Editorial

Nix: The Cardiac Styx Between Life and Death

Paula A. da Costa Martins, PhD; Leon J. De Windt, PhD

Now let Earth be my witness and the broad heaven above, and the down flowing water of the Styx . . .

—Homer, *Iliad XV*. 36–37: Greek Oath-Rituals.

In Greek mythology, the river Styx is a river that formed the boundary between earth and the underworld or Hades, the abode of the dead. The ferryman of the river Styx was called Charon, a personification of the decision-making process between life and death. According to some versions of the myth, the river Styx had miraculous powers and could make someone immortal. Achilles was said to have been dipped in it as a child, thereby becoming invulnerable, with the exception of his heel, which was held by his mother to submerge him in the flowing waters of the Styx. His exposed heel gave rise to the expression "Achilles' heel," a metaphor for a weak spot in modern meaning, as Achilles was killed in the battle for Troy by an arrow to the heel.

Article p 396

The boundary between life and death continues to intrigue humanity today as much as it did 3 millennia ago, and in our current era, with a clear need for a detailed understanding of the genetic circuits driving the onset of human disease, death pathways are becoming increasingly relevant for understanding acquired heart failure. The failure of cell-survival pathways to inhibit myocyte apoptosis seems a critical step in the initiation of heart failure that results in apoptotic cardiomyocyte dropout with replacement fibrosis. Unlike necrosis (oncosis), apoptosis is an orderly regulated process and, by inference, a logical therapeutic target if intervention occurs at an early stage.

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.[1] Two major apoptotic pathways (ie, pathways that eventually lead to activity 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%.[2] 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.[3] 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.[4–6] Even in studies in which apoptosis was not the primary focus, apoptosis is often found to correlate strongly with the extent of heart disease.[7,8] 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

(*Circulation.* **2008;117:338-340.**)

Circulation **is available at http://circ.ahajournals.org**
DOI: 10.1161/CIRCULATIONAHA.107.750125

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.[9,10] 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.[11] 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[12] and later rediscovered to be specifically upregulated by pressure-overload and Gq-mediated signals in the heart muscle by Dorn and colleagues.[13]

In this issue of *Circulation*, Diwan and colleagues[14] 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[15] 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 transgenic mice with systemic *Nix* ablation, resulting in reduced Gq-associated peripartum cardiomyopathy and decreased myocardial apoptosis. Finally, the *Nix* gene was specifically deleted from cardiac myocytes by crossbreeding mice harboring a floxed *Nix* allele with a Cre deleter strain driven by the Nkx2.5 promoter. The resulting cardiac-specific *Nix*-deficient mice displayed a remarkable level of protection against left ventricular dilation, reduced ejection fraction, and myocyte dropout after aortic banding. These beneficial effects occurred with an unchanged hypertrophic growth of the heart. Taken together, this study mechanistically links induction of *Nix* gene expression, cardiomyocyte dropout, ventricular remodeling, and functional deterioration at the transition point from compensated pressure overload hypertrophy to decompensated heart failure. These findings are suggestive for a nodal role for *Nix* gene induction as a decisive molecular switch between life and death of the myocyte, but are these findings sufficient to designate *Nix* as the one and only Styx, or do other stygian rivers flow within the (failing) heart?

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,[16] although this clearly does occur in intact cells undergoing Gq-mediated apoptosis.[17] 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.

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.[17,18] 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 provided 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.

Sources of Funding

Disclosures

None.

References

1. Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. *J Biol Chem*. 1999;274:20049–20052.
2. van Empel VP, Bertrand AT, Hofstra L, Crijns HJ, Doevendans PA, De Windt LJ. Myocyte apoptosis in heart failure. *Cardiovasc Res*. 2005;67:21–29.
3. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, Kitsis RN. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest*. 111:1497–1504.
4. Donath S, Li P, Willenbockel C, Al-Saadi N, Gross V, Willnow T, Bader M, Martin U, Bauersachs J, Wollert KC, Dietz R, von Harsdorf R. Apoptosis repressor with caspase recruitment domain is required for cardioprotection in response to biomechanical and ischemic stress. *Circulation*. 2006;113:1203–1212.
5. van Empel VP, Bertrand AT, van der Nagel R, Kostin S, Doevendans PA, Crijns HJ, de Wit E, Sluiter W, Ackerman SL, De Windt LJ. Downregulation of apoptosis-inducing factor in harlequin mutant mice sensitizes the myocardium to oxidative stress–related cell death and pressure overload–induced decompensation. *Circ Res*. 2005;96:e92–e101.
6. van Empel VP, Bertrand AT, van Oort RJ, van der Nagel R, Engelen M, van Rijen HV, Doevendans PA, Crijns HJ, Ackerman SL, Sluiter W, De Windt LJ. EUK-8, a superoxide dismutase and catalase mimetic, reduces cardiac oxidative stress and ameliorates pressure overload-induced heart failure in the harlequin mouse mutant. *J Am Coll Cardiol*. 2006;48:824–832.
7. Zhang D, Gaussin V, Taffet GE, Belaguli NS, Yamada M, Schwartz RJ, Michael LH, Overbeek PA, Schneider MD. TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. *Nat Med*. 2000;6:556–563.
8. Sadoshima J, Montagne O, Wang Q, Yang G, Warden J, Liu J, Takagi G, Karoor V, Hong C, Johnson GL, Vatner DE, Vatner SF. The MEKK1-JNK pathway plays a protective role in pressure overload but does not mediate cardiac hypertrophy. *J Clin Invest*. 2002;110:271–279.
9. Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. *Trends Biochem Sci*. 2001;26:61–66.
10. Antonsson B, Montessuit S, Sanchez B, Martinou JC. Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. *J Biol Chem*. 2001;276:11615–11623.
11. Crow MT, Mani K, Nam YJ, Kitsis RN. The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ Res*. 2004;95:957–970.
12. Chen G, Ray R, Dubik D, Shi L, Cizeau J, Bleackley RC, Saxena S, Gietz RD, Greenberg AH. The E1B 19K/Bcl-2-binding protein Nip3 is a dimeric mitochondrial protein that activates apoptosis. *J Exp Med*. 1997;186:1975–1983.
13. Yussman MG, Toyokawa T, Odley A, Lynch RA, Wu G, Colbert MC, Aronow BJ, Lorenz JN, Dorn GW II. Mitochondrial death protein Nix is induced in cardiac hypertrophy and triggers apoptotic cardiomyopathy. *Nat Med*. 2002;8:725–730.
14. Diwan A, Wansapura J, Syed FM, Matkovich SJ, Lorenz JN, Dorn GW II. Nix-mediated apoptosis links myocardial fibrosis, cardiac remodeling, and hypertrophy decompensation. *Circulation*. 2007;117:396–404.
15. Syed F, Odley A, Hahn HS, Brunskill EW, Lynch RA, Marreez Y, Sanbe A, Robbins J, Dorn GW II. Physiological growth synergizes with pathological genes in experimental cardiomyopathy. *Circ Res*. 2004;95:1200–1206.
16. Diwan A, Koesters AG, Odley AM, Pushkaran S, Baines CP, Spike BT, Daria D, Jegga AG, Geiger H, Aronow BJ, Molkentin JD, Macleod KF, Kalfa TA, Dorn GW II. Unrestrained erythroblast development in Nix−/− mice reveals a mechanism for apoptotic modulation of erythropoiesis. *Proc Natl Acad Sci U S A*. 2007;104:6794–6799.
17. Galvez AS, Brunskill EW, Marreez Y, Benner BJ, Regula KM, Kirschenbaum LA, Dorn GW II. Distinct pathways regulate proapoptotic Nix and BNip3 in cardiac stress. *J Biol Chem*. 2006;281:1442–1448.
18. Diwan A, Krenz M, Syed FM, Wansapura J, Ren X, Koesters AG, Li H, Kirshenbaum LA, Hahn HS, Robbins J, Jones WK, Dorn GW. Inhibition of ischemic cardiomyocyte apoptosis through targeted ablation of Bnip3 restrains postinfarction remodeling in mice. *J Clin Invest*. 2007;117:2825–2833.

KEY WORDS: Editorials ■ apoptosis ■ heart failure