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Rapid Communication

MK2$^{-/-}$ gene knockout mouse hearts carry anti-apoptotic signal and are resistant to ischemia reperfusion injury

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Abstract

Stress-induced mitogen-activated protein (MAP) kinases have been implicated in various forms of cardiovascular diseases. Ischemia/reperfusion potentiates activation of p38 MAP kinase (p38MAPK) leading to the activation of its downstream target MAPKAP kinase 2 (MK2). While p38MAPK has been shown to induce pro-apoptotic signal, whether MK2 also generates death signal is not known. To determine if MK2 triggers death signal, the hearts of MK2$^{-/-}$ knockout mice and genetically matched wild-type mice were subjected to 30 min ischemia followed by 2 h of reperfusion via Langendorff mode. The results indicated that the hearts of MK2$^{-/-}$ mice were resistant to myocardial ischemic reperfusion injury as evidenced by enhance recovery of post-ischemic ventricular performance, reduced myocardial infarct size and diminished number of apoptotic cardiomyocytes. We conclude that MK2, similar to p38MAPK, is involved in transmitting the death signal to the ischemic myocardium.

Keywords: p38 MAP kinase; MAPKAP kinase 2; MK2$^{-/-}$ knockout mice; Apoptosis; Ischemia/reperfusion; Heart

1. Introduction

Stress-induced mitogen-activated protein (MAP) kinases have been implicated in various forms of cardiovascular diseases including heart failure, ischemia/reperfusion and cardiac hypertrophy. For example, p38 MAP kinase (p38MAPK) activities were significantly elevated in mouse hearts after chronic transverse aortic constriction, coincident with the onset of cardiac hypertrophy [1]. Consistent with these results, over expression of MKK3bE, upstream activators of p38MAPK, resulted in increased cardiomyocyte apoptosis. Activation of p38MAPK was shown to be responsible for the development of ventricular end-systolic remodeling and restrictive cardiomyopathy [2]. In heart tissue, ischemia-induced p38MAPK activation in concert with increased DNA laddering [3]. In another related study, p38MAPK was activated by low-flow ischemia in the porcine heart [4].

MAPKAP kinase 2 (MK2) is the downstream target for p38MAPK; and MK2 is activated in response to p38 activation [5]. MK2 is stress-sensitive and can also be activated by environmental stresses including sodium arsenate, heat shock and osmotic stress [6]. In the p38MAPK pathway, MK2 serves both as an effector of p38 phosphorylating substrates and as a determinant of cellular localization of p38MAPK [7].

While, activation of p38MAPK has been linked with the apoptotic signal, it is not known if activation of MK2 can also trigger such a death signal. In the present study, we have used MK2$^{-/-}$ mouse hearts to determine the role of MK2 in ischemia/reperfusion-induced death signal. The results indicated that the MK2$^{-/-}$ mouse heart was less susceptible to ischemia/reperfusion injury, demonstrating for the first time a definitive role of MK2 in generating ischemia-induced cardiomyocyte apoptosis.

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2. Materials and methods

2.1. Development of MK2$^{-/-}$ mouse

The MK2$^{-/-}$ mice were generated at Martin Luther University, Hallem, Germany [8]. Breeding pairs of MK2$^{-/-}$ mice were transferred to the University of Connecticut Health Center where they were available for our use.

2.2. Murine ex vivo isolated Langendorff-perfused heart

Wild-type or MK2$^{-/-}$ mice weighing about 25–30 g were anesthetized with sodium pentobarbital (150 mg/kg, Abbott Laboratories, North Chicago, IL) and anticoagulated with heparin (sodium salt, 1000 U/kg, i.p. Elkins-Sinn Inc., Cherry Hill, NJ). After ensuing adequate depth of anesthesia, thoracotomy was performed; the heart was excised, and immediately immersed in ice-cold perfusion buffer. The aortic arch was quickly isolated and incised [9]. The aorta was cannulated and retrograde perfusion in the Langendorff mode through the aortic cannula was initiated at a perfusion pressure of 85 mmHg. The perfusion buffer used in this study consisted of a modified Krebs–Henseleit bicarbonate buffer ([KHB composed of (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, 1.7 $CaCl_2$, gassed with 95% O_2, 5% CO_2], filtered through a 5-mm filter to remove any particulate contaminants, pH 7.4) that was maintained at a constant temperature at 37 °C and was gassed continuously for the entire duration of the experiment.

For measurement of left ventricular systolic and diastolic pressures and, by difference, left ventricular developed pressure (LVDP), a fluid-filled balloon catheter connected to a pressure transducer was inserted into left ventricular through a mitral valve. The balloon was inflated until a left ventricular end-diastolic pressure (LVEDP) of between 4 and 8 mmHg was obtained and balloon was fitted to left ventricular wall. The balloon volume was not altered throughout each experiment. The pressure transducer was connected to the heart performance analyzer and pressure data were continuously recorded by acquisition software to calculate heart rate, the first derivative of the left ventricular pressure (LVdP/dt). Each heart was soaked in a water-jacketed chamber filled with perfusate and maintained its temperature at 37 °C throughout the experiment. Coronary flow rate was measured by timed collection using continuous drainage of excess perfusate in the water-jacketed chamber.

The preparation was allowed to attain steady state values of functional parameters, after which the baseline functional data were recorded. The hearts were then subjected to 30 min of global ischemia by arresting buffer flow through the left atrial cannula. The hearts were then reperfused for a period of 2 h.

2.3. Infarct size measurements

At the end of 2 h reperfusion period, hearts were removed from the apparatus, and the atrial tissue was dissected away. The ventricles were either fixed in 10% buffered formalin or were immersed in 1% triphenyl tetrazolium chloride (TTC) solution in phosphate buffer (Na_2HPO_4 88 mM, NaH_2PO_4 1.8 mM) at 37 °C for 10 min [10]. The hearts immersed in TTC were stored at –70 °C for later processing in infarct size calculation.

Frozen hearts were sliced transversely in a plane perpendicular to the apico-basal axis into approximately 0.5 mm thick sections, blotted dry, placed in between microscope slides and scanned on a Hewlett–Packard Scanjet 5p single pass flat bed scanner (Hewlett–Packard, Palo Alto, CA). Using the NIH Image 1.6.1 image processing software, each digitized image was subjected to equivalent degrees of background subtraction, brightness and contrast enhancement for improved clarity and distinctness. Risk as well as infarct zones of each slice were traced and the respective areas were calculated in terms of pixels. The weight of each slice was then recorded to facilitate the expression of total and infarct masses of each slice in grams in order to remove the introduction of any errors due to non-uniformity of heart slice thickness. The individual risk masses and infarct masses of each slice were summed to obtain the risk and infarct masses for the whole heart. Infarct size was expressed as a percentage of the area at risk for anyone heart.

2.4. Detection of cardiomyocyte apoptosis

The hearts kept in formalin were later embedded in paraffin following standard procedures and 3 μm thick transverse ventricular sections were obtain to perform TUNEL assays for the detection of apoptosis [10]. Immunohistochemical detection of apoptotic cells was carried out using TUNEL in which residues of digoxigenin-labeled dUTP are catalytically incorporated into the DNA by terminal deoxynucleotidyl transferase II, an enzyme that catalyzes a template-independent addition of nucleotide triphosphate to the 3′-OH ends double- or single-stranded DNA. The incorporated nucleotide was incubated with a sheep polyclonal anti-digoxigenin antibody followed by a FITC-conjugated rabbit anti-sheep IgG as a secondary antibody (Apop Tag Plus, Oncor Inc0y. The sections were washed in PBS three times, blocked with normal rabbit serum and incubated with mouse monoclonal antibody recognizing cardiac myosin heavy chain Biogenesis Ltd., UK). Followed by staining with TRIRC-conjugated rabbit anti-mouse IgG (200:1 dilution, Dako, Tokyo, Japan). The fluorescence staining was viewed with a confocal laser microscope (Olympus Co., Tokyo, Japan). For the quantitative purpose, the number of TUNEL-positive cardiomyocytes were counted on 100 high power fields (HPF, magnification ×600) from the endocardium through the epicardium of the mid portion of the left ventricular free wall in five sections from each heart.

2.5. Statistical analysis

The values for myocardial functional parameters, risk and infarct volumes, and infarct sizes are all expressed as the mean