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Unravelling mechanisms of p53-mediated tumour suppression

Kathryn T. Bieging,

Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, CCSR-South, Room 1255, 269 Campus Drive, Stanford, California 94305, USA.

Stephano Spano Mello, and

Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, CCSR-South, Room 1255, 269 Campus Drive, Stanford, California 94305, USA.

Laura D. Attardi

Department of Genetics, Stanford University School of Medicine, CCSR-South, Room 1255, 269 Campus Drive, Stanford, California 94305, USA.; Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, CCSR-South, Room 1255, 269 Campus Drive, Stanford, California 94305, USA.

Abstract

p53 is a crucial tumour suppressor that responds to diverse stress signals by orchestrating specific cellular responses, including transient cell cycle arrest, cellular senescence and apoptosis, which are all processes associated with tumour suppression. However, recent studies have challenged the relative importance of these canonical cellular responses for p53-mediated tumour suppression and have highlighted roles for p53 in modulating other cellular processes, including metabolism, stem cell maintenance, invasion and metastasis, as well as communication within the tumour microenvironment. In this Opinion article, we discuss the roles of classical p53 functions, as well as emerging p53-regulated processes, in tumour suppression.

The importance of p53 in tumour suppression is unequivocal, as shown by its inactivation in more than half of all sporadic human cancers, the susceptibility to cancer of individuals with Li–Fraumeni syndrome who inherit a mutant *TP53* allele, and the spontaneous tumour predisposition of *Trp53*-null mice^{1,2}. During tumour development, a *TP53* mutation, either sporadic or inherited, is typically followed by loss of heterozygosity, which results in complete p53 deficiency. p53 deficiency can enhance the initiation or progression of cancer, depending on the tumour type, and tumours that lack p53 are commonly characterized by more malignant characteristics, such as a lack of cellular differentiation, genetic instability, and increased invasiveness and metastatic potential^{3–10}. These effects are probably conferred both by loss of wild-type p53 function and by oncogenic gain-of-function

properties that characterize some p53 mutants (BOX 1). In addition, p53 is a member of a multiprotein family of transcription factors — also including p63 and p73 — and these factors have both overlapping and distinct cellular roles.

Although the crucial role of p53 in restraining cancer has provoked intensive investigation, the mechanisms that underlie p53-mediated tumour suppression remain incompletely understood. p53 is a cellular stress sensor that triggers transient cell cycle arrest, permanent cell cycle arrest (cellular senescence) and apoptosis in response to a host of diverse stresses, including DNA damage, hyperproliferative signals, hypoxia, oxidative stress, ribonucleotide depletion and nutrient starvation^{11,12} (FIGS 1,2). In response to such stress signals, p53 is displaced from its negative regulators MDM2 and MDM4, thereby allowing its stabilization and activation. Many of the aforementioned stresses may be encountered by incipient tumour cells in the tumour microenvironment and are therefore probably relevant for engaging p53 function in tumour suppression *in vivo*. While numerous studies have implicated the canonical p53-mediated cell cycle arrest and apoptosis responses in tumour suppression, p53 has recently been found to regulate additional diverse processes, including cellular metabolism, stem cell function, invasion and metastasis, as well as cell–cell communication within the tumour microenvironment, and these may also contribute to tumour suppression¹. Although specific p53 cellular responses depend on the function of p53 as a transcriptional activator and on the p53-mediated induction of particular target genes^{2,16} (FIG. 3), the downstream genes and pathways that are crucial for tumour suppression remain unresolved^{13–15}. In this Opinion article, we summarize our current knowledge of the cellular and molecular basis of tumour suppression by p53, highlighting lessons that have been learned from *in vivo* mouse models, and we offer insight into the pathways that may contribute to p53 tumour suppressor function.

Using mouse models to study p53

Initially, the generation of *Trp53*-knockout mice provided crucial support for the importance of p53 in tumour suppression, as these mice develop 100%-penetrant, early-onset, spontaneous tumours — primarily CD4⁺CD8⁺ T cell lymphomas — which arise during a limited developmental window before thymic involution^{17–19}. Moreover, reminiscent of patients with Li–Fraumeni syndrome, *Trp53*^{+/-} mice show an increased predisposition to cancer relative to wild-type mice; *Trp53*^{+/-} mice predominantly develop sarcomas, as well as some lymphomas and carcinomas^{17–19}. The propensity of the *Trp53*-null and heterozygous mice to develop these tumour types reflects an inherent susceptibility of mice to these tumours, which is enhanced by p53 deficiency²⁰. Early lethality from these tumours, however, precludes detection of tumour types that are more clearly associated with sporadic p53 inactivation in humans. To address this issue, more refined models have been developed to either allow conditional ablation of *Trp53* in specific tissues or to model p53 deficiency in the context of signals that are typical of human carcinogenesis, such as telomere attrition (reviewed in REF. 21). These studies have shown that, like in humans, *Trp53* inactivation promotes a range of cancer types in mice, and this underscores the importance of the mouse as a model system for unravelling p53 tumour suppressor function *in vivo*. Notably, the *Trp53*-knockout mice have also been used to show key roles for p53 in other biological processes, including development and fertility, which are reviewed elsewhere².

Tumour suppression through transcription

The most well-characterized biochemical activity of p53 is as a transcriptional activator, although it displays other activities that could contribute to tumour suppression, including repressing transcription and promoting mitochondrial membrane permeabilization to trigger apoptosis^{1,2}. Like other transcription factors, p53 comprises discrete domains that are responsible for sequence-specific DNA binding, transcriptional activation and oligomerization^{1,22} (FIG. 3a). More than 80% of *TP53* mutations in human tumours localize to the DNA-binding domain and compromise sequence-specific DNA binding, which suggests that the function of p53 as a transcription factor is crucial for tumour suppression²³. The carboxy-terminal tetramerization domain through which p53 monomers interact to form tetramers is also important for transcriptional activation²⁴. p53 tetramers bind to specific p53 response elements, which comprise two half sites of the nucleotide sequence RRRCCWWGYYY (in which R = purine, W = A or T, and Y = pyrimidine), typically separated by a spacer of 0–13 nucleotides¹⁶. Numerous direct p53 target genes involved in different cellular responses have been defined through genetic studies²⁵ (FIG. 3b). Moreover, recent genomic analyses using chromatin immunoprecipitation followed by DNA sequencing (ChIP–seq) and expression profiling have expanded the list of p53-regulated genes, and further examination of these genes will continue to elaborate the transcriptional networks involved in different p53 responses^{26–28}.

The diversity of biochemical activities ascribed to p53 necessitated a direct investigation into the importance of transcriptional activation for p53-mediated tumour suppression. Towards that end, a panel of *Trp53*-knock-in mice was generated, in which the first of two amino-terminal transcriptional activation domains (TADs) was mutated (p53^{25,26}), the second TAD was mutated (p53^{53,54}) or both TADs were mutated (p53^{25,26,53,54})^{13,29}. p53^{25,26}, with substitutions of L25Q and W26S (corresponding to amino acids 22 and 23 in human p53), is severely compromised for transactivation of most known p53 target genes, including cyclin-dependent kinase inhibitor 1A (*Cdkn1a*; encoding p21), *Puma* (also known as *Bbc3*), and *Noxa* (also known as *Pmaip1*), although it retains the ability to efficiently induce a small subset of p53 target genes, including BCL-2-associated X protein (*Bax*). Interestingly, despite its selective transactivation potential, p53^{25,26} effectively suppresses the development of tumours that are driven by different oncogenic lesions and derived from different lineages, including *Kras*^{G12D}-induced non-small-cell lung cancers, *Em-Myc*-driven B cell lymphomas, spontaneous T cell lymphomas and medulloblastomas triggered by inactivation of Patched (*Ptc*; also known as *Ptch1*)^{13,30}. Although the p53^{53,54} protein with F53Q and F54S substitutions (also residues 53 and 54 in human p53) retains intact transactivation and tumour-suppressor activity, p53^{25,26,53,54} completely lacks p53 transactivation potential, as indicated by a global gene expression profile that is indistinguishable from that of *Trp53*^{-/-} cells, and it is unable to suppress tumorigenesis in multiple mouse models. This observation underscores the importance of p53 transactivation function for effective tumour suppression.

Canonical p53 functions

p53 promotes the classical cellular functions of cell cycle arrest, senescence and apoptosis primarily through the activation of specific genes^{1,2}. As described below, the analysis of knockout mice that lack particular p53 target genes, as well as knock-in mice that express p53 separation-of-function mutants (which allow the retention of certain p53 activities but not others), has helped to establish the contribution of the canonical p53 functions to p53-mediated tumour suppression.

How does the ability of p53 to regulate cell cycle progression contribute to tumour suppression?

The earliest model to explain the mechanism that underlies p53 tumor suppressor activity was based on the description of p53 as the “guardian of the genome” (REF. 31). In this model, p53 induces a transient G1 cell cycle arrest in response to DNA-damage signals, allowing cells to repair their genomes before proceeding through the cell cycle, and thereby limiting the propagation of potentially oncogenic mutations^{31,32}. p53 triggers G1 arrest in response to DNA damage by transactivating *Cdkn1a*, as shown by the defective arrest response of cells that are derived from *Cdkn1a*^{-/-} mice upon exposure to DNA damage³²⁻³⁴. Surprisingly, however, p21-deficient mice were found to be either not at all or only mildly prone to developing spontaneous tumours³³⁻³⁵, potentially because other p53 cell cycle arrest target genes remain unperturbed. Similarly, mice that are deficient for other p53 cell cycle arrest target genes, such as growth arrest and DNA-damage-inducible 45α (*Gadd45a*), protein tyrosine phosphatase receptor type V (*Ptprv*) or promyelocytic leukaemia (*Pml*), are not susceptible to developing spontaneous tumours, although they display increased tumorigenesis in the presence of specific oncogenes or upon exposure to carcinogens³⁶⁻⁴².

Two *Trp53*-knock-in mouse strains that express separation-of-function mutants have provided evidence for the importance of p53-mediated cell cycle arrest in tumour suppression. Analysis of mouse embryonic fibroblasts (MEFs) derived from *Trp53*^{R172P} (also known as *Trp53*^{S15C}) mice showed that p53^{R172P} retains partial cell cycle arrest activity after ionizing radiation, and this correlates with some *Cdkn1a* induction⁴³ (TABLE 1); this is also the case for the human tumour-derived orthologue p53^{R175P} (REFS 44,45). Moreover, like wild-type p53, p53^{R172P} can restrict the proliferation of untreated MEFs in culture, which is an activity that may also be relevant for tumour suppression *in vivo*^{46,47}. However, diverse cell types from *Trp53*^{R172P/R172P} mice fail to undergo apoptosis in response to DNA damage or serum starvation, which indicates that p53^{R172P} has compromised apoptotic activity. Another knock-in mouse strain, which expresses *Trp53*^{E177R} (the orthologue of *TP53*^{E180R} — a mutant found both in sporadic human tumours and patients with Li–Fraumeni syndrome)⁴⁸, phenotypically resembles *Trp53*^{R172P}. Substitution of negatively charged E177 with positively charged arginine produces a ‘cooperativity mutant’ that compromises DNA binding of the p53 tetramer, particularly at apoptotic genes⁴⁹. Accordingly, p53^{E177R} cannot induce the expression of *Puma*, *Noxa* or *Bax*, or execute apoptosis in response to ionizing radiation or serum starvation⁴⁸ (TABLE 1). p53^{E177R} retains some activity in inducing cell cycle arrest and cell cycle arrest target genes, including *Cdkn1a*, in response to DNA damage, and in triggering senescence. Both

Trp53^{R172P/R172P} and *Trp53^{E177R/E177R}* mice were found to be mostly resistant to developing the early onset spontaneous T cell lymphomas that are characteristic of *Trp53^{-/-}* mice, and this correlates tumour suppression with cell cycle arrest activity. However, both strains are ultimately more prone to developing later-onset non-T cell lymphomas and sarcomas than wild-type mice, potentially because *p53^{R172P}* and *p53^{E177R}* lack apoptotic activity. Unlike *Trp53^{-/-}* tumours, however, the *Trp53^{R172P/R172P}* tumours lack evidence of aneuploidy, which suggests that the cell cycle regulation function of *p53^{R172P}* keeps genomic instability in check⁴³. Indeed, analysis of *p53^{R172P}* in a *Cdkn1a*-null background showed defective DNA-damage-induced cell cycle arrest and shorter tumour latency, which was associated with chromosome instability, than in the presence of *Cdkn1a*⁴⁷. Taken together, the ability of the cell cycle arrest-competent but apoptosis-deficient *p53^{R172P}* and *p53^{E177R}* mutants to extend tumour latency suggests that the ability of p53 to induce cell cycle arrest is important for tumour suppression. Thus, depending on the tissue, either the cell cycle arrest or the apoptosis function of p53 can be vital for tumour suppression. Interestingly, the cell cycle arrest-deficient and apoptosis-deficient *Trp53^{R172P/R172P}Cdkn1a^{-/-}* mice show longer tumour latency than *Trp53^{-/-}* mice, which suggests that other processes that are regulated by p53 may also be relevant for tumour suppression⁴⁷. Notably, *p53^{E177R}* can activate certain antioxidant target genes in response to DNA damage and can limit reactive oxygen species (ROS) accumulation and glycolysis⁴⁸, which suggests that *p53^{E177R}* may be active in regulating metabolism — a topic that we revisit below.

Beyond triggering cell cycle arrest in response to DNA damage, a fundamental component of p53 function as guardian of the genome is the active maintenance of genomic integrity⁵⁰. p53 stimulates various DNA repair mechanisms, including nucleotide excision repair, base excision repair and non-homologous end-joining^{51–53}, by activating numerous target genes that are involved in different DNA repair programmes, including *Gadd45a*, damage-specific DNA binding protein 2 (*Ddb2*), xeroderma pigmentosum, complementation group C (*Xpc*) and Fanconi anaemia, complementation group C (*Fancc*)² (FIG. 3b). Mice that lack such DNA repair genes are prone to cancer^{54–57}, and this supports the importance of p53 inducing DNA repair genes as one component of tumour suppression.

Does p53-mediated senescence contribute to tumour suppression?

Several studies have shown that p53-mediated tumour suppression correlates with cellular senescence. For example, in telomerase RNA component (*Terc*)^{-/-}*Trp53^{R172P/R172P}* mice, which sustain chronic DNA damage due to telomere erosion occurring with cell division, *p53^{R172P}* can promote senescence in different tissues and suppress spontaneous tumorigenesis⁵⁸. In addition, mice that express either oncogenic *Kras^{G12D}* or *Braf^{V600E}* develop lung cancer in which premalignant adenomas expressing p53 are positive for senescence markers, including senescence-associated β -galactosidase (SA- β gal), whereas malignant adenocarcinomas arising in the absence of p53 are negative for senescence markers^{59,60}. Similarly, in a mouse model of prostate cancer, p53-induced senescence greatly extends tumour latency in *Pten*^{-/-} mice, as reduced proliferation and increased SA- β gal levels are found in *Pten*^{-/-} prostates compared with *Pten*^{-/-}*Trp53^{-/-}* prostates⁶¹. Finally, p53 restoration in sarcomas, liver carcinomas and lung cancers that had formed in

the absence of p53 causes tumour regression, which is associated with the expression of senescence markers^{62–64}. Cellular senescence, therefore, is linked with p53-mediated tumour suppression in certain contexts. Again, because the ability of p53 to induce senescence is simply correlated with tumour suppression, these findings do not exclude that other p53 functions contribute to tumour suppression in these contexts.

The role of p53-induced apoptosis in tumour suppression

A role for the p53 apoptotic response in suppressing tumorigenesis was first shown in a choroid plexus epithelial tumour model in which retinoblastoma protein family inhibition induces slow-growing tumours that are characterized by considerable apoptosis⁶⁵. p53 inactivation in this model eliminates apoptosis and greatly accelerates tumour growth. Supporting the relevance of p53-mediated apoptosis for tumour suppression, the deletion of the pro-apoptotic p53 target gene *Bax* also attenuates apoptosis and promotes tumorigenesis in this model⁶⁶. Furthermore, in the *Eμ-Myc* transgenic B cell lymphoma model, p53 drives robust apoptosis in *Myc*-expressing B cells and suppresses lymphomagenesis in *Eμ-Myc* mice, whereas *Eμ-Myc Trp53^{+/-}* mice more rapidly succumb to lymphomas, and this is associated with the loss of the wild-type *Trp53* allele and loss of apoptosis^{67,68}. Additional experiments showed that disrupting the apoptotic pathway downstream of wild-type p53 by the overexpression of BCL-2 or dominant-negative caspase 9 promotes lymphomagenesis, which indicates that directly inactivating apoptosis substitutes for *Trp53* loss in lymphomagenesis and suggests that p53 suppresses cancer in this setting by inducing apoptosis⁶⁹.

The role of apoptosis in tumour suppression *in vivo* has also been interrogated in knockout mice lacking individual p53 target genes that are essential for p53-mediated apoptosis. Mice that are deficient for *Puma*, *Noxa*, *Bax* or p53 apoptosis effector (*Perp*) do not show an increased propensity for spontaneous tumour development^{70–73}. However, lymphomagenesis is accelerated in *Eμ-Myc Bax^{-/-}* and *Eμ-Myc Puma^{-/-}* mice, as well as in *Eμ-Myc Puma*-knockdown mice, relative to *Eμ-Myc* controls^{74–77}. Moreover, *Perp* loss enhances ultraviolet B (UVB)-induced skin carcinogenesis in mice⁷⁸. *Eμ-Myc* also promotes tumorigenesis in the presence of the apoptosis-defective p53^{R172P} and p53^{E177R} mutants, as tumour latency is greatly reduced in *Eμ-Myc Trp53^{+/E177R}*, *Eμ-Myc Trp53^{+/R172P}* and *Eμ-Myc Trp53^{R172P/R172P}* mice compared with *Eμ-Myc Trp53^{+/+}* mice^{48,186}. Taken together, these studies established the paradigm that apoptosis is a key component of p53-mediated tumour suppression.

The acute DNA damage response is dispensable for tumour suppression

An advance in our understanding of mechanisms of p53 action in tumour suppression came with a series of genetic studies directed at ascertaining the role of the DNA damage response in cancer suppression. Previous studies had provided evidence for the activation of a DNA damage response in incipient human cancers, thereby leading to a model for p53-mediated tumour suppression in which oncogene activation provokes aberrant proliferation and replication stress, hence causing DNA double-strand breaks and a DNA damage response that culminates in p53 activation and apoptosis or senescence^{79–81}. By contrast, several mouse model studies showed that the acute DNA damage response — in which treatment

with a single, high dose of a DNA-damaging agent, such as ionizing radiation or chemotherapeutic drugs, activates p53 to promote an immediate arrest or apoptosis response — is dispensable for tumour suppression^{82–84}. For example, one study investigated when p53 activity is required to suppress ionizing radiation-induced lymphomagenesis by using mice that expressed a p53–oestrogen receptor (ER) fusion protein to allow temporally-regulated, tamoxifen-inducible p53 activation⁸⁵. Relative to *Trp53*^{−/−} mice, these *Trp53*^{ER/ER} mice were protected from ionizing radiation-induced lymphomagenesis, irrespective of whether p53 was induced concurrently with ionizing radiation — when it triggered widespread apoptosis — or 8 days after ionizing radiation treatment, when no apoptosis was detected⁸². A similar analysis used conditional *Trp53* deletion in mice at time points prior to, concurrent with or after whole-body irradiation. Whether p53 was present at the time of the irradiation was inconsequential for the ultimate latency of ionizing radiation-induced lymphomagenesis, which indicates that the immediate p53 apoptotic response to ionizing radiation-induced DNA damage is irrelevant for tumour suppression⁸⁴. These results are supported by the p53^{25,26} TAD mutant, which is unable to trigger cell cycle arrest or apoptosis in the face of acute DNA damage caused by doxorubicin or ionizing radiation, but which can still suppress tumour formation in different tissues, downstream of various oncogenic signals^{13,30}. The surprising conclusion that stems from these observations is that the ability of p53 to drive responses to acute DNA damage is dispensable for p53 tumour-suppressor activity, at least in the tumour models examined here. Importantly, nascent tumour cells *in vivo* are likely to encounter more chronic, low-level DNA damage due to replication stress, telomere attrition or oxidative damage, which may promote tumour suppression through p53 pathways that are distinct from those required by acute DNA damage signalling. Therefore, these findings do not exclude a role for some type of DNA damage signalling in engaging the p53 tumour-suppressor function.

Further challenging the importance of classical p53 responses and target genes for tumour suppression

The role of classical p53-mediated responses in tumour suppression was further questioned by a study of knock-in mice expressing p53^{3KR}, in which three DNA binding domain-localized lysines that are known to be acetylated *in vivo* were mutated (K117R, K161R and K162R)¹⁴. Thymocytes and MEFs that are derived from *Trp53*^{3KR/3KR} mice fail to undergo apoptosis and cell cycle arrest, respectively, in response to ionizing radiation-induced DNA damage (TABLE 1). Moreover, p53^{3KR} is deficient in inducing the expression of *Puma* and *Cdkn1a* in DNA damage-treated cells. p53^{3KR} is also defective for transactivating the senescence target genes plasminogen activator inhibitor (*Pai1*; also known as *Serpine1*) and *Pml* after treatment with DNA-damaging agents, as well as in promoting senescence, although p53^{3KR} retains slight activity in suppressing proliferation compared with *Trp53*^{−/−} MEFs. p53^{3KR} nonetheless displays tumour-suppressor activity, as *Trp53*^{3KR/3KR} mice do not develop the 100%-penetrant spontaneous tumours that are characteristic of *Trp53*^{−/−} animals^{17–19}. Despite its inability to induce apoptosis and cell cycle arrest genes, p53^{3KR} can activate the metabolic genes glutaminase 2 (*Gls2*), TP53-induced glycolysis and apoptosis regulator (*Tigar*) and glutathione peroxidase 1 (*Gpx1*), like wild-type p53, in response to acute DNA damage. Moreover, like wild-type p53, basal levels of p53^{3KR} can suppress glucose uptake, restrain glycolysis and inhibit ROS accumulation¹⁴. These

surprising findings suggest that p53-mediated tumour suppression does not rely on cell cycle arrest, senescence and apoptosis, at least in this tumour model, and instead it may depend on other functions, such as regulating metabolism. In the future, it would be informative to use genome-wide expression profiling of p53^{3KR}-expressing cells to define additional transcriptional programmes activated by this mutant that correlate with tumour-suppressor function.

Further insight into the role of classical p53 functions in tumour suppression came from studies of the ability of p53 to suppress tumorigenesis in the complete absence of both DNA-damage-induced apoptosis and cell cycle arrest using *Cdkn1a*^{-/-}*Puma*^{-/-}*Noxa*^{-/-} triple-knockout mice¹⁵. Thymocytes from *Cdkn1a*^{-/-}*Puma*^{-/-}*Noxa*^{-/-} mice are completely deficient in DNA-damage-triggered, p53-dependent apoptosis, and activated T lymphocytes are deficient in G1 arrest after ionizing radiation (TABLE 1). Furthermore, *Cdkn1a*^{-/-}*Puma*^{-/-}*Noxa*^{-/-} dermal fibroblasts are partially compromised in undergoing senescence in response to the DNA-damaging agent etoposide. Interestingly, however, these mice do not develop spontaneous tumours when aged to 500 days, whereas all *Trp53*^{-/-} mice develop tumours by 250 days. These findings support the idea that the major target genes involved in acute DNA-damage-triggered cell cycle arrest and apoptosis responses — *Cdkn1a*, *Puma* and *Noxa* — are unnecessary for p53-dependent tumour suppression.

Analysis of the p53^{25,26} TAD mutant extends this finding by showing that robust activation of most classical p53 target genes is dispensable for tumour suppression¹³. As p53^{25,26} only efficiently activates a small subset of all p53 target genes but retains full tumour-suppressor activity, it provides a unique tool to pinpoint the most relevant target genes for p53-mediated tumour suppression. Indeed, genome-wide analysis of the genes that are efficiently activated by both wild-type p53 and p53^{25,26} in oncogene-expressing MEFs, coupled with p53 ChIP experiments, allowed the identification of a small set of direct tumour suppression-associated p53 target genes, which encode proteins that are involved in various functional processes, including signalling, regulation of the actin cytoskeleton and DNA repair. Most of these genes, which include abhydrolase domain containing 4 (*Abhd4*) and pleckstrin homology-like domain, family A, member 3 (*Phlda3*), are also regulated by p53 in human cells, and experiments using RNA interference (RNAi) in allograft tumour assays showed that the knockdown of these genes increases tumour growth. These findings show that these novel target genes have tumour-suppressor activity, and this suggests that they have importance in the p53 tumour-suppressor network¹³. Together, the *Cdkn1a*^{-/-}*Puma*^{-/-}*Noxa*^{-/-} mice, along with the p53^{25,26}- and p53^{3KR}-mutant mice, suggest that transcriptional mechanisms that are distinct from robust transactivation of the major known p53 targets that mediate apoptosis, cell cycle arrest and cellular senescence are important for effective tumour suppression by p53. Alternatively, it is possible that compensatory transcriptional programmes provide a 'backup' in the absence of classical p53 targets.

Deconvoluting p53 tumour suppression

Although much accumulated evidence has indicated that the cell cycle arrest and apoptosis functions of p53 account for its tumour-suppressor activity, several recent studies have

questioned this notion. Reconciling these conclusions requires recognition of cell-type or context-specific differences among different studies, along with other important considerations.

For p53 to limit tumorigenesis, it is logical that it would promote a programme that would ultimately restrain tumour cell proliferation, and this would be achievable by promoting cell cycle arrest and apoptosis. Accordingly, there is unequivocal *in vivo* evidence for such anti-proliferative effects of p53, in which apoptosis or senescence can be shown to occur in incipient tumours with active p53^{58–61,65,67}. Moreover, genetic studies of p53 functions in which tumorigenesis ensues after inactivation of downstream components, such as apoptotic machinery constituents⁶⁹, make a compelling argument for the importance of such functions in tumour suppression. In addition, the reactivation of p53 in tumours that had formed in its absence triggers apoptosis in lymphomas and lung cancers, as well as senescence in sarcomas, liver carcinomas and lung cancers, thereby resulting in tumour regression^{62–64,86}. Collectively, these findings can be used to argue that p53 engages cell cycle arrest and apoptosis programmes to limit tumorigenesis.

However, various converging studies, using *Trp53*^{25,26}, *Trp53*^{3KR} and *Cdkn1a*^{-/-} *Puma*^{-/-} *Noxa*^{-/-} mutant mice, support the idea that the cell cycle arrest and apoptotic pathways that have been mapped by studying acute DNA damage responses to ionizing radiation and doxorubicin are not crucial for tumour suppression, at least in the various tissues examined^{13–15} (TABLE 1). This conclusion is bolstered by studies showing that immediate p53 responses to acute DNA damage are irrelevant for suppressing tumorigenesis^{82–84}. Instead, these studies advance the idea that p53 activity is most important after acute DNA damage drives the generation of oncogenic lesions that trigger proteins such as ARF (FIG. 1) to stimulate p53 tumour-suppressor function. It is also formally possible that inactivation of p53 acute DNA damage responses can trigger compensatory mechanisms that can step in to promote tumour suppression.

The dispensability of acute DNA damage responses for p53-mediated tumour suppression need not suggest that cell cycle arrest and apoptosis responses are unimportant for p53 function. Many of the *in vitro* assays that have characterized mechanisms of DNA damage responses examine the immediate response to an acute stress, typically at one time point or one dose, rather than to a chronic stress over time, which is probably more akin to the stresses that p53 would encounter in a nascent tumour *in vivo*. Furthermore, numerous cues beyond acute DNA damage signals can stimulate p53 in the context of an incipient tumour, such as oncogenic signals, chronic low-level DNA damage that is triggered by telomere attrition, replicative stress or ROS, as well as microenvironmental stresses such as nutrient depletion or hypoxia^{11,12}. p53 may still respond to diverse signals in the tumour microenvironment by promoting cell cycle arrest and apoptosis, which we speculate could be mediated through transcriptional networks that are different from those defined in the context of acute DNA damage *in vitro*⁸⁷. Indeed, the ability of the tumour-suppression-competent p53^{25,26} TAD mutant to induce apoptosis in response to serum starvation and hypoxia²⁹ lends support to the idea that these activities could be relevant for tumour suppression.

It is also possible that so-called ‘basal’ p53 functions, including inhibiting proliferation or modulating redox state⁸⁸, are highly relevant for p53 function in tumour suppression. In fact, the ability of p53^{R172P} and p53^{3KR} to restrain proliferation compared with *Trp53*^{-/-} cells, albeit to different extents, or of p53^{E177R} and p53^{3KR} to inhibit glycolysis and limit ROS levels, is in keeping with this hypothesis^{14,47,48}.

Given the various aforementioned considerations, in the future, it will be most informative to investigate p53 mechanisms of action *in vivo*, in assays that examine the cell types of origin and the signals that are most relevant for tumorigenesis. Further insights might also be gained by analysing the activities that are retained by the p53 mutants described above, through approaches such as genome-wide transcriptomics, to better define pathways that underlie p53 tumour-suppressor function. In addition, emerging evidence underscores the importance of functions beyond the classical ones for tumour suppression, as detailed below, and it will be essential to consider these in future investigations.

Emerging p53 functions

p53 polices cellular metabolism

Metabolic reprogramming, a hallmark of cancer cells, is characterized by the Warburg effect, in which high rates of glycolysis accompanied by reduced oxidative phosphorylation occur even under aerobic conditions. This reprogramming is thought to be essential for fueling anabolic processes that are fundamental for the growth and proliferation of cancer cells⁸⁹. p53 opposes this oncogenic metabolic reprogramming (FIG. 2) by stimulating oxidative phosphorylation through the activation of synthesis of cytochrome oxidase 2 (*Sco2*), as well as by inhibiting glycolysis through the transcriptional activation of *Tigar* and the transcriptional repression of glucose transporters such as *GLUT1* (also known as *SLC2A1*) and *GLUT4* (also known as *SLC2A4*)^{90–94}. p53 also transcriptionally activates genes such as *Gls2*, sestrin 1 (*Sesn1*) and *Sesn2* to limit ROS accumulation, thereby helping to maintain cellular integrity and protect against neoplasia^{88,94–97} (FIG. 3b). Indeed, evidence suggests that limiting ROS levels contributes to p53-mediated tumour suppression, as treatment of *Trp53*^{-/-} mice with the antioxidant *N*-acetylcysteine (NAC) prevents the elevated ROS levels that are typical of *Trp53*^{-/-} cells and provides substantial protection from early onset T cell lymphomas^{88,98}. Furthermore, studies of the *Trp53*^{3KR}- and *Trp53*^{E177R}-knock-in mice support the importance of metabolic regulation in p53-mediated tumour suppression, as these mutants can activate some metabolic genes, inhibit glycolysis and restrain ROS accumulation, thereby providing a potential explanation for their effective suppression of early onset T cell lymphomas^{14,48}.

Autophagy is also crucial for metabolic homeostasis. It is a process in which cytoplasmic proteins and organelles are engulfed into autophagic vesicles that ultimately fuse with lysosomes, thereby resulting in degradation of the contents⁹⁹. Autophagy promotes both recycling of cellular components and energy production, and it therefore serves as a survival mechanism in certain settings, such as upon nutrient deprivation. Additionally, autophagy can maintain cellular integrity by removing damaged cellular components, including damaged mitochondria, which are a major source of ROS¹⁰⁰. Thus, depending on the context, autophagy can either promote or inhibit tumorigenesis. p53 transcriptional activity

is crucial for inducing autophagy²⁷, and p53 activates a host of target genes that encode proteins involved in various steps of autophagy, including upstream regulators of autophagy, core machinery components and lysosomal constituents^{27,101,102} (FIG. 3b). Furthermore, the inhibition of autophagy through ablation of autophagy related 5 (*Atg5*) — a central component of the autophagy machinery — results in defective p53-dependent apoptosis and promotes transformation of oncogene-expressing MEFs, which is similar to p53 loss²⁷. These data suggest that p53 impedes tumorigenesis at least in part through the induction of autophagy. Activation of autophagy by p53 ensures efficient apoptosis, and it potentially has other tumour-suppressive effects, such as limiting ROS accumulation. These observations additionally highlight the crosstalk between the regulation of metabolism by p53 and canonical p53 functions such as apoptosis. Similarly, p53-dependent induction of the target gene adenosine A2b receptor (*ADORA2B*) — the encoded protein of which senses the ATP metabolite adenosine — promotes apoptosis under conditions of adenosine accumulation¹⁰³.

p53 puts a brake on stem cells

In recent years, a role for p53 in regulating stem cell function has been revealed (FIG. 2). Key support for this role came from the demonstration that p53 can suppress the reprogramming of differentiated somatic cells to induced pluripotent stem cells (iPSCs)^{104–110}, which is partly attributable to restraint of the cell cycle, via induction of *Cdkn1a*, or to induction of apoptosis^{104,106,109,111}. In addition, the requirement for the p53 target genes *mir34a–c* for the p53-mediated reprogramming blockade suggests that p53 also functions by inhibiting essential pluripotency genes that are known targets of *mir-34a*¹¹². p53 further counteracts pluripotency through the direct transactivation of *mir-145*, which similarly downregulates pluripotency factors¹¹³. This p53-dependent reprogramming barrier is thought to be highly relevant for tumour suppression. Indeed, p53 loss in human cancers correlates with the development of undifferentiated, highly aggressive tumours showing gene expression profiles that are similar to embryonic stem cells (ESCs) or iPSCs, and this bolsters the idea that p53 inhibits the genesis of tumorigenic stem cells⁹. Moreover, p53 function in limiting the reprogramming of differentiated cells is in line with a more general function for p53 in stem cell biology, in which p53 inhibits self-renewal and promotes differentiation. For example, p53 impedes self-renewal of haematopoietic stem cells¹¹⁴ and leukaemia stem cells in a mouse model of acute myeloid leukaemia¹¹⁵. Additionally, studies of *Trp53^{-/-}Pten^{-/-}* mice showed that p53 and PTEN coordinately restrict neural stem cell renewal, promote differentiation and inhibit glioblastoma development¹¹⁶. Thus, p53 can also suppress tumorigenesis by inhibiting characteristics of ‘stemness’.

p53 imposes a barrier to invasion and metastasis

Studies have suggested that p53 loss also enables malignant progression (FIG. 2), as tumours are generally more aggressive and more vascularized when they are deficient for p53 (REF. 117). Indeed, the combined loss of casein kinase 1 α (CK1 α) and p53 in the mouse small intestine was used to identify a p21-dependent ‘p53-suppressed invasiveness signature’ (PSIS) that was associated with invasive carcinomas¹¹⁸. Similarly, p53 deficiency augments motility and invasiveness in primary cells *in vitro*^{119–123}. Other studies have investigated a role for p53 in opposing epithelial-to-mesenchymal transition (EMT), which is a developmental process in which cell–cell adhesion junctions are disassembled and cells

acquire more mesenchymal, migratory phenotypes. In cancer cells, EMT is tightly correlated with invasion and metastasis¹²⁴. The SNAIL, TWIST and ZEB families of transcription factors promote EMT, and their activities can be modulated by p53. For example, p53 opposes SNAIL activity by inducing *mir-34* transcription, which reduces SNAIL1 translation¹²⁵. p53 also impinges on the EMT programme by transactivating *mir-200c*, which represses the translation of *Zeb1* and *Bmi1* (REF. 126) (*Bmi1* encodes a polycomb group protein that maintains stemness and promotes EMT). Accordingly, p53 loss correlates with increased ZEB1 and BMI1 levels, as well as with tumour progression, in human breast cancer^{126,127}. p53 loss also correlates with EMT hallmarks in mouse models of skin, breast and colon cancer^{128–130}. Continued investigation of these pathways will help to further unravel how p53 prevents EMT, invasion and metastasis.

Non-cell-autonomous functions of p53

Tumour cells *in vivo* are surrounded by a heterogeneous microenvironment that comprises fibroblasts, immune cells, blood vessels and extracellular matrix. Crosstalk between tumour cells and cells of the microenvironment is crucial for regulating tumorigenesis¹³¹. Although the function of p53 as a barrier to cancer development has been extensively studied at the cell-autonomous level within nascent tumour cells, recent studies have shown non-cell-autonomous effects of p53 in stimulating an anti-tumorigenic microenvironment (FIG. 2). Initial studies showed that p53 transactivates thrombospondin 1 (*Tsp1*; also known as *Thbs1*), which encodes a secreted inhibitor of angiogenesis¹³². Moreover, p53 reactivation in liver tumours drives senescence and tumour regression through the upregulation of inflammatory cytokines and a consequent innate immune response that is characterized by the recruitment of neutrophils, macrophages and natural killer cells⁶³. Indeed, gene expression analyses have shown that p53 directly induces numerous genes involved in triggering the recruitment of immune cells and immune surveillance¹³³ (FIG. 3b). Interestingly, the senescence-associated secretory phenotype (SASP) — a hallmark of senescence characterized by the secretion of pro-tumorigenic cytokines and chemokines — can be restrained or qualitatively modified by p53 in hepatic stellate cells (HSCs), human fibroblasts, epithelial cells and tumour cell lines^{131,134,135}. For example, in contrast to senescent, p53-expressing HSCs, which secrete factors that skew surrounding macrophages towards an M1 phenotype and promote macrophage-mediated clearance of senescent cells, p53-deficient HSCs secrete factors that polarize macrophages to a tumour-promoting M2 phenotype, thereby increasing the proliferation of premalignant hepatoblasts and enhancing hepatocellular carcinoma development¹³⁴.

Evidence has also accumulated for a tumour-suppressive function for p53 in the tumour stroma. Inactivation of the retinoblastoma tumour suppressor family members in the mouse prostatic epithelium activates a p53-dependent cell cycle arrest in surrounding stromal fibroblasts¹³⁶. During tumour progression, there is a selection pressure for fibroblasts with inactive p53 in the prostate tumour stroma, and this probably further fuels carcinogenesis. In support of this model, p53 inactivation in tumour stromal cells can be detected in diverse human carcinomas^{137–140}, and subcutaneous injection of MCF7 breast cancer cells into *Trp53*^{-/-} mice produces more tumours than in wild-type controls¹⁴¹. These studies collectively show that p53 activity in one cell can influence the fate of neighbouring cells,

which suggests that the complexity of the tumour microenvironment is an important consideration in understanding p53-mediated tumour suppression.

Conclusions and perspectives

The crucial role for p53 in suppressing malignancy has sparked extensive and careful inquiry into the mechanisms of its action at the molecular, cellular and organismal levels. Studies initially focused on deducing the pathways involved in rapid p53-driven cell cycle arrest or apoptotic responses to acute DNA damage signals, whereas more recent investigations have delved deeper and have shown greater intricacies to the contexts and activities that link p53 and tumour suppression¹⁴². Unravelling the mechanisms that underlie the crucial role for p53 in tumour suppression will require conflation of these data, as well as persistent experimental inquiry.

Continued investigation to specifically interrogate hypotheses that stem from existing models will help to refine our understanding of the function of p53 in tumour suppression. For example, one hypothesis to explain differences in p53 activities associated with tumour suppression in different studies is that the mechanism of p53 action may vary with tissue context. It will therefore be important to expand mechanistic studies of p53 in mice to other models, beyond spontaneous T cell lymphoma development, particularly to epithelial cancer models, which are of paramount importance, as they represent the majority of human cancers. In addition, although we can surmise that the ability to restrain proliferation or induce apoptosis is ultimately fundamental for p53 to suppress tumour development, we can hypothesize that, in the context of the incipient tumour *in vivo*, these responses may be triggered by distinct stresses and executed by different downstream effectors than those involved in acute DNA damage responses. Hence, we should attempt to analyse the mechanisms of p53 action in response to the stresses that are most relevant in the tumour milieu and in the cell of origin for the tumour of interest. A corollary of this is that basal p53 function, in the absence of potent stressors, may also be relevant for p53 activity in tumour suppression, and this notion certainly merits further examination. The roles for p53 in dampening cellular proliferation rates or regulating metabolism exemplify such functions.

New elements of the function of p53 as a barrier to carcinogenesis have been identified in recent years, as shown by the various alterations that have been observed in p53-deficient cells. p53 loss provokes not only increased cell division and enhanced cellular survival but also more invasive behaviour, more genomic instability, metabolic transformation, augmented self-renewal and altered tumour–stromal cell crosstalk within the tumour microenvironment. These findings indicate that p53 not only keeps proliferation in check but also has a marked effect on many diverse aspects of cell behaviour — a concept that is in keeping with p53 regulating myriad transcriptional targets that are involved in a host of different cellular processes (FIG. 3b). Notably, the emerging p53 functions have not been scrutinized to the same degree as the classical cell cycle arrest and apoptosis functions, and their precise roles will need to be clarified in future studies. Furthermore, the distinction between emergent and canonical functions can be blurry, as some of the newly identified activities impinge on the canonical p53 functions of cell cycle arrest or apoptosis, and additional links such as these are likely to be identified. Ultimately, the coordinate induction

of multiple programmes by p53 suggests why it is advantageous for tumours to lose wild-type p53 activity, as many aspects of tumour suppression can be incapacitated with one fell swoop.

Deciphering the details of p53-mediated tumour suppression is important not only for deriving a better understanding of how p53 functions but also for opening up opportunities for improved early detection, prognostication and treatment of cancer. If the components that are crucial for p53-mediated tumour suppression are known, more reliable expression signatures that reflect functional p53 status can be used for diagnosis or prognostication. Moreover, identifying the key targets and pathways that are involved in the function of p53 in tumour suppression provides more flexibility for therapeutic intervention. As restoration of wild-type p53 function is not a trivial proposition, identifying a more targetable component or pathway downstream of p53 could be a key to attacking p53-deficient tumours. Future studies will provide the additional pieces required to complete the intricate molecular puzzle that underlies p53-mediated tumour suppression and will allow us to better therapeutically harness the power of this remarkable molecule.

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Box 1**Mutant p53 gain-of-function**

The abundance of *TP53* mutations found in human tumours underscores the importance of inactivating p53 during tumorigenesis. Most *TP53* mutations found in human tumours are missense mutations (80%) that reside within the DNA-binding domain (DBD), most often at six 'hot-spot' residues. These mutations are categorized into contact mutations that alter residues that are crucial for the interaction with DNA, and structural mutations that compromise the three-dimensional folding of the DBD¹⁴³ (FIG. 3a). Although *TP53* mutation clearly promotes tumorigenesis through the loss of wild-type p53 function, the retention of a mutant version of p53 is also thought to contribute to tumorigenesis. Mutant p53 not only exerts a dominant-negative effect on the wild-type protein but also displays gain-of-function (GOF) properties¹⁴⁴. This concept was originally proposed on the basis of cell culture studies in which tumour-derived p53 mutants were found to promote a host of behaviours that are characteristic of malignancy, including increased survival, proliferation, migration and invasion, among others¹⁴⁵. The GOF capacity of p53 mutants was solidified by analysis of knock-in mouse strains expressing either human or mouse equivalents of the p53^{R175H}, p53^{G245S}, p53^{R248W}, p53^{R248Q} and p53^{R273H} tumour mutants. Depending on the strain, these *Trp53*-mutant knock-in mice showed broader tumour spectra, shorter tumour latencies and/or increased frequencies of metastasis relative to *Trp53*^{-/-} mice, thereby highlighting the idea that mutant p53 actively promotes cancer^{3,4,146–149}. Given the GOF properties of p53 mutants, an interesting consideration is that specific human tumour-derived mutants, such as the p53^{R175P} and p53^{E180R} separation-of-function mutants, were actually selected for during human tumorigenesis because they have as yet undescribed GOF activities.

Several mechanisms have been proposed to account for the GOF activity of mutant p53 (REF. 150). For example, in spite of its compromised sequence-specific DNA-binding capability, mutant p53 may exert GOF effects through transcriptional regulation, by interacting with various other transcription factors, such as nuclear factor Y (NFY), vitamin D receptor (VDR), p63 and p73 (REFS 151–153). Interaction with other transcription factors can result in the recruitment of mutant p53 to the cognate sites for those factors, as well as inhibition or alteration in the DNA-binding specificity of these transcription factors, all of which can affect gene expression patterns^{154–156}. Mutant p53 can also interfere with DNA damage signalling via interactions with the MRE11–RAD50–NBS1 (Nijmegen breakage syndrome protein 1) complex⁴. Our growing understanding of the functional consequences of mutant p53 expression and the mechanisms that underlie the GOF phenotypes of p53 mutants may ultimately suggest new avenues for therapeutic intervention in advanced cancer.

Ultimately, the coordinate induction of multiple programmes by p53 suggests why it is advantageous for tumours to lose wild-type p53 activity, as many aspects of tumour suppression can be incapacitated with one fell swoop.

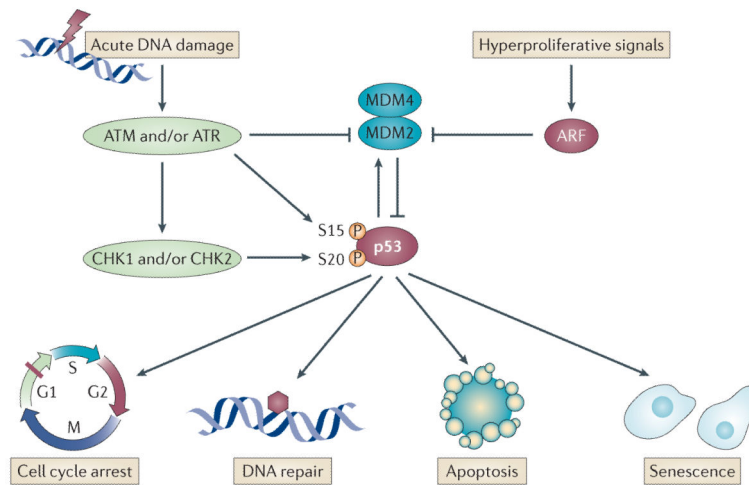


Figure 1. The classical view of p53 activation and response

The most well-elaborated molecular models for p53 activation are those in response to acute DNA damage signals and hyperproliferative signals. p53 induction by acute DNA damage begins when DNA double-strand breaks trigger activation of ataxia-telangiectasia mutated (ATM) — a kinase that phosphorylates the CHK2 kinase — or when stalled or collapsed DNA replication forks recruit ataxia telangiectasia and RAD3-related (ATR), which phosphorylates CHK1 (REF. 157). p53 is a substrate for both the ATM and ATR kinases, as well as for CHK1 and CHK2, which coordinately phosphorylate (P) p53 to promote its stabilization. Phosphorylation of p53 occurs at several sites, particularly at the amino-terminus, such as at serines 15 and 20 (REFS 158–160). These phosphorylation events are important for p53 stabilization, as some of the modifications disrupt the interaction between p53 and its negative regulators MDM2 and MDM4 (REFS 159,161–163). MDM2 and MDM4 bind to the transcriptional activation domains of p53, thereby inhibiting p53 transactivation function^{164–166}, and MDM2 has additional activity as an E3 ubiquitin ligase that causes proteasome-mediated degradation of p53 (REFS 167,168). Phosphorylation also allows the interaction of p53 with transcriptional cofactors, which is ultimately important for activation of target genes and for responses such as cell cycle arrest, DNA repair, apoptosis and senescence¹⁶⁹. Hyperproliferative signals similarly activate p53 through perturbation of the MDM2–p53 interaction. These signals can function by liberating the E2F transcription factor, which can stimulate transcription of the ARF tumour suppressor^{170,171}. ARF in turn inhibits MDM2 by antagonizing MDM2 ubiquitin ligase activity, and/or sequestering MDM2 to nucleoli^{172–175}. As a consequence, ARF activation enhances p53 stability and activity, promoting p53 responses such as apoptosis or cellular senescence¹⁷⁶.

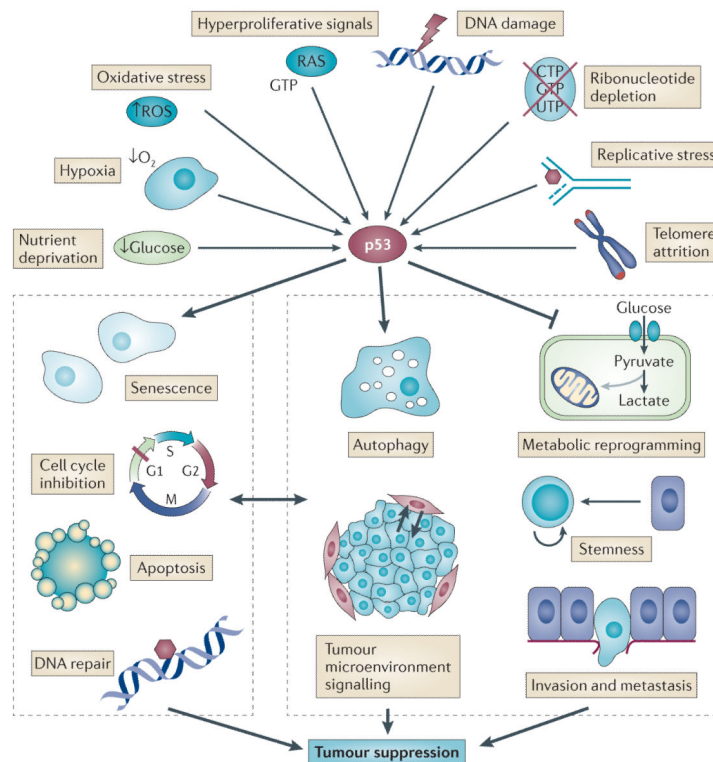


Figure 2. A revised view of p53-activating signals and responses that are important for tumour suppression

A host of different stresses can activate p53 in the context of tumour initiation or progression, including nutrient deprivation, hypoxia, oxidative stress, hyperproliferative signals (which could also promote chronic DNA damage or oxidative stress), DNA damage (which might most typically be chronic DNA damage triggered by replicative stress, telomere attrition, or oxidative stress), and ribonucleotide depletion. p53 activation by these signals, or potentially even ‘basal’ p53 action in some contexts, can consequently promote diverse responses that lead to tumour suppression. This view expands the set of stress signals that can activate p53 to promote responses of cell cycle arrest, senescence, apoptosis and DNA repair, which could potentially occur through pathways that are distinct from those used upon acute DNA damage^{29,87}. The revised view also suggests that, in addition to the ability of p53 to fully block cell cycle progression in response to a stress signal, basal p53 levels may also simply dampen the rate of progression through the cell cycle. Beyond triggering classical responses, p53 that is activated by various stress signals can modulate several additional cellular processes that are relevant to suppressing tumour development, including opposing oncogenic metabolic reprogramming and limiting the accumulation of reactive oxygen species (ROS), activating autophagy, promoting communication within the tumour microenvironment, inhibiting stem cell self-renewal and reprogramming of differentiated cells into stem cells, and restraining invasion and metastasis. Regulation of these processes by p53 may directly promote tumour suppression or may impinge on the canonical functions, such as apoptosis or senescence. For example, the inhibition of metabolic reprogramming by p53 may impede tumorigenesis by limiting proliferation or activating apoptosis, and the induction of autophagy may also suppress cancer by facilitating

apoptosis²⁷. Similarly, classical responses may affect novel functions. For example, p53-induced senescence precipitates signalling to the tumour microenvironment that ultimately provokes tumour suppression^{63,134}.

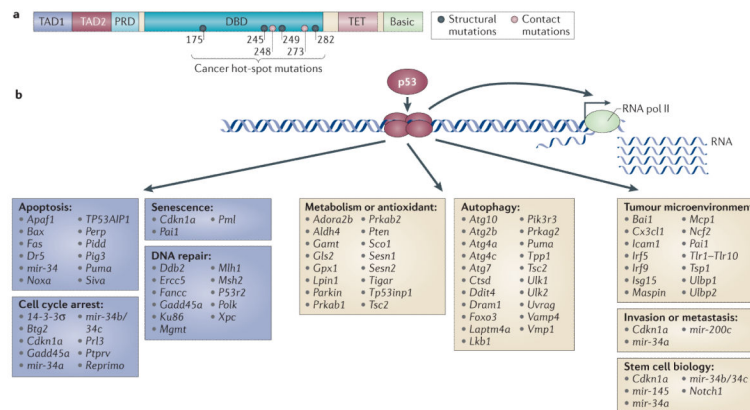


Figure 3. p53 suppresses cancer through transcriptional activation, by regulating diverse biological processes through transactivation of target genes

a | The p53 protein contains two amino-terminal transcriptional activation domains (TADs), a proline-rich domain (PRD), a DNA-binding domain (DBD), a tetramerization domain (TET) and a carboxy-terminal region that is rich in basic residues (Basic). Inactivation of p53 in human tumours typically occurs through missense mutations in the DBD of the p53 protein. Six common p53 ‘hot-spot’ mutations are categorized as either structural or contact p53 mutants, both of which disrupt the protein–DNA interaction and the transactivation of p53 target genes (see also BOX 1). **b** | Lists of key p53-induced target genes involved in processes that are important for tumour suppression, including the canonical p53-associated responses — apoptosis, cell cycle arrest, senescence and DNA repair (purple) — as well as processes that have recently been associated with p53-dependent tumour suppression, such as metabolism control, autophagy, tumour microenvironment crosstalk, invasion and metastasis, and stem cell biology^{2,13,27,133,177–185} (beige). The evidence for p53-dependent regulation of the genes on this list comes from mouse and/or human cells. Most of the genes are regulated by p53 in both human and mouse cells, but a few of the genes have currently only been identified and/or shown to be regulated by p53 in one of these species. *Adora2b*, adenosine A2b receptor; *Aldh4*, aldehyde dehydrogenase 9 family, member A1; *Apaf1*, apoptotic peptidase activating factor 1; *Atg*, autophagy related; *Bai1*, brain-specific angiogenesis inhibitor 1; *Bax*, BCL-2-associated X protein; *Btg2*, B cell translocation gene 2, anti-proliferative; *Cdkn1a*, cyclin-dependent kinase inhibitor 1A; *Ctsd*, cathepsin D; *Cx3cl1*, chemokine (C-X3-C motif) ligand 1; *Ddb2*, damage-specific DNA binding protein 2; *Ddit4*, DNA-damage-inducible transcript 4; *Dram1*, DNA-damage regulated autophagy modulator 1; *Ercc5*, excision repair cross-complementing rodent repair deficiency, complementation group 5; *Fance*, Fanconi anaemia, complementation group C; *Foxo3*, forkhead box O3; *Gadd45a*, growth arrest and DNA-damage-inducible 45a; *Gamt*, guanidinoacetate *N*-methyltransferase; *Glis2*, glutaminase 2; *Gpx1*, glutathione peroxidase 1; *Icam1*, intercellular adhesion molecule 1; *Irf*, interferon regulatory factor; *Laptm4a*, lysosomal protein transmembrane 4a; *Lkb1*, liver kinase B1 (also known as *Stk11*); *Lpin1*, lipin 1; *Mcp1*, monocyte chemoattractant protein 1 (also known as *Ccl2*); *Mgmt*, *O*-6-methylguanine–DNA methyltransferase; *Ncf2*, neutrophil cytosolic factor 2; *Pai1*, plasminogen activator inhibitor; *Perp*, p53 apoptosis effector; *Pig3*, p53 inducible protein 3 (also known as *Tp53i3*); *Pidd*, p53-induced death domain protein; *Pik3r3*,

phosphoinositide-3-kinase, regulatory subunit 3; *Pml*, promyelocytic leukaemia; *pol*, polymerase; *Polk*, DNA polymerase- κ ; *Prka*, protein kinase, AMP-activated; *Prkag2*, protein kinase, AMP-activated, γ 2 non-catalytic subunit; *Ptprv*, protein tyrosine phosphatase, receptor type, V; *Sesn*, sestrin; *Tigar*, TP53-induced glycolysis and apoptosis regulator; *Tlr*, Toll-like receptor; *TP53AIP1*, tumour protein p53 regulated apoptosis inducing protein 1; *Tp53inp1*, tumour protein p53 inducible nuclear protein 1; *Tpp1*, tripeptidyl peptidase I; *Tsc2*, tuberous sclerosis 2; *Tsp1*, thrombospondin 1; *Ulbp*, UL16 binding protein; *Ulk*, UNC-51 like autophagy activating kinase; *Uvrag*, UV radiation resistance associated; *Vamp4*, vesicle-associated membrane protein 4; *Vmp1*, vacuole membrane protein 1; *Xpc*, xeroderma pigmentosum, complementation group C.

Table 1

Functional capabilities of p53 mutants in knock-in strains or of wild-type p53 in target gene knockout mouse strains.*

	p53 ^{R172P}	p53 ^{E177R}	p53 ^{25,26}	p53 ^{53,54}	p53 ^{25,26,53,54}	p53 ^{3KR}
Apoptosis (DNA-damage induced)	-	-	-	+	-	-
Apoptosis (Non-genotoxic-stress induced)	-	-	+	ND	ND	ND
Cell cycle arrest (DNA-damage induced)	+/-	+	-	+	-	-
Senescence	+/-	+	+	+	-	-
Inhibition of proliferation	+	ND	ND	ND	ND	+/-
Other	Maintains genomic stability in tumour cells	<ul style="list-style-type: none"> Inhibits glycolysis Limits ROS accumulation 	ND	ND	ND	<ul style="list-style-type: none"> Inhibits glycolysis and glucose uptake Limits ROS accumulation
Transcriptional activity	<ul style="list-style-type: none"> Competent for transactivation of some genes, e.g. <i>Cdkn1a</i>, <i>Akl</i> and <i>Wig1</i> Deficient for transactivation of apoptosis genes 	<ul style="list-style-type: none"> Competent for transactivation of <i>Cdkn1a</i>, metabolism and antioxidant genes (e.g. <i>Gls2</i>, <i>Sesn1</i>, <i>Sesn2</i>, <i>Aldh4</i> and <i>Dram1</i>) Deficient for transactivation of apoptosis genes (e.g. <i>Bax</i>, <i>Puma</i> and <i>Noxa</i>) 	<ul style="list-style-type: none"> Competent for transactivation of certain genes: <i>Bax</i> and various new targets Deficient for transactivation of most classical genes (e.g. <i>Cdkn1a</i>, <i>Noxa</i>, <i>Puma</i> and <i>Perp</i>) 	Competent for transactivation of all genes	Deficient for transactivation of all genes	<ul style="list-style-type: none"> Competent for transactivation of some genes, e.g. <i>Mdm2</i>, <i>Gls2</i>, <i>Tigar</i> and <i>Gpx1</i> Deficient for transactivation of some genes, e.g. <i>Puma</i>, <i>Cdkn1a</i>, <i>Ccng1</i>, <i>Bax</i>, <i>Dr5</i>, <i>Noxa</i>, <i>Pml</i> and <i>Pai1</i>
Spontaneous tumour suppression	+/- Suppresses early thymic lymphomas but not other tumour types	+/- Suppresses early thymic lymphomas, but not other tumour types	+	ND	-	+
Induced tumour suppression	+/- <ul style="list-style-type: none"> Suppression of telomerase deficient tumours Partial Suppression of <i>Eμ-Myc</i> lymphomas No suppression of 	+/- <ul style="list-style-type: none"> Suppression of E1A;RAS MEF tumours Partial suppression of <i>Eμ-Myc</i> lymphomas 	+ Suppression of E1A;RAS MEF tumours; <i>Eμ-Myc</i> lymphomas; <i>Kras^{G12D}</i> NSCLCs; and <i>Ptc^{+/-}</i> MBs	+ Suppression of <i>Kras^{G12D}</i> NSCLCs	- No suppression of <i>Kras^{G12D}</i> NSCLCs and <i>Eμ-Myc</i> lymphomas	ND

	p53 ^{R172P}	p53 ^{E177R}	p53 ^{25,26}	p53 ^{53,54}	p53 ^{25,26,53,54}	p53 ^{3KR}
	DMBA-TPA-induced skin papillomas					
Refs	43,47,58,186	48	13,29,30	13	13,30	14

Akl, adenylate kinase 1; *Aldh4*, aldehyde dehydrogenase 9 family, member A1; *Bax*, BCL-2-associated X protein; *Ccng1*, cyclin G1; *Cdkn1a*, cyclin-dependent kinase inhibitor 1A; *Dram1*, DNA-damage regulated autophagy modulator 1; *Gls2*, glutaminase 2; *Gpx1*, glutathione peroxidase 1; MBs, medulloblastomas; MEF, mouse embryonic fibroblast; ND, not determined; NSCLC, non-small-cell lung cancer; *Pail*, plasminogen activator inhibitor; *Perp*, p53 apoptosis effector; *Pml*, promyelocytic leukaemia; *Ptc*, Patched; ROS, reactive oxygen species; *Sesn*, sestrin; Tigar, TP53-induced glycolysis and apoptosis regulator; *Wig1*, wild-type p53-induced gene 1.

*The “+” symbol indicates functional competency in a particular assay, similar to wild-type mice, whereas the “-” symbol indicates functional deficiency, similar to *Trp53*^{-/-} mice. “+/-” indicates partial activity.