Hypoxia, BNip3 Proteins, and the Mitochondrial Death Pathway in Cardiomyocytes

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Myocyte cell loss is a prominent and important pathogenic feature of cardiac ischemia. Limiting this loss is a desirable therapeutic goal, but the development of truly effective strategies to achieve that goal requires an understanding of the mechanisms by which ischemia triggers cell death. Investigators have turned to isolated and cultured cardiomyocytes to identify signaling pathways involved in the response to ischemia and to systematically test the effectiveness of prosurvival signaling pathways and various antideath molecules against ischemia-associated cellular insults, such as hypoxia.1–4 Despite some success in this area, it is still not totally clear how hypoxia actually triggers cell death in cardiomyocytes.

One attractive mechanism through which hypoxia might trigger cardiomyocyte cell death is explored by Regula and coworkers5 in this issue of Circulation Research. These investigators present data on the possible role of BNip3, a hypoxia-inducible member of the Bcl-2 family of apoptotic regulators, in mediating cardiomyocyte cell death. They show that (1) BNip3 expression is dramatically increased in response to hypoxia, (2) enforced expression of BNip3 causes cell death in normoxic cardiomyocytes, and (3) enforced expression of a BNip3 mutant lacking its transmembrane domain (BNip3ΔTM) partially blocks hypoxia-induced cell death. BNip3 is an attractive candidate to play a pivotal role in the cellular response to hypoxia because (1) its expression is regulated by the hypoxia-inducible factor (HIF) transcription complex, the activation of which by hypoxia is a well-characterized response, (2) its activity is tied to the Bcl-2 family of apoptosis regulators, and (3) it is localized to the mitochondria, a site where numerous cell death regulatory pathways converge. In addition, the BNip3-related protein, Nix/BNip3L, has recently been shown to play an important role in the transition from compensatory hypertrophy to heart failure in the Gaq-overexpressing transgenic mouse.6

BNip3 stands for Bcl-2 and nineteen-kilodalton interacting protein-37 and is a member of the Bcl-2 protein family. Bcl-2 proteins have been implicated in the control of both apoptotic and necrotic cell death and in guarding mitochondrial integrity. They share up to 4 conserved regions of homology known as Bcl-2 homology domains (BH1, BH2, BH3, and BH4), which mediate interactions among the various family members, and are divided functionally into antiapoptotic and proapoptotic members (see Figure, panel A). Many of these proteins normally reside in membranous cellular structures, including mitochondria, endoplasmic reticulum, and the nuclear envelope or are recruited to such structures (principally the mitochondria) during the execution of cell death signaling pathways. Antiapoptotic members, such as Bcl-2, Bcl-XL, Mcl-1, A1, Bcl-W, display sequence homology throughout all 4 BH domains. Proapoptotic members, which antagonize the activity of many prosurvival proteins and induce cell death when overexpressed, display homology to fewer BH domains. Some, like Bax and Bak, contain BH1, BH2, and BH3 domains, whereas many others (Bad, Bid, Bik, Bim, BimL, Blk, and Noxa) possess only the BH3 domain (BH3-only proteins).

By themselves, BH3-only proteins do not induce cell death, but instead act as allosteric activators of the multidomain proapoptotic proteins Bax and Bak, such that there is an absolute requirement for Bax or Bak.8 These proteins, therefore, function at the judicial rather than executioner stage of cell death, integrating information on the survival status and cellular stresses imposed from within and outside the cell, with their proapoptotic-promoting activity normally held in check by posttranslational mechanisms.9 Thus, activation of cytosolic Bid is initiated by caspase 8 cleavage, followed by its translocation to mitochondria and activation of Bak and Bax. A similar mechanism for Bax involving calpain has also been proposed. Other BH3-only proteins, such as Bad, are actively sequestered away from mitochondria through phosphorylation mediated by Akt/PKB, a prominent prosurvival signaling kinase.

BNip3 is the founding member of a small group of BH3-only proteins that includes BNip3, Nix/BNip3L, and BNip3L.10 In contrast to Bid and Bad, the proapoptotic activity of BNip3 and Nix is regulated through transcriptional mechanisms that involve the HIF complex. Thus, the promoter for BNip3 contains a functional binding site for the HIF transcriptional complex (hypoxia response element, HRE) and its mRNA and protein expression are dramatically increased in multiple cell types in response to reduced oxygen concentration.11 In cultured cells, increased expression of BNip3 appears to be part of a second wave of hypoxia-induced protein accumulation, occurring late relative to other well-characterized HIF-inducible genes that are involved in promoting angiogenesis, glycolytic metabolism, and survival (eg, erythropoietin, VEGF, heme oxygenase, hexokinase, and IGF2).12

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The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.


Circulation Research is available at http://www.circresahajournals.org
DOI: 10.1161/01.RES.0000030195.38795.CF
The study by Regula et al. extends previous observations on the expression and ability of BNip3 to induce cell death to isolated cardiomyocytes. Although they confirm an earlier report that induction of BNip3 in isolated cardiomyocytes occurs in response to hypoxia, they also show that elevated BNip3 protein levels are seen in vivo in animal models of acute ischemia and heart failure. Induction of protein expression is acute, ischemia is rapid (1 hour), and in heart failure, persistent (up to 8 weeks). The rapid accumulation during in vivo suggests that other components of the response may potentiate the transcriptional response to hypoxia or facilitate protein accumulation. Interestingly, expression of another HIF-inducible member of the BNip3 family, Nix/BNip3L, has been shown to be upregulated during cardiac hypertrophy triggered by overexpression of Gaq, a signaling intermediate for numerous hypertrophic stimulants. Together, these recent studies suggest that expression of BNip3 proteins, which apparently can be governed by stress-inducing factors in addition to hypoxia, is likely to be an important contributor not only to the pathogenesis of ischemic diseases but other cardiac disorders as well. A recent study in Circulation Research showed that the BNip2 protein, Nip21, is involved in cell death associated with experimentally induced myocarditis. Although it shares limited homology to BNip3, this protein also interacts with Bcl-2 proteins and E1B 19-kDa protein to regulate mitochondrial death pathways.

To assess the functional significance of BNip3 expression in hypoxia-mediated cell death, Regula et al. used a modified BNip3 protein lacking its transmembrane domain (BNip3ΔTM) as a potential dominant-negative mutant. The effectiveness of the mutant was established by showing that its expression blocked the stable (alkali-insensitive) incorporation of BNip3 into mitochondria (presumably BNip3ΔTM heterodimerizes with endogenous BNip3). Expression of this mutant alone had no effect on cell death, but it blocked approximately half of the total cell death (measured as loss of plasma membrane integrity) induced by hypoxia.

If BNip3ΔTM effectively sequesters BNip3 away from mitochondria, why doesn’t it completely block hypoxia-induced cell death? One possibility is that BNip3 has death-inducing effects that do not involve its direct association with mitochondria and therefore would not be expected to be inhibited by BNip3ΔTM. This notion is supported by laboratory-generated mutants of BNip3 that are redirected to extramitochondrial sites, yet still cause substantial cell death. Although further work is needed to characterize the mode of death at these extramitochondrial sites, the ultimate reconciliation of this issue might require completely removing BNip3 from the response to hypoxia, using either myocytes from BNip3 knockout mice (when and if they become available) or antisense technology to suppress BNip3 mRNA accumulation.

Another possibility for the partial effect of the BNip3ΔTM mutant is that BNip3 does not mediate the entire response to hypoxia. It is likely that expression of Nix is also upregulated during hypoxia. If its activity continues in the presence of the BNip3ΔTM mutant, then it will be necessary to use a specific dominant-negative for Nix (such a mutant in which the TM domain is partially truncated appears to occur naturally and is referred to as sNix). Together, these recent reports suggest that expression of BNip3 proteins, which apparently can be governed by stress-inducing factors in addition to hypoxia, is likely to be an important contributor not only to the pathogenesis of ischemic diseases but other cardiac disorders as well. A recent study in Circulation Research showed that the BNip2 protein, Nip21, is involved in cell death associated with experimentally induced myocarditis. Although it shares limited homology to BNip3, this protein also interacts with Bcl-2 proteins and E1B 19-kDa protein to regulate mitochondrial death pathways.

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To adequately reconcile the events linked to hypoxia-mediated cell death in cardiomyocytes, a detailed time course of the cleavage and activation for the various caspases already implicated in the response to hypoxia (caspases 3, 8, and 9) needs to be performed. This will determine when and possibly how caspase 8 is activated. These events should also be tied to events in the mitochondria and to the effects of various inhibitors of the processes (ie, CrmA, BNip3ΔTM, Bcl-XL, Nix). It is also important to determine what role both BNip3 and Nix are playing in promoting cell death at both mitochondrial and extramitochondrial sites in the cell and whether dominant-negative mutants of each protein can neutralize the effects of the other. By using these dominant-negative mutants, it should be determined whether BNip3 or Nix causes cytochrome c release and/or whether it affects caspase 8 activation/activity. Existing gene knockout mice should be used to determine the dependency of BNip3 and Nix-mediated cell death on Bax and Bak to induce cell death (Bax−/−/Bak−/− mice) and on the role of HIF in BNip3 and Nix expression during cardiac hypertrophy in vivo and the transition to heart failure (HIF-1α−/− mice). Given the remarkable induction of BNip3 by hypoxia in cultured cells and in in vivo models of cardiac ischemic disease along with recent data showing that Nix/BNip3L is involved in the progression to heart failure in the Gαq-overexpressing mouse, the answers to these questions will be eagerly awaited.

References


Key Words: BNIP3/BNIP3L/Nix ■ apoptosis ■ hypoxia ■ Bcl-2 proteins ■ mitochondria permeability transition
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Circ Res. 2002;91:183-185
doi: 10.1161/01.RES.0000030195.38795.CF
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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