No PUMA, no death: Implications for p53-dependent apoptosis

More than a decade ago, it was found that one of the two essential physiological functions of p53 is to selectively destroy stressed cells through apoptosis. Despite the large number of studies describing p53-dependent apoptosis since then, how p53 turns on the apoptotic switch has remained enigmatic. In this issue of *Cancer Cell*, Jeffers et al. report that knockout of *PUMA*, a recently identified BH3-only Bcl-2 family protein, recapitulates virtually all apoptotic deficiency in *p53* knockout mice. Their results indicate that PUMA is an essential mediator of p53-dependent and -independent apoptosis in vivo.

In the great majority of human tumors, the p53 pathway is disarmed by oncogenic mutations in *p53* itself, expression of viral oncoproteins, or defective p53 upstream regulation (Vogelstein et al., 2000). The growing understanding of its physiological function has revealed that p53 is a key regulator of apoptosis, in addition to its clear effects on cell cycle arrest and its involvement in senescence, DNA repair, differentiation, and other processes. It was a serendipitous finding that expression of wild-type p53 resulted in rapid loss of cell viability with characteristics of apoptosis (Yonish-Rouach et al., 1991). Indisputable evidence for the role of p53 in physiological cell death was later provided by studies of *p53* knockout mice (Lowe et al., 1993; Clarke et al., 1993). Thymocytes and stem cells of the intestine derived from these animals are significantly more resistant to radiation-induced apoptosis than corresponding normal cells. Cellular stresses that induce p53-dependent apoptosis include DNA damage, oncogene activation, hypoxia, and oxidative stress. Under these conditions, p53 is essential for restricting inappropriate cell proliferation and thereby suppresses neoplasia.

With the role of p53 in apoptosis established, the burning question became how p53 initiated apoptosis in response to these stresses. p53 is a transcription factor that directly binds to DNA in a sequence-specific manner to activate its downstream targets (Kern et al. 1991). There are hundreds of sites in the human genome that can be bound by p53. A number of large-scale gene expression analyses have been performed to look for the p53 targets. p21 and 14-3-3σ were thus identified and confirmed by gene-targeting experiments to be the major mediators of p53-induced cell cycle arrest (Vogelstein et al., 2000). In contrast, the search for a major apoptotic mediator of p53 has been long and difficult. At least 16 genes have been reported to play a role in p53-dependent apoptosis (Vousden and Lu, 2002). These genes are directly involved in regulation of apoptosis and can be activated by p53 in response to stresses. In each case, a consensus p53 binding site was located within the regulatory region of the gene. However, deletion of these genes in mice never recapitulated the apoptotic phenotype of the *p53* knockout mice, and the importance of these genes for p53-dependent apoptosis has remained conjectural.

A latecomer to the group of p53 targets is a protein called PUMA, or p53 upregulated modulator of apoptosis. PUMA was initially identified as a gene activated by p53 in cells undergoing p53-induced apoptosis (Yu et al., 2001; Nakano and Vousden, 2001), and as a protein interacting with Bcl-2 (Han et al., 2001). PUMA and another p53 target Noxa share homology with Bcl-2 family proteins, but only within a short stretch of amino acids termed the BH3 (Bcl-2 homology 3) domain. Proteins with similar homology, coined as "BH3-only" proteins, have been suggested to play an essential role in apoptosis initiation (Bouillet and Strasser, 2002). Several hurdles had prevented PUMA from being identified in earlier attempts, including its low expression level, GC-rich coding sequence, lack of sequence homology to known apoptotic proteins, and diversity of transcripts due to alternative splicing. Several characteristics distinguish PUMA from other p53 targets. For example, the coding and regulatory sequences of *PUMA*, including those that are directly bound by p53, are highly conserved among different species. PUMA is also extremely effective in inducing apoptosis: when expressed, it kills cancer cells within a few hours. And importantly, gene knockouts in human colorectal cancer cells showed that *PUMA* was required for apoptosis induced by p53, hypoxia, and DNA-damaging agents (Yu et al., 2003). Nevertheless, evidence validating the role of PUMA in physiological cell death in an intact animal was lacking.

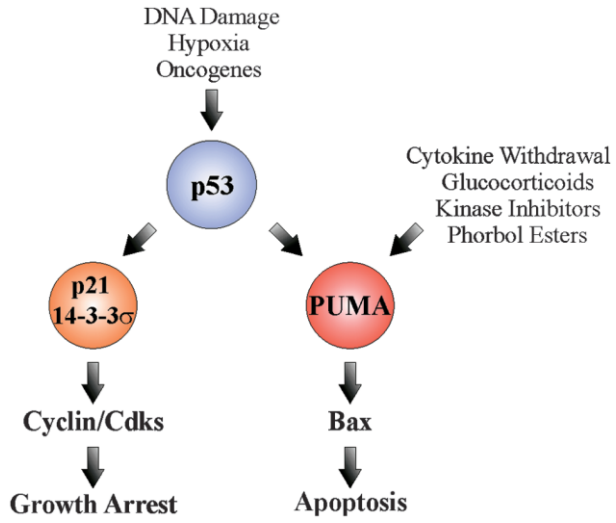

Figure 1. PUMA is essential for p53-dependent and -independent apoptosis

p53 induces either cell cycle arrest or apoptosis depending on cell type and subcellular context. PUMA is required for p53-dependent apoptosis induced by DNA damage, hypoxia, and oncogenes. PUMA is also necessary for apoptosis induced by p53-independent stimuli including serum withdrawal, glucocorticoids, kinase inhibitors, and phorbol esters. p53-dependent cell cycle arrest is mediated by p21 and 14-3-3σ.

This evidence is now provided by two studies describing striking phenotypes of *PUMA* knockout mice. One, led by Gerard Zambetti at the St. Jude Children's Research Hospital, is published in this issue of *Cancer*

Cell (Jeffers et al., 2003). The other, led by Andreas Strasser at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, is described in *Science* (Villunger et al., 2003). Both studies show that knockout of *PUMA* can recapitulate the majority, if not all, of the apoptotic deficiencies observed in *p53* knockout mice. Thymocyte apoptosis induced by γ-irradiation and DNA-damaging drugs, considered by many as the "gold standard" for analyzing p53-mediated physiological cell death, was blocked in the *PUMA* knockout mice to a similar extent as in *p53* knockout mice. PUMA is required for the p53-dependent apoptotic responses to the c-Myc and E1A oncogenes in primary embryonic fibroblast (MEF) cells, and also is necessary for the DNA damage-induced apoptosis that occurs in developing neurons. Furthermore, PUMA deficiency protects lymphocytes from p53-independent apoptotic stimuli such as cytokine withdrawal or exposure to the glucocorticoid dexamethasone, the kinase inhibitor staurosporine, or phorbol esters. The Strasser group also characterized *Noxa* knockout mice and found some effects on the apoptotic phenotype, though not as pronounced as those found with PUMA. Both PUMA and Noxa are dispensable for normal development, as is p53.

Although these studies unequivocally demonstrate the essential role of PUMA in p53-dependent apoptosis, several critical questions remain. First, do spontaneous tumors develop in the *PUMA* knockout mice? The complete loss of *p53* results in the development of tumors, predominantly lymphomas and sarcomas (Donehower et al., 1992). Neither paper has reported any tumors in the *PUMA* knockout mice, at least at their current age (6 months, a time when tumors in *p53* knockout mice are already evident). These data suggest that inactivation of the apoptotic function of p53 alone may not be sufficient for tumorigenesis and that abrogation of both the cell cycle and apoptotic effects of p53 is required for efficient tumorigenesis. The cross between PUMA-deficient mice and p21-deficient mice is therefore eagerly awaited. Second, does p53 directly activate *PUMA* transcription to initiate apoptosis in vivo, which is the case in human cancer cells (Yu et al., 2001), or induce apoptosis via transcription-independent mechanisms (Vousden and Lu, 2002)? To address this issue in the intact mouse, the p53 binding sites and other regulatory elements within the *PUMA* promoter could be manipulated using gene-targeting approaches. Third, is PUMA related to other apoptotic mediators of p53? It is possible that some proteins influence p53-dependent apoptosis by regulating PUMA or by being regulated by PUMA. For instance, PUMA functions through Bax to induce apoptosis in human colorectal cancer cells, even though Bax is not directly regulated by p53 in many cells (Yu et al., 2003). Another interesting question is whether PUMA deficiency can mimic p53 deficiency in its ability to rescue the developmental defects caused by MDM2 knockout. And finally, why do some cells undergo cell cycle arrest rather than apoptosis in response to p53, even though PUMA is induced?

Studies of *PUMA* knockout mice have several important implications for cancer biology and therapy. Although there is no evidence that *PUMA* is either mutated or abnormally expressed in tumors with normal p53, the knockout studies will undoubtedly stimulate further analysis of such tumors. More importantly, PUMA can potentially be used as a new target for anticancer therapy. Loss of apoptotic response by inactivating the p53 pathway appears to be required for malignant progression. Agents that activate PUMA via p53-independent mechanisms might restore the apoptotic response in tumor cells while sparing normal cells. It will be of interest to determine whether PUMA can sensitize p53 mutant cells to anticancer drugs.

Although many questions await answers, the identification of PUMA as an essential mediator of apoptosis is a significant step in understanding the physiological functions of p53. Hopefully, it will not take another decade to develop an effective therapy based on these discoveries.

Jian Yu* and Lin Zhang*

University of Pittsburgh Cancer Institute
Departments of Pathology and Pharmacology
University of Pittsburgh
Pittsburgh, Pennsylvania 15213
*Email: zhanglx@upmc.edu (L.Z.); yuj2@upmc.edu (J.Y.)

Selected reading

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