p53 in signaling checkpoint arrest or apoptosis

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The cell cycle arrest and apoptotic functions of p53 both contribute to the role of this tumour suppressor protein in preventing replication of cells suffering DNA damage. Although the ability of p53 to function as a sequence-specific transcription factor appears to be directly and causally linked to the implementation of an arrest at the G_1 stage of the cell cycle, the contribution of transcriptional activation to the apoptotic response is less clear. It seems likely that several p53 activities, both transcriptionally dependent and transcriptionally independent, can play a role in mediating cell death. The requirement for each of these functions appears to depend on the cell type, the cell environment and other genetic alterations already sustained by the cell in which p53 function is activated.

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Abbreviations
IGF insulin–like growth factor

Introduction

p53, one of the first tumour suppressor genes to be identified, remains the most frequent target for genetic alteration identified in human cancers. Loss of p53 function, most commonly through point mutation within one of the evolutionarily conserved domains, occurs in approximately half of most major cancers, and the essential role played by p53 in tumour suppression is illustrated by the high rate of malignancies in mice lacking functional p53. Interestingly, these mice develop more or less normally, suggesting that p53, which rather unusually is not a member of a larger protein family, plays no essential role in regulation of the normal cell cycle in most cells. Rather, the principal function of p53 appears to be in mediating a response to DNA damage, thereby preventing accumulation of potentially oncogenic mutations and genomic instability [1]. This role of p53, as guardian of the genome, provides the basis for its tumour-suppressive activities.

The cellular response to genotoxic agents initiates a rapid and substantial increase in the total p53 levels, achieved at least in part by the stabilization of the normally rapidly degraded p53 protein. Activation of p53 in this way leads ultimately to the suppression of cell growth, a function which is also evident following re-introduction of wild-type p53 into tumour cells lacking normal p53. Two mechanisms have been identified that, either individually or in combination, could account for the growth-suppression function of p53: cell cycle arrest and apoptosis. Whether these two responses are really manifestations of the same activity of p53, or whether they are entirely separate functions is one of the conundrums presently facing us. Similarly puzzling is the issue of how a cell decides which path to follow in response to p53 and, maybe most importantly, whether activation of both or either is an essential duty of p53 in its role as the molecular policeman. Evidence is being gathered in support of various hypotheses concerning these questions and already it is clear that the answers will not be simple. To further complicate matters, additional p53 functions with the potential to contribute to tumour suppression, ranging from inhibition of angiogenesis [2] to a role in differentiation [3] or senescence [4], are continually being identified.

p53 and apoptosis

Until recently, the cell cycle arrest function of p53 was considered to be its major contribution to tumour suppression. Now a large and increasing body of evidence argues for an activity of p53 in apoptosis. Indeed, the apoptotic role of p53 may be its most important contribution to the suppression of tumour cell growth, with potentially reversible cell cycle arrest serving to enable DNA repair in certain types of otherwise normal cells. Introduction of p53 into some tumour cell lines results in apoptotic death [5,6], and the induction of programmed cell death by oncogenes such as *Myc*, or DNA damage such as that induced by γ-irradiation, is dependent on wild-type p53 function [7••,8,9,10••].

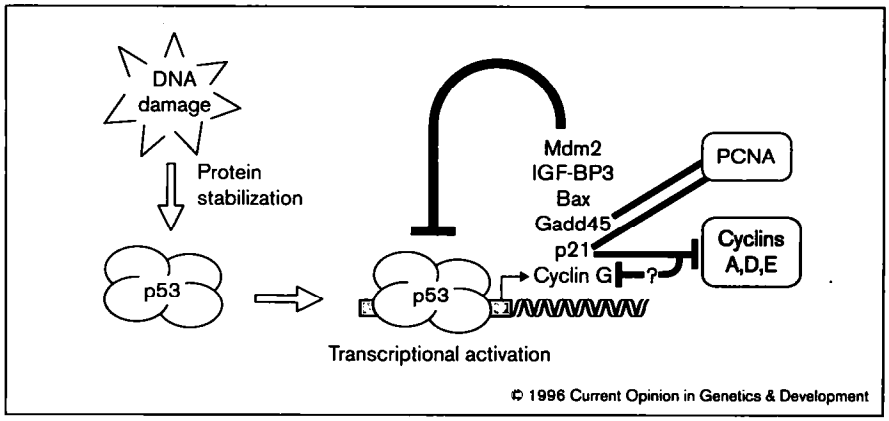
The importance of p53-mediated apoptosis to protection from transformation has been illustrated in tissue culture, where *p53* null cells fail to undergo cell death following the introduction of a variety of oncogenes, with consequent dramatic enhancement of malignant transformation (K Fukasawa, G Vande Woude, personal communication) [11]. Similarly, in mice, abrogation of the normal function of the tumour suppressor protein pRB gives rise to degenerative or slowly growing apoptotic lesions, unless p53 function is simultaneously disabled, in which case aggressively growing tumours arise [12••–15••]. Continued growth of cells that have lost the pRB checkpoint appears to depend on loss of the p53 apoptotic response, explaining why the functions of both of these tumour suppressor genes are targeted simultaneously in many examples of malignant progression.

Transcriptional activity and p53 function

Expression of p53 in many cell types results in arrest of progression through the cell cycle, with evi-

Figure 1

Sequence specific transcriptional activation by p53 in response to DNA damage. Several p53-responsive genes have been identified, and those most likely to play a role in mediating cell cycle arrest or apoptotic functions of p53 are shown here. The $p21^{CIP1/WAF1}$ protein inhibits the function of most cyclin-dependent kinases, although the effect on cyclin G, also regulated by p53, and its associated kinase has not been determined. The mdm2 protein negatively regulates the activity of p53.



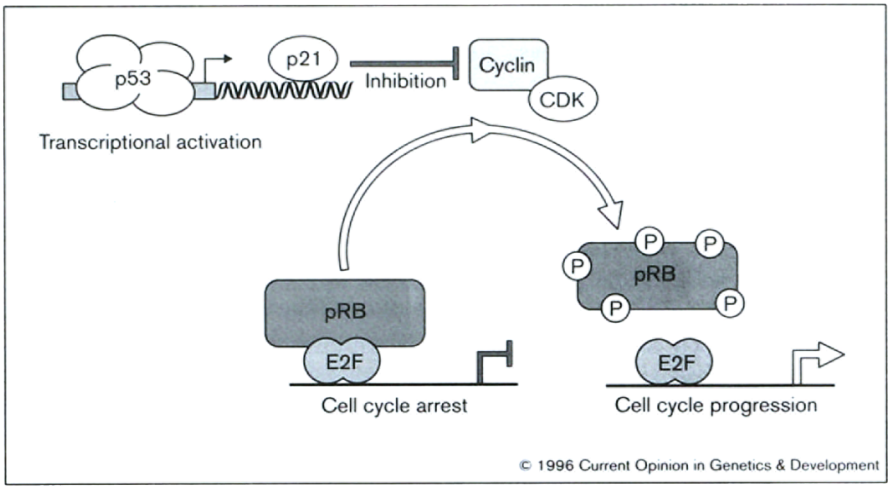
dence for both a G_1 and a G_2/M checkpoint function [3,16,17,18•,19•,20—22]. Several activities of p53 have been described, including transcriptional activation, transcriptional repression, control of DNA repair and replication, and a possible role in translational regulation. Although all of these activities are likely to contribute to p53 function, one of the least contentious observations is that the ability of p53 to induce a growth arrest is correlated with its ability to function as a sequence-specific transcriptional activator [23••,24•,25••]. A number of genes have been identified that can be induced in response to p53 expression (Fig. 1) and are likely to contribute to some, but not necessarily all, of the downstream functions of p53. The best studied of these genes encodes the cyclin dependent kinase inhibitor $p21^{CIP1/WAF1}$ (reviewed in [26]), enhanced levels of which inactivate the kinases responsible for driving cell cycle progression (Fig. 2). Inactivation of cyclin-dependent kinases by $p21^{CIP1/WAF1}$ leads to an inability to phosphorylate, and thus inactivate pRB during G_1. The hypophosphorylated pRB which persists under these conditions remains associated with transcription factors such as E2F, and failure to activate E2F-responsive genes such a B-*myb* has been shown to contribute to the p53-induced G_1 arrest [27]. Analysis of cells from $p21^{CIP1/WAF1}$ -deficient mice has shown that activation of $p21^{CIP1/WAF1}$ by p53 plays a major, but not exclusive, role in mediating the G_1 cell cycle arrest [28••,29••].

Less is known about the other p53-regulated genes, although several of these may also contribute to cell cycle arrest. Gadd45 [16] can also inhibit cell growth when overexpressed [30] and, like $p21^{CIP1/WAF1}$, associates with the polymerase delta auxiliary factor PCNA. The interactions between $p21^{CIP1/WAF1}$ or gadd45 and PCNA have been proposed to play a role during DNA repair [31–33]. Activation of cyclin G [34••,35••], a protein with the demeanor of a regulatory subunit of a protein-dependent kinase, might also contribute to the control of normal cell cycle progression. This presents the prospect of an interesting regulatory loop, in which p53-induced $p21^{CIP1/WAF1}$ might inhibit the function of p53-induced cyclin G. Negative-feedback regulation of p53 function is also accomplished by mdm2, a p53 inducible protein which binds directly to p53 to inhibit transcriptional activity [36,37].

Unlike the G_1 checkpoint function, a dependence of apoptosis on the sequence-specific transcriptional activity of p53 is not clear. It has been suggested that p53 transcriptional activity may be necessary for cell death in some systems [38•], and so far the most attractive target for a mediator of such an activity is the *Bax* gene, which is activated by p53 in some, but not all, cells [39,40,41••]. Bax plays an important role in regulating apoptosis by modulating the survival functions of Bcl-2 and related proteins [42], and it is easy to imagine how p53, through regulation of Bax, could favour the induction of cell death. Unfortunately, no clear evidence exists for a straightforward link between p53-mediated Bax expression and apoptosis. Several studies have shown that p53 mediated apoptosis can occur without detectable changes in the levels of Bax mRNA or protein [25••,43•,44], and cells from Bax-deficient mice show a normal p53-dependent apoptotic response following exposure to ionizing radiation [45••]. Overall levels of Bax expression vary significantly among different cell types, and it is conceivable that the contribution of enhanced Bax expression is only apparent in cells that, perhaps as a consequence of low normal expression levels, are sensitive to increased levels of Bax protein [38]. Other p53-inducible genes may also play a role in apoptosis. Correlations between enhanced gadd45 expression and cell death have been noted [43•] and the contribution of cyclin-dependent kinases to apoptosis [46–48] suggests a potential role for cyclin G in this pathway. Another recently identified p53 inducible gene encodes insulin like growth factor (IGF)-binding protein, an inhibitor of the mitogenic, and potentially also survival-related functions, of IGF [49•]. Activation of the expression of

Figure 2

Model for activation of a G_1 cell cycle arrest by p53. Activation of expression of the $p21^{CIP1/WAF1}$ protein by p53 inhibits the function of cyclin-dependent kinases, which contribute to progression through several stages of the cell cycle. One substrate for the cyclin-dependent kinases is the pRB protein, which blocks progress through G_1 by inhibiting expression of E2F-responsive genes. Normal release of the pRB block, following phosphorylation by the cyclin-dependent kinases, is inhibited following p53-dependent $p21^{CIP1/WAF1}$ expression.



the apoptotic trigger, fas/apo-1 [50], and repression of apoptotic protectors such as Bcl-2 [40] by p53 may also contribute to the cell death response.

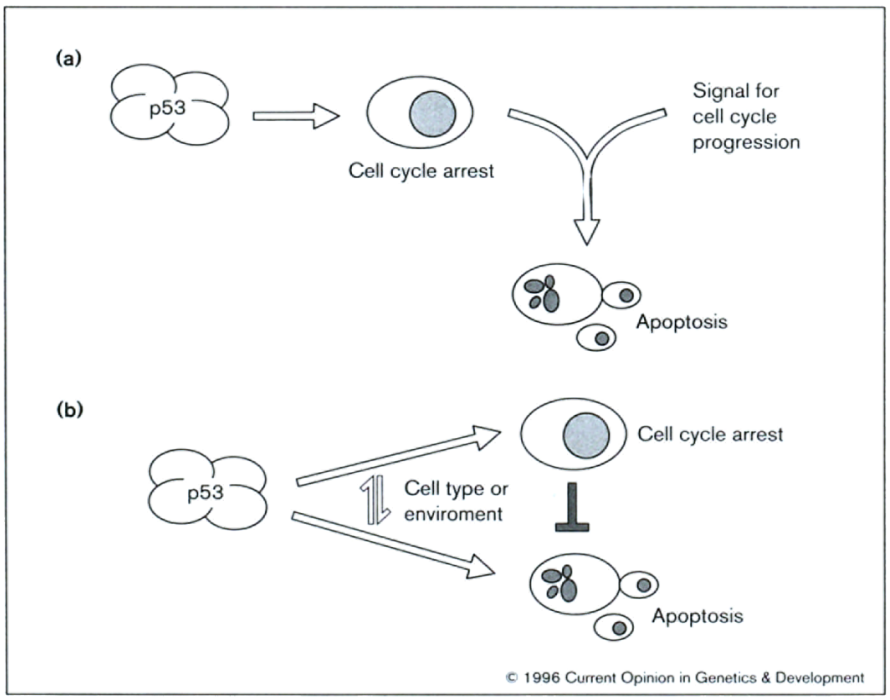
Cell cycle arrest and apoptosis: identical or different pathways?

Although at least some of the functions by which p53 induces cell cycle arrest seem to be understood, no clear consensus has been reached concerning the mechanism by which apoptosis is activated. Two models for the apoptotic function of p53, can be postulated (Fig. 3), and it seems likely that both of these operate, depending on the type and circumstances of the cell in question. Similar models have been proposed to explain the function of myc [51•], although, of course, in this case the outcome of activation is cell cycle progression or apoptosis.

In the first model, the apoptotic pathway is chosen as a direct result of the implementation, or at least the attempted implementation, of a G_1 checkpoint. In this 'conflict of signals' type of model, activation of the cell cycle arrest function of p53 in a cell which is simultaneously being driven through this checkpoint results in cell death, presumably as an emergency protective measure to prevent

Figure 3

Alternative models for the relationship between p53-mediated cell cycle arrest and p53-induced apoptosis. The models are not mutually exclusive and some evidence in different cell types suggests that both occur. **(a)** Conflicting signals. Activation of a G_1 checkpoint by p53 in a cell which is simultaneously being driven through the cell cycle activates apoptosis. In this model, the cell cycle arrest function of p53 is also an essential component of the apoptotic pathway. **(b)** Alternative pathways. Depending on cell type and environment, p53 may signal cell cycle arrest or apoptosis through independent pathways, and activation of cell cycle arrest affords protection from apoptosis. In this model, the G_1 checkpoint function of p53 is not required for the apoptotic pathway, which may require transcriptionally dependent and transcriptionally independent functions of p53.



unscheduled replication. p53-dependent apoptotic death, induced by the expression of proteins which drive cell cycle progression, such as E1A, E7, myc or E2F, could be interpreted in this way, and it is of interest to note that expression of pRB, which blocks the cell cycle, protects against p53-mediated apoptosis [52•,53•].

In general, the ability to activate transcription and the G_1 checkpoint correlates well with the ability of various p53 mutants to contribute to apoptosis, although several exceptions indicate a more complex relationship between these functions. Although apoptosis appears to proceed from the cells in the G_1 compartment in some cell types [44,54], in general there is no evidence that actual implementation of a G_1 arrest is either necessary or sufficient for activation of the apoptotic pathway. Importantly, cells from $p21^{CIP1/WAF1}$-deficient mice show a normal p53-mediated apoptotic response, illustrating that this function, unlike the G_1 arrest, is not dependent on the activation of this kinase inhibitor [28••,29••].

In the second model, cell cycle arrest and apoptosis mediated by p53 are independent and separable. This model is supported by observations that loss or perturbation of one pathway does not necessarily result in the inactivation of the other. Both pathways are likely to be engaged following p53 activation, and the ultimate outcome of the response will depend on additional factors such as cell type and environment. Although activation of the G_1 checkpoint may play a role in inducing the apoptotic pathway in some cells, it seems clear that p53 functions additional to those required for G_1 arrest are necessary for cell death to occur, and institution of a G_1 arrest is not, by itself, sufficient to activate apoptosis. A tumour-derived point mutant of p53 which retains wild-type ability to activate $p21^{CIP1/WAF1}$ and induce cell cycle arrest [25••] is nevertheless unable to participate in activating apoptosis. This mutant protein induces an apparently normal growth arrest in cells that lack p53 but shows complete loss of transformation suppression under circumstances where additional genetic events (in this case expression of HPV E7 and ras) render the cell insensitive to the impediment to cell cycle progression. p53-associated cell cycle arrest can be bypassed in a number of ways, and the particular selection for loss of apoptotic function in this p53 mutant suggests that induction of cell death, rather than G_1 arrest, is the principal tumour-suppressing function of p53. This model is supported by the observation that $p21^{CIP1/WAF1}$-deficient mice, which show a defect in p53-induced G_1 arrest but not apoptosis, have so far failed to show an enhanced incidence of tumour development [28••,29••].

Further support for the alternative pathway model is provided by the observation that transcriptional activation, and therefore the induction of a G_1 arrest, is not necessarily a prerequisite for p53-induced apoptosis [55•]. Myc-induced apoptosis, for example, can occur in the absence of new protein synthesis [10••] and transcriptionally inactive p53 mutants are not always defective for apoptosis [56•]. Interpretation of the contribution of transcriptional activation using p53 mutants is complicated by the ever growing family of p53-responsive genes and by the observation that some mutants may show selective loss of transcriptional function, retaining the ability to activate a sub-set of the responsive promoters [25••]. Thus, a transcriptionally active p53 protein which induces cell cycle arrest but not apoptosis may be specifically defective in the transactivation of apoptotic genes. In the absence of a clear p53-inducible mediator of apoptosis this question is hard to resolve, and taken together, the data suggest that p53 has at least some transcriptionally independent functions which contribute to apoptosis. Although the nature of such a function remains a mystery, direct physical interaction between p53 and a second mediator of apoptosis may be of importance. Candidate proteins that interact with p53, which to date include E2F-1, mdm2, pRB, DP-1, WT1 and abl, continue to be identified. Enhancement of p53-induced apoptosis by E2F [53,57•,58•] and protection by pRB [52•,53•] and WT1 [59•] has also been described. The interaction of p53 with mdm2 does not appear to be necessary for p53 function [60] but the exact contribution of any of these proteins to the p53 apoptotic function remains unclear.

Choice between G_1 arrest and death

The factors governing how a cell responds to p53 activation, in undergoing either G_1 arrest or apoptosis, form the subject of much interest and debate, and it seems likely that cell type and environment are major determinants of the outcome. Thymocytes, cells which undergo p53 independent cell death during the normal process of lymphoid development, also enter apoptosis in response to DNA damage through a p53-dependent pathway [8,9]. A similar dose of irradiation of normal fibroblasts, however, results in a prolonged G_1 arrest [18•] and it is possible that only some cells, such as fibroblasts, have the option of undergoing a cell cycle arrest. Transformed fibroblasts, on the other hand, in which various genetic abnormalities have accumulated to favour abnormal cell proliferation, also show a shift in response from cell cycle arrest to apoptosis [61]. Alterations in the cell's environment, particularly the presence or absence of survival factors, result in a similar shift in response to the same stimulus [5,43•,62] and the protection provided by some oncogenes which stimulate signal-transduction pathways, such as *ras* [63••], may reflect the activation of a survival, rather than a growth stimulatory, signal.

In most cases, it seems likely that the ability to mount a secure G_1 arrest in some way protects the cells from undergoing apoptosis [25••,56•,64,65]. Inhibition of apoptosis, for example by expression of death protection protein Bcl-2, leads to the establishment of cell cycle arrest [54,63••]. Similarly, the protection from apoptosis afforded by WT1 [59•] or the tyrosine kinase abl [66]

may be related to their recently described abilities to enhance p53-dependent transcriptional activation and cell cycle arrest [59•,67]. As only cell cycle arrest has the potential of being a reversible response, this choice will be intimately linked to cell survival. Another factor that may govern the choice of response is the extent of DNA damage suffered by the cell. It is tempting to speculate that modest damage, which might be repaired, activates the cell cycle arrest response whereas more catastrophic damage elicits cell death.

Conclusions

The evidence available to date supports a model in which the G_1 checkpoint function of p53 is the consequence of transcriptional activation, and in which, in most normal cells growing in an appropriate environment, induction of p53 leads to either a transient or long term cell cycle arrest. The apoptotic function of p53 is manifested in cells with genetic alterations or in the absence of survival factors, and it seems likely that several activities of p53 can contribute to this process, the importance of each being dependent on the cell type under investigation. This is clearly illustrated by a p53 protein mutated in the *trans*-activation domain, which is unable to activate transcription [68]. This mutant has been used to demonstrate both that transcriptional activity is essential for apoptosis (in transformed rat cells) [38•] and that transcriptional activity is entirely dispensable for apoptosis (in a human tumour line) [56•].

It is possible that several pathways cooperate to induce death in a normal cell, and that as these become progressively activated (during oncogenic transformation, for example), the cell becomes increasingly more sensitive to apoptotic signals. Different p53 functions, both transcriptionally dependent and transcriptionally independent, might play a role in activating these pathways; the exact combination of p53 functions necessary to induce death would depend on the type and growth conditions of the cell in question.

Although the contribution of p53 to apoptosis is complex, one emerging theme is that where the response of normal cells to p53 is cell cycle arrest, malignant versions of the same cell type are likely to be more sensitive to p53-induced apoptosis. In terms of tumour therapy, this is perhaps the most important point, giving rise to the hope that ubiquitous activation of p53 function will temporarily arrest normal cell growth, while activating programmed death specifically in the tumour cell population.

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