Outcomes of p53 activation – spoilt for choice

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Summary

The p53 tumour suppressor protein can efficiently inhibit tumour development. This activity reflects its ability to induce a number of different responses, including cell cycle arrest and apoptosis. Recent studies have revealed some interesting insights into how the choice of response to p53 is regulated, highlighting a correlation between the activation of cell cycle arrest and survival with the ability of p53 to reduce oxidative stress and protect cells from genotoxic damage. Understanding the molecular mechanisms that determine which response is selected may allow us to modulate these pathways so that therapeutic reactivation of p53 favours apoptotic cell death in tumour cells, but a reversible – and therefore far less toxic – induction of cell cycle arrest in normal cells.

Key words: p53, Apoptosis, Survival

Introduction

The *p53* tumour suppressor gene plays an important role in preventing cancer development, and loss of p53 function, or loss of the ability to activate a p53 response, appears to be a prerequisite for malignant progression. In both mice and humans, germline mutations in *p53* result in a strong predisposition to cancer (Lozano and Zambetti, 2005). The mechanisms by which p53 functions to afford us this protection appear to be related to its ability to respond to stress and contribute to either the repair of stress-induced damage or the inhibition of further proliferation of stressed cells. In this way disparate signals that could constitute oncogenic danger – such as oxidative stress, DNA damage, hypoxia, oncogene activation or loss of normal stromal support – all lead to the induction of a p53 response (Vousden, 2002). However, the ultimate response to p53 can be quite different, ranging from a reversible cell cycle arrest to the induction of a number of irreversible responses, such as cell death or senescence. This dramatic distinction in the outcome of p53 activation – death or survival – leads to obvious questions of how the choice of response is regulated and why p53 initiates these different responses. To some extent the life or death of the cell is strongly influenced by the presence or absence of p53-independent death or survival signals that cooperate with the p53-activated responses. However, the activity of p53 can also be adjusted to favour one response over another. This Commentary briefly summarises some of the recent insights into this complex system.

Transcriptional functions of p53

Probably the best understood activity of p53 is as a transcription factor that has sequence-specific DNA-binding activity and the potential to induce the expression of a large number of genes. Although bioinformatics studies suggested that there may be >4000 human genes that contain p53-binding sites (Lu, 2005), direct analysis using various chromatin-immunoprecipitation-based techniques have more recently placed this number between 500 and 1600 genes (Cawley et al., 2004; Wei et al., 2006) – still a daunting proposition. However, some order can be brought to these large numbers if we group the known genes according to their function. This reveals much broader possibilities for p53 than has been fully appreciated. While genes that might contribute to the well-established responses of cell cycle arrest, apoptosis and DNA repair are certainly well represented, the identification of groups of genes that contribute to processes such as metabolism or cell adhesion indicate that p53 might play an important role in cells beyond simply determining whether they arrest or die (Wei et al., 2006).

Of course, the ability of p53 to bind the regulatory region of any particular gene might result in the up- or down-regulation of expression, or maybe even both, depending on the circumstance. Most previous studies have suggested that genes containing a specific p53-binding site show enhanced expression in response to p53; the requirements for transcriptional repression by p53 are less well defined. In general, transcriptional repression by p53 does not require a p53-binding site but is mediated indirectly by protein-protein interactions involving p53 itself or by its downstream targets. However, examination of a small number of genes with p53-binding sites suggested that about half would be up- and half down-regulated in response to p53 activation (Wei et al., 2006). Indeed, the presence of a p53-binding site can lead to both repression and activation by p53, depending on other factors that are available. For example, the interaction of Foxo3a with p53 results in a switch from repression to activation of *SIRT1* promoter by p53 in response to nutrient starvation (Nemoto et al., 2004).

Differential activation of target genes

One of the most interesting aspects of the p53-responsive genes is that they are not all equally and coordinately regulated in response to p53. Indeed, the evidence suggests that promoter selection plays an integral part in determining the response to p53. This was initially illustrated by the identification of p53 mutants that retain the ability to induce expression of only a

subset of target genes, when compared with wild-type p53 (Friedlander et al., 1996; Ludwig et al., 1996; Menendez et al., 2006). The significance of these observations has now been supported by many studies showing that differences in the sequence and spacing of the p53-binding site, the overall levels and post-translational modifications of p53 and the presence or absence of transcriptional cofactors can all contribute to promoter selection and choice of response (Fig. 1).

Most of the studies to date examining the differential ability of p53 to activate target genes have concentrated on the regulation of cell cycle arrest versus apoptotic targets. A study examining the binding of p53 to different DNA-binding sites in yeast and mammalian systems showed a difference in the ability of p53 to bind sites derived from genes involved in cell cycle arrest and/or DNA repair compared with genes regulating mitochondrial apoptotic pathways (Quian et al., 2002). Although of course not all the potential apoptotic genes were examined, the results suggest that whereas only the binding site sequences are required for p53-dependent activation of the cell cycle arrest genes, additional sequences – and maybe additional transcription factors that bind DNA independently of p53 – are needed for the induction of expression of many of the apoptotic genes. An example of such cooperation is seen between p53 and NF-κB, another transcriptional activator that

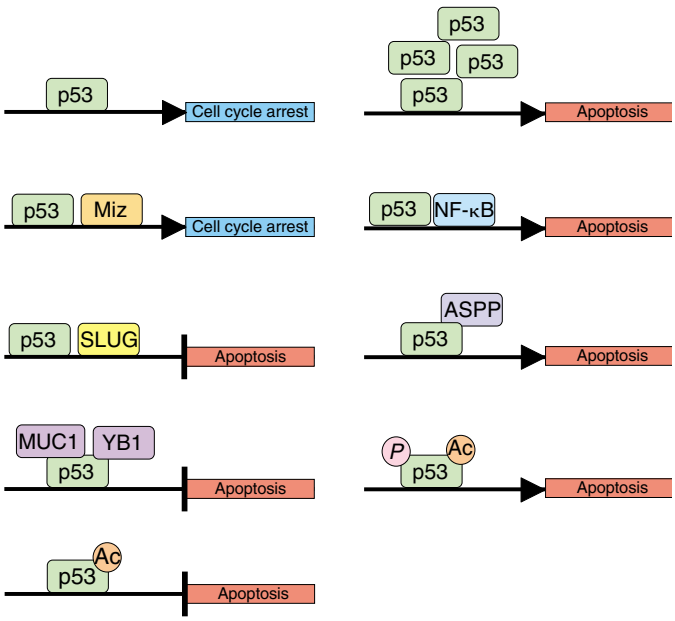

Fig. 1. Some of the possible mechanisms through which promoter selection of p53 can be regulated. On the left are mechanisms that contribute to the activation of a cell-cycle-arrest response. These include cooperation with other transcription factors, such as Miz, which may be necessary for activation of cell-cycle-arrest genes like *p21*$^{WAF1/CIP1}$, as well as factors that repress the induction of apoptotic gene expression. On the right, are mechanisms that promote cell death through activation of apoptotic target genes. These may depend on increased levels of p53 protein, cooperating transcription factors such as NF-κB, p53-binding proteins like ASPP, or post-translational modification of p53. Acetylation (Ac) has been linked to both increased and decreased expression of apoptotic target genes. These possibilities may be linked – for example, phosphorylation enhances interaction with proteins such as ASPP.

plays an important role in the regulation of apoptosis. Although NF-κB is generally associated with inhibition of death, pro-apoptotic functions of NF-κB have also been described. This duality extends to its influence on p53: NF-κB inhibits p53-induced death in some systems, but is required for efficient p53-induced apoptosis in others (Ryan et al., 2000; Tergaonkar et al., 2002). The cooperation between p53 and NF-κB is most likely to reflect their ability to function together to induce expression of apoptotic target genes regulated by promoters containing both p53- and NF-κB-binding sites, such as that of the death receptor DR5 (Shetty et al., 2005). Similarly, both p53 and Miz are required for the activation of expression of p21$^{WAF1/CIP1}$ – a cyclin-dependent kinase (CDK) inhibitor (Herold et al., 2002), although this cooperation contributes to cell cycle arrest and survival rather than death. Conversely, binding of transcriptional repressors to selected promoters that also contain p53-binding sites can play an important role in modulating p53 activity. Repression of expression of PUMA – one of the key p53-induced inducers of cell death – by SLUG is responsible for protecting hematopoetic progenitor cells from p53-induced apoptosis (Wu et al., 2005).

Clearly the cooperation between p53 and other transcription factors that interact with discrete DNA-binding sites within different promoters can hugely influence the pattern of gene expression in response to p53. However, additional complexity is provided by evidence that modulation of p53 itself may also allow the differential recognition of target-gene promoters. One interesting possibility is that changes in the conformation of p53 allow selective recognition of different p53-binding sites, a suggestion that is supported by the high degree of flexibility seen in the ability of p53 to bind DNA (Kim and Deppert, 2006). This model might mean that the mutants of p53 that only activate expression of one group of genes are locked in the conformation that only recognises the binding sites present in these promoters. Wild-type p53, by contrast, would presumably be modulated to switch between conformations, thereby allowing the recognition of different classes of promoter.

There are a number of mechanisms by which promoter recognition by p53 seems to be modulated, including post-translational modification and interaction with other proteins. In mice, the ability to phosphorylate serine residues 18 and 23 (equivalent to serines 15 and 20 in human p53) is necessary for apoptosis and tumour suppression, but not cell cycle arrest and senescence (Chao et al., 2006a). Phosphorylation of human p53 on serine 46 has been shown to contribute specifically to the activation of some apoptotic target genes, and mutation of this phosphorylation site reduces the ability of p53 to induce cell death but not proliferative arrest (Mayo et al., 2005; Oda et al., 2000). Phosphorylation of serine 46 can contribute to the interaction of the second trans-activation domain of p53 with the p62/Tfb1 subunit of the general transcription factor TFIIH (Mayo et al., 2005). Although not necessary to induce expression of all p53 target genes, this interaction may contribute to the activation of a specific subset of p53-responsive genes. Phosphorylation at serine 46 has also been linked to the ability to p53 to repress expression of galectin-3, an anti-apoptotic protein that can protect from p53-induced death (Cecchinelli et al., 2006).

Serine 46 is the target of several kinases, and phosphorylation at this site by p38 MAP kinase is regulated by

PPM1D/Wip1, a phosphatase that is itself a transcriptional target of p53 (Bulavin et al., 2002). It is possible, therefore, that selective activation of Wip1 determines the outcome of p53 induction by indirectly regulating serine 46 phosphorylation. Regulation of the apoptotic activity of p53 following differential acetylation of the C-terminus of p53 has also been shown to modulate apoptotic activity, in part by affecting the phosphorylation of N-terminal sites (Bulavin et al., 2002; Chao et al., 2006b; Knights et al., 2006).

Although phosphorylation and other modifications might directly influence the selection of binding sites by p53, they may also function less directly, by regulating the interaction of p53 with co-activator proteins. Several p53-interacting proteins may play a role in differential binding of p53 to different sets of promoters (Bulavin et al., 2002). One of the best described is the ASPP family of proteins, of which ASSP1 and ASSP2 bind to the DNA-binding domain of p53 to allow induction of apoptotic genes (Samuels-Lev et al., 2001). Inhibition of these ASPP proteins by the third family member, iASPP, can selectively prevent apoptosis in response to p53 (Bergamaschi et al., 2003). The ASPP proteins also serve a similar function in the regulation of the response to the p53 family members p63 and p73 (Bergamaschi et al., 2004). In an interesting twist, these p53 relatives are themselves required for p53-induced apoptosis in some cell types (Flores et al., 2002) and can also play an essential role in allowing the interaction of p53 with the promoters of some apoptotic genes. p53-binding proteins can also selectively impair the ability of p53 to regulate transcription. Binding to YB1 or MUC1, for example, selectively inhibits the ability of p53 to induce apoptotic target genes (Homer et al., 2005, Wei et al., 2005), whereas binding to KLF5 can abrogate p53-dependent repression of the inhibitor of apoptosis, Survivin (Zhu et al., 2006)

Transcription-independent apoptotic functions of p53

As described above, the regulation of gene expression by p53 is complex and subject to numerous levels of regulation. However, the apoptotic activity of p53 is further complicated by activities that are not related to transcriptional control, but reflect a cytoplasmic function of p53 in the regulation of mitochondrial membrane permeabilisation (Vousden, 2005). The details of this activity remain to be clarified, but so far the evidence suggest that p53 can function as a pro-apoptotic BH3-domain protein that leads to the release of cytochrome *c* from the mitochondria and induction of caspases and cell death. Although this function of p53 is independent of transcription, an elegant model has been proposed in which activation of expression of the BH3-only protein PUMA by p53 is necessary to dislodge cytoplasmic p53 from an inactivating complex it forms with the anti-apoptotic BH3 domain proteins such as BclxL (Chipuk et al., 2005). The physiological significance of this cytoplasmic function of p53 has recently been nicely supported by the identification of a small-molecule inhibitor of the p53-BclxL interaction, pifithrin-μ (Strom et al., 2006). Whereas treatment with pifithrin-μ does not affect p53-dependent transcriptional activation, the drug protects cells from p53-induced apoptosis in vitro and in vivo.

Obviously, the regulation of the cytoplasmic function of p53 – maybe through control of its subcellular localisation – could play an important role in determining the response. The export of p53 from the nucleus can be enhanced by ubiquitylation within the C-terminus of the protein, a modification that can also target p53 to the proteasome. Key to the regulation of p53 ubiquitylation is MDM2, a ubiquitin ligase that is an essential negative regulator of p53 (Vousden, 2002). The amount of MDM2 available appears to be critical in determining the outcome: mono-ubiquitylation of p53 by low levels of MDM2 allows nuclear export, whereas higher levels of MDM2 result in poly-ubiquitylation and degradation of p53 by the proteasome (Li et al., 2003). The ability of different polymorphic forms of p53 to bind MDM2 and be exported has been linked to the efficiency of apoptotic activity (Dumont et al., 2003), which suggests that the regulation of export may help balance cell cycle arrest (which appears to be primarily due to transcriptional activity of p53) with apoptosis. Cytoplasmic accumulation of p53 can also be driven by the transcription factor FOXO3a. Interestingly, despite inhibiting the transcriptional activity of p53, FOXO3a activation can induce p53-dependent apoptosis (You et al., 2006), highlighting the cytoplasmic function for p53 in the induction of cell death.

Survival functions of p53

Clearly, the differential regulation of apoptotic target genes or cytoplasmic activities that contribute to apoptosis can explain the ultimate outcome of p53 activation. However, the situation is more complex because – paradoxically – p53 induces survival as well as death signals. This function of p53 was first associated with relatively low levels of p53 expression (Lassus et al., 1996) and growing numbers of p53-induced survival genes are now being identified – the differential regulation of which may be crucial in determining the choice of response. p53-induced survival genes function through a number of diverse mechanisms. For example SLUG, the inhibitor of PUMA expression described above, is itself the product of a p53 target gene (Wu et al., 2005). Myosin VI, an unconventional motor protein, is induced by p53 to aid survival by maintaining Golgi-complex integrity (Joo et al., 2006). $p21^{WAF1/CIP1}$, which is encoded by one of the first p53-target genes to be identified (El-Deiry et al., 1993), plays a central role in the activation of both p53-dependent cell cycle arrest and survival (Polyak et al., 1996).

More recently, a p53-mediated ability to lower the levels of reactive oxygen species (ROS) in cells, and so protect from oxidative-stress-induced DNA damage and apoptosis, has been described (Bensaad et al., 2006; Budanov et al., 2004; Sablina et al., 2005). Products of a number of genes might play a role in this function of p53, including TIGAR (Bensaad et al., 2006), which reduces glycolysis and so enhances an alternative metabolic pathway, the pentose phosphate pathway. This results in the production of NADPH and thereby the generation of reduced gluthathione, which can lead to a decrease in intracellular ROS levels. Similarly, p53-induced expression of the sestrins provides an antioxidant function for p53 by protecting cells from hydrogen-peroxide-induced damage (Budanov et al., 2004). Overall, these antioxidant functions of p53 can clearly reduce the apoptotic sensitivity of cells, but also play an important role in protecting cells from DNA damage, genomic instability and cancer development (Sablina et al., 2005). p53 also plays a survival function in response to glucose deprivation, which induces a p53-response through AMP-activated protein kinase (Jones et al., 2005).

Why survive?

In the present models of p53 function, the principal activity of p53 is to prevent the outgrowth of damaged or stressed cells that may develop into malignancies if left unchallenged. Within a multicellular organism this would seem to be most efficiently achieved by eliminating any potentially aberrant cells through apoptosis, and indeed examination of the function of the p53 ortholog in more simple organisms suggests that the induction of apoptosis is the more ancient and evolutionarily conserved function. Why then would p53 adapt to induce a reversible cell cycle arrest? A hint comes from the clear role p53 plays in both preventing and repairing DNA damage, activities that are clearly useful only in a cell that is to be saved. It has recently become evident that p53 plays an important role not only under conditions of unusual or severe stress, but also in response to the milder but more constitutive types of stress encountered during normal life of the organism. Evidence for such 'non-stress' roles for p53 is growing, and these include a contribution of p53 to normal development and differentiation (Hall and Lane, 1997), regulation of the balance between mitochondrial respiration and glycolysis (Matoba et al., 2006) and the response to nutrient availability (Nemoto et al., 2004). The antioxidant activity is an example of a non-restrictive function of p53 that helps to protect cells from the accumulation of DNA damage. An attractive model is that the low levels of p53 that are induced under conditions of normal proliferation and everyday exposure to stress promote the induction of a cell cycle arrest (to temporarily halt the proliferation of the stressed cell), expression of genes that lower ROS levels (to help cells survive and reduce damage) and repair of any damage that has occurred. More severe and irreparable damage, or stress that is associated with oncogene activation or loss of survival signals, would result in conditions that allow activation of the apoptotic target genes, and elimination of the errant cell. Clearly, several other factors also contribute to the choice of response to p53 – including the cell type, other genetic lesions and cellular environment (Polyak et al., 1996; Vousden and Lu, 2002) – and is likely to be influenced by changes in transcription-independent apoptotic activities of p53. However, the model predicts that the ultimate response depends on a shift in the selection of target genes, which might be achieved through any of the mechanisms described here (Fig. 2).

The relative importance of apoptotic and other activities of p53 to tumour suppression is an interesting question. Whereas activation of apoptosis in developing cancer cells would clearly be an efficient inhibitor of tumour development, mice engineered to express p53 that retains only the cell-cycle-arrest function are remarkably tumour resistant (Liu et al., 2004). Interestingly, these animals also show genomic stability, in contrast to their p53-null siblings, suggesting that this p53 mutant retains antioxidant functions as well. Indeed, this type of p53 mutant is still able to induce expression of TIGAR – one of the effectors of p53-mediated antioxidant activity (Bensaad et al., 2006).

Cancer therapies

Therapeutic strategies that result in the activation of p53 would benefit from an ability to modulate, or at least take advantage of, the differential responses to p53 activation. One of the hopes is that stress specific to cancer cells, such as oncogene activation, hypoxia and loss of normal environment and stromal support, might result in a differential and heightened sensitivity to undergo apoptosis compared with normal tissue. A good example of at least one mechanism that might underlie such a difference between normal and tumour cells is the control of E2F1, a transcription factor that is found to be deregulated in virtually all human cancers. E2F1 can cooperate with p53 to induce cell death, reflecting its ability to drive apoptotic programmes that are both independent of and dependent on p53 (Stanelle and Putzer, 2006). Significantly, E2F1 induces expression of ASPP1, ASSP2 and p73, each of which can help p53 to induce apoptotic target gene expression. So tumour cells with increased E2F1 activity are primed to show an apoptotic response to p53, compared with normal cells, by virtue of expression of such apoptotic cofactors for p53.

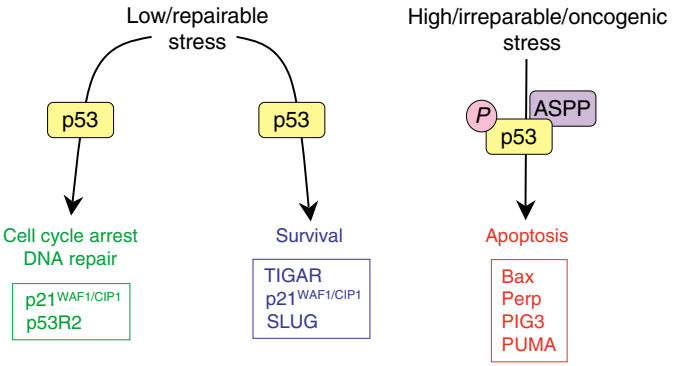

Fig. 2. Choice of response to p53 may reflect differential regulation of cell cycle arrest, and apoptotic and survival promoters. Low or repairable levels of stress or damage result in the induction of cell cycle arrest, and repair and survival signals by p53. More severe, irreparable or oncogenic stress leads to the activation of apoptotic signals, possibly accompanied by a decrease in expression of the survival genes (Bensaad et al., 2006).

How useful this differential between normal and tumour cells in their response to p53 will prove to be in practise is still somewhat a matter of faith. Clearly, activation of p53 in normal tissues following inducible loss of MDM2 can induce apoptosis in certain normal tissue types, which suggests that the tumour-to-normal-cell differential may not be as tight as one might have hoped (Marine et al., 2006). But maybe a less robust activation of p53 – possibly achieved by using inhibitors of MDM2 that are not completely effective – could reveal the hoped for differential in response. Certainly, initial studies of one class of such inhibitors, nutlin-3, have suggested that, although efficient in reducing tumour burden, such MDM2 inhibitors are not generally toxic to mice (Vassilev et al., 2004).

Unfortunately, whereas many of the current chemotherapeutic drugs efficiently activate p53, they also elicit severe collateral damage in normal tissue. This is likely to reflect both p53-dependent and -independent responses to these drugs, many of which show strong genotoxic activity leading to severe short-term and long-term side effects. The differential sensitivity of normal and tumour cells to p53-induced apoptosis is also eroded by these drugs. In just one example, it has been shown that DNA-damaging agents, such as chemotherapeutics, also directly activate E2F1 (Blattner et al.,

1999). Since deregulated E2F1 is likely to contribute to the enhanced sensitivity of tumour cells to apoptosis, activation in normal cells is likely to narrow – or even eliminate – the differential response to p53. It seems probable that specific inducers of p53, like the MDM2 inhibitors presently under development or drugs that can reactivate mutant p53, will have significant advantages over the current chemotherapeutic agents used in the clinic now. Use of p53 inhibitors may also prove effective to reduce the side effects of conventional therapeutics, which induce considerable p53-dependent toxicity in normal tissues (Komarov et al., 1999; Strom et al., 2006). As small-molecule modulators of p53 activity become clinically available, more sophisticated treatment options that involve the temporal manipulation of p53 activity may help to avoid therapy-induced toxicity while retaining p53-dependent protection from tumour development will become available (Christophorou et al., 2006). The hope is that our understanding of how outcomes to p53 activation are controlled will help us design more effective, less toxic treatment options.

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