Optical Imaging of Cardiac Mitochondrial Dysfunction in Transgenic Mice Model

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Introduction

❑ Motivation

- Heart failure is a multifactorial degenerative disease.
- It has been considered the final common pathway of several acute and chronic diseases such as:
  - Myocardial infarction
  - Diabetes
  - Obesity
  - Hypertension
- Despite the advances in treatments, more than 30 million people worldwide suffer from heart failure.

❑ Objective

- Hence it is important to understand intrinsic mechanisms responsible for the development of heart failure.
- This study focuses on identifying an intracellular target that can be responsible for the development of the heart failure.

❑ Background

- Mitochondria, the powerhouse of the cell, are considered an important node involving in the degenerative diseases (Figure 1) [1].
- They are cellular organelles that generate most of the chemical energy needed to power the cells.

Figure 1- Schematic of cell, mitochondria and the electron transport chain [2]

- This study utilizes a fluorescence cryo-imager to quantitatively assess the effect of the mitochondrial dysfunction in metabolic state.
- This approach quantifies mitochondrial bioenergetics state by imaging two auto fluorescent coenzymes in the mitochondria electron transport chain (Figure 2):
  - Reduced Nicotinamide Adenine Dinucleotide (NADH)
  - Oxidized Flavin Adenine Dinucleotide (FAD)

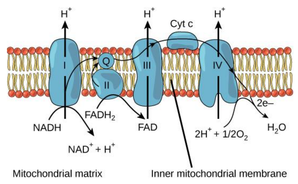

Figure 2- Mitochondrial electron transport chain [3]

Methodology

- 3D fluorescence Cryo-imager (Figure 3) is a custom designed device that enables us to image six different channels simultaneously.
- In this study we image two channels: NADH and FAD
- The sample is kept inside a freezer and cut with a blade with 25 μm thickness.
- After each cut, the sample is excited by white light passing through the excitation filters.
- Excitation wavelengths:
  - NADH (350nm ± 40 nm) , FAD (437nm ±10nm)
- The emitted light coming from the tissue is filtered by emission filters and imaged by a camera.
- Emission wavelengths:
  - NADH (460nm ± 25nm), FAD (537nm ± 25nm)
- This device designed in a way to preserve the metabolic state of tissue at the time of freezing [4].
- It provides 3D volumetric information of redox ratio (NADH/FAD) by stacking all the slices in z direction [5].

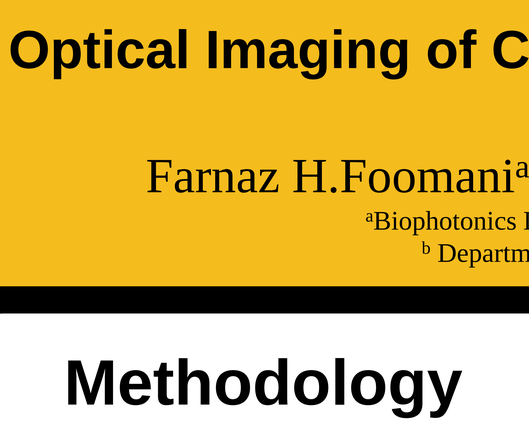

Figure 3- Schematic of the Cryo fluorescence imager

❑ Animal Model

- Transgenic mice with mutations of mitochondrial DNA would provide a model to study genetic and acquired forms of human mitochondrial diseases[6].
- We use transgenic mice as a our rodent model. These mice were modified in a way to model mitochondrial dysfunctionality with mild and severe condition.
- **Animal groups:**
  - Control (n = 6)
  - Mild (n = 14)
  - Severe (n = 11)

Results

- Figure 4 represents 3D volumetric NADH, FAD and redox ratio of three representative samples.
- Redox ratio is decreasing (blue color) in the severe group significantly (p-value = 5.56e-7 < 0.05).
- Redox ratio is decreasing in the mild group but less than the severe group.
- Lower redox ratio could cause more damage to DNA.

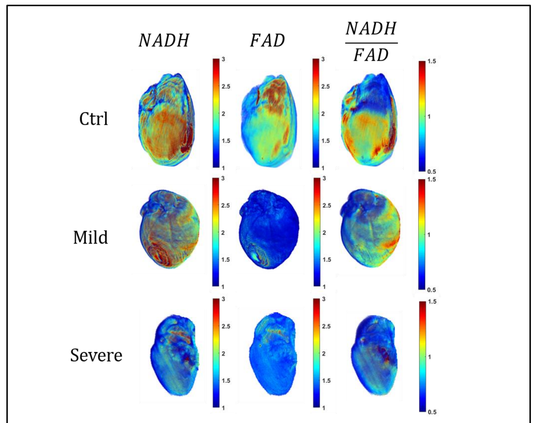

Figure 4- 3D representative data of NADH, FAD and redox ratio for Control, Mild and Severe group

- Figure 5 shows the representative redox ratio histogram distribution in the three groups.
- Due to the damage to the mitochondrial, the redox ratio decreases by 31.9% in severe group and by 12.02% in the mild group.

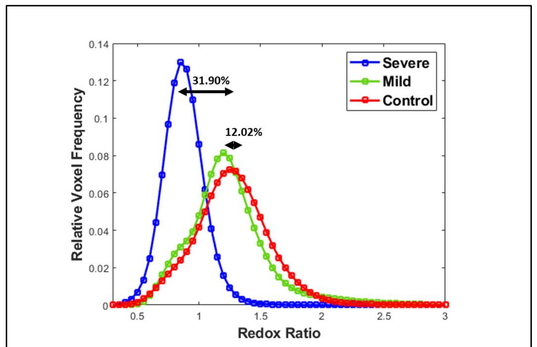

Figure 5- Representative histograms of the redox ratio voxel intensity

Conclusions

- We used novel transgenic mice to model genetic and acquired forms of the human diseases. .
- Mitochondrial redox state was successfully monitored and quantitively assessed the mitochondrial functionality.
- The significant decrease in the redox ratio suggests that any level of the mitochondrial dysfunction alters bioenergetics and energy metabolism of the heart.
- The alternation in energy production level can lead to the development of the heart diseases.
- Mitochondria redox state could be a promising therapeutic targets for the prevention of metabolic diseases.
- This study may help to develop the therapeutic strategies to reduce mitochondrial dysfunction to maintain mitochondrial quality.

ongoing study

- Our future direction is to study mitochondrial disintegrational development.
- Longitudinal study on mitochondrial dysfunctionality may show us the quality of the functionality or the growth of the dysfunctionality in mitochondria.

References

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