Project title: Mitochondrial *Disease-in-a-Dish*

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Location: NEOMED main campus, RGE building, 3rd floor.

Project Summary: Mutations in more than 250 genes are known to cause mitochondrial disease. However, the genotype-phenotype association (connection between the mutation and the manifestation of the disease) is complicated; some genetic variants may be counteracted or be aggravated by other genes or environmental factors and result in diverse clinical progressions and outcomes. This genetic diversity complicates the employment of a single preclinical model which is incapable of representing all mitochondrial diseases. Moreover, with this genetic diversity it is likely that the treatment is not a "one size fits all" therapy. An effective treatment for one patient may not be effective in other patients—even those with similar symptoms. Often this complexity, and ineffective treatment does not slow the progression of the disease leading rapid deterioration and the inability of the physician to try another treatment.

This research project focuses on the creation and the validation of *Disease-in-a-Dish* model that is tailored to simulate the complexity of individual patients with mitochondrial disease. Blood samples from patients are retrieved from our collaborator, Dr. Bruce Cohen, in the Akron Children's Hospital. After harvesting the white blood cells (with nuclei), these nucleated blood cells are reprogramed into induced Pluripotent Stem (iPS) cells for rapid proliferation. Using specific factors and conditions, the iPS cells are differentiated into Cardiomyocytes (CMs), a cell type of high energetic metabolism, which renders it ideal to study the characteristics of the mitochondrial dysfunction, e.g., excessive reactive oxygen species production, inadequate ATP production, and also devise a pharmacological and nutritional therapy to optimize mitochondrial function in each patient. A cocktail of metabolic substrates and drugs can be empirically determined to better mitochondrial function of the particular iPS-CMs. We believe this iPS cell-based platform represents a confluence of evidence-based and precision medicine and will provide in new direction in treating patients with mitochondrial diseases.

The goal is to answer the clinically relevant question: Are iPS-CMs a suitable model to study mitochondrial disease, serving a surrogate for patients with the affliction? The overreaching goal is to tailor the best cocktail for each patient.

Research Methods

The blood samples from de-identified patients and healthy donors have been retrieved and preserved with IRB approval. The summer project involves four different modules with each requiring a different approach. (1) Cellular reprogramming and differentiation, (2) Immuno-phenotyping of cell types. (3) Study of mitochondrial function, and (4) Study

of nutritional / pharmacological interventions and followed by statistical analysis. It should be noted that it will take more than two months to complete all four modules for one patient. Therefore, we employ a “distributed multithread” approach to break down the task. Multiple cell lines in the pipeline are concurrently running through different modules. Summer students will learn to master cell culture technique and can choose to engage one or more modules according to personal preference.

(1) Cellular reprogramming and differentiation: Peripheral Blood Mononuclear Cells (PBMCs) will be cultured and reprogrammed into induced Pluripotent Stem (iPS) cells and differentiated into beating Cardiomyocytes (CMs) by cell culture technique on Class II biological safety cabinet and in hypoxia chamber.
(2) Immuno-phenotyping of cell types: Cultured cell sample prepared on a glass slide will be immuno-labeled with iPS markers (Oct4 or SOX2) or CMs markers (cardiac Troponin T or Sarcomeric Alpha Actinin) and detected by fluorescence microscope.
(3) Mitochondrial function: Disease and healthy control of iPS and CMs will be compared by Seahorse Bioenergetics Flux analyzer. These measurements allow us to obtain basal oxygen consumption, maximum oxygen consumption, and oxygen consumption due to ATP production.
(4) The substrate utilization screening of surviving cells will be monitored by light absorbance on Synergy 4 microplate reader. The same microplate reader will also be used to capture fluorescence signals of the reactive oxygen species (ROS) production after different drug interventions. *In 2023 summer, we will be focusing on metabolomics/lipidomic analysis of CMs, to identify accumulated toxic metabolites as well as deficit energetic molecule in disease model, and to investigate if therapeutic agents can restore the unbalanced disease phenotype.*

Students can work in a team to learn basic lab technique, utilize research instruments, and design experiment to find out the best nutritional-pharmacological solution for CMs derived from each patient. The findings may make a difference and directly benefit the donor patient.

Student Fellow Training/Mentoring Plan

Student fellows will be working with experienced lab technicians and Dr. Kang for individually paced guidance. The student is required to attend weekly Heart and Blood Vessels Diseases group meeting as well as meet with Dr. Kang and Chilian, attend and present at a weekly journal club, and attend IMS department seminars. The student is also encouraged to present research findings during the cardiovascular group meetings. Dr. Kang will work with the student in the preparation of oral presentation and the poster session. In addition to this summer research fellowship program's poster day in NEOMED, a student fellow can also submit the abstract to the annual symposium of United Mitochondrial Disease Foundation (UMDF) Mitochondrial Medicine and can present the research discoveries during this event.