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The p53 tumour suppressor gene: a mediator of a G1 growth arrest and of apoptosis

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Abstract. The tumour suppressor gene p53 plays a major role in the protection of cells from DNA damage. Activation of the protein in response to irradiation or genotoxic agents, and possibly by other signals, results in growth arrest at the G1 phase of the cell cycle or in apoptosis. While it has been shown that the ability of p53 to function as a sequence-specific transcriptional activator is necessary for the induction of growth arrest, the mechanism of p53-mediated apoptosis is not yet clear. It appears that under some conditions activation of the G1 checkpoint will prevent apoptosis, but the cellular environment may alter the result of p53 activation towards cell death. p53 may also directly induce apoptosis through several pathways, which may be transcriptionally dependent or independent. The outcome – a G1 arrest or apoptosis – will depend on a complex network of regulatory signals.
Key words. p53; G1 arrest; apoptosis; tumour suppression.

Introduction

The p53 protein is the product of a tumour suppressor gene which has been the focus of much attention for the past few years. Loss of function of p53 occurs in over half of all human tumours, suggesting that p53 inactivation plays a central role in tumorigenesis [1]. Sequence analysis of the gene revealed five evolutionarily conserved regions [2], indicating that p53 has a significant biological role. Consistent with this notion is the fact that p53-null mice are susceptible to spontaneous tumours, and over 70% develop tumours by the age of 6 months [3–6]. A similar susceptibility is observed in humans: families with the Li-Fraumeni syndrome, which is a rare inherited autosomal condition, have been shown to transmit a mutated form of p53 in their germ line, and have a 50% probability of developing cancer by the age of 30 (for review, see ref. 7). Thus, understanding the function of p53 should provide an insight into the mechanisms of normal cell growth.

p53 is a transcription factor

p53 was shown to be a sequence-specific transcription transactivator [8]. It can interact with DNA containing the sequence 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′, typically in the context of two such sequence motifs separated by 0–13 bp [9–11]. Biochemical and functional studies of p53 have defined the domain structure of p53, and have revealed that the central 'core' of the protein is responsible for the sequence-specific binding activity [12–14]. It is within this domain that most of the residues mutated in human cancer are found [15]. The transcriptional activation domain is located at the N-terminus of the protein [16, 17]. The C-terminus of p53 contains the oligomerization domain [12, 18] which appears to stabilize the specific binding activity [19, 20]. This domain was also reported to confer on p53 the ability to bind single-stranded DNA and RNA [21], and to recognize primary DNA damage [22]. The basic region in the extreme C-terminus was shown to have negative-regulatory functions [23, 24].

p53 can also bind to many viral and cellular proteins (for review, see ref. 25), such as the TATA-box binding protein TBP. The interaction between p53 and TBP was suggested as the mechanism through which p53 can repress transcription from promoters which do not contain the p53-specific elements [26–30].

p53 can suppress cell growth: induction of a G1 arrest and/or of apoptosis

The main function of p53 appears to be in mediating the cellular response to DNA damage, and helping to maintain genomic stability [31–33]. Many forms of genotoxic stress, including ultraviolet light, g-irradiation and genotoxic chemicals, induce a rapid increase in p53 protein levels [31, 34, 35]. This is achieved mainly through stabilization of the normally short-lived p53 protein [36, 37], but also by an increase in p53 mRNA levels [38]. The main outcome of p53 activation appears to be growth suppression. However, other functions of p53 have been reported, namely a role in differentiation [39], senescence [40] and inhibition of angiogenesis [41]. The importance of these functions in the role of p53 as a tumour suppressor is still not clear.

The ability of p53 to suppress cell growth can be explained by an induction of growth arrest and/or of

apoptosis. The first reported biological activity of wt p53 was an induction of a growth arrest in the G1 phase of the cell cycle [42–45]. In these experiments, p53 was reintroduced and overexpressed in cells which had lost it. Similarly, a G1 arrest after γ irradiation was acquired following transfection of wt p53 into cells lacking it [32]. On the other hand, cells from patients with the cancer-prone disease ataxia telangiectasia were shown to be deficient in the irradiation-activated G1 checkpoint as well as in the induction of p53 protein following irradiation [36]. Thus, wt p53 can impose a G1 growth arrest under a variety of circumstances.

The involvement of wt p53 in the control of apoptosis has been reported for many systems, both in vitro and in vivo [46–51]. The first experiments that showed the ability of wt p53 to induce apoptosis were performed employing a clone of the mouse myeloid cell line M1 which is devoid of p53 expression. Cells of this clone were stably transfected with a temperature-sensitive (ts) mutant of p53, which acquires the conformation of the wt protein at the permissive temperature (32 °C). Upon down-shift to the permissive temperature the transfectants underwent a rapid loss of viability which had the characteristics of apoptosis [46]. Interestingly, although the same ts mutant imposed a reversible growth arrest in fibroblasts at the permissive temperature [44], in the M1 cells p53-induced apoptosis was not preceded by growth arrest [52]. However, the commitment to death was cell-cycle dependent and occurred in G1 [52].

Different results were obtained with mouse erythroleukemia cells DP16-1 lacking endogenous expression of p53 and which were transfected by the ts mutant [48]. In these cells, activation of wt p53 at the permissive temperature resulted in a G1 arrest, terminal differentiation and apoptosis [53]. However, in the same cells p53-mediated apoptosis was shown to be uncoupled from p53-mediated growth arrest, since the addition of cytokines inhibited the former but not the latter [54, 55].

In another cell system, the T-cell lymphoma line J3D, again using the ts p53, temperature down-shift resulted in a G1 arrest followed by apoptosis [56]. Bcl-2 inhibited the p53-triggered apoptosis but not the G1 arrest, demonstrating that the two functions of p53 are independent.

p53 was also shown to mediate E1A-induced apoptosis [57, 58]. Rodent cells transformed with E1A plus the ts p53 underwent apoptosis at the permissive temperature, and there was no growth arrest prior to apoptosis [59]. Bcl-2 prevented the p53-mediated apoptosis while keeping the cells in a predominantly growth-arrested state [59]. Thus, induction of apoptosis and induction of growth arrest by p53 were shown to be two separable functions.

p53 is required not only for irradiation-induced G1 arrest but also for irradiation-induced apoptosis in several in vivo and in vitro cell systems. Thus, thymocytes from genetically manipulated p53-null mice were resistant to irradiation-induced apoptosis [49–51]. In addition, crypt cells from the small and large intestines of these mice were resistant to γ-irradiation in vivo, while cells from normal mice underwent apoptosis [60].

Exposure of cells of the interleukin-3 (IL-3)-dependent murine lymphoid cell line Baf-3 to irradiation resulted in rapid apoptosis if IL-3 had been withdrawn, while no apoptosis after irradiation was observed in the presence of IL-3 [61]. Instead, in the presence of IL-3, Baf-3 cells arrested transiently both in the G1 and G2/M phases of the cell cycle after irradiation, and the G1 arrest was dependent on p53 function [62]. Thus, a survival factor could modify the outcome of p53 activation following irradiation from apoptosis to growth arrest.

Similarly, in the M1 cells activation of wt p53 by temperature down-shift in the presence of interleukin-6 (IL-6) did not result in cell death but rather induced a cell cycle exit into a G0-like quiescent state [63]. A G1 growth arrest was also observed in the ts p53-expressing M1 cells prior to apoptosis when ectopic expression of Bcl-2 delayed cell death following wt p53 activation at the permissive temperature [64].

Induction of p53-mediated apoptosis following DNA damage was demonstrated in Epstein-Barr virus-immortalized human B lymphoid cell lines [65]. Treatment with cisplatin rapidly induced apoptosis in these cells that was initiated in the G1 phase and was preceded by an accumulation of cells in G1. A progression of the cells through G1/S appeared to be required, and a G1 block by starvation prevented cell death to a large extent.

Transiently transfected p53 was shown to induce apoptosis in the human transformed cell lines HeLa and Saos-2 [66], and cell death was not preceded by growth arrest [67, 68]. Co-transfection of Rb could rescue HeLa cells from p53-induced apoptosis, although cells were not arrested at the G1 phase [68].

While the outcome of p53 activation can be modified from apoptosis to growth arrest, it can also be modified from growth arrest to apoptosis. In a mouse embryo fibroblast line expressing the ts p53, activation of wt p53 following temperature down-shift resulted in growth arrest in the G1 phase, but coexpression of wt p53 with transfected E2F-1 resulted in rapid apoptosis [69].

Gene involved in the induction of growth arrest

Induction of a growth arrest by p53 was shown to depend on its ability to act as a sequence-specific transcriptional activator [70, 71]. A number of genes have been identified which can be induced by p53, and an important target gene in the growth arrest pathway is WAF1/CIP1 [72, 73]. The protein product of this gene,

p21, binds to cyclin-dependent kinases and inhibits their action, thereby blocking cell proliferation [74]. In addition, p21 also binds to and inhibits the proliferating cell nuclear antigen (PCNA), a regulatory subunit of DNA polymerase δ [75]. Thus, radiation-induced G1 arrest was shown to be selectively mediated by the p53-WAF1/CIP1 pathway in human thyroid cells [76], and a correlation was observed between G1 arrest and the stability of the p53 and p21 proteins following γ-irradiation of human lymphoma cells [77]. Moreover, mice lacking p21 were shown to be defective in the G1 checkpoint control [78, 79]. However, the G1 checkpoint was only partially impaired, indicating that p21 does not play an exclusive role in this pathway. In addition, a human ts p53 mutant could arrest rat embryo fibroblasts in G1 at the permissive temperature without induced expression of WAF1/CIP1, suggesting an alternative mechanism for p53-induced growth arrest [80].

Other p53 target genes may also play a role in the induction of growth arrest. Gadd45, which was originally identified through its induction by DNA damage [81], was shown to be directly transactivated by p53 [36] and to suppress growth arrest in human tumour cell lines [82]. Like p21, Gadd45 can bind PCNA [83] and may also directly interact with p21 [84], thereby competing with it for interaction with PCNA [85]. Induction of Gadd45 by p53 may therefore play a role in the regulation of the cell cycle.

Other potential players in the control of cell cycle which are transactivated by p53 are cyclin G [86, 87], a possible subunit of a protein-dependent kinase, and mdm-2, which binds directly to the N-terminus of p53 and can serve as a negative feedback regulator by blocking transactivation and permitting re-entry to the cell cycle [88, 89].

Mechanism of induction of apoptosis

While transcriptional activation appears to be essential for the induction of growth arrest by p53, much less is known about the possible downstream elements in the apoptotic pathway. p21-null mice were not defective in p53-dependent apoptosis, indicating that this protein does not play a role in the induction of apoptosis by p53 [78, 79]. It has been suggested that p53 may induce apoptosis in the absence of transcriptional activation of target genes [90–92]. However, transcriptional activation may play a role in p53-mediated apoptosis in several cell systems [46, 67, 93]. The Bax gene, a member of the Bcl-2 family which accelerates apoptosis by forming heterodimers with Bcl-2 [94], was suggested as a possible target in this apoptotic pathway, since it was up-regulated by p53 in some cells [95–97]. However, p53-induced apoptosis was shown to occur in several cell systems without up-regulation of Bax [62, 65, 98], and analysis of cells from Bax-null mice indicated that p53-dependent apoptosis was normal in these mice [99]. Thus, the involvement of Bax as a mediator of p53-induced apoptosis is still not clear. It is also noteworthy that Bcl-2 was shown to be down-regulated by p53 in some cells [100, 101], and the equilibrium between Bcl-2 and Bax may be orchestrated by p53 in cells undergoing apoptosis.

Another potential mediator of the apoptotic pathway induced by p53 is Fas/APO-1 (CD95), a member of the tumour necrosis factor receptor superfamily. Fas/APO-1 is able to transduce a signal for apoptosis upon engagement by its ligand or specific antibodies (for review, see ref. 102), and its expression was shown to be induced by p53 [103]. In this context, irradiation and chemotherapeutic agents have been reported to enhance Fas/APO-1 expression and/or alter the sensitivity to anti-Fas mediated killing [103, 104].

The choice between growth arrest and apoptosis: why die?

Whether a cell will undergo growth arrest or apoptosis following p53 activation appears to depend on a variety of factors, such as environmental conditions and the cell type. Irradiation of normal human fibroblasts resulted in a prolonged G1 arrest [105], while a similar dose of irradiation induced apoptosis in thymocytes [49, 50]. On the other hand, fibroblasts expressing the adenovirus E1A gene were sensitized to irradiation-induced apoptosis [106], and the presence of survival factors could shift the response from apoptosis to growth arrest as demonstrated in the various examples above. The loss of the tumour suppressor pRb function may contribute to p53-induced apoptosis, since the activity of pRb and/or other pRb-related proteins was shown to be necessary for the induction of a G1 arrest by p53 following DNA damage [107–110], and pRb may have a protective effect on p53-induced apoptosis [92–111]. In cells having a functional pRb, induction of p21 would lead to inactivation of cyclin-dependent kinases and therefore to inhibition of pRb phosphorylation. The hypophosphorylated pRb retains transcription factors of the E2F family, which are necessary for the G1/S transition, thus imposing a p53-induced G1 arrest. In the absence of functional pRb, p21 will still be induced by p53 activation, but cells will be unable to growth-arrest and may therefore be 'forced' to die by entering into S phase. In this context it was reported that p53 induction by antineoplastic drugs led to apoptosis in Rb−/− mouse embryonic fibroblasts (MEFs), while Rb+/− and Rb+/+ MEFs underwent cell cycle arrest without apoptosis [112]. Along with this concept, deregulated expression of E2F was shown to induce p53-mediated apoptosis [69, 111, 113, 114]. Lacking both Rb and p53, E2F activation would stimulate cell proliferation and permit tumour formation, as was demon-

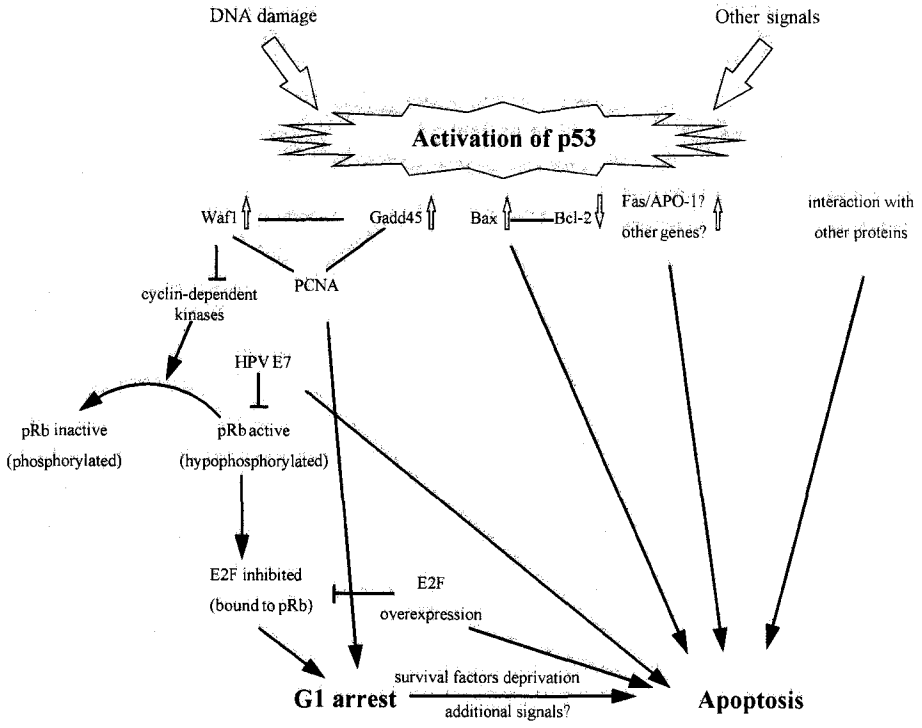

Figure 1. Possible models for p53-mediated G1 arrest and/or apoptosis. Transcriptional activation of WAF1 plays a role in activation of the G1 arrest via inhibition of cyclin-dependent kinases by the $p21^{WAF1}$ protein, activation of pRb (and related proteins) and inhibition of E2F. Transcriptional activation of Gadd45 may also contribute to G1 arrest through its interaction with PCNA and/or Wafl. Several pathways may be involved in the induction of apoptosis, some of which can relate to the G1 arrest. pRb inactivation by HPV E7, or E2F overexpression, may result in the inability to implement p53-mediated G1 arrest and result in apoptosis. Other pathways may lead to apoptosis regardless of the cell cycle function, and may involve up- or down-regulation of genes or interaction of p53 with other proteins.

strated by the development of retinal tumours in HPV E7 transgenic mice [115, 116].

However, although in some cases the cell may 'try' to growth-arrest before undergoing apoptosis if it cannot, this may not always be the case. Cells from the p21-null mice were shown to undergo a normal p53-induced apoptosis, suggesting that this function – unlike the p53-induced G1 arrest, which was impaired – does not require activation of this kinase inhibitor [78, 79]. The observation that the p21-null mice did not show an enhanced incidence of tumour development suggests that the role of p53 in the induction of apoptosis is more important for its tumour-suppressing activity than the G1 arrest. Support for this model comes from a recent study in which a tumour-derived mutant of p53 was shown to retain the ability to activate p21 and to induce growth arrest, while it was unable to induce apoptosis [98]. This mutant was also unable to suppress transformation of rat embryo fibroblasts by E7 and ras, demonstrating the correlation between induction of apoptosis and the tumour-suppressing function of p53 [98].

The reports that p53 may induce apoptosis in the absence of transcriptional activation in some cells [90–92], while activation of specific target genes may play a role in the induction of apoptosis in other cells [46, 67, 93], or even in the same cells under some other conditions [67, 92, 98], suggest that several pathways may be involved in p53-mediated apoptosis (fig. 1). Thus, p53 may activate a G1 checkpoint which will be retained in the presence of a functional pRb, or will result in apoptosis in case of signals for progression into the cell cycle. It may also directly activate apoptosis through the transactivation of specific genes, down-regulation of some other genes or interaction with proteins. Such signals may be controlled by cytokines or by members of the Bcl-2 family. The choice between a G1 arrest and one of the apoptotic pathways, and the final outcome, will depend on a complex network of regulatory signals.

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