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Investigation of Oxygen Consumption and ATP Production in Neonatal Mouse Cardiomyocytes

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. As cardiomyocyte function is directly correlated to cellular metabolism and mitochondrial respiration, we aim to explore their respiration early in life when the cells have proliferative capabilities. Based on previous work in the lab, we hypothesize that our dual cellular ATP indicator and continuous oxygen monitoring sensor will be useful in analyzing the mitochondrial respiration changes in early cardiomyocytes.

Methods: To test the hypothesis, this study used isolated neonatal mouse cardiomyocytes which are useful cell models as the cells undergo proliferation for 7 days following their isolation. To culture these cells, hearts from mouse pups were harvested at an age of 1 to 3 days. The tissue was lysed and enzymatically digested to isolate the cardiomyocytes. Following isolation, the cells were cultured for around 7 days. 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. Continuous recording of oxygen consumption of the cardiomyocytes was achieved using a Recipher cultured cell oxygen sensing system. The drugs 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor, and oligomycin A, an electron transport chain inhibitor, were applied to the cells.

Results: Evaluating changes in mitochondrial respiration and ATP production was performed by live cell imaging using a Cytation 5 imaging system. Cardiomyocytes with green and red fluorescence were identified, indicating the feasibility of the adenovirus-mediated smacATPi expression in cultured cardiomyocytes. Furthermore, the respective changes in green (cytosolic ATP) and red (mitochondrial ATP) fluorescence in response to 2-DG and oligomycin A were observed. The oxygen consumption rate of the cultured cardiomyocytes was continuously monitored over a 7-day period using the online oxygen sensing device. Analysis of the data collected by the above methods demonstrates the predicted changes in mitochondrial respiration following drug treatment in that mitochondrial respiration decreases in response to the metabolic inhibitors, 2-DG and oligomycin A.

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. For future investigations, evaluating real-time spatiotemporal cellular ATP dynamics and cellular respiration may help identify novel insights into previously unknown biochemical mechanisms in cardiomyocytes for the development of effective treatments of cardiac disorders.