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 H₂O₂ in human cardiomyocytes.

**Project Methods:** AC16 Human Cardiomyocytes (HCM) transfected with Per2 or control siRNA were subjected to stress treatment of 100ng/mL TNFα or 100μM H₂O₂. RT-PCR and Western blot were used to detect Per2 expression. After two hours of treatment, mitochondrial membrane potential (Δψₘ) 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 Δψₘ were observed in HCM treated with TNFα or H₂O₂. Knockdown of Per2 potentiated TNFα-induced cell death, TNFα- or H₂O₂-disrupted Δψₘ, and TNFα- or H₂O₂-impaired mitochondrial maximal respiration.

**Conclusion and Potential Impact:** Per2 knockdown increases apoptotic susceptibility and mitochondrial dysfunction in human cardiomyocytes exposed to TNFα or H₂O₂. 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.