Cell, Vol. 103, 691–694, November 22, 2000, Copyright ©2000 by Cell Press

p53: Death Star

Minireview

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From among the cast of thousands that appear to play a role in the regulation of programmed cell death, p53 has emerged as one of the leading stars. p53 is a protein of many talents, including activation of cell cycle arrest, senescence, and differentiation. The special appeal of p53, however, is as an apoptotic superhero, a protein that functions to selectively destroy stressed or abnormal cells, thereby protecting the organism from cancer development. The key contribution of p53's apoptotic activity to tumor suppression raises two quintessential questions: what spurs p53 into action and how does p53 implement the death sentence? Several recent publications have thrown some interesting light on both issues.

Rules of Engagement: Regulation and Activation of p53

Since p53 is such a proficient inhibitor of cell growth, restraint of p53 activity during normal growth and development is of paramount importance. p53 function is controlled through several mechanisms, one of the most effective being regulation of protein stability. Central to this process is MDM2, an E3 ligase that targets both p53 and itself for ubiquitination. This function of MDM2 has been shown to play a role in allowing export of p53 from the nucleus to the cytoplasm, and degradation of p53 by the proteasome (Vousden and Vande Woude, 2000). MDM2 is a transcriptional target of p53, creating a negative feedback loop where p53 activates expression of MDM2, which keeps p53 levels low during normal growth and development. Activation of p53 in response to cellular stress such as DNA damage, oncogene activation, telomere erosion and hypoxia is mediated, at least in part, by inhibition of MDM2 and rapid stabilization of the p53 protein. Depending on the activating signal, several mechanisms to perturb MDM2 function have now been described, including phosphorylation of p53 and MDM2 to block the interaction between the two proteins, or selective downregulation of MDM2 expression. Of particular interest has been the discovery that several oncogenes can induce stabilization of p53 by enlisting the activity of ARF, a protein that functions by binding directly to MDM2, inhibiting the ubiquitination of p53 and allowing accumulation of p53 in the nucleus. Although activation of ARF and stabilization of p53 alone is not sufficient to induce death in all cell types, upregulation of p53 in this way strongly sensitizes cells to die in response to other insults such as DNA damage, loss of survival signals, or additional apoptotic signals induced by the oncogenes themselves. Loss of ARF makes cells much easier to transform, since they are less able to induce the defensive p53 response to oncogene activation, and in mice deletion of ARF strongly accelerates tumor development (Sherr and Weber, 2000).

The ability of ARF to connect oncogene activation with the p53 pathway has prompted the search for mechanisms that regulate ARF, which, like p53, is expressed at very low levels in normal cells. ARF expression can be directly activated by the transcription factors DMP1 and E2F1, or downregulated by factors such as Twist and Bmi1. The induction of ARF following activation of oncogenes such as Myc, Ras, and E1A may therefore, in part, reflect the activation of E2F1, and both Twist and Bmi1 can counteract this oncogene-induced increase in ARF expression. Now a recent study has identified DAP kinase, another protein with the characteristics of a tumor suppressor, as an additional player in this pathway (Raveh et al., 2001). DAP kinase plays a role in the induction of ARF and stabilization of p53 in response to Myc and E2F1 overexpression (Figure 1) and both Myc and E2F1 were shown to upregulate DAP kinase activity. The identification of a kinase in the ARF pathway is extremely provocative, although so far the critical targets for DAP kinase phosphorylation remain unknown.

The ability of mitogenic signals, such as deregulated Myc, Ras, or E2F-1, to lead to the activation of p53 provides an effective mechanism to prevent abnormal proliferation associated with tumor development. However, these fail-safe pathways pose a potentially serious problem to cells that want to undergo legitimate proliferation. Clearly the activation of p53 needs to be dampened in some way to allow normal growth, and recently a couple of strategies to temper the induction of p53 by Ras have been described. In one system, the ability of Ras to directly activate expression of MDM2 provides a foil for the activation of ARF, which is also induced by Ras and inhibits MDM2 function (Ries et al., 2000) (Figure 1). Similarly JunD, which is activated by Ras, can negatively regulate ARF expression, again counterbalancing the ability of Ras to activate ARF (Weitzman et al., 2000). It would appear that the success of normal

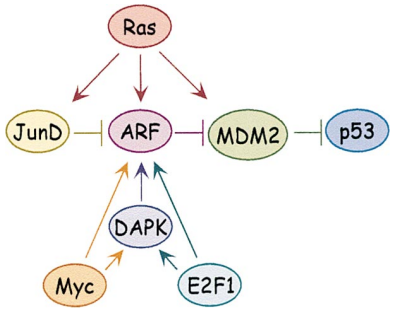

Figure 1. Activation of E2F1 and Myc Lead to Induction of ARF, in Part through DAP Kinase, thus Inhibiting MDM2 and Stabilizing p53

Mitogenic signaling by Ras can activate p53 through ARF, but in normal cells this protective response is dampened by concomitant activation of MDM2 and inhibition of ARF by JunD.

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cell division depends on the intricate coordination of signals to allow proliferation without simultaneous activation of cell cycle arrest or cell death. Disruption of this precarious balancing act, through unusually high mitogenic signals or loss of normal survival signals, activates the strong safeguards that inhibit cell growth and so protect against these types of abnormalities.

Natural Born Killer: Apoptotic Functions of p53

Having activated p53, the second burning question becomes, how does p53 function, particularly in the induction of apoptosis? The best understood activity of p53 is its ability to function as a transcription factor that can induce or repress expression of a large and growing number of genes, although less well defined transcriptionally independent activities of p53 have also been described (Bates and Vousden, 1999). The importance of transcriptional regulation by p53 has been demonstrated in many studies, most recently by the generation of mice or embryonic stem cells in which substitution of a transcriptionally inactive mutant p53 for the wild-type protein resulted in loss of cell cycle arrest and apoptotic functions (Chao et al., 2000; Jimenez et al., 2000). Although the ability of p53 to repress transcription correlates well with some activities, particularly the induction of apoptosis, the mechanisms and requirements for p53-mediated transcriptional repression remain elusive, and it is the activation of gene expression by p53 that has received the lion's share of attention. Transcriptional activation of the $p21^{WAF1/CIP1}$ cyclin-dependent kinase inhibitor plays a key role in the induction of cell cycle arrest by p53, but there does not appear to be a similar single critical apoptotic target. Bax, one of the first apoptotic genes shown to be regulated by p53, is not induced by p53 under all circumstances, and is not absolutely required for p53-mediated death. Numerous other possible apoptotic targets have been described over the past years, including several redox-related genes which led Polyak and colleagues to propose a model in which p53 regulates apoptosis through generation of reactive oxygen species (reviewed in Bates and Vousden, 1999). It is now becoming apparent that there will be an abundance of genes that can mediate p53-dependent cell death, although as yet it is unclear whether each contributes a part to the full response, or whether specific subsets of these genes are required for death in different cell types, or in response to different signals.

p53 can directly engage each of the major apoptotic pathways in the cell, stimulating both death receptor signaling and mitochrondrial perturbations, including cytochrome c release (Figure 2). Loss of caspase 9 or Apaf-1 renders mouse fibroblasts resistant to p53-dependent apoptosis (Soengas et al., 1999), and similarly, caspase 8 has been shown to play a role in transcriptionally independent apoptotic activities of p53 (Ding et al., 2000). Several recently described p53 transcriptional targets encode proteins that localize to the mitochondria and affect mitochondrial membrane potential, an activity that sends a strong apoptotic signal through this pathway. These include Noxa (Oda et al., 2000a), a protein containing a BH3 domain that interacts with the antiapoptotic Bcl2 protein, and p53AIP1 (Oda et al., 2000b), a novel protein with no known homologs. A recent addition to the death receptor pathway is PIDD, a death domain containing protein, that would be predicted to interact with the complex that transmits the apoptotic signal from the death receptor (Lin et al., 2000). Although by no means the only apoptotic genes that can be targeted by p53, these new members of the p53-inducible club have passed the acid test for bona fide mediators of p53 apoptosis, in that each of them has been shown to be required for the full apoptotic response, under some conditions at least. Most of this evidence has been gleaned from antisense experiments in tissue culture cells, and we await with interest the consequence of deleting these genes in mice.

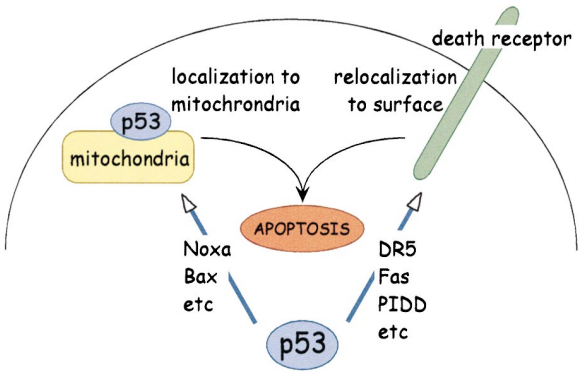

Figure 2. The Two Major Apoptotic Pathways in Cells Can Be Activated by p53

Alterations in mitochrondrial membrane potential and/or cytochrome c release may result from transcriptional activation of BH3 containing proteins such as Noxa or Bax, or even localization of p53 itself to the mitochondria (Marchenko et al., 2000). Signaling from death receptors is elevated following transcriptional activation of receptors such as DR5 and Fas (Bates and Vousden, 1999), or death domain containing proteins like PIDD. Transcriptionally independent activities of p53 such as relocalization of death receptors from the Golgi to the cell surface (Bennett et al., 1998) would also engage this pathway.

Judgement Day: Choice of Response to p53

Maybe one of the most intriguing questions about p53's apoptotic response is why do some cells show it, and others not? Or maybe even more importantly, why do transformed versions of some cell types die in response to p53, while their normal counterparts show a potentially reversible cell cycle arrest? This differential between normal and tumor cells has great potential in the development of new cancer therapies based on reactivation of p53, which might preferentially kill tumor cells, while sparing normal tissue. Clearly not all cell types require transformation to be sensitive to p53-induced death, and inhibition of the p53-mediated apoptotic response in normal gut epithelium and hematopoietic cells has been proposed as a mechanism to prevent some of the side effects of chemotherapeutic drugs (Komarov et al., 1999). Nevertheless, the notion that for some tissues, at least, p53 mediates tumor-specific killing has prompted much optimism for the success of p53-based therapies.

One interesting question to emerge from these studies concerns the contribution, if any, of p53 to the choice of response (Figure 3). Although various p53 mutants have demonstrated a separation of apoptotic and cell cycle arrest functions, it is possible that p53 plays no

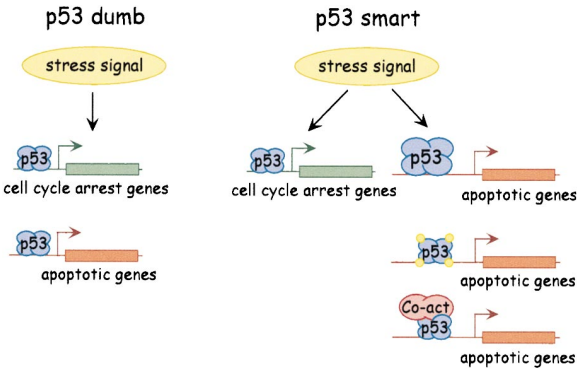

Figure 3. Activation of p53 Can Lead to the Expression of Cell Cycle Arrest and Apoptotic Target Genes

In the first model (p53 dumb), the contribution of p53 to the induction of these two groups of genes is the same under all conditions, although additional, independent transcription factors may play a role in differentially regulating expression. In the second model (p53 smart), p53 itself is responsible for the differential expression of the apoptotic genes. This could be a consequence of higher overall levels of p53 in the cell, or modifications of p53 that affect DNA binding or the interaction with transcriptional coactivators.

role in determining how the cell will respond, but always sends exactly the same signals after activation, including induction of both cell cycle arrest and apoptotic target genes. This notion is supported by the observation that inhibition of p53-induced apoptosis reveals a concomitant p53-dependent cell cycle arrest. In this "p53 dumb" model, activation of p53 always results in an apoptotic signal, which is either impeded by survival signals, or augmented to reach the threshold level required to kill the cells by additional, independent apoptotic signals. Certainly, oncogenes such as Myc or E2F1 can activate p53-independent apoptotic signals, in addition to activating p53 itself, and convergence of both oncogene- and p53-induced signals is necessary to efficiently kill many cell types. Although generally consistent with much of the available evidence, some adaptation of the p53 dumb model is required to accommodate the observation that cell cycle arrest and apoptotic target genes of p53 can be differentially regulated, and that expression of some p53-inducible apoptotic genes is activated only in cells undergoing p53-dependent death (Attardi et al., 2000; Relaix et al., 2000). In a variation of this model, p53 functions in the same way under all circumstances, but the success of transcriptional activation of some apoptotic target genes depends on the availability of additional transcription factors that also bind these promoters. It is possible, for example, that the contribution of NF-κB to p53-mediated death could reflect a requirement for both transcription factors in the activation of critical apoptotic target genes (Ryan et al., 2000), although NF-κB is not required for the induction of cell cycle arrest genes by p53.

An alternative to the p53 dumb model is one in which the choice of response to p53 activation is governed, at least in part, by activities of p53 itself. In this "p53 smart" model, p53 functions differently in cells destined to undergo cell cycle arrest than in cells doomed to die, and different forms of p53 can activate cell cycle arrest or apoptotic target genes. It has been noted that the abundance of p53 can affect the choice of response, with low amounts of p53 leading to cell cycle arrest which turns to apoptosis as p53 levels increase. One simple explanation for this observation is that the promoters driving the apoptotic target genes bind p53 with a lower affinity, and are thus only activated when p53 induction is high or prolonged. In an extension of this model, promoter binding can also be regulated by modifications of p53, and a recent report has shown that phosphorylation of p53 on serine 46 governs its ability to regulate expression of at least one apoptotic target, p53AIP1 (Oda et al., 2000b). Interestingly, phosphorylation of serine 46 was shown to correlate with the onset of apoptosis, suggesting that this modification allows p53 to function in the induction of cell death. Although the mechanism by which phosphorylation regulates p53's transcriptional activity is not known, there are several intriguing possibilities. Phosphorylation could result in slight changes in the conformation of p53, which alter DNA binding specificity. Alternatively, modification of p53 may affect its ability to interact with coactivators that are necessary specifically for expression of apoptotic genes. JMY, for example, is a cofactor that selectively contributes to the activation of only a subset of p53 responsive genes, thereby augmenting the apoptotic response to p53 (Shikama et al., 1999).

Several modifications of p53 have been shown to regulate DNA binding and transcriptional activity, including phosphorylation, acetylation, sumoylation, and glycosylation, providing plenty of mechanisms by which direct modification of p53 itself could regulate the choice of response. It is unclear whether modifications of p53 that allow expression of apoptotic targets diminish the ability to activate cell cycle arrest genes, although the apparent retention of the cell cycle arrest response in dying cells suggests that versions of p53 that can drive cell death retain the ability to activate the other responses. It is easy to conceive of models in which survival signals lead to modification of p53 so that only cell cycle arrest genes can be activated, or in which oncogenes send signals that modify p53 to allow expression of apoptotic targets. How much of this speculation is based in fact remains to be seen, but given the intense interest in all aspects of the p53 story it is unlikely that these questions will remain unanswered for long.

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