Apoptotic Mechanisms in Heart Failure

The key to understanding apoptosis is the activation and function of caspases, a group of cysteinyl-aspartate–directed proteases. In healthy cells, caspases reside in the cytosol as inactive proforms and are activated by proteolytic cleavage upon apoptosis. Two major apoptotic pathways (ie, pathways that eventually lead to activation of executioner caspases), the “extrinsic” and “intrinsic” cascades, transduce apoptotic signals in the heart muscle cell. The intrinsic pathway uses the endoplasmic reticulum and mitochondria to propel cell death through opening of the mitochondrial permeability transition pore or rupture of the outer mitochondrial membrane. This event will trigger the sudden and complete release of cytochrome c and other proteins from the intermembrane space of mitochondria into all other compartments of the cell, allowing activation of executioner caspases and subsequent proteolytic destruction of key cellular substances. The extrinsic apoptotic pathway entails the death-receptor pathway, triggered by members of the death-receptor superfamily, such as the Fas receptor or the tumor necrosis factor-α receptor, which, in turn, can activate executioner caspases.

Human failing hearts in New York Heart Association class III to IV typically display apoptotic myocyte rates ranging anywhere from 0.12% to 0.70%. If one considers that cellular apoptosis is a process that takes at most 24 hours to complete and that heart failure is a condition that only manifests itself after many years, it becomes imaginable that chronic loss of even such small numbers of the functional units of the heart (the myocytes) on a daily basis can have dramatic consequences on myocardial integrity. Moreover, the low death rate at a single point of measurement does not necessarily reflect the rate during episodes of active disease, especially at phases accompanied by (endocardial) regions with insufficient perfusion and active ischemia.

The strongest scientific evidence for a direct causal relation between the extent of myocyte apoptosis and cardiac decompensation derives from recent studies using genetically modified mice. Notably, transgenic mice that express a conditionally active caspase exclusively in the myocardium illustrate that even very low levels of myocyte apoptosis suffice to cause a lethal dilated cardiomyopathy in otherwise normal hearts. Conversely, strong genetic and pharmacological proof now is available that the primary role of endogenous proteins such as the apoptosis repressor with caspase recruitment domain or apoptosis-inducing factor is to provide protection against heart failure by actively repressing cardiac muscle death execution, albeit by fundamentally different mechanisms. Even in studies in which apoptosis was not the primary focus, apoptosis is often found to correlate strongly with the extent of heart disease. Therefore, limiting cardiac muscle loss by inhibiting apoptosis may have clear implications for the treatment of heart failure.

Despite later scientific progress, our understanding of the individual players in the molecular circuits that drive myocyte death remains primitive, nor is it known whether cardiac myocytes that are programmed to die under chronic stress situations may nevertheless die by necrotic death in case apoptosis would be therapeutically prevented. In such a case, any approach aimed at inhibiting apoptosis would likely prove ineffective in preventing cardiac decompensation. One
time-consuming but highly informative tactic to provide a more complete picture of cardiac apoptotic pathways is to discover new proapoptotic factor(s) responsible for myocyte apoptosis after cardiac pressure overloading and manipulate their expression in the heart.

**Nix: The Cardiac Styx?**
The actual transmission of death signals to the mitochondria is controlled by the so-called Bcl-2 family of proteins. This superfamily consists of death antagonists (Bcl-2, Bcl-xL) and death agonists (Bax, Bak), which either protect or disrupt the integrity of the mitochondrial membrane and subsequent release of (pro)apoptotic intermembrane proteins. Another class of death effectors, called BH3-only proteins, serves as ligands to activate proapoptotic Bcl-2 family members or inactivate antiapoptotic Bcl-2 members. BH3-only proteins are activated through transcriptional and posttranslational mechanisms and translocate to the outer mitochondrial membrane. One such nearly ubiquitous BH3-only protein is called Nix, a homolog of the E1B 19K/Bcl-2 binding and the proapoptotic Bcl2 and nineteen kilodalton interacting protein-3 (Bnip3), first described in 1999 by the group of Greenberg and later rediscovered to be specifically upregulated by pressure-overload and Gq-mediated signals in the heart muscle by Dorn and colleagues.

In this issue of *Circulation*, Diwan and colleagues exhaustively studied Nix gene function in the heart using gene (in)activating strategies in mice and gained valuable new insights into the fundamentals between dying myocytes, hypertrophy signals, and heart failure. First, transgenic Nix overexpressing mice that exhibit mild cardiac abnormalities were crossbred with Gq transgenic mice, which also exhibit a fairly modest cardiomyopathic phenotype. The combination proved lethal, with high rates of dying myocytes, demonstrating the synergy between specific cardiac growth and death pathways that resulted in a downward spiral to heart failure. Next, the authors created a novel mouse model encompassing α-myosin heavy chain–directed Gq binding and the proapoptotic Bcl2 and nineteen kilodalton interacting protein-3 (Bnip3), first described in 1999 by the group of Greenberg and later rediscovered to be specifically upregulated by pressure-overload and Gq-mediated signals in the heart muscle by Dorn and colleagues.

In Nix knockout mouse hearts, ensuing myocardial apoptosis was decreased by half, implicating that myocardial salvage was not complete, despite approximate full Nix elimination. Of course, Nix is certainly not the only mitochondrial apoptotic factor induced in cardiac hypertrophy, and it would be too simplistic to suggest that complete elimination of complex cellular programs such as apoptosis could ever be accomplished by targeting a single gene. Dorn and colleagues previously studied the related protein Bnip3 in the context of pathological hypertrophy and demonstrated that this related BH3-only protein responds more selectively to ischemic signals rather than the Gq signals that induce Nix expression. Indeed, a Bnip3 null allele affords considerable (but not complete) protection against postinfarct remodeling. It will be of interest to determine whether Nix and Bnip3 physically or functionally synergize to propel mitochondrial apoptosis. Another possibility is that extrinsic, death receptor apoptosis pathways also contribute significantly to apoptosis after pressure overload, and in such case this form of myocyte death would be less sensitive to Nix/Bnip3 ablation. Finally, the ubiquitous Nix expression pattern complicates a straightforward chemical drug approach that could benefit patients at risk to develop heart failure.

Despite these complications, the present study fully supports the premise that salvage of cardiac myocytes that were programmed to die is a safe form of myocardial protection. It does not lead to necrosis of myocytes that were destined to die from apoptosis. The ancient Greek vividly imaginative description of Styx as a physical boundary between life and death provides us an allegory for Nix, a molecular switch that, not unlike a stygian river, decides about preservation of chamber thickness, hemodynamic performance, and myocardial immortality.

**The Nix Afterlife**
Notwithstanding the impressive efforts in the study by Diwan and colleagues, many riddles still shroud Nix-induced cardiomyocyte death. For example, one remarkable feature of Nix mouse models is the relatively mild phenotype when Nix expression is induced in otherwise healthy myocardium. Overall, Nix overexpression renders the murine myocardium more sensitive to pathological remodeling and myocyte death, a result that contrasts with the impressive protection afforded in the case of combinations of pathological signals in Nix null backgrounds. Indeed, addition of recombinant Nix to isolated mitochondria does not open permeability transition pores, although this clearly does occur in intact cells undergoing Gq-mediated apoptosis. These observations suggest a number of possible explanations that may not be mutually exclusive: (1) that either Nix protein accumulation needs to reach threshold levels to allow for it to displace antiapoptotic Bcl2 members from mitochondrial pore structures; (2) that an obligatory requirement exists for a combination of pathological signals and/or proapoptotic Bcl2 members to unleash its deadly power; or (3) that Nix is part of an as of yet unappreciated larger multiprotein complex that responds to and controls mitochondrial transition pore opening. These possibilities should be more amenable to experimentally address by in vitro approaches including proteomic and biochemical analyses.

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References

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