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Control of apoptosis by p53

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The p53 tumor suppressor acts to integrate multiple stress signals into a series of diverse antiproliferative responses. One of the most important p53 functions is its ability to activate apoptosis, and disruption of this process can promote tumor progression and chemoresistance. p53 apparently promotes apoptosis through transcription-dependent and -independent mechanisms that act in concert to ensure that the cell death program proceeds efficiently. Moreover, the apoptotic activity of p53 is tightly controlled, and is influenced by a series of quantitative and qualitative events that influence the outcome of p53 activation. Interestingly, other p53 family members can also promote apoptosis, either in parallel or in concert with p53. Although incomplete, our current understanding of p53 illustrates how apoptosis can be integrated into a larger tumor suppressor network controlled by different signals, environmental factors, and cell type. Understanding this network in more detail will provide insights into cancer and other diseases, and will identify strategies to improve their therapeutic treatment.
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Tumor suppressors act to maintain tissue homeostasis, that is, to control the number and behavior of cells in a particular tissue within an organism (Hussain and Harris, 1998). To do so, they typically regulate one or more processes that prevent aberrant proliferation (Vogelstein *et al*., 2000; Vousden and Lu, 2002). p53 is the most extensively studied tumor suppressor, and acts in response to diverse forms of cellular stress to mediate a variety of antiproliferative processes. Hence, p53 can be activated by DNA damage, hypoxia, or aberrant oncogene expression to promote cell-cycle checkpoints, DNA repair, cellular senescence, and apoptosis. As a consequence, disruption of p53 function promotes checkpoint defects, cellular immortalization, genomic instability, and inappropriate survival, allowing the continued proliferation and evolution of damaged cells. Given the profound proliferative advantage produced by loss of p53 function, it is not surprising that *p53* is the most commonly inactivated tumor suppressor gene in human cancer (Hussain and Harris, 1998; Beroud and Soussi, 2003).

Although most of the attention on p53 has focused on its role in cancer, chronic activation of this key biological pathway may be equally as deleterious as its inactivation. In fact, hyperactivation of p53 has been associated with a variety of degenerative diseases such as arthritis, multiple sclerosis (Wosik *et al*., 2003), and neuropathies (Mattson *et al*., 2001), as well as with the exacerbation of ischemic damage from strokes or cardiac arrest (Komarova and Gudkov, 2001). Moreover, studies using mouse models suggest that acute p53 activation contributes to the side effects of cancer chemotherapy, whereas chronic p53 activation can contribute to aging (Komarova and Gudkov, 2001; de Stanchina and Lowe, 2002; Tyner *et al*., 2002). Together, these observations imply that p53 activity must be a tightly regulated, with too much, or too little p53 producing, or contributing to, disease.

One of the most extensively studied areas in p53 research surrounds its ability to control apoptosis. The first hint that p53 could control apoptosis came from work by Oren and co-workers who reintroduced *p53* into a *p53*-deficient myeloid leukemia cell line (Yonish-Rouach *et al*., 1991). Here, p53 induced apoptosis in a manner that could be countered by a prosurvival cytokine. Subsequently, evidence that endogenous p53 could control apoptosis was obtained from studies using thymocytes from *p53* knockout mice, which showed that p53 was required for radiation-induced apoptosis, but not cell death induced by several other stimuli (Clarke *et al*., 1993; Lowe *et al*., 1993b). These studies, together with the observation that loss of apoptosis correlated with tumor progression in *p53*-null transgenic mice (Symonds *et al*., 1994; Parant and Lozano, 2003), implied that apoptosis contributes to p53's tumor suppressor activity. Furthermore, the fundamental role apoptosis plays in the biology of p53 is emphasized by its evolutionary conservation in both *Drosophila* (Brodsky *et al*., 2000; Jin *et al*., 2000; Ollmann *et al*., 2000) and *C. elegans* (Frantz, 2001; Schumacher *et al*., 2001), where the respective orthologs are an important component of damage surveillance.

In addition to its role in suppressing tumorigenesis, p53-dependent apoptosis contributes to chemotherapy-induced cell death (see, for review, Johnstone *et al*., 2002). This was first demonstrated in studies using oncogenically transformed cells treated *in vitro* and *in*

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vivo (Lowe *et al*., 1993a), and was subsequently extended to a variety of settings. Consistent with the potential role for p53 in modulating chemotherapy in human cancers, loss of p53 function was linked to chemoresistance in certain tumor types (Wallace-Brodeur and Lowe, 1999; Johnstone *et al*., 2002). Although the overall contribution of p53 to chemosensitivity in human patients remains under debate, these studies revealed the potential importance of apoptosis in cancer chemotherapy and initiated a link between cancer development and therapy. Thus, a more complete understanding of the p53 apoptotic program presents hope for improved assays for cancer diagnosis and prognosis, and may suggest rational strategies to improve therapy. Here, we summarize the roles, regulation, and execution of the p53 apoptotic program.

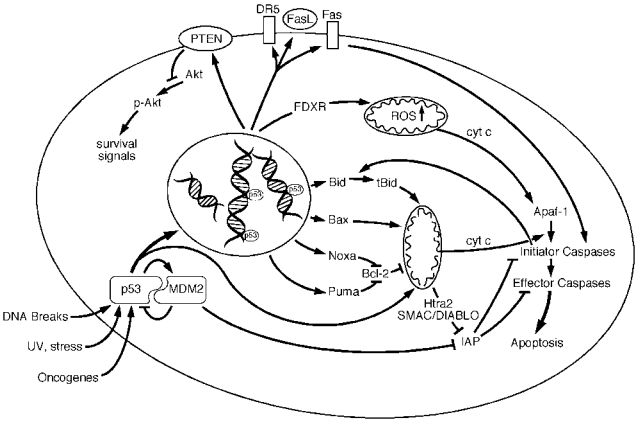

Figure 1 A model for p53-induced apoptosis by simultaneous targeting of distinct points in the apoptotic network.

The apoptotic program

Apoptosis is a complex process that proceeds through at least two main pathways (extrinsic and intrinsic), each of which can be regulated at multiple levels. The extrinsic pathway, which consists of cell surface receptors, their inhibitory counterparts ('decoy death receptors'), and their associated cytoplasmic proteins, can be modulated by altering the number of each type of receptor, thus setting the rheostat that determines the sensitivity of cells to various ligands (Peter and Krammer, 2003). Additional points of regulation include the expression levels of these activating ligands and the cytoplasmic adapter molecules (e.g. FADD) required for procaspase activation upon ligand binding, as well as the death inhibitory molecules (e.g. FLIP) (Peter and Krammer, 2003).

The intrinsic pathway centers on the mitochondria, which contain key apoptogenic factors such as cytochrome *c*, AIF, SMAC/DIABLO, Htra2/Omi (see, for review, Kroemer and Reed, 2000), and endoG (Li *et al*., 2001). Major regulators of the intrinsic pathway are the pro- and antideath members of the Bcl-2 family (Tsujimoto, 2003). These proteins reside at, or translocate to the mitochondria, controlling the release of the aforementioned factors. Furthermore, the inhibitor of apoptosis proteins (IAPs) provides another level of control for both the intrinsic and extrinsic pathways, which often cooperate – depending on cell type and stimulus – to kill a cell in an orderly way. p53 serves as a regulator of the apoptotic process that can modulate key control points in both the extrinsic and intrinsic pathways (see Figure 1 for an overview).

Downstream effectors of p53 in apoptosis

p53 is a transcription factor capable of binding DNA in a sequence-specific fashion (Ko and Prives, 1996). Interestingly, virtually all tumor-derived mutants are defective in their ability to bind DNA specifically, implying that there is a strong selective pressure to disable this p53 property during the course of tumorigenesis. Accordingly, the proapoptotic activity of p53 has been linked to its transactivation capabilities through several approaches. For example, the ability of p53 to transactivate target genes has been correlated to apoptosis in some, although not all, structure function studies (Attardi *et al*., 1996; Chen *et al*., 1996), and p53 can directly activate the transcription of genes known to promote apoptosis (el-Deiry, 1998; Yu *et al*., 1999; Sax and el-Deiry, 2003). Moreover, knockin mice expressing transcriptionally dead, but DNA-binding proficient p53 are defective in apoptosis (Jimenez *et al*., 2000). This latter result provides compelling *in vivo* evidence that transactivation is essential for p53 to promote apoptosis in normal cells.

Transcriptional control of the Bcl-2 family

The most intuitive link between p53-mediated transactivation and apoptosis comes from its ability to control transcription of proapoptotic members of the Bcl-2 family. These include the 'multidomain' Bcl-2 family member *Bax* (Miyashita *et al*., 1994), as well as the 'BH3-only' members *Puma* (Nakano and Vousden, 2001), *Noxa* (Oda *et al*., 2000a), and *Bid* (Sax *et al*., 2002). In all cases, the promoters of these genes harbor consensus p53 response elements that are capable of binding p53 *in vitro* and conferring p53 responsiveness to reporter genes *in vivo*. Precisely, how these proteins act downstream of p53 to mediate apoptosis is an active area of research, but their net effect is to increase the ratio of pro- to antiapoptotic Bcl-2 proteins, thereby favoring the release of apoptogenic proteins from the mitochondria, caspase activation, and apoptosis.

Gene targeting studies in both mice and cultured cells support the notion that proapoptotic members of the Bcl-2 family can act downstream of p53 during apoptosis. For instance, *Bax*-deficient mouse embryo fibroblasts (MEFs) are desensitized to oncogene-induced apoptosis (which is also p53 dependent), leading to increased transformation *in vitro* and tumorigenesis *in vivo* (McCurrach *et al*., 1997; Yin *et al*., 1997). Moreover, disruption of either *Bax* or *Puma* in HCT116 cells

produces various degrees of apoptotic defects (Zhang *et al*., 2000; Yu *et al*., 2003). Still, studies showing that the phenotypes of genetically targeted mice (or cells from such mice) are similar (e.g. *p53* null vs. *Bax* null MEFs) do not rule out the possibility that two gene products act in parallel pathways to produce a common phenotype. However, the fact that *p53* loss attenuates the expression of the downstream targets suggests that the phenotypes of the null effectors reflect defects in p53-mediated apoptosis.

Adding to the complexity of the program, the effects of gene disruption can vary depending on experimental setting. For example, *Bax*-deficient thymocytes are sensitive DNA-damaging signals, even though oncogene-transformed MEFs lacking *Bax* show apoptotic defects. Moreover, disruption of *Bax* does not accelerate Myc-induced lymphomagenesis, even though overexpression of Bcl-2 (which antagonizes Bax) readily does so (JSF and SWL, unpublished observations). These observations highlight a recurrent theme in studies on p53-dependent apoptosis – the program details are often context dependent.

Transcriptional control of the apoptotic machinery

A body of work indicates that p53-mediated apoptosis proceeds primarily through the intrinsic apoptotic program (e.g. Soengas *et al*., 1999; Schuler *et al*., 2000). In addition to controlling factors that act upstream of the mitochrondria, p53 can also transactivate several components of the apoptotic effector machinery. One of these components is the gene encoding Apaf-1 (Kannan *et al*., 2001; Moroni *et al*., 2001; Robles *et al*., 2001), which acts as a coactivator of caspase-9 and helps initiate the caspase cascade. In addition, p53 can upregulate expression of caspase-6, often considered an effector caspase, leading to enhanced chemosensitivity of some cell types (MacLachlan *et al*., 2002). It seems likely that this point of control is not crucial for the initiation of apoptosis, but may potentiate cell death in the presence of released cytochrome *c* (Juin *et al*., 2002). A similar model has been proposed for E2F, which both promotes apoptosis and can increase caspase expression through a direct transcriptional mechanism (Nahle *et al*., 2002).

Transcriptional control of the extrinsic pathway

The extrinsic apoptotic pathway is also regulated by p53, although the overall contribution of this regulation to p53-mediated cell death is poorly understood. For example, the *Fas/CD95* (Owen-Schaub *et al*., 1995; Muller *et al*., 1998) and *DR5* (Wu *et al*., 1997) death receptor loci, as well as the gene encoding for Fas ligand, *TNFSF6* (Maecker *et al*., 2000), are each direct p53 targets. Moreover, the ability of p53 to transactivate *Bid* may facilitate crosstalk between the extrinsic and intrinsic pathways (Sax *et al*., 2002). Consequently, p53 may sensitize cells to death receptor ligands, either inducing apoptosis directly or enhancing cell death in ligand-rich environments. Interestingly, disabling this sensitization by *p53* mutation can promote drug resistance in some contexts (Fulda *et al*., 1998; Petak *et al*., 2000), and provide a degree of immune privilege to tumor cells (Green and Ferguson, 2001).

Other transcriptional targets

Beyond the core constituents of the intrinsic and extrinsic apoptotic pathways, p53 transcriptionally activates other genes that have been linked to apoptosis. In most instances, these genes have p53 response elements in their promoters and can modulate apoptosis when over- or underexpressed. For example, *PERP* was identified in a screen for apoptosis-specific genes regulated by p53, and is capable of inducing apoptosis in *p53*-null cells, albeit not to the same extent as p53 (Attardi *et al*., 2000). In a similar fashion, *PIDD* (p53-induced protein with a death domain) was identified as a p53-responsive gene induced following shift of an erythroleukemia cell line, containing temperature-sensitive p53, to the permissive (wild-type p53) temperature (Lin *et al*., 2000). Although its precise role in apoptosis remains to be determined, suppression of *PIDD* inhibits apoptosis, whereas enforced PIDD expression induces cell death. *p53DINP1* (p53-dependent damage-inducible nuclear protein 1) (Okamura *et al*., 2001) and *p53AIP1* (p53 apoptosis-inducing protein 1) (Oda *et al*., 2000b) are both p53-inducible genes that appear to form part of a mini network in the p53 apoptotic program. p53DINP1 interacts with a multiprotein kinase complex capable of phosphorlyating p53 (Okamura *et al*., 2001) on serine 46, which correlates with the transcriptional activation of *p53AIP1*. p53AIP, in turn, disrupts mitochondrial function and is sufficient to induce cell death in a number of tumor cell lines when overexpressed (Oda *et al*., 2000b; Matsuda *et al*., 2002).

p53 targets survival signaling

In addition to its ability to transactivate genes that directly promote apoptosis, p53 can also induce genes that short-circuit antiapoptotic pathways. The most obvious example of this is the ability of p53 to regulate PTEN, a negative regulator of the PI3 kinase pathway. The PI3 kinase pathway translates signals from receptor tyrosine kinases to changes in cellular physiology. Prosurvival cytokines lead to the activation of PI3 kinase, the production of phosphotidyl inositol-3,4-P_2 and -3,4,5-P_3, and activation of downstream effectors, including Akt/PKB. Akt, in turn, phosphorylates effector molecules that can regulate survival in several ways (Vivanco and Sawyers, 2002). PTEN is a lipid phosphatase that attenuates PI3 kinase signaling by dephosphorylating 3′-phosphorylated phosphatidylinositides. Notably, p53 can transactivate the *PTEN* promoter leading to increases in PTEN expression, although the induction is relatively modest. Nevertheless, these changes can have profound effects, as disruption of *PTEN* can compromise p53-mediated apoptosis in some cell types (Stambolic *et al*., 2001). Thus, p53 can counteract survival signals from the

microenvironment, presumably reducing the threshold needed for proapoptotic factors to trigger cell death.

Redox metabolism

Induction of p53 has also been shown to produce changes in REDOX metabolism, leading to increases in reactive oxygen species (ROS) prior to the onset of apoptotic cell death (Polyak *et al*., 1997; Hwang *et al*., 2001). Although precisely how this regulates cell death is poorly understood, p53 can upregulate a number of genes that affect REDOX metabolism and certain antioxidants can suppress p53-mediated cell death (Polyak *et al*., 1997; Hwang *et al*., 2001). One gene, *ferredoxin reductase* (*FDXR*), is specifically upregulated after treatment of colon carcinoma cells with the chemotherapeutic agent 5-fluorouracil (5-FU) only in cells containing p53 (Hwang *et al*., 2001). Interestingly, disruption of *FDXR* decreases the amount of ROS and reduces apoptosis following 5-FU treatment. Furthermore, the cellular REDOX state was shown to impact the levels and activity of p53 as the two studies have implicated oxidoreductases in the regulation of p53 stability (Asher *et al*., 2001; Chang *et al*., 2003). Thus, the activation of p53 leads to an increase in ROS that, perhaps by interfering with mitochondrial function and/or integrity, contributes to cell death. In addition, the higher levels of ROS appear to be part of feedforward loop that stabilizes p53 resulting in more p53 activity.

P53-mediated transrepression

While most studies investigating the action of p53 in apoptosis have focused on its transactivation functions, p53 also has transrepression capabilities that may contribute to apoptosis (Mack *et al*., 1993; Zhang *et al*., 1998, 1999). How p53 represses transcription is not fully established, but appears to involve its ability to recruit histone deacetylases to certain genes through the mSin3a corepressor (Murphy *et al*., 1999). One of the targets of p53-mediated repression is *Survivin*, which encodes an IAP capable of inhibiting apoptosis when overexpressed (Ambrosini *et al*., 1997). In principle, *p53* mutations might contribute to the high frequency of Survivin overexpression observed in human tumors (Hoffman *et al*., 2002). Nevertheless, Survivin also has important roles in mitosis, and as such, the extent to which dysregulation of Survivin's antiapoptotic activity contributes to tumor progression is unclear. However, at least under certain circumstances, such as hypoxia, the ability of p53 to transrepress may be more important for inducing apoptosis than its transactivation function (Koumenis *et al*., 2001). How this translates to p53's role in tumor suppression is unclear.

Nontranscriptional modes of action

Although p53 can up- and downregulate gene transcription, its influence on apoptosis may not end there. Indeed, p53 may also control apoptosis through transcription-independent mechanisms (Caelles *et al*., 1994; Wagner *et al*., 1994; Haupt *et al*., 1995; Chen *et al*., 1996; Kokontis *et al*., 2001; Dumont *et al*., 2003). For the most part, studies linking apoptosis to transcription-independent functions of p53 involve overexpression of mutant p53 proteins at unphysiologic levels, and so the contribution of this mode of regulation to apoptosis induced by endogenous p53 is not well established. However, recent studies suggest that stress-induced accumulation of p53 can occur in the mitochondria (Mihara *et al*., 2003). Here, mitochondrial redistribution of p53 precedes cytochrome *c* release and caspase activation, and occurs only during p53-dependent cell death. This mitochondrial p53 appears to be proapoptotic, since direct targeting of p53 to mitochondria can promote apoptosis in *p53*-deficient cells (Mihara *et al*., 2003). Moreover, polymorphic p53 variants that have different apoptotic potential show a differential ability to localize to the mitochondria, with the least proapoptotic being deficient in this property (Dumont *et al*., 2003).

Although the transcription-independent functions of p53 are intriguing, they are unlikely to be essential for p53-mediated apoptosis. As indicated earlier, sequence-specific DNA binding appears to be the primary p53 function selected against during tumorigenesis, and normal cells harboring an endogenous p53 mutant that is defective in transactivation capabilities do not undergo apoptosis (see above). Although some tumor-derived mutants may also be defective in their ability to act at the mitochondria, the preponderance of evidence suggests that p53's role in transcriptional activation is the crucial activity in regulating apoptosis. It seems more likely that the nontranscriptional activities of p53 play an auxiliary role, potentiating p53-mediated cell death.

Coordination of the apoptotic program by p53

Why would a transcription factor evolve to use so many distinct mechanisms to produce the same biological end point? Such a scenario seems extremely inefficient and redundant for a protein that appears dispensable for normal development (Donehower *et al*., 1992). However, this paradox can be reconciled if one views the various p53 targeting mechanisms not as isolated circuits, but as part of a coordinated process that targets key nodes of the apoptotic network. By simultaneously targeting several levels of the apoptotic program, p53 increases the probability that the process goes forward and ensures a well-coordinated program once the process is initiated. Moreover, such a program builds in a variety of control points that integrate many elements of the cellular milieu.

Notably, the fact that p53 simultaneously targets multiple 'death' circuits to coordinate an apoptotic response explains, in part, why no single p53 effector molecule can account for all of p53's proapoptotic activity. Moreover, since each circuit functions as part of a larger network rather than a specific linear pathway,

they can be affected in different ways by the cell type, microenvironment, apoptotic stimulus, or genetic background. As a consequence, one or more circuits may stand out as the crucial element in a particular cell type, or at different stages during tumor progression.

Such a model may explain some of the apparently conflicting data in the literature. For example, disruption of *Bax* compromises p53-mediated apoptosis in oncogenically transformed fibroblasts (McCurrach *et al*., 1997) and developing tumors of the choroid plexus (Yin *et al*., 1997), but has no obvious effect on p53-mediated apoptosis in normal thymocytes (Knudson *et al*., 1995). Perhaps, cell type differences or changes in the apoptotic network produced by oncogene expression alter the relative importance of the Bax-regulated circuit for p53-mediated cell death. Moreover, disruption of the p53 effector *Apaf-1* attenuates p53-mediated apoptosis in transformed fibroblasts (Soengas *et al*., 1999), melanoma cells (Soengas *et al*., 2001), and the developing central nervous system and lens of the *Rb*-deficient mouse (Guo *et al*., 2001). Conversely, thymocytes lacking *Apaf-1* respond normally to irradiation (Marsden *et al*., 2002). In most instances, however, caspase-9 is activated during apoptotic cell death in the Apaf-1-expressing counterparts, implying that the p53-mediated programs are not fundamentally different, but that the ''Critical Nodes'' may vary. Here again, it is possible that aberrantly proliferating cells may rewire their apoptotic networks, leading to a greater relative reliance on the apoptosome for efficient cell death. Alternatively, thymocytes and cells of the hematopoietic compartment are hardwired to die in response to many stimuli, and may have in place more redundant or efficient death effector mechanisms. In other words, in the absence of *Apaf-1*, some cell types bypass their 'first choice' pathway and use alternative methods to activate effector caspases and induce apoptosis. Clarifying these complexities represents a challenge, but also offers hope for more selective intervention of the p53-apoptotic program.

Regulation of p53-dependent apoptosis: deciding cell fate

In addition to its ability to promote apoptosis, p53 can also induce cell cycle arrest, cellular senescence, and directly influence DNA repair. What determines whether p53 induces apoptosis rather than another outcome? Initial studies suggested that the most important determinant of this decision is cell type or tissue of origin. For example, γ-irradiation of fibroblasts engages a p53-dependent G1 cell cycle arrest, while in thymocytes, it produces a p53-mediated apoptosis (Kuerbitz *et al*., 1992; Clarke *et al*., 1993; Lowe *et al*., 1993b). However, cell type differences alone cannot explain the different outcomes, since fibroblasts expressing the E1A or Myc oncoproteins undergo p53-dependent apoptosis in response to γ-irradiation or other forms of DNA damage. Similarly, lymphoma cells typically undergo a p53-dependent apoptotic program in response to the chemotherapeutic drug cyclophosphamide; however, if these same lymphoma cells overexpress Bcl-2 (which prevents apoptosis), the cells undergo a p53-dependent program of cellular senescence (Schmitt *et al*., 2002b). Finally, enforced expression of p53 promotes apoptosis in myeloid leukemia cells in a manner that is suppressed in the presence of IL-6, despite the same levels of p53 expression (Yonish-Rouach *et al*., 1991). Therefore, both genetic background and microenvironment significantly impact p53 responses.

The outcome of p53 activation may also be influenced by the strength or nature of the p53-activating stimulus. For example, in MEFs, E1A activates p53 to promote apoptosis, whereas oncogenic Ras activates p53 to promote senescence (Lowe and Ruley, 1993; Serrano *et al*., 1997). Interestingly, in both instances, oncogene signaling to p53 requires the ARF tumor suppressor, which stabilizes p53 by interfering with its negative regulator Mdm2 (de Stanchina *et al*., 1998; Paramio *et al*., 2001). Although the molecular basis for these differences remains to be established, microarray studies show that distinct p53 activating stimuli (e.g. γ-radiation *vs* UV) can produce unique p53-dependent gene expression patterns. Thus, the upstream signal can impact the downstream response (Zhao *et al*., 2000).

Quantity vs *quality*

How might p53 interpret contextual factors and respond accordingly? One model assumes that different p53 outcomes are sensitive to the magnitude or robustness of the p53 response. In principle, the amplitude or duration of the activating signal, or a variety of factors that affect other signaling pathways in the cell, may enhance or suppress p53 activation to impact the p53 response. For example, Ras signaling can induce Mdm2 in a p53-dependent manner, thereby blunting p53 activation in response to DNA damage in Ras-expressing cells, compared to non-Ras-expressing cells (Ries *et al*., 2000). Notably, the quantitative model assumes the existence of p53-responsive genes containing promoter elements with differing binding affinities, or perhaps the engagement of nontranscriptional p53 activities dependent on p53 dose. In the case of transcriptional targets, a subset of promoters should be activated only when the expression level of p53 reaches a certain threshold. Activation of this subset of promoters would lead to unique transcription profiles altering the cellular response to p53. Such a model is consistent with studies using conditional p53 expression systems, where low p53 levels promote arrest and higher p53 levels promote apoptosis (Chen *et al*., 1996; Zhao *et al*., 2000). Nevertheless, the underlying mechanism for these effects, and whether they relate to differential affinity for certain p53-responsive promoters, remains to be determined.

It is also possible that nonquantitative (i.e. qualitative) mechanisms can influence the outcome of p53

activation. In one scenario, the downstream consequences of p53 activation are the same irrespective of biological outcome, but contextual factors ('collateral signals') influence how the cell interprets the signal. Collateral signals may differ depending on the cell or tissue type, the genetic background, or the status of other signaling pathways in the cell. As one example, enforced expression of Myc can shift the outcome of p53 activation from cell-cycle arrest to apoptosis. The underlying mechanism for this effect appears to depend on the ability of DNA damage to induce p21, the cyclin-dependent kinase inhibitor linked to p53-mediated arrest (Seoane *et al*., 2002). In cells overexpressing Myc, p53 is unable to transactivate *p21* because Miz1 (an Myc relative and binding partner) recruits Myc to the *p21* promoter, where the complex prevents p53-mediated transcription. Interestingly, Myc does not interfere with p53-mediated transcription of key apoptosis mediators and, as such, acts as a 'collateral signal' that makes apoptosis the dominant pathway upon DNA-damaging treatment.

In another scenario, p53 itself is fundamentally different depending on the activating stimulus and/or cell type, leading to qualitative differences in signal output. Such a model is consistent with the observation that gamma or UV radiation can induce different p53 target genes in the same cell type (Zhao *et al*., 2000). Interestingly, these distinct stimuli lead to different post-translational modifications on p53 (Kapoor and Lozano, 1998; Lu *et al*., 1998; Webley *et al*., 2000). In principle, p53 molecules with distinct modifications may have different promoter preferences or recruit distinct transcriptional coactivators, thus leading to the activation of a distinct population of p53 target genes and different cellular responses. In this regard, DNA damage and hypoxia produce different p53 modifications, which correlate with the ability of p53 to associate with different transcriptional coactivators and repressors (Koumenis *et al*., 2001). Furthermore, p53 modifications can influence the ability of p53 to bind its negative regulator Mdm2 (a p53-inducible E3 ubiquitin ligase capable of targeting p53 for degradation), resulting in a higher level of p53 protein and signaling (Shieh *et al*., 1997). In other words, a qualitative effect on p53 (phosphorylation) has a quantitative effect on p53 signaling.

Which post modifications might influence p53 activity?

The most well-studied p53 modification is phosphorylation, and indeed p53 can be phosphorylated on many residues (see, for review, Meek, 1999; Prives and Hall, 1999). Determining whether and how specific p53 modifications are important for distinct p53 responses represents a challenge that has been difficult to address by routine structure function analysis. Currently, only phosphorylation on serine 46 has been linked to the ability of p53 to promote apoptosis, where it has been shown that this form of p53 preferentially activates apoptotic effectors such as p53AIP1 (Oda *et al*., 2000b). Nevertheless, serine 46 is not conserved in murine p53, yet murine cells are perfectly able to undergo apoptosis. Hence, other modifications and/or mechanisms must also be important. In addition, p53 can also be post-transcriptionally modified by acetylation and sumolation of certain lysine residues, and these changes may contribute to p53 activation (see, for review, Meek, 1999; Prives and Manley, 2001; Alarcon-Vargas and Ronai, 2002). Whether these modifications qualitatively influence the outcome of p53 activation remains unclear.

Whether or not p53 activation connects to the apoptotic network has important ramifications for treating cancer, and perhaps other diseases as well. For example, as discussed previously, different cell types have different default programs following p53 activation. In principle, this may influence the utility of certain chemotherapeutic agents, as many of their dose-limiting side effects arise in tissues having apoptosis as their default p53 response (e.g. intestinal epithelium and the hematopoietic system Gudkov and Komarova, 2003). Indeed, efforts are underway to increase the therapeutic window of chemotherapeutics used to treat *p53*-deficient tumor cells by inhibiting the toxic effects to such normal tissues. Conversely, the ability of oncogenes such as Myc to sensitize cells to chemotherapy may explain, in part, the therapeutic index of certain chemotherapeutic agents to begin with (Lowe and Lin, 2000; Pelengaris *et al*., 2002). Finally, disruption of apoptosis downstream of p53 can reveal p53-dependent growth arrest programs in tumor cells that are not as effective as apoptosis at prolonging overall survival in mice treated with chemotherapy (Schmitt *et al*., 2002b). Clearly, restoring the apoptotic programs to these tumor cells would have a therapeutic benefit.

The extended p53 family

Although p53 was an orphan for many years, it is now known to be part of a larger gene family. p73 was discovered in 1997 and shortly thereafter p63 was identified (see, for review, Yang *et al*., 2002). Although both p63 and p73 share key functional domains with p53, including its N-terminal transactivation domain, C-terminal oligomerization domain, and a conserved DNA-binding domain, their gene organization and developmental roles are considerably more complex (Yang *et al*., 2002). For example, in contrast to *p53*, the *p63* and *p73* genes encode for several isoforms, including variants that lack the N-terminal transactivation domain that can function as dominant negatives when overexpressed (Moll *et al*., 2001). Moreover, studies using knockout mice reveal that p63 is required for normal epithelial stem cell function and for the proper development of several tissues (Mills *et al*., 1999; Yang *et al*., 1999), whereas p73 functions primarily in the central nervous system (survival, neurogenesis, and spinal fluid homeostasis) (Yang *et al*., 2002). This contrasts with p53, which has no overt role in normal development (Donehower *et al*., 1992).

Interestingly, both p63 and p73 have been linked to apoptosis, raising the possibility that they, like p53, may be tumor suppressors. However, the precise role of these genes in cancer development is unclear, in part, because inactivating mutations in tumors have not been identified (Yang *et al*., 2002) and *p73*-deficient mice are not tumor prone (Yang *et al*., 2000) (note that the early death of *p63*-null mice has precluded tumorigenicity studies to date). Nevertheless, recent studies suggest that p63 and p73 can modify apoptosis and perhaps tumor behavior. For example, overexpression of p63 and p73 induces apoptosis and upregulates p53 target genes in several cell types (Moll *et al*., 2001). Although it is possible that this activity merely reflects the ability of either protein to take on p53 functions when sufficiently overexpressed, emerging evidence suggests that p63 and p73 can be induced in response to certain apoptotic triggers, such as DNA damage, overexpression of E2F1 or activated oncogenes (Katoh *et al*., 2000; Soengas and Lowe, 2000). Furthermore, the transactivation domain-deficient isoforms of p63 and p73 are overexpressed in some human tumors (see, for review, Moll *et al*., 2001; Melino *et al*., 2002; Benard *et al*., 2003), where they may act as dominant negatives or interfere with normal p53 function by forming mixed complexes with p53 (Moll *et al*., 2001). Conversely, some missense p53 mutants bind p73 and interfere with chemotherapy-induced apoptosis (Bergamaschi *et al*., 2003; Irwin *et al*., 2003). These results may explain the gain-of-function activities of some p53 mutants and identify a potentially important mechanism of chemoresistance.

p63 and p73 may induce apoptosis through several mechanisms. In some settings, p63 and/or p73 may act independently of p53 to promote cell death. In principle, such programs may induce a program mechanistically similar to p53-mediated apoptosis under different circumstances or settings than p53, perhaps in developmental settings or compensatory circumstances where p53 is not expressed. Alternatively, p63 and/or p73 may act in parallel with p53 to promote apoptosis. For example, E2F-1 (which activates p53 to promote apoptosis) can induce p73, leading to apoptosis in *p53*-null cells (Irwin *et al*., 2000; Lissy *et al*., 2000; Stiewe and Putzer, 2000). Such a cooperative mechanism might explain the ability of many p53 activating stimuli to promote apoptosis in *p53*-deficient cells, albeit to a lesser extent than if p53 is present.

In some settings, p63 and p73 may be part of the central mechanism whereby p53 promotes apoptosis. Consistent with this possibility, oncogene-expressing fibroblasts and embryos from double mutant mice lacking both *p63* and *p73* are as resistant to DNA damage-induced cell death as those from animals lacking *p53* (Flores *et al*., 2002). However, double mutant cells still induce p53 in response to stress, leading to the activation of some target genes. Yet, these cells do not recruit p53 to apoptosis-specific promoters and are unable to activate p53-responsive genes linked to apoptosis (Flores *et al*., 2002). Nevertheless, while these results are provocative, the true impact of p63 and p73 on p53-dependent apoptosis in human tumors remains to be established.

Is apoptosis important for tumor suppression by p53?

The p53 tumor suppressor was initially identified as the 'guardian of the genome' based on its ability to mediate a G1 arrest following DNA damage (Kuerbitz *et al*., 1992; Lane, 1992). However, as indicated above, p53 is now known to act in many cellular processes, including cell-cycle checkpoints, DNA repair, senescence, angiogenesis, surveillance of genomic integrity, and apoptosis (Ko and Prives, 1996; Evan and Vousden, 2001). In principle, disruption of any one or combination of these may produce an advantage during tumor development and indeed, it is widely assumed that the high frequency of *p53* mutations in human tumors reflects the profound advantage a developing tumor cell receives by simultaneous loss of all p53 functions (Vogelstein *et al*., 2000).

How do we know that apoptosis is important for p53's tumor suppressor activity? Other than intuition, the importance of apoptosis for p53-mediated tumor suppression is inferred from correlative studies linking *p53* loss to apoptotic defects during the progression of murine and human tumors (Bardeesy *et al*., 1995; Attardi and Jacks, 1999), as well as by functional studies demonstrating that strictly antiapoptotic activities can accelerate tumorigenesis in transgenic mice (Strasser *et al*., 1990; Yin *et al*., 1997; Eischen *et al*., 2001). Furthermore, certain *p53* wild-type tumors harbor mutations that can suppress apoptosis downstream of p53 (Meijerink *et al*., 1998; Ionov *et al*., 2000; Soengas *et al*., 2001), and some tumor-derived p53 mutants are defective at inducing apoptosis but not cell-cycle arrest (Aurelio *et al*., 2000). Nevertheless, because of the many other defects present in *p53* mutant tumor cells, it has been difficult to assess the overall contribution of apoptosis to p53-mediated tumor suppression.

Attempts to address this issue directly have used mouse models to determine whether disruption of individual p53 effectors can recapitulate the effects of p53 inactivation during tumorigenesis. Although inactivation of a single p53 effector has not been able to phenocopy *p53* loss, it has been difficult to determine whether this observation reflects the requirement of multiple p53 effectors for apoptosis or the contribution of other p53 effector programs. This caveat has recently been addressed in Myc-induced lymphomas arising in Eμ-*myc* transgenic mice (Schmitt *et al*., 2002a). Here, the effects of *p53* deficiency on lymphomagenesis were compared to the effects of Bcl-2 expression – a potent antiapoptotic gene that acts downstream of p53 to ablate p53-mediated cell death completely. Interestingly, lymphomas arising in the presence of Bcl-2 arose with the same accelerated onset as *p53*-null lymphomas and displayed a similar disseminated pathology. Moreover, Bcl-2 overexpression prevented *p53* mutations in mice heterozygous for *p53*, indicating that disruption of apoptosis downstream of p53 could compensate for *p53* loss in this model. Interestingly, whereas *p53*-null

lymphoma cells had cell-cycle checkpoint defects and were highly aneuploidy, Bcl-2-expressing lymphomas (harboring intact *p53*) retained these checkpoints and were largely diploid. Thus, in this system, apoptosis is the only p53 function selected against during lymphomagenesis, whereas the cell cycle checkpoint defects and genomic instability are by-products of *p53* loss. Importantly, these experiments argue that not all p53 functions contribute to tumorigenesis.

While the Eμ-*myc* model represents a situation in which apoptosis is essential for p53-mediated tumor suppression, this is not to say that apoptosis is the only function of p53 that is important. In fact, there appears to be two variables that determine which p53 functions contribute to its tumor suppressor activities – context and evolution. Context – the cell type or initiating oncogenic event – can dramatically influence the response of p53 and, hence, what is the primary tumor suppressor function of p53 that must be overcome for tumor expansion. For example, the c-Myc oncoprotein drives proliferation, but also promotes apoptosis. Thus, in situations where Myc activation is the initiating oncogenic event, such as the Eμ-*myc* model described above, continued expansion is profoundly limited by ongoing apoptosis. This provides a strong selection for loss of apoptosis and, indeed, the immediate advantage these developing cells acquire from *p53* mutations is a survival advantage – that is apoptosis is the key tumor suppressor function of p53 to be circumvented. On the other hand, constitutive activation of the Ras-MAPK pathway in fibroblasts and epithelial cells can induce proliferation but, at high levels, premature senescence (Serrano *et al*., 1997; Woods *et al*., 1997; Lin and Lowe, 2001). In this setting, *p53* loss prevents senescence, allowing cell division to continue unabated. As Ras-expressing cells are not particularly sensitive to apoptosis, it seems likely that this increased propensity to undergo premature senescence produces selective pressure to inactivate *p53* during Ras-initiated tumorigenesis. Here, p53-mediated arrest is the key tumor suppressor function to be overcome.

In addition, the evolution of a cancer – driven by genetic or epigenetic changes that accompany progression or selected by cancer therapy – also provides strong selective pressure to disable p53 or its effector functions. As a consequence, selective pressure to thwart certain circuits of the p53 tumor suppressor network can vary during the course of tumorigenesis. How the tumor 'solves' specific problems early in tumorigenesis can influence tumor behavior later on. For example, as indicated above, the evolution of Eμ-*myc* lymphomas places strong selective pressure to disable p53-dependent apoptosis. The tumor can solve this problem through several mechanisms, for example, through inactivation of *Ink4a/ARF*, *p53*, or overexpression of Bcl-2 (Eischen *et al*., 1999; Schmitt *et al*., 1999, 2002a). Indeed, a substantial fraction of Eμ-*myc* lymphomas acquire spontaneous *Ink4a/ARF* or *p53* mutations during lymphoma development, and engineered lymphomas lacking *Ink4a/ARF*, *p53*, or overexpressing Bcl-2 are phenotypically indistinguishable (Eischen *et al*., 1999; Schmitt *et al*., 1999, 2002a). However, despite their similar overall pathology, mice bearing *Ink4a/ARF*-null tumors have a significantly better treatment prognosis than do mice with *p53*-null tumors when treated with chemo- or radiotherapy (Schmitt *et al*., 2002b). Presumably this reflects fact that oncogenes, but not DNA damage, signal to p53 through ARF. Hence, loss of *Ink4a/ARF* or *p53* confers the same advantage to the tumor during lymphomagenesis but not during therapeutic treatment. Similarly, tumors overexpressing Bcl-2 do not undergo apoptosis in response to therapy, but instead undergo senescence. *p53* loss disables both programs, leading to a substantially worse prognosis. Thus, while *p53* mutant Eμ-*myc* lymphomas gain no immediate advantage by disabling senescence, this 'by-product' of *p53* loss produces a more drug-resistant tumor prior to therapy. Similarly, an increased propensity for genomic instability in *p53* mutant tumors, not seen in those lacking ARF or overexpressing Bcl-2, may fuel additional mutations and the evolution of drug resistance. As such, the fact that different tumors disable the p53 network at distinct points or times may contribute to the heterogeneity of human cancers.

Conclusions

As is clear from this review, p53 biology is complex. While this is not surprising given the central role of p53 in diverse stress responses, the complexity of the p53 network presents a challenge for fully understanding its biology and using this information for diagnostic, prognostic, or therapeutic purposes. However, we do know that apoptosis is a vital part of p53's tumor suppression function, as well as a great deal concerning how p53 controls the induction of apoptosis. Largely, this is through transcriptional activation of specific target genes, although evidence implicating both its transrepressive functions as well as direct effects on the mitochondria is mounting (see above). In addition, while evading p53-mediated apoptosis can be essential for tumor evolution, the manner in which a tumor does so can have an impact on tumor behavior and patient outcome.

What remains to be determined concerning the apoptotic p53 program? More upstream regulators and downstream effectors of p53 will undoubtedly be described, but the real challenge is to determine how contextual factors influence the network and how tumor heterogeneity can be understood and exploited for therapeutic purposes. Ideally, this increased understanding will permit the p53 network to be manipulated in more selective ways. Clearly, one avenue is to restore apoptosis by reintroducing a specific p53 activity, either through gene therapy or the rational design of small molecules. At the same time, the effects of p53 in normal tissues must be taken into account, including the role of p53-dependent apoptosis in producing toxic side effects from chemotherapy, as well as the potential for blocking p53-mediated apoptosis for acute or chronic diseases involving excessive cell death (Komarova and Gudkov,

2001; Tyner *et al*., 2002). Hence, a better understanding of the p53 network may allow for custom-tailored cancer therapy, reduced therapy-induced side effects, and the ability to affect the progression of a variety of other degenerative diseases.

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