Regulated Necrotic Cell Death
The Passive Aggressive Side of Bax and Bak

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Abstract: Although the molecular effectors of apoptotic cell death have been largely annotated over the past 30 years, leading to a strong biological understanding of this process and its importance in cell biology, cell death through necrosis has only recently been accepted as a similarly regulated process with definable molecular effectors. The mitochondria are important and central mediators of both apoptosis and regulated necrosis. In apoptosis, the B-cell leukemia/lymphoma 2 (Bcl-2) family members Bcl-2-associated protein x (Bax) and Bcl-2 homologues antagonist/killer (Bak) undergo oligomerization in the outer mitochondrial membrane resulting in the release of apoptosis inducing substrates and the activation of caspases and nucleases. In contrast, during necrosis the mitochondria become dysfunctional and maladaptive in conjunction with reactive oxygen species production and the loss of ATP production, in part through opening of the mitochondrial permeability transition pore. Although regulated necrosis is caspase-independent, recent evidence has shown that it still requires the apoptotic regulators Bax/Bak, which can regulate the permeability characteristics of the outer mitochondrial membrane in their nonoligomerized state. Here, we review the nonapoptotic side of Bcl-2 family, specifically the role of Bax/Bak in regulated necrotic cell death. We will also discuss how these Bcl-2 family member effectors could be part of a larger integrated network that ultimately decides the fate of a given cell somewhere within a molecular continuum between apoptosis and regulated necrosis.

Key Words: apoptosis ■ calcium ■ mitochondria ■ necrosis

Historical Context of Cell Death
Cell death has been a recognized physiological process for >150 years, although it was not until the 1970s when the terms apoptosis and necrosis were adopted based on distinct morphological features underlying discrete forms of cell death. The first molecular breakthrough in defining the genes involved in cell death came in 1983 with the identification of cell death 1 and 2 (CED-1, -2), as genes involved in cell death in Caenorhabditis elegans (C elegans). In hindsight, C elegans are a perfect model for defining the physiological and genetic underpinnings of cell death given the ability to account for all the cells in this organism during development, with 131 of 1090 cells being destined to undergo programmed cell death. The first identified mammalian cell death gene was B-cell leukemia/lymphoma 2 (Bcl-2), which was cloned from a translocation hot spot in follicular lymphoma from hematopoietic cell lines. Bcl-2 was later shown to be an antiapoptotic protein with a conserved ortholog in C elegans, CED-9. Remarkably, expression of mammalian Bcl-2 in C elegans protected cells against apoptosis. The discovery of Bcl-2 led to the identification of many...
other key cell death regulators, such as Bcl-2 like 1 (Bcl-x), Bcl-2–associated protein x (Bax), and Bcl-2 homologues antagonist/killer (Bak), among several others.8–10

With respect to defining the molecular effectors of apoptosis, the caspases were discovered in the early to mid-1990s as cell death effector proteins. Caspases are also conserved in C. elegans where CED-3 functions as an intracellular protease to degrade key proteins to facilitate immediate cell death.11,12 Finally, also in the mid-1990s, the mitochondrion was shown to underlie the apoptotic pathway by harboring and subsequently releasing apoptogenic factors, such as cytochrome c (cyt c), which then initiates caspase activation through formation of the apoptosome.13 The mitochondrion was also suspected as being involved in apoptosis because of the localization of various Bcl-2 family members to this organelle.14–16 The evolving data subsequently connected the Bcl-2 family of proteins to the permeability and rupture of mitochondria, followed by the activation of caspases in the initiation of apoptosis, thus providing a model whereby this energy producing organelle was capable of also rapidly killing a cell through a programmed process.

Apoptotic cell death seems to be specialized for the programmed clearing of cells during development, ongoing positive and negative selection of T and B cells in the immune system, culling of cells with inappropriate cell cycle activity (cancer), and for general cellular turnover that underlies tissue homeostasis in select organs. In contrast, cells also die in adult vertebrate organisms in response to ischemic injury largely through a morphological type of cell death known as necrosis.13 Ischemia, such as after myocardial infarction injury, results in a wide-spread loss of adult cardiomyocytes through a necrotic process that is driven by the lack of oxygen, calcium overload, and the generation of aberrant reactive oxygen species (ROS).17 Calcium overload of the mitochondria during ischemic injury is perhaps the most central and damaging effect, which leads to swelling and rupture of this organelle through a process that would become known as mitochondrial permeability transition pore (MPTP) formation and opening.18–21 In 1980, this increase of mitochondrial calcium during ischemic injury was already proposed to be causative in mitochondrial dysfunction and cell death, as treatment with ruthenium red, a pharmacological inhibitor of mitochondrial calcium uptake, preserved mitochondrial function, and prevented tissue damage.22 This was the first evidence showing that mitochondrial preservation protected tissue after ischemic injury (necrotic cell death), suggesting that necrosis can be a regulated process and not simply a default pathway of cellular killing.

The first inhibitor of mitochondria permeability transition was identified in 1987 when cyclosporin A (CsA), an immunosuppressant agent, was shown to desensitize calcium-induced mitochondrial swelling without affecting calcium uptake.23,24 In addition, CsA was shown to protect against calcium- and oxidative stress-induced cell death in hepatocytes,25 providing yet another piece of evidence that necrotic cell death can be regulated. The target of CsA was determined to be cyclophilin D (CypD), a peptidyl-prolyl isomerase that resides in the matrix of the mitochondria where it regulates opening of the MPTP.26 In the late 1990s, several investigators reported data whereby the apoptosis regulating Bcl-2 family members could interact with presumed components of the MPTP and also regulate the opening of this pore, suggesting an intersection between apoptotic and necrotic cell death (more on this later).27–30 More recently, additional evidence has emerged whereby the Bcl-2 family members Bak and Bak are heavily involved in mediating multiple forms of regulated necrosis.31

Mitochondrial Effects of Apoptosis Versus Necrosis

An apoptotic cell is morphologically defined by chromatin condensation, cell shrinkage, membrane blebbing, and the removal of packaged cellular compartments by phagocytes in a noninflammatory process. Necrotic cell death is morphologically defined by cell and organelle swelling, early plasma membrane rupture, and the spilling of cellular material into the tissue with subsequent inflammation.2,32 Because of this, necrosis is considered a more harmful way for a cell to die compared with apoptosis. Although mitochondria and a shared set of molecular effectors (Bax and Bak) are at the center of both forms of cell death, activation of caspases seems to be a uniquely apoptotic process.33 Caspase activation and function during apoptosis is ATP-dependent and typically requires some degree of mitochondrial function, whereas necrosis is an ATP-independent process where it progresses in conjunction with a complete loss of mitochondrial function.33 Indeed, a hallmark of necrotic cell death is mitochondrial dysfunction itself, such that if mitochondria structure function are preserved during a necrotic insult, the cells affected typically no longer die.34 This observation further supports the hypothesis that necrosis is not purely an accidental form of cell death and that it can be regulated and prevented.

Mitochondrial dysfunction occurs during necrosis typically because of prolonged opening of the MPTP, which leads to inner membrane depolarization and loss of ATP production, swelling of the organelle, and the production of ROS.35 However, MPTP opening and mitochondrial swelling and rupture also cause the release of apoptogenic factors, such as cyt c, although in the absence of sufficient ATP caspase activation and the formation of the apoptosome is blocked so that the cell still perishes through a necrotic process.33,35 Although the outer membrane of the mitochondria is altered and engaged
during MPTP opening, the inner mitochondrial membrane opening and loss of membrane potential are the first regulated and driving aspect of MPTP-mediated necrotic cell death. However, if only a small percentage of mitochondria undergo cyt c release with MPTP opening, the remaining functioning mitochondria might still provide enough high-energy phosphate for apoptotic cell death to ensue.

During apoptosis, the regulated release of cyt c from the mitochondria is because of mitochondrial outer membrane permeability (MOMP).36 MOMP is an event initiated directly by the Bcl-2 family members, Bax and Bak.36 When Bax/Bak become activated by apoptotic signaling cues, they form expansive hetero-homo oligomers within the outer membrane of the mitochondria to directly generate large pores leading to the release of cyt c.36 Importantly, during MOMP, the mitochondria are still functional and produce ATP because the inner mitochondrial membrane is typically intact and able to maintain respiration for prolonged periods of time.36 Hence, although the mitochondria are intimately involved in both apoptotic and necrotic cell death, MOMP is a specific event for apoptosis while inner membrane opening of the MPTP is uniquely geared toward necrosis. However, as we will discuss later, Bax and Bak seem to integrate both forms of cell death at the level of the mitochondria by not only affecting MOMP, but also the ability of MPTP opening to culminate in the swelling and rupture of this organelle.37,38

The Bcl-2 Family

Bcl-2 family members are characterized by containing at least 1 Bcl-2 homology (BH) domain (Figure 1).40 There are a total of 4 different BH domains (BH1–BH4), each of which can impart functional effects to Bcl-2 family members in affecting survival or cell death.40 The other characteristic of the Bcl-2 family is that many members contain a transmembrane domain to allow their insertion into various organelle membranes, mainly the mitochondria but also the endoplasmic reticulum, lysosomes, and even the nuclear envelope (Figure 1).41,42 There are a total of 25 human Bcl-2 family members that are divided into 2 main groups, the antiapoptotic and the proapoptotic members (Figure 1).43 The proapoptotic Bcl-2 family members are further divided into 2 additional groups. The first is the BH3-only subfamily that functions to integrate signals from various prodeath stimuli and signaling pathways that either subsequently alter the activity of Bax and Bak or directly bind to and activate Bax and Bak.44 The second is the multidomain proapoptotic effectors Bax and Bak that contain BH1–BH3 domains along with a transmembrane domain.43 The Bcl-2 antiapoptotic family members, such as Bcl-2, Bcl-xL (BCL-2–related gene, long isosform), MCL-1 (myeloid cell leukemia 1), and others, contain 4 BH domains and all but 1 contains a transmembrane domain.43 The BH4 domain, which is largely exclusive to this subset of family members, is indispensable for their antiapoptotic activities.45–47 The interplay between all the Bcl-2 family members controls the equilibrium of apoptotic versus necrotic cell death in response to various stimuli.

In general, the prosurvival Bcl-2 subfamily members inhibit the activation of Bax and Bak, whereas the BH3-only subfamily members activate the Bax subfamily members. The Bcl-2 subfamily members inhibit Bax and Bak by either direct binding that results in their sequestration or by binding to and sequestering specific activators, such as the BH3-only effectors.15,48 As an example, Bid binds Bax to activate it but at the same time can bind and nullify Bcl-2 resulting in even greater Bax activity.49,50 A simplified model for the Bcl-2 proteins is that of a rheostat, with all of the prosurvival Bcl-2 subfamily members affecting the cell death equilibrium by sequestering Bax/Bak activity versus the BH3-only subfamily members that would otherwise free Bax/Bak to initiate MOMP and cell death.51

Regulated Necrosis

Historically, necrosis has been viewed as an uncontrollable form of cell death with default status, and there are certainly examples of stimuli that result in uncontrolled and unregulated necrotic cell death, such as with freeze–thaw injury. However, more recent studies have shown that under certain cellular contexts necrosis can be a highly regulated form of cell death in adult vertebrate organisms. Regulated necrosis is simply defined as caspase-independent cell death that has all the morphological hallmarks of classical necrosis but that can be inhibited or accelerated by affecting at least 1 key molecular components. As stated previously, the first identified molecular effector of regulated necrosis was CypD, which was shown to control the opening probability of the MPTP, such that inhibitors of this opening preserved cells after ischemic injury.52 In addition to

Figure 1. The B-cell leukemia/lymphoma (Bcl-2) family members and their domains. Schematic showing the 25 human Bcl-2 family members separated by their anti versus proapoptotic activities and the domains they contain. Each family member is characterized by containing at least 1 Bcl-2 homology (BH) domain. Over half of the family members contain a transmembrane (TM) domain which allows for the insertion into various organelles. Unique to Bcl-rambo is an additional domain (BHNo). Bcl2L12 contains a unique domain with 1 proline-rich (PR) region and 6 PxxP motifs. Bak indicates Bcl-2 homologues antagonist/killer; and Bax, Bcl-2–associated protein x.
ischemic injuries, inhibition of the MPTP and regulated necrosis has also been shown to mitigate muscular dystrophy, neurological degenerative disorders, and liver toxicity models.53–57

Another type of regulated necrosis is one in which cell death is induced by caspase inhibition in conjunction with apoptotic death ligand stimulation, which is referred to as necroptosis. This type of necrosis is mediated by apoptotic death receptors and the receptor-interacting protein kinases 1 and 3 (RIP1 and RIP3).58 This form of necrosis has been implicated in ischemic injuries, pancreatitis, and viral infections. An additional form of regulated necrosis involves the hyperactivation of poly (ADP-ribose) polymerase (PARP) induced by DNA damage, which was first suggested as a mechanism whereby cancer cells are killed with DNA alkylating agents.59

Taken together, these 3 independently defined forms of regulated necrosis provide strong evidence that necrosis can be a controlled form of cell death that is not purely accidental as once thought. In addition, present studies have showed that these 3 independent forms of necrosis may be more integrated than first thought because cells lacking Bax and Bak are resistant to all 3 forms of necrosis.60,61

MPTP-Dependent Necrosis

The MPTP forms within the inner membrane of the mitochondrion where it permits diffusion of molecules ≤1.5 kDa.62 MPTP opening, or something similar to it, has been shown to occur in yeast, plants, fish, amphibians, and mammals.63 The necrosis that is most prevalent in ischemic injuries is dependent on the opening of the MPTP and is because of calcium overload in the cell along with ROS production.64 MPTP opening with ischemic injury results in the diminution of cellular energy production and loss of ATP levels that secondarily prevents caspase activation and induction of apoptosis despite mitochondrial rupture and the accumulation of apoptogenic substrates like cyt c.65

The classical model of the MPTP was that of 1 contiguous pore spanning the outer and inner mitochondrial membranes, consisting of the voltage-dependent anion channel (outer membrane) and adenine nucleotide translocator (inner membrane), regulated by CypD within the mitochondrial matrix.66 Unfortunately, this model has not held up to genetic scrutiny because deletion of the gene encoding the adenine nucleotide translocator and voltage-dependent anion channel isoforms in mice or mammalian cells showed that neither were essential components of the pore, mitochondrial swelling, or necrotic cell death.67,68 However, deletion of the gene encoding CypD (Ppif) did reveal a role for this protein in regulating the opening of the MPTP, making it the first genetically defined component of this regulated necrotic process.69,70 More recently, evidence has emerged suggesting that the mitochondrial F, F, ATP synthase serves as the core component of the MPTP within the inner membrane, such that purified components of the F, F, ATP synthase reconstituted into lipid bilayers could recapitulate pore activity similar to that of the MPTP (Figure 2).65,71

Although the components that constitute the MPTP are still debated today, several older publications in the literature from the late 1990s and early 2000s showed that Bcl-2 family members could directly interact with either voltage-dependent anion channel or adenine nucleotide translocator,67,68 implying, at that time, that apoptotic regulators might affect necrotic cell death through the MPTP. Although we now know that adenine nucleotide translocator and voltage-dependent anion channel are not direct components of the MPTP, these previous observations might still hold biologically relevant insights that indeed suggest an intersection between apoptosis and necrosis through Bcl-2 family members and the MPTP. For example, Shimizu et al72 demonstrated that Bcl-2 overexpression could prevent MPTP-dependent mitochondrial swelling, and Marzoal70 showed that Bax was required to permit MPTP formation and the permeabilization of the mitochondrial membrane. Similarly, Bax overexpression in Jurkat cells resulted in cell death that was inhibited by CsA but not by caspase inhibitors.75 Finally, Bax can induce mitochondrial swelling in the presence of calcium.73 One opposing study concluding that Bax was not required for MPTP opening showed that mitochondria isolated from Bax−/− HTC116 cell lines were as susceptible to CypD-dependent mitochondrial swelling as wild-type mitochondria.74 Furthermore, mitochondria isolated from Bax and Bak1 (encodes Bak protein) null baby mouse kidney cells were also shown to undergo mitochondrial swelling in response to calcium.75 Importantly, the caveat of this result is that the swelling shown in the null baby mouse kidney cells was not inhibited by CsA; therefore, it can be argued that this was not MPTP-dependent mitochondrial swelling.74 The overall hypothesis from the older literature is that the Bcl-2 family member of proteins, including Bax and Bak, seems to regulate the MPTP.

Figure 2. Mitochondrial permeability transition pore (MPTP)-dependent necrotic pathway. When a cell receives a stress that leads to increased levels of intracellular calcium, the mitochondrial calcium uniporter (MCU) takes up the calcium into the matrix of the mitochondria where it can trigger MPTP opening through cyclophilin D (CypD). The MPTP is thought to be composed of the F, F, ATP synthase regulated by ANT and the mitochondrial phosphate carrier (PIC). On prolonged opening of the MPTP, there is an osmotic alteration and mitochondrial swelling and dysfunction occur with loss of ATP production and reactive oxygen species (ROS) generation. MPTP-dependent mitochondrial dysfunction requires the presence of Bax or Bak on the outer mitochondrial membrane. Therefore, proteins that affect the content of Bax/Bak on the outer mitochondrial membrane, such as the prosurvival the Bcl-2 family members, can secondarily affect MPTP-dependent mitochondrial dysfunction. ANT indicates adenine nucleotide translocator; BH, Bcl-2 homology; IMM, inner mitochondrial membrane; IMS, intramitochondrial membrane space; and OMM, outer mitochondrial membrane.
Recently, our laboratory and others have shown that isolated mitochondria from mouse embryonic fibroblasts, hepatocytes, and cardiomyocytes lacking Bax and Bak (DKO, double knockout) do not undergo MPTP-dependent swelling and that they have an increased calcium-holding capacity compared with wild-type mitochondria.\(^{39,75}\) However, in the absence of Bax and Bak, the inner membrane portion of the MPTP was still functional and able to induce opening that resulted in loss of membrane potential.\(^{39}\) It was simply that in the absence of Bax and Bak the outer mitochondrial membrane remained intact, thus allowing the mitochondria to persist without rupture. Importantly, this requirement of Bax and Bak to permit outer membrane rupture was independent of their activation and oligomerization that normally underlies MOMP and apoptosis because oligomeric dead mutant versions of Bax restored necrotic killing in the DKO mouse embryonic fibroblasts.\(^{39}\) Indeed, the inactive/passive state of Bax and Bak permitted necrosis by simply changing the physical properties of the outer mitochondrial membrane and making it more permeable and amenable to rupturing after inner mitochondrial membrane opening of the MPTP.\(^{96,76}\) Hence, a small increase in permeability created by inactive/passive Bax/Bak as they reside in the outer mitochondrial membrane was required for mitochondrial rupture post MPTP opening to ultimately mediate necrosis (Figure 2).\(^{39}\) Other notable findings were that the activity of Bax and Bak in the outer mitochondrial membrane were not functionally coupled to the inner membrane, suggesting that the MPTP is not a coordinated activity that directly spans the outer and inner mitochondrial membranes simultaneously.\(^{39}\) Finally, Whelan et al.\(^{75}\) showed that Bax may also function in necrotic cell death by supporting or driving fusion of mitochondria.

**Necroptosis**

Necroptosis was originally identified as a type of cell death that occurs when 1 of the 7 different apoptotic death receptors (extrinsic pathway) was stimulated with ligand, and at the same time caspases were inhibited with pharmacological agents. For example, tumor necrosis factor-\(\alpha\)–induced cell death in L929 cells was dramatically enhanced when caspase inhibitors were used, producing a morphological type of necrotic cell death.\(^{77}\) Although necroptotic cell death might seem to be fairly arbitrary and of unknown physiological relevance, subsequent work in gene-targeted mice lacking select caspase encoding genes or other genetic components of the extrinsic apoptotic death receptor have suggested a role for this type of cell death in vivo.\(^{78-80}\) The RIP1 and RIP3 kinases are part of a complex that is initiated in conjunction with death receptor stimulation where they then facilitate the necroptotic killing of cells (Figure 3). Indeed, cells lacking RIP3 protein are protected against cell death after viral infection that might normally cause necroptosis, whereas gene-targeted mice lacking RIP3 protein show reduced survival compared with wild-type mice after viral infection,\(^{81}\) but are protected from cerulein-induced necrosis of the pancreas.\(^{82}\) RIP-mediated necrosis has also been implicated in killing cells during ischemic injuries across multiple tissues because the use of a RIP1 inhibitor, such as necrostatin-1,\(^{83}\) is protective suggesting that this kinase might also be involved in other forms of regulated necrosis.\(^{84-86}\)

During necroptosis, death receptor stimulation results in caspase 8 activation where it then cleaves and inactivates RIP1 (Figure 3).\(^{87}\) It is only in the context of death receptor stimulation and caspase inactivation that RIP1 has prodeath activities in vivo. Indeed, when caspase 8 is inhibited or deleted in mice, this cleavage event does not occur and RIP1 is able to interact with RIP3 to initiate cell death, which is prevented in cells lacking RIP3 protein.\(^{81,88-90}\) These studies also determined that necrostatin-1 blocks necroptosis by inhibiting the interaction between RIP1 and RIP3.\(^{81,89}\) Finally, directly downstream of the RIPs is mixed lineage kinase like, a pseudokinase that when phosphorylated directly induces cellular necrosis, and although the mechanism of killing remains under investigation, this protein has recently been shown to directly generate pores in membranes that could directly mediate cell death.\(^{90}\)

Indeed, cells and mice lacking mixed lineage kinase like are resistant to necroptosis.\(^{91,92}\) Mixed lineage kinase like has also been shown to translocate to the plasma membrane where it induces calcium entry through interaction with transient receptor potential melastatin 7 during a necroptotic stimuli, suggesting another potential mechanism for cell death through enhanced calcium entry.\(^{93}\)

Given that necroptosis was originally thought to be a highly unique form of cell death, in conjunction with a more recent report demonstrating that mitochondria are supposedly dispensable for necroptotic killing,\(^{94}\) it came as a surprise when 2 groups independently showed that Bax and Bak are required for necroptotic cell death.\(^{88,95}\) However, it is still not clear why Bax- and Bak-deficient cells are resistant to necroptosis and to what extent mitochondria are involved as a mechanism for necrotic killing (see below).

The MPTP has been implicated in necroptosis because Ppif (CypD encoding gene) null cells are partially resistant to the treatment of tumor necrosis factor \(\alpha\) with caspase inhibitors.\(^{82}\) In contrast, necroptosis has been shown to be CypD-independent in vivo as the lethality normally observed in

![Figure 3. Necroptotic pathway.](http://circres.ahajournals.org/)

The combinatorial treatment with an apoptotic death receptor ligand and a caspase inhibitor leads to necroptosis with receptor-interacting protein kinase 1 (RIP1) activation. Without the caspase inhibitor present, caspase 8 would normally cleave and inactivate RIP1. When RIP1 is left unchecked in the presence of a caspase inhibitor, it complexes with RIP3 and together they lead to the phosphorylation and activation of mixed lineage kinase like (MLKL). MLKL is a required protein for necroptosis.
Casp8 null mice that is completely rescued with Ripk3 deletion (encodes the RIP3 protein) is not rescued with Ppif deletion. This suggests that the type of cell death that occurs in Casp8 null mice leads to their embryonic lethality does not use the MPTP type of regulated necrosis. Moreover, Ppif null mice subjected to kidney ischemia showed increased protection when also given necrostatins, suggesting that these 2 types of cell death are separate pathways. However, in the heart necrostatins provided no additional protection in Ppif null animals during cardiac ischemia–reperfusion injury, suggesting that they could be within the same linear pathway. One point that must be remembered here is that cells lacking CypD protein are still susceptible to apoptosis, and that in vivo, unlike in vitro with pan caspase inhibitors, additional caspases are still active in Casp8 null mice and hence could mediate an apoptotic pathway to effectively kill Ppif null cells.

PARP-Dependent Necrosis

PARP-mediated necrosis was first discovered through the observation that DNA alkylation agents induce efficient necrotic cell death in cancer cells. The overactivation of PARP by DNA damage results in the depletion of the NAD+ pool and the inhibition of glycolysis, which more specifically sensitizes cancer cells to death given their almost exclusive reliance on glycolysis for ATP production (Figure 4). However, cells relying on oxidative phosphorylation and not glycolysis were resistant to NAD+ depletion and death with DNA alkylation agents and PARP overactivation, suggesting that maintenance of mitochondrial function could be a protective variable to this type of necrosis. Another way, NAD+ depletion affects cell viability is by inhibition of the sirtuin family. Sirtuins are NAD+-dependent deacetylases that have been shown to play a role in cell viability and depending on the injury and cell type, sirtuin inhibition can be either maladaptive or protective. As discussed earlier, Bax/Bak DKO cells are also resistant to DNA alkylation agent–induced necrotic cell death. An attractive hypothesis is that such resistance is because of a greater level of mitochondrial protection that is observed in the absence of Bax and Bak, which preserves the ATP status of the cell more effectively with PARP overactivation. Notably, when Bax/Bak DKO cells were forced to use glycolysis for energy, they became susceptible to DNA alkylation–induced necrosis. In addition to showing that Bax is required for DNA alkylation–induced necrosis by MNNG (N-methyl-N′-nitro-N-nitrosoguanidine), Bcl-2 overexpression protected against this form of necrosis. PARP-mediated necrosis has also been implicated in...
ischemic injuries in various tissues because PARP inhibition or genetic deletion is protective.\textsuperscript{101–103}

Unified Model of Regulated Necrosis

Although the 3 types of necrosis discussed above were identified independently, it is possible that each represents part of an inter-related process. One possible integration point is the mitochondria where each form of cell death seems to require the collapse of the mitochondria at some level. This is somewhat the opposite of apoptosis as mitochondrial function is required for the maintenance of ATP generation so that cell death can occur in an orderly process to package cellular contents for removal without tissue inflammation. However, during regulated necrosis, the mitochondria swell, lose their ability to generate ATP, and subsequently rupture. As discussed throughout this review, Bax/Bak underlie both processes because DKO cells are resistant to necrotic and apoptotic cell death, and hence are likely a central integration point for potentially all forms of regulated cell death (Figure 5).

Another consideration for a unified model of regulated necrosis is that separate inhibition of PARP-mediated necrosis, necroptosis, or MPTP-dependent necrosis seems to protect tissues from cell death after ischemic injuries in vivo. Increased ROS production and calcium overload underlie ischemic injury-induced cell death in vivo, each of which impacts the mitochondria and causes MPTP opening. Indeed, the RIP1 inhibitor necrostatin-1 can delay MPTP opening in rat cardiomyocytes exposed to oxidative stress, suggesting a role for RIPS with necrotic inducers other than just death ligands and caspase inhibitors.\textsuperscript{104} Also, as stated earlier, there is no additive effect of necrostatin-1 treatment in Ppif null mice during heart ischemia–reperfusion injury.\textsuperscript{89} In addition, Ppif null mouse embryonic fibroblasts are partially resistant to RIP-mediated necrosis.\textsuperscript{89} These results suggest that RIP-mediated necrosis can function upstream of MPTP-mediated necrosis. However, other data whereby mitochondria seem dispensable for necroptosis\textsuperscript{4} might suggest that 2 distinct pathways underlie regulated necrosis, such as an extrinsic necrosis induced by death ligands and caspase inhibitors versus an intrinsic necrosis induced by calcium and ROS overload that induces MPTP opening.

ROS can also lead to DNA damage that can trigger PARP activation.\textsuperscript{105} However, for PARP activation to be deleterious and induce a form of regulated necrosis, mitochondrial ATP production has to cease.\textsuperscript{59} If mitochondrial dysfunction does not occur, the cell is able to maintain energy demands associated with PARP activation to facilitate DNA repair. This places PARP-dependent necrosis downstream of the MPTP-dependent mitochondrial dysfunction. Moreover, maintenance of mitochondrial integrity and ATP production associated with deletion of Bax/Bak protein reduces or inhibits all forms of regulated necrosis discussed above (Figure 5).

Regulated necrosis and apoptosis were previously considered as 2 separate forms of cell death controlled by unique pathways, although recent work has shown that the Bcl-2 family serves as a functional convergence point between both. The defining moment between apoptosis and necrosis seems to be at the level of the mitochondria and if MOMP occurs to drive apoptosis or if only the MPTP opens to drive a form of regulated necrosis, although both events clearly require Bax and Bak and are influenced by the activity of other Bcl-2 family members. In addition to mitochondrial permeability, Bcl-2 family members also regulate endoplasmic reticulum calcium levels, which can further affect the mitochondria and MPTP formation and necrosis through direct communication between these 2 organelles and the ultimate content of calcium within the mitochondria. Finally, Bax can also regulate lysosomal permeability adding even further levels of regulation of the necrotic pathway by the Bcl-2 family.\textsuperscript{42}

Future Directions

One important question that needs to be addressed in moving forward is if regulated necrosis underlies cellular attrition outside of injury events, such as in response to development or physiological stimuli. Although this clearly seems to be the case for necroptosis, which mediates select forms of cell death in vivo, it is less clear if the MPTP-mediated mechanism of cellular necrosis might also occur outside of ischemic injury in vivo. Another issue is defining exactly how necroptosis results in cell death because the ultimate effectors that bring about cell death downstream of death receptor activation with caspase inhibition remain mechanistically illusive. Although there are data that mitochondria are completely dispensable for necrototic cell death,\textsuperscript{94} we and others have observed a requisite role for the MPTP and Bax/Bak as potential downstream effectors of necroptosis. Finally, it will be important to continue to discover other mechanistic effectors of regulated necrotic cell killing because this form of cell death seems to be the primary way that cells die in adult vertebrate organisms after an injury event or ischemic damage, as well as in response to long-term degenerative disorders, which together underlies most forms of disease with aging in adult humans.

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Disclosures

None.

References


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