Involvement of Extracellular Signal-Regulated Kinases 1/2 in Cardiac Hypertrophy and Cell Death

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Abstract—In response to pathophysiological stress, the adult heart undergoes hypertrophic enlargement characterized by an increase in the cross-sectional area of individual myofibers. Although cardiac hypertrophy is initially a compensatory response, sustained hypertrophy is a leading predictor for the development of heart failure. At the molecular level, disease-related stimuli invoke endocrine, paracrine, and autocrine regulatory circuits, which directly influence cardiomyocyte hypertrophy, in part, through membrane bound G protein–coupled receptors and receptor tyrosine kinases. These membrane receptors activate intermediate signal transduction pathways within the cytoplasm such as mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), and calcineurin, which directly modify transcriptional regulatory factors promoting alterations in cardiac gene expression. This review will weigh an increasing body of literature implicating the intermediate signaling pathway consisting of MEK1 and extracellular signal-regulated kinases (ERK1/2) as important regulators of cardiac hypertrophy and myocyte survival. The MEK1-ERK1/2 pathway likely occupies a central regulatory position in the signaling hierarchy of a cardiac myocyte given its unique ability to respond to virtually every characterized hypertrophic agonist and stress stimuli examined to date and based on its ability to promote myocyte growth in vitro and in vivo. (Circ Res. 2002;91:776-781.)

Key Words: heart • hypertrophy • failure • signaling • mitogen-activated protein kinase

Mitogen-activated protein kinase (MAPK) signaling pathways consist of a sequence of successively acting kinases that ultimately result in the dual phosphorylation and activation of terminal kinases such as p38, e-Jun N-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERKs) (see review1) (Figure 1). The MAPK signaling cascade is initiated in cardiac myocytes by G protein–coupled receptors (angiotensin II, endothelin-1, and adrenergic receptors), receptor tyrosine kinases (insulin-like growth factor, transforming growth factor-β, and fibroblast growth factor receptors), receptor tyrosine kinases (insulin-like growth factor, transforming growth factor-β, and fibroblast growth factor receptors), cardiotoxin-1 (gp130 receptor), and by stress stimuli.2 Once activated, p38, JNKs, and ERKs each phosphorylate of a wide array of intracellular targets that includes numerous transcription factors resulting in the re-programming of cardiac gene expression as part of the hypertrophic program.

At least five different ERK proteins have been identified in mammalian cells, ERK1 to 5 (see reviews1–3). ERK5 is regulated by the upstream kinase MAPK kinase 5 (MEK5), whereas ERK3 and ERK4 are related family members with unknown upstream regulators.3 The more highly studied and abundantly expressed ERK family members, ERK1 and ERK2, are directly regulated by two MAPK kinases, MEK1 and MEK2. ERK1/2 proteins are directly phosphorylated by MEK1/2 at both a threonine and adjacent tyrosine residue within a dual specificity motif (Thr-Glu-Tyr). p38 kinases are directly activated by MKK6 and MKK3, whereas JNKs are directly activated by MKK4 and MKK7 (Figure 1). Upstream of the MAPKKs, multiple MAPKKKs exist that form a complex network of kinases that either directly sense stress stimulation (mechanism unknown), or are directly regulated by effectors such as low molecular weight G-proteins (Ras, Rac, Rho, Cdc42, etc). For example, mitogen-induced activation of a receptor-tyrosine kinase (eg, IGF-1R) results in Ras activation through the action of Grb2 and Sos, which directly leads to Raf (MAPKKK) activation and then MEK1/2 (MAPKK) activation followed by ERK1/2 activation.3

ERKs as Regulators of Cardiac Hypertrophy
In response to agonist stimulation or cell stretching, ERK1/2 become activated in cultured cardiac myocytes.4–8 More recently, ERK1/2 were shown to be activated by acute pressure overload stimulation induced by aortic banding in rodents.9,10 These observations have implicated ERK1/2 signaling factors as regulators of the hypertrophic response. In support of this notion, transfection of constitutively active MEK1 (immediate upstream activator of ERK1/2) augmented atrial natriuretic factor (ANF) promoter activity in cultured cardiomyocytes, whereas a dominant-negative MEK1 con-
Figure 1. Simplified overview of the 3 most highly characterized MAPK signaling branches: ERKs, JNKs, and p38 kinases. Growth factors, agonists, and stress stimuli facilitate MAPK activation through a network of cytoplasmic membrane bound receptors or ill-defined sensing factors that signal through G proteins to promote activation of MEKs, which in turn activate ERKs resulting in activation of ERKs, JNKs, or p38 kinases. On activation by phosphorylation, ERKs, JNKs, and p38s can translocate to the nucleus where they phosphorylate mitogenic- or stress-responsive transcription factors.

struct attenuated its activity. This described affect is consistent with a reduction in agonist-induced b-type natriuretic promoter activity in response to the MEK1 inhibitor PD98059. Using antisense oligonucleotides, Glennon et al demonstrated that ERK signaling is necessary for effective phenylephrine-induced cardiomyocyte hypertrophy in culture. Similarly, Clerk et al reported that MEK1-ERK1/2 was required for sarcomeric organization induced by hypertrophic agonists. More recently, PD98059 was shown to significantly reduce leukemia inhibitory factor (LIF)–induced cardiomyocyte hypertrophy in vitro, whereas a more selective MEK1 inhibitor, U0126, also blocked both endothelin-1 and phenylephrine-induced cardiomyocyte hypertrophy in vitro. Although these independent studies strongly suggest the hypothesis that MEK1-ERK1/2 signaling is necessary for mediating agonist-induced cardiomyocyte hypertrophy, they are largely dependent on the effectiveness and specificity of the MEK1-ERK1/2 pharmacological inhibitory agents PD98059 and U0126. However, adenoviral-mediated gene transfer of either a dominant-negative MEK1 or Raf-1 cDNA into cultured neonatal cardiomyocytes also effectively blocked endothelin-1 and phenylephrine-induced hypertrophy, strongly supporting the conclusion that MEK1-ERK1/2 activation is a requisite event in the initiation or effective progression of myocyte hypertrophy in culture.

Although convincing evidence suggests that a Ras-Raf-1-MEK1-ERK1/2 signaling pathway regulates important aspects of the hypertrophic response of neonatal myocytes in culture, a number of similarly designed studies have disputed such a conclusion. Thorburn and colleagues demonstrated that although Ras-Raf-1-ERK activation was sufficient to augment c-Fos and ANF promoter activity in cardiomyocytes, inhibition of these signaling factors did not antagonize hypertrophic morphology or cytoskeletal organization in response to agonist stimulation. Post et al also reported that neither dominant-negative ERK1/2 nor PD98059 were sufficient to block phenylephrine-induced ANF promoter activity in cultured cardiomyocytes, suggesting that ERKs are not important for inducible gene expression. In a subsequent study, transfection of an activated MEK1 expression plasmid was shown to induce c-Fos, but not ANF or myosin light chain-2V (MLC-2V) promoter activity in cultured cardiomyocytes. More recent studies with the MEK1 inhibitor PD98059 also suggested a minimal or no requirement of ERKs in cardiac hypertrophy. Intriguingly, one study even suggested that ERK activation in response to ANF treatment was associated with prevention of cardiomyocyte hypertrophy. It is perplexing that a number of equally credible pharmacological inhibitory studies failed to identify a necessary role for the MEK1-ERK1/2 signaling pathway in the regulation of agonist-induced hypertrophy in vitro. However, analysis of hypertrophy in cultured neonatal cardiomyocytes can often be influenced by cell density, media composition, the age and health of the neonatal rats, and the dosage and time course of both the pharmacological antagonist and the hypertrophic agonist used. In addition, many of the studies discussed above actually compared different indices of the hypertrophic response, such as ANF promoter expression, sarcomeric organization, or cellular growth, making direct comparisons difficult. Finally, the absolute specificity or potential toxicity of the frequently used MEK1 inhibitors PD98059 and U0126 are difficult to define, in part, given that the mammalian genome likely encodes between 500 to 1100 kinases. Given these considerations, analysis of rodent models with genetically altered MEK1-ERK1/2 signaling should permit a more definitive examination of this pathway’s role in the hypertrophic response within a more physiological setting.

**MEK1-ERK1/2 Signaling Regulates Cardiac Hypertrophy In Vivo**

To investigate the ability of MEK1-ERK1/2 signaling to induce a cardiac hypertrophic response in vivo, transgenic mice were generated containing an activated MEK1 cDNA under the transcriptional control of the cardiac-specific α-myosin heavy chain promoter. Bueno et al described the generation of 9 independent MEK1 transgenic mouse lines that each demonstrated mild concentric hypertrophy characterized by a thicker septum and left ventricular posterior wall and by smaller ventricular chambers (Figure 2). Most activated MEK1 transgenic lines demonstrated a uniform profile of increased heart-to-body weight ratio of approximately 25% at 2 and 6 months of age. These mice did not suffer from premature death and they showed very few signs of histopathology or interstitial cell fibrosis in the heart at 2 and 6 months of age (Figure 2). Lastly, echocardiography and ex vivo working heart preparations demonstrated enhanced contractile performance in MEK1 transgenic mice at both 2 and 6 months of age, suggesting an initial phenotype of compensated cardiac hypertrophy. MEK1 transgenic mice were also partially resistant to ischemia/reperfusion-induced apoptosis in vivo, further implicating this signaling pathway in regulating the compensatory phase in hypertrophic disease progression. Collectively, analysis of MEK1 transgenic mice indicated that the MEK1-ERK1/2 signaling pathway was
Downstream Targets of ERKs in the Heart

Although MEK1-ERK1/2 signaling induces the hypertrophic response in cultured cardiomyocytes and in the hearts of transgenic mice, the downstream mechanisms whereby hypertrophic growth is stimulated are less understood. In cardiac myocytes, ERK activation is associated with p70 S6 kinase 1 and 2 activation that can regulate the efficiency of translation and hence the accumulation of protein during the hypertrophic response (Figure 1). In addition to the ability of ERKs to regulate protein synthesis, ERK1/2 were recently shown to regulate ribosomal RNA transcription by directly phosphorylating the transcription factor UBF. These two reports provide a mechanism whereby ERK1/2 signaling can increase the cellular content of protein and rRNA, which characterizes the hypertrophic response.

MEK1-ERK1/2 signaling has also been implicated in regulating the transcription of polymerase II–associated genes by direct phosphorylation of cardiac-expressed transcription factors. In nonmyocytes, ERK1/2 directly phosphorylate the transcriptional effectors Elk-1, Ets1, Sap1a, c-Myc, and STAT factors (see review3). Indeed, ERK1/2 activation was recently reported to be associated with Elk-1 phosphorylation in cardiac myocytes, suggesting conservation in the mechanisms of ERK-mediated transcriptional responsiveness in multiple mammalian cell-types.33 Perhaps more importantly, ERK1/2 signaling has been associated with phosphorylation and activation of the cardiac-enriched transcription factor GATA4. GATA4 is a critical regulator of most cardiac-expressed structural genes and hypertrophy responsive genes, suggesting that alterations in its activity could have a dramatic effect on transcription within the heart (see review3). Specifically, ERK2 directly phosphorylates serine 105 in GATA4 in response to hypertrophic agonist stimulation (Figure 3A). Using a phosphospecific serine 105 antibody, GATA4 was shown to be phosphorylated in vitro and in vivo after agonist stimulation (Figures 3B and 3C). Phosphorylation of serine 105 augmented both transcriptional potency of GATA4 as well as its DNA binding activity.34 Lastly, a dominant-negative GATA4 construct attenuated MEK1-induced hypertrophy in myocytes, suggesting that MEK1-ERK1/2 requires GATA4 transcriptional activity as part of the hypertrophic response.34

More recently, cultured cardiomyocytes stimulated with phenylephrine showed increased activity of the transcriptional coactivators p300 and CBP through an ERK1/2-dependent mechanism. Specifically, ERK1/2 targeted the N-terminus of p300 and the C-terminus of CBP to enhance transcriptional potency. These data provide further insight into the transcriptional mechanisms whereby ERK1/2 signaling enhances gene expression in association with the hypertrophic response. In summary, multiple downstream effectors of MEK1-ERK1/2 signaling have been shown to regulate the progression of the hypertrophic response, further supporting the hypothesis that this pathway functions as a growth effector in the heart.

Role of ERKs in Cardiomyocyte Apoptosis

Members of the MAP kinase family have been implicated in survival signaling in response to ischemia/reperfusion, oxi-
and catecholamines were each shown to exert their antiapoptotic effects, in part, by inducing ERK signaling.26

The cardioprotective function for ERK1/2 depends in part on the presence of an activated MEK1-ERK1/2 signaling pathway in the myocardium.27–29 Although these various reports discussed above have shown that the MEK-ERK pathway may be protective or neutral against apoptosis, little is known as to how ERKs mediate the rapid nongenomic effects of estrogens.30–32 Insulin-like growth factor-1 (IGF-1), cardioprotection-1 (CT-1), and catecholamines were each shown to exert their antiapoptotic effects, in part, by inducing ERK signaling.33–35 Ischemia/reperfusion and oxidative stress induced by anthracenes activate MAPKs. Inhibition of ERK signaling was demonstrated to increase daunomycin-induced apoptosis in cultured cardiomyocytes,36 whereas in a model of ischemia/reperfusion in the intact heart, ERK1/2 activation was shown to attenuate the amount of apoptosis subsequent to reperfusion injury.37 MEK1 transgenic mice were also shown to be partially resistant to ischemia/reperfusion-induced DNA laddering, suggesting a cardioprotective function for ERK1/2 signaling.38

Although these various reports discussed have shown that stress or agonist-induced ERK1/2 activation is associated with protection from apoptosis, little is known as to how ERK signaling mediates protection in association with ERK1/2 signaling in cardiomyocytes.39 (Figure 1). In T cells, ERK1/2 activation has been associated with induction of expression of FLICE (FADD-like interleukin 1β–converting enzyme) inhibitory protein, a known inhibitor of the caspase cascade.40 It is unknown whether protection induced by ERK signaling is mediated by increasing FLICE-inhibitory protein expression in the heart. The cardioprotective effects of estrogens have been extensively documented. In addition to the classical genomic effects, estrogens have also been shown to have rapid nongenomic effects, which include NO release and ERK1/2 activation.45,46 In the heart, ERK signaling induced by estrogens results in the rapid expression of early growth response gene-1 (Egr-1) and ANF, which have been shown to have cardioprotective effects.47 Another mechanism whereby ERK1/2 may function in a cardioprotective manner is through association with protein kinase Cε (PKCε), which is a well-characterized mediator of cardiomyocyte protection (see review48). Indeed, Baines et al49 recently demonstrated that ERKs form a complex with PKCε in the mitochondria resulting in the phosphorylation and inactivation of Bad.49 As a final mechanism, ERK1/2 is known to directly phosphorylate the p90 ribosomal S6 kinases (RSKs), which in turn can augment cellular viability through phosphorylation of Bad.50,51 Future studies using MEK1, MEK2, ERK1, or ERK2 gene-targeted mice (or dominant-negative–expressing transgenic mice) subjected to cardiac ischemia/reperfusion injury will ultimately establish the overall importance of MEK-ERK signaling as an antiapoptotic effector pathway in vivo.

**Role of ERK Signaling in Heart Failure**

MAPK pathways have also been associated with the development of cardiac failure. For example, samples obtained from human patients with heart failure revealed an increase in all three MAPKs with no changes in samples obtained from hypertrophied human hearts.32 By comparison, levels of activated ERKs were unchanged in heart samples obtained from patients with heart failure secondary to ischemic heart disease, whereas levels of JNK1/2 and p38 activation were significantly increased.53 Mechanical unloading of the heart through a left ventricular assist device (LVAD) lead to significant reductions in the activity of ERKs and JNK1/2, whereas p38 activity was significantly increased after LVAD support.54 The above studies show that MAPKs are differentially regulated during heart disease and highlight the possibility that pharmacological modulation of these pathways in heart failure may restore the balance that exists among the MAPK branches. However, it remains uncertain whether ERK activation benefits or further exacerbates the phenotype of the failing human myocardium. Future studies in animal models will be required to mechanistically assess causality between MEK1-ERK1/2 signaling and the progression of human heart failure.

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**References**


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