Leading Edge
Review
Cell

Blinded by the Light: The Growing Complexity of p53

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While the tumor suppressor functions of p53 have long been recognized, the contribution of p53 to numerous other aspects of disease and normal life is only now being appreciated. This burgeoning range of responses to p53 is reflected by an increasing variety of mechanisms through which p53 can function, although the ability to activate transcription remains key to p53's modus operandi. Control of p53's transcriptional activity is crucial for determining which p53 response is activated, a decision we must understand if we are to exploit efficiently the next generation of drugs that selectively activate or inhibit p53.

Introduction

If genius is the ability to reduce the complicated to the simple, then the study of p53 makes fools of us all. Beyond the indisputable importance of p53 as a tumor suppressor, an increasing and sometimes bewildering number of new roles for p53 have recently been reported, including the ability to regulate metabolism, fecundity, and various aspects of differentiation and development. We are beginning to develop an intimate understanding of at least some of p53's functions and the mechanisms by which p53 is regulated. It is very clear, for example, that p53 is a transcription factor and regulates the expression of an array of different genes (encoding both proteins and microRNAs) that then mediate the p53 response. However, despite this intensive effort, there is still much to learn, and p53 remains a highly dynamic and rapidly expanding area of study. It is impossible to cover all aspects of p53-associated biology in one review, and so we have reluctantly passed over many fascinating topics that include the mechanisms that signal to p53; the relationships between p53 and its family members p63 and p73; the regulation of p53 expression, turnover, and localization; the effects of polymorphisms in components of the p53 pathways; the expression of different p53 isoforms; and the consequences of p53 mutations that occur during cancer development. Instead, we have chosen a few emerging themes to give a flavor of some recent advances in the p53 world.

p53 and Tumor Suppression: All that Is Good

The ability of p53 to efficiently inhibit cell proliferation—by both blocking cell cycle progression and promoting apoptotic cell death—provides a clear mechanism to stem tumor cell growth and so inhibit cancer development. Activation of p53 is driven by a wide variety of stress signals that a cell might encounter during malignant progression—genotoxic damage, oncogene activation, loss of normal cell contacts, and hypoxia to name but a few—leading to a model in which the growth inhibitory functions of p53 are normally held dormant, to be unleashed only in nascent cancer cells. But the situation is much more complex. We now understand that some p53 functions do not require activation by acute stress and that p53 can promote what appear to be entirely contradictory outcomes, although each of them may have a critical role to play in tumor suppression.

Death: The Final Frontier?

The concept that p53 can kill cancer cells is made even more pleasing by the idea that p53 might selectively induce apoptosis in developing tumor cells, while driving only a reversible cell-cycle arrest in their normal counterparts. As we will see, this is a massive oversimplification of the complex and heterogeneous responses to p53 activation, where some types of normal cells die while some types of tumor cells survive. Nevertheless, the possibility that the response to p53 activation can be modulated and that the therapeutic activation of p53 might be manipulated to promote death more efficiently in tumor cells than in normal cells is very attractive. Numerous studies have sought to reveal the molecular mechanisms that underlie the control of the response to p53 activation. But before we discuss them, let us take a step back and reconsider the real contribution of p53-induced apoptosis to tumor suppression.

Many of our models for p53 function suppose that induction of programmed cell death is the key mechanism by which p53 eliminates cancer cells. Indeed, mice that express a p53 mutant protein lacking the ability to induce cell cycle arrest but retaining apoptosis-inducing functions are still efficiently protected from spontaneous tumor development (Toledo et al., 2006). However, a growing body of evidence indicates that other functions of p53 may be equally important to prevent or stall cancer development. A clear hint that apoptosis is not the only weapon in p53's tumor suppressive arsenal comes from the identification of PUMA (p53-upregulated modulator of apoptosis) as a key mediator of p53's apoptotic activity. PUMA is a BH3 (Bcl-2 homology domain 3)-only protein that induces apoptosis through the mitochondrial pathway. The study of

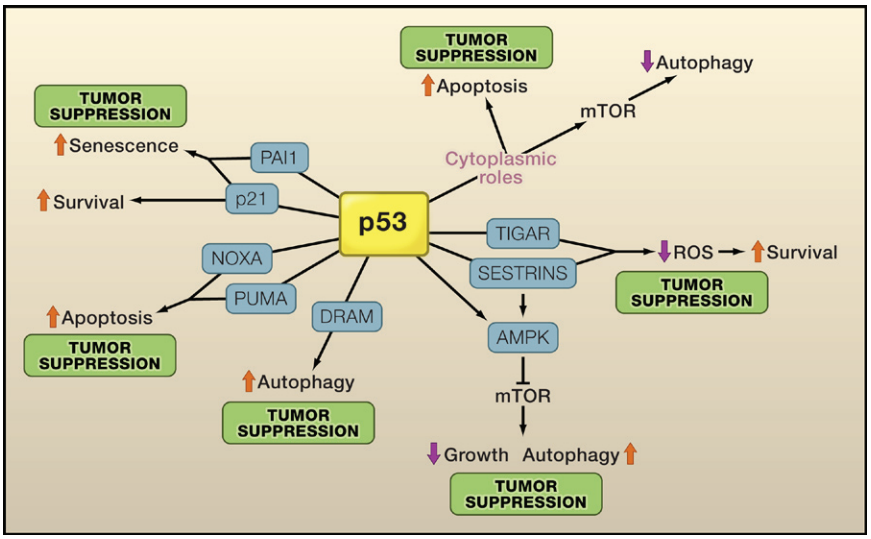

Figure 1. p53 Responses in Mediating Tumor Suppression

The control of cell survival, proliferation, and death by p53 is mediated by the regulation of expression of p53 target genes (some examples shown in blue) in the nucleus and transcriptionally independent cytoplasmic functions of p53. Most of these p53 responses have the potential to contribute to tumor suppression.

PUMA null mice showed that the induction of PUMA by p53 is necessary for the apoptotic response to p53 activation in many tissues (Yu and Zhang, 2003). However, *PUMA* null mice are not prone to developing cancer (Michalak et al., 2008), although subsequent studies have shown that the loss of PUMA can promote tumorigenesis that is driven by the Myc oncogene (Garrison et al., 2008; Hemann et al., 2004). It therefore seems clear that p53 can retain tumor suppressive functions even in the absence of a robust apoptotic response. The analysis of an unusual mutant p53 protein led to similar conclusions. Whereas most cancer-associated p53 mutations destroy all tested activities of p53, a few tumors harbor mutations in p53 that allow the protein to retain its cell cycle arrest function but selectively lose its ability to induce apoptosis (Rowan et al., 1996). The generation of mice expressing one such mutant (a single amino acid substitution of proline for arginine at residue 172 in the mouse—the equivalent of residue 175 in the human protein) revealed a very interesting phenotype. Despite being completely deficient in p53-driven apoptosis, these mice are still reasonably well protected from tumor development (Liu et al., 2004). Clearly, other functions retained by this mutant p53 protein can, at least partially, impede tumor development.

Not Dying, but Stopping

So what else might p53 be doing to prevent cancer development? There are several interesting options, including a number of different antiangiogenic activities of p53 that could limit tumor progression (Teodoro et al., 2007). But maybe the most obvious candidate for another tumor suppressor activity of p53 is the ability to inhibit cell proliferation and growth (Figure 1). p53 can effectively block cell cycle progression by activating the transcription of the cyclin-dependent kinase inhibitor p21, although several other p53-target genes such as *14-3-3 sigma* and *GADD45* also contribute to this response (El-Deiry, 1998). The induction of p21 expression is extremely sensitive to even low levels of p53 protein, leading to the idea that a temporary G1 block, as induced by mild damage or stress, allows cells to survive safely until the damage has been resolved or the stress removed (we will come back to this idea). However, a transient cell cycle arrest might be risky, if a cell with oncogenic potential that cannot be repaired is allowed to endure and ultimately resume proliferation. So how can cancer cells be permanently restrained, if not through elimination by apoptotic cell death? The answer seems to lie in the activation of senescence—an irreversible cell cycle arrest. A slew of fascinating studies have highlighted the importance of senescence in the inhibition of tumor progression and have identified a key role for p53 in this response. Several of these studies show that a pivotal point in this pathway is the induction of DNA damage, by oncogene activation or in response to telomere dysfunction, which in turn leads to the activation of p53 (Deng et al., 2008; Halazonetis et al., 2008). It would appear that precancerous lesions, which we probably all carry in abundance, are largely held back from malignant progression by p53-induced senescence. Small surprise, then, that the loss of p53 has such a dramatic effect in allowing cancer to develop. Furthermore, senescence remains an important response to p53 activation, even in established tumors. In mouse models, reactivation of p53 proves to be a potently effective therapy for cancer, resulting in the regression of many different tumor types (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007). Most interesting is the type of response to p53 activitation, which in carcinomas and sarcomas triggers senescence rather than apoptosis. Although tissue culture studies would suggest that senescence is a cytostatic response that should promote stabilization of the disease rather than induce its regression, encouraging results from in vivo studies indicate that the subsequent engagement of the immune system can result in tumor clearance (Xue et al., 2007).

As with other p53 responses, senescence is likely to result from changes in the expression of a number of proteins such as the plasminogen activator inhibitor 1 (PAI-1) (Kortlever et al., 2006; Leal et al., 2008). Intriguingly, one of the key mediators of p53-induced senescence is p21 (Brown et al., 1997), and tumor suppression by the apoptosis-defective p53 R172P mutant is accompanied by the activation of p21 and senescence (Cosme-Blanco et al., 2007; Van Nguyen et al., 2007). Furthermore, introduction of this p53 mutant into *p21* null mice results in the complete loss of the cell cycle arrest response and enhanced tumorigenicity (Barboza et al., 2006). Taken together, these results suggest that p53-dependent activation of p21 is an important axis in senescence-dependent tumor suppression. However, a recent study showed that although p21 plays an important role in mediating the p53-dependent cellular response to stress, lack of p21 does not strongly promote tumor development (Choudhury et al., 2007). In some

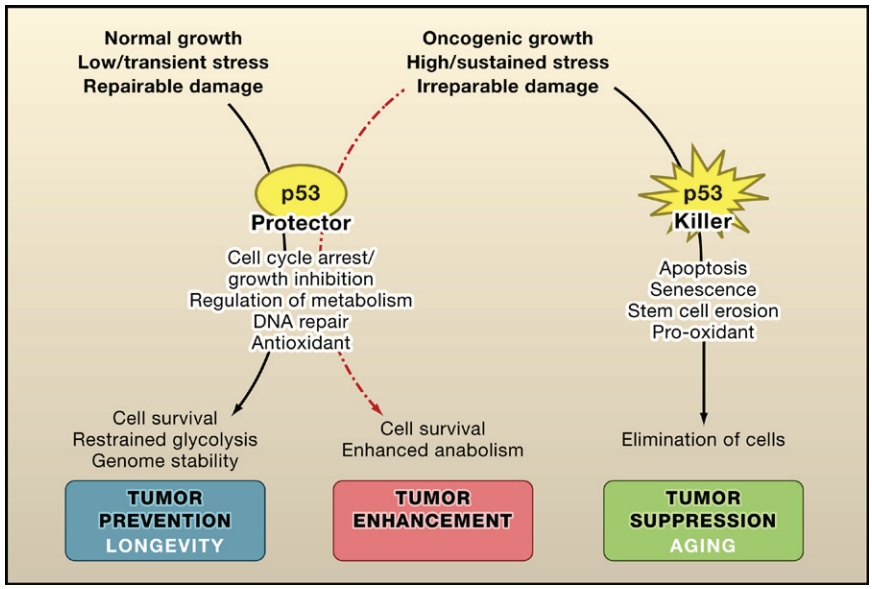

Figure 2. Dual Mechanisms of p53 Function in Tumor Suppression and Aging

p53 can help to promote the repair and survival of damaged cells, or it can promote the permanent removal of damaged cells though death or senescence. The ultimate result of p53 activation depends on many variables, including the extent of the stress or damage. In this model, basal p53 activity or that induced by low-stress elicits the protector responses that support cell survival, control glycolysis, and promote the repair of genotoxic damage. Sustained stress or irreparable damage, on the other hand, induces the killer functions of p53 to activate cell death or senescence. Notably, the protector functions of p53 could contribute to tumor development if not properly regulated (red, dashed arrow). Some of p53's protector functions may also help to enhance longevity, whereas the consequences of p53's killer functions can promote aging.

ways, this result parallels the failure of *PUMA* null mice to spontaneously develop cancer, despite a clear defect in p53-dependent apoptosis. Perhaps the senescent and apoptotic responses act as insurance for each other (a sort of tumor suppressive belt and braces) with the predominant response depending on cell type and context. An analysis of the tumor spectrum of mice lacking both *p21* and *PUMA* would be most interesting.

Choosing Life or Choosing Death

The tumor suppressive effects of p53-dependent induction of either senescence or cell death are easy to understand, as both can emphatically prevent further replication of the incipient cancer cell. In a multicellular organism, this seems to be the sensible choice—better to lose a few rogue cells to death or senescence rather than risk retaining one cell that might prove fatal. Despite the inherent logic of this argument, the cellular response to p53 is not so straightforward. In addition to eliminating damaged cells, p53 can also contribute to cell survival through a surprisingly large number of mechanisms (Figure 1). Numerous p53 target proteins function to inhibit apoptosis, including p21, decoy death receptors such as DcR1 and DcR2, the transcription factor SLUG (which represses the expression of PUMA), and several activators of the AKT/PKB (protein kinase B) survival pathways (Janicke et al., 2008). Another group of p53-inducible genes have recently also been shown to act as antioxidants by decreasing the levels of intracellular reactive oxygen species (Liu et al., 2008; Sablina et al., 2005). Although this function for p53 would help inhibit tumor progression by protecting cells against DNA damage and genome instability, downregulation of reactive oxygen species through these p53-dependent mechanisms can also result in decreased susceptibility to apoptosis (Bensaad et al., 2006).

So why would p53 contribute to cell survival when promoting death seems to be the safest option? The implication is that the wholesale elimination of every cell that is exposed to some level of stress, no matter how mild, is not always the most desirable response. It is possible that survival promotes senescence, which is simply another way to permanently remove a problematic cell. Indeed, it may be telling that p21, a key mediator of p53-induced senescence, also plays a role in cell survival. However, it also seems likely that under some conditions, this activity of p53 really does protect cells, allowing them to rejoin the normal population after the resolution of any damage. p53 engages an entire suite of responses that directly contribute to DNA repair (Gatz and Wiesmuller, 2006), so it seems only logical to assume that under some circumstances these activities of p53 will be harnessed for use—and there seems little point in repairing a cell that is doomed to die or senesce.

This brings us to the interesting question of what determines the outcome of p53 activation. Whether or not an apoptotic response is elicited is strongly dependent on the type of tissue, the nature of the stress signal, and the cell's environment. But there is also some evidence to suggest that the decision between life and death can be determined by the extent of damage or the duration of stress (Figure 2). In this model, low levels of transient stress associated with repairable damage elicit the survival response (where p53 acts as a protector), whereas high levels of sustained stress accompanied by irreparable damage lead to cell death or senescence (where p53 acts as a killer) (Bensaad and Vousden, 2007). Despite the clear difference in outcomes, both of these responses to p53 could contribute to tumor suppression, either by preventing the accumulation of oncogenic lesions or by eradicating damaged cells through cell death or senescence. This model also suggests that there are activities of p53 (as a protector) that might be extremely dangerous to sustain under conditions where repair or recovery is not possible. We will revisit this point later in the discussion.

Controlling the Engine

Oncogenic changes that promote cancer cell proliferation and survival are often accompanied by alterations in cell metabolism that also play a vital role in supporting tumor development

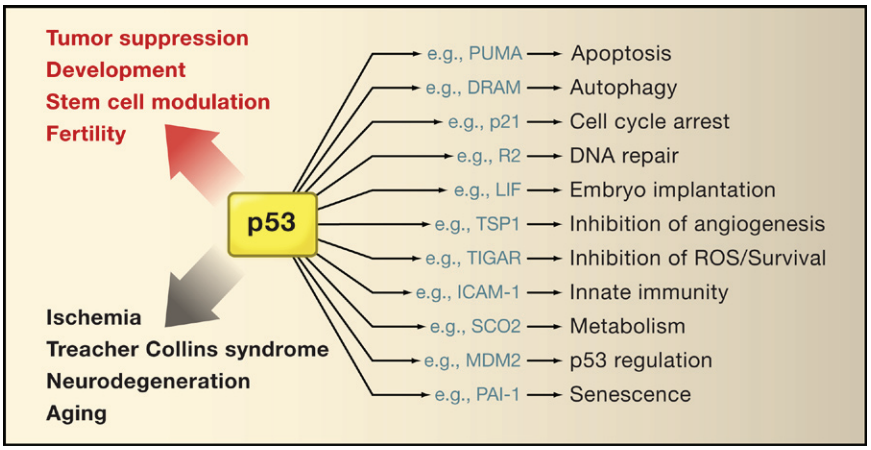

Figure 3. p53 Contributes to Multiple Normal Processes and Disease Pathologies

In addition to its well-known role as a tumor suppressor, p53 also regulates other cellular (right) and developmental processes (left). These include processes that result in positive outcomes (red arrow) and those that result in diseases or other unfavorable outcomes (black arrow). Examples of p53 target genes (blue) that are regulated by p53 to produce the indicated cellular outcomes of p53 induction are shown. Note that, in many cases, numerous targets have been identified to mediate a specific outcome, even though only one example target gene is shown here.

(DeBerardinis et al., 2008). This is a complex subject, but in essence the reprogramming of metabolic pathways provides cancer cells with numerous benefits, including the ability to survive under adverse conditions (such as low or variable oxygen availability), the ability to mobilize anabolic pathways that generate the macromolecules necessary for growth, and the ability to limit oxidative damage (DeBerardinis et al., 2008). Indeed, the dependence of cancers on metabolic transformation is highlighted by mouse models of cancer in which interfering with these altered metabolic programs profoundly limits tumor cell growth (Bonnet et al., 2007; Christofk et al., 2008; Fantin et al., 2006). A role for p53 in responding to and regulating metabolic changes is therefore an exciting and burgeoning area of study.

So how does p53 contribute to the regulation of metabolism, and how might this help it to function as a tumor suppressor? Not surprisingly, p53 can be activated by metabolic adversity (such as starvation)—a response that can be mediated through the action of AMP-activated protein kinase (AMPK), a key component of the cell's response to bioenergetic stress (Jones et al., 2005). p53 then promotes a program of gene expression (including the induction of *AMPK* expression) to negatively regulate the kinase mTOR (mammalian target of rapamycin), a central node in the control of protein synthesis (Budanov and Karin, 2008; Feng et al., 2007a) (Figure 1). This response to p53 helps to ensure the coordination of cell growth and cell proliferation, which is also regulated by p53. A role for p53 in the response to starvation and metabolic stress is also reflected in the ability of p53 to regulate autophagy, a membrane trafficking-mediated "self-eating" process that results in lysosomal digestion of cellular components. Autophagy promotes short-term cellular survival under starvation conditions and also helps to eliminate damaged proteins and organelles. The ability of p53 to promote autophagy through the induction of lysosomal proteins such as DRAM (damage-regulated autophagy modulator) (Crighton et al., 2006) or through negative regulation of mTOR signaling is certainly consistent with an observed role for autophagy in tumor suppression (Matthew et al., 2007) (Figure 1). But the relationship between p53 and autophagy remains unclear—indeed basal levels of cytoplasmic p53 have been shown to inhibit autophagy, also by regulating mTOR activity (Tasdemir et al., 2008). Similarly complicated are the consequences of autophagy for tumor suppression, as p53-associated autophagy has been reported to both contribute to apoptosis (Crighton et al., 2006) and help tumor cell survival (Amaravadi et al., 2007). How these opposing responses to p53-induced autophagy are coordinated remains to be determined.

In addition to regulating growth through modulating mTOR signaling, p53 may have even more intricate roles in the regulation of metabolic pathways (Jones and Thompson, 2009). These recently uncovered functions include modulating glucose uptake (Kawauchi et al., 2008), dampening glycolysis (Bensaad et al., 2006; Kondoh et al., 2005), and enhancing mitochondrial respiration (Ma et al., 2007). Intriguingly, some of these effects of p53 could help to curb the acquisition of enhanced aerobic glycolysis (the Warburg effect), one of the most common metabolic changes associated with oncogenic transformation. Thus, they may provide yet another route by which p53 can restrain tumor development.

p53 and Tumor Promotion: Crossing to the Dark Side

Our recent appreciation of p53's role in regulating glycolysis, oxidative stress, and cell survival leads us to a growing tangle of complexity within the p53 pathway, where p53 can be involved in disparate and even contradictory responses. These functions of p53 highlight an interesting paradox touched on earlier: if some activities of p53 that normally contribute to tumor suppression are not properly regulated, they might "switch sides" to help promote cancer development (Figure 2). Obvious examples include the prosurvival functions of p53, which might protect cells undergoing repair following mild stress but would be extremely counterproductive if maintained in irreparably damaged cells. Similarly, the ability of p53 to impede glycolysis can help control oncogenic transformation, but the consequent promotion of alternative metabolic pathways (such as the pentose phosphate pathway) might also drive the anabolism necessary for tumor cell growth (DeBerardinis et al., 2008). Nontranscriptional functions of p53 such as its role in inhibiting autophagy could also contribute to tumor development under some circumstances. Intriguingly, this particular function of wild-type p53 is retained by cancer-associated

mutant p53 proteins (Morselli et al., 2008), although whether this activity helps malignant progression is not yet known. It would appear that the tight restriction of some p53 responses may be necessary to prevent one of our principal tumor suppressors from turning from friend to foe.

p53 in Other Pathologies

Although the ability of p53 to drive processes such as cell death is clearly beneficial in the context of limiting tumor development, engaging these p53-mediated responses under all stress conditions may not necessarily be advantageous. For instance, the DNA-damage-induced p53 response is largely responsible for radiation or chemotherapy-induced sickness. Although this has often been viewed as an unfortunate but necessary side effect of activating the important genome protection functions of p53, more recent work has shown, rather surprisingly, that this initial p53 response is not required for tumor suppression (Christophorou et al., 2006; Efeyan et al., 2006). Rather, it seems that the ability of p53 to sense and respond to oncogene activation is the key to preventing cancer, suggesting that transient inhibition of p53 might be useful in protecting normal tissue from the short-term negative effects of cancer therapies (Berns, 2006). Beyond cancer, the fact that p53 responds to a myriad different types of stress without being able to distinguish when this is helpful and when not results in its involvement in a number of negative effects (Figure 3). For example, the induction of p53-driven apoptosis in response to hypoxia is clearly an asset when the hypoxia is caused by a lack of blood supply in a rapidly growing tumor. However, this response is much less desirable when the hypoxia is due to ischemia following stroke or myocardial infarction. Indeed, inhibition of p53 seems to be extremely beneficial during the early stages of ischemia or during subsequent reperfusion injury (Liu et al., 2006). The activation of p53 by ribosomal stress has been linked to tumor suppression, with abnormalities in the expression of several ribosomal proteins resulting in an increased tumor susceptibility in disorders such as Diamond Blackfan Anemia, possibly reflecting a failure to properly activate p53 (Montanaro et al., 2008). On the other hand, perturbation of ribosome biosynthesis by mutations in *TCOF1* (Treacher Collins-Franceschetti syndrome 1) can cause constitutive activation of p53 (Jones et al., 2008) and result in the congenital disorder known as Treacher Collins syndrome. Similarly, p53 appears to contribute to the pathology of various neurodegenerative diseases. Activation of p53 by mutant forms of huntingtin (which are responsible for Huntington's disease) partially mediates the neurodegeneration and neurobehavioral abnormalities observed in mouse models (Bae et al., 2005). In turn, p53 further induces the expression of huntingtin (Feng et al., 2006), an effect that might further exacerbate the progress of the disease. In animal models of Parkinson's disease, the loss of *DJ-1* expression (a gene mutated in early onset Parkinson's disease in man) leads to the activation of p53 and the death of dopaminergic neurons (Bretaud et al., 2007). p53 has also been implicated as a mediator of neuronal death in Alzheimer's disease (Culmsee and Landshamer, 2006), although recent studies suggest that expression of amyloid-beta peptides associated with Alzheimer's may induce a conformational shift in the p53 protein (Lanni et al., 2007). This effect might provide a useful additional marker for the diagnosis of Alzheimer's and also presents the intriguing possibility that the unfolding of p53 into a "mutant" conformation may contribute to the development of the disease. While these findings highlight the potentially detrimental effects of p53 activity in the nervous system, the ability of p53 to promote neural outgrowth and axon regeneration suggest that it can also have a more positive contribution to neuronal regeneration after central or peripheral nervous system injuries (Di Giovanni et al., 2006).

Everyday p53 Functions: No Stress, No Worries?

One of the most interesting shifts in our thinking about p53 is the realization that its remit may be far broader than simply to promote a tumor suppressive response to acute stress. Indeed, the ability to prevent cancer has been suggested to be an "evolutionarily late" cooption of p53 activities that had initially evolved to protect the germline and monitor development (Aranda-Anzaldo and Dent, 2007; Vousden and Lane, 2007) (Figure 3). These primordial functions of p53 as a guardian of the germline appear in lower organisms (such as flies and worms) that have no clear need for cancer suppression (Derry et al., 2001; Sutcliffe and Brehm, 2004) and are also reflected in its ability to protect mouse embryonic stem cells from DNA damage by inducing their differentiation (Lin et al., 2005). A role for p53 in differentiation and development is also observed in the frog *Xenopus laevis*, where p53 engages in complex interactions with the Smad transcriptional regulators to direct embryonic germ layer specification (Piccolo, 2008). p53 even makes a subtle but important contribution to several aspects of normal growth and development in mice, where the p53 family members p63 and p73 shoulder the bulk of the developmental functions (Aranda-Anzaldo and Dent, 2007; Vousden and Lane, 2007).

It is now becoming apparent that the manifestations of some of p53's functions in diverse aspects of health and disease (Figure 3) do not require acute stress. Rather, these p53 functions depend on basal levels of p53 or the activation of p53 by low levels of constitutive stress. One possible function for basal p53 activity is in the control of stem cell renewal. Even in the absence of obvious stress, p53 can limit the self-renewal of adult neural stem cells (Meletis et al., 2006) and regulate quiescence in hematopoietic stem cells (Liu et al., 2009). p53 also represses the expression of CD44, cell surface proteins involved in regulating many aspects of cell migration and survival (Godar et al., 2008). This may represent another contribution to tumor suppression, but the observation that basal levels of p53 can also control CD44 suggests a mechanism by which p53 could be involved in regulating normal functions of CD44, such as epithelial development or stem cell renewal. In other studies, p53 has been shown to contribute to fecundity in mice by directly regulating the expression of leukemia inhibitory factor (LIF), a protein required for blastocyte implantation (Hu et al., 2007). Most interestingly, polymorphisms known to affect p53 activity are associated with implantation failures in women (Kay et al., 2006), hinting at the conservation of this function of p53 in humans.

The ability of basal p53 activity to modulate metabolism may also have some interesting consequences beyond the control of cancer development. For example, the ability of p53 to promote aerobic respiration appears to be critical in mice to maintain endurance during exercise (Matoba et al., 2006). But maybe the most intriguing additional role for p53 is in the regulation of longevity and aging (Figure 2). In lower organisms such as worms and flies, the loss of p53 can be associated with increased longevity (Arum and Johnson, 2007; Bauer et al., 2005). Initial studies suggested that p53 could also drive premature aging in mammalian systems, although there is some indication that this effect may be the consequence of inappropriate, unregulated p53 activity—possibly reflecting enhanced oxidative stress (Matheu et al., 2008). By contrast, our more recent understanding of the antioxidant functions of basal (uninduced) levels of p53 and the ability of p53 to negatively regulate the IGF-1/mTOR growth regulation pathways suggest an alternative possibility: that p53 activity enhances longevity. Indeed, mice engineered to express additional copies of normally regulated *p53* showed resistance to cancer without any accelerated aging (Matheu et al., 2007). In fact, in combination with increased copies of the tumor suppressor *Arf*, the enhanced *p53* allele even enhances longevity in mice (Matheu et al., 2007). Thus, just as p53 can function as both protector and killer in its role as a tumor suppressor, it may also both promote and prevent aging (Figure 2). Interestingly, p53 function has been shown to decline with age (Feng et al., 2007b). This could contribute not only to the increased incidence of cancer with increased age but also possibly to the process of aging itself. Can p53 activity be somehow harnessed to allow us to both avoid cancer and enjoy increased longevity? This may be a dream, but it is certainly a question worth examining.

The Nuts and Bolts of p53 Regulation

As outlined above, the consequences of p53 activation can be dramatically different depending on numerous factors and contexts. Differences in stimuli, cellular milieu, or external environment can result in different p53-dependent outcomes. How are such decisions in cellular outcomes made? Studies performed in past 5 years have provided deeper insights into this question. Myriad transcriptional targets mediate the diverse outcomes of p53 activation (Figure 3). Although most p53 targets are only induced by types of stress that lead to increased p53 levels, some targets can be activated by basally expressed p53. p53 also may have a bona fide transcription-independent, mitochondria-associated role in inducing apoptosis (Moll et al., 2005; Schuler and Green, 2005). It is possible that both transcription-dependent and transcription-independent functions of p53 are required for promoting apoptosis and limiting tumorigenesis. Indeed, PUMA, a proapoptotic protein encoded by a p53 target gene, is required to release cytoplasmic p53 from the antiapoptotic protein Bcl-XL to facilitate mitochondrial outer membrane permeabilization (Chipuk et al., 2005). Moreover, deletion of the p53 binding sites in the endogenous *PUMA* promoter in human colorectal cancer cells (by homologous recombination) reduces both *PUMA* expression and DNA damage-induced apoptosis (Wang et al., 2007a). We discuss below recent developments in the understanding of transcriptional regulation by p53, focusing on but a few of the many notable reports that examine how p53 selects its target genes and the ensuing cellular outcome.

Thinking Globally

Elucidation of p53's function as a transcriptional regulator will require the integration of both macroscopic and microscopic views to evaluate the complete set of p53-regulated genes and to delve into the mechanisms by which it selects its target genes in different settings. From the macroscopic vantage point, it will be important to know the full repertoire of direct transcriptional targets bound and regulated by p53. One recent review lists 129 such p53 transcriptional targets that were identified as a result of either single gene discoveries or multigene screens (Riley et al., 2008). There are likely to be many more genes specifically bound and activated by p53. Furthermore, the number of genes whose expression is altered indirectly upon induction of p53 is likely to be in the thousands. To find new direct p53 targets, there is well-justified interest in the identification of sites within the human genome that are bound by p53. The original consensus site recognized by p53 consisting of two copies of the sequence—RRRCA/TT/AGYYY (R, purine; Y, pyrimidine)—has been refined by using bioinformatics analysis (Hoh et al., 2002; Miled et al., 2005; Riley et al., 2008; Sbisa et al., 2007) and experimental approaches using chromatin immunoprecipitation (chIP) in conjunction with microarrays ("chIP on chip") (Cawley et al., 2004; Hearnes et al., 2005; Smeenk et al., 2008) or chIP-paired-end (PET) sequencing (Wei et al., 2006). Such screens for p53-binding sites have provided intriguing information regarding p53 target binding. First, they have revealed that not all "good" p53-binding sites in the genome are occupied by p53 under the conditions that were used. Factors such as the spacing between half sites (Jordan et al., 2008), the location of a site within a heterochromatic locus, or the presence at the site of a p53 dominant-negative isoform or family member are all likely to be important determinants of p53 association with any particular site. Second, not all regions bound by p53 have sequences that conform to the p53 consensus site. This could be the result of p53 association with other DNA-binding proteins such as nuclear transcription factor Y (NF-Y) (Imbriano et al., 2005). Third, not all genes in the vicinity of bound p53 are transcriptionally regulated as a result of p53 binding. Eukaryotic gene promoters usually require multiple factors for activation. Moreover, the presence of corepressors at the same promoter could counteract the activity of p53. It will be important to both refine and extend the current bioinformatic and experimental approaches to obtain a more dynamic global view of p53 binding and activation. Although daunting in terms of effort and cost, it will be crucial to elucidate the extent and kinetics of p53 binding at genomic targets after different stimuli and in different types of cells.

Surveys of genes whose expression is altered upon induction of p53 always include large numbers of genes whose expression is reduced. Indeed, transcriptional repression by p53 is important in promoting cell death. p53 may reduce gene expression by several mechanisms (Laptenko and Prives, 2006). First, p53 may increase the expression of a protein (such as p21) that pre-

vents phosphorylation of the retinoblastoma protein, thereby maintaining genes regulated by the E2F transcription factors in a repressed state. Indeed, the repression of numerous p53 target genes has been shown to be mediated by p21 (Lohr et al., 2003; Tang et al., 2004; Shats et al., 2004; Baptiste-Okoh et al., 2008b). Second, p53 transcriptional repression may result from the direct association of p53 with promoters that possess binding sites for other transcription factors such as Sp1 (Esteve et al., 2007; Innocente and Lee, 2005; Sengupta et al., 2005; Zaky et al., 2008; Zhan et al., 2005), NF-Y (Imbriano et al., 2005; Matsui et al., 2004), or SMADs (found in combination with a p53 recognition sequence) (Wilkinson et al., 2008). Promoters repressed by p53 may also harbor a cell cycle-dependent element/cell cycle gene homology region (CDE/CHR element), a sequence recognized by several different transcription factors (Rother et al., 2007; St Clair et al., 2004). Third, gene repression could be mediated by unique p53 "repression" elements to which p53 binds directly (Godar et al., 2008; Johnson et al., 2001).

Gene expression microarrays have revealed that p53 regulated genes are not limited to those involved in cell cycle arrest and apoptosis. Many other gene clusters associated with diverse processes such as DNA repair, transcription, cell adhesion, cell mobility, metabolism, and membrane functions are also affected by p53 activity. The complex repertoire of p53 regulated genes further highlights the imperative need to understand how p53 selects its targets.

Acting Locally

Moving from the macroscopic to the microscopic, much attention has been paid to the mechanisms by which p53 selects some of its key target genes. We will start by discussing a number of studies that have revealed new facets of how p53 contacts its binding sites in DNA, some of which have also provided insights into p53-binding site selectivity in vitro and in cell culture. X-ray crystallographic analyses have revealed a new interface in a p53 dimer bound to DNA (Ho et al., 2006) and shown that four p53 core domains bind as a dimer of dimers to two cognate half sites in DNA (Kitayner et al., 2006). Interestingly, there are DNA-sequence-specific differences in the contacts made between the p53 protein surfaces, which could translate into the degree of induction for a given target gene (Kitayner et al., 2006). Now that Fersht and colleagues (Tidow et al., 2007) have succeeded in obtaining a structure of full-length p53 bound to DNA by using a combination of small angle X-ray scattering and nuclear magnetic resonance, it will be possible to gain a clearer view of how p53 interacts with different DNA sequences.

How does p53 identify its cognate binding sites in a vast sea of genomic DNA? Although this question is still unanswered, two studies have shown that the p53 protein is capable of diffusing two dimensionally on DNA in vitro (McKinney et al., 2004; Tafvizi et al., 2008). It is not yet known over what distances p53 can slide on DNA, whether p53 can similarly diffuse along DNA that is wrapped around a histone octamer, and whether the chromatin state of the DNA regulates how p53 binds or slides. Experimental and molecular modeling studies have revealed that the propensity of a p53 cognate binding sequence to bend has a significant impact on the stability and affinity of p53 binding (Batta and Kundu, 2007; Pan and Nussinov, 2008). A fascinating albeit somewhat exotic study has revealed that a tethered photo-oxidant, anthraquinone, can actually transmit electrical charge through the DNA to p53 protein bound at a distance, resulting in the photo-oxidation and specific release of p53 from some cognate binding sites (for example, sites in the promoter of the gene encoding Mdm2) but not others (for example, sites in the *p21* promoter) (Augustyn et al., 2007). These findings may relate to changes in the oxidative state of p53 after hydrogen peroxide treatment of cells and the ensuing selective impact on its ability to activate transcription.

Several proteins and small molecules have been shown to regulate the DNA-binding specificity of p53. Some of these work through the p53 tetramerization region. For example, a p53 isoform (p53β), that can form heterotetramers with wild-type p53, stimulates wild-type p53 binding and activation of the *Bax* (Bcl-2-associated X protein) gene, but it does not promote p53 activation of *p21* expression (Bourdon et al., 2005). The tyrosine kinase c-Abl, on the other hand, stabilizes p53 tetramerization and augments the binding of p53 to the *p21* promoter instead of the *Bax* promoter (Wei et al., 2005a). Others factors may affect p53 DNA binding by directly interacting with the central core domain of p53. Among the earliest discovered and still studied of these proteins are the apoptosis stimulating proteins of p53 (ASPPs), which selectively stimulate p53 binding and activation of the *Bax* promoter but not the *p21* promoter (Sullivan and Lu, 2007). In contrast, a zinc-finger protein called hematopoietic zinc finger (HZF), itself a p53 transcriptional target, interacts with the p53-DNA-binding domain and promotes the recruitment of p53 to the *p21* and *14-3-3* promoters, but not to the *Bax* promoter or the promoter of the proapoptotic *Noxa* gene. These findings are consistent with the observation that mouse embryonic fibroblasts lacking HZF exhibit increased *Bax* expression and decreased *p21* expression in comparison to that in wild-type mouse embryonic fibroblasts (Das et al., 2007). Even a small molecule such as nicotinamide adenine dinucleotide (NAD^+) can selectively impact p53 binding to DNA in vitro and correspondingly affect the level of p53-induced *Mdm2* expression without impacting *p21* expression in vivo (McLure et al., 2004). These are likely only a few of the ways in which p53 DNA binding and transcriptional activation can be differentially regulated.

Modifying the Regulator

Since the first discoveries revealing that p53 undergoes stress-induced phosphorylation or acetylation, there have been numerous complicated studies describing these (and other) modifications to p53 and deciphering how they affect p53 function as a transcriptional regulator. As there are a number of excellent reviews covering these aspects of p53 regulation (Appella and Anderson, 2001; Bode and Dong, 2004; Kruse and Gu, 2008; Olsson et al., 2007), we will focus on discussing findings relevant to a few key p53 modifications that selectively impact the cellular outcomes of p53 activation (Figure 4).

The Many Roles of Phosphorylation

Serine 46 (S46), an N-terminal phosphorylation site in human p53, clearly has discriminatory functions for p53 as a transcriptional activator. Phosphorylation at this residue is

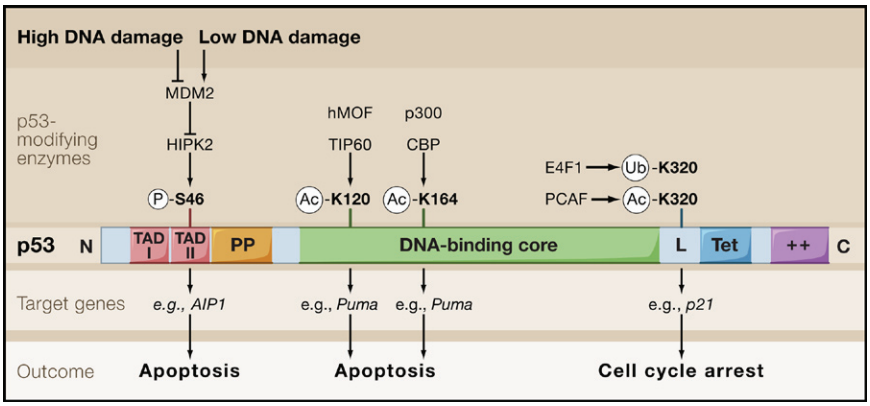

Figure 4. Selective Impact of p53 Modifications

p53 protein domains include the transcriptional activation domain I (TAD 1, residues 20–40), the transcriptional activation domain II (TAD II, residues 40–60), the proline domain (PP, residues 60–90), the sequence-specific core DNA-binding domain (DNA-binding core, residues 100–300), the linker region (L, residues 301–324), the tetramerization domain (Tet, residues 325–356), and the lysine-rich basic C-terminal domain (++, residues 363–393). A few examples are depicted of residues that when modified by phosphorylation (P), acetylation (Ac), or ubiquitination (Ub), result in a specific cellular outcome in response to p53 activation (for example, apoptosis versus cell cycle arrest) that depends on preferential activation of the indicated target genes.

correlated with an altered p53 transcriptional program that includes the induction of p53-regulated apoptosis-inducing protein 1 (p53AIP1), a proapoptotic factor that promotes the release of mitochondrial cytochrome *c* during apoptosis. One of the protein kinases that phosphorylate this site is homeodomain interacting protein kinase 2 (HIPK2) (D'Orazi et al., 2002; Hofmann et al., 2002), a protein regulated by the tumor suppressor Axin (Rui et al., 2004). Under conditions of moderate DNA damage, the p53 negative regulator Mdm2 induces HIPK2 degradation (Figure 4). In contrast, severe DNA damage results in reduced Mdm2 levels, thus allowing the now stable HIPK2 to phosphorylate p53 at S46 to induce cell death (Rinaldo et al., 2007). These findings implicate Mdm2 as a determinant of alternative cell fates that are regulated by p53 (Shmueli and Oren, 2007), a concept that we will return to later in this Review. The observations also support the previously mentioned view that the extent of cellular damage may determine whether p53 acts as a survival factor or a death factor. To make matters more complicated, p53 not only negatively regulates HIPK2 through inducing *Mdm2* expression for its degradation, but also positively regulates HIPK2 by facilitating its caspase-mediated cleavage and subsequent activation (Gresko et al., 2006). There are other protein kinases that can either directly phosphorylate S46 or are otherwise required for S46 phosphorylation. These include dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) (Taira et al., 2007), AMPK (Okoshi et al., 2008), protein kinase C delta (Yoshida et al., 2006), and p38 mitogen-activated protein kinase (Perfettini et al., 2005). That several kinases can phosphorylate S46 both supports the importance of this site in p53 function and makes the understanding of its regulation more challenging. S58 in the mouse p53 protein is likely the corresponding residue of S46 in the human protein (Cecchinelli et al., 2006), and it will be interesting to determine the physiological outcome of mutating this residue in mice.

C-terminal phosphorylation sites in p53 have also been linked to selective impacts on target gene expression and outcomes. S315 is somewhat unique among these sites in that it is phosphorylated by growth-promoting kinases. Phosphorylation of S315 regulates the ability of p53 to repress Nanog, a factor required for stem cell self-renewal, through the recruitment of the transcriptional regulator and corepressor mSin3A. Mice harboring mutant p53 where S315 is mutated to alanine (S315A) are impaired in Nanog repression. However, the interpretation of experiments where S315 is disrupted is complicated by the proximity of S315 to the major nuclear localization signal sequence of p53. The transcription factor E2F requires S315 to facilitate nuclear retention of human p53 (Fogal et al., 2005), whereas the binding of this region by the glycogen synthase kinase-β (GSK-β) after ER stress causes cytoplasmic retention and destabilization of p53 (Qu et al., 2004). Also within the C terminus of p53 are S366 and threonine 387 (T387), two sites that have been shown to be regulated by the checkpoint kinases Chk1 and Chk2. Downregulation of either Chk kinase or the mutation of these two p53 phosphorylation sites selectively affects p53 activation, promoter binding, and acetylation of C-terminal lysines on p53 (Ou et al., 2005). In mice, substitution of S389, a UV-inducible modification, with alanine results in altered expression of some p53 target genes and generally reduced repression of p53 targets in UV irradiated cells (Bruins et al., 2007, 2008). Although there is still much to learn about how different phosphorylations regulate p53, several sites clearly have discriminatory impacts on some target genes in comparison to others.

The Ever-Shifting Functions of p53 Lysines

As complex as the consequences of p53 phosphorylation are, the roles of p53 lysine residues are even more perplexing. Numerous studies have implicated the lysines within the extreme C terminus of p53 as being important for the protein's transcriptional activities. Yet, confoundingly, two knockin mice in which either six (Feng et al., 2005) or all seven (Krummel et al., 2005) of the extreme C-terminal lysines were mutated to arginines have mild (albeit somewhat different) phenotypes. A possible explanation for this puzzling result comes from the identification of two acetylation sites within the p53 central core domain. The first of these two sites, lysine 120 (K120), is acetylated in vivo in response to DNA damage and increases *PUMA* but not *Mdm2* expression. Two MYST family histone acetyl transfereases (HATs)—Tip60 (Tang et al., 2006) and hMOF (Sykes et al., 2006)—are capable of acetylating K120. Cells overexpressing a mutant form of p53 where K120 is mutated to arginine (K120R) show a partial defect in apoptosis. It should be noted that when the mutant K120A p53 protein is expressed at physiological levels, the defect in apoptosis is stronger than when the mutant protein is transiently overexpressed, indicat-

ing that abnormally high levels of p53 can produce misleading results (Zupnick and Prives, 2006). Furthermore, the loss of one copy of *Tip60* in mice impairs the Myc-induced DNA-damage response without impacting the p53 transcriptional program, suggesting that reduced Tip60 levels may not impact p53 in vivo (Gorrini et al., 2007).

The second core domain acetylation site, K164, is modified by the transcriptional coactivators p300 and CREB-binding protein (CBP) and appears to be important for the activation of the majority of p53 target genes (Tang et al., 2008). When six of the extreme C-terminal lysines in p53 are mutated in addition to mutation of K120 and K164, the resulting p53 mutant protein (p53 8KR) is virtually inert. This mutant protein lacks the transcriptional activation activity required to induce a plethora of its target genes, including those encoding p21, PUMA, Bax, and p53-inducible gene 3 (PIG3). The *Mdm2* promoter is the one notable exception: Mdm2 expression is induced by the 8KR p53 protein to a level similar to that seen with wild-type p53. What is unique about the *Mdm2* promoter and its regulation by p53? In cultured human breast cancer MCF7 cells, an ATPase component of the SWI/SNF chromatin remodeling complex called Brg1 is required for p53 binding and induction of the *p21* promoter but not the *Mdm2* promoter (Xu et al., 2007). This suggests that, compared to other p53 targets, the *Mdm2* promoter may not be as tightly associated with nucleosomes. Whether this is relevant to the observation that unacetylatable p53 can still activate expression of Mdm2 remains to be determined.

Findings implicating p300 and CBP as being critical for p53's activities in both arrest and apoptosis are supported by the observation that the F box protein Skp2 prevents p300 from binding to and acetylating p53 with consequent reduced expression of p53 targets such as *p21* and *Puma* (Kitagawa et al., 2008). However, these data will need to be reconciled with the observation that the targeted deletion of p300 in human HCT116 colon cancer cells results in reduced *p21* expression but increased *Puma* expression, with the corresponding cellular outcomes of reduced cell cycle arrest and increased apoptosis (Iyer et al., 2004).

K320 in p53 is a substrate of the transcription coactivator P300/CBP-associated factor (PCAF). It is another lysine that plays an interesting role in p53 target gene selection. Cells ectopically expressing a mutant p53 where K320 was mutated to glutamine (K320Q; Q is thought to mimic acetylation) display decreased apoptosis after some forms of DNA damage. Although the K320Q mutant protein is capable of inducing *p21* expression, it can neither induce the expression of the gene encoding apoptotic peptidase-activating factor 1 (APAF1) nor repress some p53 targets such as the gene encoding the apoptosis-inhibitor protein survivin (Knights et al., 2006). Acetylated K320 also preferentially activates two transcriptional targets of p53 (Coronin 1b and Rab13) whose gene products associate with the cellular cytoskeleton and are involved in neurite outgrowth during axonal regeneration (Di Giovanni et al., 2006). Treatment of cultured cells with a hypoxia-mimicking drug (etoposide) leads to increased association of PCAF and K320-acetylated p53 with the *p21* promoter compared to p53 acetylated at K382, despite a global decrease in the amount of p53 acetylated at K320 (Xenaki et al., 2008). The possibility that, under some conditions, acetylation of K320 predisposes p53 to activate p21 and decreases its ability to induce proapoptotic targets genes is nicely consistent with the observation that K320R knockin mice harbor several cell types that display increased apoptosis and higher expression of relevant p53 target genes (Chao et al., 2006).

Of course, lysine mutations do not exclusively reflect the loss of p53 acetylation. Other lysine modifications such as methylation, ubiquitination, sumoylation, and neddylation have the potential to also alter p53's transcriptional activity. Experimental results obtained using lysine amino acid substitutions need to be viewed as only circumstantial and not definitive. A zinc-finger protein E4F1, first identified as a cellular target of the Adenoviral E1a protein, ubiquitinates p53 at K320. Interestingly, ubiquitination at K320 does not destabilize p53. Rather, it selectively facilitates p53 activation of *p21* and *cyclin G1* expression without affecting the expression of the proapoptotic gene *Noxa*. This is consistent with the observation that *E4F1* expression markedly reduces UV-dependent p53-mediated cell death (Le Cam et al., 2006). Intriguingly, PCAF, which has been unequivocally shown to acetylate p53 at K320, has also been reported to exhibit E3 ubiquitin ligase activity toward Mdm2 (Linares et al., 2007). Future studies may reveal whether there is crosstalk between PCAF and E4F1 in the regulation of p53 and Mdm2.

Regarding alternate modifications of p53 lysines, methylation in particular has been a subject of great interest. Methylation of K372 by the SET domain methyltransferase Set9 leads to increased *p21* expression (Chuikov et al., 2004), but whether this has a selective impact on p53 target gene activation has not been determined. Methylation of K382 by Set8, however, has the interesting effect of suppressing the activation of several strong p53 targets but not others that are normally less well induced (Shi et al., 2007). K370 is methylated by the methyltransferase Smyd2 (SET and MYND domain containing 2) and causes the repression of p53 transcriptional activation, although K370 methylation is itself inhibited by Set9 methylation of K372 (Huang et al., 2006). The demethylase LSD1 removes K370 dimethylation and in doing so prevents p53 from interacting with p53-binding protein 1 (53BP1), a coactivator of p53 (Huang et al., 2007). The functional roles of p53 lysine modifications are further complicated by the crosstalk that exists between methylation and acetylation (Ivanov et al., 2007). Specifically, methylation of K372 by Set7/9 is induced by DNA damage and correlates with increased acetylation of C-terminal p53 lysines including K382.

All told, a rather daunting set of combinatorial possibilities can result from p53 lysine modifications. It is anticipated that once a full set of data has accumulated regarding the possible combination of modifications, great computational power will be needed to deconstruct their impact on p53 functions and the cellular outcomes. In the meantime, new p53 modifications continue to be uncovered. It was recently reported that the protein arginine methyltransferase 5 (PRMT5) is involved in methylation of at least two arginine residues (R333 and R335) within the p53 tetramerization domain (Jansson et al., 2008). Depletion of PRMT5 by siRNA in human cancer cell lines leads

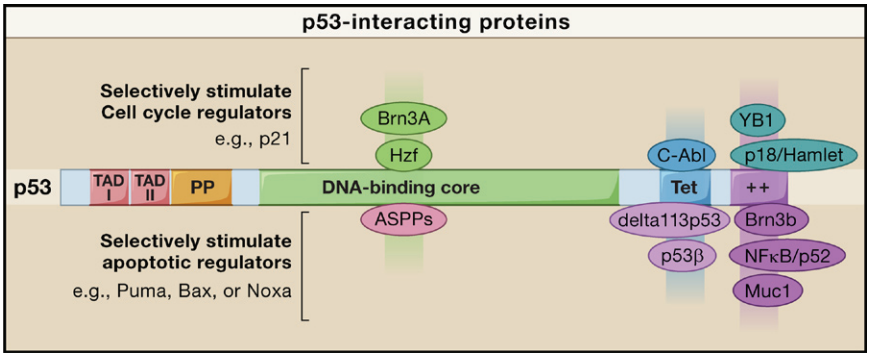

Figure 5. p53-Interacting Proteins Exert Selective Influences on p53 Target Genes and Outcomes

Different proteins can bind to p53 to induce different cellular outcomes to p53 activity. Proteins that interact with the p53 DNA-binding core (green), tetramerization domain (Tet, blue), or C-terminal basic domain (++, purple) are shown. Brn3A, Hzf, c-Abl, YB1, and p18/Hamlet selectively induce p53 activation of genes encoding cell cycle regulators such as p21 to facilitate cell cycle arrest. In contrast, ASPPs, the zebrafish p53 variant delta113p53, the p53 isoform p53β, Brn3b, NFκB/p52, and Muc1 selectively activate the expression of apoptotic regulators such as PUMA, Bax, and Noxa to promote cell death.

to increased apoptosis along with loss of p21 and a modest increase in proapoptotic Puma and Noxa proteins (Jansson et al., 2008). Whether other p53 arginines are methylated remains to be determined.

Changing Partners

Numerous noncovalent modifiers of p53 can also exert discriminatory effects on its ability to activate or repress its gene targets (Figure 5). Indeed, as one might expect, there is complex interplay between p53 modifications and its binding partners. The best studied and validated of the p53 interactors are its negative regulators Mdm2 and MdmX. A wealth of studies have delved into their interactions with p53 and have been well reviewed (Marine et al., 2006, 2007; Poyurovsky and Prives, 2006; Toledo and Wahl, 2006). Therefore, we will only highlight here a few recent findings in this aspect of the p53 field. Mechanistic understanding of how Mdm2 and MdmX repress p53 transcriptional activity is an area still requiring insight. A recent study showing that the loss of p53 rescues the early lethality in mice caused by a mutant form of Mdm2 lacking E3 ligase activity indicates the primacy of the role of Mdm2 in degrading p53 (Itahana et al., 2007). Nonetheless, Mdm2 has also been shown to reduce p53 acetylation by displacing p300 from p53 (Ito et al., 2001; Kobet et al., 2000; Teufel et al., 2007), as well as by inhibiting and degrading PCAF (Jin et al., 2004). Mdm2 can also recruit the histone deacetylases HDAC1 (Ito et al., 2002) and KAP1 (Wang et al., 2005), thus providing additional means by which Mdm2 might function to repress acetylation of either p53 or histones in the vicinity of p53-binding sites. Mdm2 expressed from its endogenous locus associates with p53 at the *p21* promoter (Arva et al., 2005; Minsky and Oren, 2004; Ohkubo et al., 2006; Tang et al., 2008; White et al., 2006). Ectopically overexpressed Mdm2 (and MdmX) can bind to several other p53 target promoters, with the exception of the *Mdm2* promoter itself (Tang et al., 2008). Having about 2-fold higher levels of Mdm2 protein in H1299 cells with tetracycline-regulated p53 expression leads to lower levels of PIG3 and 14-3-3σ but does not affect *p21* or *Bax* expression when compared to similar cells without extra Mdm2 (Ohkubo et al., 2006). Due to its ability to either displace acetylases or directly ubiquitinate histone H2B (Minsky and Oren, 2004), or possibly through other mechanisms, Mdm2 coassociation with p53 at its target gene promoters allows it to negatively regulate p53 transactivation in a selective manner. How and when MdmX negatively regulates p53 are questions being actively pursued (for example, see Wang et al., 2007b). Perhaps the most challenging aspect of this field of study is integrating the roles of Mdm2 and MdmX in the regulation of p53 turnover and localization with their respective impacts on p53 transcriptional activities.

Intrinsic to the p53 protein is its ability to select different target genes. Within its N terminus, p53 possesses transactivation domains (TADs; TAD I within residues 20–40 and TAD II within residues 40–60) and a proline-rich domain (spanning residues 60–90) that can also regulate transcription, possibly in conjunction with TAD II (Harms and Chen, 2006). Loss of TAD I function, most frequently achieved by simultaneous mutation of leucine 22 (L22) and tryptophan 23 (W23) (p53L22Q/W23S), produces a mutant p53 protein that was originally thought to be virtually bereft of all transcriptional activity. Yet, recent studies have shown that this mutant p53 protein can activate a subset of p53 proapoptotic targets in mouse cells (Johnson et al., 2005) and in human cells (Jung et al., 2006; Baptiste-Okoh et al., 2008a). These findings reveal that additional regions in p53—TAD II, the proline-rich region, or both—possess the capacity to function autonomously in transcriptional activation. Although TAD I and TAD II may be able to act independently, they also work in concert to recruit specific components of the multisubunit transcriptional activator STAGA complex, namely GCN5, Taf9, and ADA2b, in order to activate target genes such as *p21*, *Puma*, and *GADD45* (Gamper and Roeder, 2008). Furthermore, the p53-mediated transcriptional repression that is induced by hypoxia requires both TAD I and TAD II (Hammond et al., 2006). Based on data from studies examining p53L22Q/W23S, however, it is possible that some p53 targets do not require the STAGA complex. Other regions in the p53 protein with transactivation capability may be able to recruit distinct factors that are necessary to induce gene activation. Small molecules such as Nutlin (Vassilev et al., 2004) and compound 1d (Ding et al., 2005), which bind to Mdm2 and disrupt the interaction between Mdm2 and TAD I of p53, can greatly increase *p53* expression and activity (Ding et al., 2005; Vassilev et al., 2004). Intriguingly, in contrast to the effect of Nutlin in inducing cell cycle arrest, another small molecule (RITA) that also binds to p53 and prevents it from interacting with Mdm2 causes apoptosis and downregulation of *p21* expression in some cells (Enge et al., 2009). Whether these different compounds selectively affect different p53 activation regions is a question of considerable interest.

Although intrinsic features of the p53 protein are critical to its ability to induce cell cycle arrest or cell death, an increasing panoply of cellular factors have also been identified that

work with p53 to produce a specific cellular response (Figure 5). Not surprisingly, there is an intimate relationship between the ability of p53 to activate its target genes and the transcription machinery with which p53 interacts. It has been shown in recent years that the arginine methyl transferases CARM1 and PRMT1 collaborate with p300 to facilitate p53-mediated transcription from DNA assembled into chromatin in vitro (An et al., 2004). Additionally, components of a subcomplex (including the protein MED/TRAP220) of the mediator transcriptional coactivator complex can interact with p53 (Zhang et al., 2005).

A multitude of p53 binding proteins that can redirect p53 toward a specific cellular outcome have also been uncovered. For example, a well-studied p53 polymorphism at codon 72—where the residue can be either proline (P72) or arginine (R72)—results in differential cellular outputs depending on the p53 variant: the R72 variant protein is more proapoptotic than the P72 allele (Pietsch et al., 2006). The ASPP family member, iASPP, preferentially binds to the P72 variant of p53 and inhibits its activity, providing a mechanistic explanation for why the R72 variant protein is more effective at inducing apoptosis than the P72 variant protein (Bergamaschi et al., 2006). As one example of the interplay between p53 modification and its interaction with regulatory factors, the peptidyl-prolyl *cis*/trans isomerase Pin1 recognizes p53 phosphorylated at S46, leading to dissociation of iASPP from p53 and thereby promoting apoptosis (Mantovani et al., 2007). Another example of a protein that binds to p53 and directs differential cellular outcomes is the p38-regulated protein p18/Hamlet. p18/Hamlet associates with p53 and increases both p53-mediated apoptosis and activation of some p53 target gene promoters (e.g., *Noxa*) but not others (*Puma*, *Bax*, and *p21)* (Cuadrado et al., 2007). Intriguingly, cyclin G, itself a p53 target, may decrease levels of p18/Hamlet, providing another level of regulation of p53 outcomes (Cuadrado et al., 2007). Another protein, Brn3A, binds to p53 and specifically inhibits its ability to activate the *Bax* and *Noxa* promoters to promote apoptosis. However, Bm3A also cooperates with p53 to activate gene expression from the *p21* promoter (Hudson et al., 2005). It remains to be seen if there is any direct competition or cross-regulation between the activities exerted by p18/Hamlet and Brn3A. Interestingly, Brn3b, a related factor to Brn3A, functions in the opposite manner as Brn3A by assisting p53 to activate *Bax* expression and not *p21* expression (Budhram-Mahadeo et al., 2006).

An interesting regulator of p53 targets genes is the p52 subunit of the transcription factor NFκB, which inhibits *p21* expression but cooperates with p53 to increase *Puma*, *DR5*, and *Gadd45* expression; p52 also directly associates with the promoters of these genes (Schumm et al., 2006). In the case of Muc1, however, an integral membrane glycoprotein that is frequently overexpressed in cancer, it has been found that Muc1 associates with the *p21* promoter in a p53-dependent manner to facilitate *p21* transcription. Interestingly, Muc1 also associates with the *Bax* promoter independent of p53 to repress *Bax* expression. Consistent with Muc1's regulatory functions, the amount of Muc1 protein in cells shows positive correlations with cell cycle arrest and cell survival, and negative correlations with cell death (Wei et al., 2005b). The Y-box-binding protein YB1 has a similar impact on p53. YB1 associates with p53, blocking its activation of *Bax* expression, but does not impede p53 induction of *p21* expression (Homer et al., 2005). Finally, in zebrafish, another selective p53 regulator, itself a p53 variant (delta113p53), represses full-length p53 activation of arrest but not apoptosis genes (Chen et al., 2005).

In addition to myriad p53-binding proteins, several new proteins have been identified that can also bind and regulate p53 but have yet to be shown to impart any selectivity to p53 target gene activation. Among these proteins are Sug1, a component of the 19S proteasome (Zhu et al., 2007), heterogeneous ribonucleoprotein particle K (hnRNPK), which possibly acts through Mdm2 (Moumen et al., 2005), Hbo1 (Iizuka et al., 2008), KLF5 (Zhu et al., 2006), NF-Y (Imbriano et al., 2005), clathrin heavy chain (Enari et al., 2006), and the orphan receptor TR3 (Zhao et al., 2006). We will be very curious to learn whether any of these proteins exert promoter-selective effects on p53's transactivation capabilities.

Finally, not all proteins that affect p53 transcriptional activities and outcomes interact directly with p53. Notable examples of this include a somewhat mysterious regulator of p53, a noncoding RNA expressed from the *MEG3* gene locus. This noncoding RNA seems to selectively affect p53 in human cells by downregulating *Mdm2* expression, increasing p53 expression, and stimulating p53 activation of at least one target gene (*growth differentiation factor 15*; *GDF15*), all without affecting *p21* transcription (Zhou et al., 2007). Another instance of these indirect p53 regulators is the transcriptional repressor Zbt4, which forms a heterotrimeric complex with the Sin3 corepressor and the transcription factor Miz1 in order to repress p53-mediated *p21* induction and cell cycle arrest (Weber et al., 2008). The transcription repressor Slug is a beautiful case in point of a factor that profoundly affects p53 outcomes without directly interacting with p53. The gene encoding Slug is a p53 target, and Slug proteins bind to the p53-inducible *Puma* promoter to repress both gene expression and irradiation-induced apoptosis in hematopoietic cells (Wu et al., 2005).

Yet another factor that binds to a subset of p53 target genes independently of p53 (for example, *PIG3* and *AIP1* but not *p21* or *Puma*) is the nuclear transport factor hCas/Cse1L that cooperates with p53 to activate only those genes to which it binds. At those promoters, hCas/Cse1L most likely functions by facilitating downregulation of the repressive histone trimethylation mark, K27 of histone H3 (Tanaka et al., 2007).

All told, numerous transcriptional mechanisms that direct p53 toward its different cellular outcomes have been reported. The levels of p53, its modifications, the proteins that directly interact with it, and the proteins that interact independent of p53 with its target promoters can all differentially affect the outcome of p53 activation (Figure 6).

New Kids on the Block: p53 and MicroRNAs

Protein-encoding genes are not the only transcription targets of p53. No less than seven groups independently reported in 2007 that p53 can directly regulate the expression of select microRNAs (miRNAs), most dramatically the *miR-34* locus consisting of *miR-34a*, *miR-34b*, and *miR-34c* (Bommer et al., 2007; Chang et al., 2007; Corney et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tarasov et al., 2007; Tazawa et al.,

2007). Several reports showed that p53 can bind directly to response elements within the *miR-34a* and *miR-34b/c* promoters to stimulate transcription from this locus. Certainly, *miR-34a* expression is physiologically relevant to the impact of p53 activity on cells: it can induce cell cycle arrest and senescence, as well as facilitate cell death. Functional ablation of *miR-34a* reduces these p53-mediated effects on cells. Notably, the *miR-34* family is conserved in flies and worms, a rather infrequent occurrence among miRNAs. As is often the case with miRNAs, the most obvious challenge is to find the target genes of *miR-34* whose downregulation is required for cell-cycle arrest or cell death. Convincing candidates identified in some of the reports include the cell cycle regulators cyclin-dependent kinase 4 (CDK4) and cyclin E2, as well as the proto-oncogene mesenchymal-epithelial transition factor (Met) and the antiapoptotic factor Bcl2. More recently, the sirtuin SIRT1 was also shown to be a target of *miR-34a*, and its downregulation correlates with increased acetylation of p53 (Yamakuchi et al., 2008). The discovery of *miR-34a* as a key p53 target begs the question of whether its levels are altered in cancer. Satisfyingly, some of the aforementioned studies uncovering p53 regulation of the miRNA locus report markedly lower amounts of *miR-34* in some human tumors and tumor-derived cell lines (Bommer et al., 2007; Chang et al., 2007; Tazawa et al., 2007).

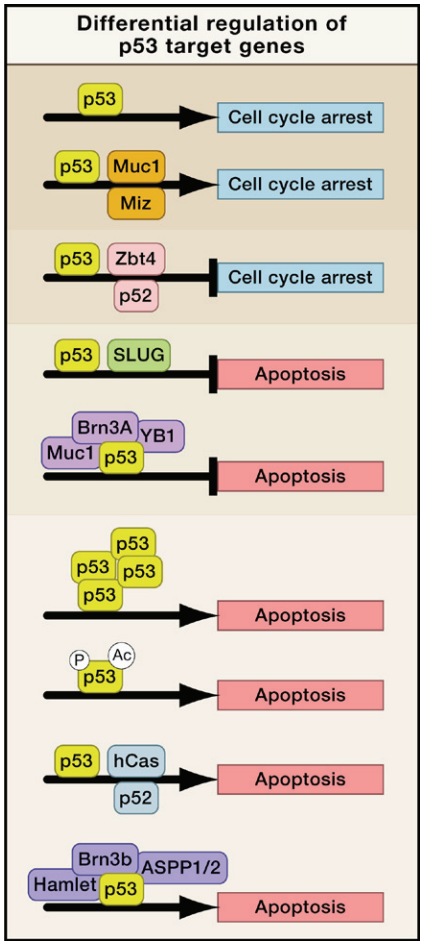

Figure 6. Multiple Mechanisms of Differential p53 Target Gene Regulation

The cellular response to p53 activation can be determined by differential target gene activation. Whether a given promoter is activated or repressed depends on the amount of p53 protein, its modification state, and the cofactors present at the promoter. p53 protein levels or modification state can also dictate which genes are targeted for transcriptional activation. The induction or repression of p53 target gene transcription can also depend on the presence of numerous coactivators or additional cooperating factors that enhance or repress p53-induced transcription.

miR-34 is not the only microRNA to be targeted by p53. Several of the first publications identifying the *miR-34* locus also found other possible miRNA targets of p53. It has now been reported that *miR-192* and *miR-215* are induced by p53 and promote increased *p21* expression (Braun et al., 2008). Moreover, *miR-145* has been implicated as a p53 target that can repress *c-myc* expression (Sachdeva et al., 2009). In fact, a number of miRNAs that target antiproliferative genes have been shown to be repressed by p53 in a manner that requires E2F, not unlike other targets of p53-mediated repression (Brosh et al., 2008). Note that although the small molecule Nutlin can induce *miR-34a* expression and senescence in some cells such as normal human fibroblasts (Kumamoto et al., 2008), it fails to induce *miR-34a* and *p21* expression in at least one tumor cell line (BV173 leukemia cells) that instead undergoes apoptosis upon Nutlin treatment (Paris et al., 2008). Thus, like other p53 target genes, *miR-34a* is not universally induced upon the activation of p53. It will be interesting for future studies to delve into the mechanism of its discriminatory regulation.

Therapeutic Applications of p53

Although there is still much to learn, it seems clear that manipulating the p53 pathway will bring considerable therapeutic benefits. As p53 activity is impaired or defective in most human cancers, regardless of type or tissue of origin, one obvious goal is to try and re-establish the growth-inhibitory functions of p53 in cancer cells. This approach is elegantly supported by animal studies where reactivation of wild-type p53 leads to efficient tumor regression (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007). So how might p53 be reactivated? One line of attack that has been successful in the clinic is the use of gene therapy to reintroduce p53 into tumor cells by means of vectors such as adenoviruses (Senzer and Nemunaitis, 2009). Our growing understanding of how p53 is regulated has also led to the development of small molecule drugs that stabilize and activate the p53 protein. Although these drugs mostly function by interfering with the ability of Mdm2 to target p53 for degradation, cell-based drug screens have also identified inhibitors of sirtuins—protein deacetylases that can restrain p53 activity—as effective p53-activating agents (Lain et al., 2008). These types of drugs, some of which are in advanced stages of preclinical development or early clinical trials (Shangary and Wang, 2009), are predicted to show efficacy in tumors that retain wild-type p53. It is worth noting that the development of such drugs has sparked a vigorous debate about the potential toxicity that a systemic activation of p53 may cause in normal tissues. In animal models, the absence of *Mdm2* in normal p53-expressing tissues is alarmingly detrimental (Ringshausen et al., 2006), although it might be hoped that Mdm2-inhibiting drugs will be less effective, and so easier to tolerate, than ablation of the *Mdm2* gene. Furthermore, because these drugs are not genotoxic, they will hopefully avoid some of the damage inflicted by conventional chemotherapies, which of course also activate p53 in all cells. Intriguingly, some studies have suggested that Mdm2-inhibiting drugs function much better in cells undergoing DNA-damage signaling, a characteristic that might further distinguish normal cells from cancer cells (Brummelkamp et al., 2006). If the use of p53 activators in cancer therapy proves to be incredibly successful, we may need to consider the possible proag-

ing effects of p53 activation during systemic long-term treatment with these drugs, although at present such concerns will likely seem trivial to most cancer patients. Restoration of p53 function in those cancers expressing mutant p53 is even more challenging, although small molecules that refold some mutant p53 proteins and thus reactivate their wild-type functions have been described (Selivanova and Wiman, 2007). This approach is technically difficult, but it may be an excellent way to selectively target cancer cells that express mutant p53 proteins.

The concept that we should be trying to reactivate p53 in cancer cells is supported by experimental data and many human studies that show a correlation between mutations in p53 and poor disease prognosis (Petitjean et al., 2007). However, as we have highlighted in this Review, the cellular response to p53 can range widely from cell death to cell survival, and the consequences of retaining p53 activity in tumor cells are similarly difficult to predict. Indeed, the retention of wild-type p53 has been shown to protect breast cancers from some forms of cytotoxic chemotherapy and so can be associated with a poor response to treatment (Bertheau et al., 2008). Possibly this effect of wild-type p53 could be exploited for therapeutic benefit. In this approach, failure of tumor cells to mount a p53-mediated response would make them particularly sensitive to cytotoxic drugs that function during S phase or the G2/M phase transition of the cell cycle. These drugs would be much less toxic to normal cells that can benefit from p53's cell cycle arrest and survival activities (Sur et al., 2009). An extension of this idea is to use drugs that activate p53 to further protect normal cells or tissue during the treatment of cancers harboring mutant p53 alleles (Carvajal et al., 2005; Kranz and Dobbelstein, 2006). Somewhat confusingly, the same concept of chemoprotection of normal tissue has also been proposed for the use of p53 inhibitors. It is clear that much of the toxicity seen in response to conventional genotoxic chemotherapies is due to the activation of p53 and the subsequent p53-induced death of radiosensitive cells in the hematopoetic system, gut lining, and other tissues. In this case, inhibition of p53 in normal cells may protect them from death, thereby increasing the patient's tolerance to higher and hopefully more effective doses of radiation or chemotherapy (Gudkov and Komarova, 2005; Strom et al., 2006). Mouse studies support this strategy, suggesting that many side effects of acute genotoxic insult might be avoided by a short-term inhibition of p53, without causing a substantial loss in tumor suppressor activity (Christophorou et al., 2006).

The potential use of p53 in therapy is not limited to cancer. For some disorders, the inhibition of p53 (or at least the inhibition of the p53-mediated apoptotic response) could be a desired therapeutic goal. This area of p53-directed therapy is still underexplored, but p53-inhibitory compounds have been used with some success in animal models of ischemia and Parkinson's disease (Duan et al., 2002; Leker et al., 2004).

As drugs are developed that can reactivate wild-type functions of mutant p53, or turn wild-type p53 on or off, our understanding of how best to use these new tools in therapy will grow. However, as we learn more about the intricacies of p53 regulation and function, predicting the outcomes of these drug treatments becomes more difficult. The response of cancer cells to p53 is clearly complicated enough, but how modulating p53 might contribute to other aspects of disease and longevity is only just beginning to be explored. Perhaps in a few years, a similar review will be able to integrate the complexity of p53 into a clearer picture to give insights into how this knowledge may be used to improve the prognosis of not only cancer patients but sufferers of other diseases as well.

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