Prime Time for JNK-Mediated Akt Reactivation in Hypoxia-Reoxygenation

James Shaw, Lorrie A. Kirshenbaum

Despite continuous advances in cardiology, heart failure still remains one of the leading causes of morbidity and mortality in the world. Heart failure can be triggered by a variety of cellular changes within the myocardium leading to a loss of contractility, arrhythmias, and ventricular remodeling. For example, myocytes in a failing heart have been shown to exhibit changes in intracellular calcium handling and altered ion channels, as well as changes in metabolism and signal transduction.1 Given that myocytes have a limited ability for self-renewal, it follows that inappropriate cell death may also contribute to permanent ventricular remodeling and irretraceable heart failure.2,3 Not surprisingly, recent evidence has indicated that cardiomyocyte cell death by an apoptotic pathway may play a causal role in development of cardiomyopathies and heart disease more generally.4–6

Apoptosis is an energy-dependent cell suicide program that requires a change in gene expression. Two predominate pathways have been identified to be important for initiating the apoptotic program: a surface death receptor-mediated “extrinsic” pathway, and a mitochondria-mediated “intrinsic” pathway, both of which result in the activation of cellular proteases known as caspases.4,7 The former is activated by ligands such as TNF-α or Fas binding to cell surface death receptors, the latter activated by internal cues that follow cellular stresses including hypoxia, oxidative stress, toxins, and environmental triggers. An important family of proteins involved in regulating apoptosis is the Bcl-2 family, which includes both cytoprotective (Bcl-2, Bcl-XL) and apoptotic (Bid, Bax, Bad, Noxa, Puma, Bnip3) members. Collectively, these proteins regulate the intrinsic mitochondrial death pathway by influencing permeability of the outer and inner mitochondrial membranes6,8.

Signal Transduction and Regulation of Apoptosis

The cell’s “decision” to live or die is derived from the dominance of either prodeath or prosurvival proteins and cellular factors. The accumulation of such molecules is the result of signaling pathways that transmit and amplify internal or external signal cues to various organelles such as the endoplasmic reticulum, mitochondrion, and nucleus to activate or prevent apoptosis.

The mitogen-activated protein kinase (MAPK) pathways are known to play both pro- and antiapoptotic roles in cardiomyocyte cell death.9 There are three principal MAPK terminal effector kinases: the extracellular signal-regulated protein kinases (ERK), p38, and the c-Jun N-terminal kinases (JNK). Historically, MAPK were initially thought to transduce growth signals that underlie cardiac hypertrophy (reviewed in reference 10). However, more recent studies have detailed a spectrum of cellular actions that include their involvement in regulating cell survival and cell death. The ERKs are cytoprotective in the heart, typically involving the activation of the Ras-Raf-MEK signaling cascade. Presumably, these impinge on components of the cell death machinery to prevent cell death. For example, ERK activation has been shown to diminish ischemia-reperfusion–induced apoptosis and injury.11

p38 MAPK and JNK are stress activated protein kinases (SAPK) that are known to regulate cell viability, though there is still considerable debate as to whether their effects are cytoprotective or proapoptotic. Both p38 and JNK are phosphorylated by MAPK kinases MKK3/6 and MKK4/7, respectively, in response to several cellular stresses including ischemia (p38), reperfusion (JNK), as well as UV radiation and cytokines. Myocyte cell culture experiments have suggested a cytoprotective role for p38 where MKK6 overexpression was shown to inhibit apoptosis via NF-κB and interleukin-6.12 In another study, p38 was shown to have an opposite effect where pharmacological inhibition of p38 decreased ischemia-induced apoptosis in cultured myocytes, implying that p38 may be proapoptotic.13 In vivo studies involving p38 and apoptosis primarily indicate that p38 is proapoptotic in the heart.14,15 The interpretation of these apparent discordant results may be explained by differences in culture conditions, experimental time points, or perhaps some undefined or nonspecific effects of the inhibitors used.

The impact of JNK signaling on cell fate is also controversial and remains an active point of inquiry particularly in the context of the heart. On one hand, JNK signaling has been shown to provoke apoptosis of cardiac myocytes16; yet on the other hand JNK has also been shown to be cytoprotective and prevent apoptosis.16–18 How can these apparent discrepancies in JNK action be explained? One plausible explanation includes the differential regulation of certain cellular factors by JNK that either promote or alternatively inhibit cell death. Further, the context of JNK activation may add to the complexity of downstream targets regulated by JNK in a cell.
specific manner. For example, JNK activation leads to the phosphorylation of a number of cellular factors including AP-1, c-jun, and most notably those proteins that intimately regulate cell survival such as Bcl-2, p53, and Akt/PKB.

**JNK Mediates Reactivation of AKT**

Among the vast signaling pathways known to regulate cell survival, considerable attention has focused on the serine/threonine kinase Akt/PKB. The paradigm for activation of cytoplasmic Akt/PKB involves the phosphorylation of critical residues Thr 308 and Ser 473 mediated by the PI3-kinase and phosphoinositide-dependent kinases 1 and 2 (PDK1 and PDK2). In particular PDK-1 is believed to phosphorylate C-terminal pleckstrin homology domain at Thr 308 resulting in partial Akt/PKB activity. However, phosphorylation of Ser 473 in the C-terminal regulatory domain is required for full Akt/PKB activation (reviewed in reference 20). The mechanism responsible for phosphorylation of the Ser 473 site is unresolved; however, autocatalytic events or an alternative PDK2 including the integrin-linked kinase (ILK) have been suggested. Perhaps best known for its involvement in promoting cardiac myocyte growth and hypertrophy, Akt/PKB activation has also been shown to suppress and/or prevent apoptosis in a number of model systems by a variety of death factors. This implies that Akt/PKB must signal through common convergence points that coordinately regulate cell growth and survival.

Indeed, constitutively active mutants of Akt/PKB have been shown to reduce hypoxia-induced apoptosis of ventricular myocytes. Further gain-of-function studies have demonstrated a reduction in apoptosis and infarct size in vivo in hearts after myocardial infarction. Recent studies have determined that the antiapoptotic properties conferred by Akt/PKB requires a functional kinase and several cellular targets involved in the regulation of cell growth and cell death have been identified. These include but are not restricted to Bad, GSK3β, FKHRl/FOXO3a, GULT, caspase 9, IKKβ, and eNOS. The fact that several proteins involved in homeostatic regulation are regulated by Akt/PKB highlights its importance as a central regulator of cell fate and potential therapeutic target.

In this issue of *Circulation Research*, Shao et al. provide new compelling in vivo and in vitro evidence that JNK prevents apoptosis of ventricular myocytes after hypoxia-reoxygenation through a mechanism that involves Akt/PKB. Using a variety of pharmacological and genetic approaches, the authors dissect the complex signaling pathways emanating downstream of p38/JNK activation. In this report Shao et al. demonstrate that dual p38/JNK as well as selective JNK inhibition promotes hypoxia-induced apoptosis. Moreover, they further show that hypoxia-reoxygenation injury in the cells defective for p38/JNK was prevented by a constitutively activated form of Akt/PKB. Perhaps most intriguing was the demonstration that selective p38 inhibition in vivo protected against myocardial damage after ischemia reperfusion with hearts exhibiting improved cardiac function. In contrast, JNK inhibition appeared to exacerbate the injury and hemodynamic recovery on reperfusion. Collectively, the data highlight the involvement and potential role of JNK as a survival factor.

In attempts to ascertain the underlying mechanism by which JNK circumvents death, the authors uncover a novel JNK phosphorylation site at Thr 450 of Akt/PKB. The significance of the Thr450 site is unknown and not well studied. Interestingly, the authors show JNK-mediated phosphorylation of the Thr 450 site is not only important for regulating the basal Akt/PKB activity but is essential for efficient reactivation of Akt/PKB on reoxygenation. Interestingly, the authors demonstrate that phosphorylation of Thr 450 by JNK was crucial for subsequent phosphorylation of Ser 473 and activation of Akt/PKB. This raises the interesting possibility that phosphorylation of the Thr450 site by JNK represents a novel nodal point for Akt/PKB activation. In this context it is possible that phosphorylation of the Thr450 by JNK “primes” other Akt/PKB phosphor-acceptor sites for complete activation and full Akt/PKB kinase activity in an analogous fashion to the Ser473 site which is presumably required for phosphorylation of the Thr308 by PKD-1 (Figure). The molecular mechanism by which the Thr450 facilitates Akt/PKB phosphorylation was not determined, nor was the impact of selective site specific phosphorylation by JNK on Akt/PKB localization or distribution within the cell. There are now several reports indicating that cytoplasmic versus nuclear Akt/PKB may differentially influence cell survival and/or cell growth. Further, the relative level and extent of Akt/PKB activation in response to a given phosphorylation signal may dramatically influence cardiac cell physiology and function.

It is tempting to speculate that the dichotomous actions of JNK may partially be explained by the relative availability and preferential site-specific phosphorylation of a given JNK.
substrate. In the case of Akt/PKB, site specific phosphorylation could potentially target or redirect Akt/PKB to organelles such as nucleus, endoplasmic reticulum, or mitochondria known to regulate cell growth or cell death. It will also be important to determine whether proteins such as Bad, GSK3β, FOXO3a, and others are equivalently affected by JNK activity on the Akt/PKB Thr450 site. It is equivalently Underscoring whether the regulation of Akt/PKB by JNK is restricted to JNK1 isoform or a universally conserved feature among other JNK proteins. Nevertheless, the data provides new intriguing information to support a cytoprotective role for JNK in the heart and importantly identify Akt/PKB residue T450 as a novel the downstream site for “priming” Akt/PKB reactivation after hypoxia-reoxygenation.

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References

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