The MEKK3/MEK5/BMK1 Signaling Cascade Regulates Calcineurin Activity by Phosphorylation of MCIP1 in Cardiac Hypertrophy

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Ca^2+–dependent and Ca^2+–independent signaling pathways are associated with cardiac hypertrophy. How these two pathways crossstalk with each other resulting in cardiac hypertrophy is poorly understood. To address this issue we designed a transfection bioassay to monitor NFAT activation in cultured rat neonatal cardiomyocytes. We determined that active MEKK3 was capable of stimulating calcineurin/Cn/NFAT signaling in cardiac myocytes. Overexpression of active MEKK3 in cardiac myocytes and mouse hearts resulted in Cn/NFAT activation and reprogramming cardiac gene expression. In contrast, small interference RNA directed against MEKK3 and a dominant negative form of MEKK3 caused the loss of NFAT activation in response to angiotensin II (Ang II) in cardiac myocytes. Likewise, MEKK3–deficient mouse embryo fibroblasts failed to activate Cn/NFAT in response to Ang II, a potent NFAT activator. Conversely, restoring MEKK3 to the MEKK3–deficient cells restored Ang II–mediated Cn/NFAT activation. Thus, MEKK3 is sufficient and essential for Cn activation. Next we determined that MEKS and BMK1 function downstream of MEKK3 to induce NFAT activation. Physical interaction assays showed that activated MEKK3/MEK5/BMK1 formed complex with MCIP1, a Cn interacting protein, resulting in its phosphorylation. In vivo labeling experiments and immunoprecipitation pulldown assays revealed that phospho–MCIP1 dissociated from Cn catalytic subunit and interacted with an unknown 31 KD protein. Sequence analysis of MCIP1 revealed a serine–proline (SP) repeat domain with the potential to bind with a 31 KD protein termed 14–3-3, a multifunctional phosphopeptide binding protein. Subsequent co-immunoprecipitation experiments demonstrated that phosphorylation of MCIP1 resulted in reduced affinity for Cn and increased affinity for 14–3-3, thereby displacing phospho–NFAT from its docking site on 14–3-3. The displaced phospho–NFAT interacted with Cn, where it was dephosphorylated allowing entry in the nucleus to contribute to the hypertrophic transcriptional response. Thus, our findings reveal a previously unrecognized novel essential regulatory role of MAP kinase signaling in Cn activation.

YY1 Protects against Pathologic Cardiac Hypertrophy through a Mechanism That Involves HDAC5 Interaction: Evidence of CaMKII Inhibition of YY1 HDAC5 Interaction

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Activation of the fetal gene program during pathologic hypertrophy is characterized by changes in gene expression that includes repression of genes that are expressed during adult development (α-Myosin Heavy Chain (α-MHC) and sarcoplasmatic reticulum ATPase 2a (SRCA2a)), and re-expression of genes that are present during the fetal development (β-Myosin Heavy Chain (β-MHC), atrial and brain natriuretic peptide (ANP and BNP), and skeletal α–actin). We have recently shown that YY1, a transcription factor capable of either repressing or activating a variety of promoters, is up-regulated in human heart failure. In cardiac myocytes, YY1 represses αMHC promoter activity, suggesting that it functions as a pro-hypertrophic agent. However, recent data from our laboratory suggest that YY1 is in fact an anti-hypertrophic agent. More specifically, we show here that the over-expression of YY1 in neonatal rat cardiac myocytes (NRCMs) results in repression of the genes involved in both adult and fetal development. We also show that β2-adrenergic–mediated induction of the fetal gene program is reversed upon over-expression of YY1. Moreover, knockdown of YY1 expression by siRNA results in up-regulation of all the genes present during fetal development and in an increase in cell size in NRMs consistent with the development of pathologic hypertrophy. We also show that YY1 interacts with HDAC5 and that this interaction increases the repressive effect of YY1. Lastly, CaMKII has been shown to phosphorylate class II HDACs, promoting their nuclear export and therefore preventing their function as repressors of transcription. Here we show that inhibition of CaMKII by KN-93 results in a dramatic increase in the repression of gene expression in cells infected with the YY1 adenovirus, suggesting that CaMKII phosphorylation of HDACs results in blockade of YY1 and HDACs interaction and consequently release of its repressive function. Our results strongly suggest that YY1 functions as an anti-hypertrophic factor and up-regulation of YY1 in human heart failure may actually be a protective mechanism against pathologic hypertrophy.

Divergent Roles for RhoA and Rho Kinase in Cardiac Hypertrophy and the Progression to Heart Failure

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Rho is a low-molecular-weight GTPase implicated in the regulation of hypertrophic cardiac muscle cell growth in vitro. There are multiple downstream effectors of RhoA, including rho kinase (ROCK) and mDia/phosphous (mDia) However, the relative in vivo role of RhoA and ROCK in mediating cardiac hypertrophy remains less clear as transgenic mice overexpressing RhoA show a prematurely lethal phenotype with development of ventricular failure but no hypertrophy. To circumvent the limitations of RhoA overexpression from birth, adult mouse hearts were transfected with an adenov-associated virus encoding a constitutively activated RhoA (AAVRhoA) or AAVROCK in vivo using a technique recently developed in our lab. Whole organ transfection with AAVRhoA led to a 6-fold increase in membrane Rho protein and a transfection with AAVROCK resulted in a 5-fold increase in ROCK activity compared to hearts transfected with a reporter gene AAVbgal. Echo-cardiography revealed significantly higher LV mass at 4 weeks in AAVRhoA mice (105 ± 4 mg) and AAVROCK mice (139 ± 6) compared to mice transfected with AAVbgal (73 ± 9, P < 0.05) with AAVRhoA mice showing a marked reduction in LV fractional shortening (42% ± 4 vs AAVROCK (69% ± 5, P < 0.01) or AAVbgal (72% ± 4, P < 0.01). At week 10, mice transfected with AAVRhoA showed dilation of the LV chamber and further reduction in fractional shortening (51% ± 7) compared to AAVROCK (58 ± 7, P < 0.05). It was not until week 16 that AAVROCK mice showed a reduction in LV FS. Given the profound difference in phenotype, we hypothesized that RhoA signaling independent of ROCK results in a heart failure phenotype. We treated AAVRhoA mice with the ROCK inhibitor (Y27632, 1 mg/kg ip bid) after transfection. Treatment with Y27632 resulted in a marked reduction in FS at week 2 (45% ± 4) with a minimal increase in cardiac mass suggesting that ROCK signaling plays a predominant role in cardiac hypertrophy and RhoA signaling results in a failure phenotype. AAVRhoA was associated with the activation of mDia and SRF activation, but not the activation of GATA4 whereas AAVROCK resulted in GATA4 activation. These data provide the first evidence for divergent roles for RhoA and ROCK signaling in cardiac hypertrophy and the progression to heart failure.
Outstanding Early-Career Investigator Award Finalists

Circ Res. 2005;97:e8
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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