HAX-1 Represses Postmitochondrial Caspase-9 Activation and Cell Death During Hypoxia–Reoxygenation

James Shaw, Lorrie A. Kirshenbaum

A delicate balance exists between cell growth and cell death. In the context of the adult myocardium, inappropriate or inordinate cell loss through an apoptotic process, coupled with the limited regenerative ability of the heart to repair after injury, has been suggested to be a contributing factor to the decline in ventricular performance in patients with heart failure. The ability to prevent or modulate untimely or inordinate cardiac cell death after myocardial injury would be of significant therapeutic value in maintaining cardiac function. For this reason, there has been considerable interest in deciphering the signaling pathways and cellular factors that govern cell survival and cell death under normal and disease conditions. Apoptosis has received considerable attention in recent years by virtue of the events leading to cell death occurring through a highly ordered, genetically regulated process. This lends versatility for the design of novel therapies against cellular targets known to activate or repress cell death.

Regulation of apoptosis in mammalian cells arises from the seminal discoveries of the ced-3, ced-4, and ced-9 genes in Caenorhabditis elegans. Mammalian counterparts to ced-3 and ced-4 have been cloned and identified. Ced-3 belongs to a large family of cellular cystine proteases, known collectively as caspases (for cysteinyl-aspartate–specific proteases) for their preferential ability to cleave cellular substrates after aspartic acid residues (reviewed previously2,3). The cleavage of caspase-specific substrates results in the biochemical destruction of the cell and phenotypic changes associated with apoptosis. To date, more than 14 caspases have been identified (reviewed previously20–22). Of these, caspase-2, -8, -9, and -10 are thought to be initiator caspases, whereas caspase-3, -6, and -7 are considered to be death effectors. Other caspases, including caspase-1, -4, -5, -11, -13, and -14, have been implicated in the inflammatory response.6,7 Caspases exist as inactive zymogens and require proteolytic cleavage for full biological activity. Initiator caspases have a long prodomain that contains either a CARD (CAspase Recruit Domain) or a death effector domain (DED) (Figure). Unlike the death effector caspases, which are activated by simple caspase cleavage, the activation of the initiator caspases, such as caspase-9, is more complex, involving autocatalytic processing by induced proximity of caspase monomers.8 Homotypic or heterotypic interactions with the DED or CARD with adaptor proteins can also facilitate proteolytic activation of caspases.9–11

One paradigm for caspase activation follows a receptor-regulated process, or “extrinsic pathway,” that includes death receptors, DR5, DR4, Fas, CD95, and tumor necrosis factor (TNF)-α receptors. In this model, binding of ligand to receptor promotes assembly of the death-inducing signaling complex (DISC),12 followed by recruitment of death domain (DD) and DED coupling proteins, which facilitates the activation of initiator caspases 8 and 10.8,13 The processing of upstream “initiator” caspases can sequentially activate prodeath factors, such as Bid, or more distal “death effector” caspases, such as caspase-3, -6, or -7.14

In addition to a receptor-based model for caspase activation, a mitochondrial-regulated or “intrinsic” pathway for caspase activation has recently been described. Mitochondrial defects leading to permeability transition pore (PTP) opening is considered a central component of the intrinsic mitochondrial death pathway.14–16 The PTP is presumed to be comprised in part by porin/voltage dependent anion channel and the adenine nucleotide translocase, as well as other outer and inner mitochondrial membrane proteins. The PTP opens in response to death signals. This results in mitochondrial swelling, generation of reactive oxygen species, and dissipation of ∆Ψm.17 Mitochondrial PTP opening has been reported during hypoxia, increased intracellular calcium, as well as oxidative stress injury.18 Furthermore, PTP opening is thought to trigger release of proapoptotic factors by mitochondria such as Smac (Second mitochondrial activator of caspases), cytochrome c,19 apoptosis-inducing factor (AIF), Htr2A/Omi, endonuclease G (Endo G), and other factors (reviewed previously20,21).

Ostensibly, interaction of the CARD domain of pro–caspase-9 with the CARD domain of the ced-4 homolog, Apaf-1 (Apoptosis-activating factor-1), together with cytochrome c, form the apoptosome and in the presence of dATP result in the activation of caspase-922 (Figure). Once activated, caspase-9 is believed to facilitate the activation of death effector caspases 3, 6, and 7, resulting in the biochemical destruction of the cell. Endogenous inhibitors of the apoptosome include the inhibitor of apoptosis proteins (IAP-1, IAP-2) and X-linked inhibitor (XIAP), which are believed to avert apoptosis by inhibiting caspase-9.23 Moreover, several lines of investigation suggest a close association between the intrinsic death pathway and members of the Bcl-2 gene family.24 For example, life-promoting Bcl-2 family members typically prevent cell death by interfering with pre- or postmitochondrial events required for caspase activation, whereas death-promoting members such as Bax,
Bak, Bim, and others, activate the intrinsic death pathway by disrupting mitochondrial function. Because inappropriate or untimely caspase activation would otherwise have catastrophic consequences to cell survival, particularly in the context of the adult myocardium, it implies that caspase activation must be a highly regulated and tightly coordinated event. However, very little is known of the cellular factors that regulate caspase activation in the absence of death signals.

In this issue of Circulation Research, Han et al provide novel information proposing that HAX-1 (HS-1–associated protein) inhibits activation of caspase-9. This is especially significant because the only class of protein previously thought to directly inhibit caspase-9 were the IAPs, which interfere with the apoptosome. HAX-1 was initially identified by Suzuki et al as a 35-kDa interacting partner with HS-1, a signal-transduction protein in hematopoietic cells. Interestingly, HAX-1 has weak sequence homology to the proapoptotic Bcl-2 family member protein Nip3, a protein known to be upregulated in response to oxidative stress in cardiac myocytes causing mitochondrial defects and apoptosis. Although HAX-1 had previously been characterized as a putative antiapoptotic factor, the underlying mechanism by which HAX-1 suppressed cell death was not determined. The report by Han et al provides exciting new evidence that HAX-1 is a novel endogenous inhibitor of apoptosis in cardiac myocytes. Furthermore, the authors provide mechanistic evidence that HAX-1 averts cell death by blocking the biological activation of caspase-9.

In brief, Kang and colleagues found that, in contrast to nonmuscle cells, cardiac myocytes were inherently resistant to apoptosis provoked by caspase-9. This sparks the exciting possibility that cardiac myocytes are innately protected from caspase-9–induced cell death by the presence of an endogenous inhibitor. In fact, by 2 hybrid analyses, Han et al demonstrate protein–protein associations between HAX-1 and caspase-9. Han et al further demonstrate that the biological processing of caspase-9 in vitro and in vivo was inhibited by HAX-1. Indeed, overexpression of HAX-1 in ventricular myocytes prevented caspase-9 activation and hypoxia–reoxygenation–induced apoptosis. In elegant structure function studies, the authors demonstrate that the amino terminus of HAX-1 amino acids 175 to 206 was critical for the interaction with caspase-9. However, both the N- and C-terminal domains of HAX-1 were found to be necessary for full antiapoptotic function. In genetic ablation studies using small interfering RNA (siRNA) direct against HAX-1, the authors show increased sensitivity of cardiac myocytes to death signals by increased caspase-9 activity. Perhaps among the most intriguing experiments to prove HAX-1 prevents cell death by antagonizing caspase-9 activation emanates from studies in which the authors test the ability of HAX-1 to prevent cell death induced by oxidative stress in cells derived from caspase-9–deficient mice. In contrast to wild-type control cells, which were rescued from oxidative stress–induced apoptosis by HAX-1, HAX-1 overexpression failed to suppress apoptosis in cells deficient for caspase-9. The fact that the antiapoptotic property of HAX-1 was lost in caspase-9−/− cells suggests that, operationally, caspase-9 is a plausible downstream target of HAX-1. Finally, the authors show by detailed confocal microscopy that caspase-9 and HAX-1 localize from cytosol to mitochondrion during hypoxia–reoxygenation, with the physical association of the 2 proteins occurring within the mitochondrial compartment.

Although the report by Han et al provides novel insight into the mechanisms by which HAX-1 inhibits caspase-9 activation and cell death in the heart, there remain several unanswered questions as to how HAX-1 prevents caspase-9 activation and whether its mode of action is direct or indirect. For example, although caspase-9 activation was shown to be inhibited by HAX-1 overexpression, it is not known whether HAX-1 blocks pro–caspase-9 by disrupting its ability for autoactivation via an induced proximity model for
involved in caspase activation, as well as the opportunity for forms of cell death, such as necrosis or autophagy, remains to be a putative HAX-1 target and suggest operationally that HAX-1 myocytes. Furthermore, the authors identify caspase-9 as a mitochondrial or expression of the IAP-1, IAP-2 or XIAP are altered in HAX-1 and Bim at the level of the mitochondria or ER. Likewise whether the IAP-1, IAP-2 or XIAP are altered in HAX-1 or expression of the IAP-1, IAP-2 or XIAP are altered in HAX-1 overexpressing cells was not determined. The localization of HAX-1 and caspase-9 to mitochondria during hypoxia–reoxygenation is interesting and, although not explored in this report, raises the possibility that HAX-1 prevents caspase-9 activation by sequestering or preventing its cytoplasmic interaction with the apoptosome.

Nevertheless, the authors provide new important information regarding the role of HAX-1 as a novel survival factor in cardiac myocytes. Furthermore, the authors identify caspase-9 as a putative HAX-1 target and suggest operationally that HAX-1 averts cell death of cardiac myocytes by preventing the activation of caspase-9. Whether HAX-1 is sufficient to prevent other forms of cell death, such as necrosis or autophagy, remains to be determined. Nonetheless, these exciting findings provide a new avenue for future studies on pre- and postmitochondrial events involved in caspase activation, as well as the opportunity for developing novel inhibitors for preventing or limiting the extent of cell death during disease conditions.

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Disclosures

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