LIVE OR LET DIE: THE CELL'S RESPONSE TO p53

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Compared with many normal tissues, cancer cells are highly sensitized to apoptotic signals, and survive only because they have acquired lesions — such as loss of p53 — that prevent or impede cell death. We are now beginning to understand the complex mechanisms that regulate whether or not a cell dies in response to p53 — insights that will ultimately contribute to the development of therapeutic strategies to repair the apoptotic p53 response in cancers.

Faced with the clinical nightmare of an invasive cancer that thwarts all therapeutic assaults, it seems hard to imagine that many tumour cells are themselves teetering on the brink of self-destruction, surviving only by virtue of mutations that allow them to evade the many death signals to which they are subjected. Yet, our increasing understanding of the molecular evolution of the cancer cell has revealed that many of the alterations that make a cell malignant and dangerous, such as oncogene-driven deregulated proliferation and invasion, are hard-wired to signals that prevent cell growth — in many cases, by signalling apoptosis[1]. It seems that the life of most cells that acquire potentially malignant characteristics is rapidly terminated, leaving only rare incipient cancer cells in which defects in the apoptotic response allow survival and further malignant progression. The net result is that virtually all cancer cells are highly sensitized to apoptosis, and repair of the lesions that prevent the implementation of the apoptotic response is likely to cause death specifically in tumour cells, sparing normal cells that do not carry such a high apoptotic burden. Components of the cell-death pathways that are altered during tumour development are therefore excellent targets for drug design, with the expectation that such therapies will show much lower toxicity in normal tissue compared with the conventional genotoxic agents that are currently in clinical use. This widening of the therapeutic window should allow for the development of more effective, better-tolerated and safer treatment options.

In order to pursue apoptotic pathways for tumour therapy, it is clearly important to understand how tumour cells become resistant to apoptosis. Tumour-associated perturbations in the components of both the mitochondrial and death-receptor-mediated apoptotic pathways have been identified[2]. Arguably, however, the most common anti-apoptotic lesion that is detected in cancers is inactivation of the p53 tumour-suppressor pathway. So, how does p53 contribute to the activation of cell death, what determines whether induction of p53 will trigger apoptosis and how might this knowledge be used to develop cancer therapies?

p53 functions

The p53 tumour suppressor belongs to a small family of related proteins that includes two other members — p63 and p73 (see the review article by Melino *et al.* on page 605 of this issue). Although structurally and functionally related, p63 and p73 have clear roles in normal development[3], whereas p53 seems to have evolved in higher organisms to prevent tumour development. p53 is activated in response to several malignancy-associated stress signals, resulting in the inhibition of tumour-cell growth[4] (FIG. 1). Several responses can be provoked by p53, including cell-cycle arrest, senescence, differentiation and apoptosis, with the option chosen being dependent on many factors that are both intrinsic and extrinsic to the cell (see below). Under some circumstances, p53 also contributes to the repair of genotoxic damage, potentially allowing for the release of the rehabilitated cell back into the proliferating pool. In most cases, however, induction of p53 leads to an irreversible inhibition of cell growth, most decisively by activating apoptosis.

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DOMINANT-NEGATIVE MUTANT
A non-functional mutant protein that competes with the normal, non-mutated protein, thereby blocking its activity.

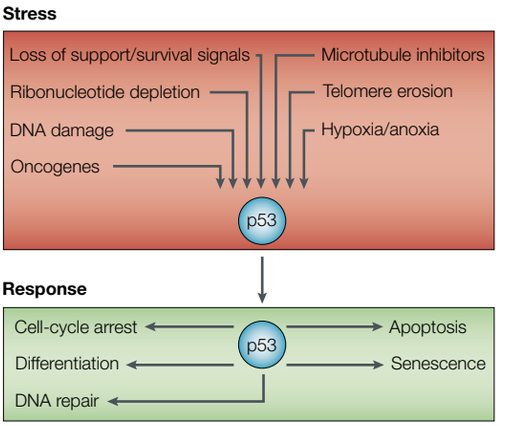

Figure 1 | **The p53 response.** p53 mediates the response to various stress signals, many of which are encountered during tumour development and malignant progression. In general, these signals induce p53 by stabilizing the p53 protein, which leads to an increase in cellular p53 levels. Several cellular responses to p53 activation have been described, and the choice of response depends on factors such as cell type, cell environment and other oncogenic alterations that are sustained by the cell. In general, however, the effect of p53 activation is to inhibit cell growth, either through cell-cycle arrest or induction of apoptosis, thereby preventing tumour development.

Mechanisms for p53 loss in cancers

Mutation of p53. p53 function in cancers can be lost by various mechanisms, including lesions that prevent activation of p53, mutations within the *TP53* gene (which encodes p53) itself or mutations of downstream mediators of p53 function (FIG. 2). Analysis of many tumours has shown that *TP53* is mutated in about half of all cancers, resulting in loss of apoptotic function. From the data available, it would seem that only 5% of *TP53* mutations are found in the regulatory domains (amino terminus, amino acids 1–99; carboxyl terminus, amino acids 301–393), whereas 95% of the mutations occur in the central region of *TP53*, which is responsible for sequence-specific DNA binding (amino acids 100–300) (FIG. 3). However, much of this information was derived from sequence analysis that included only exons 5–8 within the *TP53* gene, and examination of the whole p53 coding sequence is beginning to reveal an increasing number of p53 mutations in the amino and carboxyl termini of the protein. It is possible that the true mutation incidence for p53 in cancers is actually significantly higher than the current estimate of ~50% — indeed, recent studies that highlight the importance of mutations outside the central core[5,6] indicate that our search for important mutations within the *TP53* locus in tumours is far from over.

Tumour-associated mutations in *TP53* are predominantly point mutations (93.6%) that result in single amino-acid substitutions — a mutational spectrum that is quite different from that seen in other tumour-suppressor genes, in which large deletions or frameshift mutations tend to result in the complete loss of protein expression. Furthermore, certain *TP53* codons show an unexpectedly high mutation frequency, with 28% of the mutations affecting only six residues — 175, 245, 248, 249, 273 and 282 — of p53 (TABLE 1). The result of the mutational inactivation of *TP53* by single-amino-acid substitutions is that many tumour cells retain the ability to express the mutant p53 protein. These proteins are often more stable than wild-type p53, and are present at very high levels in the tumour cell. One explanation for the selection of such mutations is that the mutant p53 proteins can act as DOMINANT-NEGATIVE inhibitors of wild-type p53, which functions as a tetramer[7,8]. The observation that many tumours that harbour *TP53* point mutations also show loss of heterozygosity — effectively eliminating the wild-type allele[9] — indicates that the efficiency of dominant-negative inhibition might not be complete, and almost certainly depends on the nature of the initial point mutation. However, partial inactivation of wild-type p53 function by mutant p53 might allow for some selective advantage during tumour progression, and mice that are heterozygous for a dominant-negative *TP53* point mutant developed tumours without loss of the wild-type *TP53* allele[10].

In addition to the dominant-negative inactivation of wild-type p53, there is evidence that some of the tumour-associated mutant p53 proteins acquire new transforming functions that contribute to tumour development[11]. Although not all p53 mutants show this activity — for example, the expression of the potently transforming *TP53* mutant allele A135V in p53-null mice had no effect on tumour development[12] — other p53 mutants can inhibit apoptosis independently of p53 (REF. 13). The mechanism underlying the gain of function of some p53 mutants is not clear, but it might reflect the ability of these mutants to interact with and inhibit the activities of the other p53-family

Summary

- p53 is a tumour-suppressor protein that induces apoptotic cell death in response to oncogenic stress. Malignant progression is dependent on loss of p53 function, either through mutation in the *TP53* gene (which encodes p53) itself or by defects in the signalling pathways that are upstream or downstream of p53.
- Mutations in *TP53* occur in about half of all human cancers, almost always resulting in the expression of a mutant p53 protein that has acquired transforming activity.
- p53-induced apoptosis depends on the ability of p53 to activate gene expression, although transcriptionally independent activities of p53 can also contribute to the apoptotic response.
- The apoptotic and cell-cycle arrest activities of p53 can be separated, and apoptotic cofactors that play a specific role in allowing p53-induced death are being identified.
- Regulation of the apoptotic function of p53 is associated with selective activation of apoptotic target genes. Cofactors that specifically contribute to p53-mediated activation of apoptotic target genes include JMY, ASPP and the other p53-family members p63 and p73.
- Phosphorylation of p53 regulates its ability to activate the expression of apoptotic target genes, and other post-translational modifications such as acetylation might also have a role.
- In tumours that retain wild-type p53, the apoptotic response might be hindered by defects in the apoptotic cofactors. These, therefore, represent additional targets for the design of therapeutics that are aimed at reactivating p53-mediated apoptosis in cancer cells.

UBIQUITIN LIGASES
A family of enzymes that function in the final step of conjugation of ubiquitin chains to lysine residues in target proteins. Polyubiquitylated proteins are recognized and degraded by the proteasome.

APC
(Adenomatous polyposis coli). A tumour-suppressor gene that is mutated in sporadic colorectal cancers.

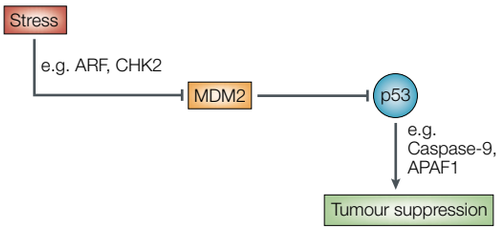

Figure 2 | **Loss of p53 activity in cancers.** In normal cells, stress signals lead to the inhibition of MDM2 — one of the main regulators of p53 stability and activity — allowing activation of the tumour-suppressor functions of p53. The ability to induce a p53 response is lost in virtually all cancer cells: by defects in the pathways that prevent downregulation of p53 by MDM2 (for example, by loss of ARF or CHK2); by mutational inactivation of p53 itself; or by disruption of the cell-growth-inhibition pathways that mediate the p53 response (for example, by loss of components of the apoptotic cascade, such as caspase-9 or apoptotic protease activating factor 1 (APAF1)).

members, p63 and p73 (REFS 14–17). These interactions — which are not seen between wild-type p53 and p63/p73 — are further regulated by a common polymorphism of *TP53* at codon 72, which influences whether or not the mutant p53 protein can bind and inactivate p73 (REF. 18). Whether mutant p53 also has a true gain of function that is independent of any dominant-negative effect on p53-family members remains unclear.

Mutations of regulators or targets of p53. Tumours that retain wild-type p53 frequently harbour defects either in the pathways that allow for the stabilization of p53 in response to stress, or in the effectors of the apoptotic activity of p53 (REF. 19; FIG. 2). Two related proteins — MDM2 and MDMX (MDM4) — have crucial roles in regulating p53 activity to allow normal growth and development[20–22]. MDM2, which participates in an autoregulatory loop with p53 (BOX 1), has been shown to function as a UBIQUITIN LIGASE that targets p53 for degradation. The activation and stabilization of p53 is generally associated with inhibition of this function of MDM2, and defects in the pathways that curb MDM2 activity are common in tumours that retain wild-type p53. Interestingly, different stress signals use different pathways to allow p53 to escape MDM2-mediated protein degradation[23], and defects in one of them might not be sufficient to prevent induction of p53 through another. This redundancy in the ability to activate p53 potentially explains the rather counter-intuitive observation that *TP53* mutations are often found in tumours at a rather late stage in malignant development, long after the acquisition of many of the oncogenic alterations that strongly induce a p53 response in experimental models. Closer examination of carcinogenic progression in colon cancers, for example, has shown that loss of ARF (also known as p14ARF in humans) — a small protein that inhibits MDM2 and mediates the induction of p53 in response to the loss of *APC* (adenomatous polyposis coli) and the deregulation of WNT-driven proliferation — occurs at early stages of tumour development, concurrent with the loss of *APC*[24]. Although this seems to be sufficient to allow the initial steps in tumour development to take place, stresses that occur later in malignant progression, such as those that accompany invasion and metastasis, might signal p53 activation by means of ARF-independent pathways. At this step, therefore, survival of the tumour depends on the acquisition of alternative lesions that inactivate p53, including mutation of *TP53* itself. The underlying advantage of this two-hit system is not clear: why not simply mutate *TP53* in the initial steps of premalignant development? The answer might lie in the tantalizing, but poorly understood, observation that loss of p53 can inhibit the initial stages of tumour development, and mice that lack p53 develop far fewer papillomas in response to carcinogen exposure[25]. The heretical suggestion that p53 might both enhance and inhibit malignant progression is consistent with the dual signals of growth and death, as proposed by Gerard Evan[26], that has become generally accepted for the proliferative oncogenes. The concept of antagonistic pleiotropy might apply to p53, as it does to oncogenes such as *MYC*[27], with the hardwiring of growth-suppressive and (as yet to be discovered) growth- or survival-promoting activities of p53. Indeed, a survival function of low levels of p53 has been described[28] that could reflect the induction of anti-apoptotic genes such as the TNF-related apoptosis-inducing ligand (*TRAIL*) decoy receptor (*TRUNDD*)[29] or heparin-binding epidermal growth factor (*HB-EGF*)[30]. Furthermore, p53-mediated induction of *CDKN1A* (which encodes WAF1, also known as p21 and CIP1) might also inhibit apoptosis[31], and these functions of p53 might actually protect cells from death during the early stages of tumour progression.

Notwithstanding the possibly equivocal role of p53 in early tumorigenesis, it seems clear that, at presentation, most cancers have lost p53 function by one mechanism

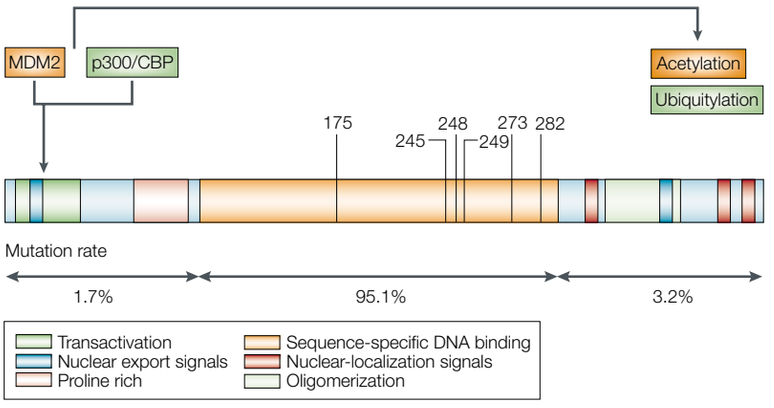

Figure 3 | **p53 structure and location of tumour-associated mutations.** p53 is a transcription factor that contains several well-defined domains, including the amino-terminal transactivation domain, a central sequence-specific DNA-binding core and a carboxy-terminal region that contains oligomerization sequences and nuclear-localization signals. Nuclear export of p53 is regulated by signals in the amino and carboxyl termini. Interaction of proteins such as MDM2 or p300/CBP with the amino terminus of p53 can lead to modifications such as acetylation or ubiquitylation in the carboxyl terminus. Almost all of the point mutations that are found in cancers occur within the central DNA-binding core of p53; the percentage of mutations within each region detected in cancers to date is indicated below. Some 28% of mutations occur within the six highlighted residues.

Table 1 | **Mutation spectra at selected codons**

Codon	% Total mutation	% At this codon
175Arg	***4.6***	***100***
175His	4.1	89
175Cys, Gln, Gly, Leu or Pro	0.5	11
245Gly	***3.3***	***100***
245Ser	1.7	52
245Asp	0.7	21
245Cys	0.4	12
245Ala, Arg, Asn, Gly or Leu	0.5	15
248Arg	***7.2***	***100***
248Gln	3.6	50
248Trp	3.0	42
248Leu	0.4	5
248Gly or Pro	0.2	3
249Arg	***3.2***	***100***
249Ser	2.2	69
249Met	0.4	12.5
249Trp	0.2	6
249Gly, Ile, Lys or Thr	0.4	12.5
273Arg	***6.8***	***100***
273His	3.2	47
273Cys	2.6	38
273Leu	0.8	12
273Gly, Ser or Tyr	0.2	3
282Arg	***2.7***	***100***
282Trp	2.33	86
282Gln, Gly, His, Leu or Pro	0.37	14

or another, and that this loss of p53 is necessary to allow continued growth and survival of the tumour cell. Tumour therapy that is based on the reactivation of p53 is therefore an attractive goal, and might be achieved by reactivating mutant p53, introducing exogenous wild-type p53 or activating endogenous p53 in those tumours that retain wild-type p53. Furthermore, accumulating evidence that the apoptotic activity of p53 is regulated separately from its other functions indicates that it might be possible to selectively induce p53-mediated apoptosis for cancer therapy.

Apoptotic activities of p53

p53 is a transcription factor that directly activates the expression of genes that contain p53-binding sites within their regulatory regions. A legion of p53-responsive genes have been identified by various means, including DIFFERENTIAL DISPLAY, SAGE and MICROARRAY[32–35]. In bioinformatic searches of the existing human genome sequence, over 4,000 putative p53-binding sites were identified[36]. Whether all of these will prove to be *bona fide* target genes of p53 is not known, and, to some extent, the criteria that we would use to verify the authenticity of a direct p53-target gene are themselves becoming less secure. We now realize, for example, that identification of p53-binding sites within promoter sequences might not reflect p53 responsiveness within intact chromatin or, conversely, that p53 responsiveness might be mediated by sequences other than the defined p53-binding sites[37]. Nonetheless, there is now a substantial number of genes that are convincingly regulated by p53 under physiologically relevant circumstances, some of which are listed in TABLE 2. Although far from complete, these examples illustrate that many of the genes that are induced by p53 can be divided into groups that might mediate a specific p53 function, such as inhibition of cell growth, DNA repair, activation of apoptosis or regulation of angiogenesis[38].

Even the most casual dip into the literature about p53 target genes reveals a rather large membership of the 'apoptosis club', indicating that p53 can induce expression of a wide array of death effectors. p53-inducible genes that might contribute to the induction

DIFFERENTIAL DISPLAY
An expression analysis method in which cDNAs from different samples are amplified by polymerase chain reaction using a combination of random primers and anchored oligo-dT primers.

SAGE
Serial analysis of gene expression that is based on the capture and analysis of a short nucleotide sequence (or tag) that is close to the 3′ end of each cDNA in the sample.

MICROARRAYS
Chips that contain arrays of oligonucleotides that correspond to known genes and that are used to analyse gene expression by hybridization with samples. In contrast to differential display and SAGE, this technique is limited to the analysis of genes that are represented on the chip.

Box 1 | **Showing some self-control**

Although many p53 target genes have a clear downstream function in mediating the response to p53 activation, there are now several examples of p53-inducible gene products that can feed back to control or modulate the activity of p53. The first — and most famous — is the ability of p53 to induce expression of MDM2, a protein that both directly inhibits p53's transcriptional activity by binding to its transactivation domain, and functions as a ubiquitin ligase to target p53 for degradation. This feedback loop contributes to the negative regulation of p53 activity during normal growth and development, and helps to switch off p53 at the end of a stress response. Layered onto this regulatory loop is the ability of p53 to induce the expression of proteins that regulate MDM2. These include WISP1 and PTEN, which could modulate MDM2 phosphorylation by controlling AKT, and cyclin G, which can recruit phosphatase 2A to dephosphorylate MDM2. As phosphorylation of MDM2 can both enhance and inhibit the degradation of p53, it is difficult at the moment to predict the effect of cyclin G on p53 activity.

p53-mediated regulation of p53 phosphorylation is developing as another mechanism for self-control. p53 induces the expression of *WIP1*, which is a phosphatase that inhibits phosphorylation of p53 on Ser46, and so prevents the induction of the apoptotic response. This activity is balanced by the p53-mediated activation of DINP1, which contributes to the phosphorylation at this site. Finally, the autoregulation of the apoptotic activity of p53 also results from the transcriptional activation of ΔNp73 — the amino-terminally truncated version of p73 — which can inhibit p53-mediated apoptosis by competing for promoter binding sites[113] (see the review article by Melino *et al.* on page 605 of this issue). Whether or not the ΔN variants of p73, or equivalent p63 isoforms, can also influence the requirement for full-length p63 or p73 in allowing the apoptotic activity of p53 is another interesting question that remains to be answered.

Table 2 | **Examples of p53-target genes**

Gene name	References
Apoptosis and survival	
APAF1	114–116
BAX	117
FAS	118
FDXR	119
IGF-BP3	120
KILLER/DR5	121
NOXA	122
p53AIP1	80
p53DINP1	86
PERP	123
PIDD	124
PIG3	32,37
PIG8/ei24	32,125,126
PTEN	102
PUMA	127,128
WIP1	87
Cell-cycle arrest and DNA repair	
BTG2	129
CDKN1A	130
14-3-3-σ	131
GADD45	132
p53R2	133,134
Angiogenesis and invasion	
TSP1 (thombospondin)	135
GD-AIF	136
BAI1	137
MMP2	138
MASPIN	139
KAI1	140
Autoregulation	
MDM2	141,142
TP73	143,144
CCNG1	145

of both death-receptor and mitochondrial apoptotic pathways have been described (TABLE 2 and FIG. 4), although studies so far indicate that the principal role of p53 is in the induction of the apoptotic cascade that is associated with mitochondrial release of factors such as cytochome *c* and SMAC[39]. In addition to inducing genes that drive apoptosis, p53 can also activate the expression of genes that inhibit survival signalling. It is possible that not all of these target genes are induced in all tissues or in response to all signals, as shown for BAX and FAS[40]. However, given the number of apoptotic target genes, it seems likely that, in most cells, the apoptotic response reflects the cumulative action of numerous p53-induced signals. The ability to engage various apoptotic pathways via several routes is likely to be particularly important for the tumour-suppressor activity of p53, as the selective pressure to lose pro-apoptotic gene function is extremely high during tumour development.

In addition to the activation of apoptotic target genes, p53 can repress gene expression and act independently of the regulation of transcription — functions that have also been implicated in the induction of the full apoptotic response. Detailed studies of tumour-derived p53 mutants showed that the tumour-suppression function of p53 is best correlated with its ability to induce apoptosis, and that the ability of p53 to repress gene expression is tightly linked to this function[41,42]. The correlation of transcriptional suppression and apoptosis is also seen in the trimeric p53–MDM2–RB (retinoblastoma) complex that retains the ability to repress gene expression and induce apoptosis, despite being defective for the transactivation of p53 target genes, such as *CDKN1A* (REF. 43). Several genes have been identified as *in vivo* targets of p53 repression, many of which have anti-apoptotic function. The identification of the transcriptional repressor protein SIN3A as an interacting protein of p53 provides a molecular explanation for how p53 might repress gene expression[44], although how p53 chooses its target genes for repression remains unclear. It is also interesting to note that SIN3A binds to and stabilizes p53 through the proline-rich region of p53, a region that is required for apoptotic function[45], again illustrating the close relationship between the transcriptional repression and apoptotic function of p53.

The transcriptionally independent activities of p53 (REFS 46–49) have been more difficult to define, and are subsequently much less well understood, with some continuing debate as to their importance — or even existence — in the physiological response to p53. Much of the cynicism surrounding the elusive transcriptionally independent activities of p53 has been engendered by elegant studies showing that the substitution of wild-type p53 for p53 proteins with point mutants in the transcriptional activation domain effectively cripples the apoptotic activity of p53 (REFS 50,51). These results strongly support the contribution of transcriptional regulation to the activation of cell death, but do not completely preclude a contributory role for other p53 functions that might also be compromised in these mutants or — if retained — might not, alone, be sufficient to reach the threshold that is necessary to activate the apoptotic response. Transcriptionally independent functions of p53 might include shuttling of death receptors to the cell surface[52] or activation of caspase-8 (REF. 53). The recent identification of p53 in the mitochondria of some cells has provided another enticing clue as to the nature of the transcriptionally independent activity of p53 (REF. 54).

Choice of response to p53

p53-independent apoptotic signals. Although p53 can be a potent activator of cell death, induction of p53 does not necessarily initiate a full apoptotic response, and the suggestion that tumour cells show a greater propensity to die in response to p53 than their normal counterparts has triggered much excitement in the use of p53 as a

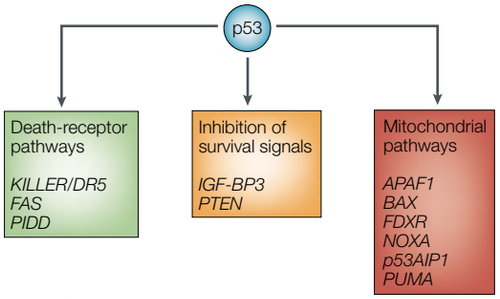

Figure 4 | **Several apoptotic pathways are activated by p53.** p53 can induce the expression of numerous apoptotic genes that can contribute to the activation of both death-receptor and mitochondrial apoptotic pathways. p53 can also affect the efficiency of survival signalling.

tumour-specific therapy. Some normal cell types do show an apoptotic response following induction of p53, and the death of thymocytes and gut epithelium in response to p53 is thought to be a significant contributor to the adverse side effects of many chemotherapeutic treatments[55]. However, other cell types, including some epithelial cells and fibroblasts, are more disposed to p53-mediated apoptosis following oncogenic transformation, and elucidating what determines the sensitivity of a cell to p53-induced death has become a high priority. To some extent, the response to p53 reflects the exposure of cells to death stimuli that can cooperate with p53 to pass the apoptotic threshold, but are not necessarily dependent on or directly part of the p53 pathway. It is clear that, although many normal cells are bolstered from the death response, the abnormal proliferation, loss of normal cellular environment and stress that accompanies malignant progression all enhance the apoptotic sensitivity of the cancer cell by either increasing death signalling or hampering survival signals[1]. The consequence of inducing the same p53-mediated apoptotic signals might therefore be quite different in normal cells that enjoy a stress-free environment that is complete with survival signals to counteract the p53 death programme, and cancer cells that are subjected to a barrage of additional apoptotic insults. The concept that apoptosis is the cumulative response to many signals, including p53, indicates that loss of any of these might enhance survival by reducing the apoptotic burden to below the threshold that is necessary for the execution of death. In effect, therefore, loss of a component of an apoptotic pathway that cooperates with p53 might inhibit the apoptotic response to p53 without necessarily participating directly in the p53 pathway.

Regulation of the apoptotic activity of p53. In the scenario described above, the eventual outcome of p53 induction is determined by cooperating or ameliorating signals, without the need to evoke any differences in the activity of p53 itself. Although it is clear that p53-independent signals are important in modulating the cell's response to p53, it seems clear that this 'p53-dumb' model is too simple to fully explain the apoptotic functions of p53. There is now accumulating evidence for the existence of 'p53-smart' mechanisms, in which p53 has a direct role in determining which response pathways are activated[56].

The initial suggestion that p53 can independently regulate cell-cycle arrest and apoptosis came from studies of tumour-derived *TP53* point mutants, which retain the ability to activate G1 arrest but fail to suppress the transformation of cells by oncogenes, such as the HUMAN PAPILLOMAVIRUS (HPV) E7 protein[57,58]. It subsequently became clear that suppression of transformation in these assays was a reflection of the ability of p53 to induce apoptosis, and that the tumour-derived mutants being studied show a selective loss of apoptotic function that correlates with a selective defect in the ability to activate expression of some of the apoptotic p53 target genes[59,60]. It is possible that these results reflect differing affinities of p53 for the binding sites in different promoters, so p53 mutants with slight alterations in conformation still bind to high-affinity sites in the promoters of cell-cycle-arrest genes, but are incapable of interacting with lower-affinity binding sites that are present in the promoters of the apoptotic target genes. This differential binding affinity could also explain the observation that low levels of p53 protein result in cell-cycle arrest, whereas higher levels of expression of p53 induce apoptosis[48], which masks the concomitant cell-cycle-arrest response. Recent studies examining the binding of p53 to promoters within the context of chromatin *in vivo* have partially supported this model[61,62] by confirming the presence of high- and low-affinity p53-binding sites in cell-cycle arrest and apoptotic promoters. However, not all known pro-apoptotic target genes are regulated by low-affinity p53-binding sites, and the binding affinity of p53 to the *PUMA* promoter, which regulates a strongly pro-apoptotic gene, was similar to the *CDKN1A* and *MDM2* promoters. It is therefore not clear whether the binding affinity of p53 to different promoters is sufficient to explain the apoptotic function of high levels of p53 protein or the selective apoptotic defects in some tumour-derived p53 mutants, and other explanations for these observations are now emerging.

Several studies have implicated p53-binding proteins as important in modulating the selection of target genes (FIG. 5). In order to function efficiently as an activator of gene expression, p53 forms complexes with other transcriptional regulators, including acetyltransferases such as p300/CBP. These interactions allow for the acetylation of histones that surround the p53-binding site, opening up the surrounding chromatin and allowing access of basal transcriptional machinery[63]. p53 is also targeted for acetylation by these binding proteins[64,65], although the consequence and importance of this modification remains equivocal[66]. Although some of the p53-associated transcription factors seem to contribute generally to the ability of p53 to induce gene expression, some of these factors have been shown to have a specific and selective role in enabling the expression of the apoptotic target genes. JMY, for example, cooperates with p300 to enhance the ability of p53 to induce the expression of genes such as *BAX*,

HUMAN PAPILLOMAVIRUS E7
A viral oncoprotein that is derived from certain human papillomavirus types that are associated with an increased risk of cervical cancer. E7 binds and inactivates retinoblastoma.

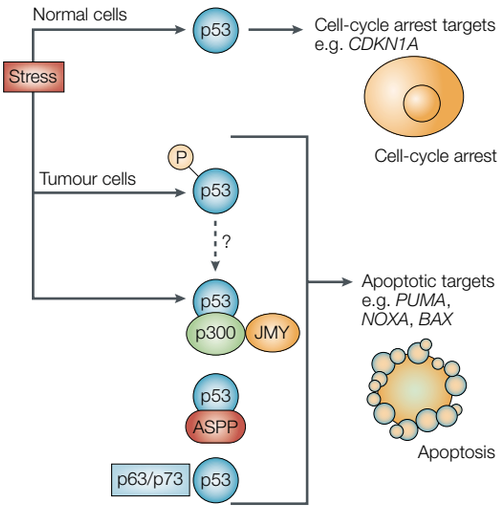

Figure 5 | **Model for the regulation of the choice of response to p53.** The choice of response to p53 activation is determined, in part, by differential regulation of p53 activity in normal and tumour cells. In this model, activation of p53 in normal cells leads to the selective expression of cell-cycle-arrest target genes (such as *CDKN1A*, which encodes WAF1), resulting in a reversible or permanent inhibition of cell proliferation. In tumour cells, phosphorylation of p53 at Ser46 (through activation of kinases, expression of co-activators such as p53DINP1 or repression of phosphatases such as WIP1) and/or functional interaction with apoptotic cofactors (such as ASPP, JMY and p63/p73) allows for the activation of apoptotic target genes. These cofactors can bind p53 (directly or indirectly) as shown for ASPP and JMY or — as shown for p63 and p73 — assist p53 DNA binding by directly interacting with p53-responsive promoters. Although not proven, it is possible that phosphorylation alters the conformation of p53 to either enhance the interaction with apoptotic cofactors, or allow binding to apoptotic target promoters.

without significantly influencing the induction of *CDKN1A* (REF. 67). The net effect of this is that *JMY* expression might be necessary for the induction of apoptosis, but not cell-cycle arrest.

Regulation of the apoptotic response can also be mediated by p53-binding proteins that directly affect the ability of p53 to interact with DNA. The ASPP family of proteins have recently been shown to interact with p53 and — in the case of full-length ASPP1 and ASPP2 — enhance the interaction with the promoters of apoptotic target genes such as *BAX*[68]. Conversely, inhibition of *ASPP* expression was shown to selectively block the apoptotic response to p53. A similar contribution to apoptotic target-gene activation by p53 was recently revealed by the unexpected observation that induction of cell death by p53 requires the presence of at least one of the other p53-family members, p63 or p73 (REF. 69). This requirement correlates with an inability of p53 to bind promoters of apoptotic targets in p63/p73 double-null cells, with evidence that the other p53-family members might be required at these sites to allow p53 binding. Remember, however, that wild-type p53 does not bind p63 or p73, indicating an indirect contribution of p63 or p73 to allow p53 binding at certain promoters.

In addition to the proteins such as ASPP, JMY and p63/p73 that have been shown to affect the ability of p53 to activate gene expression, several other factors that contribute to the induction of apoptosis by p53 by means of less-well-defined mechanisms have been described. One of these is the deregulation of the E2F family of transcription factors — an event that, like p53 inactivation, occurs in virtually all human cancers[70]. Several apoptotic pathways are induced by deregulated E2F1, including p53-independent signals that would sensitize cells to p53-mediated death[70], transcriptional activation of ARF to stabilize p53 (REF. 4) and transcriptional activation of p73 (REFS 71,72). In addition, E2F1 can interact with p53 and can directly augment the apoptotic activity of p53 (REF. 73). Although the basis for the enhancement of p53's apoptotic function by E2F1 binding is not clear, this activity can be regulated by cyclin A, which competes with p53 to bind E2F1.

The transcription factor nuclear factor of κB (NF-κB) can also influence the p53-induced apoptotic response, both positively and negatively. NF-κB shows strong anti-apoptotic activity in many systems, particularly in the inhibition of cell death in response to death-receptor activation by ligands such as tumour-necrosis factor (TNF)[74]. The role of NF-κB in modulating p53-induced apoptosis is, however, less clear. Although NF-κB can inhibit p53 function by competing for co-activators such as p300 (REFS 75,76), other studies have shown a requirement for NF-κB in p53-mediated cell death[77]. The PEUTZ–JEGHER gene product *LKB1* has also been shown to have a role in mediating p53-dependent apoptosis, and loss of LKB1 in intestinal epithelial cells seems to provide some protection against p53-induced cell death, thereby enhancing tumour development[78]. Other studies have indicated that the extreme carboxyl terminus of p53 has a role in the induction of apoptosis by interacting with DNA helicases, such as XPD, XPB, BLM and WRN1 (REF. 79). Although it is not known how these different proteins contribute to p53-mediated death, it seems likely that at least some of them will directly impinge on the apoptotic activity of p53.

Regulating the regulators

The identification of cofactors that are specifically required for p53 to induce apoptotic target genes indicates that there are several mechanisms by which the apoptotic response can be regulated. Most simply, the availability of these cofactors can be regulated at the level of expression or protein stability, allowing cells that do not express proteins such as ASPP, JMY or p63/p73 at sufficient levels to escape from the induction of the apoptotic response after p53 activation. However, more complex levels of control, including post-translational modifications that might regulate interactions between the different components of this system, are also being identified.

PEUTZ–JEGHER SYNDROME
A cancer-susceptibility syndrome that is associated with inheritance of mutation in *LKB1*, a serine/threonine kinase.

p53 is extensively phosphorylated, and modification at several residues has been specifically associated with the ability of p53 to induce an apoptotic response. Of particular interest is the phosphorylation of p53 on serine (Ser) 46, which is necessary for p53 to induce expression of apoptotic target genes such as *p53AIP1*, but not mediators of cell-cycle arrest such as *CDKN1A* (REFS 80,81) (FIG. 5). Regulation of Ser46 phosphorylation might therefore be crucial in modulating the choice of response to p53 activation, and several kinases have now been implicated as being responsible for this modification. The homeodomain-interacting protein kinase 2 (HIPK2) can mediate Ser46 phosphorylation in response to ultraviolet (UV) irradiation[82,83], although it seems that this alone is not sufficient to induce apoptosis[83]. It has also been suggested that p38 mitogen-activated protein kinase (MAPK) can mediate the phosphorylation of Ser46 in response to UV irradiation[81,84]. Interestingly, neither HIPK2 nor p38MAPK are involved in the phosphorylation of Ser46 in response to ionizing radiation, which requires both ataxia telangiectasia mutated (ATM)[85] and the p53-inducible gene *p53DINP1* (REF. 86). ATM does not directly phosphorylate p53 on Ser46, but it is likely to induce a kinase that might be co-activated by p53DINP1 to facilitate Ser46 phosphorylation. A further layer of complexity to the regulation of Ser46 phosphorylation is provided by the identification of WIP1 as a phosphatase that attenuates UV-light-induced phosphorylation of p53 on Ser46 by inactivating p38MAPK, thereby disarming the apoptotic response[84]. Most intriguingly, WIP1 is a transcriptional target of p53 (REF. 87), thereby setting up a feedback loop in which p53 can induce expression of a protein that can incapacitate its ability to induce apoptosis (BOX 1). So, it seems clear that phosphorylation of p53 can contribute to the activation of apoptosis, although the mechanism that underlies this observation is not known. Although it is possible that phosphorylation subtly alters the conformation of p53 to allow binding to the promoter elements in apoptotic target genes, it is also tempting to speculate that phosphorylation regulates the interaction of p53 with some of the apoptotic cofactors, such as ASPP.

In addition to phosphorylation, other covalent modifications of p53 might also be important in regulating p53 activity and determining the choice of response. p53 is subject to several different post-translational modifications, including sumoylation and acetylation, and — despite some conflicting reports — it seems likely that carboxy-terminal acetylation of p53 can regulate transcriptional activity under some circumstances[66]. It is, therefore, of particular interest that acetylation of p73 has recently been shown to selectively enhance the activation of apoptotic target genes[88], indicating that p53 might also be regulated in a similar way.

Subcellular localization of p53. In intact cells, the activity of p53, including post-translational modifications and the ability to interact with other proteins, can be further controlled by regulation of the subcellular localization of components of the p53-response pathways. p53 is actively transported into and out of the nucleus[89,90], and can be localized to distinct structures in both the nucleus and cytoplasm. Of particular interest is the accumulation of p53 to subnuclear structures termed nuclear bodies (NBs), which depends on the interaction of p53 with PML and acetylation of the PML protein[91–94]. This relocalization of p53 contributes to its transcriptional activation and the induction of a p53 response, and probably reflects the congregation of various transcription factors and kinases within these nuclear bodies[95]. More interestingly, the localization of p53 to NBs might specifically allow activation of apoptotic targets, indicating that apoptotic cofactors such as ASPP and JMY might also accumulate in the NBs. Supporting this idea is the observation that HIPK2 (a Ser46 kinase) co-localizes with p53 in the NBs[82,83].

Role of survival signals. The choice between cell death or survival is strongly dependent on the activity of survival signals that can be mediated by soluble ligand binding to cell-surface receptors, or direct interactions with neighbouring cells or the extracellular matrix. Rescue of p53-induced apoptosis by survival factors has been associated with activation of the AKT kinase[96], which shows anti-apoptotic activities in several systems. AKT can phosphorylate and activate MDM2, thereby enhancing the degradation of p53 (REFS 97,98), although this seems to be only one of several mechanisms by which p53-mediated death can be attenuated by AKT. Another intriguing regulator of p53-induced death is WISP1, which is induced through the WNT signalling pathway. WISP1 can also inhibit p53-mediated cell death by activation of AKT[99], but, in this case, survival is not associated with a change in p53 stability. Similarly, in neurons, AKT can inhibit p53-mediated transcriptional activation and apoptosis without reducing p53 protein levels[100]. These observations indicate that, in addition to regulating MDM2, AKT also has a role in blocking apoptotic signalling downstream of p53 activation. A hint as to how this might function is provided by the observation that serum-responsive survival signals — which would activate AKT — downregulate expression of *PUMA*, which is a mediator of the p53 apoptotic response[101]. Interestingly, the inhibition of p53 by AKT is counteracted by the ability of p53 to induce expression of *PTEN*, a phosphatase that can inhibit the activation of AKT[102]. The induction of *PTEN* has been shown to be essential for p53-mediated apoptosis in mouse cells, underscoring the importance of survival signalling in determining the final outcome of the p53 response.

Therapy and the future

So, how will our burgeoning understanding of p53 apoptosis impinge on our ability to deal with cancer? There is clear consensus that reactivation of p53 function in cancer cells would be of therapeutic benefit, and several recent studies that define small molecules or peptides that restore function to mutant p53 proteins have illustrated the tremendous potential of this approach[103–105]. The success of such ventures is based on the enhanced apoptotic sensitivity of tumour cells[1], and

the identification of cofactors that specifically contribute to p53-induced apoptosis enables us to think more precisely about why tumour cells die in response to p53. It is possible that cancer-associated alterations — such as oncogene activation — directly enhance the expression or activity of factors that are required for p53-induced death. For example, deregulated E2F1 (found in almost all cancers) can activate the expression of p73 (REFS 71,72,106), which was recently shown to have a direct role in allowing p53-mediated apoptosis[69]. Relocalization of p53 to NBs, an event that is associated with the ability to induce expression of apoptotic target genes, occurs in response to oncogene activation — in particular, the induction of RAS activity. Herein lies a complication for the model, however, because activation of p53 by RAS under these circumstances is associated with induction of premature senescence rather than apoptosis[91,94]. Of course, RAS also has the ability to activate survival signalling through induction of AKT[107], underscoring the fact that the final response of the cell to any of these stimuli is the result of the integration of a complex and interwoven network of signals. Very recently, oncogenic RAS was also shown to suppress expression of WIP1, elevating the phosphorylation of p53 at Ser46 and so enhancing apoptotic signalling[108]. Combining these p53-activating functions of oncogenes with their p53-independent apoptotic activities leads to a clearer picture of why tumour cells are so strongly primed for death.

As the apoptotic and cell-cycle-arrest activities of p53 can be independently regulated, a more subtle variation on the theme of reactivating p53 for tumour therapy becomes how specifically to activate the death-inducing functions of p53. The early studies of mutant p53 indicated strongly that loss of the apoptotic activity of p53 is crucial for allowing tumour progression, and mutation in the downstream components of the apoptotic pathways activated by p53 can substitute for loss of p53 in tumour development[109–111]. However, the identification of apoptotic cofactors that are required specifically for p53-mediated death indicates that these proteins might also be targets for inactivation in human cancers. Loss or inhibition of factors such as ASPP, or the ability to phosphorylate p53 on Ser46, would lead to profound defects in the ability of p53 to kill developing tumour cells. Indeed, the first hint of such mechanisms has been found in breast cancers, ~11% of which have an amplification of the phosphatase WIP1, which prevents phosphorylation and activation of the apoptotic function of p53 (REF. 108). Interestingly, the majority of these tumours retained wild-type p53. Similarly, many human cancers show enhanced activity of the phosphatidylinositol-3 kinase (PI3K)/AKT signalling cascade, by loss of *PTEN* or, in upper aerodigestive-tract cancers, through amplification of *PIK3CA*, the catalytic subunit of PI3K[112]. Although sustained AKT signalling has pleiotropic effects on the regulation of cell death, one consequence is the selective inhibition of p53-induced cell death. Tumour-associated expression of amino-terminally deleted isoforms of p73 might also directly impinge on the apoptotic activity of p53 (BOX 1).

The growing family of cofactors that are required for p53 to induce apoptosis is providing some auspicious new targets for the development of therapies to restore the apoptotic function of p53, and some evidence for their validation in this arena is likely to be available soon. It could be that by targeting p53's friends and associates we will ensure that p53 does the right thing in making the nascent tumour cell choose death over dishonour.

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