Activation of big MAP kinase 1 (BMK1/ERK5) inhibits cardiac injury after myocardial ischemia and reperfusion

Scott J. Cameron[a,b,1,2], Seigo Itoh[b,1], Christopher P. Baines[b], Changxi Zhang[b], Shinsuke Ohta[b], Wenyi Che[b], Michael Glassman[b], Jiing-Dwan Lee[d], Chen Yan[b], Jay Yang[a,c,*], Jun-ichi Abe[b,*]

[a]Department of Pharmacology/Physiology, University of Rochester Medical Center, Rochester, NY 14642, USA
[b]Center for Cardiovascular Research, University of Rochester Medical Center, Rochester, NY 14642, USA
[c]Department of Anesthesiology, University of Rochester Medical Center, Rochester, NY 14642, USA
[d]Department of Immunology, Scripps Research Institute, La Jolla, CA 92037, USA

Received 4 February 2004; revised 7 March 2004; accepted 23 March 2004

Available online 3 May 2004

Edited by Lukas Huber

Abstract Big MAP kinase 1 (BMK1/ERK5) plays a critical role in pre-natal development of the cardiovascular system and post-natal eccentric hypertrophy of the heart. Of the two isoforms upstream of MAPK-kinase 5 (MEK5) known to exist, only the longer MEK5α isoform potently activates BMK1. We generated cardiac-specific constitutively active form of the MEK5α (CA-MEK5α transgenic (Tg) mice), and observed a 3 to 4-fold increase in endogenous BMK1 activation and hyper-phosphorylation of connexin 43 in the ventricles of the Tg compared to wild-type mice. The CA-MEK5α-Tg-mice demonstrated a profoundly accelerated recovery of left ventricular developed pressure after ischemia/reperfusion. We propose a novel role for BMK1 in protecting the heart from ischemia/reperfusion-induced cardiac injury.

Keywords: Big MAP kinase 1; MAPK-kinase 5; MAP kinase; Transgenic; Cardiac physiology; Hypoxia/reperfusion-injury

1. Introduction

MAP kinases are serine and threonine protein kinases that are activated in response to a wide variety of extracellular stimuli. Big MAP kinase 1 (BMK1/ERK5) is part of a distinct MAP-kinase signaling pathway that is required for epithelial growth factor-induced cell proliferation and progression through the cell cycle [1]. In addition to growth factors, redox, hyperosmotic stress, and pathways involving certain G-protein coupled receptors activate BMK1. The importance of BMK1 in cardiovascular development was supported by the observation that BMK1 knock-out mice have impaired cardiac and vascular development [2]. Transgenic (Tg) mice expressing a cardiac-specific constitutively active form of MAPK-kinase 5 (MEK5), the upstream MAPK-kinase responsible for the activation of BMK1, demonstrated eccentric cardiac hypertrophy indicating the role of BMK1 in post-natal cardiac development as well [3].

MEK5 was originally cloned as splice variants MEK5α and MEK5β; both variants have distinct tissue-wide distribution and cellular localization [4]. The two proteins differ by N-terminal 89 amino acids present only in the MEK5α isoform and we recently demonstrated that MEK5α, but not MEK5β, activates BMK1 [5]. In fact, MEK5β exhibited a dominant-negative phenotype with respect to BMK1 activation. Since the prior report of MEK5 Tg mice used the MEK5β isoform [3], we created Tg mice with the cardiac-specific over expression of constitutively active form of MEK5α (CA-MEK5α-Tg). The CA-MEK5α-Tg mice hearts demonstrated activation of BMK1 and normal basal cardiac function and morphology, but enhanced recovery of contractile function and decreased post-ischemic cardiac damage. This phenotype is in contrast to the previously described cardio-specific CA-MEK5β-Tg mice.

2. Materials and methods

2.1. Transgenic mice and genotyping

Mouse CA-MEK5α (S311D/T315D) cDNA was subcloned into a pBluescript-based Tg vector between the 5.5-kb murine-αMHC promoter and 250-bp SV-40 polyadenylation sequences. The purified transgene fragment was injected into male pronuclei of fertilized mouse oocytes (University of Rochester Transgenic Core). Genotype of mouse pups was confirmed by PCR analysis of tail clippings using standard procedures.

2.2. Western blot analysis and kinase activity

After treatment, the cells were washed with PBS, harvested in 0.5 ml of MOSLB lysis buffer (50 mM sodium pyrophosphate, 50 mM NaF, 50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 100 μM Na_3VO_4, 10 mM HEPES, pH 7.4, 0.1% Triton X-100, 500 μM phenylmethanesulfonyl fluoride, and 10 μg/ml leupeptin) flash-frozen on a dry ice/ethanol bath. The heart homogenates were prepared with sonication and subcellular fractionation performed as described previously [5,6]. For Western blot analysis, cell lysates or immunoprecipitates were subjected to SDS–PAGE and proteins were transferred to nitrocellulose membranes (Hybond™-ECL, Amersham-Pharmacia, Piscataway, NJ) and blotted as previously described [12,16]. BMK1 kinase activity was measured by autophosphorylation [7].

2.3. Connexin 43 (Cx43) immunocytochemistry

For whole heart immunohistochemistry, the heart was perfused with ice-cold PBS in situ, excised, and fixed in 10% buffered formalin

* Corresponding authors. Fax: +1-212-305-0777.
E-mail addresses: jy2029@columbia.edu (J. Yang), Jun-ichi_Abe@urmc.rochester.edu (J.-i. Abe).

[1] These authors contributed equally to this work.
[2] Present address: Division of Clinical Chemistry, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA.

0014-5793/$22.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2004.03.120

overnight. After sectioning and transferring to 75% ethanol, the mounted sections were immunostained with anti-Cx43 antibody as previously described [8].

2.4. Measurement of left ventricular function by the Langendorff preparation and by trans-thoracic echocardiography

For Langendorff perfused heart studies, CA-MEK5α-Tg mice and wild type littermate mice were used for these experiments. Animals were anesthetized with ketamine (50 mg/kg) and xylazine (2.5 mg/kg), i.p., and heparinized (5000 U/kg), i.p., to protect the heart against microthrombi. The chest was opened at the sternum and the heart, after cannulation with a 23 G phalanged stainless steel cannula, quickly removed. The heart was retrogradely perfused through the aorta in a non-circulating Langendorff apparatus with KH buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 0.5 mM Na-EDTA and 11 mM glucose) at a constant pressure of 80 mm Hg. The buffer was saturated with 95% O_2/5% CO_2 (v/v, pH 7.4, 37 °C) for 50 min. A homemade water-filled balloon was inserted into the left ventricle through the left atrium and was adjusted to a left ventricular end-diastolic pressure of 5 mm Hg during initial equilibration. The distal end of the catheter was connected to an ETH-200 Bridge Amplifier (CB Sciences, Inc) and PowerLab/200 (AD Instruments) data acquisition system via a pressure transducer (DELTRAN II, Utah Medical Products, Inc). Hearts were paced at 300 beats/min except during ischemia. Pacing was reinitiated after three minutes of reperfusion in all groups. After 25 min equilibration period, hearts were subjected to 40 min of no-flow normothermic global ischemia and 40 min reperfusion.

The cardiac function in intact wild-type and CA-MEK5α-Tg mice was evaluated non-invasively by transthoracic echocardiography [9]. Mice were anesthetized with ketamine and xylazine, i.p., chest shaved and EKG leads attached to the limbs. M-mode echocardiographic studies were performed with an Accuson Sequoia Ultrasonograph (Mountainview, CA) using a dynamically focused 14-MHz annular array transducer applied to the left hemithorax through a coupling gel.

2.5. Measurement of cardiac damage

Hearts were immediately removed from the Langendorff apparatus, weighed, and frozen at −20 °C. The frozen heart was sectioned manually into 7 or 8 transverse slices of approximately equal thickness (0.8 mm) and stained by incubation in 10% triphenyl tetrazolium chloride (TTC) [10]. The risk area was the sum of total ventricular area minus cavities that equals the total left ventricular mass in this model of global no-flow ischemia. The infarct size was calculated as percent left ventricular mass. Creatine kinase and lactate dehydrogenase were measured by the University of Rochester, Department of Clinical Chemistry, and reported in clinical indices (units/L) as means ± S.D.

2.6. Caspase 3 activity

Hearts were tested for caspase 3-like activity at the following two different time points: (1) when excised and washed with KHB solution ($n = 3$) and (2) after being subjected to 40-min ischemia followed by 120-min reperfusion ($n = 3$). The hearts frozen in liquid nitrogen were homogenized with a polytron homogenizer and centrifuged at $16\,000 \times g$ for 20 min at 4 °C. Protein extracts (100 μg/sample) were used to measure caspase 3-like activity using a fluorescence substrate (CaspACE, Promega). The free 7-amino-4-methyl coumarin fluorochrome released by caspase cleavage of the fluorogenic substrate was detected by excitation at 360 nm and emission at 460 nm by an end-point assay [11]. Results were normalized for all experiments by arbitrarily setting the caspase activity of the vehicle-perfused hearts to 1.0 and reporting the data as means ± S.D.

2.7. Statistical analysis

Data are reported as mean ± S.E. except where indicated. Statistical analysis was performed with the StatView 4.0 package (ABACUS Concepts, Berkeley, CA). Differences were analyzed with one-way or at two-way ANOVA for repeated measurements as appropriate, followed by Schéffe's test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. BMK1 activation in cardiac-specific CA-MEK5α-Tg mice

To examine the effect of activation of BMK1 at the whole organ level, we created Tg mice with cardiac-specific expression of the constitutively active form of MEK5α (CA-MEK5α-Tg). The level of Tg protein expression in three different lines of Tg mice was determined by Western blots using an anti-MEK5 antibody. Because all three Tg lines showed a similar phenotype, including the response to ischemia and reperfusion in the Langendorff preparation, we describe here the data from line Tg-2 as the representative results for CA-MEK5α-Tg mice. We found a 7 to 8-fold increase in the level of total MEK5α expression relative to wild-type mice, where endogenous MEK5α protein expression is barely detectable (Fig. 1A). Endogenous MEK5β protein expression was more abundant in both wild-type and CA-MEK5α-Tg hearts in agreement with an earlier report [4] and the protein expression level did not change within the 10 weeks observation period. The CA-MEK5α-Tg lines exhibited normal feeding, activity, weight gain, and survival up to our current observational period of 30 weeks compared to the wild-type littermates.

There was a concordant 3 to 4-fold increase in BMK1 activity in CA-MEK5α-Tg mouse hearts documented by the in vitro kinase assay and autophosphorylation of BMK1 (Fig. 1B). However, there was no significant ERK1/2 activation in CA-MEK5α-Tg mouse hearts demonstrating a selective BMK1 activation in our Tg mice (Fig. 1B). To further confirm BMK1 activation in these Tg mice, we examined the level of Cx43 phosphorylation upon long-term activation of BMK1 by CA-MEK5α, since Cx43 is an excellent BMK1 substrate [12]. In wild-type mouse hearts, Cx43 was distributed between the non-phosphorylated and presumably the basally phosphorylated state, but Cx43 in CA-MEK5α-Tg hearts appeared to be entirely hyperphosphorylated (Fig. 1C, upper). Further probing for non-phosphorylated Cx43 immunoreactive signal with the monoclonal CX-1B1 antibody failed to reveal non-phosphorylated Cx43 in CA-MEK5α-Tg mouse hearts (Fig. 1C, middle) consistent with Cx43 being highly phosphorylated in vivo [12]. The total Cx43 immunoreactive signal was greater in CA-MEK5α-Tg mouse hearts compared to wild-type littermates, consistent with the well-known observation of easier detergent extraction of the phosphorylated Cx43 species [6,13].

Being acutely aware of the technical issues involved in extracting plasma membrane-bound Cx43 [14], we sought to more accurately determine Cx43 expression and subcellular localization in wild-type and CA-MEK5α-Tg mouse hearts. Left ventricular sections from wild type and CA-MEK5α-Tg were probed with an anti-pan-Cx43 antibody that detects all Cx43 isoforms. In wild-type hearts (Fig. 1D, upper), intense Cx43 immunoreactivity was seen at discrete sites of intercellular apposition, while in CA-MEK5α-Tg mouse hearts, a significant increase in the amount of Cx43 immunoreactive signal within intracellular regions of hearts was observed (Fig. 1D, lower). The immunohistochemical data suggest that BMK1 activation results in intracellular redistribution of Cx43, in vivo, consistent with our prior in vitro observation [12]. In addition, it appeared that the total Cx43 immunoreactive signal was greater than in wild-type mice, which suggests that BMK1 activation may lead to a modest upregulation of Cx43 expression. These data strongly suggest that BMK1 is highly activated in CA-MEK5α-Tg hearts.

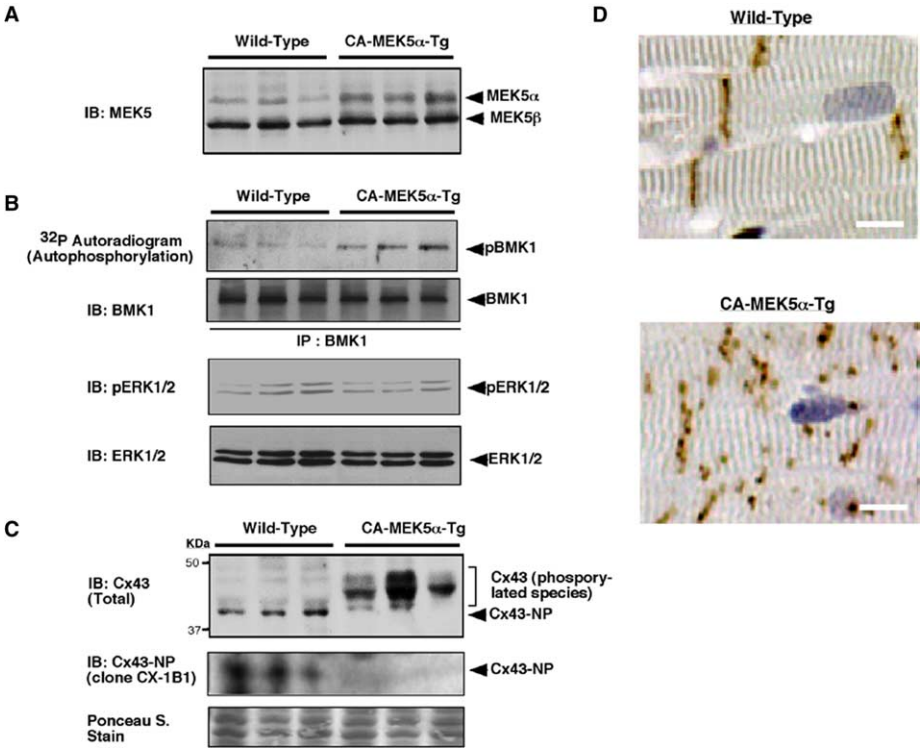


Fig. 1. Phosphorylation and localization of Cx43 in wild-type and CA-MEK5α-Tg mouse hearts. (A) Lysates were prepared from 10-week-old wild-type or Tg mice hearts and immunoblotted with a pan-MEK5 antibody. A greater expression of the MEK5α isoform was noted in the CA-MEK5α-Tg, while the MEK5β expression remained unchanged. (B) BMK1 activation in the Triton X-100 soluble cell fraction and total BMK1 protein loading was examined, *Upper two panels*. ERK1/2 activation and total ERK1/2 protein was examined, *Lower two panels*. These data are representatives of at least three independent experiments with similar results. (C) The Triton X-100 soluble cell fraction was obtained from the left ventricle and separated by SDS–PAGE. Immunoblotting with a pan-Cx43 antibody recognizes total Cx43 (phosphorylated and non-phosphorylated), *Upper panel*, or with the clonal Cx-1B1 anti-Cx43 antibody selective for non-phosphorylated Cx43, *Middle panel*, Ponceau S staining confirmed equal protein loading of all lanes, *Bottom panel*. (D) Immunoperoxidase staining of ventricular sections for Cx43 (brown precipitate, arrowhead) in wild-type hearts (upper) and CA-MEK5α-Tg hearts (lower) using a pan-Cx43 antibody. Calibration bar, 100 μm.

3.2. Functional role for activated BMK1 in ischemia/reperfusion injury in cardiac-specific CA-MEK5α-Tg mice

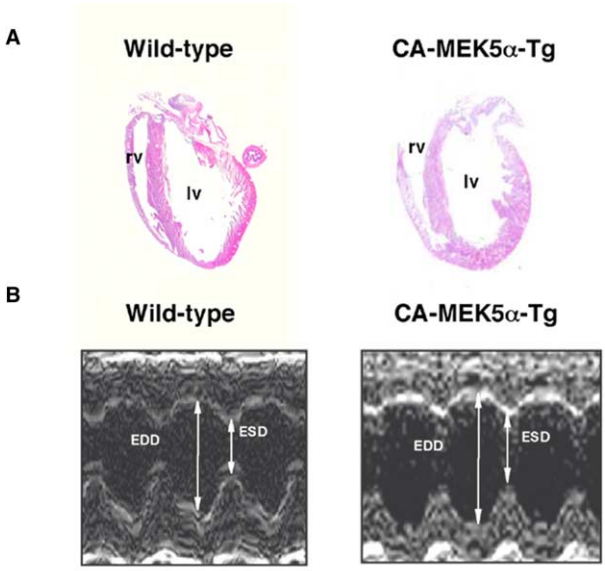
We next examined the basal phenotype and the behavior of wild-type and CA-MEK5α-Tg hearts subjected to ischemia/reperfusion. Cardiac structure and function in 10- and 30-week-old mice was normal as assessed by gross morphometric, histologic, and non-invasive echocardiographic measurements; this phenotype was stable during our measurement period extending to 60 weeks. A representative cross-section of both wild-type and CA-MEK5α-Tg hearts showed no changes in ventricular wall thickness suggestive of cardiomyopathy (Fig. 2A) and M-mode echocardiographic images (Fig. 2B) confirmed normal basal ventricular dimensions and function in live hearts (Fig. 2C). This highly specific analysis of left ventricular function suggests that constitutive BMK1 activation by the CA-MEK5α transgene had no effect on the basal cardiac function.

To examine for a potential functional consequence of BMK1-activation in myocardial ischemia/reperfusion in vivo, we assessed the contractile function of wild type and CA-MEK5α-Tg hearts in the Langendorff preparation. No difference in basal heart rate or contractile function was noted between the wild type and CA-MEK5α-Tg hearts and all hearts subjected to a 40-min period of global ischemia recovered their spontaneous heart beats. To better assess the ventricular pump function, hearts were paced at a uniform rate of 300 beats/min pre- and post-global ischemia. In wild-type hearts, the recovery of left ventricular developed pressure after ischemia and reperfusion was around 30% of the baseline level (Fig. 3A), suggestive of severe contractile dysfunction. In contrast, the developed-pressure recovered to over 70% of baseline for the CA-MEK5α-Tg hearts at all time points after ischemia and during reperfusion. A similar trend was seen in dP/dt_{max} with a significantly higher recovery of this parameter observed in CA-MEK5α-Tg hearts upon reperfusion (Fig. 3B). The results strongly suggest that although CA-MEK5α-Tg hearts are functionally normal and indistinguishable from wild type hearts basally, they display significantly greater contractile recovery even after prolonged ischemia.

3.3. Cardiac-specific CA-MEK5α-Tg mice exposed to ischemia/reperfusion are resistant to caspase 3 activation and post-ischemic damage

Caspase 3 activation has been implicated as a mediator of cardiomyocytes apoptosis [11]. Consistent with this report, we found that caspase 3-like activity was increased after 120 min of reperfusion in wild-type hearts (Fig. 3C). However, the caspase 3-like activity remained unchanged in CA-MEK5α-Tg mice, and was significantly lower compared to wild-type hearts. These observations in intact hearts suggest an inhibitory effect of BMK1 activation on caspase 3-like activity.

To assess total cardiac damage incurred by the post-ischemic/reperfused heart, we measured the cardiac enzymes, creatine kinase (CK) and lactate dehydrogenase (LDH) released from the



C

	10 weeks			30 weeks		
	Wild-type	CA-MEK5α-Tg	p	Wild-type	CA-MEK5α-Tg	p
IVSD	0.63 ± 0.1	0.68 ± 0.1	0.73	0.65 ± 0.05	0.68 ± 0.04	0.65
IVSS	0.92 ± 0.38	0.8 ± 0.2	0.78	0.68 ± 0.05	0.72 ± 0.07	0.64
LVPWD	1.13 ± 0.12	1.37 ± 0.1	0.15	1.48 ± 0.05	1.43 ± 0.08	0.6
LVPWS	1.45 ± 0.14	1.82 ± 0.2	0.15	1.73 ± 0.13	1.78 ± 0.2	0.53
LVED	2.85 ± 0.5	3.05 ± 0.6	0.8	3.35 ± 0.1	3.55 ± 0.14	0.22
LVES	1.6 ± 0.4	1.8 ± 0.6	0.78	2.03 ± 0.13	2.23 ± 0.2	0.42
FS	45.7 ± 6.5	42.5 ± 4	0.68	39.5 ± 1	37.1 ± 3.6	0.54
EF	82.5 ± 6.6	77.8 ± 9.5	0.69	76 ± 2	73.5 ± 4.7	0.64

Fig. 2. Wild-type and CA-MEK5α-Tg mice show indistinguishable myocardial anatomy and basal function. (A) Hematoxylin/Eosin stained longitudinal sections from 10 week-old wild-type and CA-MEK5α-Tg mouse hearts. lv: left ventricle, rv: right ventricle. (B) Representative M-mode echocardiographic images of intact beating hearts. Measurements are given in millimeters, except fractional shortening (FS) and ejection fraction (EF), which are given in %. EDD: end-diastolic dimension, ESD: end-systolic dimension. (C) Echocardiographic values determined from three separate measurements and averaged 7 mice from each group at both 10 and 30 weeks. IVSD, interventricular septum diastole; IVSS, interventricular septum systole; LVPWD, left ventricular posterior wall diastole; LVPWS, left ventricular posterior wall systole; LVED, left ventricular end diastolic dimension; LVES, left ventricular end systolic dimension; EF and FS.

ischemic heart which correlates with the severity of myocardial damage [15]. Perfusates collected from wild-type mouse hearts after 40 min of global ischemia and 40-min reperfusion documented elevated CK and LDH levels consistent with severe cardiac damage (Fig. 3D). CA-MEK5α-Tg mouse hearts subjected to the same insult released little CK or LDH, suggesting BMK1 activation-mediated enhanced ischemic tolerance. Since hearts subjected to ischemia in the Langendorff preparation exhibited well-defined regions of infarction, the hearts were stained with TTC and the infarct size quantified by morphometry (Fig. 3E and F). Following 40 min of ischemia and 40 min of reperfusion, the total infarct size in the CA-MEK5α-Tg hearts was smaller than in wild-type hearts (Fig. 3E and F) consistent with the difference in the recovery of ventricular pump function. These results are consistent with BMK1 activation having a protective effect after acute phase ischemia and reperfusion, with preservation of contractile function.

4. Discussion

To our knowledge, this is the first study to show that the MEK5α/BMK1 signal transduction pathway is cardioprotective in an intact heart. Nicol et al. [3] reported that cardiac-specific expression of activated MEK5β in Tg mice resulted in eccentric cardiac hypertrophy that progressed to dilated cardiomyopathy and sudden death. Our cardiac-specific MEK5α-Tg mice exhibited baseline cardiac function indistinguishable from wild-type littermates. However, the functional recovery of the Tg hearts after ischemia/reperfusion insult was profoundly accelerated compared to wild-type mice. The amount of cardiac enzyme released and the physical measurements of the infarct size were consistent with the cardio-protective effect of MEK5α overexpression. On examination, two differences between the two Tg models were apparent: (1) MEK5 isoforms: we used MEK5α, but Nicol et al. used MEK5β and (2) Mouse strain: we used FVB, but Nicol et al. used C3HB6. MEK5β is 89 amino acids shorter than MEK5α at the N-terminus and we recently reported a novel functional difference in the way full-length MEK5α and the shorter MEK5β splice variant regulate BMK1 activity [16]. In fact only MEK5α activated BMK1, while MEK5β exhibited a dominant-negative phenotype inhibiting CA-MEK5α or growth factor-induced activation of BMK1. A clear constitutive activation of BMK1 assessed by in vitro kinase assay and a parallel increase in Cx43 phosphorylation were documented in our CA-MEK5α-

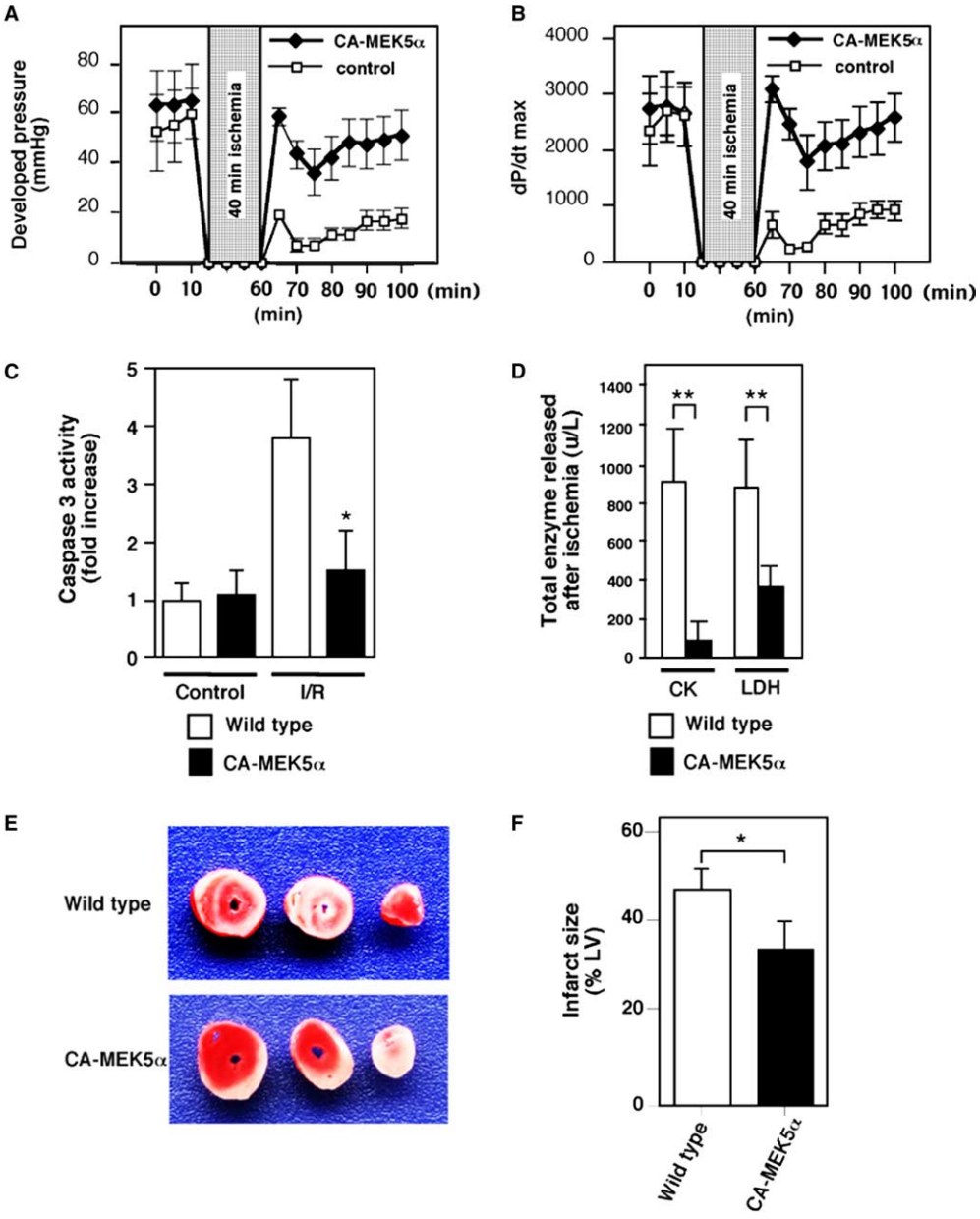


Fig. 3. Cardiac selective expression of CA-MEK5α protects the heart from ischemia/reperfusion-induced contractile dysfunction and post-ischemic cardiac injury. (A) Measurements of left ventricular developed pressure before, during, and after global (no-flow) ischemia followed by reperfusion. (B) Measurement of left ventricular dP/dt_{max} taken at the times indicated in panel (A). All experimental values calculated for CA-MEK5α-Tg mouse hearts ($n = 5$) and wild-type hearts ($n = 5$) are represented as means ± S.E. (C) Caspase 3-like activity after ischemia/reperfusion in wild-type and CA-MEK5α-Tg mouse ventricles. Results are in arbitrary units normalized to wild-type control and reported as means ± S.D. (D) CK and LDH cardiac enzymes measured in the superfusate from the heart after ischemia ($n = 3$ for wild-type, $n = 4$ for CA-MEK5α-Tg mice) and reported as mean units/L ± S.D. (E) TTC was used as an index of damaged area in the myocardium after ischemia and reperfusion. Representative images of TTC staining of coronal sections of the hearts from wild type and CA-MEK5α-Tg mice. (F) The infarct size calculated as percent of LV area and reported as mean ± S.D. * denotes $P < 0.05$ and ** denotes $P < 0.001$.

Tg mouse hearts. However, further investigation is necessary to define why the two CA-MEK5-Tg models exhibited different phenotypes.

It is well known that ischemic preconditioning protects the heart, and we previously reported that ischemic preconditioning increases the maximal BMK1 activity by approximately 3.0-fold [17]. Since we document in the present study that activation of BMK1 by expression of CA-MEK5α transgene is also cardio-protective, it raises the possibility that BMK1 activation during ischemic preconditioning may mediate the protective effect. In a more chronic model of cardiac pathophysiology, we reported that BMK1 activity is increased in ischemia and pressure overload-induced cardiac hypertrophy [18,19]. This observation also raises the interesting possibility that BMK1 activation may be a compensatory mechanism elicited by the failing heart. Further studies are necessary to clarify the role of BMK1 in preconditioning and chronic cardiac pathophysiology.

While the relative resistance of CA-MEK5α-Tg hearts to ischemia/reperfusion-injury is clear, the down-stream mechanism responsible for this phenotype is unidentified. One possible mechanism leading to cardio-protection is via phosphorylation of Cx43 by the activated BMK1 and uncoupling of the gap junction between myocytes. Gap junction intercellular communication augments cellular damage through putative calcium-dependent pathways and ischemic cardiac damage may be prevented by gap junction uncoupling agents [20]. A recent report suggests that Cx43 phosphorylation in the myocardium is a protective mechanism against subsequent ischemic episodes [21], although our study is the first to suggest this specific role to BMK1. If activated BMK1 promotes gap junction uncoupling, as supported by the circumstantial evidence reported herein, the protective effect of activated BMK1 against hypoxia/reperfusion-injury could at least partially be attributed to BMK1-mediated gap junction blockade. However, evidence for the role of gap junctions in the causal relationship between activated BMK1 and relative resistance to ischemic cardiac damage is circumstantial and further studies will be required. Nevertheless, our observation that Cx43 is predominantly phosphorylated in the CA-MEK5α-Tg mice with an apparent redistribution from the plasma membrane to cytosol is consistent with this hypothesis.

Recent reports suggest other mechanisms whereby BMK1 activation may provide protection against ischemia/reperfusion-injury. BMK1 activation phosphorylates Bad and inhibits apoptosis in endothelial cells [22]. The relation between BMK1 and Bad phosphorylation may be indirect, however, in our current study we demonstrated a phenomenological reduction in ischemia/reperfusion-induced caspase 3 activation in CA-MEK5α-Tg mice. Therefore, modulation of apoptosis could contribute to the protective effect exhibited by BMK1 activation even in the acute global ischemia/reperfusion model employed. Recently, we also found that BMK1 activation increases PPARγ activation via direct association with the hinge-helix1 region of PPARγ [23]. Since PPARγ ligands have been shown to protect against ischemia/ reperfusion injury [24,25], it is also possible that the protective effect of BMK1 activation may be due to activation of endogenous PPARγ. Further investigation is necessary to determine the exact protective mechanisms operating down-stream of BMK1 activation.

Acknowledgements: This study was supported by grants from American Heart Association Pre-doctoral Fellowship AHA 0110123T (to S.J.C.), NY State Department of Health SCRIB Grant CO16891 (to J.Y.) and NIH RO1 66919–02 (to J.A.).

References

[1] Kato, Y., Tapping, R.I., Huang, S., Watson, M.H., Ulevitch, R.J. and Lee, J.D. (1998) Nature 395, 713–716.
[2] Regan, C.P., Li, W., Boucher, D.M., Spatz, S., Su, M.S. and Kuida, K. (2002) Proc. Natl. Acad. Sci. USA 99, 9248–9253.
[3] Nicol, R.L., Frey, N., Pearson, G., Cobb, M., Richardson, J. and Olson, E.N. (2001) EMBO J. 20, 2757–2767.
[4] English, J.M., Vanderbilt, C.A., Xu, S., Marcus, S. and Cobb, M.H. (1995) J. Biol. Chem. 270, 28897–28902.
[5] Kobayashi, S., Okumura, N., Nakamoto, T., Okada, M., Hirai, H. and Nagai, K. (1997) J. Biol. Chem. 272, 16262–16267.
[6] Mograbi, B., Corcelle, E., Defamie, N., Samson, M., Nebout, M., Segretain, D., Fenichel, P. and Pointis, G. (2003) Carcinogenesis 24, 1415–1423.
[7] Abe, J., Takahashi, M., Ishida, M., Lee, J.D. and Berk, B.C. (1997) J. Biol. Chem. 272, 20389–20394.
[8] Schubert, A.L., Schubert, W., Spray, D.C. and Lisanti, M.P. (2002) Biochemistry 41, 5754–5764.
[9] Colbert, M.C. et al. (1997) J. Clin. Invest. 100, 1958–1968.
[10] Maekawa, N. et al. (2002) J. Am. Coll. Cardiol. 39, 1229–1235.
[11] Hochhauser, E. et al. (2003) Am J. Physiol. Heart Circ. Physiol. 284, H2351–H2359.
[12] Cameron, S.J., Malik, S., Akaike, M., Lerner-Marmarosh, N., Yan, C., Lee, J.D., Abe, J. and Yang, J. (2003) J. Biol. Chem. 278, 18682–18688.
[13] Musil, L.S. and Goodenough, D.A. (1991) J. Cell Biol. 115, 1357–1374.
[14] Musil, L.S. and Goodenough, D.A. (1993) Cell 74, 1065–1077.
[15] Do, E. et al. (1997) J. Mol. Cell Cardiol. 29, 1903–1913.
[16] Cameron, S.J., Abe, J., Malik, S., Che, W. and Yang, J. (2004) J. Biol. Chem. 279, 1506–1512.
[17] Takeishi, Y. et al. (2001) J. Mol. Cell Cardiol. 33, 1989–2005.
[18] Takeishi, Y., Abe, J., Lee, J.D., Kawakatsu, H., Walsh, R.A. and Berk, B.C. (1999) Circ. Res. 85, 1164–1172.
[19] Takeishi, Y. et al. (2001) J. Mol. Cell Cardiol. 33, 1637–1648.
[20] Lin, J.H. et al. (1998) Nat. Neurosci. 1, 494–500.
[21] Jain, S.K., Schuessler, R.B. and Saffitz, J.E. (2003) Circ. Res. 92, 1138–1144.
[22] Pi, X., Yan, C. and Berk, B.C. (2004) Circ. Res. 94, 362–369.
[23] Abe, J., Akaike, M., Che, W., Ohta, S. and Yan, C. (2003) Circulation 108, IV-108.
[24] Shiomi, T. et al. (2002) Circulation 106, 3126–3132.
[25] Yue, T.L. et al. (2001) Circulation 104, 2588–2594.