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News and Commentary

Renewing the debate over the p53 apoptotic response

JD Weber[1] and GP Zambetti*[,2]

[1] Department of Medicine, Washington University, St. Louis, MO, USA
[2] Department of Biochemistry, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105, USA. Tel: 901 495 3429; fax: 901 525 8025
* Corresponding author: GP Zambetti. E-mail: gerard.zambetti@stjude.org

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The context for p53 activation is widely variable with a common theme being cellular stress.[1] Once activated, p53 enforces cell cycle arrest or apoptosis; however, the factors that influence this decision process are poorly understood. We summarize here the prevailing models that represent our current understanding of these mechanisms.

Dictating cell fate by regulating the level of p53 expression

The degree to which p53 accumulates may influence whether cells cease to proliferate or undergo cell death (Figure 1a). Evidence to support such a model is derived from studies using Saos-2 cell lines that are engineered to conditionally express p53. Cells expressing low levels of p53 generally arrest in G1, while those that express high levels undergo apoptosis.[2] It is reasonable to speculate that p53-responsive genes that are involved in regulating cell proliferation, such as $p21^{Cip1}$, contain high-affinity binding sites within their promoters and these targets can be induced by low levels of p53. By contrast, apoptotic genes (e.g., *bax, noxa* and *puma*) may contain lower affinity sites that require much higher levels of p53 protein to be activated.[3–7] Under conditions where cell stress is particularly severe, p53 levels may be sufficiently induced to trigger the expression of apoptotic targets and cell death.

Paradoxically, transactivation of p53 target genes may not be absolutely necessary to induce apoptosis, since p53 fragments (amino acids 1–214 or 319–393) containing only the amino or carboxy terminus can elicit some degree of cell death despite their inability to bind DNA.[8,9] However, these studies should be considered with some reservation as they relied on the overexpression of p53 peptides to achieve an apoptotic response. This concern raises the important point that it is requisite to address p53 function(s) in a physiological manner. Indeed, mice that are genetically engineered to express a transactivation-defective mutant p53 protein from the endogenous locus as a knockin allele are defective in both growth arrest and apoptosis in response to cell stress.[10] Moreover, these animals are tumor prone. The requirement for an intact transactivation domain for p53 to function as a tumor suppressor in a physiological setting clearly supports the model that p53 must regulate the expression of genes involved in cell cycle arrest and cell death.

Cell context steers cell growth and survival responses

The induction of p53 expression in primary fibroblasts is usually associated with cell cycle arrest,[11,12] whereas the activation of p53 in hematopoietic cells (e.g., thymocytes) generally results in apoptosis.[13,14] Even within a particular cell type, the arrest and death response can be influenced by other cooperating factors. This is most evident from studies using a temperature-sensitive mutant p53 (tsp53) allele. Ectopic expression of tsp53 in immortal murine embryo fibroblasts elicits a sustainable G1 cell cycle arrest when shifted to the permissive temperature (32°C).[15] By contrast, the establishment of conflicting signals in these cells by enforced expression of E2F-1 or c-Myc, which is sufficient to promote cell cycle progression, induces an apoptotic response rather than growth arrest at the permissive temperature.[16,17] It is important to note that under these conditions all variables are held constant except for the antagonistic effects of the oncoproteins and wild-type p53 activity. Therefore, cell context significantly influences p53-mediated apoptosis and growth arrest responses (Figure 1b).

Ups and downs of p53-dependent cell death

Emerging evidence suggests that both induced and repressed target genes are required for p53-mediated cell death. Using differential display and microarray approaches, a subset of genes (*Map4* and *stathmin*) were identified that are selectively downregulated upon p53 activation, under conditions where the cells responded by undergoing apoptosis.[18–20] Consistent with these findings, enforced expression of *Map4*, which encodes a microtubule-associated protein, protects against p53-mediated cell death.[18] The Sin3a co-repressor cooperates with p53 in transrepression of *Map4* as it forms a complex at the promoter of this gene only during expression of wild-type p53.[21] Histone deacetylases (HDACs) also appear to play an essential role in this regulation as treatment with trichostatin A (TSA), which inhibits HDAC activity, blocks p53-mediated transrepression of *Map4* and promotes cell survival.[21] Interestingly, TSA has no obvious effects on p53-transactivation functions, implying that the induction of responsive genes is not sufficient to elicit a cell death response. Rather, p53-dependent cell death may occur only when the proper targets are coordinately induced (*puma, noxa* and *bax*) and repressed (*Map4* and *stathmin*) (Figure 1c).

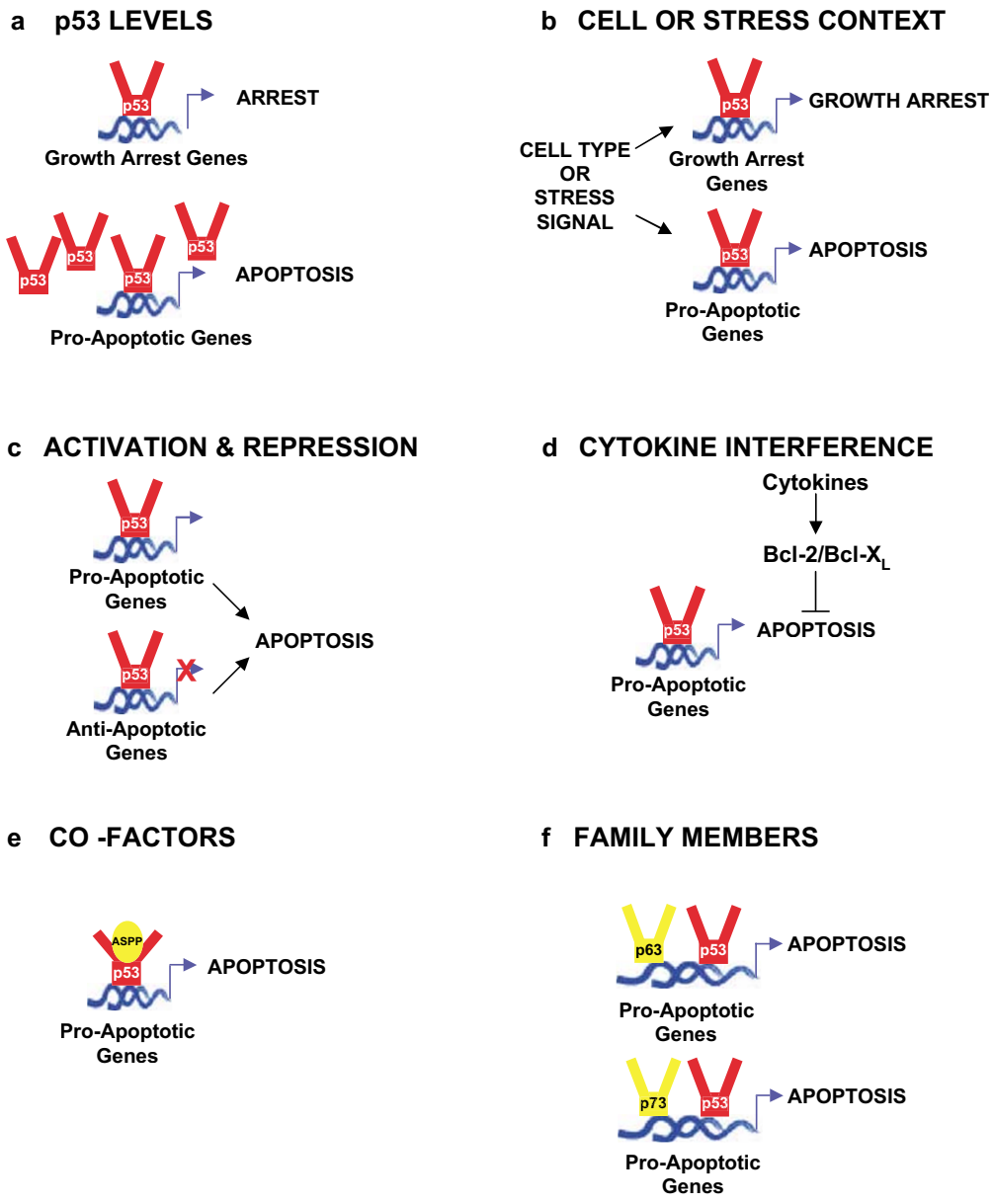

Figure 1 Existing models for the p53 apoptotic pathway. (**a**) Low levels of p53 bind to high-affinity consensus sites within genes that negatively control cell cycle progression (e.g., *p21*^{Cip1} and *Gadd45*α), whereas high levels of p53 are required to bind lower affinity sites in genes that trigger the apoptosis (e.g., Bax and Puma).[2] (**b**) Different cell types display mixed responses to p53 activation, with fibroblasts generally undergoing cell cycle arrest and hematopoietic cells undergoing apoptosis. However, intracellular signals, such as those elicited by oncogenes (e.g., Myc and E2F) can conflict with the normal p53 cell cycle responses and induce apoptosis.[16,17] (**c**) DNA microarray experiments have provided data consistent with the idea that p53 activates and represses downstream target genes. The coordinated activation of proapoptotic genes and repression of antiapoptotic genes may be required to elicit fully a p53 apoptotic response.[18,19] (**d**) Extracellular factors and environmental cues can influence p53-mediated cell fate decisions. While cytokines do not disrupt p53-targeted gene transcription, they do interfere with p53-mediated cell death by upregulating key cell survival factors, such as Bcl-2 and Bcl-X_L.[22–26] (**e**) p53 may associate with cofactors to induce synergistically proapoptotic genes. Two such cofactors, ASPP1 and ASPP2, enhance p53's ability to bind to the promoters of proapoptotic genes, but not cell cycle targets.[37] (**f**) Two additional family members, p63 and p73, act in concert with p53 to induce proapoptotic gene expression. Cells lacking either *p63* or *p73* retain p53 activity, while loss of both *p63* and *p73* results in a severe reduction in p53-dependent apoptosis, demonstrating the cooperation of p53 with each of its new family members in initiating proapoptotic gene expression.[41]

Cytokines intercept the p53 death signal

In trying to the understand the p53-dependent cell death process, especially in light of the requirement for an intact transactivation domain, a great deal of effort has been invested in defining proapoptotic downstream target genes. This linear line of investigation has been fruitful to some extent and has identified potentially important mediators of p53-dependent cell death (see above). An alternative approach that has also been rewarding is based on the early observation by Oren and co-workers,[22] as well as others,[23,24] that certain cytokines can efficiently block p53-mediated

apoptosis. For example, ectopic expression of tsp53 in murine myeloid leukemia M1 cells, which are devoid of endogenous p53, induces rampant cell death when shifted to the permissive temperature.[22] Quite remarkably, treatment of these cells with interleukin (IL)-6 efficiently protects against cell death, despite the conversion of tsp53 into the wild-type conformation and the induction of its target genes. Subsequent studies demonstrated that cytokines do not interfere directly with p53; rather, they block p53-mediated cell death, at least in part, by upregulating the expression of Bcl-2 and Bcl-X_L.[25,26] In turn, these potent survival factors can intercept the p53 death signal by binding p53-regulated BH3-containing proapoptotic proteins, such as Bax, Noxa and Puma (Figure 1d). As all the relevant variables within these cells are the same, including the level of tsp53 protein and its transcriptional activity, these findings represent yet another example of how outside factors and environmental cues can influence p53-mediated cell death responses.

Does the form of cellular stress influence the p53 response?

The nature of the upstream signaling pathways may influence p53 post-translational modifications and consequently, this could impact whether p53 affects cell proliferation or survival. Detailed genetic and biochemical analyses demonstrated that DNA-damaging events trigger p53 activation through the engagement of ATM, ATR and Chk-1 and 2.[27] Phosphorylation of p53 by these protein kinases is required to release p53 efficiently from its negative regulator Mdm2,[28] thus stabilizing p53 and activating its tumor suppressor functions. By contrast, hyperproliferative signals emanating from activated *ras* and *myc* oncogenes activate p53 indirectly through the induction of $p19^{ARF}$, an alternative reading frame gene product of the *INK4a* locus,[29] and this occurs independently of DNA damage and the ATM/ATR pathway. It is not clear how ARF is regulated by inappropriate cell growth, but when expressed at sufficient levels, ARF activates p53 by binding Mdm2, thereby blocking its E3-ubiquitin ligase activity.[30] ARF also physically relocalizes Mdm2 to the nucleolus, which allows for the nucleoplasmic activation of p53.[31,32] This process may be considered a novel topological activation of p53 requiring no direct modification of p53. However, this is clearly not the case and the sites that are modified during ARF activation differ from those that occur during DNA damage (GZ, data not shown).

Conceivably, the manner in which p53 is modified could shift the response from arrest to death, by directing p53 to specific target genes (Figure 1b). Consistent with this reasoning, emerging evidence implicates phosphorylation of p53 at serine 46 as a necessary step for apoptosis in response to severe DNA damage. Mutation of serine 46 to alanine selectively impairs the induction of *p53AIP1*(a potential proapoptotic p53-target gene), but not other responsive genes such as $p21^{Cip1}$,[33] and compromises its ability to induce apoptosis. Phosphorylation of serine 46 is regulated by the recently identified homeodomain-interacting protein kinase-2 (HIPK2) and wild-type p53-inducible phosphatase (Wip1/PPM1D).[34–36] Interestingly, Wip1/PPM1D is overexpressed in human breast cancer cell lines and primary tumors that maintain wild-type p53 status.[36] The apoptotic response is likely attenuated in these tumors because of the elevated levels of the phosphatase, which suppresses p53 phosphorylation and activation. In light of these findings, it is reasonable to speculate that various forms of cell stress as well as cell context, perhaps by regulating the expression of modifiers such as HIPK2 and Wip1, influences post-translational modifications of p53 and ultimately determines cell fate.

Role of cofactors in p53-dependent apoptosis

Although purified recombinant p53 protein binds directly to DNA in a sequence-specific manner, it does so within the cell in complex with other proteins. Some of these proteins may direct p53 to specific promoters and therefore, affect cell cycle arrest or cell survival responses (Figure 1e). Two such proteins, ASPP1 and ASPP2, have recently been identified that appear to function in this capacity.[37] Ectopic expression of wild-type p53 with ASPP1 or ASPP2 induces apoptosis of human osteosarcoma Saos-2 cells in a synergistic manner. Conversely, interference with endogenous ASPP gene expression using an antisense approach attenuates p53-dependent cell death in response to DNA damage. Consistent with its apparent role in regulating apoptosis, ASPP proteins stimulate the expression of endogenous *Bax* in cells containing wild-type p53. This response correlates well with the ability of ASPP proteins to enhance selectively p53 binding to the Bax promoter *in vivo* and to stimulate the promoters of proapoptotic responsive genes (*Bax* and *PIG3*), but not other targets ($p21^{Cip1}$, *cyclin G* and *Mdm2*).[37] As discussed above, proapoptotic p53 target genes generally have low-affinity binding sites, and ASPP proteins may stimulate p53 DNA binding activity sufficiently to trigger the expression of this subset of genes. Thus, p53-mediated cell death may be influenced by cofactors, such as ASPP1 and ASPP2, which could be expressed in a cell context- or cell stress-dependent manner.

Adding family members to the mix

In the last few years, another layer of complexity has been added to the regulation of p53 and its downstream effectors. Two additional p53 family members, p63 and p73, have been identified that are similar in sequence and biochemical properties.[38,39] While initially hailed as potentially redundant p53 family tumor suppressors, p63 and p73 have distinguished themselves from p53 in terms of their cellular functions. For example, the p73-knockout mouse is not tumor prone, although other interesting developmental abnormalities are associated with this genetic defect.[40] However, recent findings have reunited these family members at least in terms of their ability to regulate cell survival properties. Cells lacking either *p63* or *p73* display an intermediate resistance to apoptotic signals when compared to *p53*-null or wild-type cells (Figure 1f).[41] Surprisingly, cells deficient for both *p63* and *p73* resemble the apoptotic resistance seen in *p53*-null cells, demonstrating a requirement for p63 and/or p73 in p53-

mediated apoptosis.[41] Further tying together the models described above, profiles of p53-responsive genes differed in the presence and absence of p63 and p73. Induction of the cell cycle inhibitor $p21^{Cip1}$ during DNA damage was normal in the absence of p63 and p73, while the expression of proapoptotic genes, such as *bax* and *PERP*, was severely reduced.[41] The importance of these findings are two-fold: (1) additional p53 family members are required for the proper expression of p53 apoptotic effectors; and (2) cell cycle regulators and proapoptotic molecules can be separated in terms of their mechanism of induction by p53.

Final thoughts

Distinct differences between the models of p53-mediated apoptosis appear to be blurred as we learn more about this process. Indeed, a combination of all of the models seems to be warranted and recent findings are bearing this out. Cells lacking functional p53 proapoptotic effectors do not select for p53 mutations during Myc-induced lymphomagenesis.[42,43] Likewise, human breast carcinomas that are deficient in the p53 signaling pathway (e.g., overexpressing Wip1/PPM1D or lacking ASPP) can tolerate wild-type p53 expression.[36,37] It is becoming apparent that upstream signals may also drive cell fate responses. As these signals are relayed, p53 levels and post-translational modifications are changed to accommodate the setting, and different subsets of p53 effectors are transcribed to yield the appropriate cellular response. Repairable defects, in general, may require cell cycle arrest, while more serious perturbations may instill an apoptotic regimen. Collectively, these responses maintain genomic integrity and stability, and are essential to protect against tumorigenesis.

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