ELSEVIER

Pathophysiology 21 (2014) 147–151

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Akt1 expression and activity at different stages in experimental heart failure

L. Kapustian [a,*], I. Kroupskaya [a], O. Rozhko [a], V. Bobyk [a], D. Ryabenko [b], L. Sidorik [a]

[a] *Institute of Molecular Biology and Genetics, NAS of Ukraine, 150, Zabolotnogo Str., Kyiv 03680, Ukraine*

[b] *National Scientific Center "M. D. Strazhesko Institute of Cardiology, MAS of Ukraine", 5, Narodnogo Opolchenya Str., Kyiv 03151, Ukraine*

Received 28 October 2013; received in revised form 16 November 2013; accepted 19 November 2013

Abstract

Loss of function or/and death of cardiomyocytes is one of the major contributing factors in the development of heart failure. Cytosolic Hsp60 can directly interact and regulate activation of some kinases and sequestrate certain proapoptotic molecules to avoid the cardiomyocyte apoptosis. We assumed that Akt1 kinase, a downstream effector of PI3 kinase, can interact with Hsp60. Our aim was to clarify the interaction of Akt1 and Hsp60 and to investigate the Akt1 expression in normal and failing hearts in acute and chronic stress. The experimental mouse models of inducible myocarditis and DCM-like pathology were developed in our laboratory. Akt1 and phospho-Akt1 (pS473) expression were studied by Western blot analysis. Co-immunoprecipitation method was used to test complex formation of Akt1 and Hsp60. The interaction of Hsp60 and Akt1 was detected for the first time by co-immunoprecipitation method in normal myocardium and under pathology as well. There were no significant changes in the level of Akt1 expression in both myocardia. At the same time we observed significant decrease in Akt1 phosphorylation at the final stage of DCM-like pathology but not at experimental myocarditis. The final stage of heart failure in mouse model of DCM-like pathology was characterized by reduced level of phospho-Akt1/Akt1 (pS473; −26%; $P < 0.05$), whereas no differences were found in total Akt1 protein content. We suggest a possible involvement of cytoplasmic Hsp60 in regulation of Akt1 activity at heart failure progression.

Keywords: Akt kinase; Hsp60; Heart failure; DCM; Myocarditis; Mice

1. Introduction

Congestive heart failure (CHF) is a significant medico-social problem in the world. The prevalence of CHF is increasing worldwide, and the prognosis of such patients remains pessimistic [1]. Dilated cardiomyopathy (DCM) is common heart disease and leads to heart failure. Etiology of DCM is unknown; the contributory factors are thought to be prior viral infections, cardiac specific autoantibodies, toxic agents, genetic factors, sustained alcohol consumption and other kinds of chronic stress. Some investigators consider that in many cases DCM is consequence of myocarditis progression [2]. Death of cardiomyocytes or their impaired functioning is thought to be the major contributing factor in the development of DCM. The understanding of the mechanisms implicated in cardiomyocyte cell death is needed for the development of successful treatment.

Many signal transduction cascades which control cell cycle, homeostasis and apoptosis are dependent on the assistance by molecular chaperones, which can maintain their target proteins in active or inactive conformations. Molecular chaperone Hsp60 is one of the highest scientific interests due to lately revealed its antiapoptotic function in the cytoplasm of cardiomyocytes. Hsp60 is primarily a mitochondrial protein, but significant amount of it (25–30%) is extramitochondrial [3].

Previously we have observed an increase in total Hsp60 level in human DCM hearts as well as in the hearts of mice affected with experimental DCM-like pathology [4]. We observed the increase in Hsp60 level in mitochondria and decrease in cytoplasm, which could be one of the reasons for apoptotic response in cardiomyocytes at heart failure associated with chronic stress. Cytosolic form of Hsp60 was demonstrated to sequestrate proapoptotic molecules Bax and

* Corresponding author at: Laboratory of Molecular Mechanisms of Autoimmunity, Institute of Molecular Biology and Genetics, NAS of Ukraine, 150, Zabolotnogo Str., Kyiv 03680, Ukraine.
Tel.: +380 44 526 55 89; fax: +380 44 526 07 59.
E-mail address: lyuda-kap@mail.ru (L. Kapustian).

0928-4680/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pathophys.2013.11.005

Bak avoiding apoptosis and promoting cardiomyocyte survival [3]. In addition Hsp60 can directly interact and regulate activation of some kinases [5,6].

Akt, a serine-threonine kinase, is a critical enzyme in signal transduction pathways involved in multiple cellular processes. There are three Akt isoforms in mammals – Akt1, Akt2 and Akt3. In the heart, Akt1 activation has been demonstrated to increase cell size and decrease apoptosis and it is thought to be beneficial for the failing heart. The role of Akt1 in regulation of cell survival and apoptosis in experimental models of heart failure has been a major recent interest.

The aim of our study was to investigate the Akt1 expression in the heart in murine experimental myocarditis and DCM-like pathology as models of acute and chronic heart failure. We also assumed possible interaction of Hsp60 and Akt1 kinase, which was previously reported to bind to another molecular chaperone Hsp90 [7].

2. Materials and methods

2.1. Antibodies

Polyclonal anti-Akt1and anti-phospho-Akt1 (pS473) antibodies produced in rabbit were obtained from company Sigma (USA). The polyclonal anti-GroEL antibody produced in rabbit and polyclonal anti-GAPDH antibody produced in mouse were developed in our laboratory. Immunologic cross-reactivity of GroEL with Hsp60 was demonstrated previously [8].

2.2. Animal models

Myosin-induced myocarditis was developed in BALB/c mice as described previously [9] with modifications. Experimental model of autoimmune myosin-induced injury similar to human DCM was developed in BALB/c mice as described previously [10]. There were 3–5 animals in every experimental group.

2.3. Immunoprecipitation

Myocardium cell lysates were obtained by homogenization in buffer containing 20 mM Tris HCl (pH 7,5), 150 mM NaCl, 5 mM EDTA, 0.1% protease inhibitor cocktail (Sigma, USA). Supernatant obtained after 20 min centrifugation at $20{,}000 \times g$ was used as total cell lysate which was incubated with polyclonal anti-GroEL(Hsp60) antibody (5 μg of antibodies for 6 mg of total protein) at 4 °C overnight. Then 30 μl of protein A-Agarose beads (Sigma, USA) were added and incubation was continued for 1.5 h at 4 °C. Immunocomplexes were collected by centrifugation and washed 4 times with cold buffer (137 mM NaCl, 20 mM Tris–HCl, pH 7.5, 1% triton X-100, 2 mM EDTA, pH 8.0, 2 mM PMSF). The final products were briefly boiled with SDS-PAGE sample buffer and analyzed by SDS-PAGE and Western-blotting with specific antibodies.

2.4. Western blot analysis

Heart samples were prepared by homogenization in ice-cold RIPA buffer (20 mM Tris–HCl, pH 7.5; 0.15 M NaCl; 1 mM EDTA; 1% NP-40; 1% sodium deoxycholate; 0.1% SDS and 0.1% protease inhibitor cocktail (Sigma, USA)). After 30 min extraction at 4 °C, the samples were centrifuged at $10{,}000 \times g$ for 20 min at 4 °C. The supernatants were collected and the protein concentration was determined by Bradford assay. Protein samples (80 μg per well) were electrophoresed in 12% SDS polyacrylamide gel. The semi-dry electrotransfer of proteins to the nitrocellulose membranes was held at 180 mA for 45 min. The membranes were blocked for 1 h at room temperature with blocking buffer (5% non-fat milk in PBST buffer) and then probed with specific primary antibodies overnight at 4 °C. After washing in PBST buffer (10 min × 3) the membranes were incubated with peroxidase-conjugated secondary anti-rabbit or anti-mouse (in case of GAPDH) antibodies for 1 h at room temperature. The treatment of the membranes with secondary antibodies was followed by washing in PBST buffer (10 min × 3) and chemiluminescence detection according to manufacturer's instructions (Pierce). Then the membranes were exposed to autoradiography film (Agfa, Belgium) for 0.5–1 min. Digital images of immunoblots were analyzed using densitometric scanning analysis program TotalLab Quant.

GAPDH was used as internal control of protein expression. The level of Akt1 was calculated as the ratio of protein values (Akt1 to GAPDH) and presented as relative units. The level of phospho-Akt1 (pS473) was calculated as the ratio of protein values (phospho-Akt1 to Akt1) and presented as relative units.

2.5. Statistical analyses

Statistical analyses were performed using STATISTICA 8.0 (StatSoft Inc. 2007, USA). Data were analyzed using Mann–Whitney *U*-test. A $P < 0.05$ was considered significant.

3. Results and discussion

Cardiomyocyte loss via necrotic, apoptotic, and autophagic cell death is thought to be one of key processes in initiation and progression of heart failure, worsening in cardiac contractile function and left ventricular remodeling. It can be induced by different kind of stresses like ischemia-reperfusion, hypoxia, anticancer drugs, etc.

Molecular chaperones are known as cardioprotective proteins, playing a role of key regulators of apoptosis or survival of cardiomyocytes [11]. Molecular chaperone Hsp60, the main functional compartments of which are mitochondria,