials [7,8]. We successfully identified several compounds that both increase and reduce mitochondrial mass. Moving forward, we will further test lead compounds from our screen, examine how they function and determine whether they can be validated as anti-cancer drugs based on their role in either mitophagy or mitochondrial biogenesis.

Results: We performed a high throughput screen for small molecules contained within the Prestwick library (1,120 compounds) and the NCI Cancer Compound library (101 compounds) to identify compounds that elicited an effect on mitochondrial mass in tumor cells engineered to stably express Mito-dsRed, a mitochondrially targeted fluorophore. This screen was initially performed with U2OS cells (a human osteosarcoma cell line) due to their large size and easily imaged mitochondrial reticulum and their ability to undergo functional mitophagy. Results were then validated in MCF7 cells representing ER-positive/luminal breast cancers and HCC38 cells representing ER-negative/basal like breast cancers. Cells were screened by robotics for changes in red fluorescence in multiple distinct areas in each well at 16 hours and at 36 hours following addition of 10 μM of each drug and cell number per well was monitored following the 36 hour imaging by staining with DAPI to count cell nuclei and measure inter-cell distance (an indirect measure of cytoplasmic volume). This time-scale has been optimized to detect robust effects on mitochondrial mass that are directly due to addition of the drug library (the 16 hour timepoint) and not secondary to adaptive responses, such as increased proliferation or cell death (36 hour timepoint). Results showed good concordance between triplicate plates. We performed a dose-response analysis for lead compounds at decreasing doses that allowed us to narrow down the compounds eliciting strong effects on mitochondrial mass.
Validated lead compounds will be pursued for their effects on tumor growth.

Future Directions. Moving forward, we will further examine effects of validate lead compound treatment on mitochondrial mass, mitophagic flux, rates of mitochondrial biogenesis, mitochondrial metabolism and function (respiration, carbon flux through the Krebs cycle, ultra-structure, membrane potential, citrate synthase activity etc), production of ROS, cell growth rate (cell death versus proliferation), as well as effects on invasion/migration of cells in classical Boyden chamber assays. *These are all approaches that we have used successfully in the past* [12-13]. We postulate that acute inhibition of mitophagy will be most effective at blocking tumor cell growth in combination with drugs such as these that induce mitochondrial dysfunction, as we described previously in a recent review of the field [6].

Summary: Our studies have focused on a high throughput drug screen used to identify compounds that modulate mitochondrial mass that could then be tested for their efficacy in tumor cell killing and extend patient survival. This is based on the growing realization that targeting mitochondria is a viable anti-cancer approach given the dysfunctional nature of mitochondria in cancer. The screen and follow-up analyses focus on agents that are different from the current emphasis on respiratory chain inhibitors that can have undesirable side effects. These studies are designed to provide important proof-of-principle results that targeting mitochondrial mass, and mitophagy specifically is a valid approach to stopping cancer cell growth. Using the data obtained from the work supported by the Fletcher Scholar award, I recently applied to the NIH/NCI for a RO1 grant that leverages the data obtained using the resources provided by the Fletcher Award and thus I hope to be able to extend the research and pursue this work to its logical conclusion.

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