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Disruption of calpain reduces lipotoxicity-induced cardiac injury by preventing endoplasmic reticulum stress

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ABSTRACT

Diabetes and obesity are prevalent in westernized countries. In both conditions, excessive fatty acid uptake by cardiomyocytes induces cardiac lipotoxicity, an important mechanism contributing to diabetic cardiomyopathy. This study investigated the effect of calpain disruption on cardiac lipotoxicity. Cardiac-specific *capns1* knockout mice and their wild-type littermates (male, age of 4 weeks) were fed a high fat diet (HFD) or normal diet for 20 weeks. HFD increased body weight, altered blood lipid profiles and impaired glucose tolerance comparably in both *capns1* knockout mice and their wild-type littermates. Calpain activity, cardiomyocyte cross-sectional areas, collagen deposition and triglyceride were significantly increased in HFD-fed mouse hearts, and these were accompanied by myocardial dysfunction and up-regulation of hypertrophic and fibrotic collagen genes as well as pro-inflammatory cytokines. These effects of HFD were attenuated by disruption of calpain in *capns1* knockout mice. Mechanistically, deletion of *capns1* in HFD-fed mouse hearts and disruption of calpain with calpain inhibitor-III, silencing of *capn1*, or deletion of *capns1* in palmitate-stimulated cardiomyocytes prevented endoplasmic reticulum stress, apoptosis, cleavage of caspase-12 and junctophilin-2, and pro-inflammatory cytokine expression. Pharmacological inhibition of endoplasmic reticulum stress diminished palmitate-induced apoptosis and pro-inflammatory cytokine expression in cardiomyocytes. In summary, disruption of calpain prevents lipotoxicity-induced apoptosis in cardiomyocytes and cardiac injury in mice fed a HFD. The role of calpain is mediated, at least partially, through endoplasmic reticulum stress. Thus, calpain/endoplasmic reticulum stress may represent a new mechanism and potential therapeutic targets for cardiac lipotoxicity.

1. Introduction

Diabetes and obesity are prevalent in westernized countries. In both conditions, excessive fatty acid uptake by cardiomyocytes may alter cellular structures and activate downstream pathways in the heart resulting in cardiomyocyte apoptosis and/or impaired insulin signaling followed by decreased glucose use and myocardial dysfunction, a condition described as cardiac lipotoxicity [1–3], eventually leading to adverse myocardial remodeling and heart failure. In addition to glucotoxicity, lipotoxicity is also an important mechanism contributing to diabetic cardiomyopathy [3], a condition independent of coronary artery disease and hypertension. However, the underlying mechanisms responsible for cardiac lipotoxicity remain partially understood.

Calpains are Ca^{2+}-dependent intracellular proteases [4]. Amongst 15 family members, two of the best characterized calpain species are calpain-1 and calpain-2. These isoforms are heterodimers consisting of ~80 kDa catalytic subunits, encoded by the *capn1* and *capn2* genes, respectively, and a ~30 kDa small regulatory subunit encoded by *capns1* (also known as *capn4*). The small regulatory subunit is essential for the stability and catalytic activity of calpain-1 and calpain-2. Thus,

Abbreviations: ANP, atrial natriuretic peptide; ATF4, activating transcription factor 4; β-MHC, beta-myosin heavy chain; *capns1*, small regulatory subunit of calpain; CHOP, C/EBP homologous protein; ER, endoplasmic reticulum; GRP78, chaperones glucose-regulated protein-78; GRP94, chaperones glucose-regulated protein-94; HDL-C, high-density lipoprotein cholesterol; HFD, high fat diet; IL-1β, interleukin-1 beta; IPGTT, intraperitoneal glucose tolerance test; LDL-C, low-density lipoprotein cholesterol; LV, left ventricle; ND, normal diet; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-alpha.

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deletion of *capns1* in mice abolished calpain-1 and calpain-2 activity [5]. Calpain-1 and calpain-2 activities are also tightly controlled by the endogenous inhibitor calpastatin. Calpain activation has been implicated in myocardial remodeling and heart failure [6]. We have recently demonstrated that calpain activation mediates apoptosis in high glucose-stimulated cardiomyocytes [7] and genetic inhibition of calpain reduces adverse cardiac remodeling and myocardial dysfunction in hyperglycemic mice [5], indicating a critical role of calpain in cardiac glucotoxicity. However, it remains to be determined whether calpain plays a role in cardiac lipotoxicity.

Endoplasmic reticulum (ER) stress is induced by accumulation of unfold proteins, resulting from oxidative stress, ischemia, disturbance of calcium homeostasis, and over-expression of normal and/or incorrectly folded proteins [8]. ER stress-associated unfolded protein response activates ER transmembrane sensors including protein kinase-like ER kinase, inositol-requiring kinase 1, and activating transcription factor 6. These tree branches of ER transmembrane sensors initiate adaptive response through phosphorylation of eukaryotic translation initiation factor 2α, transcription factor ATF4 translation, X-Box Binding Protein 1 splicing, and finally the induction of the unfolded protein response related genes, including chaperones glucose-regulated protein-78 (GRP78) and -94 (GRP94), and C/EBP homologous protein (CHOP). If ER stress is prolonged or overwhelming, however, it can induce apoptotic cell death through CHOP and/or other pathways. Recent animal and human studies have demonstrated that ER stress is implicated in the pathophysiology of various cardiovascular diseases [9,10]. ER stress is induced and has been implicated in cardiac lipotoxicity [11–13]. We reported that calpain activation induces ER stress in cardiomyocytes following ischemia/reperfusion [14]. However, it remains to be determined whether calpain regulates ER stress in cardiac lipotoxicity.

In this study, we analyzed the impact of calpain disruption on ER stress, myocardial hypertrophy, fibrosis and pro-inflammatory response in a mouse model of high fat diet (HFD)-induced cardiac lipotoxicity. We further investigated the direct relationship between calpain, ER stress, and apoptosis and pro-inflammatory response in palmitate-stimulated cardiomyocytes.

2. Materials and methods

2.1. Animals

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23). All experimental procedures were approved by the Animal Use Subcommittee at the Soochow University, China. Breeding pairs of C57BL/6 mice were purchased from the Jackson Laboratory. Mice with cardiomyocyte-specific disruption of *capns1* (*capns1*-ko) were generated by breeding mice bearing the targeted $Capn4^{PZ}$ allele containing *loxP* sites flanking essential coding exons and mice with cardiomyocyte-specific expression of Cre recombinase under the control of alpha-myosin heavy chain as we recently described [5]. All of the mice used in this study, including controls, were littermates of the same generation. A breeding program was implemented at Soochow University's animal care facilities.

2.2. Experimental protocol

Male capns1-ko mice and their wild-type littermates (mice bearing the targeted $Capn4^{PZ}$ allele containing *loxP* sites flanking essential coding exons) at age of 4 weeks were fed a high fat diet (HFD) or normal diet (ND) for 20 weeks. This HFD contains 26.2% of protein, 26.3% of carbohydrate and 34.9% of fat (% by weight) (Research Diets Inc., USA). The calculated caloric intake from these nutrients (% kcal) is 20%, 20% and 60%, respectively. At the end of each experiment, mice were anaesthetized with ketamine (100 mg/kg)/xylazine (5 mg/kg, i.p.) and blood was taken from anaesthetized mice *via* the cardiac puncture. Mice were then euthanized by cervical dislocation right away.

2.3. Blood pressure measurement

Blood pressure was measured using a tail-cuff method. Briefly, mice were gently restrained in a holder and warmed to 37 °C by a heating platform under the holder. Animals were acclimated in the environment for 5–10 min after occlusion cuffs and pulse transducers (MRBP system, IITC Life Science, USA) were placed on the tail. The values of at least 10 readings for each mouse were used for blood pressure.

2.4. Intraperitoneal glucose tolerance

For intraperitoneal glucose tolerance test (IPGTT), 16-hour fasted mice were given a glucose load (2 g/kg, i.p.). Blood samples were taken from the tail vein at 0, 15, 30, 60, 120 min after glucose injection, and blood glucose was measured using a glucose meter (LifeScan, USA).

2.5. Blood lipid profiles

Blood samples were obtained in mice after 5-hour fasting. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured by using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instruction.

2.6. Echocardiography

Animals were lightly anaesthetized with inhalant isoflurane (1%) and imaged using a 40-MHz linear array transducer attached to a preclinical ultrasound system (Vevo 2100, FUJIFILM VisualSonics, Canada) with nominal in-plane spatial resolution of 40 μm (axial) × 80 μm (lateral). M-mode and 2-D parasternal short-axis scans (133 frames/s) at the level of the papillary muscles were used to assess changes in left ventricle (LV) end-systolic inner diameter, LV end-diastolic inner diameter, and fractional shortening (FS%) [15,16].

To assess diastolic function, we obtained apical four-chamber views of the left ventricle. The pulsed wave Doppler measurements of maximal early (E) and late (A) transmitral velocities in diastole were obtained in the apical view with a cursor at mitral valve inflow [16].

2.7. Mouse cardiomyocyte cultures

Neonatal mice (born within 2 days) were euthanized by decapitation and the neonatal cardiomyocytes were prepared and cultured according to methods we described previously [14].

Adult cardiomyocytes were isolated and cultured as we described previously [15].

2.8. Treatment of siRNA

Cholesterol-conjugated siRNAs for *capn1* and *capn2* were purchased from Guangzhou Ribobio Co., Ltd. (China). The sequences for *capn1* and *capn2* siRNA are as follows: 5′CCAGCTACCTTCTGGGTAA3′ (*capn1* siRNA) and 5′GGAGAAAGGTTCTCTGCTT3′ (*capn2* siRNA). A scrambled siRNA conjugated with cholesterol served as a control. Cardiomyocytes were incubated with siRNA (50 nmol/L) in normal culture medium.