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Programmed cell elimination is an important pathological mediator of disease. Multiple pathways to programmed cell death have been delineated, including apoptosis, autophagy and programmed necrosis. Cross-talk between the signaling pathways mediating each process has made it difficult to define specific mechanisms of in vivo programmed cell death. For this reason, many "apoptotic" diseases may involve other death signaling pathways. Recent advances in genetic complementation using mouse knockout models are helping to dissect apoptotic and necrotic cell death in different pathological states. The current state of research in this area is reviewed, focusing upon new findings describing the role of programmed necrosis induced by the mitochondrial permeability transition in mouse models of heart failure and diabetes.

Introduction

Like people, cells are born, they live for a time and (with a few notable exceptions) they die. Indeed, cell death is an essential part of tissue renewal. The linkage between cell death and birth is bi-directional. An unexpected loss of cells accelerates growth of similar cells in a healing process that is essential for functional homeostasis, whereas the need to replace senescent cells with new cells requires programmed elimination of older, damaged or less functional tissue. An appreciation of these two pathophysiological contexts led to the classical paradigm of cell death being either mandated or elective. Mandated cell death (often referred to as "necrosis") is typically the consequence of trauma or tissue damage, and has therefore been anthropomorphically labeled cell murder; like murder, mandated cell death is not tidy. The cellular response to lethal injury involves cell swelling, loss of membrane integrity and release of chemical mediators that can cause collateral damage. From the victim's (cell/tissue/organ) perspective, this form of cell death is random and therefore unplanned. For the organism there is a crime scene to clean and cellular corpses to remove. Critical functions normally performed by the murdered cells are interrupted by their absence, and can be further disrupted by first responders (i.e., inflammation).

By contrast, the conventional view is that elective cell death is much cleaner for all concerned. This is certainly the case for apoptotic cell death, as originally conceived by Vogt in the 19th century and more strictly defined by Kerr, Wyllie and Curie in 1972.[1] The best anthropomorphism for apoptosis is probably "assisted cell suicide" as there is extensive molecular and cellular infrastructure that supports this form of elective cell termination. Apoptotic cell death is the deliberate result of processes initiated in response to developmental cues or external factors that are, themselves, non-lethal. In either case, the cell decides to remove itself, packs up its constituent proteins, organelles and DNA for easy removal and essentially "turns off and fades away". As a consequence of this planning and effort, the untoward collateral effects associated with mandated cell death are largely avoided.

Elective cell death is essential for normal tissue development and homeostasis, and is therefore tightly regulated. Apoptotic cell death is an evolutionarily conserved process resulting from cascade activation of caspase cysteine proteases downstream of cytokine death receptors (the extrinsic apoptosis pathway) or transcriptionally and post-translationally regulated mitochondrial-localized Bcl2 family members (the intrinsic apoptosis pathway; reviewed in ref. 2). Dysregulation of programmed cell death perturbs normal tissue homeostasis and is implicated in cancer (too little apoptosis), Alzheimer disease (inappropriate apoptosis) and other chronic degenerative conditions.[3-5]

In recent years, this comfortable and straightforward paradigm of mandated versus elective cell death has been complicated by the recognition of multiple apoptotic and non-apoptotic pathways for programmed cell elimination. In particular, it is clear that cell death also occurs via a phenomenon of "programmed necrosis", which is elective like apoptosis, but untidy like necrosis. To extend the previous anthropmorphisms, programmed necrosis is neither cell murder, nor assisted cell suicide, but is more like the cell figuratively throwing itself in front of an oncoming bus. The result is elective cell elimination in a manner that is functionally disruptive and activates inflammatory mechanisms. The molecular and biochemical pathways that regulate, initiate and mediate programmed cell necrosis are only now being defined, and it likely to be mediated by multiple non-apoptotic mechanisms leading to abrupt metabolic shut-down. It is ironic that John Foxton Ross Kerr, the father of modern apoptosis, originally used the term "programmed cell necrosis" to describe his concept

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of natural cell death. The various terms currently being used to describe non-apoptotic elective cell death (programmed necrosis, regulated necrosis, necroptosis) continue to be confusing as none are mechanistically based.[6,7]

Apoptosis and programmed necrosis are mediated by distinct signaling pathways, but the two processes can be difficult to differentiate in vivo. There is no specific histochemical marker for programmed necrosis. Dystrophic calcification and complement activation have been used to identify necrotic tissue, but these markers do not differentiate between standard and programmed tissue necrosis. On the other hand, conventional markers for apoptosis (cytochrome *c* release, activate caspases and TUNEL labeling) do not exclude programmed necrosis because mitochondrial swelling and outer membrane rupture that are the primary cause of programmed necrosis secondarily release cytochrome *c* into the cytosol. Although this activates apoptosis signaling, reversal of ATP production in cells undergoing necrosis prevents the orderly (and energy-requiring) dismantling of the cellular machinery, and so they don't die of apoptosis. Concomitant activation of apoptotic markers in cells undergoing programmed necrosis seems likely to have implicated apoptosis in diseases where programmed necrosis is a primary mechanism of elective cell elimination.

Here, new findings linking programmed necrosis mediated via the mitochondrial permeability transition with diabetes and heart failure are explored. The striking benefits that accrued from genetic interruption of programmed necrosis in mouse models of these two human conditions should prompt a re-evaluation of widely held notions that apoptotic cell death is the major effector of pathological cell loss.

Pancreatic β-cell Deficiency in Diabetes; Apoptosis or Programmed Necrosis?

A simple operational definition of diabetes is: any condition in which endogenous insulin production and secretion are insufficient to meet metabolic demands. Mechanistically, this includes conditions where there is an absolute paucity of insulin in individuals of normal body mass (type 1 diabetes),[8] or where there is an abundance of insulin in individuals whose need is still greater, due to increased body mass and/or insulin resistance (type 2 diabetes).[9,10] Insulin is produced exclusively by β-cells located within the pancreatic Islets of Langerhans, and these cells have the ability to undergo hyperplasia in response to metabolic stress.[11] Both forms of diabetes are causally linked to (absolute or relative) β-cell deficiency,[12] and in many instances attributed to increased β-cell apoptosis.[13-15] However, targeting β-cell apoptosis has either been incompletely successful in preventing diabetes,[16] or has unmasked latent oncogenic potential.[17] These results point to a parallel programmed death pathway in pancreatic β-cells.[18]

We recently used genetic complementation in a mouse diabetes model to interrogate the role of programmed β-cell necrosis caused by opening of the mitochondrial permeability transition pore (MPTP). The MPTP is a non-selective pore in the mitochondrial inner membrane that opens in response to increased local calcium concentration.[19] Proton diffusion through the MPTP dissipates the normal mitochondrial pH gradient and inner membrane electrochemical potential ($\Delta\psi_m$) that are required for ATP production through oxidative phosphorylation.[20] MPTP opening therefore reverses ATP synthesis, and the mitochondrion becomes a net ATP consumer. The loss of cellular ATP subsequent to MPTP opening ignites a cascade of metabolic dysfunction that can ultimately lead to cell necrosis.

Another consequence of MPTP opening is osmotic mitochondrial swelling that disrupts outer mitochondrial membranes, permitting cytochrome *c* to be released into the cytosol from the mitochondrial inter-membranous space. Although MPTP-dependent cytochrome *c* release can activate the apoptotic caspase cascade, cell death by apoptosis requires ATP that is critically deficient after the mitochondrial permeability transition. Thus, cell death subsequent to the mitochondrial permeability transition proceeds via metabolic shutdown and programmed necrosis, rather than apoptosis. Because cytochrome *c* release and its sequelae occur in both apoptosis and MPTP-dependent programmed cell necrosis, they do not reliably indicate one or the other cell death pathway.

The structural components of the MPTP are not completely clear.[21,22] Core MPTP elements consist of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT) and cyclophilin D (CyP-D). The VDAC was long considered to be an essential component of the MPTP,[23-25] but genetic models have convincingly refuted this notion; the VDAC is dispensable for both Bcl2 family member-mediated apoptosis and MPTP-dependent necrosis.[26] Likewise, gene ablation studies in mice showed that mitochondria lacking ANT can undergo permeability transition.[27] ANTs may function as phosphate- and calcium-sensitive MPTP regulators. In contrast, three independent groups have reported that genetic ablation of CyP-D is sufficient to eliminate the mitochondrial permeability transition without affecting conventional mitochondrial-mediated apoptosis.[6,28,29] For this reason, genetic *Ppif* ablation has been used to demonstrate the role of MPTP opening and programmed cell necrosis in mouse models of Alzheimer's disease, muscular dystrophy, diabetes mellitus and heart failure.[30-33]

Our studies of MPTP-mediated programmed β-cell death in diabetes used a genetic mouse model, the *Pdx1* hemizygous mouse.[32] PDX1 (pancreatic and duodenal homeobox factor 1; also called insulin promoter factor 1, IPF1) is a necessary transcription factor for pancreas development. Loss of function human *Pdx1* mutations are linked with pancreatic agenesis, heritable maturity onset diabetes of the young and type 2 diabetes.[34-36] Likewise, germ-line homozygous *Pdx1* gene ablation in mice produces lethal pancreatic agenesis,[37] whereas heterozygous germ-line or β-cell specific *Pdx1* null mutations result in a diabetes phenotype.[38,39] Several cellular mechanisms have been proposed for loss of insulin-producing pancreatic β-cells in the Pdx1 deficient model of diabetes, including decreased proliferation,[40] and increased programmed cell death through apoptosis and autophagy.[13,41,42] The prevailing opinion has been that increased β-cell death, and not decreased proliferation, is the more important mechanism, with apoptosis most often implicated.[14] As indicated above however, the standard markers of apoptosis do

not discriminate between apoptosis and programmed necrosis. Therefore, it was possible that programmed β-cell necrosis in *Pdx1* insufficiency had been mis-identified as apoptosis based on findings of cytochrome *c* release and β-cell TUNEL positivity. We examined this possibility in cultured mouse insulinoma-derived β-cells (MIN6 cells), using shRNA to suppress Pdx1 expression, TUNEL as a measure of apoptosis signaling, rhodamine 123 to measure integrity of the MPTP and propidium iodide to assess cell death in a pathway-independent manner.[32] As expected, Pdx1 suppression increased TUNEL positivity and β-cell death. Importantly, it also opened β-cell MPTPs, which is not a direct effect of apoptosis. To determine if MPTP opening was a primary or secondary effect of Pdx1 suppression, we treated the cells with cyclosporin A, a pharmacological inhibitor of CyP-D (the essential protein for MPTP opening). Cyclosporin A prevented MPTP opening and significantly reduced Pdx1-deficient β-cell TUNEL positivity and death. This result suggested that MPTP opening could be responsible for as much as half of cultured β-cell death induced by Pdx1 deficiency.

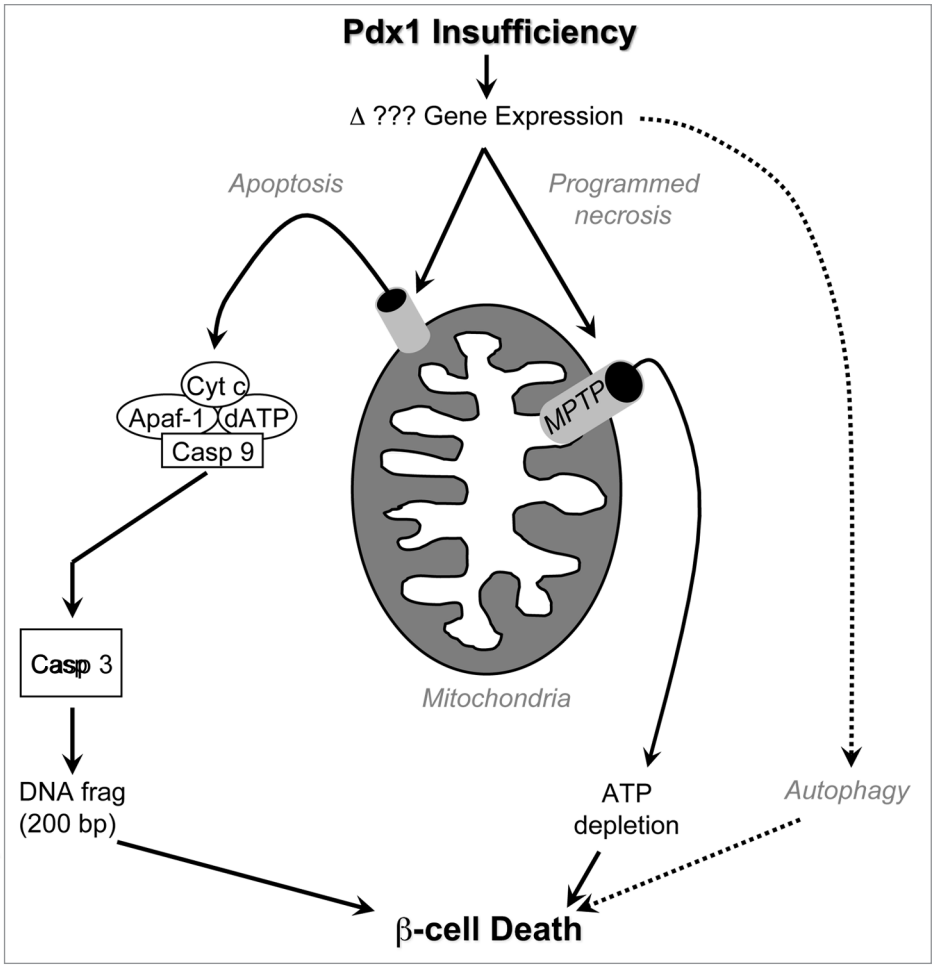

Figure 1. Schematic depiction of programmed β-cell death pathways implicated in diabetes caused by Pdx1 haplo-insufficiency. Deficiency of transcription factor Pdx1 likely alters gene expression, activating pathways that induce apoptosis via mitochondrial outer membrane permeabilization (left) and programmed necrosis via the mitochondrial permeability transition (right). Pancreatic β-cell autophagy is also induced by Pdx1 insufficiency, but its role in programmed cell death is currently unclear.

We used genetic complementation with *ppif* null mice (the *ppif* gene encodes CyP-D) to assess the role of MPTP-induced β-cell necrosis in murine diabetes caused by *Pdx1* haplo-insufficiency. Pancreatic islets of *Pdx1* haplo-insufficient mice are characteristically small, with strikingly reduced numbers of insulin-containing β-cells. Eliminating functional MPTPs through *ppif* ablation increased islet size and decreased TUNEL labeling. We also stained the pancreata for complement 9, a component of the complement membrane attack complex that is part of the inflammatory response to cell necrosis. Complement 9 staining was increased in *Pdx1* haplo-insufficient islets, but was normalized by concomitant *ppif* ablation. This histological "rescue" was observed in both adult and neonatal pancreata, revealing a role for MPTP-mediated programmed β-cell necrosis during embryonic development and for β-cell homeostasis in the adult pancreas.

Finally, we determined the consequences of preventing MPTP-mediated programmed β-cell necrosis on the diabetic phenotype of adult *Pdx1* deficient mice. Diabetes in this model is manifested by decreased circulating insulin and increased blood glucose levels measured either at baseline or in response to an acute glucose challenge. Ablation of *ppif* in Pdx1 haplo-insufficient mice almost completely normalized both of these parameters. Together, these studies implicate MPTP-mediated β-cell necrosis as an important component of pathological programmed β-cell death caused by Pdx1 deficiency, and support a co-equal role for conventional apoptosis through the mitochondrial pathway (**Fig. 1**). Prevention of diabetes with only partial normalization of β-cell mass in *Pdx1*-deficient mice lacking functional MPTP likely reflects physiological pancreatic insulin reserve.

Programmed Cardiac Myocyte Death in Heart Failure

Because adult cardiac myocytes are terminally differentiated and replication-deficient, normal tissue homeostatic mechanisms do not apply. Although these cells proliferate during cardiac development, shortly after birth the rate of cell division drops to levels that are difficult to quantify, but are certainly inadequate for meaningful tissue regeneration. Because there is no cardiac myocyte replacement, there is no normal level of programmed cardiac myocyte death. Even after tissue injury or under conditions of

non-lethal stress, programmed cardiac myocyte elimination is pathological. Nevertheless, apoptosis is observed in cardiac myocytes of failing, ischemic and pressure overloaded hearts, and is implicated in progression to cardiomyopathy.[43] Indeed, a genetic program for programmed cell death is one of the hallmarks of the "compensatory" hypertrophic reaction to cardiac overload.[44,45]

Using microarray-based RNA profiling of Gq transgenic mice, our laboratory first described a genetic program for cardiac myocyte death in hypertrophied hearts. Gαq (the alpha sub-unit of the heterotrimeric Gq signaling protein) transduces signaling through cardiac myocyte hypertrophy pathways stimulated by angiotensin II, α1-adrenergic and endothelin receptors. The Gq mouse expresses four to five times the normal amount of myocardial Gαq, resulting in ligand-independent Gq signaling and autonomous cardiac hypertrophy with many of the pathological characteristics of pressure overload hypertrophy (but without pressure overload).[46] Shortly thereafter we further described progression to dilated cardiomyopathy mediated by Gαq-stimulated cardiomyocyte apoptosis,[47,48] suggesting a link between cardiomyocyte hypertrophic growth and programmed death. Specifics of this linkage were established by transcriptional profiling, which identified increased levels of several death pathway genes in Gq hearts.[49,50] One of these death genes encoded Nix (also called BNip3L), a member of the small BH3-like only subclass of mitochondrial-targeted Bcl2 apoptosis regulating proteins.

Because they are the sites of action for Nix and other Bcl2 family proteins, mitochondria have been called the "gatekeepers" of programmed cell death.[51] Bax and Bak are the essential pore-forming proteins that permeabilize mitochondrial outer membranes, leading to cytochrome *c* release and intrinsic apoptosis signaling. Pore-formation by Bax and Bak is facilitated by pro-apoptotic BH3 domain-only factors, including Nix/BNip3L that we identified in the heart. Nix and other BH3-only factors hetero-dimerize with anti-apoptotic Bcl2 and $Bclx_l$, which prevents mitochondrial pore formation by Bax and Bak.[2] Dynamic regulation of Nix, Bax, Bak and other pro- or anti-apoptotic Bcl2 family proteins is characteristic of heart failure (reviewed in ref. 52). A protein kinase C/SP1-dependent mechanism for transcriptional upregulation appears to be responsible for increased Nix gene expression in cardiac hypertrophy.[53]

We used cardiac transgenesis to determine the in vivo consequences of Nix upregulation in hearts, independent of Gq, cardiac hypertrophy or any hemodynamic stress or cardiac injury. Nix expression was directed specifically to cardiac myocytes using the *MYH6* promoter, which is transcriptionally activated only after birth. The result of post-natal cardiac-specific Nix expression was mice that were born normal, but succumbed to progressive dilated cardiomyopathy on the seventh to tenth day of life. TUNEL staining was positive in 15–20% of cardiac myocytes, implicating massive programmed cardiac myocyte death in this form of fulminant heart failure.[50] A subsequent study in which Nix was conditionally expressed in the heart revealed synergism between Nix and pressure overload stress for inducing apoptotic heart failure in adult hearts,[54] uncovering functional coordination by Nix of transcriptional and physiological stimuli for programmed cardiomyocyte elimination.

Based on the above results, we hypothesized that Nix (and the programmed cardiac myocyte death it produces) mediates progression to heart failure in pressure overload cardiac hypertrophy. To test this idea, we created cardiac-specific *Nix* gene knockout mice, subjected them to surgical pressure overload and compared cardiomyocyte TUNEL labeling, ventricular remodeling and cardiac function to identically pressure overloaded hearts of mice with intact *Nix* genes. Cardiac *Nix* knockout mice exhibited only half the typical rate of cardiomyocyte TUNEL positivity and myocardial fibrosis after pressure overloading and exhibited almost no ventricular dilation, wall thinning or decrease in contractile systolic function.[55] These data identified Nix as a critical inducible factor mediating programmed cardiac myocyte death in pressure overload hypertrophy and established a causal role for Nix in adverse ventricular remodeling and development of heart failure.

Delineation of Multiple Nix-Stimulated Cell Death Pathways

Like all pro-apoptotic Bcl2 family members, Nix stimulates caspase-dependent apoptosis resulting from mitochondrial outer membrane permeabilization. In cardiac myocytes and other cell types, Nix localizes to mitochondria and induces cytochrome *c* release that is followed by caspase activation and oligonucleosomal DNA degradation.[50] However, we recently observed that only ~80% of transfected Nix localizes to mitochondria; the remainder is localized to endoplasmic reticulum (ER) or the analogous structure in cardiac myocytes, sarcoplasmic reticulum (SR).[56] We further observed a direct relationship between cardiac myocyte Nix levels and cardiomyocyte SR calcium stores: Nix overexpression increased SR calcium content by ~20%, whereas *Nix* gene ablation decreased SR calcium content by ~20%. Although SR calcium release is a critical regulator of myocyte contraction, we postulated that SR-localized Nix did not meaningfully regulate cardiac contractility. Rather, we felt that SR calcium might play a role in Nix-mediated cell death. Indeed, restoration of normal SR calcium levels in *Nix* knockout mice by genetic complementation with the phospholamban null mouse reversed the cardio-protective effects observed with *Nix* ablation, increasing programmed cardiomyocyte death, exaggerating the cardiomyopathy and increasing mortality. These results linked SR calcium to programmed cardiac myocyte death and heart failure produced by Nix.

We considered that reticular-mitochondrial calcium cross-talk[57] stimulated by SR-localized Nix could induce the "necrotic" pathway to programmed cardiomyocyte death. If this were the case, cardiomyocyte "apoptosis" we and others have reported based on TUNEL staining might instead be a secondary effect of mitochondrial rupture after MPTP opening, as we had observed in pancreatic β-cells (see above). However, little was known about the role of calcium and MPTP opening in programmed cardiac myocyte death. While calcium is used to stimulate MPTP opening in laboratory studies using isolated mitochondria, it has been

suggested that the MPTP is relatively calcium insensitive in vivo due to stabilization by cytosolic factors.[58] Because dual Nix localization to mitochondria and reticulum confounds experimentation designed to identify calcium-stimulated non-apoptotic death pathways induced by reticular Nix, we created mitochondria-directed and ER/SR-directed Nix mutants and analyzed their effects after recombinant expression in *Nix* null embryonic fibroblasts.[33] Mitochondrial-directed Nix stimulated cell death marked by caspase activation, but normal $\Delta\psi_m$, i.e., *apoptosis*. In contrast, reticular-directed Nix stimulated cell death preceded by MPTP opening and dissipation of $\Delta\psi_m$, i.e., *necrosis*, but in which caspase activation attributed to cytochrome *c* release after MPTP opening and outer mitochondrial membrane rupture was also observed. Using the same experimental design as in our diabetes studies, we found that pharmacological (cyclosporin A) or genetic (CyP-D, *Ppif* ablation) inhibition of the MPTP prevented cell death induced by reticular Nix, but not by mitochondrial Nix.[33] Importantly, apoptosis induced by mitochondrial-directed Nix required Bax or Bak, whereas programmed cell necrosis induced by reticular-directed Nix showed no requirement for Bax and Bak. These results demonstrated that mitochondrial-directed Nix specifically activates conventional Bax/Bak-dependent mitochondrial pathway apoptosis, whereas reticular-directed Nix specifically induces MPTP-dependent programmed cell necrosis by increasing reticular calcium concentration and calcium delivery to mitochondria (**Fig. 2**).

To determine the relative contribution of Nix-mediated apoptosis versus programmed necrosis to in vivo heart failure we translated the above experimental design to the in vivo murine system and created cardiac-specific conditional transgenic mice expressing mitochondrial-directed or reticular-directed Nix mutants.[33] The in vivo dilated cardiomyopathy phenotypes and extent of cardiac myocyte TUNEL labeling (which does not differentiate between apoptosis and programmed necrosis) were similar in mitochondrial-directed and reticular-directed Nix mutant mice. However, cardiac myocyte necrosis visualized by anti-complement 9 staining was observed only in reticular-directed mutant Nix mice. Transmission electron microscopy likewise revealed mitochondrial swelling, matrix degeneration and outer membrane disruption only in cardiomyocytes from reticular-directed Nix mice. A causal link between MPTP opening in reticular-directed Nix-induced cardiac myocyte death was established by concomitant ablation of *ppif* (encoding CyP-D) in the Nix mutant cardiomyopathy models. *ppif* ablation prevented cardiomyocyte death (TUNEL labeling) and complement 9 staining in reticular-directed Nix expressing mice, normalizing mitochondrial ultrastructure and preventing the dilated cardiomyopathy. In contrast, *ppif* ablation did little to improve the apoptotic cardiomyopathy produced by mitochondrial-directed Nix. These findings show that an important aspect of Nix-mediated cell death is programmed necrosis mediated by SR-mitochondrial crosstalk. As other reports have implicated cardiac myocyte or sarcoplasmic reticular calcium levels in cardiac injury and heart failure progression,[59-61] MTPT opening stimulated by reticular-mitochondrial calcium cross-talk may play a greater role than previously suspected in hypertrophy decompensation and the progression to overt heart failure.

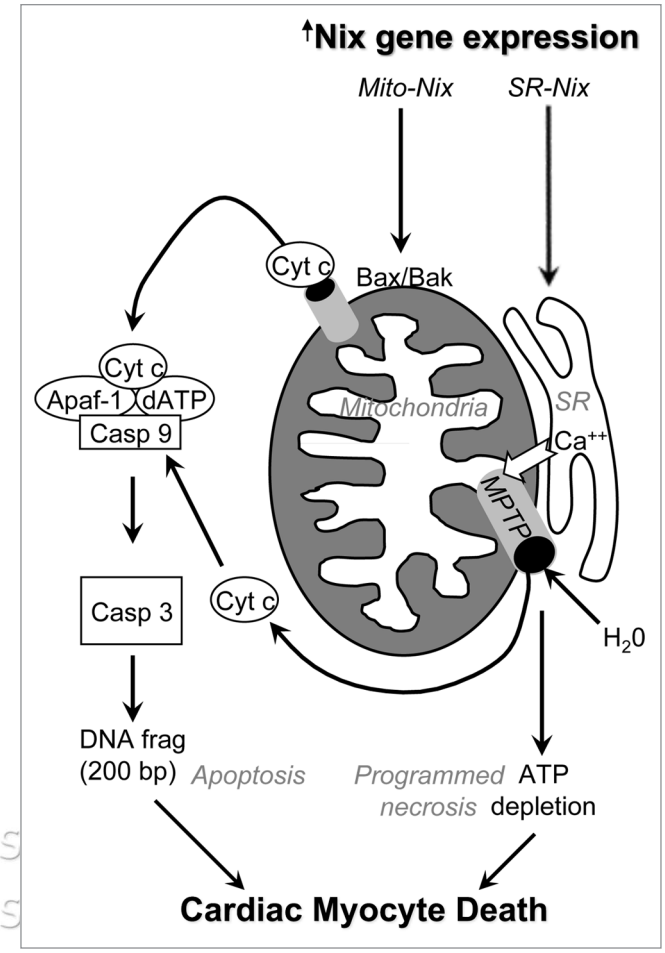

Figure 2. Schematic depiction of multiple Nix-induced pathways for cell death in heart failure. Transcriptionally upregulated Nix localizes either to mitochondria, where it interacts with Bax and Bak to induce apoptosis via outer membrane permeabilization, or to calcium-containing sarcoplasmic reticulum (SR), where it increases calcium stores and activates the mitochondrial permeability transition pore (MPTP) independent of Bax and Bak. Mitochondrial disruption subsequent to MPTP opening leads to metabolic shutdown and death via programmed necrosis.

Conclusions

It may be time to revise our perceptions of the role for apoptosis in chronic disease. In general, apoptosis seems to be a good thing. It is an essential feature of normal embryonic tissue development and adult tissue homeostasis. When normal apoptotic mechanisms are interrupted by natural or experimental mutation, the consequences of unopposed cell growth can be tumor or malignancy. Because the assays used to define apoptosis are not specific for that mechanism, "apoptosis" has been implicated and vilified in variety of chronic diseases associated with abnormal cell death. An increased appreciation that all programmed cell death is not apoptotic, and especially for the role of the mitochondrial

permeability transition and programmed necrosis, may reveal that non-apoptotic programmed cell death plays a major role in pathological programmed cell elimination. Chronic diseases in which MPTP-dependent programmed cell death has been implicated include Alzheimer's disease,[30] muscular dystrophy,[31] amyotrophic lateral sclerosis,[62] heart failure,[33] and diabetes.[32] It is likely that this list will expand further.

The dichotomy between apoptotic and programmed necrotic cell death is mechanistically useful, but is almost certainly blurred in the in vivo context. As noted previously, a cell may initiate the process for apoptosis via permeabilization of mitochondrial outer membranes by Bax and Bak, but if there is insufficient ATP to complete the process, the ultimate consequence may be necrotic death from metabolic shutdown, or even a state of severe, but non-lethal dysfunction that some have describes as a "zombie" cell. It is also likely that some mitochondria within a single cell will undergo outer membrane permeabilization (apoptosis), others will undergo the permeability transition (necrosis), and some fraction will continue to function normally for at least a while. Under these circumstances, the determination of whether the cell will die, and the mechanism for programmed cell elimination, would be the aggregate of all relevant external factors and intracellular conditions.

As our understanding of programmed cell death continues to evolve, the following aspects deserve attention: First, researchers and reviewers should bear in mind that conventional markers of apoptosis are not specific for apoptosis. Mechanistic implications from TUNEL labeling may be no more valid than those from propidium iodide staining. Both label dead or dying cells without unambiguously defining a particular mechanistic pathway. Even observation of apoptotic bodies, oligonucleosomal DNA cleavage, and nuclear chromatin condensation (each of which is widely considered pathognomonic for apoptosis) does not exclude concomitant activation of alternate pathways to programmed cell elimination. Complicating this issue, there is no satisfactory marker for programmed cell necrosis, although complement activation is more abundant with necrosis than apoptosis. Second, we need to determine if there is a physiological role for MPTP opening and programmed cell necrosis, or if it is a purely pathological process. Finally, we need to identify the proximal causes of cell death after MPTP opening. Certainly ATP depletion starts the process, but is there a role for mitochondrial release of reactive oxygen species and calcium in addition to metabolic shutdown in programmed cell necrosis? Further examination of these issues may suggest novel approaches to interdict programmed cell death in disease.

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