This Review is part of a thematic series on **Mitochondrial Dysfunction in Ischemia**, which includes the following articles:
Role of the Mitochondrial Permeability Transition in Myocardial Disease
Primary and Secondary Signaling Pathways in Early Preconditioning That Converge on the Mitochondria to Produce Cardioprotection
Evidence for Mitochondrial K^+ Channels and their Role in Cardioprotection

The Mitochondrial Death Pathway and Cardiac Myocyte Apoptosis

Elizabeth Murphy, Guest Editor; Roberto Bolli, Editor

The Mitochondrial Death Pathway and Cardiac Myocyte Apoptosis

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Abstract—Apoptosis has been causally linked to the pathogenesis of myocardial infarction and heart failure in rodent models. This death process is mediated by two central pathways, an extrinsic pathway involving cell surface receptors and an intrinsic pathway using mitochondria and the endoplasmic reticulum. Each of these pathways has been implicated in myocardial pathology. In this review, we summarize recent advances in the understanding of the intrinsic pathway and how it relates to cardiac myocyte death and heart disease. **(*Circ Res*. 2004;95:957-970.)**

Key Words: apoptosis ■ necrosis ■ cell death ■ mitochondria ■ Bcl-2 ■ caspase ■ death-inducing signaling complex ■ apoptosome ■ ischemia ■ heart failure

Over the past decade, interest in cell death has intensified among scientists in multiple areas of biology and medicine. This fascination has been driven by the discovery that apoptosis is mediated by an ancient program that is hard-wired into all metazoan cells. Renewed attention in the cardiovascular field has been fueled by the notion that cell death is often an active process that, in principle, can be inhibited in various disease states.

Types of Cell Death

Three modes of death are currently defined by morphological criteria: apoptosis, autophagy, and necrosis. Work over the past 20 years has provided a molecular framework for apoptosis,[1] the subject of this review. Autophagy is an important cellular process in which proteins and organelles are degraded in the lysosomal pathway so that their constituents can be used as energy substrates by the cell.[2] There exists considerable debate, however, as to whether autophagy functions as a death process.[3] The mechanisms that mediate necrosis in mammals are poorly understood. Recent studies in *Caenorhabditis elegans*, however, demonstrate that necrosis is carried out by specific cellular pathways,[4–6] suggesting that this form of cell death may be more "programmed" than initially thought. With further understanding of the mechanisms that mediate various types of cell death, it is likely that future classification systems will be based on molecular, rather than morphological, criteria.

Apoptosis

Apoptosis is an evolutionarily conserved suicide process that plays critical roles in embryonic development and in the homeostasis, remodeling, surveillance, and host defenses of

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postnatal tissues.[7] The pathways that mediate apoptosis are more than one billion years old and are central to such fundamental biological processes as growth, proliferation, differentiation, death, inflammation, and immunity. Hence, apoptosis is essential for life itself. Conversely, dysregulation of apoptosis, resulting in either too little or too much cell death, has been implicated in human disease.[8] For example, insufficient apoptosis may contribute to carcinogenesis, whereas excessive apoptosis may be a component in the pathogenesis of stroke, myocardial infarction, and heart failure. It is interesting to speculate why a pathological role for apoptosis has persisted in the face of evolutionary change. One possibility is that the importance of apoptosis in basic biological functions outweighs its potentially detrimental effects. Another likely explanation is that most diseases involving excessive apoptosis occur in postreproductive life.

Cardiac myocytes undergo apoptosis in response to a myriad of stimuli including hypoxia,[9] especially followed by reoxygenation,[10] acidosis,[11] oxidative stress,[12] serum deprivation,[13,14] glucose deprivation and metabolic inhibition,[15,16] β_1-adrenergic agonists,[17–19] stretch,[20] angiotensin II,[21] tumor necrosis factor-α,[22] Fas ligand,[23,24] and anthracyclines.[25] In intact animals, cardiac myocyte apoptosis occurs during myocardial infarction,[26–29] especially followed by reperfusion,[30,31] heart failure[32–38] and various cardiomyopathic states,[39] myocarditis,[40] and transplant rejection.[41] The strength of the data differ for these syndromes but are most compelling for ischemia-reperfusion injury and heart failure.

During ischemia-reperfusion in rodents and humans, 5% to 30% of cardiac myocytes in the area at risk undergo apoptosis within 16 hours.[26,29,31,42] Although controversy exists as to whether this cell death occurs within the central infarct or border zones, most data suggest that both areas are involved. In contrast to this large burst of death during ischemia-reperfusion that takes place over a short period of time, heart failure involves low, but abnormal, rates of cardiac myocyte apoptosis that persist for months (0.023% in rodents and 0.08% to 0.25% in humans compared with 0.001% to 0.002% in normal rodent and human controls).[32,35–37]

To elucidate whether cardiac myocyte apoptosis plays a causal role in the pathogenesis of any cardiac syndrome, it is necessary to determine whether inhibiting this death process ameliorates the expected pathological changes in cardiac structure and function. Genetic approaches (discussed below) have established that inhibition of cardiac myocyte apoptosis reduces infarct size ≈50% to 70% and decreases cardiac dysfunction after ischemia-reperfusion.[23,43–46] Although pharmacological studies have been less consistent, caspase inhibitors have reduced infarct size by 21% to 52%.[47–50] These data suggest that cardiac myocyte apoptosis plays a critical role in the pathogenesis of ischemia-reperfusion injury.

Rodent studies have also implicated low rates of cardiac myocyte apoptosis in the pathogenesis of heart failure. An apoptotic rate as low as 0.023% is sufficient to cause a lethal, dilated cardiomyopathy within 8 to 24 weeks in transgenic mice with cardiac-restricted expression of an inducible caspase-8 allele.[32] Of note, this apoptotic rate is 5- to 10-fold lower than that measured in cardiac tissue from patients with end-stage heart failure, suggesting that apoptosis may also be a causal mechanism of human heart failure.[35–37] In addition, the structural and functional abnormalities in this model can largely be rescued by caspase inhibition. Caspase inhibition has also been demonstrated to decrease apoptosis, improve cardiac function, and, most strikingly, ablate mortality in the peripartum cardiomyopathy resulting from myocardial Gαq overexpression,[51] a model of hypertrophic signaling.[52] These data suggest that cardiac myocyte apoptosis plays a causal role in the pathogenesis of heart failure. A caveat pertaining to both the ischemia-reperfusion and heart failure studies is that they are limited to rodent models. Whether the data "translate" to humans remains to be shown. If so, however, cardiac myocyte apoptosis may constitute a novel target for therapies directed against myocardial infarction and heart failure.

Central Apoptotic Pathways

Apoptosis is mediated by two central pathways (Figure 1): the extrinsic (or death receptor) pathway and the intrinsic (or mitochondrial) pathway.[1] The immediate objectives of apoptotic signaling are the activation of procaspases and the disabling of mitochondrial function. Caspases, a subclass of cysteine proteases that cleave substrates after aspartic acid residues, are central to the execution of apoptosis.[53] These proteases are synthesized as largely inactive zymogens (procaspases) containing an N-terminal prodomain and a C-terminal catalytic domain consisting of ≈20 kDa (p20) and ≈10 kDa (p10) subdomains. Human caspases are subdivided into upstream (apical, signaling) caspases (caspases 2, 8, 9, 10, and 12) and downstream (effector, executioner) caspases (caspases 3, 6, and 7). Upstream procaspases are activated by dimerization.[54–56] In contrast, downstream procaspases, which exist as preformed inactive dimers, are activated by proteolytic cleavage usually performed by already activated upstream caspases. The cleavage events take place after aspartic acid residues located in small linker regions between the prodomain, p20, and p10 subunits.[57] This allows two p20 and two p10 subunits to reassemble noncovalently into the active downstream caspase. After activation, downstream caspases perform the proteolytic destruction of the cell.[58]

Extrinsic Pathway

Although the focus of this review is the intrinsic pathway, the extrinsic pathway will be considered briefly because the two pathways are intimately entwined. Extrinsic signaling is initiated by the binding of a death ligand trimer to its cognate cell surface receptor,[59] which also exists as a preformed trimer.[60] The ligand may be an integral membrane protein on the surface of a second cell (eg, Fas [CD95/Apo-1] ligand) or a soluble extracellular protein (eg, tumor necrosis factor-α). Ligand binding initiates the formation of a multiprotein complex termed the death-inducing signaling complex (DISC).[61–63] Using the Fas death receptor as an example, the binding of Fas ligand is presumed to induce a conformational change in Fas. This results in the recruitment of the adaptor protein FADD (Fas-associated via death domain) to Fas through interactions involving death domains in each of the proteins.[64,65] FADD, in turn, recruits procaspase-8 through

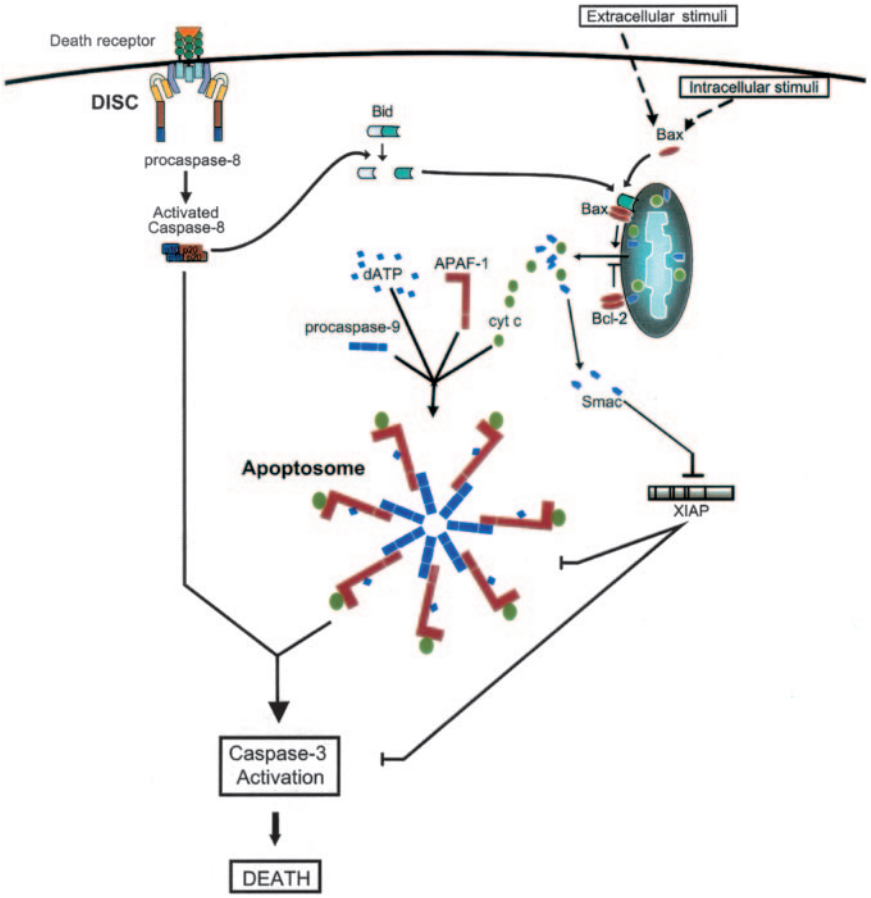

Figure 1. Central apoptosis pathways. In the extrinsic pathway, death receptor activation stimulates DISC assembly (see text) leading to procaspase-8 activation. Activated caspase-8 then cleaves downstream procaspase-3, which proteolyses cellular substrates killing the cell. In the intrinsic pathway, intracellular and extracellular death signals are transmitted to the mitochondria through BH3-only proteins (eg, Bid) and Bax, which translocates to the outer mitochondrial membrane. Bax and Bak (not shown) stimulate the release of cytochrome c (green), Smac/DIABLO (blue), and other apoptogens (not shown). This process is opposed by Bcl-2 and Bcl-x_L (not shown). Cytochrome c, dATP, Apaf-1, and procaspase-9 assemble into the apoptosome (see text) leading to procaspase-9 activation, which subsequently activates procaspase-3. XIAP inhibits activation of procaspase-9 as well as active caspases-9, 3, and 7 (not shown). Smac/DIABLO and Omi/HtrA2 (not shown) bind XIAP, relieving this inhibition. Bid, a direct substrate of caspase-8, connects the extrinsic and intrinsic pathways. After cleavage, its C-terminus translocates to and inserts into the outer mitochondrial membrane triggering activation of Bax and Bak and cytochrome c release.

death effector domains in each protein.[62,63] Thus, the net result of death receptor activation is the recruitment of procaspase-8 into the DISC. This, in turn, results in procaspase-8 dimerization and activation.[56] Once activated, caspase-8 cleaves and activates downstream procaspase-3 and Bid (BH3 [B cell leukemia/lymphoma-2 {Bcl-2} homology domain 3] interacting domain death agonist),[66–68] a proapoptotic Bcl-2 protein, which links the extrinsic and intrinsic pathways.

Intrinsic Pathway: Premitochondrial Events

In contrast to the extrinsic pathway that transduces a specialized set of death stimuli, the intrinsic pathway integrates a broad spectrum of extracellular and intracellular stresses. Extracellular stimuli include deficiencies in survival/trophic factors/nutrients, radiation, and other chemical (eg, drugs) and physical stresses, whereas intracellular stimuli include oxidative stress, DNA damage, and protein misfolding. Intrinsic death signaling is mediated by a complex interplay between stimulus, upstream pathways, and sometimes the endoplasmic reticulum. This myriad of signals converges on the mitochondria leading to dysfunction of this organelle, the release of apoptogenic proteins, and the activation of caspases.

The link between apoptotic stimuli and the central apoptotic machinery is provided by a multitude of peripheral signaling pathways, which are not the subjects of this review. Moreover, in many instances, the precise connections linking these pathways to the central death machinery remain sketchy. It is clear, however, that most, if not all, apoptotic stimuli eventually funnel into the proapoptotic Bcl-2 proteins.[1] The Bcl-2 family can be divided into the following: (1) antiapoptotic members (eg, Bcl-2 and Bcl-x_L [Bcl-x protein long isoform]) and (2) proapoptotic members, which are further subdivided into (a) multidomain proapoptotics (eg, Bax [Bcl-2–associated X protein] and Bak [Bcl-2–antagonist/killer]) and (b) BH3-only proapoptotics (eg, Bid, Bad [Bcl-2-antagonist of cell death], Bim [Bcl-2 interacting mediator of cell death], Bmf [Bcl-2 modifying factor], Noxa [for damage], Puma [p53 upregulated modulator of apoptosis], BNip3 [Bcl-2/adenovirus E1B nineteen kDa-interacting protein 3], and Nix [Nip3-like protein X]/BNip3L [BNip3-like protein]). The antiapoptotics and multidomain proapoptotics contain BH1–3 domains. In addition, some, but not all, of the antiapoptotics contain a BH4 domain. The BH3-only proteins are homologous to these two groups only in the 10 to 16 residue BH3 domain. Surprisingly, the three-dimensional structures of all 3 groups of proteins are similar, with each containing 8 to 9 α-helices.[69]

Multidomain Proapoptotic Bcl-2 Proteins

Bax[70] and Bak[71–73] are multidomain proapoptotic Bcl-2 proteins. Either Bax or Bak is required for all instances of apoptosis mediated via the intrinsic pathway.[74] Cells lacking both proteins are resistant to activators of this pathway. Thus, these proteins control access of upstream apoptotic signals to

the mitochondria. Conformational changes regulate the activation of both Bax and Bak. Bax is held in an inactive state in the cytosol by the insertion of its most C-terminal α-helix (α9) into a hydrophobic cleft created by its BH1–3 domains.[75] In response to apoptotic stimuli, this helix is presumed to move out of the cleft causing a conformational change that also affects the N-terminus. These events trigger Bax translocation to mitochondria, oligomerization, and insertion into the outer mitochondrial membrane via its C-terminal tail, which includes α9. The precise sequence of these events is unclear. Bax then stimulates the release of cytochrome c and other apoptogenic mitochondrial proteins into the cytosol. Although the precise molecular events that mediate Bax conformational activation are not understood, caspase-2[76] and several Bax-binding proteins are involved. The latter include proteins that inhibit Bax activation (Ku70,[77] Humanin,[78] and ARC [apoptosis repressor with a CARD {caspase recruitment domain}[79,80]), as well as promote Bax activation (ASC [apoptosis-associated speck-like protein containing a CARD][81]). In addition, p53, whose abundance increases in response to genotoxic insults, hypoxia, and other cellular stresses, conformationally activates Bax through mechanisms that are not understood because the two proteins do not appear to interact.[82] In addition to these examples of nonproteolytic conformational activation, Bax activity may also be augmented by removal of its inhibitory N-terminus by calpain.[83,84] As calpain has been implicated in necrosis,[3] this event may provide a connection between necrotic and apoptotic pathways. Bax may also be transcriptionally upregulated during apoptosis by p53.[85] Bax plays a critical role in ischemia-reperfusion injury as illustrated by the 50% reductions in infarct size exhibited by isolated hearts from Bax knockout mice.[86]

Bak also undergoes conformational activation, but the mechanism differs from that of Bax. In the case of Bak, the C-terminal tail does not mediate intramolecular inhibition because it is already involved in anchoring Bak constitutively to the outer mitochondrial membrane. Instead, Bak inhibition is provided by VDAC 2 (voltage-dependent anion channel 2), an outer mitochondrial membrane protein that may be a component of the mitochondrial permeability transition pore (MPTP),[87] and Mcl-1 (myeloid cell leukemia sequence 1 isoform 2), an antiapoptotic Bcl-2 protein.[88] On initiation of apoptosis, VDAC2 is displaced from Bak by activated (truncated) Bid, Bim, and Bad, whereas Mcl-1 binding to Bak is disrupted by p53. Bak conformational activation stimulates its intramembranous homoligomerization and triggers the release of apoptogenic mitochondrial proteins into the cytoplasm.

BH3-Only Bcl-2 Proteins

BH3-only proteins function as sentinels that survey upstream apoptotic signals and transmit them directly or indirectly to Bax and Bak. In contrast to Bax and Bak, however, the BH3-only proteins are often specialized to transduce only a subset of specific stimuli. For example, Bim senses cytokine deprivation in lymphocytes,[89] whereas Puma responds to p53.[90,91] In general, the ability of BH3-only proteins to kill is dependent on the BH3 domain. The activation of the BH3-only proteins involves diverse mechanisms. Bid[92] is cleaved by caspase-8[66–68] in the death receptor pathway, calpain,[93] and granzyme B[94,95] to expose its BH3 domain. Caspase-3 can also cleave and activate Bid after the onset of apoptosis as part of a positive feedback loop.[96] After cleavage, the truncated C-terminal portion of Bid (tBid) translocates to the mitochondria and inserts into the outer membrane via its tail.[68,97] This leaves its BH3 domain facing the cytosol, where it can bind to Bak,[97] resulting in the displacement of VDAC2 from Bak[87] and Bak activation.[97] The BH3 domain of Bid also interacts with Bax,[92] facilitating its insertion into the outer mitochondrial membrane.[98] Bid thus functions as a direct conduit between the extrinsic and intrinsic pathways. This link is important in the pathogenesis of myocardial infarction as evidenced by 53% reductions in infarct size and improved cardiac function in $Bid^{-/-}$ mice subjected to ischemia-reperfusion (P. Lee, S.J. Korsmeyer, and R.N. Kitsis, unpublished data).

Other post-translational mechanisms that activate BH3-only proteins include release from the microtubule and actin cytoskeletons in the case of Bim[99] and Bmf,[100] respectively. Bad[101] is regulated by phosphorylation of specific serine residues by a variety of survival kinases, leading to its sequestration by the 14 to 3-3 protein.[102] Loss of survival factors, such as insulin-like growth factor-1, lead to Bad dephosphorylation, resulting in its translocation to the mitochondria, where Bad activates Bak by displacing VDAC2.[87] Interestingly, a dominant-negative mutant of 14 to 3-3 predisposes cardiac myocytes to pressure overload-induced apoptosis in vivo,[103] although this effect cannot be attributed specifically to Bad release, because 14 to 3-3 also binds other proapoptotic molecules.

Transcriptional induction of gene expression is central to the regulation of other BH3-only proteins including Noxa, Puma, BNip3, and Nix/BNip3L. The transcription factor p53 activates the expression of *Noxa*[104] and *Puma,*[90,91] whereas the transcription of *BNip3*[105,106] and *Nix/BNip3L*[105] is upregulated by hypoxia-inducible factor-1α. Levels of BNip3 increase in response to hypoxia and acidosis in cardiac myocytes and in failing hearts.[107,108] Moreover, BNip3 is required for hypoxia–acidosis-induced cardiac myocyte death.[107] The abundance of Nix/BNip3L is increased by hemodynamic overload and the transgenic overexpression of Gαq,[109] a signaling molecule important in the hypertrophic program.[52] Furthermore, Nix/BNip3L is necessary for the peripartum cardiomyopathy of Gαq transgenic mice.[109] Both BNip3 and Nix/BNip3L are situated at the mitochondria during apoptosis, and the transmembrane domain is required for this localization and the subsequent killing.[110] In contrast to other BH3-only proteins, however, the "BH3-like" domain of BNip3 is not needed for killing,[110] raising the possibility that another effector motif or distinct mechanism mediates its cytotoxicity.

Antiapoptotic Bcl-2 Proteins

Multidomain and BH3-only proapoptotic Bcl-2 proteins are antagonized by antiapoptotic family members, including Bcl-2[111–113] and Bcl-x_L.[114] Although the latter were among the first apoptotic regulators to be discovered, their precise

mechanisms of action, including how they inhibit mitochondrial cytochrome c release,[115,116] remain unclear. Bcl-2 is constitutively localized at the outer mitochondrial membrane, endoplasmic reticulum (ER), and nuclear envelope,[117] and is thought to face the cytoplasm. Although Bcl-x_L is also found at these membranes, a significant pool is not membrane-bound.[118] The rheostat hypothesis holds that the ratio of Bcl-2 or Bcl-x_L to Bax or Bak determines whether the cell lives or dies.[70] The physical basis for this hypothesis was thought to be the direct interaction of Bcl-2 or Bcl-x_L with Bax or Bak. Some data, however, raise the possibility that previously demonstrated interactions between these proteins may be an artifact of the nonionic detergents in the buffers used.[118] Moreover, genetic data show that Bcl-2 and Bax are able to exert their effects independently of one another.[119] These observations suggest that Bcl-2 and Bcl-x_L may exert their prolife effects through mechanisms other than direct interactions with Bax and Bak. One postulated mechanism is that Bcl-2 and Bcl-x_L function as "sinks" that sequester BH3-only proteins, such as tBid, preventing these BH3-only proteins from activating Bax and Bak.[120] Consistent with this model, a Bcl-x_L mutant, which is defective for Bax and Bak binding but competent to bind tBid, is still cytoprotective. In contrast, a mutant defective for binding tBid is unable to protect.[120] Other BH3-only proteins, such as Bad, may facilitate apoptosis by binding to Bcl-2 and displacing tBid.[121] Antiapoptotic Bcl-2 proteins can markedly affect myocardial ischemia-reperfusion injury. For example, transgenic mice with cardiac-restricted overexpression of Bcl-2 demonstrate 48% to 64% decreases in infarct size.[43,44]

Tumor Suppressor p53

The transcription factor p53 mediates apoptosis in response to diverse stimuli including hypoxia, oxidative stress, and DNA damage.[122] The abundance and activity of p53 are controlled at multiple levels by these and other stimuli. In turn, p53 induces apoptosis by transactivating the expression of multiple proapoptotic genes, including *bax*, *noxa*, *puma*, *bid*, *asc*, *apaf-1*, *caspase-6*, and *fas*.[81,122] It is also well-known that transcriptionally defective p53 mutants retain the ability to kill, potentially through their activation of Bax[82] and Bak[88] proteins, as described.

In cardiac myocytes, hypoxia increases p53 levels, and p53 alone suffices to kill normoxic cardiac myocytes.[123] Despite this, p53 deficiency does affect ischemia-reperfusion–induced cardiac myocyte apoptosis.[11,29] One important caveat concerning this conclusion, however, is that changes in infarct size, the bottom-line parameter of myocardial damage, were not measured in these early studies. For this reason, a re-evaluation of the role of p53 in ischemia-reperfusion injury might be warranted. Stretch of cardiac myocytes has also been noted to increase p53 levels through a mechanism involving secreted angiotensin II and activation of the type 1 angiotensin II receptor.[124] In turn, p53 transactivates the expression of the genes encoding angiotensinogen and the type I angiotensin II receptor.

ER Pathway

The mitochondria are appropriately viewed as the central organelle in the intrinsic pathway. In some circumstances, however, the ER (or sarcoplasmic reticulum in muscle cells) plays an important role in the mitochondrial death pathway, as well as perhaps mediating cell death independently of mitochondria. For example, stimuli, including ceramide, arachidonic acid, and, interestingly, oxidative stress, appear to require the ER death pathway for efficient killing.[125]

Although the mechanisms by which the ER brings about cell death are poorly understood, increases in intracellular Ca^{2+} appear to be central. ER Ca^{2+} stores are thought to be increased by Bax and Bak,[125] which are located at ER, as well as mitochondrial, membranes.[126] Conversely, Bcl-2, which also resides at the ER membrane,[117] decreases steady-state ER Ca^{2+} stores.[127,128] Increased ER Ca^{2+} facilitates a more robust release of Ca^{2+} into the cytoplasm on delivery of an apoptotic stimulus.

Increased cytoplasmic Ca^{2+} may activate several apoptotic mechanisms. First, mitochondrial Ca^{2+} overload can trigger MPTP opening and cytochrome c release.[129] Interestingly, this mechanism may be amplified by a positive feedback loop in which cytochrome c binds the inositol 1,4,5-trisphosphate (IP3) receptor, one of the ER Ca^{2+} release channels, to further stimulate Ca^{2+} release.[130] Second, increased intracellular Ca^{2+} can activate calpain. As discussed previously, calpain can cleave Bid, providing another mechanism for cytochrome c release.[93] An additional consequence of calpain activation is cleavage of procaspase-12.[131] Caspase-12, which is present in some, but not all, human populations (attributable to polymorphisms with non-sense mutations[132]), has been shown in knockout mice to be required for apoptosis induced specifically by ER stress.[133] How caspase-12 signals downstream to bring about cell death is not certain, but recent data suggest that cleaved caspase-12 translocates to the cytoplasm and activates caspase-9 independently of apoptosome formation.[134,135] These events provide a mitochondria-independent mechanism for ER-mediated apoptosis. In addition to its multiple roles in apoptotic signaling, increased intracellular Ca^{2+} has also been implicated in necrotic pathways through its effects on mitochondria[136] and calpains.[3] Thus, Ca^{2+} may be a point of convergence between apoptotic and necrotic pathways. The putative role of Ca^{2+} in cell death raises an important question, however. How do cardiac myocytes avoid mitochondrial dysfunction and cell death in the face of the relentless wide cyclic swings in cytoplasmic Ca^{2+} concentrations (10^{-7} M in diastole to 10^{-6} M in systole) with each contraction?[137]

Some signals that activate the ER death pathways originate within this organelle itself, where a complex array of pathways mediate the unfolded protein and other ER stress responses.[138] In addition, given their roles in carrying upstream apoptotic stimuli to Bax and Bak at the mitochondria, BH3-only proteins would be anticipated to perform an analogous function in the ER pathway. In fact, the BH3-only proteins Bik (Bcl-2–interacting killer)[139] and Puma[140] have been implicated in the ER death pathway. It remains unclear, however, whether these proteins function to relay signals from the periphery to the ER and/or from the ER to mitochondria. However, upstream signals originating in the extrinsic pathway are known to be linked with the ER by Bap31 (B-cell receptor–associated protein 31), an integral ER

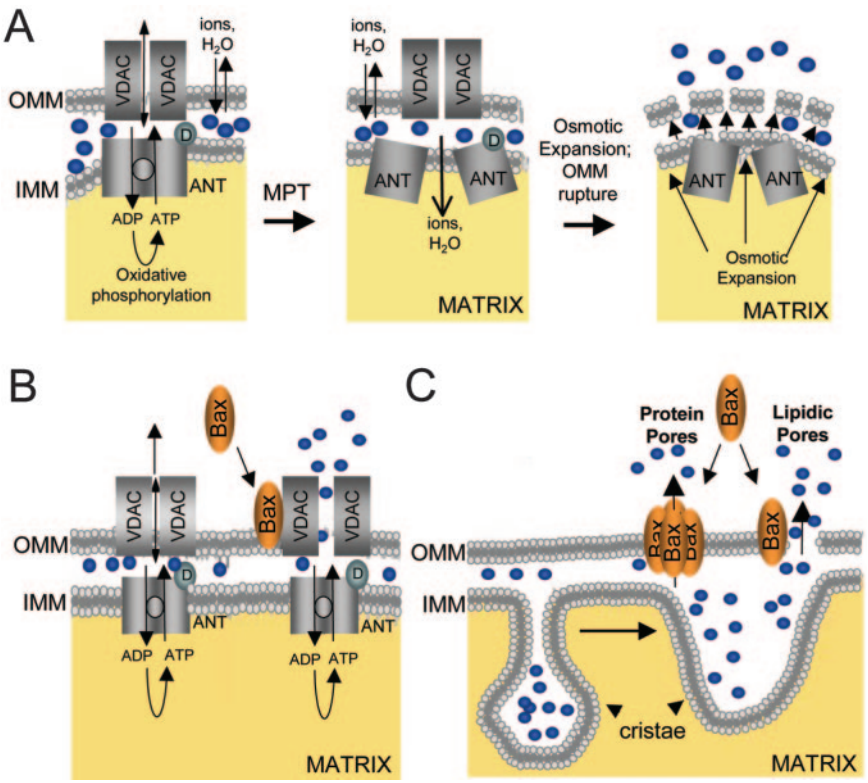

Figure 2. Models of cytochrome c release. A, MPT model. In healthy cells (left panel), the MPTP functions as a nucleotide exchanger. In response to stress signals, ANT undergoes a conformational change that converts it from a ligand-specific translocase to a nonspecific pore that collapses the inner mitochondrial membrane (IMM) potential and allows the entry of water down its osmotic gradient (middle panel). The subsequent swelling of the IMM ruptures the outer mitochondrial membrane (OMM), releasing cytochrome c (blue) (right panel). D denotes cyclophilin D. B, Bax–VDAC model. In healthy cells (left), VDAC functions as a component of the nucleotide exchange complex and restricts the passage of cytochrome c. During apoptosis (right), Bax binds VDAC changing its properties to permit cytochrome c release. C, Bax pore models. In healthy cells (left), ≈15% of cytochrome c is in the mitochondrial intermembrane space and ≈85% in cristae that are sequestered from the intermembrane space because of narrow junctions. During apoptosis, remodeling of cristae widens the junctions, allowing the majority of cytochrome c to access the intermembrane space. Bax is postulated to form protein pores or to stimulate the formation of lipid pores in the OMM, through which cytochrome c translocates into the cytosol.

membrane protein that is cleaved by caspase-8 resulting in ER Ca^{2+} release.[141]

Relatively few studies have explored the role of the ER pathway in cardiac myocyte apoptosis. Transgenic overexpression of heat shock protein-70 (Hsp-70) in the heart decreases infarct size after ischemia-reperfusion.[142] It remains to be determined whether cardioprotection results from the role of Hsp-70s as a chaperone (reducing ER stress) and/or to its inhibition of apoptosome assembly[143,144] or apoptosis-inducing factor (AIF).[145] Transverse aortic constriction has been shown to induce an ER stress response in the heart that is associated with apoptosis, and inhibition of the angiotensin II type 1 receptor decreases both the ER stress response and apoptosis.[146] Future work will be required to precisely define the role of the ER death pathway in heart disease.

Intrinsic Pathway: Intramitochondrial Events

Mitochondria play an important role in transmitting and amplifying death signals. In the intrinsic pathway, they function as the interface between upstream apoptotic pathways and the caspases and other downstream death machinery. The most critical mitochondrial events during apoptosis are the structural and functional remodeling of this organelle and subsequent release of apoptogenic proteins into the cytosol. These proteins include cytochrome c,[116,147] Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI),[148,149] Omi/HtrA2 (high temperature requirement protein A2),[150,151] AIF,[152] and EndoG (Endonuclease G).[153] The release of these proteins activates apoptotic events in the cytosol and nucleus. Although cytochrome c participates in respiration, the functions of the other apoptogens under normal conditions have not been established definitively.

Mechanisms Underlying Mitochondrial Apoptogen Release

The mechanisms that mediate the release of the apoptogenic mitochondrial proteins are poorly understood. Cytochrome c has been studied most intensively in this context. Although it has been known for some time that cytochrome c release is stimulated by proapoptotic Bcl-2 proteins (eg, Bax) and inhibited by antiapoptotic Bcl-2 proteins (eg, Bcl-2), the precise mechanisms by which this apoptogen gains access to the cytosol are not known. A number of models have been proposed. One of the earliest postulated that cytochrome c release is linked to the mitochondrial permeability transition (MPT) (Figure 2A), an event triggered by changes in the permeability of the inner mitochondrial membrane. In healthy cells, the inner mitochondrial membrane is relatively impermeable, as it must be to maintain the proton gradient driving respiration and the osmotic gradient created by the high concentration of metabolites in the mitochondrial matrix. Under stress conditions (eg, death signals), the MPTP opens to allow the passage of molecules <1.5 kDa, including protons and water. The proton gradient and electrical potential across the inner mitochondrial membrane collapses, leading to uncoupling of oxidative phosphorylation. The influx of water, attributable to the osmotic pressure resulting from the protein- and metabolite-dense mitochondrial matrix, leads to swelling of the matrix and expansion of the highly convoluted inner mitochondrial membrane. Although the inner mitochondrial membrane possesses sufficient surface area to accommodate this expansion without rupturing, the

ability of the outer mitochondrial membrane to expand is limited. Thus, expansion of the inner mitochondrial membrane causes the outer mitochondrial membrane to rupture and release cytochrome c and other apoptogenic proteins into the cytosol.

Although the precise molecular composition of the MPTP is currently undergoing investigation, its core components are thought to be the adenine nucleotide translocase (ANT) located in the inner mitochondrial membrane and cyclophilin D, a peptidyl prolyl *cis-trans* isomerase that interacts with ANT.[129] Other components may include VDAC and the peripheral benzodiazepine receptor, which are located in the outer mitochondrial membrane. In healthy mitochondria, the close association of VDAC and ANT create a macromolecular complex that shuttles adenine nucleotides between the ATP-producing matrix and ATP-consuming cytosol. MPTP opening can be triggered under stress conditions, however, by increases in Ca^{2+}, oxidative stress, depletion of adenine nucleotides, increases in inorganic phosphate, and depolarization of the inner mitochondrial membrane, stimuli that operate during ischemia-reperfusion.[129] Although proapoptotic (Bax and Bak) and antiapoptotic (Bcl-2 and Bcl-x_L) Bcl-2 proteins have been reported to bind ANT and VDAC,[87,154–157] the functional significance of these interactions remains controversial.

Cytochrome c release caused by MPT-mediated rupture of the outer mitochondrial membrane may occur during necrosis and forms of apoptosis associated with mitochondrial calcium overload including ischemia-reperfusion.[136] It fails, however, to account for many instances of apoptosis in which cytochrome c release occurs before or without mitochondrial swelling or collapse of the inner mitochondrial membrane potential.[158,159] In addition, whereas MPT is often blocked by caspase inhibition, cytochrome c release is not.[158] In fact, caspase activation resulting from cytochrome c release can stimulate MPT,[160] which acts as an amplifying mechanism for further cytochrome c release. These data argue against a role for MPT in the initiation of cytochrome c release in many instances of apoptosis.

Given the limitations of the MPT to explain the first steps in cytochrome c release, other models have focused primarily on more selective permeabilization of the outer mitochondrial membrane. Bax and Bak play central roles in these models by inducing other proteins to form channels for cytochrome c release, forming channels themselves, or inducing lipid channel formation. Regarding the first of these models, an interaction between Bax and VDAC has been noted previously.[157] Moreover, addition of Bax to VDAC-containing liposomes changes the properties of VDAC from that of a voltage-dependent and anion-selective channel to a voltage-independent and nonselective pore sufficient in size to allow the release of cytochrome c (Figure 2B).[156] Questions about the importance of VDAC in this process have been raised, however, by yeast studies in which the absence of VDAC does not affect Bax-stimulated cytochrome c release.[161] In addition, as discussed, VDAC2 may actually be antiapoptotic through its interaction with and inhibition of Bak.[87] Of course, the possibility remains that Bax may modulate cytochrome c release via other yet to be identified proteins, an area of active investigation.

In the second model, Bax and Bak themselves are postulated to form pores in the outer mitochondrial membrane (Figure 2C). The first hint that Bcl-2 proteins might be capable of pore formation was provided by the three dimensional structure of Bcl-x_L, which resembles diphtheria toxin and the colicins.[162] In fact, Bax, Bcl-2, and Bcl-x_L are able to form ion channels in artificial membranes,[163–165] and under some conditions, Bax channels are inhibited by Bcl-2.[163] Moreover, Bax can form pores in liposomes capable of releasing cytochrome c.[166]

Some studies, however, demonstrate that Bax-induced pores do not show discrete conductance states characteristic of protein-lined pores[167,168] and are sensitive to the lipid composition of the planar membrane in which they reside.[169] These properties suggest a third model in which Bax induces the formation of lipid pores (Figure 2C). In support of this model, Bax reduces the linear tension of membranes, a property that could facilitate the rearrangement of membrane phospholipids into pores or pore-like structures.[167] Monomeric Bax and tBID together or oligomerized Bax on its own have been shown to produce pores in outer mitochondrial membranes and liposomes that allow the release of molecules up to 2 MDa in size.[169] These Bax-induced pores are also sensitive to the source of phospholipids used to produce the liposomes, with lipids from the outer mitochondrial membrane markedly more effective than those from microsomal membranes. This preference may be attributable to the presence of cardiolipin. When incorporated into chemically defined liposomes, cardiolipin markedly increases Bax-induced permeabilization.[169] These data suggest a role for Bax-induced lipid remodeling in the permeabilization of the outer mitochondrial membrane.

The mechanisms that mediate release of the other mitochondrial apoptogens, all of which are larger than cytochrome c, are even less well-understood. Caspase inhibition differentially affects the release of these proteins suggesting the involvement of different release mechanisms. In contrast to cytochrome c release, which is caspase-independent,[158] translocation of AIF and EndoG is sensitive to caspase inhibition.[170,171] Smac/DIABLO release was also originally thought to be caspase-dependent,[172] but additional work suggests that its mitochondrial release as well as that of Omi/HtrA2 is caspase-independent.[171] Clearly, further work is needed to understand the mechanisms that regulate the release of these apoptogens.

Mechanisms of Mitochondrial Remodeling During Apoptosis

Although permeabilization of the outer mitochondrial membrane could explain how cytochrome c gains access to the cytosol, it fails to account for the completeness of its release observed in many apoptotic models. This is because only 15% of cytochrome c resides in the mitochondrial intermembrane space, where it is freely available for discharge.[173] The remainder of the cytochrome c is located in the cristae. This pool of cytochrome c is prevented from accessing the mitochondrial intermembrane space under normal conditions

because of the narrowness of cristae junctions. During apoptosis, mitochondria undergo a remodeling process in which these junctions widen from 19 to 57 nm (shown schematically in Figure 2C).[159] In addition, remodeling results in increased interconnectivity among cristae. These changes facilitate a redistribution of the majority of cytochrome c to the mitochondrial intermembrane space so that after outer membrane permeabilization, most cytochrome c can be released into the cytoplasm.[174]

Remodeling of the cristae is mediated by a mechanism that is distinct from that responsible for permeabilization of the outer mitochondrial membrane.[159] Using tBid to induce apoptosis, remodeling has been shown to be inhibited by cyclosporin A but to occur independently of Bax, Bak, and the BH3 domain of tBid. Because cyclopsporin A inhibits MPTP opening, these characteristics suggest involvement of the MPTP in the mobilization of intracristal cytochrome c stores. In fact, transient opening of the MPTP occurs in this process but does not progress to mitochondrial swelling or loss of the inner mitochondrial membrane potential. In contrast to remodeling of the inner mitochondrial membrane, tBid-induced permeabilization of the outer mitochondrial membrane requires the BH3 domain of tBid, as well as Bax or Bak.

Similar remodeling changes have been reported in cardiac myocytes treated with hydrogen peroxide.[175] Despite this, the rapidity and completeness of cytochrome c release in cardiac myocytes may be less than that in cell lines induced with ultraviolet radiation[174] or in isolated mitochondria stimulated with tBid.[159] Further work will be required to define the dynamics of cytochrome c release in cardiac myocytes.

Intrinsic Pathway: Postmitochondrial Events

Apoptosome Formation

Once cytochrome c is released into the cytoplasm, it binds WD40 repeats in the C-terminus of the adaptor protein Apaf-1 (apoptotic protease activating factor-1).[176,177] In addition, dATP and/or ATP, which are already present in the cytoplasm, interact with the nucleotide binding domain in the central portion of Apaf-1. These events are thought to stimulate a conformational change in Apaf-1 that results in its homo-oligomerization (mediated through the nucleotide binding domain) and the recruitment of procaspase-9 (mediated through caspase recruitment domains [CARD] in Apaf-1 and procaspase-9).[177–187] This >1 MDa complex, termed the apoptosome, appears as a wheel with heptad symmetry (Figure 1).[181,187] The forced approximation of the upstream procaspase-9 in the apoptosome leads to its activation. As with procaspase-8 activation in the DISC, dimerization of procaspase-9 appears to be the major activating event, with the subsequent proteolytic processing playing a less important role.[54–56] Once activated, caspase-9 cleaves and activates downstream procaspase-3, which is also recruited into the apoptosome.[188] Caspase-3, in turn, feed backs on and further activates procaspase-9.[189]

Cells that are genetically deficient for cytochrome c,[190] Apaf-1,[191] or procaspase-9[192,193] show marked resistance to activators of the mitochondrial pathway. Transgenic mice with cardiac-restricted overexpression of either of two procaspase-9 dominant-negative mutations exhibit 53% and 68% reductions in infarct size, respectively, and improved cardiac function after ischemia-reperfusion (C.-F. Peng, G. Tremp, A. Silberstein, and R.N. Kitsis, unpublished data). These data demonstrate that postmitochondrial events in the intrinsic pathway are important in the pathogenesis of myocardial ischemia-reperfusion injury.

Inhibitors of Apoptosis

Several endogenous proteins, which contain *baculovirus* inhibitor of apoptosis repeats (BIR),[194] antagonize the postmitochondrial steps in the intrinsic pathway. One such protein, XIAP (X-linked inhibitor of apoptosis), binds to and inhibits caspases-9, 3, and 7.[195] These interactions involve BIR3 in the case of caspase-9, and the BIR1–2 linker in the case of caspases-3 and 7.[196–199] Inhibition of these already activated caspases is achieved by blocking substrate access to the active sites. XIAP also inhibits procaspase-9 activation in the apoptosome by preventing its dimerization.[200] In this manner, XIAP protects against the destruction of the cell by the accidental activation of caspases. Other BIR proteins cIAP1 and 2 (cellular inhibitor of apoptosis 1 and 2) act in a similar fashion. Thus, inhibitor of apoptosis (IAP) proteins inhibit both upstream caspase-9, the major initiator caspase in the intrinsic pathway, and downstream caspases-3 and 7.

The release of Smac/DIABLO and Omi/HtrA2 from mitochondria to the cytosol opposes the inhibition of caspases by XIAP.[148,149,151] This relief of inhibition is mediated by binding of Smac/DIABLO and Omi/HtrA2 to XIAP, displacing the caspases. In addition to this mechanism, Omi/HtrA2 cleaves and irreversibly inactivates XIAP through its serine protease activity.[201] Thus, Smac/DIABLO and Omi/HtrA2 act as "inhibitors of the inhibitor," permitting activation of these caspases. The roles of Smac/DIABLO and Omi/HtrA2 emphasize that although release of cytochrome c is necessary, it is not sufficient in many situations to bring about cell death.[202] Inactivation of the "cell death brakes" by these other apoptogens is also often necessary for apoptosis to occur.

Interestingly, the IAPs also possess a RING domain, which functions as a ubiquitin E3 ligase that mediates ubiquitin-dependent protein degradation.[203] Targets of this ligase include proapoptotic proteins such as caspases-3 and 7, as well as Smac.[204–207] This provides an additional mechanism for IAP-mediated cytoprotection. IAPs, however, also target themselves for degradation through self-ubiquitination,[203] presumably as a mechanism to remove themselves rapidly and efficiently, so as to allow apoptosis to proceed in the face of an overwhelming cellular insult. The mechanisms that differentially direct IAP E3 ligase activity toward proapoptotic versus antiapoptotic proteins remain to be determined.

Apoptosis-Inducing Factor

Another mitochondrial protein that promotes cell death when released into the cytoplasm is AIF, a flavoprotein with oxidoreductase activity.[152] In response to death signals, AIF translocates from the mitochondria to the nucleus in a manner dependent on PARP (poly[ADP-ribose] polymerase) activation.[152,208] Moreover, AIF is required for cell death mediated

by PARP, which is activated by genotoxic and oxidative stresses.[208] In the nucleus, AIF triggers the degradation of DNA to 50 Kb fragments,[152] which subsequently undergo internucleosomal cleavage by endonucleases. AIF itself does not have endonuclease activity.[152] Wah-1 (worm apoptosis-inducing factor homolog-1), the *C elegans* ortholog of AIF, binds to and cooperates with Cps-6 (Ced-3 [cell death abnormality gene-3] protease suppressor-6), the worm ortholog of the mammalian endonuclease EndoG, a protein that is also released from mitochondria during apoptosis.[153,209] These observations suggest that AIF may cooperate with EndoG or another endonuclease, but this mechanism remains to be demonstrated. It has been shown, however, that AIF interacts with cyclophilin A to degrade DNA, and the peptidyl prolyl *cis-trans* isomerase activity of cyclophilin A is not needed for this function.[210] In addition to its nuclear effects, AIF also triggers mitochondria to release cytochrome c.[152] $AIF^{-/-}$ embryonic stem cells exhibit defects in apoptosis, and embryoid bodies generated from these cells undergo inadequate cavitation attributable to decreased cell death.[211] Although most data support a role for AIF in apoptosis, cells derived from the *harlequin* mouse, a naturally occurring mutant with 80% reduction in AIF levels, exhibit increased sensitivity to oxidative stress.[212] The explanation for this seemingly paradoxical observation may relate to a dual function of AIF in the metabolism of reactive oxygen species as well as its role in apoptosis. Although AIF release from mitochondria is caspase-dependent in some models,[170,171] its actions after release occur independently of caspase activation.[152] Moreover, AIF-induced killing does not require Apaf-1 or procaspase-9.[211] Thus, the AIF pathway is distinct from, but intricately involved with, caspase-mediated cell death. The reduction in infarct size noted in PARP knockout mice subjected to ischemia-reperfusion suggests that AIF may play a role in this process.[213]

The net result of apoptotic signaling is mitochondrial dysfunction and caspase activation, the consequences of which have been considered here. The activation of downstream caspases leads to the cleavage of numerous structural and regulatory cellular proteins.[58] These proteolytic events bring about the implosion of the cell in ways that are incompletely understood. This execution phase and the engulfment of apoptotic bodies by phagocytes have been the subjects of recent reviews.[58,214]

Conclusion

The preceding discussion highlights progress over the past decade in defining the molecular circuitry that mediates the mitochondrial apoptotic pathway, as well as its relevance to aspects of heart disease. In contrast, relatively little is known about necrosis at the molecular level. Future efforts to further delineate necrotic pathways and their points of convergence with the apoptotic program will be critical to achieve a more complete understanding of the role of the mitochondrial death pathway in disease pathogenesis.

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References

1. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell*. 2004; 116:205–219.
2. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. *Science*. 2000;290:1717–1721.
3. Yuan J, Lipinski M, Degterev A. Diversity in the mechanisms of neuronal cell death. *Neuron*. 2003;40:401–413.
4. Hall DH, Gu G, Garcia-Anoveros J, Gong L, Chalfie M, Driscoll M. Neuropathology of degenerative cell death in Caenorhabditis elegans. *J Neurosci*. 1997;17:1033–1045.
5. Xu K, Tavernarakis N, Driscoll M. Necrotic cell death in C. elegans requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. *Neuron*. 2001;31:957–971.
6. Syntichaki P, Xu K, Driscoll M, Tavernarakis N. Specific aspartyl and calpain proteases are required for neurodegeneration in C. elegans. *Nature*. 2002;419:939–944.
7. Metzstein MM, Stanfield GM, Horvitz HR. Genetics of programmed cell death in C. elegans: past, present and future. *Trends Genet*. 1998; 14:410–416.
8. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science*. 1995;267:1456–1462.
9. Tanaka M, Ito H, Adachi S, Akimoto H, Nishikawa T, Kasajima T, Marumo F, Hiroe M. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. *Circ Res*. 1994;75:426–433.
10. Kang PM, Haunstetter A, Aoki H, Usheva A, Izumo S. Morphological and molecular characterization of adult cardiomyocyte apoptosis during hypoxia and reoxygenation. *Circ Res*. 2000;87:118–125.
11. Webster KA, Discher DJ, Kaiser S, Hernandez O, Sato B, Bishopric NH. Hypoxia-activated apoptosis of cardiac myocytes requires reoxygenation or a pH shift and is independent of p53. *J Clin Invest*. 1999;104: 239–252.
12. von Harsdorf R, Li PF, Dietz R. Signaling pathways in reactive oxygen species-induced cardiomyocyte apoptosis. *Circulation*. 1999;99: 2934–2941.
13. Fujio Y, Kunisada K, Hirota H, Yamauchi-Takihara K, Kishimoto T. Signals through gp130 upregulate bcl-x gene expression via STAT1-binding cis-element in cardiac myocytes. *J Clin Invest*. 1997;99: 2898–2905.
14. Sheng Z, Knowlton K, Chen J, Hoshijima M, Brown JH, Chien KR. Cardiotrophin 1 (CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for myocardial cell hypertrophy. *J Biol Chem*. 1997;272:5783–5791.
15. Bialik S, Cryns VL, Drincic A, Miyata S, Wollowick AL, Srinivasan A, Kitsis RN. The mitochondrial apoptotic pathway is activated by serum and glucose deprivation in cardiac myocytes. *Circ Res*. 1999;85: 403–414.
16. Malhotra R, Brosius FC, 3rd. Glucose uptake and glycolysis reduce hypoxia-induced apoptosis in cultured neonatal rat cardiac myocytes. *J Biol Chem*. 1999;274:12567–12575.
17. Shizukuda Y, Buttrick PM, Geenen DL, Borczuk AC, Kitsis RN, Sonnenblick EH. beta-adrenergic stimulation causes cardiocyte apoptosis: influence of tachycardia and hypertrophy. *Am J Physiol*. 1998;275: H961–H968.
18. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. *Circulation*. 1998;98:1329–1334.
19. Xiao RP. Beta-adrenergic signaling in the heart: dual coupling of the beta2- adrenergic receptor to G(s) and G(i) proteins. *Sci STKE*. 2001; 2001:RE15.
20. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed myocyte cell death. *J Clin Invest*. 1995;96:2247–2259.

21. Kajstura J, Cigola E, Malhotra A, Li P, Cheng W, Meggs LG, Anversa P. Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. *J Mol Cell Cardiol*. 1997;29:859–870.
22. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, Glembotski CC, Quintana PJ, Sabbadini RA. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. *J Clin Invest*. 1996;98:2854–2865.
23. Lee P, Sata M, Lefer DJ, Factor SM, Walsh K, Kitsis RN. Fas pathway is a critical mediator of cardiac myocyte death and MI during ischemia-reperfusion in vivo. *Am J Physiol Heart Circ Physiol*. 2003;284:H456–H463.
24. Jeremias I, Kupatt C, Martin-Villalba A, Habazettl H, Schenkel J, Boekstegers P, Debatin KM. Involvement of CD95/Apo1/Fas in cell death after myocardial ischemia. *Circulation*. 2000;102:915–920.
25. Wang L, Ma W, Markovich R, Chen JW, Wang PH. Regulation of cardiomyocyte apoptotic signaling by insulin-like growth factor I. *Circ Res*. 1998;83:516–522.
26. Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert SR, Cigola E, Anversa P. Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. *J Mol Cell Cardiol*. 1996;28:2005–2016.
27. Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest*. 1996;74:86–107.
28. Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Voipio-Pulkki LM. Apoptosis in human acute myocardial infarction. *Circulation*. 1997;95:320–323.
29. Bialik S, Geenen DL, Sasson IE, Cheng R, Horner JW, Evans SM, Lord EM, Koch CJ, Kitsis RN. Myocyte apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. *J Clin Invest*. 1997;100:1363–1372.
30. Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest*. 1994;94:1621–1628.
31. Fliss H, Gattinger D. Apoptosis in ischemic and reperfused rat myocardium. *Circ Res*. 1996;79:949–956.
32. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, Kitsis RN. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest*. 2003;111:1497–1504.
33. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. Apoptosis in myocytes in end-stage heart failure. *N Engl J Med*. 1996;335:1182–1189.
34. Condorelli G, Morisco C, Stassi G, Notte A, Farina F, Sgaramella G, de Rienzo A, Roncarati R, Trimarco B, Lembo G. Increased cardiomyocyte apoptosis and changes in proapoptotic and antiapoptotic genes bax and bcl-2 during left ventricular adaptations to chronic pressure overload in the rat. *Circulation*. 1999;99:3071–3078.
35. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. *N Engl J Med*. 1997;336:1131–1141.
36. Saraste A, Pulkki K, Kallajoki M, Heikkila P, Laine P, Mattila S, Nieminen MS, Parvinen M, Voipio-Pulkki LM. Cardiomyocyte apoptosis and progression of heart failure to transplantation. *Eur J Clin Invest*. 1999;29:380–386.
37. Guerra S, Leri A, Wang X, Finato N, Di Loreto C, Beltrami CA, Kajstura J, Anversa P. Myocyte death in the failing human heart is gender dependent. *Circ Res*. 1999;85:856–866.
38. Hirota H, Chen J, Betz UA, Rajewsky K, Gu Y, Ross J, Jr., Muller W, Chien KR. Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. *Cell*. 1999;97:189–198.
39. Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. *N Engl J Med*. 1996;335:1190–1196.
40. Saraste A, Arola A, Vuorinen T, Kyto V, Kallajoki M, Pulkki K, Voipio-Pulkki LM, Hyypia T. Cardiomyocyte apoptosis in experimental coxsackievirus B3 myocarditis. *Cardiovasc Pathol*. 2003;12:255–262.
41. Narula J, Acio ER, Narula N, Samuels LE, Fyfe B, Wood D, Fitzpatrick JM, Raghunath PN, Tomaszewski JE, Kelly C, Steinmetz N, Green A, Tait JF, Leppo J, Blankenberg FG, Jain D, Strauss HW. Annexin-V imaging for noninvasive detection of cardiac allograft rejection. *Nat Med*. 2001;7:1347–1352.
42. Palojoki E, Saraste A, Eriksson A, Pulkki K, Kallajoki M, Voipio-Pulkki LM, Tikkanen I. Cardiomyocyte apoptosis and ventricular remodeling after myocardial infarction in rats. *Am J Physiol Heart Circ Physiol*. 2001;280:H2726–H2731.
43. Brocheriou V, Hagege AA, Oubenaissa A, Lambert M, Mallet VO, Duriez M, Wassef M, Kahn A, Menasche P, Gilgenkrantz H. Cardiac functional improvement by a human Bcl-2 transgene in a mouse model of ischemia/reperfusion injury. *J Gene Med*. 2000;2:326–333.
44. Chen Z, Chua CC, Ho YS, Hamdy RC, Chua BH. Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice. *Am J Physiol Heart Circ Physiol*. 2001;280:H2313–H2320.
45. Miao W, Luo Z, Kitsis RN, Walsh K. Intracoronary, adenovirus-mediated Akt gene transfer in heart limits infarct size following ischemia-reperfusion injury in vivo. *J Mol Cell Cardiol*. 2000;32:2397–2402.
46. Matsui T, Tao J, del Monte F, Lee KH, Li L, Picard M, Force TL, Franke TF, Hajjar RJ, Rosenzweig A. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation*. 2001;104:330–335.
47. Yaoita H, Ogawa K, Maehara K, Maruyama Y. Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. *Circulation*. 1998;97:276–281.
48. Holly TA, Drincic A, Byun Y, Nakamura S, Harris K, Klocke FJ, Cryns VL. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. *J Mol Cell Cardiol*. 1999;31:1709–1715.
49. Huang JQ, Radinovic S, Rezaiefar P, Black SC. In vivo myocardial infarct size reduction by a caspase inhibitor administered after the onset of ischemia. *Eur J Pharmacol*. 2000;402:139–142.
50. Yang W, Guastella J, Huang JC, Wang Y, Zhang L, Xue D, Tran M, Woodward R, Kasibhatla S, Tseng B, Drewe J, Cai SX. MX1013, a dipeptide caspase inhibitor with potent in vivo antiapoptotic activity. *Br J Pharmacol*. 2003;140:402–412.
51. Hayakawa Y, Chandra M, Miao W, Shirani J, Brown JH, Dorn GW, 2nd, Armstrong RC, Kitsis RN. Inhibition of cardiac myocyte apoptosis improves cardiac function and abolishes mortality in the peripartum cardiomyopathy of Galpha(q) transgenic mice. *Circulation*. 2003;108:3036–3041.
52. Adams JW, Sakata Y, Davis MG, Sah VP, Wang Y, Liggett SB, Chien KR, Brown JH, Dorn GW, 2nd. Enhanced Galphaq signaling: a common pathway mediates cardiac hypertrophy and apoptotic heart failure. *Proc Natl Acad Sci U S A*. 1998;95:10140–10145.
53. Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science*. 1998;281:1312–1316.
54. Stennicke HR, Deveraux QL, Humke EW, Reed JC, Dixit VM, Salvesen GS. Caspase-9 can be activated without proteolytic processing. *J Biol Chem*. 1999;274:8359–8362.
55. Renatus M, Stennicke HR, Scott FL, Liddington RC, Salvesen GS. Dimer formation drives the activation of the cell death protease caspase 9. *Proc Natl Acad Sci U S A*. 2001;98:14250–14255.
56. Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR, Salvesen GS. A unified model for apical caspase activation. *Mol Cell*. 2003;11:529–541.
57. Stennicke HR, Jurgensmeier JM, Shin H, Deveraux Q, Wolf BB, Yang X, Zhou Q, Ellerby HM, Ellerby LM, Bredesen D, Green DR, Reed JC, Froelich CJ, Salvesen GS. Pro-caspase-3 is a major physiologic target of caspase-8. *J Biol Chem*. 1998;273:27084–27090.
58. Fischer U, Janicke RU, Schulze-Osthoff K. Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ*. 2003;10:76–100.
59. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science*. 1998;281:1305–1308.
60. Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, Tsien RY, Lenardo MJ. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science*. 2000;288:2354–2357.
61. Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J*. 1995;14:5579–5588.
62. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell*. 1996;85:803–815.

63. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell*. 1996;85:817–827.
64. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell*. 1995;81:505–512.
65. Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J Biol Chem*. 1995;270:7795–7798.
66. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell*. 1998;94: 481–490.
67. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell*. 1998;94: 491–501.
68. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P, Korsmeyer SJ. Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. *J Biol Chem*. 1999;274:1156–1163.
69. Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. *Biochim Biophys Acta*. 2004;1644:83–94.
70. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*. 1993;74:609–619.
71. Farrow SN, White JH, Martinou I, Raven T, Pun KT, Grinham CJ, Martinou JC, Brown R. Cloning of a bcl-2 homologue by interaction with adenovirus E1B 19K. *Nature*. 1995;374:731–733.
72. Chittenden T, Harrington EA, O'Connor R, Flemington C, Lutz RJ, Evan GI, Guild BC. Induction of apoptosis by the Bcl-2 homologue Bak. *Nature*. 1995;374:733–736.
73. Kiefer MC, Brauer MJ, Powers VC, Wu JJ, Umansky SR, Tomei LD, Barr PJ. Modulation of apoptosis by the widely distributed Bcl-2 homologue Bak. *Nature*. 1995;374:736–739.
74. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science*. 2001;292:727–730.
75. Suzuki M, Youle RJ, Tjandra N. Structure of Bax: coregulation of dimer formation and intracellular localization. *Cell*. 2000;103:645–654.
76. Lassus P, Opitz-Araya X, Lazebnik Y. Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. *Science*. 2002;297:1352–1354.
77. Sawada M, Sun W, Hayes P, Leskov K, Boothman DA, Matsuyama S. Ku70 suppresses the apoptotic translocation of Bax to mitochondria. *Nat Cell Biol*. 2003;5:320–329.
78. Guo B, Zhai D, Cabezas E, Welsh K, Nouraini S, Satterthwait AC, Reed JC. Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature*. 2003;423:456–461.
79. Nam Y-J, Mani K, Ashton AW, Peng CF, Krishnamurthy B, Hayakawa Y, Lee P, Korsmeyer SJ, Kitsis RN. Inhibition of Both the Extrinsic and Intrinsic Death Pathways Through Non-homotypic Death-fold Interactions. *Mol Cell.* 2004;15:901–912.
80. Gustafsson AB, Tsai JG, Logue SE, Crow MT, Gottlieb RA. Apoptosis Repressor with Caspase Recruitment Domain Protects against Cell Death by Interfering with Bax Activation. *J Biol Chem*. 2004;279: 21233–21238.
81. Ohtsuka T, Ryu H, Minamishima YA, Macip S, Sagara J, Nakayama KI, Aaronson SA, Lee SW. ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. *Nat Cell Biol*. 2004;6:121–128.
82. Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science*. 2004;303: 1010–1014.
83. Wood DE, Newcomb EW. Cleavage of Bax enhances its cell death function. *Exp Cell Res*. 2000;256:375–382.
84. Gao G, Dou QP. N-terminal cleavage of bax by calpain generates a potent proapoptotic 18-kDa fragment that promotes bcl-2-independent cytochrome C release and apoptotic cell death. *J Cell Biochem*. 2000; 80:53–72.
85. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell*. 1995;80:293–299.
86. Hochhauser E, Kivity S, Offen D, Maulik N, Otani H, Barhum Y, Pannet H, Shneyvays V, Shainberg A, Goldshtaub V, Tobar A, Vidne BA. Bax ablation protects against myocardial ischemia-reperfusion injury in transgenic mice. *Am J Physiol Heart Circ Physiol*. 2003;284: H2351–H2359.
87. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science*. 2003; 301:513–517.
88. Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol*. 2004;6:443–450.
89. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science*. 1999;286:1735–1738.
90. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol Cell*. 2001;7: 673–682.
91. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell*. 2001;7:683–694.
92. Wang K, Yin XM, Chao DT, Milliman CL, Korsmeyer SJ. BID: a novel BH3 domain-only death agonist. *Genes Dev*. 1996;10:2859–2869.
93. Chen M, He H, Zhan S, Krajewski S, Reed JC, Gottlieb RA. Bid is cleaved by calpain to an active fragment in vitro and during myocardial ischemia/reperfusion. *J Biol Chem*. 2001;276:30724–30728.
94. Barry M, Heibein JA, Pinkoski MJ, Lee SF, Moyer RW, Green DR, Bleackley RC. Granzyme B short-circuits the need for caspase 8 activity during granule-mediated cytotoxic T-lymphocyte killing by directly cleaving Bid. *Mol Cell Biol*. 2000;20:3781–3794.
95. Sutton VR, Davis JE, Cancilla M, Johnstone RW, Ruefli AA, Sedelies K, Browne KA, Trapani JA. Initiation of apoptosis by granzyme B requires direct cleavage of bid, but not direct granzyme B-mediated caspase activation. *J Exp Med*. 2000;192:1403–1414.
96. Slee EA, Keogh SA, Martin SJ. Cleavage of BID during cytotoxic drug and UV radiation-induced apoptosis occurs downstream of the point of Bcl-2 action and is catalysed by caspase-3: a potential feedback loop for amplification of apoptosis-associated mitochondrial cytochrome c release. *Cell Death Differ*. 2000;7:556–565.
97. Wei MC, Lindsten T, Mootha VK, Weiler S, Gross A, Ashiya M, Thompson CB, Korsmeyer SJ. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. *Genes Dev*. 2000;14: 2060–2071.
98. Eskes R, Desagher S, Antonsson B, Martinou JC. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Mol Cell Biol*. 2000;20:929–935.
99. Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A. The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol Cell*. 1999;3:287–296.
100. Puthalakath H, Villunger A, O'Reilly LA, Beaumont JG, Coultas L, Cheney RE, Huang DC, Strasser A. Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science*. 2001;293:1829–1832.
101. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell*. 1995;80:285–291.
102. Datta SR, Ranger AM, Lin MZ, Sturgill JF, Ma YC, Cowan CW, Dikkes P, Korsmeyer SJ, Greenberg ME. Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell*. 2002;3:631–643.
103. Xing H, Zhang S, Weinheimer C, Kovacs A, Muslin AJ. 14–3-3 proteins block apoptosis and differentially regulate MAPK cascades. *Embo J*. 2000;19:349–358.
104. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T, Tanaka N. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science*. 2000; 288:1053–1058.
105. Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. *Cancer Res*. 2001;61:6669–6673.
106. Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. *Proc Natl Acad Sci U S A*. 2000;97: 9082–9087.

107. Kubasiak LA, Hernandez OM, Bishopric NH, Webster KA. Hypoxia and acidosis activate cardiac myocyte death through the Bcl-2 family protein BNIP3. *Proc Natl Acad Sci U S A*. 2002;11:11.
108. Regula KM, Ens K, Kirshenbaum LA. Inducible expression of BNIP3 provokes mitochondrial defects and hypoxia-mediated cell death of ventricular myocytes. *Circ Res*. 2002;91:226–231.
109. Yussman MG, Toyokawa T, Odley A, Lynch RA, Wu G, Colbert MC, Aronow BJ, Lorenz JN, Dorn GW, 2nd. Mitochondrial death protein Nix is induced in cardiac hypertrophy and triggers apoptotic cardiomyopathy. *Nat Med*. 2002;8:725–730.
110. Ray R, Chen G, Vande Velde C, Cizeau J, Park JH, Reed JC, Gietz RD, Greenberg AH. BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. *J Biol Chem*. 2000;275:1439–1448.
111. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. *Cell*. 1985;41:899–906.
112. Cleary ML, Sklar J. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. *Proc Natl Acad Sci U S A*. 1985;82:7439–7443.
113. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. *Science*. 1985;228:1440–1443.
114. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell*. 1993;74:597–608.
115. Kharbanda S, Pandey P, Schofield L, Israels S, Roncinske R, Yoshida K, Bharti A, Yuan ZM, Saxena S, Weichselbaum R, Nalin C, Kufe D. Role for Bcl-xL as an inhibitor of cytosolic cytochrome C accumulation in DNA damage-induced apoptosis. *Proc Natl Acad Sci U S A*. 1997;94:6939–6942.
116. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science*. 1997;275:1132–1136.
117. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC. Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. *Cancer Res*. 1993;53:4701–4714.
118. Hsu YT, Youle RJ. Nonionic detergents induce dimerization among members of the Bcl-2 family. *J Biol Chem*. 1997;272:13829–13834.
119. Knudson CM, Korsmeyer SJ. Bcl-2 and Bax function independently to regulate cell death. *Nat Genet*. 1997;16:358–363.
120. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol Cell*. 2001;8:705–711.
121. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell*. 2002;2:183–192.
122. Fridman JS, Lowe SW. Control of apoptosis by p53. *Oncogene*. 2003;22:9030–9040.
123. Long X, Boluyt MO, Hipolito ML, Lundberg MS, Zheng JS, O'Neill L, Cirielli C, Lakatta EG, Crow MT. p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. *J Clin Invest*. 1997;99:2635–2643.
124. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. *J Clin Invest*. 1998;101:1326–1342.
125. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science*. 2003;300:135–139.
126. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J, Thompson CB. Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J Cell Biol*. 2003;162:59–69.
127. Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Di Virgilio F, Pozzan T, Rizzuto R. Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells. *J Cell Biol*. 2000;148:857–862.
128. Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demaurex N, Krause KH. Bcl-2 decreases the free Ca2+ concentration within the endoplasmic reticulum. *Proc Natl Acad Sci U S A*. 2000;97:5723–5728.
129. Halestrap AP, McStay GP, Clarke SJ. The permeability transition pore complex: another view. *Biochimie*. 2002;84:153–166.
130. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH. Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat Cell Biol*. 2003;5:1051–1061.
131. Nakagawa T, Yuan J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. *J Cell Biol*. 2000;150:887–894.
132. Saleh M, Vaillancourt JP, Graham RK, Huyck M, Srinivasula SM, Alnemri ES, Steinberg MH, Nolan V, Baldwin CT, Hotchkiss RS, Buchman TG, Zehnbauer BA, Hayden MR, Farrer LA, Roy S, Nicholson DW. Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. *Nature*. 2004;429:75–79.
133. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature*. 2000;403:98–103.
134. Morishima N, Nakanishi K, Takenouchi H, Shibata T, Yasuhiko Y. An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J Biol Chem*. 2002;277:34287–34294.
135. Rao RV, Castro-Obregon S, Frankowski H, Schuler M, Stoka V, del Rio G, Bredesen DE, Ellerby HM. Coupling endoplasmic reticulum stress to the cell death program. An Apaf-1-independent intrinsic pathway. *J Biol Chem*. 2002;277:21836–21842.
136. Kim JS, He L, Lemasters JJ. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. *Biochem Biophys Res Commun*. 2003;304:463–470.
137. Marks AR. Calcium and the heart: a question of life and death. *J Clin Invest*. 2003;111:597–600.
138. Rao RV, Ellerby HM, Bredesen DE. Coupling endoplasmic reticulum stress to the cell death program. *Cell Death Differ*. 2004;11:372–380.
139. Germain M, Mathai JP, Shore GC. BH-3-only BIK functions at the endoplasmic reticulum to stimulate cytochrome c release from mitochondria. *J Biol Chem*. 2002;277:18053–18060.
140. Reimertz C, Kogel D, Rami A, Chittenden T, Prehn JH. Gene expression during ER stress-induced apoptosis in neurons: induction of the BH3-only protein Bbc3/PUMA and activation of the mitochondrial apoptosis pathway. *J Cell Biol*. 2003;162:587–597.
141. Breckenridge DG, Stojanovic M, Marcellus RC, Shore GC. Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol. *J Cell Biol*. 2003;160:1115–1127.
142. Marber MS, Mestril R, Chi SH, Sayen MR, Yellon DM, Dillmann WH. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest*. 1995;95:1446–1456.
143. Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, Green DR. Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol*. 2000;2:469–475.
144. Saleh A, Srinivasula SM, Balkir L, Robbins PD, Alnemri ES. Negative regulation of the Apaf-1 apoptosome by Hsp70. *Nat Cell Biol*. 2000;2:476–483.
145. Ravagnan L, Gurbuxani S, Susin SA, Maisse C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger JM, Garrido C, Kroemer G. Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol*. 2001;3:839–843.
146. Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S, Hirata A, Fujita M, Nagamachi Y, Nakatani T, Yutani C, Ozawa K, Ogawa S, Tomoike H, Hori M, Kitakaze M. Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. *Circulation*. 2004;110:705–712.
147. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell*. 1996;86:147–157.
148. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell*. 2000;102:33–42.

149. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell*. 2000;102:43–53.
150. Faccio L, Fusco C, Chen A, Martinotti S, Bonventre JV, Zervos AS. Characterization of a novel human serine protease that has extensive homology to bacterial heat shock endoprotease HtrA and is regulated by kidney ischemia. *J Biol Chem*. 2000;275:2581–2588.
151. Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, Takahashi R. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol Cell*. 2001;8:613–621.
152. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature*. 1999;397:441–446.
153. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature*. 2001;412:95–99.
154. Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, Prevost MC, Xie Z, Matsuyama S, Reed JC, Kroemer G. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science*. 1998;281:2027–2031.
155. Belzacq AS, Vieira HL, Verrier F, Vandecasteele G, Cohen I, Prevost MC, Larquet E, Pariselli F, Petit PX, Kahn A, Rizzuto R, Brenner C, Kroemer G. Bcl-2 and Bax modulate adenine nucleotide translocase activity. *Cancer Res*. 2003;63:541–546.
156. Shimizu S, Narita M, Tsujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature*. 1999;399:483–487.
157. Narita M, Shimizu S, Ito T, Chittenden T, Lutz RJ, Matsuda H, Tsujimoto Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc Natl Acad Sci U S A*. 1998;95:14681–14686.
158. Bossy-Wetzel E, Newmeyer DD, Green DR. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *EMBO J*. 1998;17:37–49.
159. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, Korsmeyer SJ. A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. *Dev Cell*. 2002;2:55–67.
160. Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, Green DR. Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. *Cell*. 2004;117:773–786.
161. Priault M, Chaudhuri B, Clow A, Camougrand N, Manon S. Investigation of bax-induced release of cytochrome c from yeast mitochondria permeability of mitochondrial membranes, role of VDAC and ATP requirement. *Eur J Biochem*. 1999;260:684–691.
162. Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL, Ng SL, Fesik SW. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature*. 1996;381:335–341.
163. Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod JJ, Mazzei G, Maundrell K, Gambale F, Sadoul R, Martinou JC. Inhibition of Bax channel-forming activity by Bcl-2. *Science*. 1997;277:370–372.
164. Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, Waksman G, Korsmeyer SJ. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. *Proc Natl Acad Sci U S A*. 1997;94:11357–11362.
165. Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB. Bcl-x(L) forms an ion channel in synthetic lipid membranes. *Nature*. 1997;385:353–357.
166. Saito M, Korsmeyer SJ, Schlesinger PH. BAX-dependent transport of cytochrome c reconstituted in pure liposomes. *Nat Cell Biol*. 2000;2:553–555.
167. Basanez G, Nechushtan A, Drozhinin O, Chanturiya A, Choe E, Tutt S, Wood KA, Hsu Y-T, Zimmerberg J, Youle RJ. Bax, but not Bcl-xL, decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. *PNAS*. 1999;96:5492–5497.
168. Melikov KC, Frolov VA, Shcherbakov A, Samsonov AV, Chizmadzhev YA, Chernomordik LV. Voltage-Induced Nonconductive Pre-Pores and Metastable Single Pores in Unmodified Planar Lipid Bilayer. *Biophys J*. 2001;80:1829–1836.
169. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR, Newmeyer DD. Bid, Bax, and Lipids Cooperate to Form Supramolecular Openings in the Outer Mitochondrial Membrane. *Cell*. 2002;111:331–342.
170. Arnoult D, Parone P, Martinou JC, Antonsson B, Estaquier J, Ameisen JC. Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome c release in response to several proapoptotic stimuli. *J Cell Biol*. 2002;159:923–929.
171. Arnoult D, Gaume B, Karbowski M, Sharpe JC, Cecconi F, Youle RJ. Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. *EMBO J*. 2003;22:4385–4399.
172. Adrain C, Creagh EM, Martin SJ. Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. *EMBO J*. 2001;20:6627–6636.
173. Bernardi P, Azzone GF. Cytochrome c as an electron shuttle between the outer and inner mitochondrial membranes. *J Biol Chem*. 1981;256:7187–7192.
174. Goldstein JC, Waterhouse NJ, Juin P, Evan GI, Green DR. The coordinate release of cytochrome c during apoptosis is rapid, complete and kinetically invariant. *Nature Cell Biology*. 2000;2:156–162.
175. Akao M, O'Rourke B, Teshima Y, Seharaseyon J, Marban E. Mechanistically Distinct Steps in the Mitochondrial Death Pathway Triggered by Oxidative Stress in Cardiac Myocytes. *Circ Res*. 2003;92:186–194.
176. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell*. 1997;90:405–413.
177. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell*. 1997;91:479–489.
178. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol Cell*. 1998;1:949–957.
179. Hu Y, Ding L, Spencer DM, Nunez G. WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation. *J Biol Chem*. 1998;273:33489–33494.
180. Saleh A, Srinivasula SM, Acharya S, Fishel R, Alnemri ES. Cytochrome c and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. *J Biol Chem*. 1999;274:17941–17945.
181. Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem*. 1999;274:11549–11556.
182. Qin H, Srinivasula SM, Wu G, Fernandes-Alnemri T, Alnemri ES, Shi Y. Structural basis of procaspase-9 recruitment by the apoptotic protease-activating factor 1. *Nature*. 1999;399:549–557.
183. Vaughn DE, Rodriguez J, Lazebnik Y, Joshua-Tor L. Crystal structure of Apaf-1 caspase recruitment domain: an alpha-helical Greek key fold for apoptotic signaling. *J Mol Biol*. 1999;293:439–447.
184. Zhou P, Chou J, Olea RS, Yuan J, Wagner G. Solution structure of Apaf-1 CARD and its interaction with caspase-9 CARD: a structural basis for specific adaptor/caspase interaction. *Proc Natl Acad Sci U S A*. 1999;96:11265–11270.
185. Day CL, Dupont C, Lackmann M, Vaux DL, Hinds MG. Solution structure and mutagenesis of the caspase recruitment domain (CARD) from Apaf-1. *Cell Death Differ*. 1999;6:1125–1132.
186. Jiang X, Wang X. Cytochrome c promotes caspase-9 activation by inducing nucleotide binding to Apaf-1. *J Biol Chem*. 2000;275:31199–31203.
187. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol Cell*. 2002;9:423–432.
188. Bratton SB, Walker G, Srinivasula SM, Sun XM, Butterworth M, Alnemri ES, Cohen GM. Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *Embo J*. 2001;20:998–1009.
189. Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR, Martin SJ. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9- dependent manner. *J Cell Biol*. 1999;144:281–292.
190. Li K, Li Y, Shelton JM, Richardson JA, Spencer E, Chen ZJ, Wang X, Williams RS. Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. *Cell*. 2000;101:389–399.

191. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell*. 1998;94:739–750.
192. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW. Differential requirement for caspase 9 in apoptotic pathways in vivo. *Cell*. 1998;94:339–352.
193. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell*. 1998;94:325–337.
194. Liston P, Fong WG, Korneluk RG. The inhibitors of apoptosis: there is more to life than Bcl2. *Oncogene*. 2003;22:8568–8580.
195. Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J*. 1998;17:2215–2223.
196. Sun C, Cai M, Meadows RP, Xu N, Gunasekera AH, Herrmann J, Wu JC, Fesik SW. NMR structure and mutagenesis of the third Bir domain of the inhibitor of apoptosis protein XIAP. *J Biol Chem*. 2000;275:33777–33781.
197. Chai J, Shiozaki E, Srinivasula SM, Wu Q, Datta P, Alnemri ES, Shi Y, Dataa P. Structural basis of caspase-7 inhibition by XIAP. *Cell*. 2001;104:769–780.
198. Huang Y, Park YC, Rich RL, Segal D, Myszka DG, Wu H. Structural basis of caspase inhibition by XIAP: differential roles of the linker versus the BIR domain. *Cell*. 2001;104:781–790.
199. Riedl SJ, Renatus M, Schwarzenbacher R, Zhou Q, Sun C, Fesik SW, Liddington RC, Salvesen GS. Structural basis for the inhibition of caspase-3 by XIAP. *Cell*. 2001;104:791–800.
200. Shiozaki EN, Chai J, Rigotti DJ, Riedl SJ, Li P, Srinivasula SM, Alnemri ES, Fairman R, Shi Y. Mechanism of XIAP-mediated inhibition of caspase-9. *Mol Cell*. 2003;11:519–527.
201. Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. *Genes Dev*. 2003;17:1487–1496.
202. Potts PR, Singh S, Knezek M, Thompson CB, Deshmukh M. Critical function of endogenous XIAP in regulating caspase activation during sympathetic neuronal apoptosis. *J Cell Biol*. 2003;163:789–799.
203. Yang Y, Fang S, Jensen JP, Weissman AM, Ashwell JD. Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. *Science*. 2000;288:874–877.
204. Huang H, Joazeiro CA, Bonfoco E, Kamada S, Leverson JD, Hunter T. The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7. *J Biol Chem*. 2000;275:26661–26664.
205. Suzuki Y, Nakabayashi Y, Takahashi R. Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. *Proc Natl Acad Sci U S A*. 2001;98:8662–8667.
206. MacFarlane M, Merrison W, Bratton SB, Cohen GM. Proteasome-mediated Degradation of Smac during Apoptosis: XIAP Promotes Smac Ubiquitination in Vitro. *J Biol Chem*. 2002;277:36611–36616.
207. Hu S, Yang X. Cellular inhibitor of apoptosis 1 and 2 are ubiquitin ligases for the apoptosis inducer Smac/DIABLO. *J Biol Chem*. 2003;278:10055–10060.
208. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science*. 2002;297:259–263.
209. Wang X, Yang C, Chai J, Shi Y, Xue D. Mechanisms of AIF-mediated apoptotic DNA degradation in Caenorhabditis elegans. *Science*. 2002;298:1587–1592.
210. Cande C, Vahsen N, Kouranti I, Schmitt E, Daugas E, Spahr C, Luban J, Kroemer RT, Giordanetto F, Garrido C, Penninger JM, Kroemer G. AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. *Oncogene*. 2004;23:1514–1521.
211. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida H, Kong YY, Mak TW, Zuniga-Pflucker JC, Kroemer G, Penninger JM. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature*. 2001;410:549–554.
212. Klein JA, Longo-Guess CM, Rossmann MP, Seburn KL, Hurd RE, Frankel WN, Bronson RT, Ackerman SL. The harlequin mouse mutation downregulates apoptosis-inducing factor. *Nature*. 2002;419:367–374.
213. Pieper AA, Walles T, Wei G, Clements EE, Verma A, Snyder SH, Zweier JL. Myocardial postischemic injury is reduced by polyADP-ripose polymerase-1 gene disruption. *Mol Med*. 2000;6:271–282.
214. Ravichandran KS. "Recruitment signals" from apoptotic cells: invitation to a quiet meal. *Cell*. 2003;113:817–820.

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