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News and Commentary

p53-dependent pathways of apoptosis

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The ability of p53 to control passage through the cell cycle (in G1 and in G2) and to control apoptosis in response to abnormal proliferative signals and stress including DNA damage is considered to be important for its tumor suppression function.[1] p53 is a transcription factor that binds to DNA in a sequence-specific manner to activate transcription of target genes. The consensus DNA binding sequence for p53 consists of two repeats of the 10 bp motif 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3′ separated by 0–13 bp.[2] Mutated p53 alleles typically found in tumors encode defective products no longer capable of binding to DNA or activating transcription. There is compelling evidence that the transcriptinal activity of p53 is required for its growth suppressing and tumor suppressing activity.[3,4] p53 has also been implicated as a transcriptional repressor;[5] however, neither the physiological significance nor the mechanism of p53-mediated repression is known.

The ability of p53 to promote cell cycle arrest is fairly well understood in terms of its ability to transactivate three critical target genes: *p21*^{WAF1}, *GADD45* and *14-3-3*σ.[6–8] p21 induction arrests cells in G1 and prevents S-phase entry while *GADD45* and *14-3-3*σ control the G2/M transition.[9,10] None of these genes appears to be involved in p53-dependent apoptosis.

The pathway through which p53 promotes apoptosis involves transcriptional regulation of target genes as well as transcription-independent functions of p53, possibly reflecting distinct mechanisms of p53 action in different cell types.[11–20] p53-dependent apoptosis is dependent on the Apaf-1/caspase-9 pathway[21] and involves mitochondrial cytochrome *c* release.[22] How p53 elicits the release of cytochrome *c* to promote caspase activation remains elusive. A number of p53-regulated genes containing p53 responsive elements have been identified, and some of these represent potential downstream mediators of p53-dependent apoptosis (Figure 1). These include: *Bax*,[23] *CD95 (Fas/APO-1)*,[24,25] *Killer/DR5*,[26] *Ei24/PIG*8,[27–29] *Noxa*,[30] *PERP*,[31] *Pidd*,[32] *p53AIP1*,[33] and *PUMA*.[34,35]

p53-regulated genes encoding cell surface proteins

Killer/DR5[26] and *CD95 (Fas/APO-1)*[24,25] two members of the TNF receptor family, are induced by DNA damage in a p53-dependent manner and in some systems seem to be sufficient to induce apoptosis. Both proteins contain a death domain and provide a potential link between DNA damage-mediated activation of p53 and caspase activation. PERP is a plasma membrane protein whose induction by doxorubicin is correlated with activation of the p53-dependent apoptotic pathway in transformed mouse embryo fibroblasts. When overexpressed, PERP was shown to cause cell death in fibroblasts.[31]

p53-regulated genes encoding mitochondrial proteins

The *Bax* gene promoter contains a p53-binding site and was shown to be p53 responsive.[23] The proapoptotic Bax protein is known to accumulate in mitochondria in response to death signals. *Noxa* mRNA is induced by ionizing radiation in a p53-dependent manner. It encodes a 103-amino acid protein and contains a BH3 motif that is found on Bcl-2 family members. Noxa localizes to the mitochondria and, like Bax, was shown to interact with anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-X_L, Mcl-1) through its BH3 domain. Overexpression of Noxa induces apoptosis in a number of cancer cell lines.[30] *PUMA* (for p53 upregulated modulator of apoptosis) encodes a BH3-containing protein that also localizes to the mitochondria. PUMA protein interacts with Bcl-2 and Bcl-X_L through its BH3 domain. PUMA expression inhibits cell growth and rapidly induces apoptosis through a pathway involving cytochrome *c* release and activation of caspase 3 and 9.[34,35] p53AIP1 (for p53-regulated apoptosis-inducing protein 1) protein is located in mitochondria and its overexpression results in growth suppression and apoptosis. The induction of *p53AIP1* transcription in response to DNA damage is dependent on phosphorylation of p53 at Ser-46.[33]

p53-regulated genes encoding cytoplasmic proteins

A number of p53-induced genes (*PIG*s) may be involved in apoptosis through the generation of reactive oxygen species.[28] Ei24/PIG8 was initially isolated from mouse cells undergoing etoposide-induced cell death and later from human cells undergoing apoptosis in response to ectopic expression of p53.[27,28] Overexpression of Ei24/PIG8 suppresses cell growth and induces aopoptosis.[29] *Pidd* (for p53 induced protein with a death domain) encodes a protein of 915 amino acids in mice (910 amino acids in humans) and contains seven tandem leucine rich repeats (LRR) in the amino terminus and a death domain in the carboxy terminus. *Pidd* mRNA is induced by γ-irradiation in a p53-dependent manner and the basal level of *Pidd* mRNA is dependent on p53 status. Overexpression of *Pidd* inhibits cell growth in a p53-like manner by inducing apoptosis.

Antisense inhibition of *Pidd* expression attenuated p53-mediated apoptosis suggesting that *Pidd* expression is required for apoptosis.[32] A nearly identical molecule was isolated independently on the basis of its similarity to the death domain of hRIP and named LRDD (leucine repeat death domain containing protein).[36]

The identification of various proteins with apoptosis potential that act immediately downstream of p53 supports the notion of p53 as an apoptotic regulator and offers hope that elucidation of the p53-dependent apoptosis pathway is attainable. So far, however, no single molecule can be considered to be the principal mediator of p53-dependent apoptosis. This raises the question – why is p53 so prolific? Does this property of p53 betray a level of molecular uncertainty and indecision unworthy of the mighty 'guardian' or does it reveal a transcriptional activator's functional perfection?

The following observations may be useful in examining this conundrum.

(1) The p53-mediated transcriptional response to DNA damage is extremely complex. p53-regulated gene expression patterns differ not only in different cell types but also in response to different induction signals. There is substantial heterogeneity in the kinetics (timing and extent) of gene induction as well as on the dependency on p53 for gene induction. This heterogeneity is seen even in related cell lines derived from the same lineage.[37,38] The complexity of the p53 response may reflect the distinct pathways through which p53 can be activated in response to various stimuli.[39,40] Selectivity among different p53 target promoters could reflect differences in the affinity of various promoters for p53, such that some are responsive only to high levels of p53 or to certain modified forms of p53.[41]

(2) In adult mice exposed to whole body γ-irradiation, cells within certain tissues accumulate p53, and some of these cells undergo p53-dependent apoptosis (splenic and thymic lymphocytes, intestinal crypt cells) while other cells (in the lung, salivary gland, choroid plexus, adrenal gland, kidney) do not undergo apoptosis. Moreover, little or no p53 protein is detected in liver, skeletal muscle and brain. Hence, not all cells that accumulate p53 *in vivo* in response to DNA damage undergo p53-dependent apoptosis.[42–44] The restriction of p53-dependent apoptosis to certain tissues is likely related to the finding of selective transactivation of endogenous p53 target genes in different organs from irradiated mice.[45]

(3) Transgenic mice with p53-responsive reporter constructs demonstrate that p53 transcriptional activity is tightly controlled *in vivo* in response to γ-irradiation.[46,47] p53-dependent transgene induction is seen in the adult spleen, thymus and intestine as well as in most cells of the early but not late embryo. In general, cells which exhibit the strongest p53 transcriptional response belong to the highly proliferative, relatively undifferentiated compartment and it is these cells which are most sensitive to p53-dependent apoptosis.

(4) A transcription factor, like p53, will have many targets, and many of these will be codependent on other transcription factors that may or may not be coexpressed with p53. For example, the transcriptional regulation of *Bax* by p53 requires the cooperation of Sp1 or a Sp1-like factor through a 6 base pair motif (5′-GGGCGT-3′) adjacent to the p53 response element.[48]

(5) It remains unclear why certain cells undergo apoptosis in response to p53 activation while other cells undergo p53-dependent cell cycle arrest. Differences in response have been attributed to the presence of survival factors in the extracellular environment[49–52] and to intrinsic factors including cell type and genotype. For example, normal fibroblasts undergo p53-dependent G1 arrest in response to DNA damage whereas hyperproliferative fibroblasts such as those expressing E1A, c-*myc* or E2F-1 undergo p53-dependent apoptosis.[12,53–57] Another model proposes that the level of p53 determines whether a cell undergoes cell cycle arrest or apoptosis; growth arrest occurring at low p53 levels and apoptosis occurring with higher levels of p53.[58]

(6) p53 was shown to suppress tumor growth in a transgenic mouse model in which expression of a truncated form of SV40 T antigen (T_{121} – consisting of the amino-terminal 121 residues of T antigen) is directed to the brain choroid plexus epithelium.[59] T_{121} binds and sequesters Rb but is lacking the p53 binding domain. This transgenic model provides evidence that p53 acts to suppress tumor growth by mediating apoptosis of abnormally proliferating cells *in vivo*.[59] Crossing the TgT_{121} mice with *Bax* deficient mice showed an attenuation of p53-induced cell death.[60] However, deficiency in *Bax* resulted in a 50% reduction in apoptosis and accelerated tumor

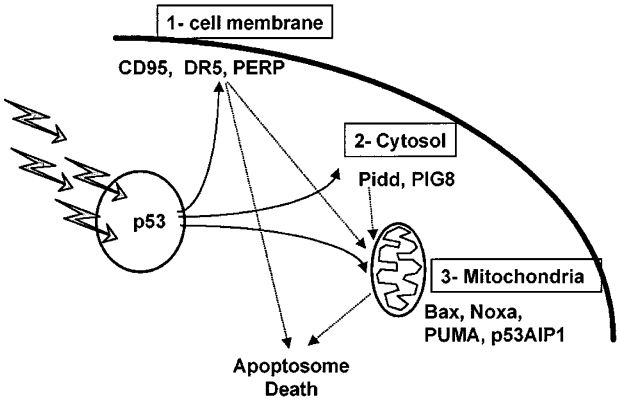

Figure 1 In response to a variety of stress stimuli, p53 protein becomes activated and acquires the ability to bind site specifically to DNA and to promote transcription of genes containing a p53-response element. A number of p53 target genes with pro-apoptotic activity have been identified. These fall into one of three categories. The first group includes genes that encode proteins that localize to the cell membrane (CD95, DR5, PERP); the second group encode proteins that localize to the cytosol (Pidd, PIG8); the third group encode proteins that localize to the mitochondria

growth threefold whereas deficiency in p53 resulted in 85% reduction in apoptosis and a sevenfold increase in tumor growth rate. These findings suggest that Bax mediates only part of the p53-induced cell death effect. In contrast, p53-dependent apoptosis occurs normally in irradiated thymocytes derived from *Bax*-deficient or *Fas*-deficient mice suggesting that *Bax* and *Fas* may be more relevant in some cellular contexts than others.[61–63]

Animal studies have played an important role in helping to define p53 as a tumor suppressor and in demonstrating the importance of p53-mediated apoptosis to tumor suppression. Not all tissues in p53-null mice are susceptible to tumor formation and not all tissues in normal mice respond to DNA damage or to abnormal proliferative signals by activating p53 and undergoing p53-dependent apoptosis. Tumor suppression by p53 is likely restricted to certain tissues and it is possible that efficient induction of apoptosis in different cells requires the activation of several apoptotic genes perhaps acting in concert or acting independently. Thus, p53 appears to induce apoptosis by multiple pathways in a manner that is regulated in a cell type and signal-specific fashion.

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