Puma joins the battery of BH3-only proteins that promote death and infarction during myocardial ischemia

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Puma (p53-upregulated modulator of apoptosis) is a member of the Bcl-2 homology 3 (BH3)-only subfamily of Bcl-2-related proapoptotic proteins that are implicated in numerous apoptotic pathways responding to developmental signals and cellular stress. Puma was originally identified as a target for the tumor suppressor transcription factor p53 and was shown to play the leading role in p53-mediated apoptosis (for review, see Ref. 20). Puma can bind and sequester all the prosurvival Bcl-2 proteins and initiates the intrinsic death pathway by activating proapoptotic Bax and Bak (18). Death by Puma typically involves increased permeability of the outer mitochondrial membrane, leakage of cytochrome c and apoptosis-inducing factor-1, and activation of caspases. Puma is activated at the transcriptional level by p53 and is induced by all stimuli that activate p53, including DNA damage and oxidative stress (19). In addition, Puma mediates multiple p53-independent death pathways initiated by diverse cytotoxic stimuli, including cytokine deprivation and exposure to glucocorticoids, the kinase inhibitor staurosporine, phorbol esters, and conditions that induce endoplasmic reticulum (ER) stress and the unfolded protein response (2, 19). As an essential target for p53, Puma is credited with the tumor suppressor function of p53 that protects organs against cancer by eliminating cells that have suffered DNA damage or proliferate in an uncontrolled manner (20). As such, Puma has become an important target for anticancer drugs. Recently, Puma was implicated in the apoptotic death of neurons resulting from ischemia-reperfusion and copper and arsenite toxicity and as a component in the proapoptotic death of neurons resulting from ischemia-reperfusion and copper and arsenite toxicity (20). As such, Puma has become an important target for p53-null mice are similar to those of wild-type mice (16). Therefore, p53 does not appear to be the principal mediator of Puma during ischemic or hypoxic stress in the heart. In contrast to BNIP3 and Noxa, which are induced by hypoxia through promoter elements that bind the transcription factor hypoxia-inducible factor-1α, Puma has not been reported to possess such a function and probably does not respond directly to hypoxia. So how is Puma induced by ischemia? One possibility is that it is regulated indirectly through components of the ER stress pathways, which have been described in ischemic heart and brain (13, 19). ER stress during ischemia is predicted to occur under conditions of ATP depletion, acidosis, and abnormal ER/sarcoplasmic reticulum Ca2+ handling. In isolated hippocampal neurons, Puma is induced by treatment of cells with the ER stress mediators tunicamycin, a protein glycosylation inhibitor, and thapsigargin, an ER Ca2+-ATPase inhibitor (13). Transcriptional activation of Puma coincides with induction of the early growth response factor-1 and the ER stress-specific transcription factor C/EBP homologous protein (CHOP). In forebrain ischemia, Puma activation was reported to occur late (48–120 h) after ischemia-reperfusion and coincided with apoptotic death (13). The delayed induction occurred, despite early induction of CHOP, and was attributed to a requirement for prolonged ER stress. The molecular mediators of Puma induction by such prolonged ER stress have not been identified. If, as suggested by Toth et al., Puma has a predominant role in apoptosis and necrosis in the heart, it will be extremely important to identify the upstream regulation.

Two other factors that have been implicated in the transcriptional activation of Puma are p73, a homolog of p53, and E2F1, a transcription factor that binds the retinoblastoma protein and controls cell cycle regulatory genes (5, 9). The relations are complex: E2F1 and p73 transactivate Puma directly by binding and activating the promoter. E2F1 also transactivates p73, and this may amplify the induction of Puma (11) (Fig. 1). Similarly, p73 has multiple and complex roles in apoptosis. In addition to inducing Puma, p73 also stimulates the transcription of Bax (proapoptotic) and Scotin, a protein required for initiating the ER stress pathway (3, 15). Brains of E2F-null mice are resistant to focal ischemia, and at least one study reported that apoptosis of cardiac myocytes exposed to simulated ischemia also requires active E2F (4, 8).

If E2F and/or p73 contribute to the induction of Puma in the heart by hypoxia and/or ischemia, these conditions must activate these factor(s). This could occur by changes in the post-translational regulatory pathways. There is some evidence for this. Both p53 and p73 are subject to ubiquitination and proteasomal degradation. Degradation of p53 is determined by its binding to the murine double minute-2 ubiquitin ligase and p73 by binding to the HECT ubiquitin ligase Ich (14). Binding of p73 to Itch promotes ubiquitination and rapid proteasome-dependent degradation. This process normally keeps p73 at very low levels in most cells. Under conditions of stress, such
Bid (tBid) with calpain inhibitors reduced infarct size by 50% in rabbits. Similarly, Bad is dephosphorylated during reperfusion and translocates to the mitochondria to activate apoptosis. Inhibition of Bad by blocking PKC-δ was reported to reduce infarction by >80% (6, 10). BNIP3 is induced by hypoxia in cardiac myocytes and by ischemia in the heart (17). BNIP3 also translocates to mitochondria, where it activates an atypical programmed death pathway. The contribution of BNIP3 to reoxygenation damage as determined by small interfering RNA knockdown might also account for up to 50% of cardiac myocyte death (unpublished observations). Puma, Bid, Bad, and Noxa pathways converge on the intrinsic mitochondrial pathway through direct or indirect interactions with Bax and/or Bak (18). Puma and Bid bind and neutralize all Bcl-2 prosurvival proteins, whereas Bad and Noxa recognize only a subset and, as a consequence, are weaker killers; therefore, complementary family members may be required for efficient killing. To account for the contributions of multiple BH3-only proteins to death and infarction, it is probable that they act in concert in wild-type hearts, neutralizing prosurvival Bcl-2 proteins and maximizing the activation of Bax and Bak. BNIP3 appears to be capable of activating caspase-dependent and -independent death pathways, but both involve mitochondrial signaling and outer membrane puncture, which may complement the activities of the other BH3-only proteins. Further work is required to determine the precise signaling pathways that mediate Puma induction during ischemia-reperfusion, to define the interrelations between the different BH3-only proteins, and to identify the optimal interventions for protecting the heart against these combined death pathways.

GRANTS

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REFERENCES

9. Melino G, Bernassola F, Ranalli M, Yee K, Zong WX, Corazza M, Knight RA, Green DR, Thompson C, and Vousden KH. p73 Induces...


17. Webster KA, Graham RM, and Bishopric NL. BNip3 and signal-specific programmed death in the heart. J Mol Cell Cardiol 38: 35–45, 2005.


