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αB-crystallin is phosphorylated during myocardial infarction: Involvement of platelet-derived growth factor-BB

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Abstract

αB-crystallin is the most abundant low-molecular-weight heat shock protein in heart and recent studies have demonstrated that it plays a cardioprotective role during myocardial infarction both in vivo and in vitro. On the other hand, platelet-derived growth factor (PDGF), a potent serum mitogen, has been reported to improve cardiac function after myocardial infarction. In the present study, using a mouse myocardial infarction model, we investigated whether αB-crystallin is phosphorylated during myocardial infarction and the implication of PDGF-BB. Phosphorylation of αB-crystallin at Ser-59 was time dependently induced and plasma PDGF-BB levels were concomitantly increased. Moreover, PDGF-BB-stimulated phosphorylation of αB-crystallin was suppressed by SB203580, a specific inhibitor of p38 mitogen-activated protein (MAP) kinase, in primary cultured cardiac myocytes. Our results indicate that PDGF-BB induces phosphorylation of αB-crystallin via p38 MAP kinase during myocardial infarction.
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The α-crystallins (αA and αB) are major lens structural proteins of the vertebrate eye that are related to the low-molecular-weight heat shock protein (HSP) family. For a while, it has been generally recognized that the α-crystallins are lens-specific. However, reports have indicated that α-crystallins also exist in many other tissues including heart, lung, spinal cord, skin, muscle, brain, kidney, and retina [1–4]. Among the non-lenticular tissues, αB-crystallin is present at high levels in heart [5], where it comprises up to 3% of total myocardial protein [5,6], making it the most abundant low-molecular-weight HSP in heart [3,7,8].

αB-crystallin expression could be induced by heat shock and oxidative stress [9,10]. It is well known to function as a molecular chaperone in protein biosynthesis to facilitate protein folding and translocation [11], and undergoes posttranslational modifications [12]. In human, an R120G missense mutation in the αB-crystallin gene has been reported to cause a desmin-related myopathy [13]. Recent studies have demonstrated that overexpression of αB-crystallin protects cardiomyocytes against ischemic damage [14]. In addition, transgenic mouse hearts overexpressing αB-crystallin are resistant

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to ischemic injury compared to those from wild-type mice [15]. However, the exact mechanism of this cardioprotective effect of αB-crystallin has not been fully elucidated.

On the other hand, platelet-derived growth factor (PDGF) is one of the most potent serum mitogens [16]. PDGF exists as a dimer, having three active isoforms: AA, AB, and BB [17] as well as two inactive isoforms: CC and DD [18,19]. PDGF has an angiogenesis activity and is required for recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation [20]. In addition, PDGF increases the rate of myocardial development [21]. Recent studies have revealed that PDGF improves cardiac function in rodent myocardial infarction models [22,23]. It is shown that the effect of PDGF-BB increasing embryonic cardiac development is greater than PDGF-AA does [21]. In addition, angiogenic and cardiac effects of gene transfer of PDGF-BB after myocardial infarction have been reported [24,25]. Thus, it is of great interest for us to know how PDGF-BB improves cardiac function.

In the present study, we examined the relationship between myocardial infarction and phosphorylation of αB-crystallin, and the involvement of PDGF-BB. We showed that αB-crystallin is phosphorylated during myocardial infarction in vivo. In addition, PDGF-BB induced the phosphorylation of αB-crystallin at Ser-59 via p38 mitogen-activated protein (MAP) kinase in vitro.

Materials and methods

Animals

C57BL/6 mice (SLC, Japan) weighing 25–30 g were used. All experiments were performed in accordance with institutional guidelines.

Reagents

PDGF-BB was obtained from Invitrogen (Carlsbad, CA). Antibodies specifically recognizing αB-crystallin and phosphorylated residue at Ser-59 were prepared as previously described [26]. Antibodies specifically recognizing phosphorylated residue of αB-crystallin at Ser-45 and Ser-19 were purchased from Affinity Bioreagents (Golden, CO, USA) and Stressgen Biotechnologies (Victoria, BC, Canada), respectively. PD98059, SB203580, and SP600125 were purchased from Calbiochem–Novabiochem (La Jolla, CA). An ECL Western blotting detection system was obtained from Amersham Japan (Tokyo, Japan). PD98059, SB203580, and SP600125 were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which did not affect Western blot analysis.

Cell culture

Mouse neonatal ventricular myocytes were prepared as previously described [27], with a minor modification. In brief, apical halves of cardiac ventricles from 1 to 2-day-old mice were separated, minced, and dispersed with 80 U/ml collagenase IV and 0.6 mg/ml pancreatin. The myocytes were then incubated on uncoated 90-mm-diameter dishes for 30 min to remove any nonmyocytes and the nonattached viable cells were seeded into gelatin-coated 60-mm-diameter dishes (2×10^6) in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. The cells were cultured at 37 °C under a humidified atmosphere of 5% carbon dioxide and 95% air. After 5 days, the medium was exchanged for serum-free DMEM. The cells were used for experiments 24 h thereafter.

Myocardial infarction model

Experimental acute myocardial infarction in mice was performed by the ligation of the left anterior descending (LAD) coronary artery under anesthesia with an intraperitoneal injection of pentobarbital at a dose of 44 mg/kg. Mice were placed in a supine position with paws taped to the operating table and the chest wall was shaved. Endotracheal intubation was performed under direct laryngoscopy and mice were ventilated with a small animal respirator (Harvard Model 683; volume 1.0 ml, rate 140 breaths/minute; South Natick, MA). Proper intubation was confirmed by observation of chest expansion and retraction during ventilated breaths. The chest was opened by a lateral cut with tenotomy scissors along the left side of the sternum, cutting through the ribs to approximately midsternum. Ligation was done with an 7-0 silk suture passed with a tapered needle underneath the LAD branch of the left coronary artery a few millimeters from the tip of the normally positioned left auricle. The chest cavity was closed in layers with 5-0 silk, and the mouse was gradually weaned from the respirator. Once spontaneous respiration resumed, the endotracheal tube was removed and the mouse was placed on a heating mat (Model K-20; American Pharmaseal, Valencia, CA). The mice remained in a supervised setting until fully conscious, at which stage they were returned to individual cages and given standard chow and water ad libitum. Mice were euthanized 3, 6, 9, 12, 24, and 48 h after coronary ligation and the hearts were harvested and frozen in liquid nitrogen. Transverse sections of mouse hearts below the nylon suture were prepared.

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