1. Project title: *Roles of mitochondrial HCCS in the regulation of healthspan by enhancing cardiac resilience to heart failure*

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2. Abstract of Project:

The objective of this proposed research project is to study the role of mitochondrial health in cardiac adaptation to pathological conditions of chronic ischemia and reperfusion in controlling the disease development of heart failure. The focus of this project will be on one disease model of heart failure caused by chronic myocardial infarction in the aging process, one major upstream biosynthetic enzyme, cytochrome c heme lyase (HCCS) in the cardiac mitochondria, and cardiac resilience to ischemia and reperfusion injury during aging process. The mitochondrial HCCS as the major upstream enzyme of redox signal pathway in heart and a major target of oxidative attack occurred during chronic myocardial infarction and consequent heart failure complicated by aging. Impairment and down-regulation of cardiac HCCS is closely related to myocardial infarction-caused heme defect, associated mitochondrial dysfunction and declining of cardiac resilience to development of heart failure during aging process. The disease model of chronic infarction associated heart failure will be created by the animal surgery of in vivo occlusion of mouse heart for 30-min and followed by in vivo chronic reperfusion for 4 weeks. Cardiac-specific HCCS transgenic mice (HCCS-tg) will be employed to evaluate if gain of HCCS function in the myocardium can promote myocardial adaptation to chronic ischemia and reperfusion and enhance cardiac mitochondrial function, preserve mitochondrial health, and improve healthspan via preserving heme integrity. Mitochondrial function in the mouse heart will be measured using oxygen polarography and kinetic assays of the enzymatic activities from electron transport chain. Echocardiography will be used to measure the cardiac function and determine cardiac resilience to chronic ischemia and reperfusion. The progress of this project will advance our knowledge toward understanding cardiac adaptation to pathological conditions of ischemia and reperfusion and promote cardiac resilience to chronic post-ischemic reperfusion as well as aging-induced heart failure with benefits of improving human healtspan.

3. The significance of overall research:

Myocardial infarction is known colloquially as heart attack. More than 0.7 million Americans suffer a heart attack every year with a 50% mortality rate. Chronic infarction complicated by aging process is the leading cause of heart failure. Mitochondria as the major source of energy generation are essential for proper cellular function in heart. There is considerable evidence supporting the key role of mitochondrial dysfunction and decreasing cardiac resilience in the disease pathogenesis of heart failure and progress of aging. At the myocardial level of the post-ischemic and aging heart, there is a marked defect in energy metabolism associated with mitochondrial dysfunction. Downregulation of mitochondrial HCCS has been further detected in the mitochondria of the disease model of acute myocardial infarction. Deregulated HCCS is closely associated to heme impairment and waning ability of myocardial adaptation to chronic post-ischemic reperfusion and aging complication and eventual vanishing of cardiac resilience in the disease progress of heart failure. This project will test the hypothesis that enhancement of heme biogenesis in the heart via transgenic overexpression of HCCS in the mitochondria in mouse heart mediates myocardial adaptation, promotes cardiac resilience, and alleviates the risk of heart failure in the physiological progress of ischemia and reperfusion and aging.

4. Goals and objectives

The objectives of this research are to assess the role of HCCS and mitochondrial health in myocardial adaptation to ischemia and reperfusion injury and aging and to explore new avenues into promoting cardiac resilience in order to more fully understand the mechanisms of cardioprotection and alleviate the risk of developing heart failure. As mitochondrial dysfunction caused by heme impairment and associated HCCS defect in the mitochondria is likely to have an impact on myocardial ability to resist pathological stress, it is desirable to obtain further information on how normalizing heme integrity via

gaining HCCS function in mitochondria in vivo affects the myocardial adaptation and stimulates cardiac resilience, overall cardiac function, and eases heart remodeling and related pathogenesis of heart failure development. To partially address this question in 8 weeks and optimize the efficacy of the summer fellowship training in biomedical research, the proposed studies narrow the scope of investigation to focus specifically on measuring the phenotypes of cardiac and mitochondrial functions from the disease model of chronic ischemia and reperfusion using echocardiographic and oxygen polarographic approaches.

5. The research methods to be used

In vivo disease model of chronic myocardial infarction using mouse and cardiac function assessed by echocardiography - The procedure for in vivo myocardial ischemia and reperfusion to create the disease model of heart attack will be the same technique reported in our previous publications [Chen, Y-R (2007) J. Biol. Chem., 282, 32640-54, and Chen, C-L (2008) J. Biol. Chem. 283, 27991-28003]. The mice of HCCS-tg and wild type control (11-12 weeks old and FBV background) will be anesthetized and subjected to 30-min of in vivo coronary artery ligation followed by 4 weeks reperfusion. At 4 weeks post-ischemic reperfusion the mice will be placed under anesthesia and subjected to measurement of cardiac function with echocardiography [Kang, PT (2015) Journal of Molecular and Cellular Cardiology, 88, 14-28]. The mouse hearts will then be excised. The infarct area of heart will be identified by 2,3,5-triphenyltetrazolium chloride (TTC) staining. The risk region of the myocardium will be excised for mitochondrial preparation and analyses with polarographic oxygen consumption.

Mitochondria preparation, oxygen consumption measurements, and enzymatic activity assay of mitochondrial electron transport chain - Mitochondria will be prepared from the non-ischemic and infarct tissues by differential centrifugation described in the published method [Chen, Y-R (2007) J. Biol. Chem., 282, 32640-54; Chen, C-L (2008) J. Biol. Chem. 283, 27991-28003; Lee, H-L (2012) AJP-Heart and Circulatory Physiology 302, H1410-1422]. Mitochondrial respiration will be measured by the polarographic method using a Clark-type oxygen electrode (Oxytherm, Hansatech Instruments) at 30 °C. The NADH-linked respiration will be induced by malate/glutamate and the enzymatic activity of ETC complexes will be assayed according to published approaches [Kang, PT (2015) Journal of Molecular and Cellular Cardiology, 88, 14-28, and Kang PT (2015) Free Radical Biology and Medicine, 79: 56-68].

6. The proposed method of data analysis

(1) Data analysis of echocardiographic assessment of cardiac function will be done by measuring the parameters of ejection fraction, fractional shortening, left ventricle internal dimension under systole and diastole, left ventricle volume of systole and diastole, relative wall thickness, ratio of heart weight to body weight, mitral valve E/A ratio based on the m-mode echo image [Kang, PT (2015) Journal of Molecular and Cellular Cardiology, 88, 14-28].

(2) Data analysis of mitochondria-mediated oxygen consumption rate and the enzymatic activities of mitochondrial ETC complexes will be accessed by the first derivative of kinetic curve of oxygen consumption rate, ubiquinone 2-mediated DCPIP reduction (complex II), and ubiquinol 2-mediatied ferricytochrome c reduction (complex III) [Chen, C-L (2021) Journal of Molecular and Cellular Cardiology, 161, 23-38, and Kang PT (2015) Free Radical Biology and Medicine, 79: 56-68].

(3) All data will be reported as group averages ± SEM. Statistical analyses and comparison between two groups (sham control hearts vs post-ischemic hearts, and health tissue/mitochondria vs post-ischemic tissue/mitochondria) will be assessed by One-Way ANOVA.

7. Significance of anticipated findings

Combining the biochemical and echocardiographic approaches with unique genetically modified mice of HCCS-tg and in vivo animal disease model will allow us to gain a new understanding of the phenotype of mitochondrial health in regulating cardiac resilience in aging as well as in the disease process of heart failure. The project will serve as a pilot study which may establish a useful platform available in the key bioenergetic pathway of heme biogenesis responsible for cardiac resilience in the mitochondria

of the cardiac system. Results from these studies will increase the depth of understanding of cardiac adaptation to the stress imposed by ischemia and reperfusion and aging, which could potentially translate to clinical intervention for cardioprotection, easing the risk of heart failure and improving human healthspan.

Summer Research Fellow Training/Monitoring Plan.

1. Requirements and procedure for the student fellow is as follows:

    a. First, the student will meet and familiarize themselves with Dr. Chen, and the members of his lab, who will explain project rationale, teach the skills necessary to follow protocol: learning in vivo myocardial ischemia and reperfusion as well as aging system, how to properly conduct echocardiography, mitochondrial preparation, the assay procedures, data analysis and interpretations.

    b. Echocardiography will be conducted in the facility available in the Department of Integrative Medical Sciences/NEOMED under the supervision of Dr. Vahagn Ohanyan.

    c. There will be 1:1 meetings between the student fellow and the mentor (Dr. Chen) as well as 2:1 meeting with both Dr. Chen and Dr. Ohanyan (Assistant professor of Integrative Medical Sciences).

    d. Disease model training will be completed within the first 3 weeks. Training in polarographic analysis and mitochondrial biology will be completed following first 3 weeks. Training in the final two weeks will be focused on data analysis of echocardiography and bioenergetic analysis.

    e. The student trainee will attend weekly lab meetings of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research including Dr. Chen, Dr. Chilian, Dr. Dong, Dr. Kang, Dr. Ohanyan, Dr. Raman, Dr. Goodwill, Dr. Yin, and Dr. O'Leary), and will present findings or related research article during meeting.

    f. The student will participate in a summer journal club that involves all the summer research students and faculty members. Each summer student will be expected to make a presentation and participate in discussion.

    g. The student will be expected to present a poster at the research day when all summer fellows present a synopsis of their summer work.

2. The mouse model and strain of HCCS-tg is available in my lab. All the necessary resources and equipment are available. Furthermore, a research technician is available to assist in training the student fellow.

3.All research will be completed at the RGE and facility of Echocardiography (C building) on NEOMED's campus.