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Complex inhibition of autophagy by mitochondrial aldehyde dehydrogenase shortens lifespan and exacerbates cardiac aging☆

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ABSTRACT

Autophagy, a conservative degradation process for long-lived and damaged proteins, participates in a cascade of biological processes including aging. A number of autophagy regulators have been identified. Here we demonstrated that mitochondrial aldehyde dehydrogenase (ALDH2), an enzyme with the most common single point mutation in humans, governs cardiac aging through regulation of autophagy. Myocardial mechanical and autophagy properties were examined in young (4 months) and old (26–28 months) wild-type (WT) and global ALDH2 transgenic mice. ALDH2 overexpression shortened lifespan by 7.7% without affecting aging-associated changes in plasma metabolic profiles. Myocardial function was compromised with aging associated with cardiac hypertrophy, the effects were accentuated by ALDH2. Aging overtly suppressed autophagy and compromised autophagy flux, the effects were exacerbated by ALDH2. Aging dampened phosphorylation of JNK, Bcl-2, IKKβ, AMPK and TSC2 while promoting phosphorylation of mTOR, the effects of which were exaggerated by ALDH2. Co-immunoprecipitation revealed increased dissociation between Bcl-2 and Beclin-1 (result of decreased Bcl-2 phosphorylation) in aging, the effect of which was exacerbated with ALDH2. Chronic treatment of the autophagy inducer rapamycin alleviated aging-induced cardiac dysfunction in both WT and ALDH2 mice. Moreover, activation of JNK and inhibition of either Bcl-2 or IKKβ overtly attenuated ALDH2 activation-induced accentuation of cardiomyocyte aging. Examination of the otherwise elderly individuals revealed a positive correlation between cardiac function/geometry and ALDH2 gene mutation. Taken together, our data revealed that ALDH2 enzyme may suppress myocardial autophagy possibly through a complex JNK-Bcl-2 and IKKβ-AMPK-dependent mechanism *en route* to accentuation of myocardial remodeling and contractile dysfunction in aging. This article is part of a Special Issue entitled: Genetic and epigenetic control of heart failure - edited by Jun Ren & Megan Yingmei Zhang.

1. Introduction

Aging is an irreversible biological process with decreased organ function leading to high morbidity and mortality in the elderly [1]. Cardiac aging is manifested as cardiac remodeling, loss of cardiac contractile reserve, and increased prevalence of heart failure [1–4]. A number of cellular processes are speculated to contribute to cardiac aging including oxidative stress and compromised protein quality control such as autophagy, an intracellular bulk degradation process for clearance of long-lived proteins and organelles [4–8]. Autophagy process is initiated with the formation of phagophores prior to elongation and engulfing of a portion of cytoplasm to form mature autophagosomes [9]. Autophagosomes then fuse with lysosomes to form autolysosomes, where engulfed cellular contents are degraded by acidic lysosomal hydrolases [9,10]. Autophagy has been demonstrated to participate in a variety of physiological and pathological processes, including development, immunity, cancer, longevity, aging and neurodegenerative diseases [10–12]. It is conceived that the serine/threonine kinase, mammalian target of rapamycin (mTOR), serves as the main governing body to negatively regulate autophagy through the mTOR complex 1 (mTORC1) [9,13,14]. mTORC1 then functions to regulate autophagy in response to rapamycin, starvation, as well as changes in nutrients and energy status via a complex consist of mammalian Atg13, ULK1 and FIP200 [9,10,14]. Ample of evidence has indicated regula-

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tion of autophagy through activation of the stress signal molecule c-Jun N-terminal kinase 1 (JNK1), which in turn phosphorylates Bcl-2 to disengage the association (interaction) between Bcl-2 and the autophagy initiating protein Beclin-1, an essential part of the class III phosphatidylinositol 3-kinase (PI3K)/hVps34 complex for autophagosome formation, leading to autophagy induction [15,16]. Existence of mTOR-independent autophagy pathways are also reported including IKK (IKB kinase), which is capable of regulating autophagy independently of its effects on NFKB [11]. In particular, IKKβ has been reported to facilitate autophagy through JNK1-mediated Bcl-2 phosphorylation [11,17].

The mitochondrial aldehyde dehydrogenase (ALDH2) is a gene located on chromosome 12. All Caucasians carry homozygous ALDH2 while nearly 50% of Asians are heterozygous and possess one mutant copy encoding an inactive mitochondrial isozyme [18]. Recent evidence from our lab and others has depicted a pivotal role for ALDH2 in cardiac homeostasis under both physiological and pathological conditions [19,20]. For example, genome-wide association data have revealed a role for ALDH2 mutation in hypertension in Asian decedents [21]. More clinical and experimental evidence has depicted a beneficial role for ALDH2 in the regulation of cardiac function in pathological settings including diabetes mellitus, alcoholism, endoplasmic reticulum stress, arrhythmias and ischemia-reperfusion injury [19,20,22–28]. A number of theories have been postulated for ALDH2-offered effect in the cardiovascular system including oxidative stress and mitochondrial integrity [25,29,30]. More recent evidence has favored a role for autophagy in ALDH2-offered beneficial effect in cardiovascular homeostasis [24,25,31,32]. Given that genetic factors represents one-fifth of heart failure incidence [33], this study was designed to evaluate the role of ALDH2 in lifespan and cardiac aging, an irreversible biological process with a high prevalence of heart failure and compromised autophagic self-repair [2,8,13,34]. Aging is characterized by adaptive changes in the heart including cardiac remodeling, altered diastolic filling such as prolonged diastole, and compromised myocardial contractile capacity, contribute to the high morbidity and mortality in the elderly [35,36]. Our recent data noted a tie between ALDH2 gene and advanced aging-induced cardiac contractile and mitochondrial anomalies [37] although the underlying mechanism remains poorly defined. Changes in autophagy as well as mTOR-dependent (IKKβ-AMPK-TSC-Rheb-mTOR) and -independent (such as the JNK-Bcl-2-Beclin-1 cascade) autophagy regulatory machineries were examined in wild-type (WT) and ALDH2 transgenic mice at young and old age. Furthermore, therapeutic effect of autophagy induction using rapamycin was evaluated in aged WT and ALDH2 mice. An *in vitro* H9C2 myoblast senescence model was employed using doxorubicin to evaluate the role of autophagy in senescence [8,38], which is associated with cell cycle arrest, changes in telomere length, as well as cell cycle regulatory proteins p53, p16 and p21 [2].

2. Methods and Materials

2.1. ALDH2 mice, Kaplan-Meier curve and rapamycin treatment

The animal procedures described here were approved by our Institutional Animal Care and Use Committees at the University of Wyoming (Laramie, WY) and Fudan University Zhongshan Hospital (Shanghai, China). Production of ALDH2 overexpression transgenic mice in Friendly virus B (FVB) mouse background using the chicken β-actin promoter was described [23]. Four month-old (young) and 24–26 month-old (old) male ALDH2 mice and wild-type littermates were maintained with a 12/12-light/dark cycle with free access to lab chow and tap water. At the time of sacrifice, blood glucose, plasma insulin and triglyceride levels were measured using glucose meter and ELISA commercial kits. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin resistance based on the following equation: fasting insulin (μU/ml) × fasting blood glucose (mM)/22.5 [39]. All mice used for the lifespan analysis (Kaplan-Meier survival curve) were assigned to a longevity cohort at birth and were not used for any biochemical or immunoblotting tests. Only male mice were used for this study. To evaluate the effect of autophagy induction in cardiac aging, a cohort of old WT and ALDH2 mice (24–26 month-old) were treated with the autophagy inducer rapamycin (2 mg/kg/d, i.p.) every other day for 8 weeks [40] prior to assessment of cardiac contractile function.

2.2. Mouse heart perfusion

After intraperitoneal administration of a sedative (ketamine 80 mg/kg and xylazine 12 mg/kg), mouse hearts were removed and retrogradely perfused with a Krebs-Henseleit buffer containing 7 mM glucose, 0.4 mM oleate, 1% BSA and a low fasting concentration of insulin (10 μU/ml). Hearts were perfused at a constant flow of 4 ml/min (equal to an aortic pressure of 80 cmH_2O) at baseline for 60 min. A fluid-filled latex balloon connected to a solid-state pressure transducer was inserted into the left ventricle through a left atriotomy to measure pressure. LVDP, the first derivative of LVDP (± dP/dt) and heart rate were recorded using a digital acquisition system at a balloon volume which resulted in a baseline LV end-diastolic pressure of 5 mm Hg [41].

2.3. Histological examination

After anesthesia, hearts were excised and immediately placed in 10% neutral-buffered formalin at room temperature for 24 h after a brief rinse with PBS. The specimens were embedded in paraffin, cut into 5-μm sections and stained with fluorescein isothiocyanate (FITC)-conjugated wheat germ agglutinin. Cardiomyocyte cross-sectional areas were calculated on a digital microscope (× 400) using the Image J (version1.34S) software [29].

2.4. Isolation of mouse cardiomyocytes and in vitro drug treatment

Hearts were mounted onto a temperature-controlled (37 °C) Langendorff system. After perfusion with a modified Tyrode's solution, the heart was digested with a Ca^{2+}-free KHB buffer containing liberase blendzyme 4 (Hoffmann-La Roche Inc., Indianapolis, IN) for 20 min. The modified Tyrode solution (pH 7.4) contained the following (in mM): NaCl 135, KCl 4.0, $MgCl_2$ 1.0, HEPES 10, NaH_2PO_4 0.33, glucose 10, butanedione monoxime 10, and the solution was gassed with 5% CO_2/95% O_2. The digested heart was then removed from the cannula and left ventricle was cut into small pieces in the modified Tyrode's solution. Tissue pieces were gently agitated and pellet of cells was resuspended. A yield of 60–70% viable rod-shaped cardiomyocytes with clear sarcomere striations was achieved. Only rod-shaped myocytes with clear edges were selected for contractile studies [23]. To elucidate the role for JNK, Bcl-2 and IKKβ in ALDH2-induced response on cardiac in aging, cardiomyocytes from young or aged WT mice were treated with the ALDH2 activator Alda-1 (20 μM) for 6 h in the presence or absence of the JNK phosphorylation inducer anisomycin (50 ng/ml) [42], the Bcl-2 inhibitor ABT-737 (10 μM) [43] or the IKKβ inhibitor (phosphorylation stimulator) sodium salicylate (5 mg/ml) [44] prior to the assessment of cardiomyocyte function or protein levels.

2.5. Cell shortening/relengthening

Mechanical properties of cardiomyocytes were assessed using a SoftEdge MyoCam® system (IonOptix Corporation, Milton, MA) [23]. Cells were placed in a Warner chamber mounted on the stage of an inverted microscope (Olympus, IX-70) and superfused (1 ml/min at 25 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, at pH 7.4. The cells were field stimulated with supra-threshold voltage at a frequency of 0.5 Hz (unless otherwise stated), 3 msec duration. The myocyte being studied was displayed on