Activation of mitogen-activated protein kinases and p90 ribosomal S6 kinase in failing human hearts with dilated cardiomyopathy

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Abstract

Objective: A new member of the MAP kinase family, big MAP kinase-1 (BMK1), has been recently identified to promote cell growth and attenuate apoptosis. P90 ribosomal S6 kinase (p90RSK), one of the potentially important substrates of extracellular signal regulated kinase (ERK), regulates gene expression in part via phosphorylation of CREB and the Na+/H+ exchanger. Recently, we have demonstrated that the activity of BMK1, Src (the upstream regulator of BMK1) and p90RSK was increased in hypertrophied myocardium induced by pressure-overload in the guinea pig. However, the abundance and activity of these kinases in human hearts are unknown.

Methods: In addition to the three classical MAP kinases (ERK, p38 kinase, and c-Jun NH2-terminal kinase (JNK)), we examined the protein expression and activity of Src, BMK1, and p90RSK in explanted hearts from patients with dilated cardiomyopathy (n=9). Normal donor hearts, which were not suitable for transplant for technical reasons, were used as controls (n=5).

Results: There were no significant differences in the levels of protein expression of these kinases between normal and failing hearts. ERK1/2 and p90RSK were activated in heart failure compared to control (P<0.01 and P<0.03, respectively), while the activity of p38 kinase was decreased (P<0.05) and the activity of JNK was unchanged in heart failure. By contrast, the activities of Src and BMK1 were significantly reduced in end-stage heart failure compared to normal donor hearts (P<0.05).

Conclusion: These data suggest that multiple MAP kinases, p90RSK, and Src are differentially regulated in human failing myocardium of patients with idiopathic dilated cardiomyopathy and may be involved in the pathogenesis of this complex disease.

Keywords: Cardiomyopathy; Heart failure; Protein kinases; Signal transduction

1. Introduction

Heart failure is an increasingly important public health problem with a high mortality rate. Although a variety of metabolic and/or neurohumoral factors are implicated in the progression of this syndrome, the precise mechanisms responsible for this complex condition are poorly understood. Activation of the Gαq signaling pathway, which includes protein kinase C (PKC), appears to play a critical role in the progression of heart failure [1]. Recently, we found that translocation of PKC isoforms from cytosolic to membranous fractions were increased in a conventional animal model of heart failure that was induced by pressure-overload [2,3] and in myocardium from patients with end-stage heart failure [4]. As downstream phosphorylation targets of PKC activation, the mitogen-activated protein (MAP) kinase family plays an important role in cardiac hypertrophy and failure [5]. Four subfamilies of
MAP kinases have been identified, including extracellular signal-regulated kinase (ERK1/2), c-Jun NH₂ terminal kinase (JNK), p38 kinase, and big MAP kinase 1 (BMK1 or ERK5) [5]. BMK1 is a recently identified MAP kinase family member, which shares the TEY activation motif with ERK1/2 but is activated by MEK5 [6]. It has been reported that oxidative stress using H₂O₂ activates ERK1/2. JNK and p38 kinase in isolated coronary-perfused rat hearts [8]. In addition, we recently showed that oxidative stress activates ERK1/2 and BMK1 in coronary-perfused isolated adult guinea pig hearts [9]. Each MAP kinase subfamily may be regulated by different signal transduction pathways that modulate specific cell functions [10]. A potentially important downstream effector of ERK1/2 is p90 ribosomal S6 kinase (p90RSK), which plays a pivotal role in cell growth by activating several transcription factors as well as the Na⁺/H⁺ exchanger (NHE-1) [11].

Although there are considerable data pertaining to MAP kinases in animal models of cardiac hypertrophy and heart failure, data regarding these kinases in the failing human heart are limited, particularly for p90RSK and BMK1. Accordingly, the purpose of this study was to characterize the activity of multiple MAP kinases including BMK1 and p90RSK in explanted failing hearts from patients with idiopathic dilated cardiomyopathy. Of note, we excluded patients with ischemic cardiomyopathy from the present study, since it is known that ischemia itself activates several MAP kinases.

2. Material and methods

2.1. Patient population

Left ventricular myocardium was obtained from nine hearts explanted from patients with end-stage heart failure who were undergoing orthotopic cardiac transplantation (all men, mean age 41±15 years). All patients were diagnosed with New York Heart Association class IV congestive heart failure secondary to idiopathic dilated cardiomyopathy. Non-failing human myocardium was obtained from five donors (four men and one woman, mean age 44±19 years), who had sustained traumatic brain death; these donors had normal cardiac function (as assessed echocardiographically) and had not been prescribed cardiovascular medications. The normal donor hearts were not used for transplantation because of technical reasons, such as death of the recipient, logistic problems, signs of chest trauma after explantation, etc. The investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3).

2.2. Protein preparation

Myocardial samples were immediately frozen in liquid nitrogen after dissection and were stored at −80°C until use [4,12]. The sampling conditions were identical for both failing and non-failing hearts. Fibrotic or adipose tissue, endocardium, epicardium, or great vessels were carefully excised, and remaining tissue was homogenized with 4 vols of ice-cold lysis buffer (50 mM sodium pyrophosphate, 50 mM NaF, 50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 100 μM Na VO₄, 10 mM HEPES, pH 7.4, 1% Triton X-100, 0.1% SDS, 500 μM phenylmethylsulfonyl fluoride (PMSF), and 10 μg/ml leupeptin). The heart homogenates were centrifuged at 14,000×g (4°C for 30 min), and protein concentration was determined using the Bradford protein assay (Bio-Rad, Hercules, CA, USA). Immunoreactive bands were visualized with horse radish peroxidase (KPL laboratories, Gaithersburg, MD, USA). Immunoreactive bands were visualized using enhanced chemiluminescence (ECL, Amersham Life Science).
2.4. Activity for ERK1/2, p38 kinase and Src

To examine phosphorylation of ERK1/2 and p38 kinase, the blots were incubated for 12 h with anti phospho-specific ERK1/2 (mouse anti-human; New England Biolabs, Beverly, MA, USA) and phospho-specific p38 kinase (mouse anti-human; New England Biolabs) antibodies, respectively. We and other investigators have previously reported that immunoblotting with phospho-specific ERK1/2 antibody has a good correlation with an immune complex kinase assay [14,15]. Activity of Src was measured using anti activated-Src antibody clone 28 (H. diomyocytes [5]. To determine the role of p38 kinase and JNK in cultured cardiomyocytes [5]. JNK activity was determined immunologically in cell lysates using an antibody that specifically recognizes activated JNK (H. diomyocytes [5]). We also examined the activity of JNK in failing human hearts with idiopathic dilated cardiomyopathy [5]. To determine the role of p38 kinase and JNK in failing human hearts with idiopathic dilated cardiomyopathy, we examined the protein expression and activity of p38 kinase and JNK as shown in Fig. 2, although neither the protein expression of p38 kinase, JNK1, and JNK2 nor the activity of JNK were significantly different in normal and failing myocardium, the p38 kinase activity (0.4±0.3-fold, P<0.05) was significantly reduced in myocardium from failing hearts.

2.5. p90RSK, JNK, and BMK1 kinase assays

p90RSK kinase activity was measured by glutathione S-transferase (GST)-NHE-1 phosphorylation and BMK1 kinase activity was measured by autophosphorylation as described previously [9]. JNK activity was measured with a commercially available kit (New England Biolabs, Beverly, MA, USA) based on phosphorylation of recombinant c-Jun [9]. We could not detect ERK1/2, p38, p90RSK, or BMK1 in c-Jun fusion protein beads immunoprecipitates, indicating that JNK is the dominant c-Jun kinase present (data not shown).

2.6. Statistical analysis

Data are reported as mean±S.D. Statistical analysis was performed with the StatView 4.0 package (Abacus Concepts, Berkeley, CA, USA). Differences between failing and non-failing hearts were analyzed by un-paired t-test. P values less than 0.05 were considered significant.

3. Results

3.1. ERK1/2 expression and activity: comparison with p90RSK

As determined by quantitative immunoblotting, there were no significant differences in the level of ERK1/2 protein expression between failing human hearts with end-stage dilated cardiomyopathy and non-failing human hearts (Fig. 1A). By contrast, the activity of ERK1/2 was increased in failing hearts with dilated cardiomyopathy compared to non-failing control hearts (4.2±0.1-fold, P<0.01).

We also evaluated p90RSK activation in the same samples, since p90RSK is one of the important downstream substrates of ERK1/2. As shown in Fig. 1B, there was no significant difference in p90RSK protein expression between failing and non-failing hearts. However, p90RSK activity was significantly increased (2.1±0.7-fold, P<0.03) in failing myocardium.

3.2. Determination of p38 kinase and JNK expression and activity

It has been reported that cellular stresses such as hyperosmotic shock, protein synthesis inhibitors (e.g. anisomycin), hypoxia/reoxygenation, and reactive oxygen species activate p38 kinase and JNK in cultured cardiomyocytes [5]. To determine the role of p38 kinase and JNK in failing human hearts with idiopathic dilated cardiomyopathy, we examined the protein expression and activity of p38 kinase and JNK. As shown in Fig. 2, activity (0.4±0.3-fold, P<0.05) was significantly reduced in myocardium from failing hearts.

3.3. Src and BMK1 expression and activity in human failing hearts

We previously demonstrated that Src kinase regulates BMK1 activity in part by oxidative stress in adult guinea pig hearts [8]. Since reactive oxygen species are known to be increased in failing myocardium, we also examined here Src and BMK1 kinases activity. As shown in Fig. 3, Src and BMK1 protein expression was not different between failing and non-failing myocardium. Src activity, which was evaluated by anti-activated Src-clone 28 antibody, was decreased significantly in failing human hearts (0.7±0.2-fold, P<0.05). As shown in Fig. 3B, BMK1 activity was also decreased in failing human hearts compared to non-failing hearts (0.4±0.2-fold, P<0.05).

4. Discussion

In the present study, we examined whether the expression and activity of multiple MAP kinases, p90RSK, and Src were altered in ventricular tissue from failed human hearts with end-stage dilated cardiomyopathy. We found no differences in protein abundance of these kinases between failing and non-failing human hearts. The mean activities of ERK1/2 and p90RSK were significantly greater in failing hearts compared to non-failing donor hearts. In contrast, p38 kinase, Src, and BMK1 activities were reduced in human myocardium with end-stage heart failure. We could not detect any significant changes in JNK activity between failing and non-failing hearts. These data suggest that the MAP kinase family activity is differentially regulated in human dilated cardiomyopathy without any alterations in protein abundance.
Fig. 1. ERK1/2 and p90RSK in failing and non-failing human hearts. (A) ERK1/2 activity in whole heart extracts was measured by Western blot analysis with a phospho-specific ERK antibody (upper). No difference in the amount of ERK1/2 protein was observed in lysates from any of the heart samples by Western blot analysis with anti-ERK1/2 (lower). For densitometric analysis of ERK1/2 activity, results were normalized for all experiments by arbitrarily setting the densitometry of control heart samples (non-failing hearts) to 1.0 (shown is mean ± S.D.). (B) p90RSK activity was measured by an in vitro kinase assay using GST-NHE-1 (625–747) as a substrate. p90RSK protein level was examined by Western blot analysis with anti-p90RSK antibody. Representative autoradiogram showing p90RSK kinase activity (upper panel) and Western blot analysis showing p90RSK protein levels (lower panel). Densitometric analysis revealed that kinase activity of ERK1/2 and p90RSK was significantly increased in explanted human failing hearts compared to normal control hearts. IB, immuno-blotting; * P<0.05.

Cook et al. [16] have reported that ERK1/2 activity was unchanged and p38 kinase activity was increased in failing human hearts secondary to ischemic cardiomyopathy. In our study, the patients were diagnosed as idiopathic dilated cardiomyopathy, while in that study they selected patients with heart failure secondary to ischemic cardiomyopathy. It has been reported that ischemia and ischemia/reperfusion induced multiple MAP kinase activation [5,8,9]. Therefore, it is likely that the presence of ischemia itself, in addition to LV dysfunction and heart failure, modulates the activity of multiple MAP kinases in these patients.

To our knowledge, this is the first report of p90RSK, Src and BMK1 activity in human tissues. We have reported that protein expression and activity of PKC isoforms are elevated in guinea pig model of heart failure by chronic pressure-overload [2,3] and in human explanted hearts with end-stage heart failure [4,12]. We and others have reported that ERK1/2 and p90RSK activation are at least in part PKC-dependent [17]. ERK1/2 were the first MAP kinases described and it has been reported that ERK1/2 activation protects against apoptotic cell death [18,19]. Among the substrates of ERK, p90RSK is a versatile mediator of ERK signal transduction [11,17]. These include: (1) regulation of gene expression via phosphorylation of transcription factors including c-fos, cAMP-response element-binding protein (CREB) and CREB-binding protein, (2) regulation of protein synthesis by phosphorylation of polyribosomal proteins and glycogen synthase kinase-3, and (3) stimulation of the Na⁺/H⁺ exchanger by phosphorylating serine 703 of NHE-1 [10]. Recently Bonni et al. [19] and Tan et al. [20] reported that p90RSK phosphorylated the pro-apoptotic protein BAD at serine 112. Phosphorylation of BAD at serine 112 specifically suppressed BAD-mediated apoptosis. Further in-
Fig. 2. p38 kinase and JNKs in failing and non-failing hearts. (A) p38 kinase activity in whole extracts was measured by Western blot analysis with a phospho-specific p38 antibody (upper). No difference in the amount of p38 kinase was observed in lysates from any of the heart samples by Western blot analysis with anti-p38 kinase antibody (lower). Densitometric analysis showed that p38 kinase activity was reduced in failing human hearts. Results were normalized for all experiments by arbitrarily setting the densitometry of control heart samples (non-failing hearts) to 1.0 (shown is mean ± S.D.). (B) JNK activity was measured by in vitro kinase assay using c-Jun (1–89) fusion protein as a substrate. Western blots were performed with an anti phospho-specific c-Jun antibody for JNK activity assay (upper). No difference in the amount of JNK1/2 protein was observed in samples by Western blot analysis with anti-JNK (lower) and c-Jun substrate by Ponceau staining (data not shown).

Investigation is needed to define the precise role of ERK1/2 and p90RSK activation in failing human hearts.

Recently, Kato et al. [21] have reported that BMK1 activation is also protective against apoptotic cell death. BMK1 is highly expressed in cardiac myocytes and activated by reactive oxygen species in the adult guinea pig heart [8]. Recently, we have demonstrated that BMK1 is activated in hypertrophied myocardium induced by chronic pressure-overload [22]. MEK5-dependent BMK1 activation results in the phosphorylation of MEF2A and MEF2C, which are transcription factors belonging to the myocyte enhancer factor-2 (MEF2)-family [23]. Since BMK1 activation was significantly decreased in failing human hearts in the present study, it will be interesting to evaluate critically the role of this MAP kinase in diminished contractility of the failing heart.

In conclusion, multiple MAP kinases, p90RSK, and Src were differentially regulated in human failing myocardium of patients with idiopathic dilated cardiomyopathy and may be involved in the pathogenesis of this clinical syndrome.

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Fig. 3. Src and BMK1 in failing and non-failing hearts. (A) Src kinase activity in whole extracts was measured by Western blot analysis with a Src antibody clone 28 which recognizes the activated form of Src (upper). No difference in the amount of Src was observed in lysates from any of the heart samples by Western blot analysis with anti-Src antibody (lower). (B) BMK1 activity was analyzed by autophosphorylation in an immune complex kinase assay (upper). BMK1 protein level was assayed by Western blot analysis with anti-BMK1 antibody. No difference in the amount of BMK1 was observed in immunoprecipitates from any of the heart samples with anti-BMK1 antibody (lower). Densitometric analysis indicated that Src and BMK1 kinase activity was decreased in failing hearts compared with normal hearts (shown is mean ± S.D.).

References


