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14-3-3 protein regulates Ask1 signaling and protects against diabetic cardiomyopathy

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ABSTRACT

Mammalian 14-3-3 proteins are dimeric phosphoserine-binding proteins that participate in signal transduction and regulate several aspects of cellular biochemistry. Diabetic cardiomyopathy is associated with increased oxidative stress and inflammation. In order to study the pathogenic changes underlying diabetic cardiomyopathy, we examined the role of 14-3-3 protein and apoptosis signal-regulating kinase 1 (Ask1) signaling by using transgenic mice with cardiac-specific expression of a dominant-negative 14-3-3η protein mutant (DN 14-3-3η) after induction of experimental diabetes. The elevation in blood glucose was comparable between wild type (WT) and DN 14-3-3η mice. However, a marked downregulation of thioredoxin reductase was apparent in DN 14-3-3η mice compared to WT mice after induction of diabetes. Significant Ask1 activation in DN 14-3-3η after diabetes induction was evidenced by pronounced de-phosphorylation at Ser-967 and intense immunofluorescence observed in left ventricular (LV) sections. Echocardiographic analysis revealed that cardiac functions were notably impaired in diabetic DN 14-3-3η mice compared to diabetic WT mice. Marked increases in myocardial apoptosis, cardiac hypertrophy, and fibrosis were observed with a corresponding up-regulation of atrial natriuretic peptide and galectin-3, as well as a downregulation of sarcoendoplasmic reticulum Ca^{2+} ATPase2 expression. Furthermore, diabetic DN 14-3-3η mice displayed significant reductions of platelet-endothelial cell adhesion molecule-1 staining as well as endothelial nitric acid synthase and vascular endothelial growth factor expression. In conclusion, our data suggests that enhancement of 14-3-3 protein could provide a novel therapeutic strategy against hyperglycemia-induced left ventricular dysfunction and can limit the progression of diabetic cardiomyopathy by regulating Ask1 signaling.

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1. Introduction

Diabetic cardiomyopathy is now well documented and is characterized by left ventricular (LV) remodeling, which involves both diastolic and systolic dysfunction [1,2]. The development of diabetic cardiomyopathy is multifactorial and regulated by dynamic and complex mechanisms on cellular and molecular levels [3]. Hyperglycemia-induced oxidative stress is a major risk factor for the development of microvascular pathologies in the diabetic myocardium that result in myocardial cell death, hypertrophy, fibrosis, abnormalities of calcium homeostasis and endothelial dysfunction [2–4]. However, the possible molecular/genetic mechanisms involved in diabetic cardiomyopathy are not well characterized; translational studies with transgenic animals are limited and only partly explain the mechanisms of cardiomyopathy and heart failure in diabetic patients.

14-3-3 protein belongs to a class of highly conserved proteins involved in regulating apoptosis, adhesion, cellular proliferation, differentiation, survival, and signal transduction pathways [5]. Apoptosis signal-regulating kinase 1 (Ask1), a mitogen-activated protein kinase (MAPK) kinase kinase, is involved in biological responses such as apoptosis, inflammation, differentiation and survival in different cell types. Activated Ask1 relays signals to c-jun NH_2 kinase (JNK) and p38 MAPK [6,7]. Recent evidence suggests that oxidative stress activates Ask1 by dissociating its inhibitor, 14-3-3 protein, from Ask1 Ser-967 in Cos7 cells [8]. 14-3-3 protein and thioredoxin are reported to limit Ask1 activity by guarding the C-terminal and N-terminal of Ask1 kinase, respectively [9]. Moreover, Ask1 is now emerging as a potential target for cardiac diseases [10]; hence the specific role of Ask1 in the development of diabetic cardiomyopathy should be examined. We have recently reported that transgenic mice with cardiac-specific expression of a dominant-negative mutant of 14-3-3η protein (DN 14-3-3η) exhibit enhanced cardiomyocyte apoptosis, hypertrophy, and fibrosis after induction of experimental diabetes [11,12]. However, the specific involvement of the Ask1 signaling pathway in the development of diabetic cardiomyopathy has been not directly demonstrated. Based on our previous findings, we postulated that DN 14-3-3η mice could provide a model for the investigation of Ask1 signaling in the streptozotocin (STZ)-induced cardiac remodeling process.

We here demonstrate that 14-3-3 protein acts as an endogenous cardioprotector and limits the development of diabetic cardiomyopathy by limiting myocardial apoptosis, hypertrophy, fibrosis, and endothelial dysfunction via inhibition of Ask1 activation after induction of experimental diabetes.

2. Materials and methods

2.1. Generation of DN 14-3-3η transgenic mice

Transgenic DN 14-3-3η mice were generated as described previously [13]. Briefly, the coding region of human DN (R56A and R60A) 14-3-3η cDNA with a 5′-Myc-1 epitope tag was subcloned into a vector containing the α-myosin heavy chain promoter and an SV40 polyadenylation site. Linearized DNA was injected into the pronuclei of one-cell C57BL/6 XSJL embryos at the Neuroscience Transgenic Facility at Washington University School of Medicine. Progeny were backcrossed into the C57BL/6 genetic background and were analyzed by polymerase chain reaction to detect transgene integration using mouse-tail DNA as template. Age-matched C57BL/6 JAX mice (obtained from Charles River Japan Inc., Kanagawa, Japan) were used as wild type (WT) controls.

2.2. Diabetes induction

Diabetes was induced by a single intraperitoneal injection (150 mg/kg) of STZ (Sigma–Aldrich Inc., St. Loius, USA) dissolved in vehicle (20 mM sodium citrate buffer, pH 4.5) to 8–10-week-old male WT and DN 14-3-3η mice. Age-matched WT and DN 14-3-3η mice were injected with 100 μl of citrate buffer and used as non-diabetic controls. Animals were studied twice after streptozotocin administration, i.e. on day 3 for acute measurement and on day 28 for chronic measurement. Mice were maintained with free access to water and chow throughout the period of study, and animals were treated in accordance with the guidelines for animal experimentation of our institute.

2.3. Blood glucose measurement and survival rate

Blood glucose levels of animals were measured at 0, 1, 3, 7 and 28 days after STZ injection. Blood glucose level was determined using Medi-safe chips (Terumo Inc., Tokyo, Japan). Four separate groups comprised of control WT mice (vehicle treated, $n = 10$), diabetic WT mice (STZ treated, $n = 14$), control DN 14-3-3η mice (vehicle treated, $n = 10$) and diabetic DN 14-3-3η mice (STZ treated, $n = 12$) were utilized for Kaplan–Meier survival analysis.

2.4. Transthoracic echocardiography

Two-dimensional echocardiography studies were performed in anesthetized mice (pentobarbital, 50 mg/kg, i.p.) to evaluate cardiac function using an echocardiographic machine with 7.5 and 12 MHz transducers linked to an ultrasound system (SSD-5500; Aloka, Tokyo, Japan). The short-axis view of the LV was recorded to measure the LV dimension in systole (LVDs) and diastole (LVDd) as well as the percent fractional shortening (% FS). Hearts were harvested for analysis from control and diabetic mice. The LV was quickly dissected and cut into two parts. One part was immediately transferred into liquid nitrogen and then stored at −80 °C for protein analysis. The other part was either stored in 10% formalin or stored at −80 °C after the addition of Tissue-Tek OCT compound (Sakura Co. Ltd., Tokyo, Japan) for histopathological and immunohistochemical analysis.

2.5. Protein analysis

Protein lysate was prepared from heart tissue as described previously [11]. The total protein concentration in samples was measured by the bicinchonic acid method. For western blotting experiments, 100 μg of total protein was loaded and proteins were separated by SDS-PAGE (200 V for 40 min) and

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