

## Mitogen-activated protein kinases and cardiomyocyte hypertrophy

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### ABSTRACT

Because p38 Mitogen-activated protein kinases is a stress-activated kinase, its activation and function has been studied in cardiomyocyte hypertrophy. Both p38a and p38b activity were increased during the progression of hypertrophy. Activation of the p38 pathway by the introduction of constitutively active MKK6- and MKK3-elicited hypertrophic responses, including an increase in cell size, enhanced sarcomeric organisation and elevated atrial natriuretic factor expression. Furthermore, p38a and p38b appear to have different functions in cardiomyocytes. p38b seems to be more potent in inducing hypertrophy, whereas p38a appears to be more important in cardiomyocyte apoptosis. In this review, we summarize the characteristics of the major components of the p38 signalling transduction pathway and role of them in cardiomyocyte hypertrophy.

**Key words:** Mitogen-activated protein kinases, cardiomyocyte hypertrophy

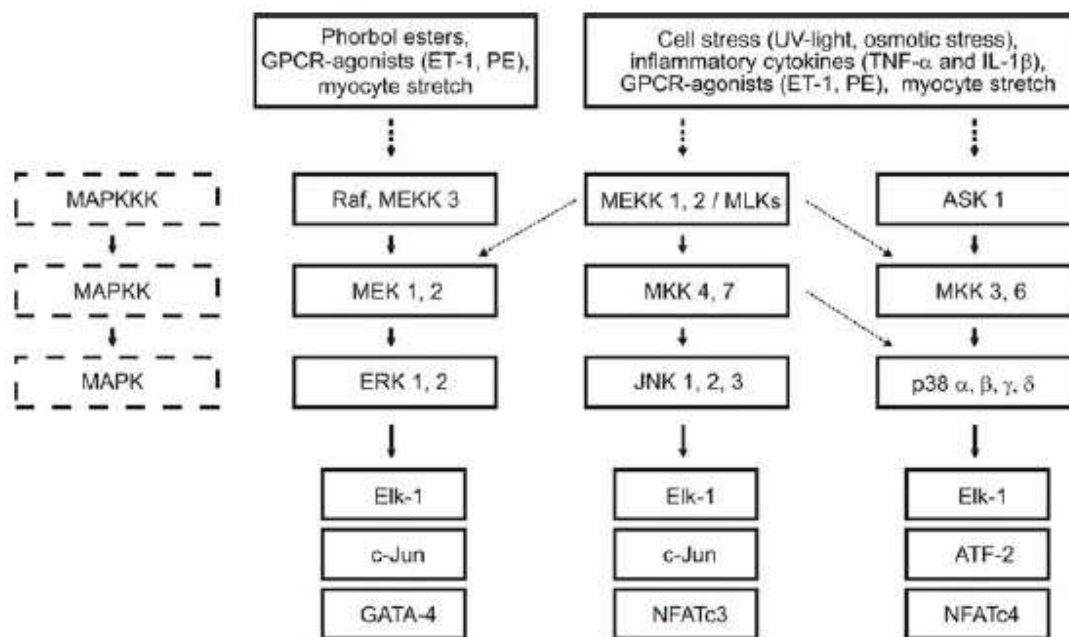
### Mitogen-activated protein kinases

There are multiple MAPK pathways in all eukaryotic cells, which allow the cells to respond differently to divergent inputs. MAPK signaling cascades are usually divided into three parallel pathways: ERK, JNK and p38 MAPK pathways [1]. All MAPK pathways include three signaling levels, i.e. MAPKKK activating MAPKK, which in turn activates MAPK. Activation of MAPKKKs results from translocation, oligomerization and phosphorylation by upstream kinases [2]. Active MAPKKKs phosphorylate serine and threonine residues in MAPKKs, which in turn activate tyrosine and threonine residues in the activation loop of the MAPKs. Most physiological substrates of the MAPKs possess specific binding sites for MAPKs that allow strong interactions with selectivity for MAPK subfamilies [3]. MAPKs, in turn, also possess complementary docking sites, which allow them to interact with MAPK binding domains on substrate proteins. The signaling mechanism is coordinated by the interaction of components of the protein kinase cascade with scaffold proteins, such as JNK interacting protein-1 and MEK1 partner [4]

### Extracellular signal-regulated kinase

The ERK family of MAPKs [ERK1,2], also known as p42/44 MAP kinase, is activated by mitogenic stimuli and generally associated with cell growth and survival. It was the first of the MAPK pathways proposed to participate in hypertrophic signaling in cardiac myocytes [5]. ERKs and the upstream activators of the MEKs were first identified from yeast [Fig. 1]. The kinases in all levels are activated by Gq/G11 protein 29 coupled receptors and PMA in the heart [6]. Activation of c-Raf and A-Raf occurs in response to PMA, ET-1 and PE, involving activation of PKC. In fact, several studies have found ERK as a downstream target of PKC [7]. PMA and ET-1 activate also MEKs and ERKs, whereas activation by  $\alpha$ -adrenergic agonist PE is less effective [8].

**Fig1. overview of MAP kinase cascades activated by diverse hypertrophic stimuli and regulating phosphorylation and activation of numerous cardiac transcription factors.**

**Table1. Nomenclature of MAP kinases and MAP kinase substrates**

| Name | Alternate names | Substrates   |
|------|-----------------|--|
| ERK1 | p44 –MAPK       | MAPKAP-K1, Elk-1, c-Jun, GATA4<br>MAPKAP-K1, Elk-1, c-Jun, GATA4 |
| ERK2 | p42 –MAPK       | c-Jun, ATF-2, Elk-1, NFATc3<br>c-Jun, ATF-2, Elk-1, NFATc3       |
| JNK1 | SAPK1c, SAPK-γ  | c-Jun, ATF-2, Elk-1, NFATc3                                      |
| JNK2 | SAPK1a, SAPK-α  | MAPKAP-K2/3, ATF-2, NFATc4,                                      |
| JNK3 | SAPK1b, SAPK-β  | MEF2   |
| P38α | SAPK2α, CSBP1   | MAPKAP-K2/3, ATF-2, NFATc4<br>MAPKAP-K2/3, ATF-2                 |
| P38β | SAPK2β, p38-2   | MAPKAP-K2/3, ATF-2   |
| P38γ | SAPK3, ERK6     |  |
| P38δ | SAPK4           |  |

As noted, ERKs are activated by various neurohumoral stimuli, and also by myocyte stretching in cultured cardiac myocytes and *in vivo* [9]. Transfection of constitutively active MEK1 into cultured cardiac myocytes results in increased ANP promoter activity, whereas inhibition of ERK cascade with dominant negative mutant of MEK1 abolishes PE induced ANP promoter activity [2]. Transgenic mice with cardiac restricted expression of activated MEK1 demonstrated concentric hypertrophy with an approximately 25% increase in heart to body ratio. Antisense oligonucleotide inhibition of ERK1 and ERK2 has been shown to attenuate PE induced sarcomeric organization and increase in cell size, the morphological changes seen in hypertrophy, as well as PE induced increase in ANP promoter activity and ANP mRNA levels [8]. Since then, inhibitors of ERK pathway, PD 98059 and U0126 have become commercially available. ERK inhibition with PD 98059 has been shown to attenuate the myofibrillar organization induced by PE or ET-1, supporting the role of ERKs in the development of cardiac hypertrophy [7]. However, contradicting data also exist, showing that dominant-negative ERK1 or ERK2, or PD 98059 are not sufficient to block PE induced ANP promoter activity or ET-1 induced increase in protein synthesis. Although the exact role of ERKs in cardiac hypertrophy is not fully clear at present, there are convincing data indicating involvement of ERK in mechanical stretch induced increase in c-fos and  $\alpha$ -SkA gene expression, and hypertrophic agonist induced increase in ANP promoter activity [9]. In fact, ERKs were the first MAPKs shown to phosphorylate transcription factor c-Jun, although later studies proved JNKs to be principally responsible for c-Jun phosphorylation [Fig. 1]. Recent studies have also shown a role for ERKs in regulation of transcription factor GATA4 phosphorylation [Table 1][10]. Previously, Elk-1 transcription factor has been shown to contain a phosphorylation

motif for ERK2, and ERK pathway has also been implicated in the regulation of Elk-1 dependent transcriptional activation [11].

#### ***c-Jun N-terminal kinase***

Molecular cloning of p54, JNK2, has revealed a family of SAPKs or JNKs, encoded by at least three genes [Table 1]. JNK activity is induced by a number of upstream kinases [Fig. 1]. The JNKs are serine/threonine kinases, which also, similarly to ERKs, require tyrosine and serine/threonine phosphorylation for their activation. Interest in these kinases was heightened when they were found to phosphorylate c-Jun, a component of the AP-1 transcription factor [6]. JNKs are preferentially activated by cellular stresses, such as UVlight and osmotic stress, and by inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$  [12]. In the heart, activation of JNKs, primarily JNK1 and JNK2, results from myocyte stretch or activation of GPCRs. A number of studies have implicated JNKs in the regulation of cardiac hypertrophy *in vitro* and *in vivo*. Adenovirus mediated gene transfer of dominant negative mutant of MKK4, an upstream kinase of JNKs, blocks ET-1 induced increase in protein synthesis, sarcomere organization and ANP mRNA levels. Activated MEKK1 has been shown to stimulate ANP reporter gene expression, while a dominant negative MEKK1 mutant inhibits PE induced ANP expression [13]. Promoter studies have further revealed that activated MEKK1 induces  $\beta$ -MHC, ANP and  $\alpha$ -SkA expression in cultured neonatal ventricular myocytes. The results from studies using dominant negative or activated MEKK1 are not clear, since MEKK1 has also been shown to induce activation of both ERK and p38 MAPK pathways [14]. Crosstalk between MAPK pathways is also known to exist at other levels, i.e. MEKK3, a MAPKKK of ERK pathway, activating MAPKKs in ERK, JNK and p38 MAPK pathways, as well as MKK4 activating JNKs and p38 MAPKs [Fig.1]. Also, specific activation of p38 $\alpha$  by adenovirusdelivered constitutively active MKK3 $\beta$  has been shown to result in potent inhibition of the activity of ERK1,2 and its upstream activator MEK1,2 [13].

There are also contradictory results concerning the role of JNKs in hypertrophic signaling. Activated MEKK1 induced ANP expression was inhibited by activation of JNK pathway, whereas in an earlier report MEKK1 induced ANP expression was JNK dependent. Recently, studies with MEKK1 deficient mice showed that JNK signaling via MEKK1 is not required for aortic banding induced cardiac hypertrophy and ANP expression, but protects the heart from apoptosis [12]. Adenovirus mediated gene transfer of dominant negative MKK4 has been shown *in vivo* to inhibit pressure overload induced cardiac hypertrophy, characterized by a decrease in LV thickness, LV to body weight ratio and ANP expression. Primary targets for JNKs are transcription factors c-Jun, Elk-1 and ATF-2 [Fig. 1, Table1]. ATF-2 and c-Jun have been shown to form heterodimers that transactivate at cAMP response element -like consensus sequences within various promoters [5]. In the future, studies with selective inhibitors are expected to further enlighten the JNK dependent mechanisms of hypertrophic gene expression.

#### ***p38 mitogen-activated protein kinase***

The p38 MAPK was also identified as a protein kinase cascade activated by IL-1 $\beta$  and physiologic stress [1]. Like other MAPKs, p38 is activated by dual 32 tyrosine/threonine phosphorylation in catalytic domain. Four p38 isoforms have been described to date: p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$  and p38 $\delta$ . The MAPKKs for p38 are MKK3 and MKK6, which specifically activate p38 MAPKs [5]. As mentioned, MKK4, a MAPKK for JNK pathway, also phosphorylates and activates p38 *in vitro*. The primary MAPKKK is MAPKKK5 [ASK1], which also activates MKK4 and MKK7 in the JNK pathway. MEKK1 is also capable of inducing the p38 pathway, but not until MEKK1 expression robustly exceeds that required for maximal JNK activation [Fig. 1]. The p38 MAPKs are activated by a variety of stimuli, including chemical stress, physical stress, osmotic stress and radiation, and by GPCR-agonist activation [14].

In cardiac myocytes p38 $\alpha$  and p38 $\beta$  are the most important isozymes, whereas p38 $\gamma$  and p38 $\delta$  are hardly detectable [13]. In the heart, p38 MAPKs are activated by GPCR ligands, such as ET-1, PE, Ang II and by myocyte stretch [5]. Activated p38 isoforms are shown to phosphorylate and activate a number of transcription factors, including MEF2, ATF-2, ATF-6, nuclear factor $\kappa$ B [NF- $\kappa$ B] and Elk-1 [Fig. 1, Table 1]. In addition to transcription factors, p38 MAPKs phosphorylate and activate several other protein kinases. These include MAPK-activated protein kinases 2 and 3 [MAPKAP-K2 and MAPKAP-K3] and p38-regulated/activated kinase, which phosphorylates the small heat shock protein Hsp25/27 [Table 1] [7]. MAPKAP- 2 is expressed in adult cardiac ventricular myocytes as 66 kd alpha and 61 kd beta subunits. Other known substrates for p38 MAPKs are Mnk1 and Mnk2, and mitogen- and stress-activated protein kinases [10].

In cultured cardiac myocytes p38 is rapidly activated by ET-1, PE and stretching of the myocytes [10]. *In vivo* pressure overload activates p38 MAPK, and increased p38 activation has also been observed in human hearts with failure secondary to coronary artery disease [1]. *In vitro* overexpression of activated MKK3 or MKK6 induces hypertrophic growth of myocytes and increased expression of hypertrophic genes, such as ANP, BNP and  $\alpha$ -SkA [14]. Adenoviral overexpression of wild type p38 $\beta$  results in increased cell size and ANP mRNA levels. Two

relatively specific inhibitors exist for p38 $\alpha$  and p38 $\beta$ , SB203580 and SB202190 [10] *In vitro* pharmacological inhibition of p38 with SB 203580 [20  $\mu$ M] blocks PE induced increase in ANP and BNP promoter activity, cell size and sarcomeric organization [14]. SB 202190 at 20  $\mu$ M concentration is sufficient to suppress PE induced ANP promoter activity and ET-1 induced increase in cell size and sarcomeric organization [3]. Role of p38 is not fully clear, since 10  $\mu$ M concentration of SB 203580 failed to inhibit ET-1 and PE induced increase in  $\beta$ -MHC expression and cell size, and only partially inhibited agonist induced myofibrillar organization [7].

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