Oxygen Consumption and ATP Production in Neonatal Mouse Cardiomyocytes

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Introduction

Cardiomyocytes are the contractile muscle cells of the heart. A key developmental feature of mammalian cardiomyocytes is their terminal differentiation early in life and inability to proliferate following myocardial injury. Cardiomyocyte function is directly correlated to cellular metabolism and mitochondrial respiration, and more specifically ATP production. A small amount of ATP is made in the cytosol through glycolysis while the majority of ATP necessary to power cells is made through oxidative phosphorylation in the mitochondria. In the cytosol, ATP production occurs via substrate level phosphorylation. In the mitochondria, the generation of ATP occurs through a series of oxidative reactions to generate a proton motive force that drives the flow of protons into the matrix through ATP synthase, Complex V, of the electron transport chain (ETC). Understanding the dynamic changes in cellular respiration and ATP production in the cytosol and mitochondria could allow for key insights into how the cardiomyocyte is functioning. To do this, our lab uses a continuous oxygen monitoring system and a novel genetically encoded ATP indicator (smacATPi) that can be transduced into cells for visualization of the real time changes in ATP production (Figure 1).

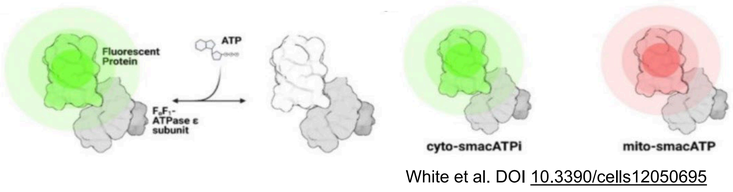

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Figure 1 – Genetically encoded dual ATP indicator smacATPi.

Hypothesis

The dual cellular ATP indicator and continuous oxygen monitoring sensor will analyze the mitochondrial respiration changes in early cardiomyocytes.

Methods

To test the hypothesis, this study used isolated neonatal mouse cardiomyocytes (NMCMs). Following isolation, the cells were cultured for around 7 days (Figure 2). A novel genetically encoded fluorescent ATP indicator of cytosolic and mitochondrial ATP (smacATPi) was transduced in the cultured cardiomyocytes to monitor the dynamic changes of ATP in response to metabolic inhibitors. Following transduction, cells were imaged using the Cytation 5 live cell imager, and the drug 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor, was applied to the cells. Continuous recording of oxygen consumption of the cardiomyocytes was achieved using a Resipher cultured cell oxygen sensing system.

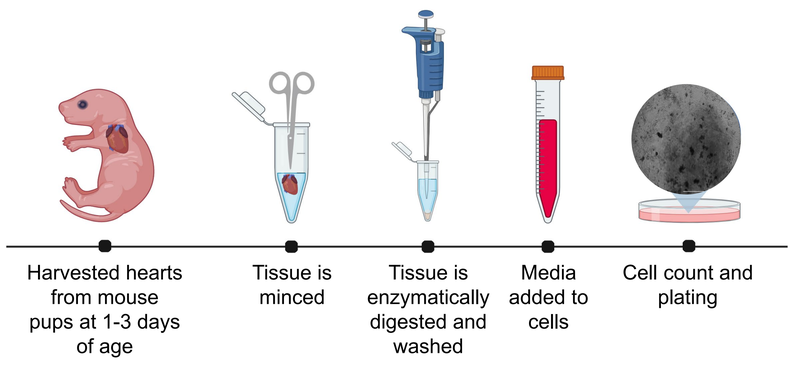

Figure 2 – Schematic for neonatal cardiomyocyte isolation.

Data

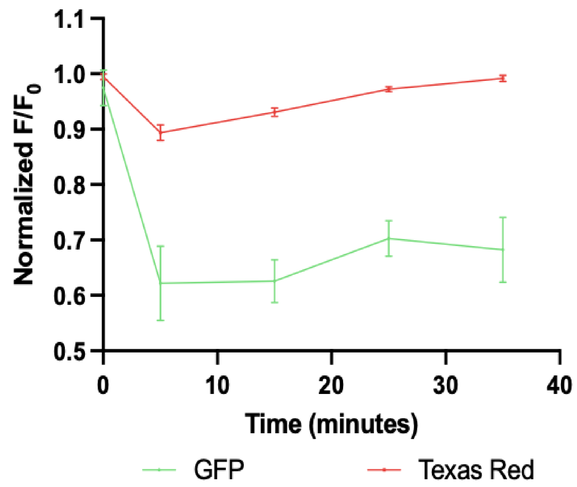

Figure 3 – Normalized fluorescence change for both GFP and Texas Red following drug treatment with 50mM 2DG at 0 minutes.

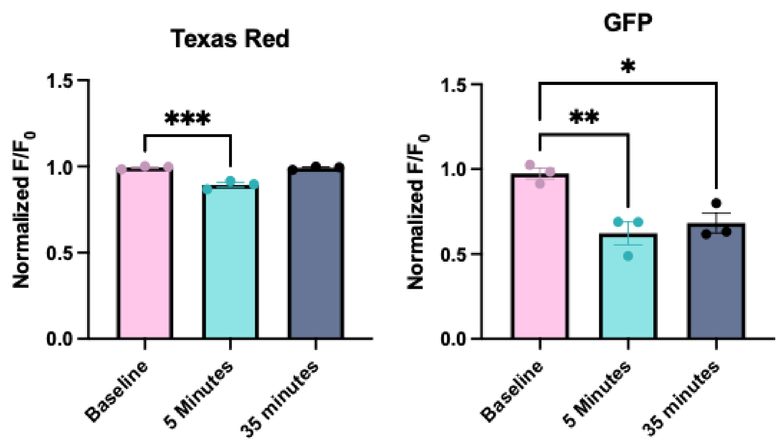

Figure 4 – Normalized fluorescence intensity following 50mM 2DG exposure at 5 minutes and 35 minutes for cytosolic GFP and mitochondrial Texas Red.

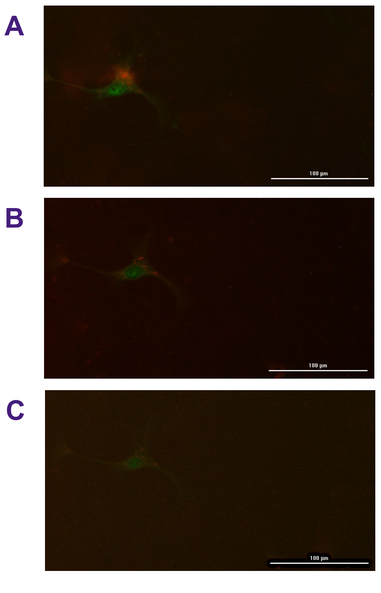

Figure 5 – 20x fluorescence images of NMCMs transduced with smacATPi. A) Baseline image prior to drug treatment. B) Image taken 5 minutes after drug treatment with 50mM 2DG. C) Image taken 35 minutes after drug treatment with 50mM 2DG.

Data

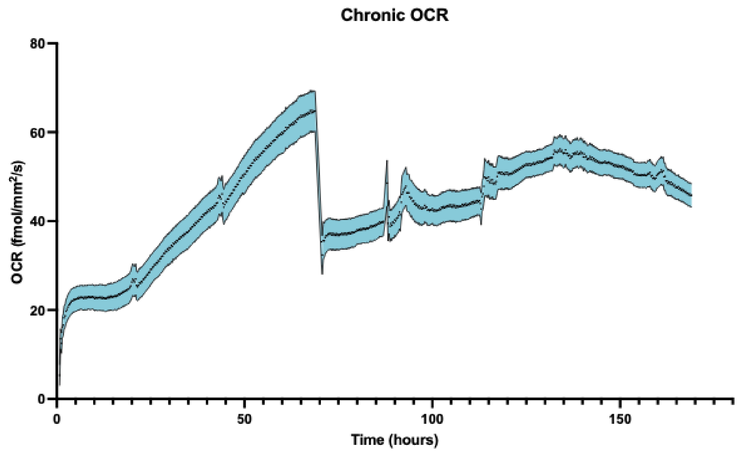

Figure 6 – Oxygen consumption rate of Neonatal Mouse Cardiomyocytes (NMCMs) starting at isolation day 0 until day 7 post isolation.

Figure 7 – 20x phase contrast video of NMCMs taken with Cytation 5 on day 7 following isolation.

Results

- Cardiomyocytes with green and red fluorescence were identified via Cytation 5 imaging, indicating the feasibility of the adenovirus-mediated smacATPi expression in cultured cardiomyocytes.
- The respective changes in green (cytosolic ATP) and red (mitochondrial ATP) fluorescence in response to 2-DG were observed (Figures 3-5).
- The oxygen consumption rate of the cultured cardiomyocytes was continuously monitored over a 7-day period using the online oxygen sensing device (Figure 6).
- Analysis of the data demonstrates the predicted changes in mitochondrial respiration in response to drug treatment in that ATP production decreases in response to the metabolic inhibitor, 2-DG.

Conclusion

Experimentation demonstrates that the methods employed in this study provide insight into mitochondrial respiration changes in early cardiomyocytes and how cellular respiration responds to different metabolic inhibitors. Future investigations will focus on evaluating real-time spatiotemporal cellular ATP dynamics and cellular respiration to help identify novel insights into previously unknown biochemical mechanisms in cardiomyocytes for the development of effective treatments of cardiac disorders.