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 cysteine proteases, known collectively as caspases (for cysteinyl-aspartate–specific proteases) for their preferential ability to cleave cellular substrates after aspartic acid residues (reviewed previously). 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 previously). 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 (reviewed previously). The ability for the design of novel therapies against cellular targets known to activate or repress cell death.

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Bak, Bim, and others, activate the intrinsic death pathway\textsuperscript{25} 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 \textit{Circulation Research}, Han et al provide novel information proposing that HAX-1 (HS-1–associated protein)\textsuperscript{26} 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\textsuperscript{27} 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.\textsuperscript{28,29} Although HAX-1 had previously been characterized as a putative antiapoptotic factor,\textsuperscript{30} 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\textsuperscript{26} 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. In contrast to wild-type control cells, which were rescued from 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\textsuperscript{−/−} 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\textsuperscript{26} 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

\textbf{Intrinsic death pathway in cardiac myocytes.} Death signals provoke mitochondrial perturbations resulting in the liberation of proapoptotic factors including cytochrome c, Smac, Htr2A/Omi, endonuclease G, and procaspases. Left, The interaction of cytochrome c with Apaf-1 and pro–caspase-9 in the presence of dATP forms the apoptosome and the resultant activation of caspase-9. Right, HAX-1 blocks the activation of caspase-9, downstream death effector caspases, and cell death.
caspase-9 activation, or by disrupting homotypic or heterotypic interactions with adapter proteins required for caspase-9 activation. It is equally unknown whether the CARD domain of caspase-9 is conformationally altered or affected by HAX-1 overexpression, as this would presumably interfere with caspase-9 activity. One likely scenario not tested is that HAX-1 simply disrupts the apoptosome formation by displacing Apaf-1 or influencing one of its components. Furthermore, as discussed by the authors, there is currently no evidence to support a model in which HAX-1 exclusively interacts with caspase-9. Therefore, the inhibition of caspase-3 in cells overexpressing HAX-1 could result from a direct effect of HAX-1 on caspase-3, in addition to its effects on caspase-9. If this supposition is true, then inhibition of the death effector caspases 3, 6, or 7 could account, in part, for the partial antiapoptotic effect of HAX-1 in the caspase-9−/− cells. The other point of interest that was not addressed in the present study was the potential influence of HAX-1 on the Bcl-2 gene family, given their reported ability to influence mitochondrial function. Clearly, one could argue that HAX-1 prevents cell death and postmitochondrial defects leading to caspase-9 activation by preventing the actions of Bax, Bak, Bad, and Bim at the level of the mitochondria or ER. Likewise whether the expression and/or cellular distribution of Bcl-2 survival factors 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 as a novel survival factor in cardiac myocytes. Furthermore, the authors identify caspase-9 as a potential 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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None.

**References**


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