Importance of Per2 in Cardiac Mitochondrial Function during Stress

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Background and Hypothesis: Ischemic heart disease is the worldwide leading cause of death. Cardiac cellular damage from ischemia is mainly inflicted in the form of mitochondrial dysfunction by inflammatory cytokines and reactive oxidative species (ROS). Mitochondria are critical for metabolic function to maintain cardiac activity. Interventions against inflammatory cytokines and ROS are therefore cardioprotective during ischemic damage. Period Circadian Regulator 2 (Per2) is a circadian rhythm protein involved in metabolic regulation as a time-responsive gene in cardiomyocytes during ischemic damage. Overexpression of Per2 has been shown to decrease infarct size following myocardial infarction. In this study, we hypothesize that Per2 protein plays a regulatory role in the mitochondrial response to inflammatory cytokine TNFα and oxidative stressor H_2O_2 in human cardiomyocytes.

Project Methods: AC16 Human Cardiomyocytes (HCM) transfected with Per2 or control siRNA were subjected to stress treatment of 100ng/mL TNFa or 100μM H_2O_2. RT-PCR and Western blot were used to detect Per2 expression. After two hours of treatment, mitochondrial membrane potential ($\Delta\psi_M$) was detected using JC1 fluorescence probe and mitochondrial respiration capacity was evaluated via Seahorse Mito Stress Test. After four hours of treatment, cell death was measured using Annexin V and propidium iodide (PI) apoptosis kit via flow cytometry.

Results: Per2 siRNA significantly reduced Per2 mRNA and protein levels in HCM. Increased cell death and decreased $\Delta\psi_M$ were observed in HCM treated with TNFa or H_2O_2. Knockdown of Per2 potentiated TNFa-induced cell death, TNFa- or H_2O_2 -disrupted $\Delta\psi_M$, and TNFa- or H_2O_2-impaired mitochondrial maximal respiration.

Conclusion and Potential Impact: Per2 knockdown increases apoptotic susceptibility and mitochondrial dysfunction in human cardiomyocytes exposed to TNFa or H_2O_2. Delivery of Per2 may serve as a promising therapeutic strategy to protect cardiomyocyte mitochondrial function during periods of stress, such as myocardial infarction, organ transplantation, and cardiac surgery.