COLLEGE OF HEALTH SCIENCE FACULTY GRANT-IN-AID REPORT

PROJECT TITLE: CARD9 Regulates Obesity-Induced Cardiac Mitochondrial Dysfunction

INVESTIGATOR: Guanglong He

YEAR PROJECT WAS SUPPORTED: 2014

Participants/Subjects PI: Guanglong He; Co-investigators: Jun Ren, D Paul Thomas

Methods: A novel gene mutation *CARD9*$^{-/-}$ mouse model was employed to dissect the signaling pathways responsible for cardiac mitochondrial dysfunction in obese mice. Additionally, echocardiography was used to assess cardiac dysfunction.

Results:

1) **A high fat diet (HFD) increases macrophage infiltration in the heart.** Hearts from WT and *CARD9*$^{-/-}$ mice fed a ND or HFD for 5 months were excised and frozen. The frozen heart tissue was cryosectioned, permeabilized, and incubated with antibody against CD68, which is a marker of macrophages, followed by secondary antibodies and DAPI staining. Fluorescence was visualized using a microscope. A HFD increased macrophage infiltration in the heart of WT mice. The absence of CARD9 led to a significant decrease in the number of infiltrated macrophages in the heart of HFD-fed *CARD9*$^{-/-}$ mice.
2) **A HFD increases p38 MAPK phosphorylation and suppresses autophagy in the heart.** Heart tissue from ND- and HFD-fed mice was homogenized and analyzed by immunoblotting with antibodies against p38 MAPK, p-p38 MAPK, LC3BI/II, p62, α-tubulin, and β-actin. The ratio of p-p38 MAPK/p38 MAPK was significantly increased in the heart of HFD-fed WT mice, and *CARD9* KO completely ameliorated this increase. A HFD was associated with a decreased ratio of LC3BII/LC3BI and increased p62 expression, indicating dysfunctional initiation and maturation of myocardial autophagy. *CARD9* KO completely restored these markers of autophagy signaling in the heart of HFD-fed *CARD9*$^{-/-}$ mice, suggesting a potential mechanistic link between CARD9 signaling and HFD-suppressed myocardial autophagy.
3) **A HFD induces myocardial contractile dysfunction.** Cardiomyocytes were isolated from HFD- and ND-fed mice, and their contractile properties were measured with the SoftEdge MyoCam system. The lengthening and re-lengthening indices were assessed: resting cell length, peak shortening, time to peak shortening (TPS), time to 90% re-lengthening (TR_{90}), and maximal velocity of shortening (+dL/dt) and re-lengthening (–dL/dt). Cardiomyocytes from HFD-fed WT mice showed a significant reduction in peak shortening and ±dL/dt and a significant increase in TR_{90}, with little change in resting cell length and TPS, indicating diastolic and systolic dysfunction. Importantly, *CARD9* KO totally ameliorated myocardial anomalies associated with HFD-induced obesity.

Limitations: None

Conclusions: In conclusion, the current study demonstrated that CARD9 knockout protected against HFD-induced and obesity/diabetes-associated myocardial dysfunction through attenuation of macrophage infiltration and restoration of cardiac autophagy signaling. These results may provide a potential new therapeutic target for the management of HFD-induced metabolic syndrome.

Future Research & Dissemination: The overall objective of our research is to determine the signals that activate the CARD9 signaling complex and the consequences of activated CARD9 signaling on myocardial function in HFD-induced obesity. With the outcome from this grant support, we will continue to investigate the role of CARD9 on obesity-associated myocardial dysfunction. A R01 grant proposal was planned for the deadline of 10/5/2016.