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Mechanisms of Cell Death in Heart Disease

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Abstract

The major cardiac syndromes, myocardial infarction and heart failure, are responsible for a large portion of deaths worldwide. Genetic and pharmacological manipulations indicate that cell death is an important component in the pathogenesis of both diseases. Cells die primarily by apoptosis or necrosis, and autophagy has been associated with cell death. Apoptosis has long been recognized as a highly regulated process. Recent data indicate that a significant subset of necrotic deaths is also highly programmed. In this review, we discuss the molecular mechanisms that underlie these forms of cell death and their interconnections. Because of their regulated nature, the possibility is raised that small molecules aimed at inhibiting cell death may provide novel therapies for these common and lethal heart syndromes.

Keywords

cell death; apoptosis; necrosis; myocardial infarction; heart failure

INTRODUCTION

Cells die primarily by apoptosis or necrosis. Apoptosis is a highly regulated mode of cell suicide.¹ While necrosis has traditionally been regarded as passive and unregulated, data accumulated over the past decade indicates that a substantial proportion of necrotic deaths are actively executed by the cell in a highly regulated manner. This form of necrosis is sometimes referred to as regulated or programmed. Both apoptosis and necrosis play critical roles in normal biology including prenatal development and postnatal homeostasis.² Accordingly, when increased, decreased, or mislocalized, cell death plays major roles in human diseases, including cardiovascular disease, cancer,^{3,4} diabetes,^{5,6} sepsis,⁷ and some neurological disorders.^{8,9}

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DISCLOSURES

None.

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Apoptosis is characterized by cell shrinkage,¹⁰ fragmentation into membrane-enclosed “apoptotic bodies”, and phagocytosis of these corpses by macrophages, or occasionally, neighboring cells.¹¹ When this clean-up operation is efficient, inflammation is avoided. ATP levels in apoptotic cells are reasonably well maintained both because of continued production and decreased expenditures.^{12, 13} The net result of apoptosis is the stealth deletion of individual cells within a tissue. In contrast, necrosis is characterized by loss of plasma membrane integrity, cellular and organellar swelling, and marked inflammation. ATP levels are dramatically reduced in necrotic cells, both because of severe mitochondrial damage that cripples ATP generation as well as unrestrained energy expenditures.¹⁴ The chicken and egg relationships between ATP deficits and loss of plasma membrane integrity remain unclear. Similarly, while it is tempting to speculate that the decision of a doomed cell to undergo apoptosis versus necrosis is determined by energetics, this possibility has not yet been definitively established.

MECHANISMS OF CELL DEATH

Apoptosis and necrosis are mediated by distinct, but highly overlapping central pathways (Figure). The extrinsic pathway involves cell surface death receptors and the intrinsic pathway utilizes the mitochondria and endoplasmic reticulum (ER). These pathways, which mediate both apoptosis and necrosis, are linked by multiple biochemical and functional connections. Extrapolating this degree of connectivity, the possibility is raised that these cell death mechanisms comprise a single unified death machinery. However, given the morphological differences among types of cell death and the presumption that each arose at a specific time in evolution for a specific purpose, the notion of a unified model remains to be established.

Extrinsic (death receptor) pathway: apoptosis and necrosis

In the death receptor pathway, a variety of death ligands bind their cognate receptors to trigger cell death. Some of these ligands are soluble (e.g. tumor necrosis factor (TNF)- α), and some are bound to the surface of other cells (e.g. Fas ligand). The efficiency with these ligands to induce death varies with cell type. Recent work has shown that the same death ligands may induce apoptosis or necrosis, the choice mediated by downstream events.

Binding of ligand to receptor induces the formation of either of two multiprotein complexes: the Death Inducing Signaling Complex (DISC) and complex I.^{15, 16} The DISC signals apoptosis, while Complex I can signal either apoptosis, necrosis, or cell survival. The DISC has been studied most intensively in the context of Fas ligand/Fas signaling, and complex I in the setting of TNF/TNF receptor 1 signaling. However, which ligand/receptor combinations employ the DISC versus complex I, or both, is incompletely understood.

In DISC formation, the binding of death ligand induces a conformational change in the cytosolic domain of the death receptor, which recruits an adaptor protein (e.g. Fas-associated via death domain (FADD), TNF receptor-associated death domain (TRADD)).¹⁷ This adaptor protein, in turn, binds upstream procaspases-8 or -10 to form the DISC.^{15, 17} Procaspases are the zymogen form of caspases, cystenyl proteases that cut following aspartic acid residues.¹⁸ Within the DISC, procaspases-8 and -10 are activated through a forced proximity mechanism.^{19, 20} Once activated, these caspases cleave and activate downstream procaspases-3 and -7. Caspases-3 and -7 then cut hundreds of cellular proteins to bring about apoptotic death through mechanisms that are incompletely understood. In most cells, activation of the extrinsic pathway alone is insufficient to kill, and requires amplification through the intrinsic pathway. One means by which amplification is achieved is through the cleavage of the Bcl-2 family protein BH3-interacting domain death agonist (Bid) by

caspase-8, following which truncated Bid translocates to the mitochondria and contributes to outer mitochondrial membrane (OMM) apoptotic events described below.²¹

In the assembly of complex I, the binding of death ligand to receptor recruits TRADD, which recruits receptor interacting protein 1 (RIP1, a serine/threonine kinase), cellular Inhibitor of Apoptosis Proteins (IAP) 1 and 2, and TNF receptor-associated factor 2 and 5 (TRAF 2 and 5).¹⁶ RIP1 undergoes K63-polyubiquitination by cIAP1 and -2.^{22, 23} This provides a platform for the recruitment of additional kinases that activate NF- κ B, resulting in the transcription of survival proteins.²⁴ However, following dissociation of death receptor, endocytosis, deubiquitination of RIP1, and recruitment of a FADD-RIP3 complex, complex I morphs into complex II.^{25, 26} Complex II signals apoptosis when FADD recruits procaspase-8 leading to its activation by forced proximity.^{16, 19} Caspase-8 not only activates downstream caspases to bring about apoptosis, it also cleaves RIP1 and RIP3 abrogating their ability to signal necrosis (see below).²⁷ If caspase-8 activity is inhibited experimentally or by certain viral or cancer proteins, apoptosis is blocked, obligating the cell to undergo necrosis in this pathway.^{28, 29} Necrosis is triggered by the interaction of RIP1 with RIP3, a second serine/threonine kinase, resulting in a complex series of cross-phosphorylation events. Necrostatin-1, a small molecule inhibitor of the kinase activity of RIP1, ablates necrosis in the death receptor pathway.³⁰

Events in this pathway downstream of RIP1 and RIP3 are incompletely understood, but include phosphorylation by RIP3 of mixed lineage kinase domain-like protein (MLKL),³¹ phosphoglycerate mutase (PGAM5, a mitochondrial phosphatase),³² and certain catabolic enzymes (glutamate dehydrogenase 1 (GLUD1), glutamate ammonia ligase (GLUL), and glycogen phosphorylase (PYGL)), the latter potentially eliciting necrosis through the generation of ROS.³³ The effects of ROS at the mitochondria will be discussed below. In addition, ROS-mediated DNA damage leads to overactivation of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme that consumes NAD⁺ leading to significant ATP consumption, a key feature of necrosis.³⁴ Other downstream events that have been implicated in death receptor necrosis signaling include activation of calpains, phospholipases, lipoxygenases, and sphingomyelinases and permeabilization of lysosomes. For further details, the reader is referred to a recent review.³⁵

Intrinsic (mitochondrial/ER) pathway: apoptosis and necrosis

Mitochondria and ER are central to both apoptotic and necrotic signaling, and the intrinsic pathway mediates a more diverse array of death stimuli than does the death receptor pathway. These include deprivation of nutrients, oxygen, and survival factors, oxidative stress, DNA damage, proteotoxic stress, and chemical and physical toxins. Current understanding suggests that the pathways and events that mediate apoptosis and necrosis at the mitochondria are spatially and mechanistically distinct. The primary event in apoptosis is permeabilization of the OMM resulting in the release of apoptogens.¹ In contrast, the defining event in primary necrosis is the early opening of a channel in the inner mitochondrial membrane (IMM) termed the mitochondrial permeability transition pore (mPTP).³⁶

Mitochondrial signaling: apoptosis—The main regulators of the mitochondrial apoptosis pathway are the Bcl-2 family proteins.³⁷ In addition, as will be discussed below, recent data also implicates these proteins in the regulation of necrosis. The Bcl-2 family is comprised of both antiapoptotic (e.g. Bcl-2, Bcl-x_L, Mcl-1) and proapoptotic members, and the proapoptotics are further divided into multidomain (e.g. Bax, Bak) and BH3-only proteins (multiple members).³⁷ In healthy cells, Bax resides primarily in the cytosol. In response to death stimuli, Bax undergoes conformational activation and translocates to the

mitochondria, where it inserts into the OMM.³⁸ Apoptotic signals also stimulate the conformational activation of Bak, which is constitutively localized to the OMM.³⁹ Within the OMM, Bax and Bak homo- and hetero-oligomerize to bring about OMM permeabilization through poorly understood mechanisms.⁴⁰ The noxious stimuli that activate Bax and Bak are transduced from various locations in the cell via specific BH3-only proteins. For example, loss of the survival signals insulin and IGF-1 leads to activation of the BH3-only protein Bad by decreasing Bad phosphorylation and permitting its release from the 14-3-3 protein.⁴¹ The means by which BH3-only proteins activate Bax and Bak is complex. Certain BH3-only proteins called “activators” (e.g. Bim, Bid) bind directly to Bax (and possibly Bak) to conformationally activate these proteins. Other BH3-only proteins called “sensitizers” displace the activator BH3-only proteins from anti-apoptotics such as Bcl-2 and Bcl-x_L. Conversely, anti-apoptotic Bcl-2 proteins inhibit Bax and Bak by sequestering the BH3-only activators, and possibly also through direct interactions with Bax and Bak.³⁸

Permeabilization of the OMM leads to the release of apoptogens, including cytochrome c, Smac/Diablo, Omi/HtrA2, Apoptosis Inducing Factor (AIF), and Endonuclease G (Endo G) from the mitochondria to the cytosol. Cytosolic cytochrome c and dATP bind to the adaptor protein Apaf-1 resulting in a presumed conformational change that stimulates Apaf-1 oligomerization and its recruitment of upstream procaspase-9 into a complex termed the apoptosome.^{42, 43} Procaspase-9 is activated by forced proximity within this complex and goes on to cleave and activate procaspases-3 and -7. Apoptosis is opposed by IAP family members, the same proteins that act in the death receptor necrosis pathway to signal survival through their K63-polyubiquitination of RIP1. In the mitochondrial apoptosis pathway, these IAPs inhibit already activated downstream caspases by occluding access of substrates to the active sites of these caspases.⁴⁴⁻⁴⁶ The apoptogens Smac/Diablo and Omi/HtrA2 reverse caspase inhibition by IAPs through binding to IAPs and displacing the caspases.⁴⁷⁻⁵⁰ In addition, Omi/HtrA2 possesses serine protease activity that cleaves XIAP, an IAP.⁵¹ AIF, which in combination with perhaps EndoG, causes fragmentation of DNA from ~200 to 50 kb fragments, has been hypothesized to mediate a form of caspase-independent cell death.^{52, 53} However, it is possible that the primary role of AIF-induced DNA damage is to further augment activation of PARP-1 leading to ATP depletion during necrosis.

A host of inhibitors oppose these apoptosis pathways. These include FADD-like IL-1 - converting enzyme inhibitory protein (FLIP) which inhibits DISC assembly and/or function,⁵⁴ anti-apoptotic Bcl-2 proteins that block release of mitochondrial apoptogens, and IAP family members that inhibit already activated downstream caspases as described. While these inhibitors act on either the death receptor or mitochondrial apoptosis pathways, Apoptosis Repressor with Caspase recruitment domain (ARC) inhibits both pathways by disrupting DISC assembly and inhibiting Bax activation.⁵⁵ ARC expression was initially believed to be limited to cardiac and skeletal myocytes and neurons, but recent data shows that it is also induced at high levels in cancer cells⁵⁶⁻⁵⁸ and hypoxic pulmonary artery smooth muscle cells *in vivo*.⁵⁹

Mitochondrial signaling: necrosis—In contrast to OMM permeabilization in apoptosis, the defining event of necrosis at the mitochondria is opening of the mPTP, a pore in the IMM. In healthy mitochondria, the IMM is impermeable to water, ions, and even single protons. As substrates are metabolized in the mitochondrial matrix resulting in the transport of electrons along the respiratory chain, protons are pumped from the matrix to the intermembrane space. This creates an electrochemical gradient ($\Delta\psi_m$) between the intermembrane space and matrix, which provides the potential energy necessary to drive ATP synthesis. Necrotic stimuli, such as Ca²⁺, trigger opening of the mPTP.⁶⁰ Ca²⁺-induced mPTP opening can be potentiated by ROS, alkalosis and depletion of ATP or ADP.^{61, 62}

Opening of the mPTP causes abrupt loss of $\Delta\psi_m$ leading to cessation of mitochondrial ATP synthesis. In addition, mPTP opening allows water to rush down its osmotic gradient into the matrix, leading to mitochondrial swelling, and sometimes frank rupture of the OMM. Although rupture of the OMM can cause release of cytochrome c and activate caspases,⁶³ it is unclear how much engagement of downstream apoptosis signaling contributes to cell death in the mitochondrial necrosis pathway given the other cataclysmic events precipitated by mPTP opening. However, as will be discussed below, potential caspase activation during necrosis complicates interpretation of assays such as TUNEL, which are traditionally assumed to be specific to apoptosis.

Despite extensive research in the field, the components of the mPTP remain unknown. The adenine nucleotide translocase (ANT) and phosphate carrier in the IMM, voltage-dependent anion channel (VDAC) and peripheral benzodiazepine receptor in the OMM, hexokinase which is loosely attached to the cytosolic face of the OMM, and cyclophilin D (a peptidyl prolyl *cis-trans* isomerase) in the matrix have been proposed to be components of the pore.³⁶ However, genetic studies have excluded ANT,⁶⁴ VDAC,⁶⁵ and cyclophilin D^{63, 66} as core pore components, although ANT and cyclophilin D are important positive regulators of pore opening.^{63, 64, 66}

Necrosis can occur as a primary event or secondary to apoptosis, the latter when the disposal of apoptotic bodies is delayed. Delayed clean-up occasionally occurs *in vivo*, and is almost always observed at late time points in cell culture.¹¹ In primary necrosis, mPTP opening occurs early, before cytochrome c release. If mPTP opening takes place during apoptosis, it occurs coincident with or following cytochrome c release. In this case, mPTP opening may result from caspase-dependent events.⁶⁷ Although the kinetics differ markedly, these observations explain why loss of $\Delta\psi_m$ may provide a marker for both necrosis and apoptosis.

How cell death stimuli connect with the mitochondrial necrosis machinery is incompletely understood. Some classic activators of this pathway, such as ischemia and ischemia-reperfusion, induce mPTP opening through Ca^{2+} and ROS. In addition, activators of the death receptor necrosis pathway may ultimately engage the mitochondrial necrosis pathway through links that were previously discussed. It is likely, however, that additional connections/pathways exist.

ER-mediated apoptosis and necrosis—The ER mediates the synthesis and proper folding of multiple proteins, some post-translational modifications, trafficking of newly synthesized proteins to the Golgi apparatus, lipid biosynthesis, and Ca^{2+} homeostasis. These effects are critical for normal cellular functioning. Under certain conditions, however, the ER can also mediate cell death – both apoptosis and necrosis. Considerable controversy exists as to the precise mechanisms by which the ER contributes to cell death and the mechanisms that mediate the switch from adaptation to death. While adaptive and death responses could be mediated by parallel pathways, the involvement of shared signaling components implicates the same pathways in both outcomes. For example, misfolded proteins in the ER lumen elicit a response mediated by ER transmembrane sensors PKR-like ER kinase (PERK), inositol-requiring protein 1 (IRE1), and activating transcription factor 6 (ATF6). These proteins activate complex transcriptional and post-transcriptional cascades to re-establish ER homeostasis. However, it is thought that, when various ER stresses (e.g. misfolded proteins, oxidative stress, certain lipids) fail to be resolved in a timely manner, death may result.⁶⁸

While the precise ER-specific machinery by which cell death is promoted remains incompletely understood, the transcription factor C/EBP homologous protein (CHOP) has

been clearly implicated. CHOP, which is activated downstream of the ER transmembrane sensors, induces the expression of pro-apoptotic proteins Bim,⁶⁹ TREB3,⁷⁰ and DR5⁷¹ and represses that of Bcl-2.⁷² Another important death mediator is Ca²⁺, which transits from the ER lumen to the mitochondria, to trigger apoptosis or necrosis through mechanisms that are discussed in the section on cross-talk between mitochondrial apoptosis and necrosis pathways. Less clear are potential roles for various caspases,^{73, 74} JNKs,⁷⁵ other ER membrane proteins,⁷⁶ and cleavage of multiple mRNAs by IRE1,⁷⁷ which also possesses endonuclease activity.

Autophagy-associated cell death

Autophagy is a process in which the cell breaks down its own proteins and lipids. This provides energy during periods of starvation and stress, a means for the disposal of long-lived proteins, and a mechanism for protein quality control.⁷⁸ Accordingly, in organisms ranging from yeast to mammals, autophagy is a survival mechanism. That said, too much autophagy has been hypothesized to cause cell death, a process referred to as autophagic cell death or, more accurately, as autophagy-associated cell death. It is plausible that self-cannibalization could result in cell death. However, at this point in time, a direct causal link between autophagy and cell death has not been definitively demonstrated. One impediment in establishing this connection is the absence of markers for autophagy-associated death - in distinction to the existence of abundant markers for autophagy itself. In most experiments, an intervention is used to alter rates of autophagy, the success of which is confined with autophagy markers, and this manipulation is then correlated with histological markers of cell death (e.g. TUNEL). While it is possible that autophagy kills cells indirectly through another form of cell death (see below), an autophagy-specific mode of killing has not been identified. Questions remain even regarding the interpretation of electron micrographs showing presumably dead or dying cells that contain autophagic vacuoles because it is unclear whether autophagy in this situation represents a pathogenic mechanism, a compensatory process, or is unrelated to the presumed cell death.⁷⁹ There are, however, some convincing data supporting a role for autophagy in cell death, e.g. during regression of the salivary gland in *Drosophila* development.⁸⁰ In addition, we will highlight studies linking autophagy to cell death during myocardial infarction and heart failure in the section on heart disease below.

Although a dedicated machinery for autophagy-associated cell death has not been identified, physical and functional connections between key autophagy and cell death proteins have been recognized and might provide insights into interrelationships between these processes.⁸¹ In the discussion to follow, the reader is referred to a number of comprehensive reviews dealing with autophagy.^{78, 82, 83} Beclin-1, a protein involved in autophagosome formation, contains a BH3 domain analogous to those in BH3-only proteins, which as discussed above promote apoptosis. The Bcl-2-Beclin-1 interaction inhibits the pro-autophagic function of Beclin-1 in response to starvation without interfering with anti-apoptotic function of Bcl-2. Moreover, multiple BH3-only proteins can displace Beclin-1 from Bcl-2 to promote autophagy.⁸¹

Connections between cell death pathways

We have previously discussed connections that link (a) death receptor apoptosis with mitochondrial apoptosis pathways (e.g. Bid); and (b) death receptor apoptosis with death receptor necrosis pathways (caspase-8 activity as a decision point in apoptosis versus necrosis in this pathway). In this section, we consider molecules/pathways connecting (a) necrosis signaling at death receptors with that at the mitochondria; and (b) mitochondrial apoptosis and necrosis pathways.

Cross-talk between death receptor and mitochondrial necrosis pathways—As previously discussed, activation of the death receptor pathway signals necrosis when caspase-8 is inhibited.^{28, 29} Induction of necrosis in this paradigm is abrogated by the absence of Bax/Bak or cyclophilin D, genetically linking death receptor and mitochondrial necrosis events.^{84, 85} Second, RIP1 translocates to the mitochondria when activated in the death receptor necrosis pathway, although its mitochondrial actions are not yet understood.⁸⁶ Third, activation of RIP1 and RIP3 in the death receptor pathway stimulates ROS production through NOX1 and GLUD1/GLUL/PYGL1 activation respectively,^{33, 87} and as discussed, ROS is a strong potentiator of Ca²⁺-induced mPTP opening. Fourth, as discussed previously, RIP3 activation in the death receptor pathway also triggers cell death through phosphorylation of the mitochondrial phosphatase PGAM5.³² Other connections are likely to become evident as these pathways are understood in more detail.

Cross-talk between mitochondrial apoptosis and necrosis pathways—We have previously discussed some connections between these pathways including how OMM rupture (not permeabilization) in necrosis may result in cytochrome c release, and how caspase activation in apoptosis may trigger late mPTP opening. Another important connection involves Bcl-2 proteins, which unite apoptosis and necrosis signaling at the mitochondria through their effects on Ca²⁺ handling at the ER.⁸⁸ Bax, which induces OMM permeabilization during apoptosis, also increases the concentration of Ca²⁺ in the ER lumen, such that a larger Ca²⁺ bolus is released when the ER is presented with a death stimulus. ER Ca²⁺ transits to the mitochondria either through the cytoplasm or via direct connections between mitochondria and ER.^{89, 90} Increases in mitochondrial Ca²⁺ can trigger mPTP opening and necrosis or apoptosis through mechanisms that have not yet been defined. Bcl-2 opposes these Bax-induced effects at both the mitochondria and ER.

CELL DEATH IN HEART DISEASE

Myocardial infarction

Surgical occlusion of the left coronary artery is used as a surrogate for acute thrombosis in animal models of ST-segment elevation (STEMI) myocardial infarction. This process is usually studied in the context of reperfusion (“ischemia-reperfusion”, I/R) because of the clear benefit of restoring blood flow in human myocardial infarction. It should be noted, however, that despite the net effect of reperfusion to reduce infarct size, the introduction of blood into an ischemic zone generates ROS, Ca²⁺, and alkalosis, all inducers of mPTP opening.³⁶ For this reason, significant research is directed toward reducing “reperfusion injury”.⁹¹ Another point relevant to interpreting data from rodent models of ischemia-reperfusion is that, despite rare reports to the contrary, it is unusual for genetic or pharmacological manipulations to reduce infarct size in the setting of prolonged ischemia without reperfusion (“permanent occlusion”), another reason why most studies employ I/R.

Cell Death in myocardial infarction—In both permanent occlusion and I/R models of myocardial infarction, a large burst of cell death takes place within the area rendered ischemia over the first 6-24 hours.⁹² Lesser amounts of cell death takes place in the peri-infarct zone, initially the result of residual ischemia, but persisting due to cardiac remodeling driven by the loss of contractile units in the infarct. A yet lower magnitude of cell death continues for months in the remote myocardium as remodeling progresses.⁹³ In this section, we focus on cardiac myocyte death in the ischemic zone.

During myocardial infarction, cardiac myocytes in the ischemic zone die by both apoptosis and necrosis. Surprisingly, the magnitudes of each form of cell death remain unclear. The impediment has been limitations of current assays to definitively distinguish between apoptosis and necrosis in tissue from animals subjected to myocardial infarction. For

example, although the primary consequence of mPTP opening during necrosis is cessation of ATP synthesis, the accompanying mitochondrial swelling can result in OMM rupture and cytochrome c release. It is unclear how often OMM rupture occurs in this situation, but the potential release of cytochrome c confounds the interpretation of assays based on caspase activation and DNA fragmentation (e.g. TUNEL). Solutions include the direct evaluation of plasma membrane integrity *in vivo* using a variety of approaches and electron microscopy, although the latter is limited by differential sensitivities for the detection of necrotic versus apoptotic cells. While these techniques have been employed to some extent, a rigorous quantification of apoptosis and necrosis during myocardial infarction is needed.

Apoptosis in myocardial infarction—Multiple studies have demonstrated a causal connection between cardiac myocyte apoptosis and myocardial infarction. Both the death receptor and mitochondrial pathways have been shown to be critical. Hearts of mice lacking Fas (*Ipr* mice) exhibit smaller infarcts in response to I/R, when studied as isolated preparations or *in vivo*.^{94, 95} Given that death signals related to I/R potentially activate the mitochondrial pathway, the reasons underlying the importance of the death receptor pathway in this process are not obvious. One explanation may be that death ligands themselves are important mediators of I/R, and in support of this, Fas ligand appears in the coronary effluent of isolated hearts during the reperfusion phase. Another possibility may be that activation of the death receptor pathway provides another input into activation of the mitochondrial apoptosis pathway through truncated Bid.

Cardiac-specific overexpression of Bcl-2 decreases infarct size and cardiac dysfunction following I/R *in vivo*.^{96, 97} In addition, deletion of Bax reduces infarct size in isolated hearts subjected to I/R.⁹⁸ Bax deletion has also been reported to cause mild reductions in infarct size following permanent occlusion *in vivo*.⁹⁹ Absence of PUMA, a p53 responsive BH3-only protein, reduces infarct in isolated, perfused hearts subjected to I/R.¹⁰⁰ Thus, Bcl-2 family members modulate infarct size.

Cardiac overexpression of cIAP2 results also in smaller infarcts in isolated perfused hearts subjected to I/R.¹⁰¹ This effect may result from the inhibition of already activated downstream caspases by IAPs by cIAP2 and/or its K63-polyubiquitination of RIP1 which activates the death receptor survival pathway. UCF-101, a small molecule inhibitor of the serine protease activity of Omi/HtrA2, decreases infarct size following I/R.^{102, 103} Pancaspase inhibitors provide varying degrees of reduction in the size of infarcts elicited by I/R.¹⁰⁴⁻¹⁰⁷ Overexpression of ARC, which inhibits both death receptor and mitochondrial apoptosis pathways, also decreases infarct size after I/R.¹⁰⁸ The fact that multiple manipulations of apoptosis pathways affect infarct size provides confidence that this form of cell death is involved in myocardial infarction.

Necrosis in myocardial infarction—Regulated necrosis has also been demonstrated to play a role in the development of myocardial infarction. Necrostatin, the inhibitor of the kinase activity of RIP1, reduces infarct size in response to I/R *in vivo*. Interestingly, its cardioprotective effect is dependent on the presence of cyclophilin D, suggesting connections between RIP1 and mitochondrial necrosis events.¹⁰⁹

Bax and Bak have recently been shown to regulate necrosis. In addition to reducing infarct size, deletion of Bax and Bak markedly reduces the degree of necrotic injury in the hearts of mice subjected to I/R. These effects occurs through a pathway distinct from the regulation of apoptosis by Bax and Bak, as evidenced by the ability of oligomerization-deficient Bax mutants, which cannot support apoptosis, but retain the ability to mediate necrosis.¹¹⁰

Mice lacking cyclophilin D, a positive regulator of mPTP opening, demonstrate decreased infarct size following I/R.^{63, 66} Pharmacologic inhibition of cyclophilin D, using cyclosporine A or sangliferin A, also reduces infarct size.¹¹¹⁻¹¹⁴ A pilot study has translated this work to a small number of patients with ST-segment elevation myocardial infarction. When superimposed on angioplasty and stenting, cyclosporine A resulted in statistically significant reduction in infarct size as measured by serum levels of creatine kinase, but not troponin I, and by magnetic resonance imaging.¹¹⁵ While significant reductions in infarct size persisted at 6 months post-myocardial infarction, only a non-statistically significant trend toward preserved cardiac function was observed.¹¹⁶ Thus, further work is needed to assess the efficacy of this cardioprotective strategy in humans.

Taken together, these studies demonstrate that both apoptosis and necrosis contribute to the pathogenesis of myocardial infarction.

Autophagy-associated death in myocardial infarction—Autophagy is induced during both I/R and permanent occlusion. However, the mechanisms and the consequences of this induction appear to be different.¹¹⁷ During permanent occlusion, AMPK is activated and inhibits mTOR, a potent inhibitor of autophagy. Consequently, autophagy is induced. Inhibition of autophagy by transgenic overexpression of dominant negative AMPK resulted in worsening of infarct size in response to permanent occlusion.¹¹⁷ Similar results were obtained when autophagy was inhibited by overexpression of Rheb,¹¹⁸ overexpression of a dominant negative form of GSK3 β , or deletion of one allele of GSK3 β .¹¹⁹ Thus, consistent with the survival role of autophagy during starvation, these data suggest that autophagy protects the myocardium during prolonged ischemia. During I/R, however, Beclin-1 levels increase to activate autophagy. Mice in which one Beclin-1 allele has been inactivated exhibit smaller infarcts in this situation.¹¹⁷ Similar results were found when autophagy was decreased by loss of function manipulations of GSK3 β as described above.¹¹⁹ These and other studies^{120, 121} suggest that autophagy is associated with a protective role during ischemia and a pathogenic role during I/R. Further investigation is needed, however, to determine the extent to which alterations in autophagy explain these changes in infarct size.

Cell death and heart failure

Apoptosis in heart failure—In contrast to myocardial infarction in which there is an explosive and short-lived burst of cell death, the absolute percentage of apoptotic cardiac myocytes in failing human hearts is quite low (0.08-0.25% as assessed by TUNEL). However, this percentage of cardiac myocyte apoptosis is ~10-100-fold higher than that observed in control hearts (0.001-0.01%).¹²²⁻¹²⁴ These data suggest the hypothesis that low, but elevated levels of cardiac myocyte apoptosis, result over time in cumulative loss of cardiac myocytes and heart failure. This possibility was first tested in transgenic mice with a conditionally-activatable procaspase-8 allele, which showed that rates of cardiac myocyte apoptosis as low as 0.023% elicit a lethal dilated cardiomyopathy. Control mice overexpressing an enzymatically-dead procaspase-8 remained normal.¹²⁵ These data establish the sufficiency of clinically-relevant levels of apoptosis to induce heart failure.

Conversely, the necessity of cardiac myocyte apoptosis for heart failure was tested using pancaspase inhibition in a model of peripartum cardiomyopathy.¹²⁶ This was induced by cardiac-specific overexpression of G β q, a surrogate for humoral stimuli relevant to heart failure. Pregnancy precipitated lethal heart failure in 30% of G β q transgenic mice. Pre-treatment with a pancaspase inhibitor reduced cardiac myocyte apoptosis, preserved heart function, and completely rescued mortality. These data demonstrate the necessity of cardiac myocyte apoptosis for heart failure in this model. These concepts have also been extended to other models. For example, following myocardial infarction, deletion of Bcl-2/adenovirus

E1b 19kD interacting protein 3 (Bnip3), a BH3-like protein, reduced pathological remodeling in the perinfarct zone and resultant heart failure.¹²⁷

Necrosis in heart failure—Cardiac myocyte necrosis may also play a role in heart failure. Cardiac myocyte-specific transgenic overexpression of the α_2 -subunit of the L-type Ca^{2+} channel resulted in Ca^{2+} overload, mPTP opening, necrosis, and cardiac dysfunction.¹²⁸ This phenotype was rescued by deletion of *ppif* encoding cyclophilin D, but not overexpression of Bcl-2, suggesting that heart failure in this model is attributable to cardiac myocyte necrosis. Similarly, doxorubicin-induced cardiomyopathy was ameliorated by knockout *ppif*. In contrast to myocardial infarction, involvement of necrosis in heart failure is somewhat unexpected. While this interpretation may be correct, it is important to also consider recently discovered effects of cyclophilin D on cardiac metabolism.¹²⁹ Future work will be needed to determine the magnitude of cardiac myocyte necrosis in failing hearts and the general applicability to pathogenesis of this syndrome.

Autophagy-associated death in heart failure—A previous study of failing human hearts has suggested that autophagy-associated cell death is the most common form of cellular demise during heart failure.¹³⁰ However, the markers used to diagnose various forms of cell death in this study were not specific. Stronger data concerning the relationship of autophagy and heart failure have been provided by genetic loss- and gain-of-function studies. Atg5 deletion in the heart precipitates ventricular enlargement and cardiac dysfunction after hemodynamic overload implying that autophagy is a compensatory mechanism during heart failure.¹³¹ In contrast, Beclin-1^{+/-} mice subjected to pressure overload exhibited decreased pathological remodeling and cardiac dysfunction, while Beclin-1 overexpression resulted in the opposite.¹³² The explanation for the conflicting results in the Atg5 and Beclin-1 studies is not known, but may be related to differences in the genetic manipulations or apparent severity of pressure overload. Therefore, the role of autophagy in the pathogenesis of pressure overload-induced heart failure is not clear. On the other hand, deletion of one allele of Beclin-1 worsens cardiac remodeling and function and mortality in response to proteotoxic stress induced by transgenic overexpression of the R120G mutant of α -crystallin, a model of desmin-related cardiomyopathy.¹³³ Thus, in keeping with its role in disposing of defective proteins, autophagy plays a protective role in heart failure initiated by proteotoxicity. Taken together, these data highlight that autophagy may be protective in response to some cardiomyopathic stimuli and pathogenic in response to others.

CONCLUDING REMARKS

This review discusses the role of cell death in the major syndromes that affect the heart: myocardial infarction and heart failure. Although myocardial infarction and heart failure are complex and involve multiple cellular processes, the data indicate that cell death plays a critical role in the pathogenesis of both syndromes. The regulated nature of much of the cell death in these diseases opens up the possibility of manipulating death pathways to therapeutic advantage. Given its acute nature, myocardial infarction is currently the most attractive target. An important issue in this setting is how the drug will access tissue in which the blood supply is compromised. One possibility is drug delivery at the time of reperfusion. However, administration even prior to reperfusion may have beneficial effects on the peri-infarct region as well as potentially extending the window for effective reperfusion. Heart failure may also be a viable target, but potential oncogenic effects of chronic cell death inhibition are a concern. To circumvent this obstacle will require the development of approaches to target drug to the myocardium. The hope is that, in combination with therapies directed at atherosclerosis and plaque rupture, small molecule

approaches to decrease the susceptibility of the myocardium to cell death will limit tissue damage and ultimately reduce mortality.

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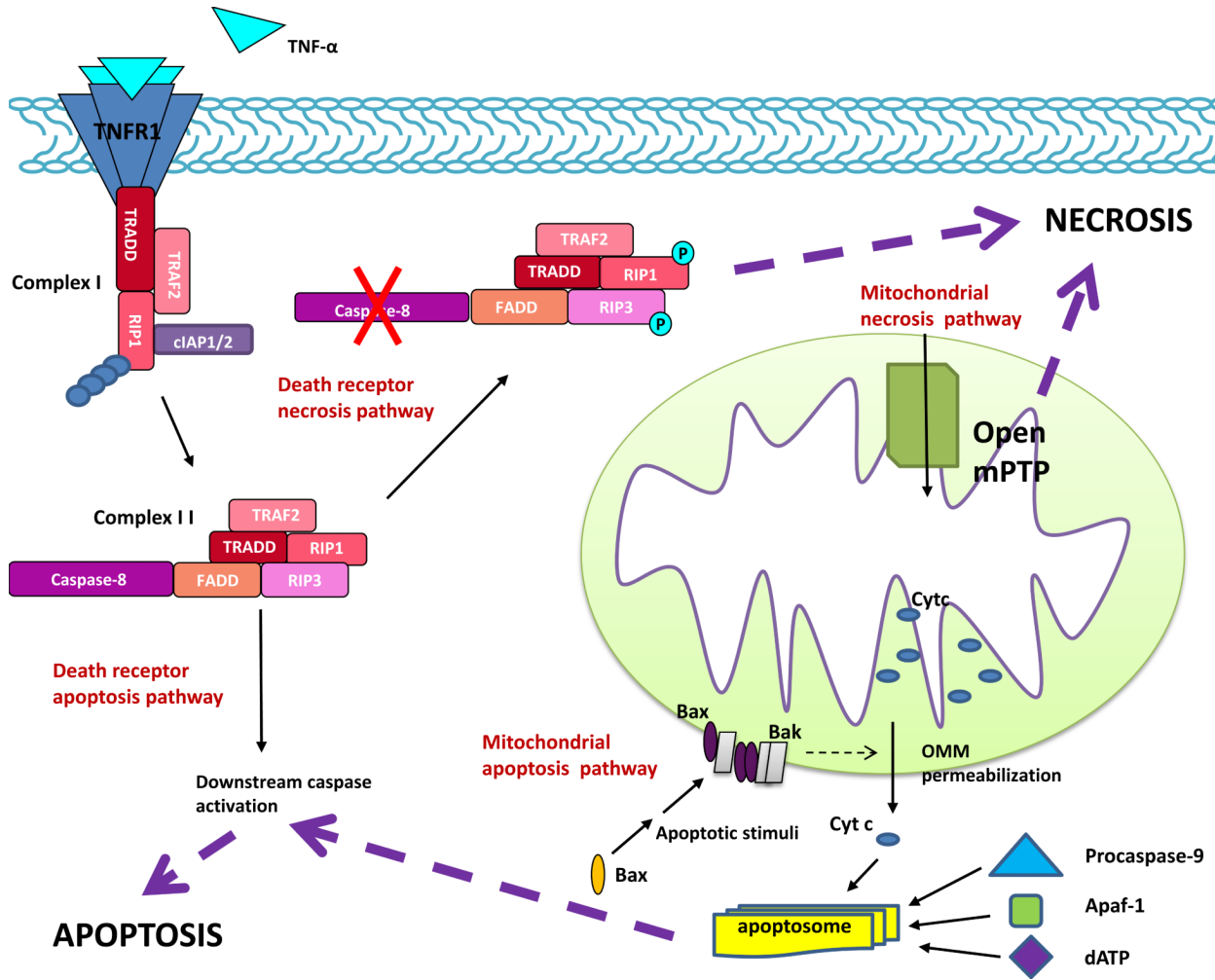


Figure. Cell death pathways

Apoptosis and necrosis are mediated by death receptor (extrinsic) and mitochondrial (intrinsic) pathways. In the death receptor pathway, a death ligand (e.g. TNF- α) binds its cognate death receptor to trigger assembly of either the DISC (not shown) or complex I. When RIP1 is K63-polyubiquitinated by cIAP1/2, complex I signals survival through NF- κ B activation (not shown). If (a) death receptor dissociates from complex I, (b) the complex is endocytosed, (c) RIP1 undergoes deubiquitination, and (d) a FADD-RIP3 complex is recruited, complex II is formed. This complex signals apoptosis or necrosis depending on procaspase-8 activity. Activation of procaspases-8 leads to cleavage and activation of downstream procaspases that proteolyze cellular proteins to bring about apoptosis. Procaspase-8 also cleaves RIP1 and RIP3, to preclude necrosis. In contrast, with caspase-8 inhibition, RIP1 and RIP3 undergo a series of cross-phosphorylation events that trigger necrosis by a variety of mechanisms (see text). In the mitochondrial pathway, the critical event in apoptosis is permeabilization of the outer mitochondrial membrane (OMM), which results in release of mitochondrial apoptogens (e.g. cytochrome c) to the cytoplasm. Complex interactions among Bcl-2 family members (e.g. Bax and Bak) mediate OMM permeabilization (see text). Once in the cytoplasm, cytochrome c stimulates assembly of the apoptosome, a multiprotein complex in which procaspase-9 is activated. Procaspase-9 goes on to activate downstream procaspases. In contrast, the defining event in necrosis is opening of the mitochondrial permeability transition pore (mPTP) in the inner membrane, which (a)

collapses the electrical gradient across the IMM leading to cessation of ATP synthesis and (b) promotes the influx of water into the mitochondrial matrix resulting in severe mitochondrial swelling. Multiple connections exist between these pathways. TNF- α , tumor necrosis factor α , TNFR1, tumor necrosis factor receptor 1, RIP1, receptor interacting protein 1, cIAP1/2, cellular inhibitor of apoptosis 1 and 2, FADD, Fas-associated via death domain, RIP3, receptor interacting protein 3, TRADD, TNF receptor-associated death domain, TRAF2, TNFR-associated factor 2, Cyt c, cytochrome c, Bax, Bcl-2 associated X protein, Bak, Bcl-2 homologous antagonist/killer, Apaf-1, apoptotic protease activating factor-1.