CMLS, Cell. Mol. Life Sci. 55 (1999) 28–37
1420-682X/99/010028-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Mechanisms of p53-mediated apoptosis

S. Bates and K. H. Vousden*

ABL Basic Research Program, NCI-FCRDC, Building 560, Room 22-96, West 7th Street, Frederick (Maryland 21702-1201, USA), Fax +1 301 846 1666, e-mail: vousden@ncifcrf.gov

Abstract. The loss of p53-mediated apoptosis (programmed cell death) has been implicated as an important event in tumour progression in a number of systems. p53 can induce or potentiate apoptosis through several mechanisms, both by regulating the expression of genes which can participate in the apoptotic response and through transcriptionally independent means. There appears to be cell type variability in both the response to p53 expression and in the requirement for p53 transcriptional transactivation for the induction of apoptosis. It seems clear, however, that the induction of p53 in untransformed cells is more likely to result in cell-cycle arrest, whereas the expression of p53 in their transformed counterparts is more likely to result in the induction of apoptosis, and this may, in part, reflect the deregulated expression of E2F-1 in tumour cells. The synergistic action of p53 and E2F-1 in the induction of apoptosis has raised the possibility that the reactivation of p53 in transformed cells can be an effective tumour therapy.

Key words. p53; apoptosis; transcription; Bax; death receptors; tumours.

Introduction

A role for the p53 protein has been implicated in many and varied cellular processes, including DNA repair, recombination, differentiation and senescence. Our fascination with p53 stems, however, from its frequent loss or mutation in human cancers and the dramatic demonstration in knockout mice of the importance of p53 and its tumour suppressor function.

Many of the p53 functions described so far could play important roles in monitoring, preventing or eliminating tumour cells. It seems clear, however, that the ability of p53 to prevent cell growth is pivotal to its tumour suppressor functions. A large body of data now suggests that p53 suppresses growth through two mechanisms, namely, cell-cycle arrest and apoptosis. While cell cycle arrest can function to inhibit the growth of normal cells, it seems that cells that have attained oncogenic activation are less susceptible [1, 2]. The ability of p53 to induce apoptosis, by contrast, appears to correlate well with its ability to suppress transformation, and there is evidence that the apoptotic activity of p53 is of central importance to its tumour suppressive activity [3]. The suggestion that p53 will preferentially kill tumour cells, while inducing a reversible growth arrest in normal cells, presents many tantalizing therapeutic possibilities.

Mechanisms of apoptosis

Apoptosis is a programmed cell death pathway that is essential in the development of multicellular organisms and functions in the maintenance of tissue homoeostasis. The apoptotic pathway is characterized by the activation of a family of cell death proteases called the caspases, which are themselves activated by catalytic cleavage, thereby setting up a cascade of proteolytic cleavage that disrupts the function of essential regulatory proteins and commit the cell to the suicide pathway [4]. Caspase activation is followed by nuclear condensation, and the activation of an apoptotic nuclease results in the destruction of the nuclear DNA [5]. The final

* Corresponding author.

stages of the process are characterized by membrane blebbing, the loss of cellular volume and finally the loss of membrane integrity. While much is understood about the machinery of the apoptotic pathway, comparatively less is known about the regulation of mechanisms by which the cell commits to programmed cell death. It has become clear, however, that under some circumstances p53 can play a major role in the activation of cell death.

Apoptosis has been noted as the normal response of some cell types, notably T lymphocytes, to the induction of DNA damage, and this has been shown to be dependent on the presence of a wild-type p53 gene [6, 7]. A role for p53 in the apoptotic response to DNA damage was further highlighted by the fact that the introduction of exogenous p53 into some tumour cell lines is able to activate programmed cell death [8, 9]. p53 has also been implicated in playing a role in the apoptotic response initiated by the oncogenes *E2F-1* [10] and c-*myc* [11] and the tumour necrosis factor death receptor (TNF) [12, 13]. p53, therefore appears to activate a programme of apoptotic cell death in response to stress or potentially oncogenic abnormalities, and a great deal of research has been focused on identifying the exact mechanisms involved.

Transcriptional activation of apoptotic target genes

One of the most certain facts about p53 is that it is a transcription factor, and a great number of genes have been identified, with greater or lesser degrees of certainty, as targets for transcriptional regulation by p53 [14]. The importance of this activity to tumour suppression is manifest by the observation that the vast majority of p53 mutations found in cancers prevent DNA binding and thus transcriptional activity of the mutant protein [15]. Primary amongst the targets identified to date is the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ [16], which is able to bind and inhibit cyclin/cyclin-dependent kinase (cdk) complexes [17–19]. Homozygous deletion of $p21^{WAF1/CIP1}$ in mice and in human cell lines has shown it to be a critical component of p53-induced cell-cycle arrest, implicating transcriptional transactivation as an essential component of these p53-mediated effects in vivo [20–24].

Despite the overwhelming evidence for a role for transcription transactivation in the cell-cycle arrest pathway, some controversy has surrounded the role of p53 transactivation in the apoptotic pathway, and it is likely that there are cell type differences underlying this requirement. It seems clear, however, that p53 transactivation can contribute to the induction of apoptosis in some cell types, and a number of genes transactivated by p53 have been implicated in the apoptosis response. Not all p53 target genes have been linked with a role in apoptosis, however, and there is overwhelming evidence to suggest that $p21^{WAF1/CIP1}$ plays no role in the ability of p53 to induce apoptosis. For other targets such as *Cyclin G1*, *Gadd45*, and *Mdm2* there is also little evidence for a direct role in apoptosis.

Mitochondrial events

One of the first signs of apoptosis is loss of the mitochondrial membrane potential, believed to result from the opening of multiprotein pores located at the contact sites of inner and outer mitochondrial membranes. This so-called permeability transition uncouples the respiratory chain leading to acidification of the cytosol, oxidation of mitochondrial proteins and the release of intermembrane proteins, such as cytochrome c, into the cytosol [25]. In the cytosol, cytochrome c functions as an activator of caspase cleavage, and in this way the release of cytochrome c from the mitochondrion initiates the proteolytic cascade characteristic of apoptosis [26]. The induction of mitochondrial damage can be achieved through many and varied mechanisms, and many of these are implicated in p53-mediated cell death.

A family of proteins, related to *Bcl-2*, have been shown to function, at least in part, by regulating the release of cytochrome c from the mitochondria, an event that is seen in both p53-dependent and -independent death [27–29]. There are two groups of regulatory genes: the antiapoptotic genes, such as *Bcl-2*, and the proapoptotic genes, such as *Bax*. The two classes of proteins are localized to intracellular membranes, particularly mitochondria, and have been shown to interact with each other, although the significance of their association with respect to their activity remains to be seen [30]. Also unclear at present is the mechanism by which they regulate cytochrome c release, but structural homology to bacterial pore-forming proteins suggests that ion transport may be important to their function [31]. Indeed, both Bcl-2 and Bcl-X can serve as ion channels in synthetic lipid membranes in vitro [32, 33].

The death-promoting gene *Bax* has been shown to contain p53-binding sites in its promoter and be up-regulated in response to DNA damage and p53 in a number of systems [34]. Introduction of *Bax* into cells results in rapid cell death, and this can be inhibited by simultaneous expression of the Bax-binding and death-inhibiting proteins Bcl-2 and Bcl-X. Similarly, p53-mediated cell death can be inhibited by Bcl-2/Bcl-

X consistent with a role for Bax in the p53 death pathway (see fig. 1). Whereas T lymphocytes from mice homozygously deleted for the *Bax* gene are still able to undergo p53-dependent cell death in response to ionizing radiation [35], recent reports have noted that p53-mediated cell death in fibroblasts induced to die by expression of E1A [36] and in SV40 T antigen-induced brain tumours [37] is impaired in Bax null cells. These data are consistent with a role for *Bax* in some forms of p53-mediated apoptosis. Nevertheless, since *Bax* deletion is only able to inhibit, at best, 50% of the apoptosis in these systems, it cannot represent the only mechanism of p53-mediated cell death.

Reactive oxygen species (ROS) are powerful activators of both mitochondrial damage and apoptosis. The ability of a number of antioxidants, which eliminate ROS, to inhibit p53-mediated apoptosis in some systems has suggested a role for ROS in the p53 death response [38, 39] (see fig. 1). This concept was greatly advanced by the identification of a number of genes that can induce oxidative stress as p53-induced genes (PIGs), one of which, *PIG3* (a quinone oxidoreductase homologue), was shown to contain p53-binding sites in its promoter [40]. Transcripts of the PIG genes were induced to an extent similar to $p21^{WAF1/CIP1}$ when a colon cell line was induced to undergo programmed cell death in response to p53. Closer analysis of these cells confirmed that the production of ROS was increased following p53 expression and that cell death could be blocked by inhibitors of the mitochondrial permeability transition [25]. However, it should be noted that these genes are also induced by p53 in cells that do not undergo apoptosis, suggesting that simple induction of PIGs cannot account for the fact that some cells die with p53 and others do not. Furthermore, simply expressing of one of these genes, *PIG3*, failed to initiate apoptosis, implying that these genes function in combination with other signals.

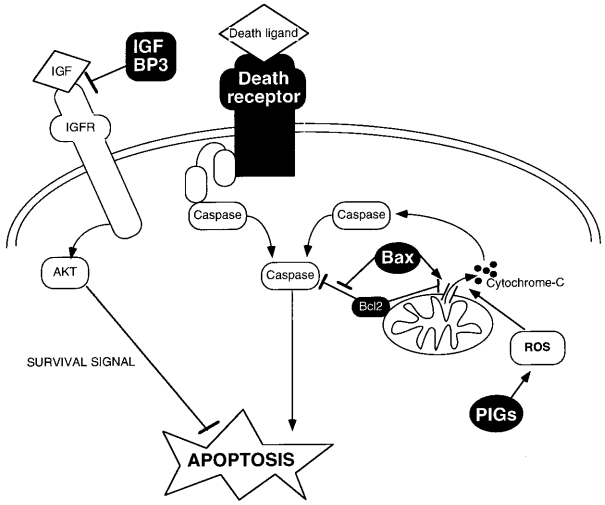

Figure 1. Induction of apoptosis by p53 transcriptional target genes. In response to genotoxic stress, p53 levels are elevated and p53 functions to transactivate genes involved in the apoptotic pathway (indicated in black). Both upregulation of death receptors (Fas and Trail receptor/DR5/Killer) and Bax results in the activation of caspases either through indirect association via adaptor molecules or in the latter case through the release of cytochrome c from mitochondria. Death signals are potentiated by inhibition of the survival signals provided by the IGF receptor/AKT/PKB pathway by the increased expression of the secreted IGF binding protein IGF-BP3.

Death receptors

Fas/Apo is a death receptor molecule that has important roles in the immune system, allowing the removal of autoantibodies and the elimination of virally infected and tumorigenic cells [41]. When activated by Fas ligand (*FasL*), the receptor (*Fas*) trimerizes, resulting in the cleavage of a caspase that binds to its intracellular domain via death domain proteins. The activated caspase then initiates the cascade of proteolytic cleavage, although mitochondrial damage and the release of cyctochrome c has also been implicated as a downstream event in death receptor signalling [29]. A number of studies have shown that *Fas* levels are elevated in response to DNA damage, and although p53 has been implicated, no p53-binding sites have been identified in the *Fas* gene to date [42]. There are conflicting reports as to the extent to which T lymphocytes from mice with functionally inactivated *Fas* are resistant to DNA damage, and thereby p53-induced apoptosis [43, 44], and similarly the extent to which Fas-induced death can be protected by *Bcl-2* [45, 46]. However, the recent identification of a role of *Fas* in *Myc*-induced apoptosis [47], which has also been reported to be p53-dependent, suggests a potential contribution of *Fas* to p53-mediated apoptosis. Consistent with a role for death receptors in the apoptotic response to genotoxic stress, another receptor molecule has also recently been shown to be induced in response to DNA damage, and activation was shown to correlate with p53 status. The *Trail receptor/DR5/Killer* protein is a member of the TNF superfamily of death receptor molecules, and it can cause apoptosis when overexpressed in a manner similar to *Fas* [48]. One arm of the p53 apoptotic response may therefore be in the general upregulation of death receptor expression, which might not trigger an apoptotic response alone but would certainly sensitize the cell to other death signals (see fig. 1).

Survival factors

Although the ultimate executioners of the apoptotic signal, the caspases, are central to all programmed cell death, it is clear that the signal to die can be regulated at many different levels. In many systems the presence of specific survival factors is required to protect cells from apoptotic cell death [49]. Indeed, the response of cells to DNA damage can be dramatically modulated by the addition or removal of such survival factors [50–52]. The exact mechanism by which survival factors function is not clear, although a role for signalling through the kinase AKT/PKB has been strongly implicated in several systems [53]. In other systems, survival factors may directly lead to the inhibition of p53 function through induction of Mdm2 [54]. Insulin-like growth factor (IGF) has been shown to function as both a mitogen and a survival factor under a number of conditions, and it was therefore of great interest to identify the IGF binding and inhibiting protein *IGF-BP3* as a target for transcriptional activation by p53. Overexpression of *IGF-BP3* can inhibit IGF mitogenicity in some systems, while in others it is able to inhibit IGF survival signals and thus potentiate apoptotic signals [55, 56] (see fig. 1). The simultaneous expression of genes that can inhibit survival signals as well as proapoptotic genes would be an efficient way of inducing a p53-mediated apoptotic response.

Other p53-inducible genes may also play a role in the apoptotic response, although their function is much less well understood at present. The newly isolated gene *PAG608* can induce apoptosis when introduced into cells, although the mechanism it might employ is unknown [57]. *Mcl-1* and *Bcl-X*, genes known to function in the regulation of apoptosis, have also been reported to be induced in response to DNA damage, but a direct link to p53 has yet to be identified [58–60].

Transcriptional repression

In addition to transcriptional activation, p53 has also been shown to specifically repress transcription from a number of genes such as *Bcl-2*, *MAP4* and a number of viral promoters [61, 62]. Repression of the death-inhibiting gene *Bcl-2* could be imagined to push the cells towards death and, together with induction of the death-promoting gene *Bax*, provides an attractive mechanism for p53-mediated apoptosis [63]. In support of this view, inhibition of p53-mediated cell death by E1B 19 kDa also inhibits the repression of *Bcl-2* [64]. These genes do not contain p53-binding sites in their promoters, however, and the mechanism of repression remains unclear. Indeed, in some cases it would seem that p53-induced repression is a consequence of the induction of apoptosis, rather than a direct role of p53, complicating the assessment of the role of transcriptional repression by p53.

Transcription-independent apoptotic functions of p53

Unlike the clear correlation between transactivation and growth arrest, p53 has been shown to induce apoptosis in a manner independent of transcription in a number of systems. A dispensable role for transcription in p53-mediated apoptosis is shown by the ability of cells to die in the presence of inhibitors of RNA and protein synthesis [65, 66]. The interpretation of these data is complicated, however, by reports showing that transcription is required for p53-mediated apoptosis in other systems [67–69] and the ability of these drugs to function as inducers of apoptosis in their own right.

More direct evidence of a transcriptionally independent form of p53-mediated apoptosis comes from the analysis of deletion and point mutants engineered into the p53 sequence itself. Deletion of the C-terminal 30 amino acids of human p53, activating p53 for DNA binding but not impinging on p53's ability to oligomerize, has been shown in a number of systems to impair p53's ability to efficiently induce apoptosis, while induction of G1 arrest is unimpaired [70, 71]. p53 binds to XP-B and XP-D, two helicases which function in DNA repair, through the same region, and it has been speculated that inhibition of the enzymatic activity of these two proteins is essential to p53-mediated apoptosis [71].

By contrast, a p53 mutant affecting key residues in the transactivation domain and a large C-terminal deletion mutant have been shown to induce apoptosis in the absence of significant transcriptional transactivation in HeLa cells [72], although this activity appears to be cell-type dependent. A number of studies have implicated a proline-rich region, situated between the transactivation domain and the DNA-binding domain at the N-terminus of p53, in having a role in growth suppression [73, 74]. Deletion of this region impairs p53's ability to induce apoptosis in response to E1A [74], but the same region is also required to mount a secure G1 arrest in response to the G0 specific gene *Gas1* [75]. This region contains five repeats of the PXXP motif, which are found in many signalling molecules and serve as docking sites for other proteins containing SH3 domains. It seems likely that any transcriptionally independent ability of p53 might reflect an ability to directly complex with another protein, and p53 has been reported to interact with many other cell proteins, including kinases, other tumour supressors and positive regulators of cell growth [14]. To date, however, there is no firm evidence that any of these interactions contribute to the induction of apoptosis.

To die or not to die: choice of response to p53

The observation that p53 can induce both cell-cycle arrest and apoptosis has given rise to the interesting question of how the cell decides which outcome will prevail. It seems likely that there is no simple explanation, and rather the outcome depends on balancing signals from a variety of sources. While we may be unable to predict the outcome of p53 induction in every situation, we are gaining insight into some of the many cellular and environmental factors that contribute to the choice.

As mentioned earlier, p53 is able to transactivate genes that are associated with cell-cycle arrest (e.g., $p21^{WAF1/CIP1}$) or death (e.g., *Bax*), and one obvious way to distinguish between growth arrest and apoptosis is to preferentially activate only one subset of genes. Indeed, examination of certain p53 point mutants revealed that they were able to distinguish between different p53 target promoters, remaining active towards $p21^{WAF1/CIP1}$ but no longer able to activate *Bax* and *IGF-BP3* [76, 77]. The consequence of this bias is that these mutants activate predominately a cell-cycle arrest, rather than the combination of G1 arrest and apoptosis seen with the wild-type protein. Could such a promoter choice be made in response to DNA damage in vivo, and if so what regulates it? Differential promoter usage has been reported for wild-type p53 in some systems with a potential role for phosphorylation [78]. However, putative death effector genes are often induced irrespective of the outcome, and the cellular response to them is likely to be regulated at a later step.

It seems that the extent of the DNA damage can also regulate the outcome, with low levels of damage inducing a cell-cycle arrest, allowing the damage to be repaired, and more extensive damage inducing apoptosis. In agreement with this idea, the level to which p53 is induced seems to play a role in the choice between cell-cycle arrest and death, with apoptosis correlating with higher levels of p53 induction [70]. This may represent a differential ability of p53 to efficiently transactivate target genes, with some genes being maximally induced following relative modest p53 accumulation, whereas others are only induced in response to a more robust elevation. This is an attractive idea; under conditions of modest genomic damage, genes that induce cell-cycle arrest (e.g., $p21^{WAF1/CIP1}$) are activated to halt cell growth and allow DNA repair to initiate, whereas genes that initiate an apoptotic cascade (e.g., *Bax*) are only required when the damage is beyond repair. This model also accommodates the point mutants which retain $p21^{WAF1/CIP}$/cell-cycle arrest function but lose *Bax*/*IGF-BP3*/apoptotic activity. In these cases, the mutations (which are in the sequence-specific DNA-binding domain) reduce DNA-binding activity, so only the high-affinity (cell-cycle arrest) promoters remain sensitive to activation. Upstream events which activate p53 can also play a role in determining the choice of response. ATM, a protein kinase which is deleted in patients with ataxia telangiectasia, is necessary for the normal activation of p53-dependent cell-cycle arrest in response to some signals. However, lack of ATM does not impede the p53-dependent apoptotic response [79], again highlighting the independence of these two activities of p53.

In systems where apoptosis is the predominant outcome, inhibition of cell death results in a G1 arrest [80, 81], suggesting that this is a constant response which is in some cases obscured by overlying apoptosis. Indeed, $p21^{WAF1/CIP1}$ is induced in response to p53 irrespective of whether the final outcome will be growth arrest or apoptosis. Therefore, it seems that the choice the cell makes is to die or not, rather than to arrest or die, and that inhibition of apoptosis naturally results in cell-cycle arrest under these conditions. In agreement with this idea, fusion of cells that normally die in response to p53 with cells that arrest results in a cell that is sensitive to p53-mediated apoptosis, that is, death is the dominant pathway [22]. An interplay between the two responses has also been noted; deletion of the $p21^{WAF1/CIP1}$ gene by homologous recombination results in cells that cannot arrest and are more sensitive to p53-induced apoptosis [22, 23].

Although the response of different cell types to genotoxic damage and p53 can be similar in terms of the molecular events, the eventual outcome can be dramatically different. These differences likely reflect the varied origin and environmental factors that regulate cells. In particular, the presence of survival factors can inhibit p53-induced apoptosis and reveal an underlying cell-cycle arrest [50–52]. The origin of the cell also seems to be critical, as not all cells respond to DNA damage in a similar way. This is highlighted by the contrasting response of fibroblast and T cells to ionizing radiation; whereas T cells respond by undergoing extensive apoptosis [6, 7], fibroblasts enter a cell-cycle arrest [82, 83].

p53, E2F and tumour therapy

Although inhibition of tumour cell growth by cytostatic mechanisms may be of some use in tumour therapy, far preferable would be the induction of tumour cell death. One of the most exciting concepts in p53 research is the possibility that p53-dependent apoptosis may be prefer-

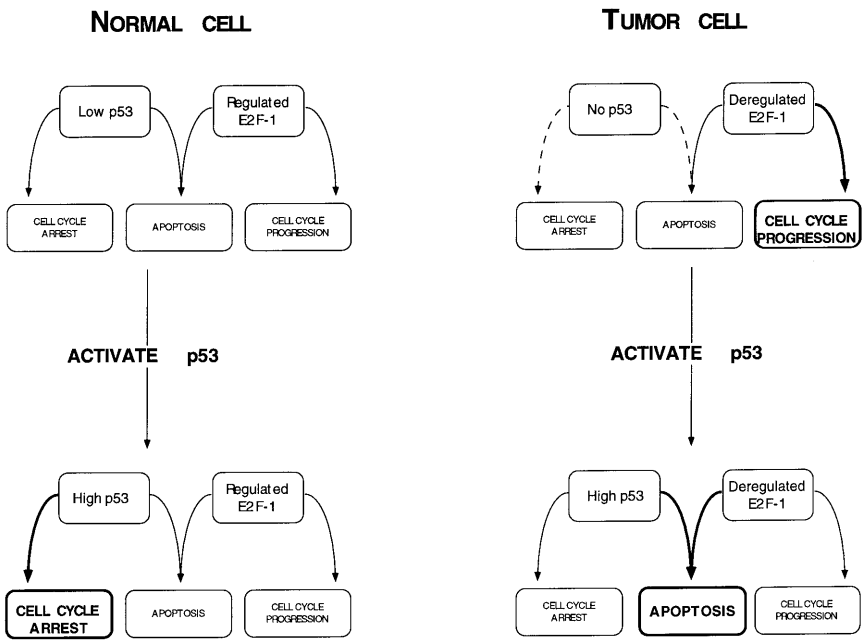

Figure 2. Cooperation between E2F-1 and p53 in the induction of apoptosis. In normal cells where the pRB pathway remains intact and E2F-1 activity is consequently regulated, the induction of p53 in response to genotoxic insult is most likely to result in cell-cycle arrest. In tumour cells, by contrast, E2F-1 activity is deregulated as a result of loss of pRB function. To compensate for the increased E2F-1 apoptotic activity, p53 function is impaired either through mutation or expression of the regulatory proteins Mdm2 or HPV E6 and as a result, tumour cells rapidly divide. Reactivation of wild-type p53 in tumour cells, rather than inducing a cell-cycle arrest, synergizes E2F-1 to induce apoptotic cell death.

entially induced in tumour cells which show other oncogenic alterations or loss of normal checkpoints. Indeed, a recent study has indicated that the presence or absence of the $p21^{WAF1/CIP1}$-dependent cell-cycle arrest checkpoint can determine the success of irradiation treatment of tumours [84].

Tumorigenesis in many cell types in vivo involves the inactivation of pRB function either through direct mutation or loss of the *pRB* gene product itself or through modulation of regulatory proteins such as p16 and cyclin D1. In fact, direct or indirect loss of normal pRB function is likely to be as common a genetic alteration in human tumorigenesis as loss of p53 function [85]. One of the primary consequence of pRB inactivation is the deregulated activity of the E2F family of transcription factors, which drive the cell through G1 and into DNA synthesis. In addition to this growth-promoting activity, one member of the E2F family, E2F-1, also shows apoptotic activity, and the recent generation of mice homozygously deleted for E2F-1 provided the exciting results that E2F-1 can function as a tumour supressor gene in vivo [86, 87]. Although the apoptotic activity of E2F-1 is not dependent on p53, E2F can synergize with p53 to induce high levels of cell death [10, 88–90], and thus there is strong selective pressure for the loss of p53 from tumours with deregulated E2F. How this synergy is visualized at the molecular level will be of great interest, but for the moment this concept goes some way towards explaining the specific combinations of genetic changes seen in tumorigenesis and the increased sensitivity of tumours and tumour cell lines to p53-mediated cell death. Perhaps more important, the model also suggests that the reactivation of p53 in a tumour cell harbouring deregulated E2F-1 would be more likely to lead to apoptotic cell death than similar p53 activation in normal cells. The notion that p53 preferentially kills tumour cells is very attractive and could be of immense therapeutic potential (see fig. 2).

A great deal of effort is currently being expended in the search for mechanisms which might reactivate p53 in tumour cells. Several approaches can be envisioned, depending on the defect which originally led to the loss of p53 function. The most common mechanism for inactivation of p53 is through point mutation in one of the conserved DNA-binding regions [15]. Mutant p53 takes on a conformation that is unable to associate with

DNA, transactivate target genes and ultimately induce apoptosis. In vitro studies have shown, however, that about half of the point mutants identified in tumours, like wild-type p53, can be activated for DNA binding by modification of the C-terminal regulatory domain. This can be achieved by phosphorylation, or by the binding of C-terminal antibodies or peptides [91–94]. Furthermore, the delivery of antibody or peptide to cells in vivo can induce a similar activation of some mutant p53 molecules, and the induction of apoptosis has been reported [95–97].

By contrast, in other tumour types p53 is rarely mutated, although its activity is impaired. This is exemplified in cervical carcinomas, where the presence of the human papilloma virus (HPV) is tightly linked to both the inactivation of p53 function and the induction of tumorigenesis. The tumour-associated HPVs encode two major oncoproteins: E7, which binds to pRB family members thereby disrupting the normal regulation of E2F and pushing the cells into the cell cycle, and E6, which binds to p53 and targets its destruction by a ubiquitin-mediated pathway. A number of tumour model systems have shown that expression of both E6 and E7 results in aggressively growing tumours, whereas only degenerate apoptotic lesions are detected following E7 expression alone [98, 99]. Expression of E7 in wild-type p53-expressing tumour cells results in high levels of apoptosis, and the cells can be rescued by the expression of E6 [100–102]. These data suggest that the disruption of E6 function, without compromising E7 activity, would allow p53/E2F-1-mediated apoptosis and might be an effective therapy for cervical carcinoma.

Maintenance of wild-type p53 in most other human tumours cannot be explained by the presence of viral proteins, and it seems likely that p53 function is impaired by alterations in either the upstream events leading to p53 activation or the downstream mediators of p53 function. One example is the recent identification of the cellular Mdm2 protein as a regulator of p53 stability. As discussed by Freedman and Levine, Mdm2 appears to play a key role in the maintenance of low levels of p53, and overexpression of Mdm2, or loss of the mechanisms which might normally allow p53 to become stabilized following genotoxic stress, could result in a functional loss of p53 activity similar to that seen in HPV E6-expressing cells. Activation of p53 by inhibiting degradation by Mdm2 is therefore another attractive goal in wild-type p53-expressing cancers, and preliminary studies have indicated that inhibition of p53/Mdm2 binding in cells can result in the stabilization of functional p53 [103].

Such approaches may be thwarted, however, in those tumours which have lost the protection of p53 by failing to respond to p53 activity. An example of wild-type p53-expressing tumours which show defects in Bax illustrates the potential for a class of cancers which might not be susceptible to a p53-based therapy [104].

Conclusions

A large body of work over many years has established a role for p53 in the induction of apoptotic cell death. It is likely that p53 utilizes a combination of transcription-dependent and -independent mechanisms, depending on cell type and environment, to initiate cell death. While we are still some way from completely understanding the molecular mechanisms by which p53 can induce apoptosis, the role of p53-mediated cell death in inhibition of tumour progression cannot be denied. A general increase in the tendency of cells to choose apoptosis rather than growth arrest as they become more transformed also points to the great therapeutic potential for p53. The challenge in the future will be to use our burgeoning knowledge of p53-mediated apoptosis to design and produce the tumour therapies for the next century.

Acknowledgments. This work was supported by the National Cancer Institute under contract with ABL.

1 Crook T., Marston N. J., Sara E. A. and Vousden K. H. (1994) Transcriptional activation by p53 correlates with suppression of growth but not transformation. Cell **79:** 817–827
2 Pietenpol J. A., Tokino T., Thiagaligam S., El-Deiry W., Kinzler K. W. and Vogelstein B. (1994) Sequence-specific transcriptional activation is essential for growth suppression by p53. Proc. Natl. Acad. Sci. USA **91:** 1998–2002
3 Symonds H., Krall L., Remington L., Saenzrobles M., Lowe S., Jacks T. et al (1994) p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell **78:** 703–711
4 Martin S. and Green D. (1995) Protease activation during apoptosis: death by a thousand cuts? Cell **82:** 349–352
5 Enari M., Sakahira H., Yokoyama H., Okawa K., Iwamatsu A. and Nagata S. (1998) A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature **391:** 43–50
6 Clarke A. R., Purdie C. A., Harrison D. J., Morris R. G., Bird C. C., Hooper M. L. et al. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature **362:** 849–852
7 Lowe S. W., Schmitt E. M., Smith S. W., Osborne B. A. and Jacks T. (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature **362:** 847–849
8 Shaw P., Bovey R., Tardy S., Sahli R., Sordat R. and Costa J. (1992) Induction of apoptosis by wild type p53 in a human colon tumor-derived cell line. Proc. Natl. Acad. Sci. USA **89:** 4495–4499
9 Yonish-Rouach E., Borde J., Gotteland M., Mishal Z., Viron A. and May E. (1994) Induction of apoptosis by transiently transfected metabolically stable wt p53 in transformed cell lines. Cell Growth Differ. **1:** 39–47

10 Wu X. W. and Levine A. J. (1994) p53 and E2F-1 cooperate to mediate apoptosis. Proc. Natl. Acad. Sci. USA **91:** 3602–3606
11 Hermeking H. and Eick D. (1994) Mediation of c-myc-induced apoptosis by p53. Science **265:** 2091–2092
12 Cai Z., Capoulade C., Moyret-Lalle C., Amor-Gueret M., Feunteun J., Larsen A. K. et al. (1997) Resistance of MCF-7 human breast carcinoma cells to TNF-induced cell death is associated with loss of p53 function. Oncogene **15:** 2817–2826
13 Klefstrom J., Arighi E., Littlewood T., Jäättela M., Saksela E., Evan G. I. et al. (1997) Induction of TNF-sensitive cellular phenotype by c-Myc involves p53 and impaired NF-kB activation. EMBO J. **16:** 7382–7392
14 Levine A. J. (1997) p53, the cellular gatekeeper for growth and division. Cell **88:** 323–331
15 Harris C. (1996) Structure and function of the p53 tumour suppressor: clues for rational cancer therapeutic stratagies. J. Natl. Cancer Inst. **88:** 1442–1455
16 El-Deiry W., Tokino T., Velculescu V. E., Levy D. B., Parson V. E., Trent J. M. et al. (1993) WAF1, a potential mediator of p53 tumour suppression. Cell **75:** 817–825
17 Harper J. W., Adami G. R., Wei N., Keyomarsi K. and Elledge S. J. (1993) The p21 CDK-interacting protein cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell **75:** 805–816
18 Gu Y., Turck C. W. and Morgan D. O. (1993) Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. Nature **366:** 707–710
19 Zhang H., Hannon G. J. and Beach D. (1994) p21-containing cyclin kinases exist in both active and inactive states. Genes Dev. **8:** 1750–1758
20 Deng C., Zhang P., Harper J. W., Elledge S. J. and Leder P. (1995) Mice lacking $p21^{CIP1/WAF1}$ undergo normal development, but are defective in G1 checkpoint control. Cell **82:** 675–684
21 Brugarolas J., Chandrasekaran C., Gordon J. I., Beach D., Jacks T. and Hannon G. J. (1995) Radiation-induced cell-cycle arrest compromised by p21 deficiency. Nature **377:** 552–556
22 Polyak K., Waldman T., He T.-C., Kinzler K. W. and Vogelstein B. (1996) Genetic determinants of p53-induced apoptosis and growth arrest. Genes Dev. **10:** 1945–1952
23 Waldman T., Kinzler K. W. and Vogelstein B. (1995) p21 is necessary for the p53-mediated G1 arrest in human cancer cells. Cancer Res. **55:** 5187–5190
24 Brown J. P., Wei W. and Sedivy J. M. (1997) Bypass of senescence after disruption of $p21^{CIP1/WAF1}$ gene in normal diploid human fibroblasts. Science **277:** 831–834
25 Kroemer G., Zamzami N. and Susin S. A. (1997) Mitochondrial control of apoptosis. Immunol Today **18:** 45–51
26 Liu X., Kim C., Yang J., Jemmerson R. and Wang X. (1996) Induction of apoptotic programme in cell-free extracts: requirement for dATP and cytochrome c. Cell **86:** 147–157
27 Yang J., Liu X., Bhalla K., Kim C. N., Ibrado A. M., Cai J. et al. (1997) Prevention of apoptosis by bcl-2: release of cytochrome c from mitochondria blocked. Science **275:** 1129–1132
28 Kluck R. M., Bossy-Wetzel E., Green D. R. and Newmeyer D. D. (1997) The release of cytochrome c from mitochondria: a primary site for bcl-2 regulation of apoptosis. Science **275:** 1132–1136
29 Vander Heiden M. G., Chandel N. S., Williamson E. K., Shumacker P. T. and Thompson C. B. (1997) Bcl-X_L regulates the membrane potential and volume homeostasis of mitochondria. Cell **91:** 627–637
30 Reed J. C. (1997) Double identity for proteins of the Bcl-2 family. Nature **387:** 773–776
31 Muchmore S. W., Sattler M., Liang H., Meadows R. P., Harlan J. E., Yoon H. S. et al. (1996) X-ray and NMR structure of human Bcl-x_L, an inhibitor of programmed cell death. Nature **381:** 335–341
32 Minn A., Velez P., Schendel S., Liang H., Muchmore S., Fesik S. et al. (1997) Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature **385:** 353–357
33 Schendel S., Xie Z., Montal M., Matsuyama S., Montal M. and Reed J. (1997) Channel formation by antiapoptotic protein Bcl-2. Proc. Natl. Acad. Sci. USA **94:** 5113–5118
34 Miyashita T. and Reed J. C. (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell **80:** 293–299
35 Knudson M. C., Tung K. S. K., Tourtellotte W. G., Brown G. A. J. and Korsmeyer S. J. (1995) Bax-deficient mice with lymphoid hyperplasia and male germ cell death. Science **270:** 96–98
36 McCurrach M. E., Connor T. M. F., Knudson M. C., Korsmeyer S. J. and Lowe S. W. (1997) *bax* deficiency promotes drug resistance and oncogenic transformation by attenuating p53-dependent apoptosis. Proc. Natl. Acad. Sci. USA **94:** 2345–2349
37 Yin C., Knudson C. M., Korsmeyer S. J. and Van Dyke T. (1997) Bax suppresses tumorigenesis and stimulates apoptosis in vivo. Nature **385:** 637–640
38 Lotem J., Peled-Kamar M., Groner Y. and Sachs L. (1996) Cellular oxidative stress and the control of apoptosis by wild-type p53, cytotoxic compounds, and cytokines. Proc. Natl. Acad. Sci. USA **93:** 9166–9171
39 Johnson T. M., Yu Z., Ferrans V. J., Lowenstein R. A. and Finkel T. (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc. Natl. Acad. Sci. USA **93:** 11848–11852
40 Polyak K., Xia Y., Zweier J. L., Kinzler, K. W. and Vogelstein B. (1997) A model for p53-induced apoptosis. Nature **389:** 300–305
41 Nagata S. (1997) Apoptosis by death factor. Cell **88:** 355–365
42 Owen-Schaub L. B., Zhang W., Cusack J. C., Angelo L. S. Santee S. M., Fujiwara T. et al. (1995) Wild-type human p53 and a temperature sensitive mutant induce Fas/APO-1 expression. Mol. Cell Biol. **15:** 3032–3040
43 Reinke V. and Lozano G. (1997) The p53 targets *mdm2* and *Fas* are not required as mediators of apoptosis in vivo. Oncogene **15:** 1527–1534
44 Reap E. A., Roof K., Maynor K., Borrero M., Booker J. and Cohen P. L. (1997) Radiation and stress-induced apoptosis: a role for Fas/Fas ligand interactions. Proc. Natl. Acad. Sci. USA **94:** 5750–5755
45 Strasser A., Harris A. W., Huang D. C. S., Krammer P. H. and Cory S. (1995) Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. EMBO J. **14:** 6136–6147
46 Itoh N., Tsujimoto Y. and Nagata S. (1993) Effects of bcl-2 on Fas antigen-mediated cell death. J. Immunol. **151:** 621–627
47 Hueber A.-O., Zörnig M., Lyon D., Suda T., Nagata S. and Evan G. I. (1997) Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. Science **278:** 1305–1309
48 Wu G. S., Burns T. F., McDonald E. R., Jiang W., Meng R., Krantz I. D. et al. (1997) KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nature Genet. **17:** 141–143
49 Harrington E. A., Bennett M. R., Fanidi A. and Evan G. I. (1994) c-myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J. **13:** 3286–3295
50 Yonish-Rouach E., Resnitzky D., Lotem J., Sachs L., Kimchi A. and Oren M. (1991) Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature **353:** 345–347
51 Canman C. E., Gilmer T. M., Coutts S. B. and Kastan M. B. (1995) Growth factor modulation of p53-mediated growth arrest versus apoptosis. Genes Dev. **9:** 600–611
52 Lin Y. and Benchimol S. (1995) Cytokines inhibit p53-mediated apoptosis but not p53-mediated G1 arrest. Mol. Cell. Biol. **15:** 6045–6054
53 Kauffmann-Zeh A., Rodriguez-Viciana P., Ulrich E., Gilbert C., Coffer P., Downward J. et al. (1997) Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. Nature **385:** 544–548
54 Shaulian E., Resnitzky D., Shifmen O., Blandino G., Amsterdam A., Yayon A. et al. (1997) Induction of Mdm2 and enhancement of cell survival by bFGF. Oncogene **15:** 2717–2725
55 Buckbinder L., Talbott R., Velasco-Miguel S., Takenaka I., Faha B., Seizinger B. R. et al. (1995) Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature **377:** 646–649

56 Rajah R., Valentinis B. and Cohen P. (1997) Insulin-like growth factor (IGF)-binding protein-3 induced apoptosis and mediates the effects of transforming growth factor-β1 on programmed cell death through a p53- and IGF-independent mechanism. J. Biol. Chem. **272:** 12181–12188
57 Israeli D., Tessler E., Haupt Y., Elkeles A., Wilder S., Amson R. et al. (1997) A novel p53-inducible gene, *PAG608*, encodes a nuclear zinc finger protein whose expression promotes apoptosis. EMBO J. **16:** 4384–4392
58 Zhan Q., Alamo I., Yu K., Boise L. H., Cherney B., Tosato G. et al. (1996) The apoptosis-associated γ-ray response of *BCL-X$_L$* depends on normal p53 function. Oncogene **13:** 2287–2293
59 Merchant A. K., Loney T. L. and Maybaum J. (1996) Expression of wild-type p53 stimulates an increase in both Bax and Bcl-XL protein content in HT29 cells. Oncogene **13:** 2631–2637
60 Zhan Q., Bieszczad C., Bae I., Fornace A. J. and Craig R. (1997) Induction of the BCL2 family member MCL1 as an early response to DNA damage. Oncogene **14:** 1031–1039
61 Ko L. J. and Prives C. (1996) p53: puzzle and paradigm. Genes Dev. **10:** 1054–1072
62 Murphy M., Hinman A. and Levine A. J. (1996) Wild-type p53 negatively regulates the expression of a microtubule-associated protein. Genes Dev. **10:** 2971–2980
63 Miyashita T., Harigai M., Hanaka M. and Reed J. C. (1994) Identification of a p53-dependent negative response element in the bcl-2 gene. Cancer Res. **54:** 3131–3135
64 Sabbatini P., Chiou S.-K., Rao L. and White E. (1995) Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. Mol. Cell. Biol. **15:** 1060–1070
65 Caelles C., Helmberg A. and Karin M. (1994) p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature **370:** 220–223
66 Wagner A. J., Kokontis J. M. and Hay N. (1994) Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell-cycle arrest and the ability of p53 to induce p21waf1/cip1. Genes Dev. **8:** 2817–2830
67 Yonish-Rouach E., Deguin V., Zaitchouk T., Breugnot C., Mishal Z., Jankins J. R. et al. (1995) Transcriptional activation plays a role in the induction of apoptosis by transiently transfected wild-type p53. Oncogene **11:** 2197–2205
68 Attardi L. D., Lowe S. W., Brugarolas J. and Jacks T. (1996) Transcriptional activation by p53, but not induction of the p21 gene, is essential for oncogene-mediated apoptosis. EMBO J. **15:** 3639–3701
69 Sabbatini P., Lin J., Levine A. J. and White E. (1995) Essential role for p53-mediated transcription in E1A-induced apoptosis. Genes Dev. **9:** 2184–2192
70 Chen X., Ko L. J., Jayaraman L. and Prives C. (1996) p53 levels, functional domains and DNA damage determine the extent of the apoptotic response of tumor cells. Genes Dev. **10:** 2438–2451
71 Wang X. W., Vermeulen W., Coursen J. D., Gibson M., Lupold S. E., Forrester K. et al. (1996) The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev. **10:** 1219–1232
72 Haupt Y., Rowan S., Shaulian E., Vousden K. H. and Oren M. (1995) Induction of apoptosis in HeLa cells by trans-activation deficient p53. Genes Dev. **9:** 2170–2183
73 Walker K. K. and Levine A. J. (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc. Natl. Acad. Sci. USA **93:** 15335–15340
74 Sakamuro D., Sabbatini P., White E. and Prendergast G. C. (1997) The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene **15:** 887–898
75 Ruaro E. M., Collavin L., Del Sal G., Haffner R., Oren M., Levine A. J. et al. (1997) A proline-rich motif in p53 is required for transactivation independent growth arrest as induced by Gas1. Proc. Natl. Acad. Sci. USA **94:** 4675–4680
76 Friedlander P., Haupt Y., Prives C. and Oren M. (1996) A mutant p53 that discriminated between p53 responsive genes cannot induce apoptosis. Mol. Cell. Biol. **16:** 4961–4971
77 Ludwig R. L., Bates S. and Vousden K. H. (1996) Differential transcriptional activation of target cellular promoters by p53 mutants with impaired apoptotic function. Mol. Cell. Biol. **16:** 4952–4960
78 Wang Y. and Prives C. (1995) Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. Nature **376:** 88–91
79 Barlow C., Brown K. D., Deng C.-X., Tagle D. A. and Wynshaw-Boris A. (1997) Atm selectively regulates distinct p53-dependent cell-cycle checkpoint and apoptotic pathways. Nature Genet. **17:** 453–455
80 Guillouf C., Grana X., Selvakumaran M., De Luca A., Giordano A., Hoffman B. et al. (1995) Dissection of the genetic programmes of p53-mediated G1 growth arrest and apoptosis: blocking p53-induced apoptosis unmasks G1 arrest. Blood **85:** 2691–2698
81 Chiou S. K., Rao L. and White E. (1994) Bcl-2 blocks p53-dependent apoptosis. Mol. Cell. Biol. **14:** 2556–2563
82 Di Leonardo A., Linke S. P., Clarkin K. and Wahl G. M. (1994) DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev. **8:** 2540–2551
83 Lowe S. W. and Ruley H. E. (1993) Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. Genes Dev. **7:** 535–545
84 Waldman T., Zhang Y., Dillehay L., Yu J., Kinzler K., Vogelstein B. et al. (1997) Cell-cycle arrest versus cell death in cancer therapy. Nature Med. **3:** 1034–1036
85 Hall M. and Peters G. (1996) Genetic alterations of cyclins, cyclin-dependent kinases and cdk inhibitors in human cancers. Adv. Cancer Res. **68:** 67–108
86 Field S. J., Tsai F.-Y., Kuo F., Zubiaga A. M., Kaelin W. G., Livingston D. M. et al. (1996) E2F-1 functions in mice to promote apoptosis and suppress proliferation. Cell **85:** 549–561
87 Yamasaki L., Jacks T., Bronson R., Goillot, E., Harlow E. and Dyson N. (1996) Tumor induction and tissue atrophy in mice lacking E2F-1. Cell **85:** 537–548
88 Phillips A. C., Bates S., Ryan K. M., Helin K. and Vousden K. H. (1997) Induction of DNA synthesis and apoptosis are separable functions of E2F-1. Genes Dev. **11:** 1853–1863
89 Qin X.-Q., Livingston D. M., Kaelin W. G. and Adams P. D. (1994) Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc. Natl. Acad. Sci. USA **91:** 10918–10922
90 Morgenbesser S. D., Williams B. O., Jacks T. and DePinho R. A. (1994) p53-dependent apoptosis produced by *Rb*-deficiency in the developing mouse lens. Nature **371:** 72–74
91 Hupp T. R., Meek D. W., Midgley C. A. and Lane D. P. (1993) Activation of the cryptic DNA binding function of mutant forms of p53. Nucleic Acids Res. **21:** 3167–3174
92 Hupp T. R. and Lane D. P. (1994) Regulation of the cryptic sequence-specific DNA-binding function of p53 by protein kinases. CSH Symp. Quant. Biol. **59:** 195–206
93 Hupp T. R., Sparks A. and Lane D. P. (1995) Small peptides activate the latent sequence-specific DNA binding function of p53. Cell **83:** 237–245
94 Halazonetis T. D. and Kandil A. N. (1993) Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants. EMBO J. **12:** 5057–5064
95 Selivanova G., Iotsova V., Okan I., Fritsche M., Ström M., Groner B. et al. (1997) Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nature Med. **3:** 632–638

96 Abarzúa P., LoSardo J. E., Gubler M. L., Spathis R., Lu Y.-A., Felix A. et al. (1996) Restoration of the transcription activation function to mutant p53 in human cancer cells. Oncogene **13:** 2477–2482
97 Abarzúa P., LoSardo J. E., Gubler M. L. and Neri A. (1995) Microinjection of monoclonal antibody PAb421 into human SW480 colorectal carcinoma cells restores the transcriptional activation function to mutant p53. Cancer Res. **55:** 3490–3494
98 Pan H. C. and Griep A. E. (1994) Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: implications for tumor suppressor gene function in development. Genes Dev. **8:** 1285–1299
99 Howes K. A., Ransom L. N., Papermaster D. S., Lasudry J. G. H., Albert D. M. and Windle J. J. (1994) Apoptosis or retinoblastoma: alternative fates of photoreceptors expressing the HPV-16 E7 gene in the presence or absence of p53. Genes Dev. **8:** 1300–1310
100 Demers G. W., Foster S. A., Halbert C. L. and Galloway D. A. (1994) Growth arrest by induction of p53 in DNA damaged keratinocytes is bypassed by human papillomavirus 16 E7. Proc. Natl. Acad. Sci. USA **91:** 4382–4386
101 Slebos R. J. C., Lee M. H., Plunkett B. S., Kessis T. D., Williams B. O., Jacks T. et al. (1994) p53-dependent G(1) arrest involves pRB-related proteins and is disrupted by the human papillomavirus 16 E7 oncoprotein. Proc. Natl. Acad. Sci. USA **91:** 5320–5324
102 Hickman E. S., Bates S. and Vousden K. H. (1997) Perturbation of the p53 response by human papillomavirus type 16 E7. J. Virol. **71:** 3710–3718
103 Bottger A., Bottger V., Sparks A., Liu W., Howard S. and Lane D. (1997) Design of a synthetic Mdm2-binding mini protein that activates the p53 response in vivo. Curr. Biol. **7:** 860–869
104 Rampino N., Yamamoto H., Ionov Y., Li Y., Sawai H., Reed J. et al. (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science **275:** 967–969