Biochemical Pharmacology, Vol. 58, pp. 1089–1095, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00153-7

COMMENTARY

Mechanisms of p53-Induced Apoptosis

*Martin R. Bennett**

UNIT OF CARDIOVASCULAR MEDICINE, UNIVERSITY OF CAMBRIDGE SCHOOL OF CLINICAL MEDICINE, DEPARTMENT OF MEDICINE, ADDENBROOKE'S HOSPITAL, CAMBRIDGE, CB2 2QQ, U.K.

ABSTRACT. The *p53* tumour suppressor gene functions in both cell cycle arrest and apoptosis. Despite considerable advances in understanding as to how p53 regulates growth arrest, the mechanisms by which p53 regulates apoptosis are only just emerging. In particular, there appears to be a structural and functional separation between the ability of p53 to induce growth arrest and apoptosis. This review examines the interactions between p53-induced growth arrest and apoptosis, and the mechanisms of p53-induced apoptosis, both via induction of p53 transcriptional targets and via nontranscriptional mechanisms. BIOCHEM PHARMACOL **58**;7:1089–1095, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. apoptosis; p53; cancer; oncogene; transcription; cell cycle

p53 is a tumour suppressor gene, whose loss or inactivation is the most common single lesion in human neoplasia [1–3]. Thus, lack of p53 expression, although frequently a late event in tumourigenesis, is seen as a major step towards the development of the neoplastic phenotype. p53 exerts its tumour suppressor effect via the regulation of cell proliferation [4], regulating cycle checkpoints and mediating growth arrest, and also by mediating apoptosis, controlling the propagation of damaged DNA. These seemingly contradictory actions of p53, although both control the cell mass of a tissue, appear to occur via pathways that are in part overlapping, and in part distinct. This commentary attempts to summarise the current position of p53 in the control of cell death.

P53—THE SWITCH BETWEEN GROWTH ARREST AND APOPTOSIS

p53 regulates apoptosis following a variety of stimuli, with apoptosis being dependent predominantly upon the cell type under study. Thus, p53 can prime cells to die following (a) DNA damage, due to cytotoxic drugs, free radicals, or irradiation, (b) growth factor withdrawal [5], (c) hypoxia [6] or metabolic change, (d) virus infection, (e) cytokines [7], (f) deregulated expression of cell cycle genes, or (g) the biological end-of-life of cultured mesenchymal cells.

The fact that p53 induces both growth arrest and apoptosis suggested at first that the same pathways might mediate both responses. However, the mechanisms by which p53 mediates apoptosis appear to be distinct from those mediating growth arrest. For instance, p53-dependent apoptosis does not require induction of p53 target proteins such as $p21^{waf/cip1}$ [8], which is a major effector of p53-mediated growth arrest [9]. Furthermore, p53 can induce apoptosis without apparent growth arrest, for example, in cells having deregulated expression of the proto-oncogenes c-*myc* or adenovirus 12S E1A, or the transcription factor E2F-1. c-*myc*, E1A, and E2F-1 all cause accumulation of p53 [10–12], although E1A and E2F-1 also can induce apoptosis independent of p53 induction or status [13–16].

The separation of p53-dependent pathways for growth arrest and apoptosis may be based on selective induction of gene products specific to each pathway. For example, p53 has been shown to up-regulate pro-apoptotic genes such as *Bax* and to suppress anti-apoptotic genes such as *bcl-2*, by transcriptional activation or repression, thus altering the relative quantities of Bax to Bcl-2 and shifting the balance towards apoptosis [17–19]. p53 also directly induces transcription of IGF-bp3†, a protein that can inhibit both the mitogenic and anti-apoptotic activity of IGF-1 [20], via a p53 consensus motif in the promoter region. IGF-bp3 can induce apoptosis, which may be via sequestration of IGF-1, a potent anti-apoptotic signal, although IGF-bp3 also can induce apoptosis independent of both p53 status and IGF-1/IGF-1R signalling [21]. However, a number of p53 mutants can transactivate p21 and induce arrest, but fail to induce IGF-bp3 or Bax, and therefore show impaired pro-apoptotic activity [22, 23]. This indicates that promoter selectivity of p53 mutants can determine whether p53 induces growth arrest or apoptosis.

Another level of regulation of p53-mediated actions is

* Correspondence: Dr. Martin R. Bennett, Division of Cardiovascular Medicine, Addenbrooke's Centre for Clinical Investigation, Level 6, Box 110, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, U.K. Tel. +44 1223-331504; FAX +44 1223-331505; E-mail: mrb@mole.bio.cam.ac.uk

† *Abbreviations*: IGF(-bp3), insulin-like growth factor 1 (binding protein 3); IGF-1R, IGF-1 receptor; DR-5, TRAIL receptor death receptor 5; TNF, tumour necrosis factor; TNF-R, TNF receptor; SAGE, serial analysis of gene expression; FADD, Fas-associated protein containing death domain; TRADD, TNF-R-associated protein containing death domain; and RB, retinoblastoma protein.

demonstrated by certain human tumour-derived or Li–Fraumeni p53 mutants, which retain the ability to transactivate p53-responsive promoters or induce growth arrest, but are defective in apoptosis [23, 24]. Importantly, some tumour-derived p53 mutants retain pro-apoptotic function despite the inability to induce Bax or IGF-bp3, implying that they possess additional pro-apoptotic activities, which may be nontranscriptional in nature [23] (see below). Although there is a lack of correlation between apoptosis and G_1 arrest, there appears to be a correlation between the ability of p53 to arrest cells in G_2 and apoptosis [25]. The implication of this correlation, specifically whether it indicates that the same mechanisms may be responsible for both outcomes, is presently unclear.

The outcome of p53 activation, either growth arrest or apoptosis, also depends upon proteins that regulate p53 action. The tumour suppressor gene *BRCA1* can bind to p53 and cooperatively promotes both p53-induced transcription of target genes, such as p21 and *Bax*, and apoptosis [26]. In addition, p300, a transcriptional co-activator that complexes with p53 [27], can promote p53-dependent induction of p21 [28], but can inhibit p53-induced apoptosis [29]. It has been suggested that inhibition of p53-induced apoptosis is mediated by mdm2, which is up-regulated by p300, but which inhibits p53 function [30]. mdm2 binds the transcriptional activation domain of p53, induces rapid p53 degradation, and blocks its ability to regulate target genes and to exert antiproliferative effects [31]. However, as mdm2 is a transcriptional target of p53, this implies that there is an autoregulatory feedback loop in the control of p53-mediated apoptosis. The interval between p53 activation and consequent Mdm2 accumulation, therefore, would define a time window during which p53 exerts its effects. A further autoregulatory loop occurs at the level of mdm2 degradation. mdm2 is cleaved by caspase 3 [32], which is frequently activated in p53-induced apoptosis, although mdm2 binding to p53 and inhibition of p53 may not be affected by mdm2 cleavage [32]. Thus, activation of p53-induced apoptosis potentially would amplify p53 signalling within the cell. The anti-apoptotic genes adenovirus *E1B 19k* and *bcl-2* also can increase mdm2, which may explain, in part, their inhibition of p53-induced apoptosis [30].

In addition, the presence of survival factors determines whether a cell line such as interleukin-3-dependent M3 cells will undergo growth arrest or apoptosis [33]. In the absence of interleukin-3, M3 cells undergo apoptosis following p53 activation, although cells undergo arrest in the presence of interleukin-3. In haematopoietic cells, which undergo both apoptosis and growth arrest following irradiation, distinct members of the JAK kinases family signalling to bcl-2 can block p53-induced apoptosis but not growth arrest [34], implying that the apoptotic and growth arrest responses to DNA damage in haematopoietic cells are modulated by distinct, cytokine-specific signal transduction pathways.

In summary, the response to p53 activation depends upon cell type, nature of arrest/apoptotic stimulus, and a number of downstream gene products, both transcription targets of p53 and p53 regulatory proteins. Although growth arrest and apoptosis are both distinct outcomes of p53 induction, prior growth arrest can influence p53-induced death profoundly. For example, arrest via activation of p21 protects cells from p53-induced apoptosis [35].

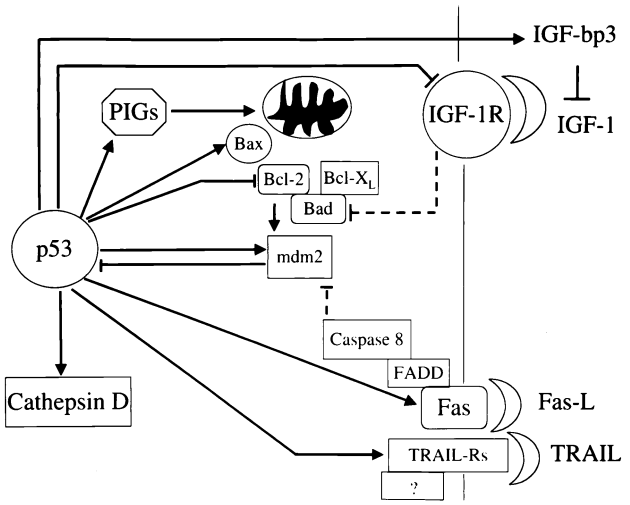

FIG. 1. Transcriptional targets of p53, and their interactions with apoptosis signalling proteins. p53 transcriptionally activates a number of target genes, including several p53-induced genes (PIGs), which may function in redox-related activities in association with mitochondria; Bax, which also functions at the mitochondrion; IGF-bp3, which binds to and prevents the anti-apoptotic signalling of IGF-1; mdm2, which acts in an autoregulatory role; the death receptors Fas and DR-5 (a TRAIL-receptor), which signal via adapter molecules (FADD and unknown for DR-5) to activate caspases; and cathepsin D. The dashed lines indicate an indirect effect.

P53-INDUCED APOPTOSIS VIA TRANSCRIPTIONAL REGULATION

p53 transcriptionally activates a number of target genes that function in apoptosis, including *bax*, IGF-bp3, and PAG608, a novel nuclear zinc finger protein that localises preferentially to nucleoli and promotes apoptosis [36] (Fig. 1). Fas and DR-5 are induced by p53, with increased surface expression of both after DNA damage induced by ionising radiation or by ectopic p53 expression [37]. Cathepsin D, a serine protease that may be involved in macrophage-mediated apoptosis, is also a transcriptional target for p53, containing two p53-binding sites in its promoter [38]. Cells undergoing p53-dependent apoptosis up-regulate cathepsin D, and cathepsin D-negative cells are resistant to cytotoxic drugs, implying that cathepsin D may be part of p53 signalling for apoptosis [38]. p53 also induces the protein p85, a regulator of the signalling protein phosphatidyl-3-OH kinase, which participates in the cell death process that is induced in response to oxidative stress [39]. Both p53 and p85 are required for apoptosis induced by oxidative stress [39].

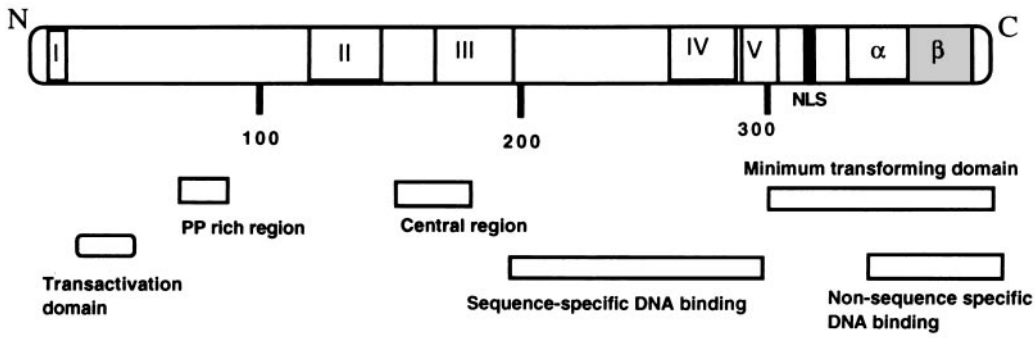

FIG. 2. Structure of the p53 protein. Key: I–V, conserved domains; α and β, oligomerisation motifs; NLS, nuclear localisation signal; and PP-rich, polyproline-rich region.

A more systematic analysis of p53-induced transcripts by SAGE has identified a number of p53-induced redox-related genes [40] that may regulate apoptosis. Many of these targets are predicted to encode proteins that could generate or respond to oxidative stress or are redox-related genes. Indeed, expression of many of these genes results in the formation of reactive oxygen species, leading to the oxidative degradation of mitochondrial components, culminating in cell death.

Consistent with the requirement for transcription, the transcriptional activation domain (amino acids 20–42) of p53 is necessary for apoptosis of many cells [41–43] (Fig. 2). This has been interpreted as indicating that transcription itself is required for p53-induced apoptosis, since no other function has been mapped to this domain. Recent studies also have indicated that the N-terminus of p53 is necessary for the efficient induction of apoptosis in murine GHFT1 cells, although this is irrespective of its ability to bind to the transcription co-activators hTAF [11] 31 and 70 [44]. In contrast, other studies have indicated that p53-induced apoptosis requires sequence-specific DNA binding, but the N-terminal 42 amino acids are not required. In particular, mutants lacking the N-terminal 42 amino acids still can induce apoptosis that is transcription-independent [45], although the region between amino acids 43 and 63 is required for apoptosis [45]. Another region of p53 that appears to be required for apoptosis is the polyproline-rich region located between the N-terminal transactivation domain and the DNA binding domain (amino acids 62–91). This polyproline-rich region contains putative SH3 binding motifs, suggesting that protein:protein interactions may mediate p53-induced apoptosis. Indeed, these studies found that the polyproline-rich region was dispensable for DNA binding and was not required for growth arrest [46]. The C-terminus is dispensable for the induction of p53-induced apoptosis, although it may stabilise p53 protein and thereby alter the kinetics of apoptosis [47].

NONTRANSCRIPTIONAL MECHANISMS OF P53-INDUCED APOPTOSIS

p53 mutants exist that are defective in apoptosis but retain normal ability to bind DNA and activate p53 reporter constructs and physiological promoters [48], or that are transactivation-defective but still can induce apoptosis or suppress colony formation of tumour cells [49, 50]. p53-mediated apoptosis also can occur in the absence of *de novo* RNA and protein synthesis in some cell types [8, 51] and after exposure to some inducers of apoptosis [52]. These observations imply that p53-induced apoptosis can occur through transactivation-dependent and -independent mechanisms, according to cell type. This suggestion is supported by observations that no individual p53 transcriptional targets, neither Bax, mdm2, nor Fas, are required for p53-induced apoptosis of thymocytes following irradiation *in vivo* [53].

How p53 induces apoptosis without transcription of target genes has not been elucidated, although direct protein:protein interactions and transcriptional repression both have been implicated. p53 can interact with the xeroderma pigmentosa complementation groups B and D (XPB and XPD) helicases, components of the basal transcription/DNA repair TFIIH complexes, to induce apoptosis [54]. Cells deficient in these helicases do not undergo p53-mediated apoptosis. p53 also binds the protein 53BP2. This protein may have a role in p53-mediated apoptosis, as the apoptosis inhibitor Bcl-2 competes with p53 for 53BP2 binding. However, the role of this binding protein is uncertain, as 53BP2 expression *per se* does not induce apoptosis [55].

It is also possible that p53 represses genes necessary for cell survival. Both Bcl-2 and IGF-1 receptor expression are reduced by wild-type p53, and p53 also inhibits signalling from the IGF-1R to induce apoptosis [56, 57]. The finding that genes that abrogate p53-dependent apoptosis, such as *bcl-2* and $E1B^{19k}$, also abrogate p53-mediated repression of promoters that lack a p53 binding site, raises the possibility that p53 might induce apoptosis, at least in part, by repressing transcription. In contrast, transcriptional activation by p53 still occurs in the presence of both bcl-2 and $E1B^{19k}$. This idea is substantiated by studies of the adenovirus $E1B^{55k}$ protein, where mutants lacking intrinsic transcription repression activity are defective for apoptosis [58]. Inhibition of p53-dependent apoptosis appears to depend on the transcriptional repression function of the 55-kDa

protein, which can be regulated by phosphorylation at the carboxy terminus [58].

A further nontranscriptional mechanism of action of p53-induced apoptosis has come from studies examining the cross-talk between p53 and the Fas/TNF superfamily of death receptors. Fas or TNF-R1 bind to their cognate ligands (Fas-L, TNF-α), which results in the recruitment of adapter proteins (FADD to Fas, TRADD and FADD to TNF-R1) to their cytoplasmic domains, via shared protein motifs known as death domains. Death domain interactions then can recruit upstream caspases such as caspase 8 to the death complex, resulting in activation of a caspase cascade that irreversibly cleaves proteins required for cellular architecture or homeostasis, resulting in cell death. Fas-induced apoptosis is rapid and does not require new protein or RNA synthesis. In recent studies we have found that p53 activation rapidly causes translocation of an intracellular pool of Fas located in the Golgi complex to the cell surface [59]. This increased cell surface Fas induces FADD recruitment to Fas, and caspase activation and trafficking of death receptors do not require new protein or new RNA synthesis. In addition, p53-induced apoptosis has been shown to require Fas/Fas-L and FADD [59]. This mechanism of p53-induced trafficking of death receptors is also apparent for TNF-R1, and in a variety of different mesenchymal cells, implying that it may have widespread applicability. In other studies, Fas/APO1-induced death is accompanied by massive translocation of p53 from the cytoplasm to the nucleus in human B-lymphocytes [60], and Bcl-2 inhibition of apoptosis is associated with failure of p53 to translocate into the cell nucleus [61]. A similar mechanism may explain the findings that UV irradiation induces Fas clustering on the cell surface and activation of Fas-mediated apoptosis [62], and that Fas-induced apoptosis is impaired in cells expressing mutant p53 [63]. Indeed, Fas induction after irradiation is impaired in p53-null tumour cells [64]. Similarly, tumour cells that are resistant to chemotherapy-induced apoptosis show cross-resistance to Fas-induced apoptosis [65], and chemotherapy can increase both Fas mRNA and Fas surface expression, rendering cells susceptible to Fas-induced apoptosis [66]. These studies indicate that p53-induced apoptosis may be signalled through Fas/Fas-L.

p53 also interacts with signalling from the Fas receptor via other pathways. Apoptosis induced by deregulated expression of c-*myc* is promoted by signalling from Fas [67], and thymocytes expressing a dominant negative FADD, which effectively blocks all signalling from death receptors that use FADD as an adapter molecule, show impaired proliferation, which is p53-dependent [68]. In contrast, irradiation of thymocytes induces apoptosis that requires both p53 and caspase 3, but is independent of Fas/Fas-L signalling [69]. Thus, apoptosis via both p53 and c-*myc* may be augmented by signals through Fas, but conversely, p53 may regulate signals originating from Fas.

A novel mechanism of p53-induced apoptosis occurs via the ability of p53 to facilitate RB cleavage during apoptosis [33]. RB is cleaved by caspases during apoptosis, rendering it inactive to suppress apoptosis or cell proliferation. In the absence of growth factors, p53 promotes RB cleavage, probably via an increase in activity of caspases [33]. Elimination of a presumptive anti-apoptotic effect of RB then may facilitate conversion of p53-mediated growth arrest into apoptosis.

P53—SENSITISATION OR INDUCTION OF APOPTOSIS?

Although p53 activation undoubtedly is involved in apoptosis, a major controversy exists as to whether p53 is a direct mediator of apoptosis or just sensitises cells to apoptosis induced by a variety of other agents. Whereas the induction of different gene products may mediate p53-dependent apoptosis or growth arrest, high levels of p53 expression alone are frequently insufficient to induce apoptosis, and other signals appear to be required [70]. As discussed above, p53-dependent apoptosis is regulated by the presence of cell-specific survival factors, presumably acting downstream from p53. For example, c-*myc*-induced apoptosis of fibroblasts, which is dependent upon p53 expression, also is regulated by the presence of IGF1 [71]. Thus, p53 may be only one of many pro- or anti-apoptotic signals that the cell integrates before deciding whether or not to undergo apoptosis. The exception to this appears to be cells that do not express wild-type p53, which undergo apoptosis rapidly in response to reintroduction of p53 [72–74]. However, the reintroduction of wild-type p53 into M1 myeloid cells can be inhibited by interleukin-6 [75].

The most convincing evidence that p53 is a sensitiser but not a direct mediator of apoptosis comes from $p53^{(-/-)}$ mice. These mice develop normally with no apoptotic phenotype at birth. Thus, the widespread apoptosis that occurs in embryogenesis is not critically dependent upon p53. However, it should be noted that the elimination of p53 in a pluripotent stem cell, which may have multiple redundant pathways for mediating proliferation and apoptosis, might be very different from elimination in a differentiated somatic cell. Indeed, embryonic stem cells do not up-regulate p53 or undergo p53-dependent apoptosis in response to DNA damage [76]. However, p53-null animals are prone to develop a variety of tumours in later life [77], and numerous cell types from these animals are resistant to apoptosis induced by some, but not all agents [78, 79]. Furthermore, loss of p53-dependent apoptosis in tumours markedly increases their growth and progression *in vivo* [80]. This suggests that p53 plays a critical, nonredundant role in the prevention of apoptosis in adult cells.

The interaction of p53 and oncogenes that also promote cell proliferation has suggested that the induction of apoptosis by p53 is due to an aborted attempt to suppress cell proliferation in the presence of deregulated expression of the oncogene, the so-called "conflict of signals." Against this hypothesis is evidence indicating that cells induced to die by deregulated expression of c-*myc* do so without

suffering cell cycle arrest [71]. Furthermore, the ability of E2F-1 to induce apoptosis, which clearly is regulated by p53, does not require transactivation, and does not correlate with its ability to promote DNA synthesis [13], indicating a separation between the abilities of p53 to promote apoptosis or suppress cell cycle progression.

The distinction between the abilities of p53 to induce apoptosis directly or to sensitise cells to apoptosis induced by other agents may actually be a simplification, as there is evidence of considerable cross-talk between apoptosis signalling pathways. For example, evidence that p53 causes trafficking of death receptors to the cell surface, and that c-*myc*-induced apoptosis (which requires p53) also requires signals through Fas, suggests that a considerable degree of cross-talk occurs between p53 expression and components of the apoptosis signalling machinery. Indeed, the fact that many pro-apoptotic gene products are direct transcriptional targets of p53 implies that p53 may play more than a generalised "priming" role.

In summary, there are multiple mechanisms by which p53 promotes or signals apoptosis. These mechanisms involve both transcription or target genes, and nontranscriptional mechanisms. The most important mechanism will depend upon the cell type and the inducer of apoptosis, and, clearly, more than one mechanism can operate in the same cell with different kinetics of action, the summated effect of which will result in the final response of the cell to p53 activation.

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