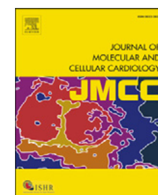
Journal of Molecular and Cellular Cardiology 102 (2017) 53–60

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc



Original article

Cardiac proteasome functional insufficiency plays a pathogenic role in diabetic cardiomyopathy

Jie Li [a,b], Wenxia Ma [a], Guihua Yue [a,c], Yaoliang Tang [a], Il-man Kim [a], Neal L. Weintraub [a], Xuejun Wang [b], Huabo Su [a,b,d,*]

[a] *Vascular Biology Center, Medical College of Georgia, Augusta University, Augusta, GA, United States*
[b] *Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD, United States*
[c] *Department of Cardiology, Guangxi University of Chinese Medicine, Nanning, Guangxi, China*
[d] *Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta University, Augusta, GA, United States.*

ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form 21 November 2016
Accepted 24 November 2016
Available online 30 November 2016

Keywords:
PA28α
Ubiquitin
Proteasome
Diabetic cardiomyopathy

ABSTRACT

Background: Diabetic cardiomyopathy is a major risk factor in diabetic patients but its pathogenesis remains poorly understood. The ubiquitin-proteasome system (UPS) facilitates protein quality control by degrading unnecessary and damaged proteins in eukaryotic cells, and dysfunction of UPS is implicated in various cardiac diseases. However, the overall functional status of the UPS and its pathophysiological role in diabetic cardiomyopathy have not been determined.
Methods and results: Type I diabetes was induced in wild-type and transgenic mice expressing a UPS functional reporter (GFPdgn) by injections of streptozotocin (STZ). STZ-induced diabetes progressively impaired cardiac UPS function as evidenced by the accumulation of GFPdgn proteins beginning two weeks after diabetes induction, and by a buildup of total and lysine (K) 48-linked polyubiquitinated proteins in the heart. To examine the functional role of the UPS in diabetic cardiomyopathy, cardiac overexpression of PA28α (PA28αOE) was used to enhance proteasome function in diabetic mouse hearts. PA28αOE diabetic mice displayed exhibited restoration of cardiac UPS function, as demonstrated by the diminished accumulation of GFPdgn and polyubiquitinated proteins. Moreover, PA28αOE diabetic mice exhibited reduced myocardial collagen deposition, decreased cardiomyocyte apoptosis, and improved cardiac systolic and diastolic function.
Conclusion: Impairment of cardiac UPS function is an early event in STZ-induced diabetes. Overexpression of PA28α attenuates diabetes-induced proteotoxic stress and cardiomyopathy, suggesting a potential therapeutic role for enhancement of cardiac proteasome function in this disorder.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Diabetes mellitus remains a major health problem worldwide and is associated with a high rate of mortality and morbidity due cardiovascular complications [1]. Diabetic cardiomyopathy, defined as ventricular dysfunction in the absence of coronary artery disease and hypertension, is one major risk factor among others in diabetic patients [2,3]. Manifestations of diabetic cardiomyopathy include myocyte hypertrophy, interstitial fibrosis, cardiomyocyte dropout, and decreased diastolic and systolic function [4,5]. Research into the pathophysiology underlying the progression of diabetic cardiomyopathy to heart failure has identified increased oxidative stress, altered substrate metabolism, mitochondrial dysfunction, impaired calcium homeostasis and upregulation of the renin-angiotensin system as possible contributory factors [4–6]. However, the precise molecular and cellular mechanisms underpinning its pathogenesis remain to be elucidated.

The ubiquitin proteasome system (UPS) maintains protein homeostasis by timely degradation of unnecessary and/or damaged proteins such as terminally misfolded proteins and oxidized proteins. UPS-mediated protein degradation generally involves two steps, the ubiquitin-specific E1-E2-E3 enzyme-mediated covalent attachment of ubiquitin to protein targets (i.e., ubiquitination) and degradation of the modified targets by the proteasome [7,8]. Eukaryotic cells have several types of proteasome complexes, which are generally comprised of a 20S core proteasome (20S) and different regulatory complexes such as the 19S proteasome (19S) and the 11S proteasome (11S), to carry out protein degradation. While the 19S-20S-19S complexes, known as the 26S proteasome, are responsible for selective degradation of most intracellular proteins in a ubiquitin- and ATP-dependent manner, the 11S-activated proteasome is generally believed to mediate protein degradation in a

* Corresponding author at: Vascular Biology Center, Medical College of Georgia, Augusta University, 1459 Laney Walker Blvd, Augusta, GA 30912, United States.
E-mail address: HSU@augusta.edu (H. Su).

http://dx.doi.org/10.1016/j.yjmcc.2016.11.013
0022-2828/© 2016 Elsevier Ltd. All rights reserved.

ubiquitin- and ATP-independent manner [7]. The 11S proteasome can be formed by either PA28α and PA28β heteroheptamers (α3β4 or α4β3) or by PA28γ in homoheptamers (γ7).

As a major player of intracellular protein quality control, the UPS is essential to the survival and function of most eukaryotic cells including cardiomyocytes. Dysfunction of the UPS disrupts protein homeostasis and has been implicated in human cardiomyopathies and various forms of murine cardiomyopathies including desmin-related, hypertrophic and ischemic cardiomyopathy [9–11]. UPS dysfunction in these diseased hearts is typically manifested by increased ubiquitinated proteins, decreased proteasomal peptidase activities, oxidative damage to proteasome subunits and aberrant protein aggregates, indicating a proteolytic demand-supply imbalance. Emerging evidence suggests that impairment of UPS function is sufficient to cause cardiomyopathy and heart failure or to expedite maladaptive cardiac remodeling [12–15]. Moreover, improvement of proteasome function by overexpression of 11S proteasome subunit PA28α was shown to protect the heart against pathological stresses in experimental models of myocardial ischemia reperfusion, desmin-related cardiomyopathy and right ventricle hypertrophy [16,17]. These lines of compelling evidence suggest that UPS dysfunction may be a common mechanism in the pathogenesis of diverse cardiac diseases.

Circumstantial data links UPS dysfunction to diabetic cardiomyopathy. Alterations in the expression of UPS components, changes in proteasomal peptidase activities and increased ubiquitinated and oxidized proteins have been detected in the hearts of different diabetes models [18–20]. However, the effects of diabetes on the overall cardiac UPS function and its pathophysiological role in diabetic cardiomyopathy have not been examined. Using an in vivo UPS functional reporter in combination with other biochemical analyses, we have unveiled here that cardiac UPS function is impaired soon after the onset of type I diabetes and further deteriorates with diabetes progression. Moreover, enhancement of cardiac proteasome function by overexpression of PA28α alleviated the proteotoxic stress in diabetic hearts and attenuated cardiac dysfunction. Our findings suggest that insufficient proteasome function is a novel mechanism underlying the development of diabetic cardiomyopathy.

2. Methods

2.1. Animals

The transgenic mouse models expressing a tetracycline-controlled transactivator protein (tTA), PA28α or GFPdgn were previously described [17]. These mice were maintained in the FVB/N inbred background for our studies. Eight- to 10-week-old male mice were injected intraperitoneally with streptozotocin (STZ, 50 mg/kg, Sigma-Aldrich) for five consecutive days to induce type I diabetes. Animals with fasting blood glucose levels greater than 250 mg/dL at day 7 post the first STZ injection were deemed diabetic and included in the studies. All animal experiments were approved by the Augusta University Institutional Animal Care and Use Committee.

2.2. Immunoblotting, fluorescence confocal microscopy and histology staining

Proteins were extracted from ventricular myocardium tissues or cultured cells and subjected to SDS-PAGE as previously described [21]. Membranes were incubated overnight at 4 °C with antibodies against GFP (sc-9996, 1:5000, Santa Cruz), ubiquitin (#04-263, 1:1000, Sigma-Aldrich), K48-ubiquitin (#8081, 1:1000, Cell Signaling Technology), GAPDH (SAB1405848, 1:6000, Sigma-Aldrich), β-tubulin (E7, 1:5000, Developmental Studies Hybridoma Bank) and PA28α (customized [22]).

Fluorescence confocal microscopy was performed as described previously [21]. Direct GFP fluorescence on O.C.T.-embedded ventricular myocardium sections (5 μm) was visualized using a Zeiss LSM 510 upright confocal microscope (Zeiss). The images were captured with the same PMT voltage setting among groups and digitalized using the associated software.

Histological analyses were performed using 5-μm paraffin-embedded ventricular myocardium sections. Cardiac fibrosis was assessed by Masson's Trichrome staining using the Trichrome Stain kit (American MasterTech). Data were quantified by Image-Pro Plus (Media Cybernetics).

2.3. Determination of proteasome peptidase activity

Proteasome peptidase activity was quantified as previously described [23]. Briefly, myocardial tissues were homogenized at 4 °C in 10 volumes of HEPES buffer (50 mmol/L, pH 7.5). The supernatants of the homogenates were immediately used for protein concentration assay followed by determination of peptidase activities. The following synthetic fluorogenic peptides: Suc-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin (AMC) (18 μmol/L, Enzo Life Sciences), *Z*-Leu-Leu-Glu-AMC (45 μmol/L, Enzo Life Sciences), and Ac-Arg-Leu-Arg-AMC (40 μmol/L, Enzo Life Sciences) were used respectively for measuring chymotrypsin-like, caspase-like, and trypsin-like peptidase activities in the absence or presence of a proteasome inhibitor, MG-132 (20 μmol/L, for chymotrypsin-like and caspase-like activity) or Epoxomicin (5 μmol/L, for trypsin-like activity), and in the absence or presence of ATP (28 μmol/L), respectively. Measurements of each specimen were performed in triplicates. Twenty micrograms of crude protein extracts were added to 200 μL of the HEPES buffer containing the fluorogenic substrate to each well in 96-well plates, and incubated at 37 °C. The fluorescence intensity was measured after 60 min of incubation using a Synergy H4 plate reader (Bio-Tek) with excitation wave length of 380 nm and emission wave length at 460 nm. The portion of peptidase activity inhibited by the proteasome inhibitor was attributed to the proteasome.

2.4. Pressure-volume loop analysis

For the invasive assessment of left ventricular (LV) pressure–volume (P-V) relationship, mice were anesthetized with isoflurane (2%) in medical grade oxygen, intubated and artificially ventilated. Body temperature was monitored with a rectal thermometer and maintained at 37 °C. Retrograde catheterization of the LV was performed via the right carotid artery with a 1.2-F mouse P-V catheter (Millar). The instrumented animal was stabilized for 10 min, data were recorded at a sampling rate of 1500 Hz during steady-state conditions, and P-V loops were constructed using LabChart8 (ADInstruments). The raw conductance volumes were corrected for parallel conductance by the hypertonic saline bolus.

2.5. RNA preparation and quantitative real-time polymerase chain reaction (qRT-PCR)

Isolation of total RNA and reverse transcription into single-stranded cDNA were performed as previously described [21]. Gene expression levels were measured in triplicate per biological sample by real-time PCR (StepOnePlus Real-Time PCR system, Life Technologies) using the SYBR-Green assay with gene-specific primers at a final concentration of 200 nM. The following primers were used: GFP-forward: GGGCACAAGCTGGAGTACAACT, GFP-reverse: ATGTTGTGGCGGATCTTGAAG. Relative gene expression was calculated using the $2^{-\Delta\Delta ct}$ method against a mouse house-keeping gene hypoxanthine guanine phosphoribosyl transferase (Hprt).

2.6. Terminal dUTP nick end-labeling (TUNEL) assays

Myocardial cryosections were fixed with 4% paraformaldehyde and rinsed with cold PBS. TUNEL staining was performed using the In Situ

Download English Version:

https://daneshyari.com/en/article/5533594

Download Persian Version:

https://daneshyari.com/article/5533594

Daneshyari.com