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Undermining the endothelium by ablation of MAPK-MEF2 signaling

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Numerous stimuli activate Big MAPK-1 (BMK1), an MAPK that activates the myocyte enhancer factor-2 (MEF2) transcription factor. Conditional gene deletion showed BMK1 to be required for survival of endothelial cells (see the related article beginning on page 1138). An active form of MEF2C could partially bypass the requirement for BMK1 for endothelial cell survival in vitro. These findings reveal an essential role for BMK1-MEF2 signaling in an endothelial cell survival pathway and raise interesting questions about the molecular basis of this response.

A variety of extracellular stimuli transmit signals from the cell membrane to the nucleus via a cascade of MAPKs (1). MAPK pathways contain three distinct types of MAPKs that are activated sequentially (Figure 1). The most upstream kinase, MAPK kinase kinase (MEKK), is activated by G protein-coupled receptors and phosphorylates a subordinate MAPK kinase (MEK), which phosphorylates an MAPK. The final MAPK in the cascade phosphorylates various transcription factors, leading to activation of specific programs of gene expression.

Nonstandard abbreviations used: big MAPK-1 (BMK1); embryonic day (E); endothelial cell (EC); MAPK kinase (MEK); MAPK kinase kinase (MEKK); myocyte enhancer factor-2 (MEF2); polyinosinic-polycytidylic acid (pIpC).

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Big MAPK-1 (BMK1; also called ERK5) is the terminal MAPK that is activated by MEK5, which is activated by MEKK2/3 (Figure 1). BMK1 is unique among MAPKs because of its large size and bifunctionality. The N-terminal region of BMK1 contains the kinase domain, while the C-terminal region functions as a transcription activation domain (2). One of the best-characterized targets of BMK1 is the myocyte enhancer factor-2 (MEF2) family of transcription factors (3-6). There are four mammalian *MEF2* genes, *MEF2A*, *-B*, *-C*, and *-D*, which are expressed in overlapping patterns in numerous cell types (7). MEF2 factors bind DNA as homo- and heterodimers and activate or repress transcription by recruiting positive or negative cofactors, many of which are cell type-specific and signal-responsive. First discovered as regulators of muscle development, MEF2 factors are now

known to play diverse roles in the control of cell growth, survival, and apoptosis (7).

BMK1 acts in an endothelial cell survival pathway

Previous studies showed that KO mice lacking BMK1 die around embryonic day (E) 10 from severe abnormalities in cardiovascular development that are remarkably similar to defects seen in KO mice lacking MEKK3 or MEF2C, consistent with the sequential actions of these signaling molecules (8-13). However, the severity of the defects in these mutant embryos, and the associated abnormalities in yolk sac development, complicated the interpretation of the phenotypes and precluded the identification of the precise cell type responsible for embryonic lethality.

To further define the role of BMK1 in mouse development, Hayashi and coworkers generated mice harboring a conditional *BMK1* allele (14). In this issue of the *JCI*, they report that excision of *BMK1* after birth, using an inducible Cre recombinase transgene controlled by polyinosinic-polycytidylic (pIpC) acid, resulted in degeneration of the cardiovascular system, accompanied by multifocal hemorrhages, distended capillaries, and ruptures in the normally seamless endothelial lining of the vessels and the

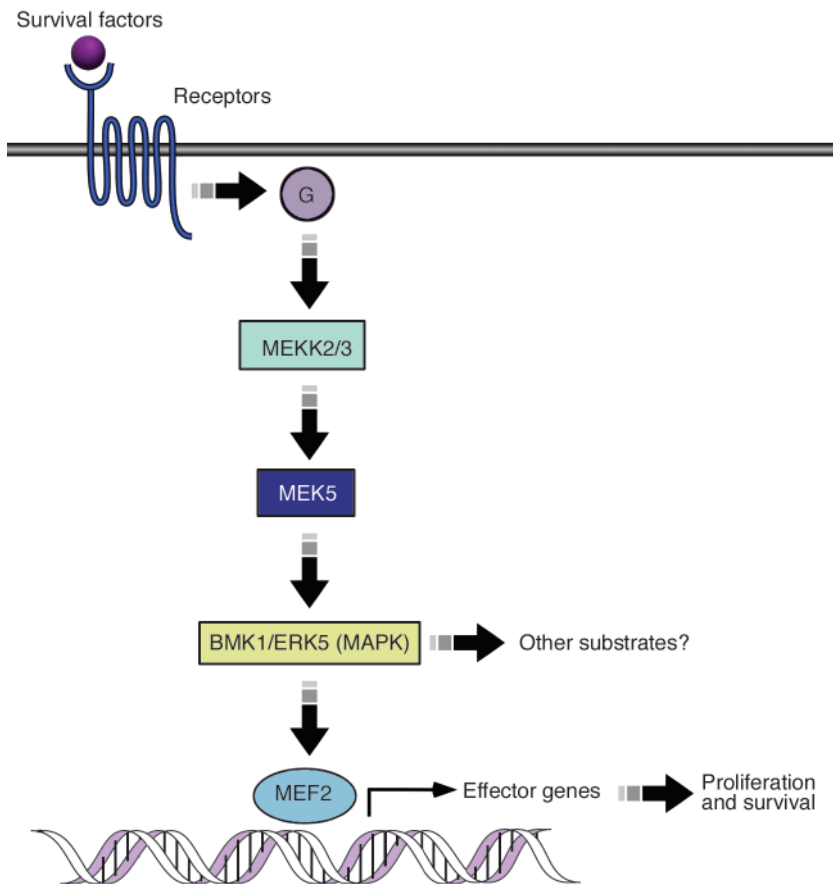


Figure 1

MAPK signaling pathways. Survival signals activate MEKK2/3, which activates MEK5, which activates BMK1. BMK1 stimulates the transcriptional activity of MEF2C by phosphorylating the transcription activation domain and by interacting directly with MEF2C and contributing its own transcriptional activation domain. MEF2C is required for cell survival and proliferation by activating downstream target genes that remain to be identified.

heart. Vascular demise following *BMK1* deletion was attributable to apoptosis of endothelial cells (ECs). Subsequent analysis of the expression pattern of the pIpC-inducible Cre transgene using a floxed alkaline phosphatase reporter showed strong expression in endothelial and endocardial cells. In agreement with the conclusion that the lethal phenotype reflected an essential role of BMK1 in the endothelium, *BMK1* deletion using a Cre transgene controlled by the endothelial-specific *Tie2* promoter caused embryonic lethality at E9.5–10.5 with a phenotype indistinguishable from that of global *BMK1*-KO mice.

ECs normally proliferate and undergo a mesenchymal transformation in cardiac explant cultures. In contrast, explanted ECs from *BMK1*-KO mice failed to proliferate in vitro (14). The specific dependence of ECs on *BMK1* signaling for proliferation

and survival was further demonstrated by the finding that ECs isolated from conditional *BMK1*-KO mice stopped dividing and underwent apoptosis upon deletion of *BMK1* with a Cre-expressing adenovirus in vitro, whereas fibroblasts were unaffected by *BMK1* deletion.

VEGF and other growth factors that promote EC survival and proliferation activate *BMK1* in cultured ECs. The possibility that *MEF2C* is a target of *BMK1* in an EC survival pathway is supported by the finding that serum, a source of survival signals, stimulated activity of an *MEF2C* reporter in cultured ECs, and this stimulatory effect was lost upon deletion of *BMK1* (14). Moreover, infection of *BMK1*-KO ECs with an adenovirus encoding a constitutively active form of *MEF2C* fused to the VP16 activation domain partially protected cells from apoptosis following *BMK1* deletion.

A *BMK1*-*MEF2* connection in endothelial survival

Together, these findings reveal a cell-autonomous requirement for *BMK1*-*MEF2* signaling for EC proliferation and viability. The endothelial phenotype of *BMK1*-KO mice raises interesting questions about the cellular specificity of this response and the targets of *BMK1* and *MEF2* in the EC survival pathway. For example, it is unclear why ECs are so dependent on the *BMK1*-*MEF2C* signaling module for survival, whereas fibroblasts and hepatocytes have been shown to be unaffected by deletion of *BMK1* (14). Previous studies have shown that *BMK1* and *MEF2C* also promote survival of neurons in vitro (15–18). Perhaps other MAPK and *MEF2* family members play redundant roles in other cell types, or perhaps ECs and neurons are more dependent on continual antiapoptotic signals for survival than other cell types. It will be of interest to identify the target genes of *MEF2C* in the EC survival pathway and to determine the extent to which *BMK1* substrates other than *MEF2C* contribute to the EC phenotype of *BMK1*-KO mice. The fact that *MEF2C*-VP16 protected *BMK1*-KO ECs only partially from cell death suggests the existence of additional antiapoptotic *BMK1* targets.

Other cardiovascular functions of *BMK1*-*MEF2*

The vascular endothelium is a seamless, yet dynamic, tissue required for multiple functions of the cardiovascular system, including maintenance of vascular tone, regulation of blood circulation, coagulation, inflammatory responses, and proper growth and development of vascular smooth muscle and cardiac myocytes. Perturbation of the vascular endothelium is responsible for a variety of cardiovascular disorders, including atherosclerosis, thrombosis, and hypertension. It will be of interest to determine whether *BMK1* and *MEF2* are involved in the pathogenic mechanisms associated with these endothelial disorders. In this regard, *BMK1* is potentially activated by fluid shear stress within the vessel wall, which is atheroprotective (19). Conversely, lack of shear stress has been shown to trigger EC apoptosis. Shear stress and activated *MEK5* stimulate phosphorylation of the pro-apoptotic factor *Bad*, which prevents it from translocating to the mitochondria and activating caspase-3. *BMK1* is also activated by and plays a protective role against oxidative stress. The apparent requirement



of MEF2C for maintenance of endothelial integrity is also intriguing in light of the recent association of premature coronary artery disease and myocardial infarction with a mutation in the human *MEF2A* gene (20). Since MEF2A is highly expressed in the endothelium and is a substrate for BMK1, it is likely to act within the same EC survival pathway as MEF2C.

A remarkable number of processes within the cardiovascular system are dependent on signaling from MAPKs to MEF2 (7). In addition to its requirement for EC survival, this signaling pathway is important for differentiation and morphogenesis of cardiac and smooth muscle cells, and has been implicated in numerous cardiovascular disorders, including cardiac hypertrophy, dilated cardiomyopathy, coronary artery disease, and myocardial infarction. Further insights into the functions and mechanisms of action of this signaling module promise to provide new opportunities for its therapeutic manipulation in the settings of human disease.

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Insig: a significant integrator of nutrient and hormonal signals

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Lipogenesis is regulated by sterols and by insulin through the regulated expression and activation of the sterol regulatory element-binding proteins (SREBPs). A new study shows one way in which sterol and insulin regulation can be decoupled (see the related article beginning on page 1168). In transgenic mice overexpressing a protein that regulates SREBP activation, lipogenesis is more sensitive to cholesterol and less sensitive to insulin.

Nonstandard abbreviations used: insulin receptor substrate-2 (IRS-2); SREBP cleavage-activating protein (SCAP); sterol regulatory element-binding protein/adipocyte differentiation factor-1 (SREBP/ADD1).

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Animal cells, like nations, have a domestic and a foreign policy. “Domestic policy” involves local control of metabolism, responses to energy charge, redox status, or the concentration of key allosteric regulators (e.g., malonyl-CoA and fructose 2,6-bisphosphate). The world of a multicellular organism imposes a “foreign policy” on its cells. They must

respond to global signals in the interest of the whole organism.

Global and local signals invariably converge (as former Congressman Tip O’Neil famously quipped, “All politics is local.”). Lipogenesis is a case in point. Feeding, fasting, and refeeding cause large changes in the rates of fatty acid and cholesterol synthesis. These changes are evoked by both the carbohydrate and the lipid components of the diet. We now have a molecular window into some of the key molecules that integrate nutrient-derived signals.

A key breakthrough was the simultaneous discovery by Tontonoz et al. (1) and Yokoyama et al. (2) of the sterol regulatory ele-