UROP Proposal

Title of Proposal

The role of Fibroblast Growth Factor 21 (FGF21) in Mitochondrial Disorders (MDs)

Problem/Topic of Research or Creative Work

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the leading causes of death in the world. Consequently, medical device companies invest a large amount of money in building devices that would sustain patients' lives. Targeted treatments have the potential to improve outcomes, but additional specific biomarkers need to be developed. CVDs often feature mitochondrial dysfunction, which in heart failure is associated with mitochondrial DNA damage and deletions. One biomarker associated with mitochondrial stress responses is fibroblast growth factors 21 (FGF21). High levels of FGF21 were detected in children with mitochondrial mutation induced mitochondrial dysfunctions. Similarly, in dilated cardiomyopathy (DCM), the most common type of heart failure, mitochondrial dysfunction is associated with mitochondrial DNA damage and deletion. According to Ahuja et al., mitochondrial DNA damage in DCM are resulted from oxidative stress during progression of the disease. Dr. [redacted]'s lab studies how cardiac metabolism is altered in heart failure. As such, the proposed research is interested in investigating how to determine when cardiac muscle cells (cardiomyocytes) are under metabolic stress. Based on what is known about FGF21 in patients with mitochondrial mutation induced mitochondrial dysfunction, we hypothesize that the level of FGF21 will serve as a biomarker of metabolic stress related heart failure.

The second part of the project investigates the pathway that leads to the production and effects of FGF21 during heart failure. The levels of serum FGF21 measured in our lab were higher than what has been determined by other labs, leading us to believe other organs, besides the heart, might be producing FGF21 in response to oxidative stress in cardiomyocytes. Therefore, we hypothesize that when the heart is under stress, it signals other organs to produce FGF21. As such, the proposed research will investigate which organs are secreting FGF21 in heart failure patients. Furthermore, the research will also investigate the pathway leading to effects.

Relevant Background/Literature Review

FGF21 is a subfamily of fibroblast growth factor proteins responsible for regulating metabolism. It plays a role in controlling energy homeostasis, lipid metabolism, and glucose regulation. For the longest time, it was believed that the liver, adipose tissue, and skeletal muscle were the only production sites of FGF21. However, recent studies have indicated FGF21 may also be a cardiomyokine (CMK), a protein secreted by the heart that acts in auto/para/endocrine manner to affect cardiovascular function. Multiple studies have shown that FGF21 provides cardioprotective effects in response to cardiac and oxidative stress, as well as diabetes. In cardiomyocytes, secreted FGF21 activates the MAPK/ERK pathway that results in the activation of antioxidant genes, which have the ability to protect cells from oxidative damage. Furthermore, FGF21 regulates energy supply in the heart by regulating myocardium PGC-alpha to promote fatty acid β oxidation (FAO), which is the major source of energy for cardiomyocytes (Tanajak, et al., 2015). However, the origin and the pathway of FGF21 and its numerous effects in the maintenance of homeostasis in heart failure is still to be determined.

A 2013 study by Ahuja et al. summarizes the link between oxidative stress, mitochondrial dysfunction, and mitochondrial mutation in heart failure. It was suggested that mitochondrial

dysfunctions leads to reduced ATP production and progression of left ventricular failure. During left ventricular failure, cardiomyocytes go under oxidative stress, leading to mitochondrial mutation. Furthermore, a study by Lehtonen et al. provides evidence that show serum FGF21 could be used as a biomarker for muscle-manifesting mitochondrial myopathy (MM), such as: defects affecting mitochondrial translation (675 pg/mL), mitochondrial DNA (mtDNA) deletion (347 pg/mL), and respiratory chain (RC) subunits/assembly mutations. The serum/plasma levels of FGF21 were measured using ELISA kit and the gene expression was assessed using quantitative polymerase chain reaction (qt-PCR). This study also investigated FGF21's ability to determine disease progression. It was noted that FGF21 was directly related to ejection fraction, showing that FGF21 is induced during heart failure.

Although other studies have measured levels of FGF21, there has not been a study indicating the link between metabolic stress and elevated FGF21 levels in DCM. This research forms a link between what has been discovered about FGF21 in children with mitochondrial dysfunction to establish the use of FGF21 as a biomarker in determining disease progression in heart failure patients.

Specific Activities to be Undertaken and Timeframe for Each Activity

To induce heart failure, transverse aortic constriction (TAC) combined with coronary artery ligation was performed in mouse models. This procedure involves partial occlusion of the transverse aorta leading to myocardial infarction along the anterior wall of the left ventricle. Ultimately, the pressure overload and dysfunctional contraction led to development of heart failure. After performing this procedure, tissue samples were collected from 11 different organs and marked as experimental group. Similarly, a control group was created from mice that did not undergo the TAC procedure. Therefore, at the beginning of this semester (Spring 2019), I was given a total of 88 tissue samples (experiment and control groups) to analyze. I have managed to homogenize the tissue samples and isolate RNA from each sample. The isolated single-stranded RNA was then used to synthesize a complementary DNA (cDNA) via reverse transcription polymerase chain reaction (RT-PCR). RT-PCR was then followed by quantitative PCR (qPCR), which involves fluorescent labeling to monitor the quantification of amplified DNA during each cycle. Quantitative PCR is used to quantify gene expression and isolate the production of FGF21 in various organs in the body. Since qPCR does not provide information beyond gene expression, Protein expression will be investigated to determine the pathway of FGF21 (origin of production and effect), throughout summer 2019. Therefore, the plan for summer 2019 is as follows:

- 5/6/2019-5/31/2019: Finish qPCR experiments and analyze the data.
  - Conduct literature reviews to obtain abstracts similar to the research conducted.
  - Perform data comparisons with other labs that have conducted similar experiments.
  - Update the lab on whether or not the qPCR data supports the hypothesis.

- 6/3/2019-6/21/2019: Learn how to perform Western blots, to investigate protein expression in the experimental and control groups.

- 6/24/2019-8/2/2019: Perform Western blots on the 88 samples obtained from various organs of experiment and control groups. This helps support the qPCR data.

- 8/1/2019-8/30/2019: Analyze data from western blots.

○ If a mistake has been made during any of the procedures, use this time to redo the tests and analyze the data.
○ Draw conclusion based on the combination of the qPCR data and protein expression analysis.
○ Present findings to the lab.

In addition to performing experiments outlined above, I will also be attending weekly lab meetings and presenting my research findings to the lab at least twice (June 2019 and August 2019) during the summer.

Relationship of the Proposed Work to the Expertise of the Faculty Mentor

Dr. [redacted] is an assistant professor of internal medicine at the University of Utah School of Medicine. He received his MD and PhD from John Hopkins University and completed his training in Internal Medicine and Cardiovascular Diseases. Dr. [redacted] joined the [redacted] in 2016. He was a recipient of the Gilead Sciences Research Scholars Program and an R01 from the National Heart Lung and Blood Institute of National Institutes of Health (NIH) in 2017. His research focuses on how mitochondrial dysfunction in the heart leads to cardiomyopathy. The use of FGF21 to measure cardiac metabolic stress in human heart failure was established within the lab shortly before I joined. Members of the lab have quantified FGF21 levels in human hearts and serum, and the finding that these are elevated prompted my proposal, as we aim to see where the FGF21 is being made.

Dr. [redacted] has over 12 years of experience in this field and has published over 10 scientific articles. In addition, he has been mentoring undergraduate and graduate students throughout his time in CVRTI. Currently, he serves as my mentor in my BIOEN 4990(Bioengineering Research or Internship I) class, a pre-bioengineering thesis writing class. His willingness to share his knowledge and expertise, provide constructive criticism, and regulate my progress makes him an ideal mentor.

Relationship of the Proposed Work to Student's Future Goals

I am currently in my junior year of college studying biomedical engineering (BME) with a biomaterial emphasis. In addition to full time enrollment, I also work as a Research and Development (R&D) intern in one of the world's leading medical device companies, BD Medical. As an R&D intern, my work involves manufacturing and testing central venous access devices, which are used for diagnostic/therapeutic purposes in a clinical setting.

Designing medical devices is not only about building great products, but also identifying and addressing patient needs. Working at BD Medical allows me to use my skills to produce devices that are biocompatible and safe to use. In doing research however, I am able to directly impact the quality of patient testing in clinical laboratories. As previously stated, the goal of the proposed research is to find a biomarker that will allow clinicians to determine disease progression in heart failure patients via non-invasive methods. By conducting molecular studies in animals, we are able to help delineate those patient who are most likely to receive benefit from a treatment. As such, in the future I would like to combine my interest in medical device development with the research topic at hand to further advance personalized medicine.

References

Ahuja, Preeti et al. "Divergent mitochondrial biogenesis responses in human cardiomyopathy" Circulation, vol. 127, no. 19, 2013.

Bhatti, Jasvinder Singh et al. "Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies" Biochimica et biophysica acta. Molecular basis of disease, vol. 1863, no. 5, 2016.

Jung, Tae Woo et al. "Implication of hepatokines in metabolic disorders and cardiovascular diseases" BBclinical, vol. 5, no. 5, 2016.

Lehtonen, Jenni M et al. "FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders" Neurology vol. 87, no. 22, 2016.

Tanajak, Pongpan, et al. "Effects of Fibroblast Growth Factor 21 on the Heart." Journal of Endocrinology, vol. 227, no. 2, 2015.