**Cell Death and Differentiation (2018) 25,** 104–113
 1350-9047/18

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REVIEW

# How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression?

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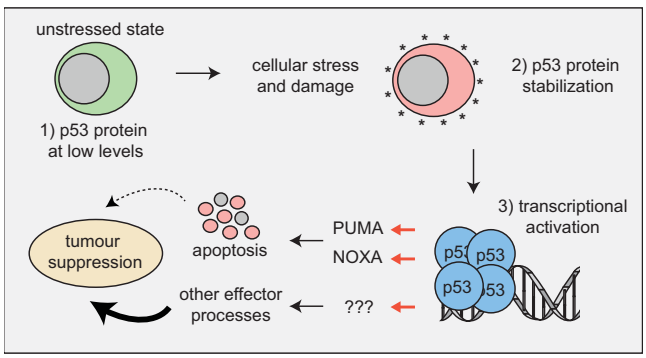
**The tumour suppressor gene *TP53* is mutated in ~50% of human cancers. In addition to its function in tumour suppression, p53 also plays a major role in the response of malignant as well as nontransformed cells to many anticancer therapeutics, particularly those that cause DNA damage. P53 forms a homotetrameric transcription factor that is reported to directly regulate ~500 target genes, thereby controlling a broad range of cellular processes, including cell cycle arrest, cell senescence, DNA repair, metabolic adaptation and cell death. For a long time, induction of apoptotic death in nascent neoplastic cells was regarded as the principal mechanism by which p53 prevents tumour development. This concept has, however, recently been challenged by the findings that in striking contrast to *Trp53*-deficient mice, gene-targeted mice that lack the critical effectors of p53-induced apoptosis do not develop tumours spontaneously. Remarkably, even mice lacking all mediators critical for p53-induced apoptosis, G1/S boundary cell cycle arrest and cell senescence do not develop any tumours spontaneously. In this review we discuss current understanding of the mechanisms by which p53 induces cell death and how this affects p53-mediated tumour suppression and the response of malignant cells to anticancer therapy.**

*Cell Death and Differentiation* (2018) **25,** 104–113; doi:10.1038/cdd.2017.169; published online 17 November 2017




Graphical Abstract

## Facts

- *TP53* is a critical tumour suppressor that is mutated in ~50% of human cancers.
- In unstressed cells p53 protein levels are very low because it is targeted for proteasomal degradation by the E3 ubiquitin ligase MDM2.
- *TP53* is activated in response to many stress stimuli, including activation of oncogenes and DNA damage.
- Upon activation, p53 directly regulates the transcription of ~500 genes and indirectly regulates many additional genes and thereby controls diverse cellular processes.
- P53 induces apoptosis in nontransformed cells mostly by direct transcriptional activation of the pro-apoptotic BH3-only proteins PUMA and (to a lesser extent) NOXA.
- Combined loss of the p53 effectors of apoptosis (PUMA plus NOXA) and cell cycle arrest/cell senescence (p21) does not cause spontaneous tumour development.
- Apoptosis induction via PUMA and NOXA is critical for the killing of malignant cells by anticancer drugs that activate *TP53* but other effectors contribute also.

## Open Questions

- Which processes and target genes activated by p53 are critical for the prevention of cancer?
- Loss of which p53-induced processes cooperate with loss of p53-induced apoptosis to cause cancer?
- Why do certain malignant as well as nontransformed cells undergo apoptosis upon *TP53* activation, whereas others do not die, but instead undergo cell cycle arrest and/or senescence?
- What are the differences in p53-induced apoptosis between nontransformed and malignant cells?
- How do the hot spot p53 mutant proteins inhibit wild-type p53-induced apoptosis in nascent neoplastic as well as malignant cells?

## Discovery of p53 and Discovery of Mutations in the *TP53* Gene in Human Cancer

The p53 protein (also called TP53) was discovered as a protein bound to the SV40 large T antigen in transformed cells (reviewed in Levine *et al.*[1] and Lane and Benchimol[2]).

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Received 29.5.17; revised 05.9.17; accepted 08.9.17; Edited by F Pentimalli; published online 17.11.17

Inadvertently, some of the first experiments in which p53 was overexpressed in cell lines used constructs encoding cancer-derived mutant *TP53*. Such enforced mutant p53 expression enhanced cell growth, and it was therefore concluded that p53 functions as an oncoprotein (reviewed in Levine *et al.*[1] and Lane and Benchimol[2]). Subsequent studies found, however, that enforced expression of wild-type (WT) *TP53* actually impaired the growth of transformed cells in culture, providing the first evidence that *TP53* can function as a tumour suppressor.[3,4] At about the same time, it was discovered that many sporadic human cancers of diverse origins carried mutations in *TP53*, usually a point mutation in one allele that permitted expression of mutant p53 protein (often at abnormally high levels; see below) accompanied by a deletion that removed the other allele, including adjoining regions.[5,6] Moreover, individuals with the Li–Fraumeni syndrome, who carry germline heterozygous mutations in *TP53*, usually develop multiple cancers over their lifetime, often from a young age.[7,8]

Most of the mutations in *TP53* detected in cancer cells are point mutations in the DNA-binding domain. These mutant p53 proteins are thought to be unable to regulate the transcription of WT p53 target genes (loss of function (LOF)) (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). Interestingly, many mutant p53 proteins are detected at high levels in malignant cells. Therefore, by forming mixed tetramers with WT p53, mutant p53 proteins can exert dominant negative effects (DNEs) that are likely to play critical roles early during transformation when nascent neoplastic cells still retain their WT *TP53* allele (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). In addition, certain p53 mutants have been reported to exert gain-of-function (GOF) effects by binding to and thereby modulating the functions of other tumour suppressors and transcriptional regulators (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). It remains unclear which of the LOF, the DNE or the GOF effects of mutant p53 are most important during the development and sustained growth of a cancer, and it appears likely that this may vary depending on both the cell of origin undergoing transformation and the nature of the cooperating oncogenic lesions that drive the neoplastic transformation of these cells.

## Control of *TP53* Activation and Cellular Responses Activated by p53

Unstressed, nontransformed cells contain very low (often undetectable) levels of WT p53 protein despite readily detectable mRNA expression.[11] The main reason for this is that p53 is targeted for proteasomal degradation by the E3 ligase, MDM2 (Figure 1).[12–14] In response to diverse stress stimuli, including activation of oncogenes, DNA damage or nutrient deprivation, the levels of p53 protein rise substantially because several signalling pathways that are activated in response to the aforementioned stressors converge upon the inhibition of MDM2, whereas some lead to modifications (e.g., acetylation, phosphorylation) in the p53 protein itself (Figure 1) (see reviews[9,10,15]). Upon activation, p53 binds as a homotetramer to specific sequences in the regulatory regions of its target genes (~500).[16–20] Studies using enforced expression or conditional activation (e.g., using temperature-sensitive mutants) of p53 in cell lines revealed that p53 can activate diverse cellular effector processes, including cell cycle arrest, cellular senescence, coordination of various DNA damage repair pathways, metabolic adaptation and apoptotic cell death (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). Gene expression studies and functional assays using gene-targeted mice soon identified genes that are essential for certain p53-activated cellular responses. For example, the cyclin-dependent kinase inhibitor (CDKi) p21 is critical for p53-mediated G1/S boundary cell cycle arrest and cell senescence[21] (although additional p53 target genes also play a role in the latter process). Moreover, several genes implicated in various DNA repair processes were found to be either direct targets of p53 or indirectly regulated by p53.[22]

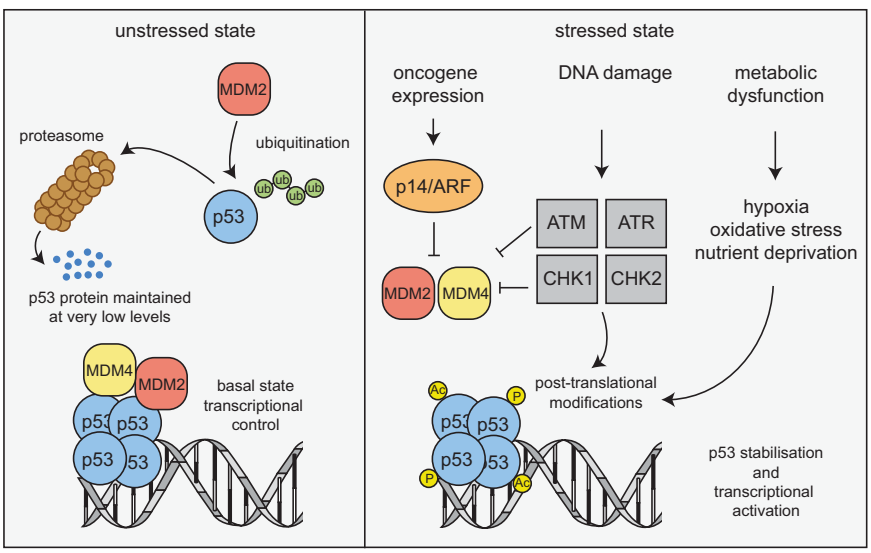


**Figure 1** **Regulation of p53 protein level and activity in unstressed *versus* stressed cells.** Models depicting the mechanisms that regulate p53 protein levels and activity in unstressed cells and in cells undergoing stress, for example, due to the activation of oncogenes or DNA lesions that they have sustained. (Ub, ubiquitin; P, phosphorylation; Ac, acetylation)

Finally, direct transcriptional induction of *Mdm2* by p53 was recognised as a major negative feedback loop in p53 signalling.[23] This is most spectacularly demonstrated by the finding that loss of MDM2 causes excess p53 activation, resulting in early embryonic lethality in mice, and that this lethality can be prevented by concomitant loss of *Trp53*.[24,25]

## P53-Mediated Induction of Apoptosis

The first clue that p53 can induce apoptotic cell death came from studies using a myeloid leukaemia cell line expressing a temperature-sensitive conditionally active mutant of p53 (i.e., at 37 °C this protein behaves as mutant p53 but at 32 °C it assumes WT p53 structure and function).[26] The observation that p53 can induce apoptosis was confirmed and extended by similar experiments in which a temperature-sensitive p53 or WT p53 was also enforcibly expressed in erythroleukaemia cells,[27] a colon cancer cell line[28] and a Burkitt lymphoma line.[29]

There are two distinct, although ultimately converging, pathways to apoptosis in mammalian cells:[30] the so-called BCL-2-regulated (also called intrinsic, mitochondrial or stress) pathway that is activated by stress conditions, such as cytokine deprivation, ER stress or DNA damage, and the so-called death receptor (also called extrinsic) pathway that is activated by ligation of members of the tumour necrosis factor receptor (TNFR) family bearing an intracellular death domain.[31–33] In the BCL-2-regulated apoptotic pathway, cell death is initiated by the transcriptional and/or post-transcriptional upregulation of the so-called pro-apoptotic BH3-only members of the BCL-2 protein family (BIM, PUMA, BID, BMF, BAD, BIK, NOXA, HRK). The BH3-only proteins bind and inhibit the pro-survival BCL-2 proteins (BCL-2, BCL-XL, MCL-1, BCL-W and A1/BFL1), thereby unleashing the cell death effectors BAX and BAK (the pro-apoptotic multi-BH domain members of the BCL-2 family that may also include BOK[34–37]). Certain BH3-only proteins were reported to also activate BAX/BAK directly (see reviews[32,38]). Activation of BAX/BAK causes mitochondrial outer membrane permeabilisation (MOMP), the point of no return in apoptosis signalling, with consequent activation of the cascade of aspartate-specific cysteine proteases (caspases; in this pathway initiated by caspase-9[39–41] and its activator APAF-1[42,43]) that dismantle the cell (Figure 2) (reviewed in Green[32]). Conversely, the death receptor pathway activates apoptosis by recruitment and activation of the pro-form of caspase-8 via the adaptors FADD, and in some cases also TRADD, at the ligated death receptors at the plasma membrane.[44,45] In so-called type 1 cells (e.g., thymocytes), such caspase-8 activation with consequent activation of the effector caspases (caspase-3 and -7) is sufficient for effective induction of apoptosis. In contrast, in the so-called type 2 cells (e.g., hepatocytes), efficient cell killing requires amplification of the caspase cascade by crossover activation of the BCL-2-regulated apoptotic pathway that is achieved by caspase-8-mediated proteolytic activation of the otherwise inert BH3-only protein BID.[46–50]

Studies using cell lines with enforced expression of WT p53 or temperature-sensitive p53 revealed that overexpression of anti-apoptotic BCL-2 could prevent p53-induced apoptosis.[51–53] Notably, the cells rescued from p53-induced death by expression of BCL-2 still underwent cell cycle arrest,[52] demonstrating that p53 was fully functional (i.e., BCL-2 does not directly block all p53 functions). Thus, p53 must induce cell cycle arrest and apoptosis through distinct pathways, and BCL-2 (or other pro-survival BCL-2 family members) inhibit p53-induced apoptosis at a downstream point in apoptosis signalling (Figure 2).

The caveat with the aforementioned experiments is that the levels of p53 used to induce apoptosis were abnormally high. Hence, it was not yet proven that p53 could induce apoptosis under physiological conditions, that is, when expressed at normal levels. This was established when it was shown that thymocytes and other lymphoid cell subsets from *Trp53* knockout mice are completely resistant to apoptosis induced by $\gamma$-radiation and treatment with chemotherapeutic drugs that induce DNA damage (e.g., etoposide, cyclophosphamide, cisplatin).[52,54,55]

## Discovery of the p53-Activated Inducers of the BCL-2-Regulated Apoptotic Pathway

The demonstration that p53-induced apoptosis can be blocked by BCL-2 overexpression launched the hunt to identify the p53-activated initiators of the cell death pathway that is regulated by BCL-2. Many candidates were identified by searching for genes that were upregulated in response to overexpression of p53 at highly nonphysiological levels. Perhaps predictably, most of these candidates have still not been proven to have roles in apoptosis. The two notable exceptions are *Puma/Bbc3* and *Noxa/Pmaip*: both of these genes are direct p53 targets and encode pro-apoptotic BH3-only proteins.[56–59] These genes are directly upregulated by p53 and their enforced expression causes rapid apoptosis in cell lines, whereas their knockdown protects cells against cytotoxic stimuli that trigger apoptosis in a p53-dependent manner.[56–59] Studies with gene-targeted mice revealed that PUMA and to a lesser extent NOXA are critical for p53-mediated apoptosis (e.g., apoptosis induced by $\gamma$-radiation or chemotherapeutic drugs that cause DNA damage) in a broad range of cell types, including lymphoid as well as myeloid cells, fibroblasts and skin keratinocytes, both in culture and *in vivo*.[60–63] Remarkably, thymocytes from *Puma/Noxa* double knockout mice are as resistant to $\gamma$-radiation *in vivo* as those from *Trp53* knockout mice (Figure 2).[64] Loss of PUMA generally affords many cell types with much greater protection against cytotoxic agents that trigger apoptosis via p53 activation (e.g., $\gamma$-radiation in lymphoid cells) than loss of NOXA.[61,64] Curiously, however, in certain cell types and under certain conditions the impact of loss of NOXA is more pronounced. For example, NOXA deficiency protects skin keratinocytes and fibroblasts more potently against UV radiation (a p53-dependent apoptotic stimulus) than loss of PUMA.[63] This suggests that the relative contributions of PUMA *versus* NOXA to the induction of apoptosis may vary depending on the cytotoxic insult, the nature of the responding cell or both. Of note, NOXA preferentially inhibits MCL-1 and (in contrast to other BH3-only proteins that can bind to MCL-1) promotes the degradation of this pro-survival protein.[65,66] The prominent role of NOXA in UV radiation-induced apoptosis

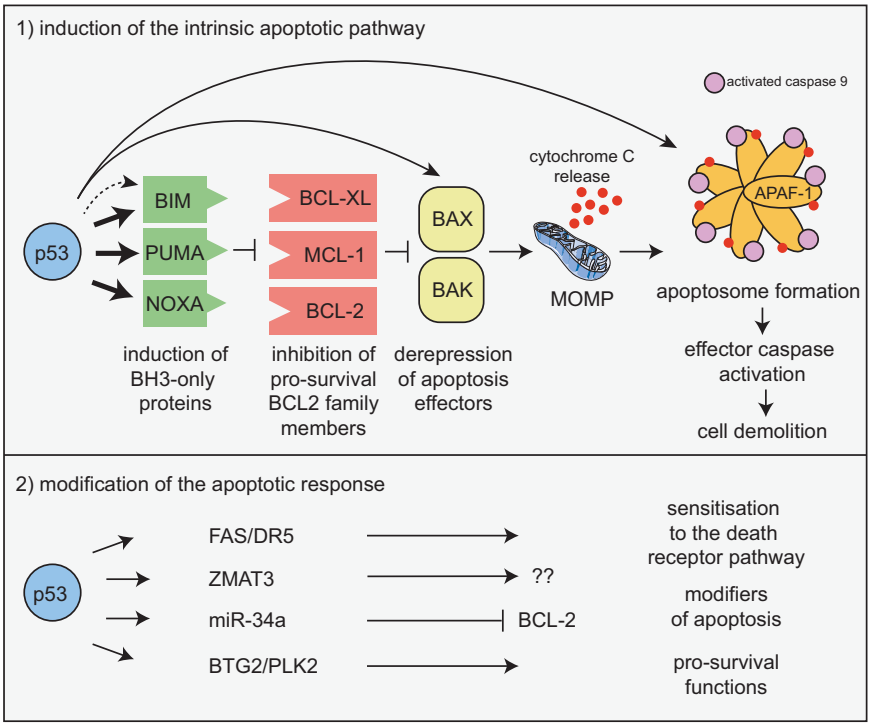


**Figure 2** Mechanisms of p53-induced apoptosis. Model depicting the mechanism by which activated p53 induces apoptosis through the BCL-2-regulated pathway. Fat arrows indicate p53-induced targets that are essential for p53-induced apoptosis. Thin arrows indicate p53-induced targets that are constituents of the BCL-2-regulated apoptotic pathway but are still expressed at levels sufficient for apoptosis induction in the complete absence of p53; that is, their induction by p53 may make the pathway work more efficiently, but this induction is not a *sine qua non* for p53-induced apoptosis, at least in haematopoietic cells. The broken arrow indicates that p53 may also activate BIM expression indirectly. The possible scenario that activation of targets that are not constituents of the apoptosis machinery *per se* can impact on apoptosis indirectly is also depicted

may therefore be explained if MCL-1 is the critical pro-survival protein protecting skin keratinocytes and fibroblasts against UV radiation.

Even though in non-transformed cells combined loss of PUMA and NOXA provides full protection (i.e., as potent as loss of *TP53* itself) against apoptosis induced by γ-radiation or chemotherapeutic drugs that induce DNA damage,[64] this is not the case in malignant lymphoma and leukaemia cells. For example, mouse *Eμ-Myc* lymphoma cells lacking both PUMA and NOXA are much less resistant to cyclophosphamide, etoposide or nutlin-3a (that activates p53 in a nongenotoxic manner by blocking its major inhibitor, the MDM2 E3 ubiquitin ligase[67]) than loss of p53 or overexpression of anti-apoptotic BCL-2.[68,69] Interestingly, additional loss of the BH3-only protein BIM[70,71] (i.e., combined loss of PUMA, NOXA plus BIM) provided as potent protection against nutlin-3a and etoposide as loss of p53 (Figure 2).[68,69] After treatment with etoposide or nutlin-3a, BIM expression was upregulated in *Eμ-Myc* lymphoma cells at a considerably later time compared with the induction of PUMA and NOXA.[68,69] Therefore, p53 may indirectly regulate BIM expression, perhaps through repression of a microRNA that regulates BIM. However, evidence for direct activation of *Bim* transcription by p53 has also been reported.[72–74] Collectively, these findings reveal that p53-induced apoptosis is likely to be more complex in malignant cancer cells compared with nontransformed cells (Figure 2).

It is also noteworthy that two additional constituents of the BCL-2-regulated apoptotic pathway, the pro-apoptotic effector BAX and APAF-1 (the scaffold protein for caspase-9 activation) have been convincingly shown to be transcriptionally regulated by p53.[75–77] However, p53 is not a *sine qua non* for BAX and APAF-1 expression. This is best demonstrated by the observations that *Trp53*-deficient haematopoietic cells express normal levels of BAX and APAF-1 and undergo apoptosis as readily as control (wild-type) cells after exposure to cytotoxic insults that induce BAX/BAK-dependent apoptosis,[78] involving APAF-1,[42,43,79] in a p53-independent manner (e.g., cytokine deprivation, treatment with glucocorticoids).[52] Accordingly, we conclude that p53-mediated transcriptional induction of BAX and APAF-1 is not essential for induction of apoptosis, at least in haematopoietic cells, but may serve in certain other cell types, as a mechanism to make the system work more efficiently or even allow this pathway to operate. This may relate to the observation that the levels of BAX, APAF-1 and other constituents of the apoptosis machinery are much lower in many tissues (e.g., heart, kidney, brain) in adult mice and humans compared with newborns. This may account for the reduced sensitivity to apoptotic stimuli of cells from these tissues in adults compared with newborns.[80]

## Mechanisms of Induction of Apoptosis by p53-Related Proteins

For many years p53 was thought to have no relatives, but then within a short time frame, two closely related proteins, called p63[81] and p73,[82] were discovered. P63 and p73 share

similarity with p53 in their DNA-binding and transactivation domains and it is therefore widely assumed that many recognised p53 target genes, and hence the cellular processes they control, can also be regulated by p63 and p73.[83] Some early studies showed that overexpression (although at clearly nonphysiological levels) of p63 or p73 can cause cell death with morphological and biochemical features of apoptosis.[81,84] The first demonstration that p63 can induce apoptosis under physiological conditions came from elegant studies in the mouse ovary. Even very low dose (0.5 Gy) γ-radiation kills all primordial follicles in 5-day-old female mice and renders these animals permanently infertile. This cell death is completely prevented by loss of p63, but loss of p53 has no protective effect.[85] Combined loss of PUMA and NOXA protected the primary follicles in the ovary from γ-radiation to the same extent as loss of p63.[86] This demonstrates that p63 induces apoptosis in the same way as p53. Remarkably, PUMA/NOXA double knockout and even PUMA single knockout females, when irradiated either as pups or as adults, retained normal fertility. Of note, among several hundred offspring of such irradiated *Puma*$^{-/-}$*Noxa*$^{-/-}$ or *Puma*$^{-/-}$ mothers, none were found to exhibit developmental abnormalities or cancer predisposition.[86] This means that primordial follicles that are protected from γ-radiation-induced apoptosis due to the absence of PUMA or PUMA plus NOXA must be able to repair their DNA lesions highly efficiently.

Of note, the nematode *C. elegans* homologue of p53/p63/p73 also has a function in DNA damage-induced killing of female germ cells and it induces a pathway to apoptosis that is initiated by the pro-apoptotic BH3-only protein, EGL-1, and can be inhibited by the pro-survival BCL-2 homologue, CED-9.[87] This demonstrates that DNA damage-induced, p63-induced apoptosis via the BCL-2-regulated pathway in female germ cells is evolutionarily highly conserved and is likely to play a critical role in safeguarding genomic stability in the germline.[88]

### Impact of p53 on the Death Receptor Apoptotic Pathway

Importantly, p53 can also regulate the expression of components of the extrinsic apoptotic pathway. The p53 can transcriptionally induce the genes encoding FAS (also called APO-1 and CD95) and possibly other genes encoding related death receptors.[89] Some studies have reported that cytotoxic drugs that cause activation of p53 (e.g., etoposide, cyclophosphamide) and γ-radiation can induce apoptosis in leukaemic cells through the death receptor pathway.[90] However, experiments using a panel of transgenic and gene knockout mice demonstrated beyond doubt that DNA damage-inducing anticancer drugs and γ-radiation kill normal as well as transformed cells by activating the BCL-2-regulated apoptotic pathway[91,92] in a p53-dependent manner. In striking contrast, complete loss of the death receptor apoptotic pathway (e.g., due to loss of function of caspase-8 or its activator FADD) does not protect these cells from these agents.[44,93] Although we would conclude that p53-mediated upregulation of death receptors is not essential for cell killing, it may serve to sensitise cells to so-called death ligands (e.g., FASL, TNF; the ligands for the death receptors FAS and TNFR1) expressed on neighbouring cells. This would allow for paracrine killing by cytotoxic T cells or NK cells and such a process may contribute to the effectiveness of cancer therapy in certain cancers.

### Indirect Effects of p53 on Apoptosis Signalling

To further add to the complexity of p53-mediated control of apoptosis, p53 also drives the expression of several genes whose functions do not lie within the two apoptotic pathways *per se* but may modulate the cellular response to cell death-inducing insults. For example, p53 drives expression of a number of microRNA species, including miR-34,[94] that is known to target the pro-survival *Bcl-2* gene.[95] Thus, p53-induced miR34a expression may sensitise cells to apoptotic stimuli by reducing the levels of BCL-2. Another well-characterised transcriptional target of TP53 is *Zmat3*[96] that has a poorly defined function but has been shown to impact on the response of cells to apoptotic stimuli.[97] Of note, p53 can also drive the expression of various genes that may serve pro-survival functions, such as *BTG2* and *PLK2*.[96] Thus, p53 signalling should not be viewed as exclusively inducing apoptosis, but in certain situations p53 activation may preferentially activate processes that enhance cell survival and cell growth. This may well be pertinent to tumour development and cancer therapy.

### Reported Roles of p53 in Other Forms of Cell Death

Although the role of p53 (and p63) in the induction of apoptosis is widely accepted, there are also reports that p53 can regulate additional non-apoptotic cell death pathways. For example, p53 was reported to open the mitochondrial permeability transition pore to thereby induce necrotic cell death.[98] Moreover, p53 has also been reported to sensitise cells to ferroptosis, a non-apoptotic form of cell death,[99] by repressing expression of SLC7A11, a key component of the cystine/glutamate antiporter.[100] However, the relevance of these processes to normal physiology (e.g., the death of nontransformed cells with DNA lesions) or cancer therapy-induced killing of tumour cells has not been established.

### Future Directions in Research on p53-Induced Cell Death

A 'holy grail' in research on p53 is to understand the mechanistic basis determining the strongly context-dependent functional output following p53 activation. For example, p53 activation by nutlin-3a results in apoptosis in some cells but cell cycle arrest and senescence in others (both malignant and nontransformed).[67] Moreover, restoration of wild-type p53 in cancers driven by loss of p53 (plus additional oncogenic lesions) causes apoptosis in lymphoma cells but cell senescence and cell cycle arrest in solid organ cancers.[101–104] Importantly, there is evidence that p53 activation and expression may occur without necessarily resulting in apoptosis or senescence, as has been observed in stem cells, where p53 activation may drive differentiation rather than exerting antiproliferative effects.[105] Finally, dramatic differences are seen in the sensitivity of different cell types to p53 activation. For example, cells within the gastrointestinal tract[106] and the haematopoietic system[54,55,91] are particularly vulnerable to p53-induced apoptosis that

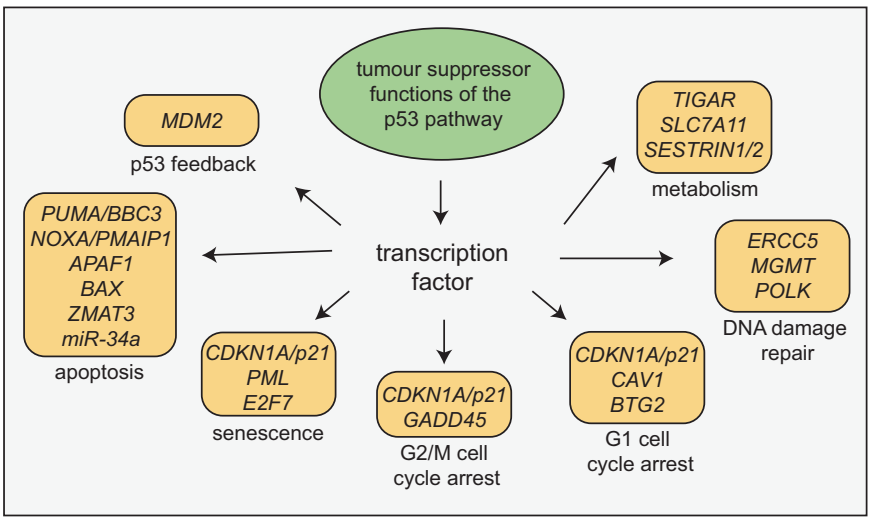


**Figure 3** P53 activates a multitude of cellular effector processes. Model showing a selected cellular effector processes that can be activated by p53. Some, but not all, of the p53 target genes that are critical for the execution of these processes are indicated. The challenge remains to understand of which of these processes are critical for tumour suppression in which setting; that is, cell of origin undergoing neoplastic transformation and nature of the oncogenic lesions driving their transformation

underlies their prominent involvement in toxicity associated with DNA damage-inducing chemotherapy. We can speculate on factors that may differentiate the p53 response: post-translational modifications on the p53 protein (e.g., acetylation, phosphorylation) that alter the function of p53 as a transcription factor, functional interaction between p53 and other transcription factors that may vary according to cell type, context-dependent regulation of specific target genes (e.g., altered regulation in the setting of oncogene expression), epigenetic regulation of p53 target genes (e.g., p53 regulation may vary depending on whether certain target genes are epigenetically silenced) as well as post-transcriptional and post-translational regulation of p53 target genes or their protein products (e.g., a role for p53-independent microRNA-mediated regulation). These are all fascinating possibilities that require much further investigation.

## The Role of p53-Induced Apoptosis in p53-Mediated Tumour Suppression

The finding that p53 can induce apoptosis led to the widely accepted assumption that out of all the cellular effector processes activated by p53 (Figure 3) this is the most critical, possibly even the sole, process by which p53 suppresses tumour development (reviewed in Vousden and Lane[9]). This made sense: after all, if cells during the early stages of neoplastic transformation are killed through p53-induced apoptosis, no fully transformed malignant cells will emerge from this clone. However, matters are not that simple. If apoptosis is the critical process for p53-mediated tumour suppression, it would be predicted that *Puma*$^{-/-}$*Noxa*$^{-/-}$ mice should be as prone to spontaneous or oncogene-driven tumour development as *Trp53*$^{-/-}$ mice themselves, as combined loss of PUMA and NOXA abrogates p53-induced apoptosis in many (possibly all) cell types.[64] Contrary to this prediction, no spontaneous tumour development was observed in a large cohort of *Puma*$^{-/-}$*Noxa*$^{-/-}$ mice that were monitored over a long time period.[64,107] Induction of G1/S boundary cell cycle arrest and cell senescence are also thought to be processes that could be critical for p53 tumour suppression (Figure 3). The CDK inhibitor p21 is essential for cell cycle arrest and also a major contributor to cellular senescence.[108] It is therefore remarkable that mice with mutations in *Trp53* that impair its ability to transcriptionally induce *Puma*, *Noxa* and *p21* and even mice completely deficient for these genes (i.e., *Puma*$^{-/-}$*Noxa*$^{-/-}$*p21*$^{-/-}$ mice) do not spontaneously develop any tumours (Figure 4).[107,109,110] Notably, the cells from all of these mutant mice are unable to undergo apoptosis, cell cycle arrest or senescence upon p53 activation. This demonstrates that p53 is capable of preventing spontaneous development of cancer in the complete absence of its ability to induce apoptosis, G1/S cell cycle arrest and cell senescence. Moreover, combined loss of PUMA and p21 or mutations in the two transactivation domains of p53 that are critical for the transcriptional induction of *Puma*, *Noxa* and *p21* accelerate c-MYC-driven lymphoma development and mutant RAS-driven lung cancer development to a much lesser extent than loss of p53 (Figure 4).[111,112] The observation that loss of PUMA (or combined loss of PUMA and NOXA or PUMA and p21) can accelerate c-MYC-driven lymphoma development[112,113] does, however, show that p53-mediated apoptosis (via PUMA and NOXA) can exert significant tumour suppressive function. The relative importance of the induction of apoptosis to overall TP53-mediated tumour suppression is likely to vary depending on the type of cell undergoing neoplastic transformation and the nature of the oncogenic lesions that drive tumorigenesis.

Important insight into the mechanisms that are critical for TP53-mediated tumour suppression also came from experiments using an elegant genetically engineered mouse model in which p53 activity can be turned on or off at will.[114] These studies used the γ-radiation-induced thymic T-cell lymphoma model and showed that the presence of p53 during the acute response to DNA damage (characterised by extensive apoptosis of many haematopoietic

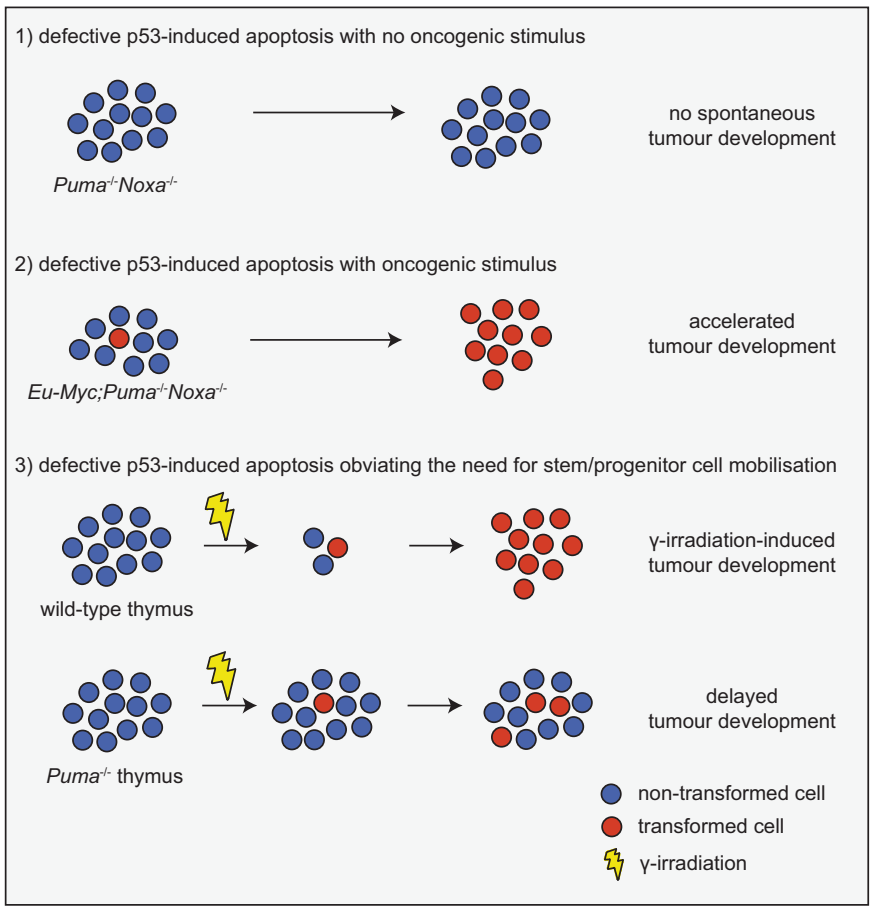


**Figure 4** Impact of p53-induced apoptosis on tumour development. Models showing the impact of loss of p53-induced apoptosis on tumour development in different cancer models/settings

cell types) was not needed for tumour suppression. Instead, p53 function was required during the later recovery phase. Accordingly, p53-mediated tumour suppression was not activated through the DNA damage sensing process but instead through p19/ARF[114] that is activated in response to the expression of oncogenes (e.g., deregulated c-MYC expression). P19/ARF inhibits MDM2, the major negative regulator of p53. This indicates that in this model, thymic T-cell lymphomas emerge from the stem/progenitor cells that have sustained potentially oncogenic DNA lesions and are mobilised to replenish the haematopoietic system that was depleted by γ-radiation. The very rapid proliferation of progenitor cells bearing oncogenic lesions, which is likely to also be the basis of the development of many other cancers, may facilitate the acquisition of mutations in oncogenes or suppressor genes that further drive neoplastic transformation. Some initially paradoxical findings are consistent with this. In the aforementioned γ-radiation-induced thymic T-cell lymphoma development mouse model, loss of pro-apoptotic PUMA or overexpression of pro-survival BCL-2 completely prevented tumour development (Figure 4).[115,116] The explanation for this is that loss of pro-apoptotic PUMA or pro-survival BCL-2 overexpression prevented the γ-radiation-induced death of diverse leukocyte populations and this obviated the need for mobilisation and excess proliferation of haematopoietic stem/progenitor cells in the bone marrow that are thought to be the lymphoma/leukaemia-initiating cancer stem cells in this model.[117] This concept may extend to many additional cancers, including epithelial ones, as overexpression of pro-survival BCL-2 paradoxically delays liver cancer development,[118] whereas, conversely, loss of pro-survival MCL-1 promotes its development.[119]

## Impact of Mutant p53 on WT p53-Induced Apoptosis and on WT p53-Mediated Tumour Suppression

The p53 is unusual among tumour suppressors. In tumours that are driven by mutations in the tumour suppressors PTEN or RB, the expression of these proteins is usually lost completely because of the nature of the mutations selected for during tumorigenesis (reviewed in Knudsen and Knudsen[120] and Yin and Shen[121]). In contrast, many tumours that are driven by mutations in *TP53* express high levels of the mutant p53 protein and show a loss of the other allele of *TP53* (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). In fact, the high-level mutant p53 protein expression can be used as a diagnostic marker for cancers driven by mutations in the *TP53* gene (reviewed in Liu and Gelmann[122]). The highly expressed mutant p53 protein can promote tumorigenesis in three ways: (1) loss of the WT p53 activity, (2) DNEs over the WT p53 protein early in transformation

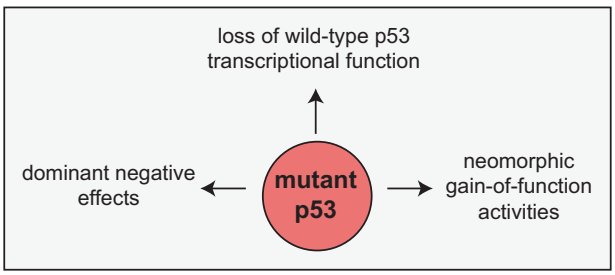


**Figure 5** Impact of mutant p53 proteins on tumour development. Model depicting the mechanisms by which mutant p53 proteins, which are frequently highly overexpressed (compared with the wild-type p53 protein), on tumour development

before loss of the WT *TP53* allele, through the formation of mixed tetramers containing both wild-type and mutant p53 proteins and (3) *de novo* GOFs that are mediated through interactions of mutant p53 protein with other transcription factors and tumour suppressors (e.g., p63, p73) (Figure 5) (reviewed in Vousden and Lane[9] and Freed-Pastor and Prives[10]). As early in transformation, mutant p53 levels are often variable and low,[123] it appears likely that the GOF effects may only come into play at a late stage of transformation. It is obvious how loss of the WT p53 function contributes to the tumour promoting action of mutant p53 but the mechanisms by which the DNE and GOF effects of mutant p53 drive tumour development are not established. A detailed understanding of the role of these processes in the development as well as the sustained growth of tumours is anticipated to identify targetable vulnerabilities for the development of novel cancer therapies.

## Conclusions

In conclusion, *TP53* is arguably one of the most important (if not the most important) genes in human cancer. It appears that p53 is critical for tumour suppression not during the acute response to cellular stress, such as DNA damage (e.g. caused by *γ*-radiation and reliant on the CHK1/CHK2 kinases) that is characterised by extensive apoptosis, but for the killing or silencing of the cancer-initiating (often thought to be stem/progenitor) cells that have acquired oncogenic lesions that drive the neoplastic transformation. The p53 transcription factor activates several effector processes, apoptotic cell death being one of them. Contrary to long-held perceptions, loss of p53-induced apoptosis (via PUMA and NOXA) even when combined with additional loss of induction of G1/S boundary cell cycle arrest and cell senescence (via p21) does not lead to spontaneous tumour development, in striking contrast to loss or mutation of p53. Thus, additional cellular processes, either by themselves or in a manner overlapping with the aforementioned mechanisms, must account for the potent tumour suppressive action of p53. Identifying these signalling pathways and how they are integrated will provide exciting research opportunities for several years. A better understanding of p53-mediated apoptosis and p53-mediated tumour suppression more generally holds promise for various potential clinical applications. These include improving the efficacy of anticancer therapies that rely on p53 activation, reducing the toxicities associated with chemotherapy and radiotherapy and improving haematopoietic stem cell transplant conditioning regimens and perhaps also in nonmalignant settings where abnormal induction of cell death pathways (that may in part be driven by p53) contributes to tissue damage, such as myocardial infarction and cerebral ischaemia.

## Conflict of Interest

The authors declare no conflict of interest.

**Acknowledgements**. We thank all of our current and past colleagues at The Walter and Eliza Hall Institute and elsewhere. Our work is supported by the Australian National Health and Medical Research Council (Program Grant 1016701 to AS and Fellowship 1020136 1020363 to AS), the Leukemia and Lymphoma Society of America (LLS SCOR 7001-13 to AS), the Cancer Council of Victoria (1052309 to AS) as well as operational infrastructure grants through the Australian Government Independent Research Institute Infrastructure Support Scheme (9000220) and the Victorian State Government Operational Infrastructure Support Program.

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