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 and is induced by all stimuli that activate p53, including DNA damage and oxidative stress (19).

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Whereas Puma appears to be essential for p53-mediated apoptosis, p53 is not essential for Puma activation. Indeed, Toth et al. (15a) observed that Puma-null mice were resistant to ischemia-reperfusion injury. Infarct sizes in the Puma-null hearts were reduced by 50% compared with wild-type or heterozygous mice. Apoptotic indexes were dramatically reduced, and posts ischemic recovery was significantly improved. Toth et al. further report that Puma levels were induced in cardiac myocytes exposed to hypoxia under glucose starvation, and Puma promoted necrosis as well as apoptosis if energy was depleted. This study aligns Puma alongside other BH3-only proteins, including BNIP3, Bid, Bad, and, possibly, Noxa, which are also implicated in the death pathways that are activated by ischemia.

Whereas Puma appears to be essential for p53-mediated apoptosis, p53 is not essential for Puma activation. Indeed, Toth et al. (15a) observed that Puma-null mice were resistant to ischemia-reperfusion, but p53-null mice were not. This latter result confirms previous reports that ischemia-related infarcts of 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 Iitch (14). Binding of p73 to Iitch promotes ubiquitination and rapid proteasome-dependent degradation. This process normally keeps p73 at very low levels in most cells. Under conditions of stress, such
Fig. 1. Ischemia-reperfusion mediates hypoxic and oxidative stress in cardiac myocytes. Multiple pathways linked to hypoxia and reoxygenation may induce p53-upregulated modulator of apoptosis (Puma). In one pathway, Puma is induced by endoplasmic reticulum (ER) stress caused by hypoxia and glucose depletion, reoxygenation, and abnormal Ca²⁺ flux. Transcription factor C/EBP homologous protein (CHOP) is a major transcription factor activated by ER stress, but it is not known whether CHOP is directly involved in Puma induction. Oxidative stress may also inactivate Itch and/or the proteasome, both of which may promote p73 accumulation. Puma is directly activated by E2F and p73, and E2F also induces p73, creating a positive-feedback loop. Scotin is a factor required for activation of the ER stress pathway and is induced by p73, creating another positive-feedback loop. Once induced, Puma translocates to mitochondria, where it joins forces with other Bcl-2 homology 3 (BH3)-only proteins to bind and neutralize prosurvival Bcl-2 proteins and activate proapoptotic Bax and Bak. PKC-δ also translocates during ischemia-reperfusion and activates Bad. BNIP3 may function in combination with or independently of the other BH3-only proteins. ROS, reactive oxygen species; AIF-1, apoptosis-inducing factor-1.

as DNA damage or oxidative stress, Itch is downregulated, and p73 protein levels rise. Because this p73 regulatory pathway was discovered very recently, it is not yet known whether Itch or p73 levels change during ischemia in the brain or heart. If Itch levels do not change, another pathway that could mediate an increase of p73 and Puma in the ischemic heart is reduced activity of the proteasome. Proteasomal activity in the heart is significantly inhibited by ischemia-reperfusion (12).

Toth et al. (15a) report that infarction due to ischemia-reperfusion decreased by 50% in mouse hearts with targeted disruption of Puma. The reduced infarcts presumably involved protection from Puma-mediated apoptosis and necrosis. These observations prompt additional questions, in particular, the contributions of other BH3-only proteins to infarction and the extent to which each pathway is responsive to cardioprotective interventions such as ischemic preconditioning and kinase (PKC and JNK) inhibitors. Other BH3-only proteins that have been causally linked to ischemic injury include Bid, Bad, and BNIP3. Additionally, pathways that activate Puma, including ER stress, EF2, and p73, also activate Noxa (15). If it is assumed that the Noxa gene is expressed in the heart, it should be induced in parallel with Puma. Noxa is also directly induced by hypoxia through hypoxia-inducible factor-1α and may be activated before Puma during ischemia (7). Bid is cleaved and activated during ischemia-reperfusion, and Chen et al. (1) report that blocking activation of the caspase-activated form of Bid (tBid) with calpain inhibitors reduces 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 reoxygengenation 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 Bak and/or Bak (18). Puma and Bid bind and neutralize all Bcl-2 prosurvival proteins, whereas Bid and Noxa recognize only a subset and, as a consequence, are weaker killers; therefore, complementarity 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


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