Tumour suppression by p53: a role for the DNA damage response?

David W. Meek

Abstract | Loss of p53 function occurs during the development of most, if not all, tumour types. This paves the way for genomic instability, tumour-associated changes in metabolism, insensitivity to apoptotic signals, invasiveness and motility. However, the nature of the causal link between early tumorigenic events and the induction of the p53-mediated checkpoints that constitute a barrier to tumour progression remains uncertain. This Review considers the role of the DNA damage response, which is activated during the early stages of tumour development, in mobilizing the tumour suppression function of p53. The relationship between these events and oncogene-induced p53 activation through the ARF pathway is also discussed.

Innate tumour suppression
Cellular mechanisms that detect and eliminate incipient tumour cells.

Senescence
The irreversible withdrawal of cells from the proliferative cycle into a terminally non-proliferative state. When this state is promoted by oncogenes, it is often termed oncogene-induced senescence.

Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, UK.
e-mail: d.w.meek@dundee.ac.uk
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p53 is a short-lived transcription factor that has been most extensively studied in its capacity to mediate innate tumour suppression[1–3]. In animal models, loss or mutation of p53 predisposes to a range of spontaneous and induced tumours[4–7], highlighting its protective role as a barrier to tumour development. This barrier is disabled during the pathogenesis of most, if not all, human cancers, either through sporadic *TP53* mutations[8] or through alterations in genes encoding crucial regulators of p53 (REFS 9–12). The evidence to date suggests that p53 does not influence the rate of tumour initiation or mutation but prevents the malignant progression of tumour cells (for example, see REFS 13–15). In support of such a role, restoration of p53 expression can promote tumour regression and clearance *in vivo*[16,17].

p53 induction is pivotal to innate tumour suppression and can lead to different biological outcomes, depending on the context. For example, the continued expression of dominant oncogenes *in vivo* can lead to the irreversible withdrawal of cells from the proliferative cycle into a terminal state termed oncogene-induced senescence[13,18,19]. Such a mechanism has been observed to protect against prostate tumour development in mice[13] and to occur in human fibroblasts and mammary epithelial cells[20,21]. Moreover, senescence may be assisted by autophagy, another p53-mediated event in which cellular components undergo controlled lysosomal degradation[22,23]. p53 can also suppress tumour development by initiating apoptosis, the major form of programmed cell death, which involves the ordered and rapid destruction of the cell in the absence of an inflammatory response[24,25]. For example, p53-mediated apoptosis is thought to protect against the development of lymphoma[24]. Key factors that determine the outcome of p53 induction, at least in cultured cells, are: the type and intensity of stress, the cell type and the genetic background[26,27]. Crosstalk with other pathways, such as survival signalling[28] or the retinoblastoma pathway[29,30], can tip the balance between growth arrest or apoptosis. Other mechanisms, such as the prevention of metastasis, are likely to contribute to tumour suppression[31]. However, given the many hundreds of genes that are thought to be regulated by p53 (REFS 32–34) and the many varied biological functions to which it is now known to contribute[35–45], we do not have a complete picture of how tumour suppression is mediated mechanistically in all instances. The common principle is the protection of the organism either by maintaining the integrity of the cell and its genome or by preventing the proliferation of incipient cancer cells.

Fundamental to the initiation of most tumours is DNA damage, which, if inaccurately or inappropriately repaired, can lead to the activation, deregulation and/or overexpression of oncogenes that drive cell proliferation and/or survival in the absence of physiological stimuli[1]. How p53 is alerted to these changes is still uncertain but accumulating evidence suggests that, at least in some cases, the DNA damage response pathways might mediate tumour suppression by activating p53 in response to the persistent DNA damage and genomic instability that accompanies tumour progression. This Review will examine the evidence supporting this model and consider how it might fit with the long-accepted view that the ARF tumour

At a glance

- The p53 pathway mediates innate tumour suppression in cells that have sustained genetic changes that drive tumour initiation and progression. p53 functions principally as a transcription factor that alters gene expression in favour of biological events, such as senescence or apoptosis, and the outcome of these events blocks the proliferation of or eliminates the tumour cell.
- Early-stage human tumours show evidence of DNA damage, suggesting that this could be the signal by which p53 recognizes the incipient tumour. This notion is supported by the finding that oncogenes can induce DNA damage in cultured cells.
- By contrast, some animal models show that induction of p53 in response to DNA damage has little protective effect against tumour formation. The induction of p53 by the ARF tumour suppressor pathway in these animals seems to be crucial for mediating p53-dependent tumour suppression.
- p53 knock-in mice lacking key p53 phosphorylation sites that are modified through the DNA damage pathways but not through the ARF pathway show increased tumour susceptibility, but in a limited number of tissues. These mice provide evidence to support the idea that DNA damage pathways can, at least partially, influence tumour suppressor function.

suppressor pathway is principally responsible for driving p53-mediated tumour suppression independently of DNA damage.

The induction and activation of p53

p53 is stabilized and activated in response to a range of cellular stresses, including DNA damage and hyperproliferation[3,46]. Interestingly, the p53 pathway is extremely sensitive to a very small number of DNA strand breaks or single-stranded gaps[47], a factor which could be important in the early detection of DNA lesions in tumours. Once induced, p53 regulates the expression of a wide range of genes, leading to the biological outcomes of repair, growth arrest or apoptosis[32]. The crucial event in the induction of the p53 pathway, regardless of the activating stimulus, is the uncoupling of p53 from its key negative regulators, principally MDM2 and MDM4, which leads to the accumulation of stable active p53 (FIG. 1). Small molecules that interfere with the p53–MDM2 interaction are sufficient to robustly induce p53 in the absence of a stress stimulus, underscoring the central importance of this event[48–50]. Physiologically, however, different stresses target this interaction through different and often overlapping mechanisms, and might have additional context-dependent regulatory features.

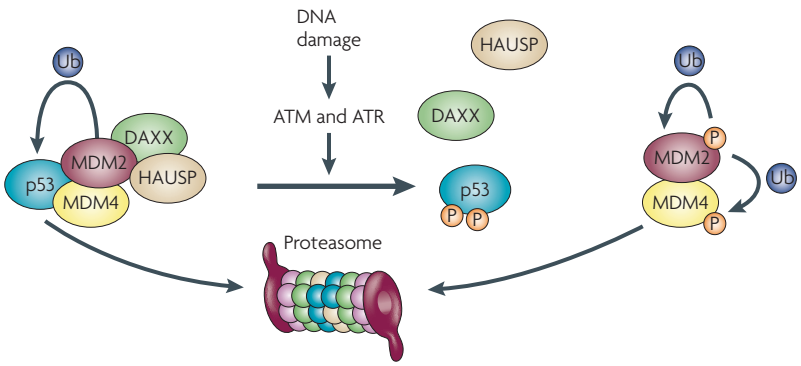

Figure 1 | **Mechanism of p53 turnover.** p53 is normally kept at low levels through ubiquitylation and proteasomal degradation, which are mediated by several E3 ubiquitin ligases, but mainly by MDM2 (REFS 115–118). p53 stimulates the expression of MDM2 and thus operates in a negative feedback loop with its principal inhibitor[119]. p53 is also restrained by other regulators, such as MDM4 (also known as MDMX), which inhibits p53-mediated transcription[120,121]. In addition to ubiquitylating p53, MDM2 mediates the ubiquitylation of both itself and MDM4 (REFS 122–125). p53 turnover involves the actions of additional proteins, including herpesvirus-associated ubiquitin-specific protease (HAUSP; also known as USP7) and the adaptor protein DAXX[46,51,126–130]. HAUSP can deubiquitylate MDM2 and p53, both of which compete for the same binding site[131]. Under normal, unstressed conditions DAXX acts as an adaptor that interacts simultaneously with HAUSP and MDM2 and directs the activity of HAUSP principally towards MDM2 and MDM4 (REF 129). This minimizes MDM2 auto-ubiquitylation and promotes p53 ubiqutylation and turnover. The induction of p53 in response to DNA strand breaks is mediated by the ataxia–telangiectasia mutated (ATM) and ataxia–telangiectasia and Rad3-related (ATR) protein kinases (see FIG. 2), and leads to disruption of the MDM2–DAXX–HAUSP complex[129] and the rapid destruction of MDM2 and MDM4 (REFS 51–53). P, phosphate; Ub, ubiquitin.

The induction of p53 in response to DNA damage is coordinated by the ataxia–telangiectasia mutated (ATM) and ataxia–telangiectasia and Rad3-related (ATR) protein kinases, which mediate the rapid destruction of MDM2 and MDM4 (REFS 51–53) (FIG. 2). ATM and ATR are members of the phosphatidylinositol-3 kinase-like kinase family and coordinate a complex signalling network in response to various forms of DNA damage[54]. ATM plays a crucial part in the immediate response to double-strand breaks by coordinating the activation and execution of checkpoint pathways and repair pathways. Consistent with this role, cells from patients with ataxia–telangiectasia lack functional ATM activity and show defective double-strand break repair, defective cell cycle checkpoint control and radiation sensitivity. The response to other forms of DNA damage, such as replication stress and DNA crosslinking, is coordinated mainly by ATR. However, there is substantial interplay between the pathways governed by these molecules, and they share downstream targets in the repair and checkpoint pathways, including the transducer kinases CHK1 and CHK2 and components of the p53 pathway.

DNA damage signalling mediated by ATM and ATR induces a range of differential posttranslational modifications of p53 that can tailor the p53 response in an appropriate and proportionate manner according to the nature of the damage and intensity of the stress (these modifications have been reviewed in depth elsewhere[26,27,55]). The roles of some of these modifications and their relationship to tumour suppression have been investigated through the generation of p53 knock-in mice that carry alanine substitutions at major sites of posttranslational modification in p53 (see below). Serine 15, threonine 18 and S20 are key phosphorylation sites, and are involved not only in stimulating the interaction of p53 with the transcriptional machinery, but can also inhibit the interaction of p53 with MDM2 (FIG. 3). Phosphorylation of p53 might therefore contribute to p53 induction and could be important in the detection of developing tumour cells, possibly in a context-dependent manner.

p53 is also induced through the ARF tumour suppressor pathway[12,56], which has been considered to function independently of the DNA damage pathway (FIG. 4). ARF is an important inhibitor of MDM2 that is normally present at low levels[57–62]. Induction of ARF by

Checkpoint pathway
A signal transduction pathway that is activated by stresses such as DNA damage, leading to the halting of a crucial biological process, such as DNA replication or cell division.

Ataxia–telangiectasia
An inherited disease in which the absence of a functional ATM protein kinase gives rise to many disabilities, including a substantially increased risk of developing cancer.

Focus
A sub-nuclear location at which DNA damage has occurred and to which DNA damage-associated proteins are specifically recruited.

Fragile site
A chromosomal region that is highly susceptible to double-strand breaks under conditions of replication stress.

activated oncogenes (which does not seem to involve substantial increases in the levels of p53 phosphorylation[57,63–65]) has classically been considered to be the mechanism by which p53 responds to abnormally sustained proliferation. Mice lacking ARF are highly prone to tumour development[66], underscoring the role of ARF in tumour suppression. As with p53, spontaneous inactivation of *Cdkn2a*^{ARF} through deletion, mutation or epigenetic silencing is a common feature during tumour progression that eliminates its protective function, at least in mice[67–69]. In humans, however, mutations at the *CDKN2A* locus (which encodes INK4A, also known as p16, and ARF in overlapping reading frames) target mainly INK4A and rarely target ARF[70,71] suggesting that ARF may be less crucial to tumour suppression in humans.

p53 is a member of a family of proteins that includes p63 and p73, both of which can interact with the p53 pathway in addition to their own functions. p63 and p73 may therefore contribute to tumour suppression through crosstalk with p53, although growing evidence raises the possibility that they may also influence tumour development independently of p53 (BOX 1).

The DNA damage response in tumour suppression

In addition to inducing ARF, recent studies have indicated that the increased expression of oncogenes can induce the p53 pathway through ARF-independent mechanisms that require ATM and ATR and involve the phosphorylation of p53 at S15 (REFS 72,73). These observations blur the boundaries between the classical models of p53 activation and raise the possibility that DNA damage checkpoints may respond to the effects of oncogene activation during the early stages of tumour progression. Evidence for such a role comes from the analyses of numerous early-stage human tumours[20,21,72,74,75].

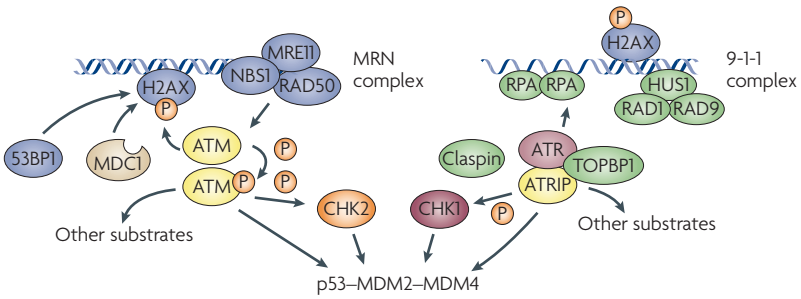

Figure 2 | **DNA damage response signalling pathways target p53 and its key regulators.** Double-strand breaks are recognized by the MRN (MRE11–RAD50–NBS1) complex and lead to the activation of ataxia–telangiectasia mutated (ATM) and subsequent amplification of the response through the recruitment of other DNA damage response proteins. Activated ATM phosphorylates a range of substrates, including p53, MDM2, MDM4 and CHK2, which in turn phosphorylates p53 and other substrates. Other forms of DNA damage lead to the generation of single-stranded regions that become coated with replication protein A (RPA). This attracts the ataxia–telangiectasia and Rad3-related (ATR)–ATR-interacting protein (ATRIP) complex, which phosphorylates complexes, such as the 9-1-1 complex (comprising RAD9, RAD1 and HUS1), that feed forward and further stimulate ATR. ATR associates with claspin and phosphorylates downstream substrates (some of which overlap with ATM substrates), including p53, MDM2 and CHK1 (which, in a similar manner to CHK2, also phosphorylates p53). 53BP1, p53-binding protein 1; MDC1, mediator of DNA damage checkpoint 1; P, phosphate; TOPBP1, DNA topoisomerase 2-binding protein 1.

These studies show that cells in the earliest precursor lesions — which show no signs of chromosomal instability or mutation of *TP53* — often show constitutive activation of DNA damage signalling pathways as measured by the presence of activated forms of ATM, CHK2, phosphorylated p53, phosphorylated histone H2AX and foci containing DNA damage-associated proteins, such as p53-binding protein 1 (53BP1). Notably, these markers are not detectable even in highly proliferative normal tissues, such as the intestinal epithelium, suggesting that incipient tumour-driven but not normal cell cycles give rise to DNA damage.

The activation of checkpoint proteins is also observed in cultured cells following a controlled increase in the expression of oncogenes that deregulate DNA replication[72]. In addition, the induction of hyperplasia in human skin xenografts in nude mice leads to the appearance of DNA damage response markers, notably in the absence of telomere erosion but coincident with genomic instability at common fragile sites[75]. These various studies support the idea that DNA damage-induced checkpoints might act as a barrier to sustained proliferation by inducing apoptosis or senescence in early-stage tumour cells[76,77]. Notably, the activation of DNA damage-associated proteins in tumours persists in more developed and malignant tumours but, in many cases, advanced tumours gradually lose the expression of these proteins. This might reflect a selective pressure to eliminate components of the DNA damage response system, including p53. These observations are also consistent with the idea that acquired defects in the DNA damage response may underlie the genetic instability seen in tumours and increase the mutation rate, thereby accelerating cancer progression.

The proposed explanation for the occurrence of DNA damage in developing tumours is that oncogenes, by driving aberrant proliferation and the untimely activation of cyclin-dependent kinases, lead to DNA replication stress that might result from impaired or inappropriately activated origins of replication. In support of this model, Bartkova and colleagues[20] showed that the increased expression of cyclin E in human cultured cells induced stalled and prematurely terminated replication forks. They also observed phosphorylated H2AX, a marker of DNA damage, at sites of DNA replication, as indicated by the presence of proliferating cell nuclear antigen (PCNA). The expression of oncogenic *HRAS* in diploid human fibroblasts also leads to foci that contain numerous DNA damage-associated proteins together with markers of stalled or impaired replication[21]. In addition, cells that are blocked from entering S phase of the cell cycle do not show markers of DNA damage, confirming that this DNA damage is a replication-associated phenomenon[20,21].

The model also predicts that the DNA damage response initiates oncogene-induced senescence, acting as a barrier to tumour progression. In support of these ideas, Bartkova and colleagues[20] have shown that various oncogenes induce a senescence phenotype that is suppressed following small-interfering RNA (siRNA)-mediated elimination of ATM but not

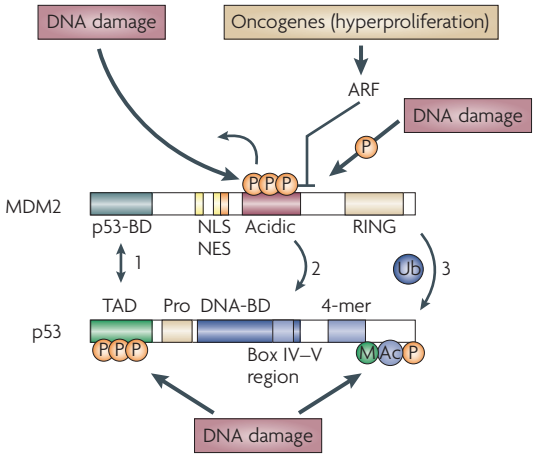

Figure 3 | **Molecular events mediating p53 induction** Under unstressed conditions, MDM2 and p53 associate through their amino-termini (step 1), which leads to the acidic domain of MDM2 making contact with the Box IV–V region in p53 (step 2). This allows the subsequent ubiquitylation of p53 (REFS 132–135) (step 3). DNA damage stimulates ataxia–telangiectasia mutated (ATM)-dependent and ataxia–telangiectasia and Rad3-related (ATR)-dependent phosphorylation of MDM2, leading to MDM2 degradation[51–53]. In addition, ATM and ATR directly phosphorylate serine 15 of p53 (REFS 136–139), protein kinase CK1 phosphorylates threonine 18 (using phosphorylated S15 as a priming site)[140,141] and S20 is phosphorylated by CHK2 (which is activated by ATM)[142–144]. Many other modifications of p53 are dependent either indirectly upon ATM or occur sequentially following the phosphorylation of S15 (REFS 141,145–147). Biochemical analyses and studies using cultured cells indicate that the phosphorylation of these p53 sites stimulates the recruitment of key transcriptional proteins, such as p300 and CBP[148–155], leading to the acetylation of several key lysine residues in the carboxy-terminus of p53 that are normally targets for ubiquitylation: this process is thought to help stabilize p53 (REFS 147,156). Phosphorylation of T18 and S20 also inhibit the association of p53 with MDM2 (REFS 140,141,157–160). Several stresses target the crucial acidic domain of MDM2: DNA damage-mediated hypophosphorylation inhibits MDM2-mediated p53 degradation[161,162], and interaction with the ARF tumour suppressor inhibits MDM2 function (see FIG. 4 and REFS 163,164). Ac, acetyl; DNA-BD, DNA-binding domain; M, methyl; NES, nuclear export signal; NLS, nuclear localization signal; P, phosphate; p53BD, p53-binding domain; Pro, proline-rich region; TAD, transactivation domain.

following knockdown of *CDKN2A*^{INK4A}. The coincidence of activated DNA damage checkpoint proteins with several senescence markers in colon adenomas and early urinary bladder lesions reinforces the idea that DNA damage-dependent senescence might block malignant progression. In a related study, Di Micco and colleagues[21] showed that expression of oncogenic *HRAS* in human diploid fibroblasts induces senescence-associated DNA damage foci that contain a range of activated forms of DNA damage- and checkpoint-associated proteins. Moreover, chemically induced *HRAS*-dependent early benign skin papillomas in mice also show evidence of DNA damage and senescence, which suggests there is a link between these events. The induced senescence observed in human fibroblasts is independent of telomere erosion but does not occur in cells that lack functional CHK2, ATM or p53, again suggesting the existence of a causal link between oncogene-induced DNA damage and senescence. Consistent with the idea that the DNA damage response forms a barrier to tumour progression, elimination of *Chek2* allows immortalized mouse embryonic fibroblasts (MEFs) that express an oncogenic form of *HRAS* to form tumours in immunocompromised mice[21]. Notably, *Chek2* knockout mice do not succumb to spontaneous tumour formation but they have a significantly increased susceptibility to carcinogen-induced skin tumour formation[78], suggesting that CHK2, and by implication the DNA damage response, can contribute to the suppression of at least some types of tumour.

Evidence from animal models for dependence on the DNA damage pathways. The contribution of DNA damage signalling to p53-mediated tumour suppression could theoretically be addressed by using an appropriate mouse model in which a key component(s) of the pathway is genetically eliminated. Targeting ATM (or its downstream kinases CHK1 and CHK2) would be unsatisfactory for addressing this issue given the multitude of different substrates of these kinases in the DNA repair and checkpoint pathways that would be affected. In addition, p53 activation also requires the input of ATR so, although knocking out ATM will impair the p53 response, it does not eliminate p53 induction by DNA damage. A different approach for uncoupling p53 from the DNA damage response would be to eliminate the targets of DNA damage signalling in the p53 pathway in a manner that does not interfere with ARF signalling. One way of achieving this would be to incorporate mutations in p53 at crucial sites of DNA damage-induced modification that do not play a part in the activation of p53 by ARF. Phosphorylation sites merit attention because of their specific association with DNA damage pathways, whereas acetylation events seem to be common to both the DNA damage- and ARF-mediated pathways[79,80]. If the mobilization of p53 by DNA damage has a crucial role in tumour suppression, one might expect abrogation of tumour suppressor activity following the elimination of one or more of these modifications in animal models.

To date, only a few of these p53 modification sites have been examined in mice by substituting them with amino acids that cannot be modified. Mice that carry homozygous alanine substitutions of S18 and S23 (which are equivalent to S15 and S20, respectively, in human p53) seem normal but succumb to various spontaneous late-onset tumours, principally B-cell lymphomas, and show increased hyperplasia in certain tissues[81–84]. The analysis of S18A–S23A double-substitution mice suggests that these two phosphorylation sites, which are modified simultaneously following DNA damage, act in a synergistic manner. Nevertheless, these mice still develop late-onset tumours and show an altered

tumour spectrum compared with *Trp53*$^{-/-}$ mice[85]. Mice expressing p53 substituted at the UV-responsive S389 (human S392) phosphorylation site also show selective tumour susceptibility but, in this case, to UV-induced skin tumours[86] and bladder tumours induced by agents that generate DNA damage by covalently attaching large chemical groups to the DNA[87]. These observations support the idea that the DNA damage pathways responsible for modifying these phosphorylation sites have a positive effect on tumour suppression. However, the long latency period for tumour development suggests that posttranslational modifications of p53 itself (as opposed to the DNA damage pathways, which also target other components of p53 signalling) play only a contributory part in tumour suppression.

One striking feature to emerge from the study of these mice is that the contributions of the phosphorylation sites to p53 function are cell-type specific. This might reflect a context-dependent role of p53 in tumour suppression. MEFs from the animals with S18A and/or S23A substitutions show no differences in their growth rates or their ability to induce p53 following DNA damage[81–83,85], yet in other cell types, such as splenocytes and thymocytes, DNA damage-induced apoptosis is impaired[82,83] or abolished[85]. Moreover, the S23A and S18A–S23A mice fail to induce p53 effectively in splenocytes and thymocytes through the DNA damage response pathways, and the S18A mice show significantly reduced expression of p53 upregulated modulator of apoptosis (PUMA; also known as BBC3)[81], which is the crucial mediator of apoptosis in haematopoietic cells[88]. In addition, expression of the p53 S18A–S23A double mutant, but not wild-type p53, can rescue the embryonic lethality of *Xrcc4* deficiency (a mutation in *Xrcc4* results in extensive DNA damage owing to failure of the non-homologous end joining pathway), in which the mice undergo massive p53-dependent neuronal apoptosis. From these studies, it seems that, at least in certain cell types, p53-mediated apoptosis is tightly coupled to DNA damage through the S18 and S23 phosphorylation sites, possibly through their ability to induce p53 by interrupting the p53–MDM2 interaction (FIG. 3). It is therefore possible that, for example, in the haematopoietic system, p53 might be alerted through a DNA damage-independent route (such as the ARF pathway[67]), but in a manner that is influenced by, or requires a contribution from, the DNA damage response pathways. Similarly, cells from the S389A mice show impaired UV-mediated p53 induction and apoptosis but show no differences in oncogene-induced apoptosis or ionizing radiation-induced arrest in the G1 phase of the cell cycle[89]. These analyses also suggest that UV-responsive DNA damage pathways potentially contribute to tumour suppression but, again, their influence is subtle and context-dependent.

Evidence from animal models for dependence on ARF. The studies described above provide a strong case for the DNA damage checkpoint pathways in mediating oncogene-induced tumour suppression. However, this issue is far from settled, and two groups have provided equally compelling evidence for the dependence of tumour suppression on the ARF pathway independently of the DNA damage response[90,91]. Using a knock-in mouse that expresses a wild-type p53–oestrogen receptor fusion protein (p53ER^{TAM}) which is dependent upon 4-hydroxytamoxifen (4-OHT) for activity, Christophorou and colleagues[90] showed that the restoration of p53 function six days before administering a single whole-body dose of ionizing radiation led to widespread p53-dependent cell death in radiosensitive tissues in a manner similar to that observed in wild-type mice. However, although there was a substantial p53 response, it provided no protection against the subsequent onset of lymphoma development. By contrast, when p53 function was absent during irradiation but was restored for a six-day period eight days after administering the radiation (that is, at a time when the observable radiation response had acquiesced but, presumably, as malignant cells were emerging), a significant level of protection from tumour formation was observed. Notably, this acquired protection was lost when the mice were crossed onto an ARF-null background. This analysis suggests that the massive apoptotic response mediated by the DNA damage response pathways in sensitive tissues offers little prevention of tumorigenesis. It also suggests that, even if oncogenes activated by ionizing radiation cause persistent DNA damage, the pathways that detect the damage cannot mediate tumour suppression in the absence of ARF, at least in this mouse model.

A similar conclusion has been reached by Serrano's group[91], who studied the role of ARF in tumour suppression in transgenic mice that expressed an additional copy of *Trp53* (known as p53^{super} mice), which is known to provide added protection against the development of

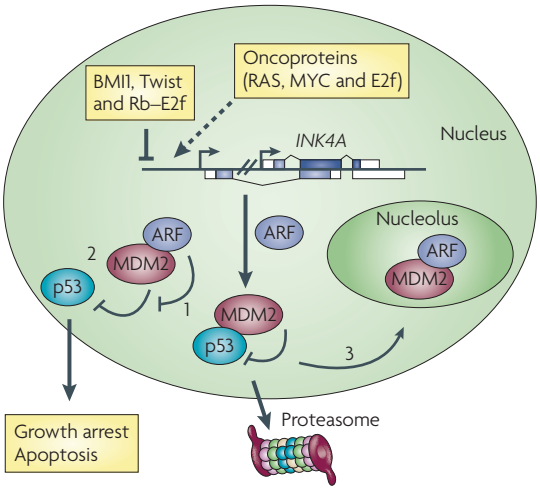

Figure 4 | **Induction of p53 by the ARF pathway.** The *CDKN2A* locus encodes ARF in an overlapping reading frame with the tumour suppressor INK4A (also known as p16), and ARF is normally expressed at low levels in cells. Hyperproliferative signals lead to the increased expression of ARF, which inhibits MDM2 by blocking its E3 ubiquitin ligase activity[58] (mechanism 1), uncoupling the p53–MDM2 interaction[57] (mechanism 2) and sequestering MDM2 in the nucleolus, thereby segregating it from nucleoplasmic p53 (REFS 60–62) (mechanism 3).

Non-homologous end joining
A method of DNA repair in which the ends arising from a double-stranded break are recognized by specialized proteins and religated.

Box 1 | **Contribution of p53 family members to tumour suppression**

The p53 family members p63 and p73 have tissue-specific and essential roles in normal development[103]. Their complex expression as a series of alternatively spliced full-length and amino-terminally truncated isoforms that have opposing activities has made it difficult to fully assess their contribution to tumour suppression[104]. Experiments using mouse embryonic fibroblasts from knock-out mice that are primed to undergo apoptosis by the expression of the adenoviral E1A oncogene have shown that p63 and p73 can cooperate with DNA damage-induced p53 (REF. 105). However, the deletion of *Trp63* and/or *Trp73* seems to have little influence on the p53-mediated apoptosis of T cells *in vivo*[106]. These observations suggest that the contribution of p63 and p73 to tumour suppression might be influenced by factors such as the cell type or by oncogenic signals. Full-length transactivation-competent isoforms of p63 and p73 (TAp63 and TAp73) may contribute to the DNA damage response independently of p53. For example, p63 is crucial for the protection of the female germline[107]. Similarly, the p73–E2F1 pathway is involved in DNA damage-induced apoptosis and tumour chemosensitivity[105,108–112]. Ageing *Trp63*$^{+/-}$ *Trp73*$^{+/-}$ heterozygotes (carrying deletions that inactivate all isoforms of p63 and p73) spontaneously succumb to the development of a range of small but detectable tumours in specific tissues[109], which is consistent with the idea that p63 and p73 contribute to tumour suppression. Recently, p63 has been identified as a potent transforming growth factor-β (TGFβ)-dependent suppressor of metastasis that is inhibited by mutant p53 during tumour progression[113]. Therefore, although mutation of p63 or p73 is not a common feature of tumour development[104], other mechanisms might impair their contributions to tumour suppression[114]. Additionally, some tumours upregulate the ΔN isoforms (which lack the transactivation domain) of these proteins, which can act as dominant-negative inhibitors of the p53 family[104].

cancer[92]. They showed that wild-type and p53^{super} mice, regardless of whether they are in an ARF-competent or ARF-null background, respond normally to DNA damage as measured by the number of apoptotic thymocytes detected following a high dose of ionizing radiation. Notably, the p53^{super} mouse is more effective than wild-type mice at mediating apoptosis. However, although the p53^{super} mice have extra protection against spontaneous and drug-induced tumour development, they are not protected in the absence of ARF. Moreover, when MEFs from these animals were used in a two-oncogene focus assay, focus formation was detectable only in the absence of either p53 or ARF, suggesting that ARF is required to suppress the transformed phenotype arising from oncogene expression.

These studies therefore provide strong support for the idea that ARF is the key mediator of p53-dependent tumour suppression, at least in mice. However, it would also be of interest to know whether crossing the mice from these studies onto an *Atm*-deficient background would lead to a lower level or absent tumour protection in a manner similar to the ARF-knockout. At least, this experiment would be an interesting control.

Two paradigms: common ground?

How might these two seemingly irreconcilable models of p53-mediated tumour suppression be resolved? Several observations suggest that these two paradigms might not necessarily be mutually exclusive.

Crucially, the processes of p53 induction by DNA damage and by oncogenes cannot be completely separated, at least in cultured cells: ARF-null MEFs are partially defective in the DNA damage response and show reduced levels of p53 following ionizing radiation[93]. In addition, ARF levels are increased by some forms of DNA damage[93,94]. Moreover, ARF itself can activate the ATM pathway through a mechanism that involves the stabilization of TIP60 and the consequent acetylation-dependent activation of ATM[94]. The induced acetylation of key regulatory lysines in p53 is also common to p53 induction by both DNA damage response pathways and ARF-mediated pathways[79,80]. Therefore, collectively the available data support the idea that there is a substantial degree of crosstalk between different pathways that induce p53 expression.

It is also clear that ARF functions in some but not all p53-mediated tumour suppression pathways. For example, ARF is dispensable for suppression of SV40 T antigen-induced choroid plexus tumours, suggesting that additional pathways might operate in tumour suppression[95]. Similarly, p53-mediated suppression of medulloblastoma in mice that are heterozygous for patched (*ptch*) is independent of ARF[96]. p53 also retains a substantial capacity to suppress spontaneous tumour formation on an ARF-null background[97,98]. Moreover, in some cases, the tumour spectrum that is obtained with the p53 knockout differs from that observed when ARF is knocked out[98]. Therefore, given the examples cited above, it is possible that the ARF pathway and the DNA damage response pathways may have differential contributions to tumour suppression, or might even function in an overlapping or cooperating manner, depending on the context (such as cell or tissue type).

As discussed above, the principal and common event in the p53 induction process is the uncoupling of p53 from its negative regulators. What differences between the induction by ionizing radiation and the detection of incipient tumour cells through the ARF pathway might explain why only the ARF pathway leads to tumour suppression in some studies[90,91]? One possibility is that the duration of the p53 response is important. In the case of ionizing radiation, p53 is induced by a single short-lived intense stimulus (which is also the initiator event in tumorigenesis). In cells that avoid apoptosis and survive, it is possible that the DNA damage is repaired and that the p53 induction process is attenuated in a short time (although the possibility of a low level of persistent DNA damage cannot be ruled out[99]). Given that different p53-responsive genes show varied kinetics in their expression profiles after p53 induction[34], it is plausible that such a short-lived, albeit intense, induction of p53 may not achieve the necessary changes in the expression levels of particular genes that are required for tumour suppression. By contrast, surviving cells that have acquired mutations that activate oncogenes will undergo a prolonged and sustained p53 response during which appropriate changes in gene expression might be achieved or maintained.

Another potential issue is the growth status of the cells. In the studies by Christophorou, Efeyan and colleagues[90,91], DNA damage is induced in normal (possibly non-cycling) cells, but ARF function has an effect once the cells have acquired incipient tumour

Focus assay
A cell culture-based measurement of the neoplastic transformation of cells with respect to their ability to overcome contact inhibition.

characteristics. Is the DNA damage response qualitatively or quantitatively different under these conditions compared with the ionizing radiation-induced damage in normal cells? Moreover, might the involvement of the DNA damage response require cooperation with ARF? If this suggestion is true, this might explain why an apparently intact DNA damage response does not affect tumour suppression in an ARF-null background[90,91]. It would be interesting to know whether persistent DNA damage markers are detectable in or completely absent from incipient tumours that arise in the irradiated p53ER^{TAM} or p53^{super} mice. A further point that should be considered is the question of whether the requirement for ARF is essential for mounting a barrier to the development of all or most types of tumours, or whether it is restricted to a subset of tumour types. In this sense, it would be interesting and valuable to investigate the responses of the p53ER^{TAM} or p53^{super} mice in the backgrounds of other murine models that have been designed to lead to tumour formation in certain tissues.

Finally, a key issue that cannot be overlooked is that interspecies differences could influence the mechanism by which p53 is alerted to tumour initiation. For example, although ARF has a key role in preventing tumour development in mice, it is rarely mutated in human cancer, which suggests that it does not constitute a major barrier to human cancer progression[70,71]. Although mutations occur at a high frequency in the *CDKN2A* locus that encodes ARF and the INK4A tumour suppressor in overlapping reading frames, these affect mainly INK4A and not ARF. Moreover, ARF can induce p53-mediated senescence in response to oncogenic Ras in murine fibroblasts but not in human fibroblasts[100]. It is therefore possible that the murine animal models might not faithfully represent or predict p53 responses in humans.

Conclusions and perspectives

Understanding the routes by which the p53 tumour suppressor detects the earliest stages of tumour development (FIG. 5) should not only improve our knowledge of the mechanisms of carcinogenesis, but also lead to a better appreciation of how early tumours can be detected, monitored and even eradicated. The evidence that DNA damage occurs very early in tumour development and correlates with tumour-suppressive events, such as senescence, is powerful and persuasive but still circumstantial on the issue of whether DNA damage is causal in stimulating tumour suppression[20,21,72,75]. Unquestionably, oncogenes can induce DNA damage in cultured cells and the ATM pathway can mediate tumour suppression in xenograft models. However, unlike the ARF-null mouse, there is currently no robust animal model that can be used to conclusively prove that these early DNA damage events mediate p53-dependent tumour suppression. A definitive answer to the question of whether the DNA damage response is fundamental for mediating p53-dependent tumour suppression could be provided if the appropriate knock-out or knock-in mouse model were available. As discussed above, such a mouse should have an intact ARF pathway but the p53 response to DNA damage should be eliminated in such a way that only p53 induction should be affected and all other aspects of the DNA damage response (such as the activation of effector and mediator kinases and mechanisms of DNA repair) should remain intact. Given that ATM-targeted phosphorylation of MDM2 is crucial to the induction of p53 by DNA damage, the generation of a mouse with alanine substitutions of the appropriate phosphorylation sites in MDM2 might provide an interesting and informative approach to addressing this issue. At present, however, we still do not fully understand how phosphorylation mediates MDM2 self-destruction, and the production of such a mouse model might be some distance away. Incidentally, as ATR-null cells and animals are not viable[101], it is unlikely that there would be a selection for loss of ATR function during tumour development. Individuals with Seckel syndrome have substantially impaired but not abolished ATR function; however, they do not seem to show an increased susceptibility to cancer[102]. It is possible that the low but detectable levels of functional ATR in such individuals might still protect against tumour development.

We have perhaps been trying to achieve a unified model of p53 regulation and tumour suppression — or a 'one-size-fits-all' model. The data reviewed above suggest that such a concept is not necessarily correct and there are indications that particular p53 induction pathways are dominant or ancillary, depending on the given cell types or tissues and the given set of circumstances. For example, as discussed above, DNA damage-induced modifications seem to have little effect in some cell types but can influence

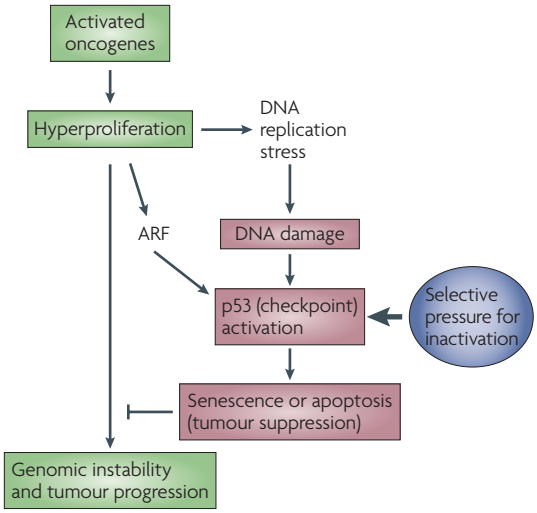

Figure 5 | **p53-mediated tumour suppression mediated by two distinct pathways.** Oncogene activation is thought to lead to the induction of ARF and consequent activation of p53 and tumour suppression[90,91]. Oncogene-induced DNA damage has been proposed as an alternative mechanism through which p53 tumour suppressor function is alerted to the presence of aberrant proliferative factors[72,75]. In both cases, there is a selective pressure for the inactivation of checkpoint components to allow developing tumours to progress to malignancy.

p53-mediated apoptosis and tumour suppression in others. Moreover, although some oncogenes can generate reactive oxygen species, others might stimulate the DNA damage response pathways by causing DNA replication stress. Therefore, the way in which p53 is induced (that is, the intensity of the stimulus, the duration of the response, the nature or type of activated or dysregulated oncogenes driving the tumour, the interacting factors in any given cell type, and the absence or presence of a specific combination of posttranslational modifications on p53) could have a substantial and context-dependent effect on tumour suppression. Efforts over the next few years might more definitively resolve these issues.

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DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Cdkn2a^{ARF} | *HRAS* | *ptch* | *Xrcc4*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ataxia–telangiectasia | Seckel syndrome
UniProtKB: http://www.uniprot.org
53BP1 | ATM | ATR | CHK1 | CHK2 | H2AX | INK4A | MDM2 | MDM4 | p53 | p63 | p73 | PCNA | PUMA | TIP60

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