Journal of Molecular and Cellular Cardiology 97 (2016) 56–66

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original Article

DJ-1 protects the heart against ischemia–reperfusion injury by regulating mitochondrial fission

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ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form 2 April 2016
Accepted 16 April 2016
Available online 22 April 2016

Keywords:
DJ-1
Myocardial ischemia
Mitochondrial fission
SUMOylation

ABSTRACT

Recent data indicates that DJ-1 plays a role in the cellular response to stress. Here, we aimed to examine the underlying molecular mechanisms mediating the actions of DJ-1 in the heart following myocardial ischemia–reperfusion (I/R) injury. In response to I/R injury, DJ-1 KO mice displayed increased areas of infarction and worsened left ventricular function when compared to WT mice, confirming a protective role for DJ-1 in the heart. In an effort to evaluate the potential mechanism(s) responsible for the increased injury in DJ-1 KO mice, we focused on SUMOylation, a post-translational modification process that regulates various aspects of protein function. DJ-1 KO hearts after I/R injury were found to display enhanced accumulation of SUMO-1 modified proteins and reduced SUMO-2/3 modified proteins. Further analysis, revealed that the protein expression of the de-SUMOylation enzyme SENP1 was reduced, whereas the expression of SENP5 was enhanced in DJ-1 KO hearts after I/R injury. Finally, DJ-1 KO hearts were found to display enhanced SUMO-1 modification of dynamin-related protein 1, excessive mitochondrial fission, and dysfunctional mitochondria. Our data demonstrates that the activation of DJ-1 in response to myocardial I/R injury protects the heart by regulating the SUMOylation status of Drp1 and attenuating excessive mitochondrial fission.

1. Introduction

DJ-1, also knows as Park7 (Parkinson's Disease autosomal recessive, early onset 7), is an evolutionarily conserved 189-amino acid protein [1]. Although originally identified as an oncogene [2], it is now recognized that DJ-1 promotes cytoprotection in response to various pathological stimuli. For instance, in the brain, the deletion or loss of function of DJ-1 is associated with autosomal recessive, early-onset Parkinson's Disease [3]. Additionally, in vitro and in vivo models show that over-expression of DJ-1 protects cells against oxidative stress-induced injury, whereas knockdown or knockout of DJ-1 increases susceptibility to oxidative injury in models of cerebral ischemia and neuronal cell death [4,5]. In regard to mechanisms of action, these studies indicate that DJ-1 plays an important role in multiple cellular processes, including oxidative stress response, protein quality control, anti-apoptotic signaling, and transcriptional regulation [1,5,6]. Due in large part to the association of DJ-1 with Parkinson's Disease, most studies aimed at investigating its role in response to pathological stimuli have been confined to the brain or neuronal cells. However, DJ-1 is expressed in many other tissues, including the heart [7]. Two recent studies report that mice deficient in DJ-1 develop more severe heart failure in response to aortic banding [8] and display exaggerated myocardial injury in response to ischemia [9]. While these studies provide evidence that DJ-1 plays a protective role in the heart, its mechanism of action remains unclear.

Post-translational modifications (PTMs) are essential for controlling the function and stability or proteins. As such, PTMs regulate cell fate under physiological and pathological conditions. SUMOylation is a PTM process in which small ubiquitin-like modifier (SUMO, also called Sentrin) proteins are covalently and reversibly conjugated to target proteins [10]. In recent years, SUMOylation has been shown to regulate and influence a number of cellular processes, including cell cycle regulation, apoptosis, epigenetic regulation, and transcription [11]. It has also been reported to play a role in several disease states, such as cancer and cerebral ischemia [12]. In the heart, SUMOylation contributes to normal cardiac development and function [13]. It also plays a role in the adaptation of the heart to pathological stress [14,15]. Mammalian cells express

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http://dx.doi.org/10.1016/j.yjmcc.2016.04.008
0022-2828/

three isoforms of SUMO that can be covalently conjugated to proteins: SUMO-1, SUMO-2, and SUMO-3 [16]. Because SUMO-2 and SUMO-3 are nearly homologous (~97% identical) and cannot be distinguished from each other under most contexts, they are collectively referred to as SUMO-2/3. In contrast, SUMO-1 shares very little homology to SUMO-2/3 (~47% identical) [13]. Whereas SUMO-1 and SUMO-2/3 share some overlap in the modifications of certain proteins, each has a distinct pool of targets, suggesting that they may play different role in cellular processes [13,17]. A key feature of protein SUMOylation is its reversibility by a family of Sentrin/SUMO-specific proteases (SENPs), whose activity is relatively specific for distinct SUMO proteins [18]. For instance, SENP1 targets all SUMO isoforms for deconjugation, whereas SENP5 preferentially targets SUMO-2/3 isoforms [19]. Importantly, cellular homeostasis is dependent on balancing SUMOylation with de-SUMOylation as evidenced by studies demonstrating that tipping the balance either way results in aberrant signaling and pathological conditions [14,15]. Therefore, understanding the cellular mechanisms by which the SUMO machinery is regulated under physiological and pathological conditions has become an important area of research.

DJ-1 has been implicated as a cellular inhibitor of SUMO-1 modifications in human dopaminergic cell lines [10]. However, the mechanism(s) responsible for this inhibition are not known and it is not known if DJ-1 alters SUMO modifications in vivo. Moreover, the cellular mechanisms that underlie the reported cardioprotective actions of DJ-1 remain largely unknown. To clarify some of these issues, we examined the actions of DJ-1 in the heart using an established in vivo murine myocardial I/R model.

2. Materials and methods

2.1. Animals

Male mice with a global deficiency in DJ-1 (B6·Cg-*Park7*^{tm1Shn}/J; DJ-1 KO) maintained on a C57BL/6J background, were purchased from Jackson Labs (Bar Harbor, ME). In all experiments, Wild-Type (WT) littermates were used as controls. All experimental protocols were approved by the Institute for Animal Care and Use Committee at Emory University School of Medicine and conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996), and with federal and state regulations.

2.2. Materials

Mitochondrial division inhibitor, Mdivi-1 (Sigma Aldrich; St. Louis, MO) was dissolved in di-methyl sulfoxide (DMSO) and given as intraperitoneal injection at a dose of 50 mg/kg (final volume 50 μL) 15 min before the onset of myocardial ischemia. DMSO was administered in the same manner for the vehicle group.

2.3. Myocardial I/R protocol and myocardial injury assessment

Surgical ligation of the left coronary artery, myocardial infarct size determination, Troponin-I measurements, echocardiography and invasive hemodynamics were performed similar to methods described previously [20,21].

2.4. Subcellular fractionation, Western blot analysis

Whole cell and mitochondrial fractions were obtained from the hearts excised from separate groups of mice. Whole cell homogenates were obtained as previously described [20]. Mitochondrial fractions were obtained using the Mitochondria Isolation Kit (MITOISO1, Sigma). Western blot analysis was performed as described previously [20].

2.5. Immunoprecipitation

Heart homogenates were immunoprecipitated with antibodies to either DJ-1 or dynamin-related protein 1 (Drp1) using the Dynabeads® Protein G Immunoprecipitation Kit according to the manufacturer's instructions. The samples were then subjected to standard Western blot techniques and the membranes probed with antibodies to SENP1, SENP5, SUMO-1, or SUMO-2/3.

2.6. Isolation of mRNA and Taqman qPCR

RNA was isolated using the RiboPure kit according to the manufacturer's instructions (Ambion). Reverse transcription was performed in a standard fashion with QuantiTect Reverse Transcription Kit (QIAGEN) supplemented with DNase treatment. Taqman qPCR was carried out according to the manufacturer's instructions using probe sets for senp1 and senp5. Analysis was carried out using the ΔΔ-CT method with 18S correction and reported as relative fold change versus WT Sham.

2.7. Electron microscopy

Heart tissue was dissected along the muscle fiber while immersed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2). Samples were stored in the same fixative overnight at 4 °C. Samples were washed with the same buffer and post-fixed in 1% buffered osmium tetroxide, dehydrated through a graded ethanol series to 100%, and embedded in Eponate 12 resin. Ultrathin sections were cut on a Leica UC6rt ultra-microtome at 70–80 nm and counter-stained with 4% aqueous uranyl acetate and 2% lead citrate. Sections were examined using a Hitachi H-7500 transmission electron microscope equipped with a Gatan BioScan CCD camera.

2.8. Myocyte isolation and immunohistochemistry

Adult cardiomyocytes were isolated and fixed as previously described [22]. Cardiomyocytes were stained with primary antibodies to α-MHC and DJ-1 followed by incubation with secondary antibodies. Coverslips were mounted using Vectashield H-1500-4′,6-Diamidino-2-phenylindole (DAPI)-containing medium (Vector Laboratories). Images were acquired on a Leica DM6000.

2.9. Neonatal cardiomyocyte isolation and siRNA transfection

Neonatal rat ventricular cardiomyocytes (NRVMs) were isolated from 1- to 2-day-old neonatal rat pups and cultured as a monolayer as described previously [23]. The entire ventricles were excised without contaminating atrial myocytes. After 24 h of culture, monolayers of NRVMs were transfected with siRNA (10 μM) against: DJ-1 (Ambion, ID:s128737), SENP5 (Ambion, ID:s192872), both DJ-1 and SENP5, or scrambled sequence (negative control) (Ambion, cat#4390843). Transfections were carried out with Lipofectamine 2000 (Life Technologies, Carlsbad, CA) by incubating the NRVMs with the respective siRNA for one day. Afterwards, media containing the transfecting siRNA agent was removed from the cell monolayers, washed twice with cold PBS, and replenished with fresh 2% FBS-containing NRVM media. For all in vitro experiments, NRVMs were plated at a density of 210,000 cells per each cm^2 of surface area. Unless indicated otherwise, the NRVMs were plated and transduced in 96-well plates. Transfections were performed in a routine NRVM culture media, based on M199 with the following components: 10 mM HEPES, 0.1 mM non-essential amino acids, 3.5 mg/ml glucose, 2 mM L-glutamine, 4 μg/ml vitamin B12, 100 U/ml penicillin and heat-inactivated FBS at 10% (first two days of culture) or 2% (after two days of culture) final concentration.