Cell Death and Differentiation (2018) 25, 93–103

www.nature.com/cdd

REVIEW

Deconstructing networks of p53-mediated tumor suppression *in vivo*

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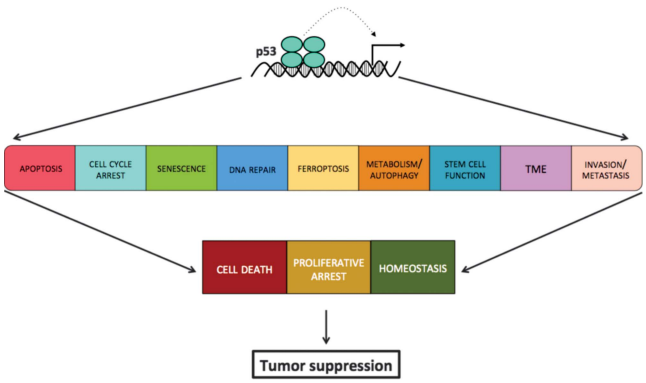
The transcription factor p53 is a vital tumor suppressor. Upon activation by diverse stresses including oncogene activation, DNA damage, hypoxia and nutrient deprivation, p53 activates a panoply of target genes and orchestrates numerous downstream responses that suppress tumorigenesis. Although early studies of p53 suggested that its ability to induce cell cycle arrest, senescence and apoptosis programs accounted for its tumor-suppressor activity, more recent studies have challenged this notion. Moreover, p53 regulates a suite of additional processes, such as metabolism, stem cell function, invasion and metastasis. The processes p53 coordinately regulates to enact tumor suppression, and how such regulation occurs, thus remain elusive. In this review, we will summarize our current knowledge of p53-mediated tumor-suppressive mechanisms gleaned from *in vivo* studies in mouse models.
Cell Death and Differentiation (2018) **25,** 93–103; doi:10.1038/cdd.2017.171; published online 3 November 2017



Graphical Abstract

Facts

- p53-mediated cell cycle arrest and apoptosis in response to acute DNA damage are dispensable for the suppression of diverse tumor types.
- Robust transactivation of the p53 target genes *Cdkn1a*, *Puma* and *Noxa* is not required for suppressing cancer in several mouse models.
- Non-canonical p53 functions, including regulating ferroptosis, metabolism, stem cell function and invasion, have been implicated in tumor suppression.

Open Questions

- Which p53 target genes are most important for mediating p53 tumor-suppressor function?
- Which are the critical cellular processes regulated by p53 during tumor suppression?
- What are the most relevant p53-activating stresses in incipient tumors *in vivo*?
- How does the stress and cell type affect which p53 responses are induced and contribute to tumor suppression?

Using Mouse Genetics to Understand p53-Mediated Tumor Suppression

The importance of tumor suppression by the p53 transcription factor is indisputable; over half of sporadic human cancers have mutations in the *TP53* gene, and >90% of Li–Fraumeni patients, who harbor a mutant allele of *TP53*, develop cancer in their lifetimes.[1–3] The critical role of p53 in tumor suppression was unequivocally shown, however, by experiments performed in mouse models. *Trp53* null mice develop cancer with complete penetrance in their first year of life.[4,5] These mice overwhelmingly develop T-cell lymphomas, although sarcomas are also observed. *Trp53* heterozygous mice are also predisposed to tumor development, but with longer latency than their *Trp53* null counterparts, and they predominantly succumb to sarcomas, some lymphomas, and occasionally carcinomas. The increased cancer susceptibility of $Trp53^{+/-}$ mice is reminiscent of those individuals with Li–Fraumeni syndrome who carry a mutant allele of *TP53*.[3,6] In addition, *Trp53* loss in numerous genetically engineered mouse models (GEMMs) for different cancer types accelerates tumor development.[7–10] Together, these observations provide key *in vivo* experimental evidence of the importance of p53 in tumor suppression.

Since these original observations, mouse models have continued to be instrumental for understanding p53 tumor-

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Received 06.6.17; revised 18.8.17; accepted 31.8.17; Edited by F Pentimalli; published online 03.11.17

a

b

Marker	Genotype	p53 mutation	p53 function	Phenotype	Reference
△	*Trp53*^{L25Q,W26S}	Mutations that inactivate TAD1 in reporter assays.	- Deficient in transactivation of many target genes, including classical targets such as *Cdkn1a*, *Puma*, and *Noxa*.	- p53-dependent apoptosis and cell cycle arrest in response to acute DNA damage are abrogated. - Suppresses lung adenocarcinoma, B- and T-cell lymphomas, fibrosarcoma, and medulloblastoma in mice. - Constitutive expression causes embryonic lethality.	18,19,23
△	*Trp53*^{P47S}	Single nucleotide polymorphism in TAD2 seen in some African populations.	- Deficient in transactivation of a subset of target genes, including *Gls2*, *Noxa*, and *Sco2*.	- Induction of ferroptosis in MEFs is reduced. - Mice display an increased rate of spontaneous cancer.	72
△	*Trp53*^{F53Q,F54S}	Mutations that inactivate TAD2 in reporter assays.	- Proficient in transactivation of classical p53 target genes.	- No compromised phenotype compared to wild-type mice.	18
△△	*Trp53*^{L25Q,W26S,F53Q,F54S}	Mutations that inactivate each TAD in reporter assays.	- Deficient in transactivation of all p53 target genes.	- Mice mirror the phenotypes of *Trp53*$^{-/-}$ mice, including no tumor suppressor activity.	18,23
△	*Trp53*^{K117R,K161R,K162R} (*Trp53*^{3KR})	Lysine to arginine mutations introduced block acetylation of 3 residues in the DBD.	- Deficient in transactivation of some genes, such as *Cdkn1a*, *Puma*, *Noxa*, *Bax*, *Pml*, and *Pai1*.	- p53-dependent apoptosis and cell cycle arrest in response to acute DNA damage are abrogated. - Replicative and oncogene-induced senescence are impaired. - Suppresses spontaneous tumor development in mice.	65
△	*Trp53*^{R172P}	Human tumor mutation in the DBD.	- Deficient in transactivation of apoptosis genes such as *Puma* and *Noxa*. - Proficient in activation of *Cdkn1a*.	- p53-dependent apoptosis is abrogated. - Cell cycle arrest and senescence responses are partially intact. - Maintains genomic stability in cancer cells. - Suppresses early onset T cell lymphomas but not other cancer types in mice.	42
△	*Trp53*^{R172H}	"Hotspot" human tumor mutation in the DBD.	- Incapable of binding DNA (conformational mutant).	- No tumor suppressor activity.	16,17
△	*Trp53*^{E177R}	Human tumor mutation in the DBD.	- Cooperative DNA binding is compromised. - Deficient in transactivation of apoptosis genes such as *Puma*, *Noxa*, and *Bax*. - Proficient in transactivation of some genes such as *Cdkn1a* and *Gls2*.	- p53-dependent apoptosis is abrogated. - Cell cycle arrest and senescence responses are intact. - Suppresses early onset T cell lymphomas but not other cancer types in mice.	43
△	*Trp53*^{R270H}	"Hotspot" human tumor mutation in the DBD.	- Incapable of binding DNA (contact mutant).	- No tumor suppressor activity.	16

Figure 1 p53 mutant mouse models. (**a**) Domain structure of p53. The arrowheads depict the location of mutations introduced into knock-in mouse models discussed in this review. PRD, proline-rich domain. Basic, basic amino-acid-rich region. (**b**) Descriptions of the mouse models with mutated forms of p53 discussed in this review

suppressor function. Although findings from cell culture models have defined p53 target genes, responses and signaling pathways, these models cannot directly address the contribution of these functions to tumor suppression *per se*, as these *in vitro* systems lack several key components that shape tumor behavior *in vivo* such as the 3D tissue architecture, the host immune system and the tumor microenvironment. To understand pathways and mechanisms of p53 action *in vivo*, numerous strains of mice harboring genetic alterations in *Trp53*, p53 regulators and p53 target genes have been generated and analyzed for tumor predisposition.[2,11] Collectively, these mouse model experiments have begun to reveal the mechanisms underlying p53 tumor-suppressor function.

Transcriptional Activation Function is Critical for Tumor Suppression

p53 is a transcription factor that can induce the expression of hundreds of target genes. In response to diverse stresses, including hyperproliferative signals, DNA damage, hypoxia and nutrient deprivation – all of which may be experienced by emerging cancer cells – p53 is activated by displacement from its negative regulators, Mdm2 and Mdm4.[12–14] Activated p53 can bind to consensus sites in the genome and induce transcription of a host of target genes to ultimately impede tumorigenesis.[1,2] In addition, transactivation-independent functions have been ascribed to p53, including promoting apoptosis through mitochondrial membrane permeabilization and directly repressing transcription.[15]

To define molecular mechanisms of p53 action in tumor suppression, several *Trp53* mutant knock-in mouse models were developed to interrogate the contribution of different p53 domains to cancer suppression (Figure 1). p53 has discrete domains critical for its function as a transcription factor (Figure 1a):[1] two amino-terminal transactivation domains (TADs), a sequence-specific DNA-binding domain (DBD) and a tetramerization domain (TET). Over 80% of *TP53* mutations found in human cancers are in the DBD, suggesting that p53 binding to and activating its transcriptional targets is essential for its tumor-suppressor function.[3] Two of the most common sites of mutations in human cancers (termed 'hotspots') are in the DBD at codons 175 and 273 and prohibit p53 from binding DNA.[3] Knock-in mice expressing *Trp53* mutated at the equivalent murine residues (*Trp53*^{R172H} and *Trp53*^{R270H}, respectively) were generated to assess how these DBD mutations affect tumorigenesis.[16,17] *Trp53*$^{+/M}$ mice ('M' designates either hotspot mutation) are just as susceptible to

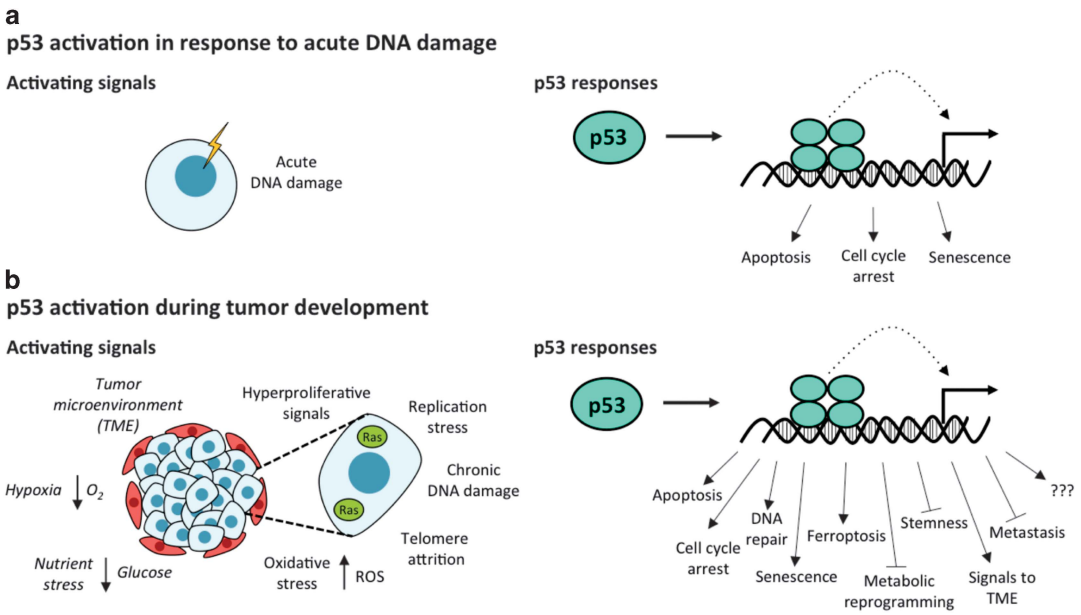

Figure 2 p53-activating stresses and responses. (**a**) The classical view of p53 can induce and response. In response to acute DNA damage, p53 can induce apoptosis, cell cycle arrest and senescence. These responses are dispensable for the suppression of numerous diverse tumor types. (**b**) A revised view of p53 activation and response during tumor development. A suite of stresses in the context of a developing tumor can activate p53. These stresses may be cell intrinsic or cell extrinsic. Cell-intrinsic stresses include hyperproliferative signals and chronic DNA damage arising from events such as replication stress, telomere attrition and oxidative stress. Cell-extrinsic stresses, denoted by italics, include signals from the tumor microenvironment (TME), such as poor oxygen and nutrient availability. In addition, p53 may be active in the 'basal' state to maintain homeostasis. Upon activation, p53 can function in numerous cellular pathways to oppose tumorigenesis. Which functions are activated by a given stress and contribute to tumor suppression is still under investigation

cancer as *Trp53*$^{+/-}$ mice, indicating that the introduced mutations incapacitate p53 tumor-suppressor function. These mutant mouse models thus demonstrated that the ability of p53 to bind DNA is essential for tumor suppression.

The necessity of the p53 DBD for tumor suppression suggests that transactivation is also essential for this biological function. To directly assess the contribution of each TAD to tumor suppression, a panel of p53 TAD mutant mouse strains was created.[18,19] Point mutations known to severely compromise TAD function *in vitro* were introduced into mice to create strains with mutations in TAD1 (*Trp53*^{L25Q,W26S}), TAD2 (*Trp53*^{F53Q,F54S}) or both TADs (*Trp53*^{L25Q,W26S,F53Q,F54S}).[20–22] Interestingly, mutating either TAD is not sufficient to compromise p53 tumor suppression, despite the fact that the p53^{L25Q,W26S} mutant (referred to hereafter as p53^{25,26}) is severely impaired in the transactivation of many p53 target genes, including *Cdkn1a*, *Puma* and *Noxa*. However, mutating both TADs renders the protein transcriptionally inert and unable to suppress tumorigenesis, demonstrating that p53 transactivation function is essential for tumor suppression.[18,23]

These mouse models have thus revealed the necessity of DNA binding and transactivation for effective tumor suppression. However, these findings do not address the downstream programs by which p53 suppresses cancer. Which responses and target genes are critical for tumor suppression? As we describe below, both knock-in mice with *Trp53* mutations and p53 target gene knockout mice have helped to begin to delineate the contribution of specific p53 responses to its tumor-suppressor function (Figure 1b).

'Classical' p53 Functions

The earliest biological outcomes ascribed to p53 were the induction of cell cycle arrest, senescence and apoptosis, the so-called 'classical' p53 responses. p53 was initially discovered to block proliferation by inducing a transient G1 cell cycle arrest in response to acute DNA damage (Figure 2a), which could allow cells time to pause and resolve any sustained damage that could promote cancer development, a role known as 'guardian of the genome'.[24–26] p53 can halt cell cycle progression in response to DNA damage by inducing transcription of *Cdkn1a*, which encodes the cyclin-dependent kinase inhibitor p21, although additional target genes such as *Gadd45a* can contribute to p53-mediated cell cycle arrest.[27–31] In addition to inhibiting proliferation, p53 can induce apoptosis in response to DNA damage and hyperproliferative signals,[32–34] a response envisioned to prevent tumorigenesis by removing damaged or inappropriately proliferating cells. p53 can trigger apoptosis through the direct induction of target genes, including pro-apoptotic Bcl-2 family members such as *Bax*, *Puma* and *Noxa*.[35,36] As described below, multiple mouse models have helped to define the contribution of these classical responses to tumor suppression.

The role of cell cycle arrest/senescence in tumor suppression. The contribution of cell cycle arrest to tumor suppression was investigated initially using knockout mice lacking p53 target genes. Surprisingly, *Cdkn1a*$^{-/-}$ mice do not display early-onset tumorigenesis seen in *Trp53*$^{+/-}$ and

Trp53$^{-/-}$ mice, although a mild cancer susceptibility is observed beginning at 16 months,[28,37] suggesting that p21 loss does not significantly compromise p53-mediated suppression of spontaneous tumors. Similarly, deletion of other p53 target genes that promote cell cycle arrest *in vivo*, including *Gadd45a* and *Ptprv*, does not enhance spontaneous tumorigenesis.[29,31] However, loss of these cell cycle regulators can augment tumor development in specific contexts. For example, p21 deficiency cooperates with oncogenic Ras to promote mammary tumorigenesis[38] and sarcoma formation,[39] and *Gadd45a* loss accelerates Ras-driven mammary tumor formation.[40,41] Although these studies demonstrate the importance of p53 target genes encoding cell cycle regulators in tumor suppression, they do not show that direct activation of these genes by p53 is necessary for tumor suppression.

Knock-in mouse strains expressing mutant p53 variants have also implicated cell cycle arrest in p53-mediated tumor suppression. One such strain – the *Trp53*^{R172P} strain (also known as *Trp53*^{515C}) – harbors a human tumor-derived mutation at the orthologous murine residue.[42] p53^{R172P} is defective in mounting an apoptotic response to DNA damage and oncogene activation, but retains partial activity in irradiation-induced cell cycle arrest. Another model – the *Trp53*^{E177R} mouse strain – similarly expresses a mouse analog of a human tumor-derived *TP53* mutant that cannot induce apoptosis but can induce cell cycle arrest and some metabolic responses.[43] Despite being compromised in apoptosis, p53^{R172P} or p53^{E177R} suffice to partially protect mice from early-onset lymphomas that characterize *Trp53*$^{-/-}$ mice, suggesting that cell cycle arrest contributes to suppressing early-onset spontaneous tumors. Indeed, *Trp53*$^{R172P/R172P}$*;Cdkn1a*$^{-/-}$ mice display accelerated tumor onset compared with *Trp53*$^{R172P/R172P}$ mice,[44] suggesting that p21 and cell cycle arrest are important for tumor suppression in *Trp53*^{R172P} mice.

Inducing a transient proliferative arrest can be beneficial if cells restore homeostasis and repair oncogenic lesions. However, at times cells may need to be permanently restrained, which p53 achieves by inducing senescence, an irreversible cell cycle arrest. p53 activates transcription of multiple genes involved in senescence, including *Cdkn1a*, *Pml* and *Pai1*.[2] Several *in vivo* studies have correlated senescence and p53-mediated tumor suppression. For example, late-generation *Terc*$^{-/-}$ mice lack the RNA component of telomerase and exhibit telomere erosion and DNA damage signaling.[45] These mice are cancer prone, and *Trp53* deficiency accelerates cancer-associated mortality. *Terc*$^{-/-}$*;Trp53*$^{R172P/R172P}$ and *Terc*$^{-/-}$*;Trp53*$^{+/+}$ mice are protected from tumor development to a similar extent, and tumor suppression is accompanied by senescence *in vivo*.[46] Similarly, in B-cell lymphomas driven by the *Eμ-Myc* transgene, tumor suppression by p53^{R172P} is associated with the activation of cellular senescence markers.[47] Wild-type p53 is associated with senescence in several tumor models, including murine models of non-small cell lung cancer and prostate cancer.[48,49] For example, in a murine prostate cancer model, prostates in *Pten*$^{-/-}$ mice display p53-dependent growth arrest and SA-βgal positivity, whereas prostates in *Pten*$^{-/-}$*;Trp53*$^{-/-}$ mice are more proliferative and lack signs of senescence.[50] These *Pten*$^{-/-}$*;Trp53*$^{-/-}$ mice display a drastically reduced tumor latency, suggesting that p53-mediated senescence constrains tumor development.

Apoptosis contributes to tumor suppression. Initial evidence implicating the p53 apoptotic response in tumor suppression came from studies of oncogene-expressing fibroblast tumor xenografts and a choroid plexus epithelial tumor GEMM.[51–53] In the latter model, highly apoptotic tumors arise after inactivation of retinoblastoma family proteins, and *Trp53* inactivation diminishes tumor cell apoptosis and accelerates tumor growth. Moreover, deletion of the pro-apoptotic p53 target gene *Bax* accelerates tumor development, directly implicating apoptosis as a tumor-suppressive mechanism in this model.[52] Similarly, in *Eμ-Myc*-driven B-cell lymphomas, p53 induces significant apoptosis.[7] Genetically blocking apoptosis (i.e., through Bcl-2 overexpression) mimics the phenotype of *Trp53* loss in this model, suggesting that apoptosis is a primary mechanism by which p53 suppresses lymphomagenesis.[54] In addition, loss of the p53 pro-apoptotic targets *Bax, Puma,* or *Puma* and *Noxa*, accelerates tumor development.[35,55–57] However, tumor latency in *Eμ-Myc;Puma*$^{-/-}$*;Noxa*$^{-/-}$ mice is longer than that in *Eμ-Myc;p53*$^{+/-}$ mice, suggesting that p53 functions other than apoptosis contribute to tumor suppression in this model.[35] Notably, the importance of p53-dependent apoptosis has not been well characterized in epithelial cancers, although loss of the p53 target gene *Perp* does compromise ultraviolet B-induced apoptosis and enhance UVB-induced skin carcinogenesis in mice.[58] Again, an important consideration with experiments examining p53 target gene knockout mice is that p53-independent activation of these genes could contribute to tumor suppression. Nonetheless, these *in vivo* studies collectively suggest that p53-mediated apoptosis contributes to suppression of several tumor types.

The Acute DNA Damage Response is Dispensable for Tumor Suppression

The aforementioned studies implicated the p53 responses of cell cycle arrest, senescence, and apoptosis as essential for p53-mediated tumor suppression, and given the critical, well-established role of p53 downstream of acute DNA damage signals, it was envisioned that the p53 responses to acute genotoxic stress mediated its tumor-suppressor function (Figure 2a). This paradigm was strengthened by findings that the DNA damage response is engaged during incipient tumor development: nascent tumors frequently display markers of DNA damage signaling, including γH2AX, pCHK2 and pATM, and often express p53.[59,60]

This model has been overturned as multiple studies challenged the importance of p53 acute DNA damage responses for tumor suppression. The initial evidence came from mouse models where p53 activation was temporally regulated after DNA damage. In one model, mice expressing a tamoxifen-inducible p53–estrogen receptor fusion protein were used to study when p53 activity is required to suppress lymphomagenesis induced by ionizing radiation.[61,62] Restoring p53 activity concurrently with irradiation induced widespread p53-dependent apoptosis, but this response offered no

protection from cancer development compared with mice without p53 restoration. Interestingly, activating p53 8 days after irradiation did not induce any detectable apoptosis but extended lymphoma-free survival. In a separate study, *Trp53* was deleted using a conditional allele before, concurrent with, or after whole-body irradiation[63] Notably, the presence of p53 at the time of DNA damage offered no protection from tumorigenesis. Finally, mice deficient for $p19^{Arf}$, an upstream regulator of p53, displayed accelerated development of DNA damage-induced fibrosarcomas, despite the fact that p53 responds normally to DNA damage in cells derived from $p19^{Arf\,-/-}$ mice.[64] These *in vivo* observations provided the first evidence that p53 responses to acute DNA damage are dispensable for the suppression of at least some cancers.

Further studies have extended this conclusion to a broad range of cancer types. As mentioned above, $p53^{25,26}$ is severely compromised in the transactivation of many target genes, including canonical targets like *Cdkn1a* and *Puma*, which mediate cell cycle arrest and apoptosis.[18,19] As a result, various cell types, including those of the thymus and small intestine, in $Trp53^{25,26}$ mice fail to undergo apoptosis in response to γ-irradiation *in vivo*. Furthermore, $p53^{25,26}$ MEFs cannot undergo cell cycle arrest when treated with DNA-damaging agents. However, $p53^{25,26}$ robustly suppresses various cancer types, including lung adenocarcinoma, medulloblastoma, fibrosarcoma, and T- and B-cell lymphomas.[18,23] The studies of the $p53^{25,26}$ mice elaborate the idea that p53 acute DNA damage responses are dispensable for tumor suppression by demonstrating that they are expendable for the suppression of numerous, diverse tumor types arising from different tissues. In addition, studies of $p53^{25,26}$ have uncovered transcriptional programs important for tumor suppression. Although robust transactivation of canonical p53 targets is not required for tumor suppression, $p53^{25,26}$ activates transcription of a set of primarily novel p53 target genes. Further characterization of these genes will help to elucidate the transcriptional programs required for tumor suppression.

Support for the conclusions derived from studies of the $p53^{25,26}$ mice came from knock-in mice expressing $p53^{3KR}$, in which lysines 117, 161 and 162 were mutated to arginine to block acetylation.[65] These mutations disrupt the ability of p53 to induce select target genes, including *Cdkn1a* and *Puma*, and as a consequence, $p53^{3KR}$ cannot induce cell cycle arrest or apoptosis in MEFs and thymocytes, respectively, in response to irradiation. In addition, senescence is impaired in $p53^{3KR}$-expressing MEFs. However, $p53^{3KR}$ can still transactivate some metabolic targets like *Tigar* and *Gls2* and suppress both spontaneous tumor formation and pro-B-cell lymphomas in $Xrcc4^{-/-}$ mice, furthering the notion that p53 responses to DNA damage are not essential for tumor suppression.[66]

Although studies of $p53^{25,26}$ and $p53^{3KR}$ suggest that potent transcriptional activation of the canonical target genes *Cdkn1a*, *Puma* and *Noxa* is dispensable for p53-mediated tumor suppression, residual activation of these genes by these mutants could be contributing to suppressing cancer development. To investigate this possibility, $Cdkn1a^{-/-}$;$Puma^{-/-}$;$Noxa^{-/-}$ mice were generated.[67] Similar to cells derived from $p53^{25,26}$ and $p53^{3KR}$ mice, thymocytes from this triple knockout strain were deficient in p53-dependent apoptosis triggered by DNA-damaging agents. In addition, T lymphocytes did not arrest in G1, and dermal fibroblasts displayed impaired senescence after DNA-damaging agent treatment. $Cdkn1a^{-/-}$;$Puma^{-/-}$;$Noxa^{-/-}$ mice were not predisposed to spontaneous tumor development, supporting the idea that these three major mediators of the p53 acute DNA damage response are not required for p53-dependent suppression of spontaneous tumorigenesis. *Puma* and *Noxa* are, however, required for robust suppression of *Eμ-Myc* lymphomas, suggesting that the mechanisms of p53-mediated tumor suppression are context dependent.[35]

Together, these mouse models demonstrate that canonical p53 target genes and responses downstream of acute DNA damage are dispensable for tumor suppression. Yet these studies beg more questions than they answer. How do we reconcile prior data showing the importance of cell cycle arrest, senescence and apoptosis for tumor suppression with the data just described?

Reconciling Old and New Studies

Although studies of $Trp53^{25,26}$, $Trp53^{3KR}$, and $Cdkn1a^{-/-}$;$Puma^{-/-}$;$Noxa^{-/-}$ mice have revealed that p53 responses to acute DNA damage are dispensable for tumor suppression, we should not conclude that cell cycle arrest and apoptosis are unimportant to tumor suppression. Rather, these studies suggest that the p53 pathways mapped by studying acute DNA damage responses are dispensable for tumor suppression. Indeed, it seems logical that p53 is engaged by stresses other than acute DNA damage in the context of tumor suppression, such as oncogene activation, nutrient and oxygen deprivation, and chronic, low-level DNA damage (Figure 2b).[2,12,13] Supporting the notion that apoptosis triggered by non-genotoxic stresses could be relevant for tumor suppression is the observation that the tumor suppression-competent $p53^{25,26}$ mutant can induce apoptosis in response to hypoxia and serum starvation.[19] In addition, the cellular response to chronic DNA damage is mechanistically distinct from that to acute DNA damage, and therefore the response of p53 to chronic DNA damage may be relevant for tumor suppression.[68] It is thus plausible that cell cycle arrest and/or apoptosis triggered by the diverse stresses present in incipient tumors could account for p53 tumor-suppressor function, via downstream pathways distinct from those involved in acute DNA damage responses. Alternatively, DNA damage responses could appear dispensable for p53-mediated tumor suppression because compensatory mechanisms exist in $Trp53^{25,26}$, $Trp53^{3KR}$, and $Cdkn1a^{-/-}$;$Puma^{-/-}$;$Noxa^{-/-}$ mice that allow for tumor suppression.

Although these three mouse models have not proven that cell cycle arrest and apoptosis as a whole are dispensable for tumor suppression, they have prompted the exploration of alternative transcriptional programs and cellular processes that could mediate tumor suppression downstream of p53, which we will describe next (Figure 3).

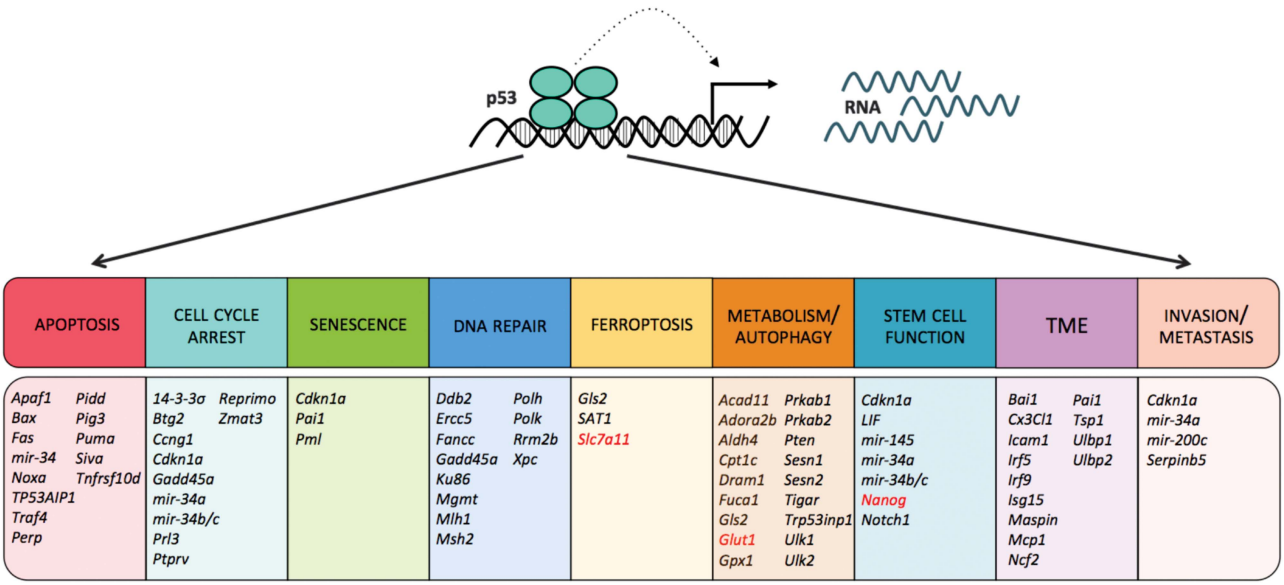

Figure 3 p53 suppresses cancer development through transcriptional regulation of target genes that regulate diverse cellular processes. p53 has been proposed to regulate a plethora of functions including apoptosis, cell cycle arrest, senescence, DNA repair, ferroptosis, metabolism, autophagy, stem cell function, invasion and metastasis. In addition, p53 is proposed to have non-cell autonomous functions that allow communication within the tumor microenvironemt (TME). Multiple target genes have been implicated in each response, and those that are activated by p53 are indicated in black. Of note, p53 has also been suggested to act as a transcriptional repressor. Select genes to which p53 binds and represses transcription are indicated in red. The murine gene annotation is shown unless the gene has no mouse ortholog

Emerging p53 Functions

Ferroptosis as a new mechanism of tumor suppression. A recent function ascribed to p53 is the regulation of ferroptosis, an iron-dependent, non-apoptotic form of cell death.[69] Ferroptosis is triggered by the inhibition of cystine uptake, which depletes glutathione levels and leads to the iron-dependent accumulation of reactive oxygen species (ROS) that trigger cell death.[70] The first study to suggest that p53 regulates ferroptosis showed that p53 directly represses transcription of *SLC7A11,* a component of the cystine/glutamate transporter whose downregulation reduces cystine uptake and primes cells for ferroptosis.[71] p53 has since been reported to activate the expression of genes involved in ferroptosis, including *Gls2* and *SAT1*.[72,73] Notably, although deficient for some p53 functions, the tumor suppression-competent $p53^{3KR}$ mutant retains the ability to repress *SLC7A11* and to induce ferroptosis.[71] Mutating a fourth acetylation site in p53 lysine 98 to create a $p53^{4KR}$ mutant abolishes *SLC7A11* repression, ferroptosis, and tumor suppression in a xenograft model,[74] further correlating ferroptosis with p53-mediated tumor suppression. *SLC7A11* overexpression enhances the growth of xenograft tumors expressing $p53^{3KR}$, but not *p53*-null tumors, suggesting that ferroptosis could be important for tumor suppression. An additional correlation between ferroptosis and tumor suppression came from knock-in mice expressing $p53^{P47S}$, a *TP53* polymorphic variant enriched in African and African–American populations.[72] $p53^{P47S}$ is impaired in regulating select p53 target genes, including the ferroptosis genes *SLC7A11* and *Gls2*, and in triggering ferroptosis. $Trp53^{P47S}$ mice are susceptible to spontaneous tumorigenesis, again linking defective ferroptosis and tumorigenesis. However, the finding that p53 induces ferroptosis, and that this activity contributes to tumor suppression, is challenged by a recent study which suggests that p53 antagonizes ferroptosis in colorectal cancer cell lines.[75] It will be interesting to assess how context affects p53 regulation of ferroptosis and to extend these studies to various GEMMs.

p53 regulates stemness. Another emerging function of p53 is in regulating stem cell function and differentiation. Initial observations suggested a potential role for p53 in embryonic stem cells, where p53 promotes differentiation after DNA damage by directly suppressing *Nanog* expression.[76] The importance of p53 in inhibiting 'stemness' was bolstered by the discovery that p53 suppresses reprogramming of somatic cells into induced pluripotent stem cells.[77–81] p53 promotes maintenance of the differentiated state at least in part by inducing *Cdkn1a* and subsequent cell cycle arrest. In addition, by inducing transcription of the microRNA *miR-34a*, p53 can indirectly repress pluripotency genes such as *Nanog* to restrain somatic cell reprogramming.[82] These studies helped to establish the general paradigm that p53 opposes stemness and promotes differentiation. These functions could contribute to tumor suppression by promoting a more differentiated, less plastic phenotype in incipient cancer cells or by limiting the proliferation of cancer stem cells.

A role for p53 in regulating cancer stem cells could reflect a physiological role in maintaining normal stem cell homeostasis to limit the inappropriate expansion of these cell populations. Indeed, the importance of p53 for stem cell homeostasis *in vivo* was shown by studies in which *Trp53* loss promoted the expansion and blocked the differentiation of stem cell populations. For example, p53 limits the expansion of hematopoietic stem cells (HSCs), as $Trp53^{-/-}$ mice have approximately two- to threefold more HSCs than wild-type

mice.[83] In addition, the mammary epithelium of *Trp53*$^{-/-}$ null mice displays increased numbers of mammary stem cells.[84] Finally, *Trp53* loss in the airway epithelium promotes the expansion of club cell populations and dampens differentiation into ciliated cells.[85] Similarly, p53 limits the proliferation and promotes the differentiation of cancer stem cells. *Trp53* inactivation in a mouse model of acute myeloid leukemia promotes expansion of leukemia-initiating myeloid progenitor cells.[86] In addition, analysis of murine glioblastomas driven by *Trp53* and *Pten* inactivation suggests that these tumor suppressors cooperate to restrict neural stem cell self-renewal and promote differentiation.[87] Future *in vivo* investigations will reveal how p53 regulates both normal and cancer stem cell function and how this activity contributes to tumor suppression.

p53 controls diverse aspects of metabolism. To fuel their enhanced growth and proliferation, neoplastic cells undergo metabolic reprogramming, typically by enhancing glycolytic function and dampening oxidative phosphorylation.[88] Although the role of p53 in metabolism is complex, p53 is thought to oppose this reprogramming. p53 limits glycolysis and enhances oxidative phosphorylation through mechanisms such as restricting glucose uptake by *Glut1* repression and modulating key TCA cycle enzymes via transactivation of genes like *Sco2.*

How does this transcriptional response contribute to tumor suppression *in vivo*? Interestingly, in several mouse models, transactivation of metabolic targets correlates with tumor suppression. In *Trp53*^{3KR} mice, where suppression of spontaneous tumors is intact, p53 induction of certain metabolic target genes like *Tigar* is preserved.[65] In models where transactivation of metabolic targets like *Gls2* is compromised, such as *Trp53*^{P47S} mice and p53^{4KR}-expressing cells, p53-mediated tumor suppression is defective.[72,74] These observations establish a correlation between the activation of some p53 metabolic target genes and tumor suppression. However, direct evidence that these genes contribute to tumor suppression is lacking, and in some cases is contradictory. For example, *Tigar*$^{-/-}$ mice display less intestinal adenoma burden than *Tigar*$^{+/+}$mice.[89] Furthermore, p53 targets such as *Cpt1c* and *Acad11* promote tumor cell survival under metabolic stress.[90,91] Perhaps individual target gene ablation does not mimic its deregulation in cancer; alterations in multiple targets may be required to compromise tumor suppression. It is also possible that regulation of some metabolic genes by p53 may be most important in untransformed cells and serve to maintain homeostasis.

In addition to altering patterns of fuel metabolism, cancer cells experience an increase in ROS, which can promote protumorigenic events such as proliferation.[92] p53 exerts antioxidative effects by driving expression of target genes including *Sesn1*, *Gls2* and *Trp53inp1*.[2] Interestingly, loss of *Trp53inp1*, which encodes a nuclear protein that promotes an antioxidant response, accelerates spontaneous tumor development in *Trp53*$^{+/-}$ mice and pancreatic cancer in a *Kras*^{G12D}-driven GEMM.[93,94] Although the contribution of other antioxidant target genes to p53-mediated tumor suppression has not been rigorously tested *in vivo*, the importance of the antioxidant response as a whole has been demonstrated. Treating *Trp53*$^{-/-}$ mice with the antioxidant *N*-acetylcysteine significantly delayed spontaneous lymphoma development.[95] These data suggest that the p53 antioxidant response contributes to tumor suppression. However, as with p53 regulation of fuel metabolism, the contribution of the p53 antioxidant response to tumor suppression is complex. For instance, p53 preserves antioxidant capacity and thus promotes viability in cancer cell lines experiencing serine deprivation.[88] However, this response could potentially promote homeostasis and prevent incipient tumor development in noncancerous cells.

Another process critical for metabolic homeostasis is autophagy, a cellular process through which cytoplasmic components are degraded in the lysosome.[96] By eliminating damaged proteins and organelles, autophagy promotes cellular integrity and survival, as well as mobilizing energy during nutrient deprivation. p53 can induce autophagy through transcriptional activation of genes such as *Ulk1, Ulk2* and various *Atg* genes.[97] Studies in oncogene-expressing fibroblasts demonstrated that loss of autophagy promotes transformation, and autophagy deficiency in lung and pancreatic tumors enhances tumor initiation, suggesting that autophagy may be tumor suppressive.[97–100] However, the role of autophagy in cancer is complex, as it can also promote tumor progression.[96] Thus, further studies are needed to fully understand how p53-activated autophagy impacts tumor development.

p53 maintains genomic integrity. By inducing a temporary G1 arrest upon DNA damage, p53 allots cells time to repair potentially oncogenic mutations in their genomes.[2] p53 can additionally directly stimulate DNA repair, including base excision repair, nucleotide excision repair, and mismatch repair, to maintain genomic integrity. Several p53 target genes, including *Ddb2*, *Gadd45a*, *Mlh1* and *Xpc,* encode proteins that participate in DNA repair. Mouse strains lacking such genes, including *Xpc*$^{-/-}$*, Gadd45a*$^{-/-}$ and *Ddb2*$^{-/-}$ *mice,* are tumor-prone, suggesting that activation of DNA repair pathways could contribute to p53-mediated tumor suppression.[40,41,101,102–104] Additional support for a role of DNA repair in tumor suppression comes from *Cdkn1a*$^{-/-}$*; Puma*$^{-/-}$*;Noxa*$^{-/-}$ mice.[67] Fibroblasts from these mice rapidly resolve DNA damage after irradiation, similarly to wild-type fibroblasts but unlike *Trp53*$^{-/-}$ fibroblasts, thus correlating intact DNA repair with p53-mediated tumor suppression.

p53 may also have a role in maintaining genomic stability at the chromosomal level. Abnormalities in chromosome number and structure are hallmarks of cancer, and the loss of *Trp53* permits propagation of polyploid and aneuploid cells.[105,106] Whether this instability observed in the absence of p53 is a cause or consequence of malignant progression is debated, and only correlative data are available from mouse models. For example, *Trp53*$^{R172P/R172P}$ mice develop late-onset tumors that are typically diploid or tetraploid, whereas *Trp53*$^{-/-}$ mice develop tumors that are almost exclusively aneuploid.[42] In addition, spontaneous T-cell lymphomas arising in *Trp53*$^{-/-}$ mice display numerous copy number alterations in putative driver mutant alleles.[107] Overall, data from mouse models suggest that intact p53 correlates with

enhanced genomic integrity, but the contribution of this response to tumor suppression warrants further investigation.

p53 opposes invasion and metastasis in cancer. p53 is proposed to impede malignant progression by inhibiting tumor cell invasion and metastasis.[108] *In vitro,* p53 restricts the motility and invasiveness of diverse primary and cancer cell types. In addition, p53 opposes epithelial-to-mesenchymal (EMT), which is associated with the dissolution of cell–cell adhesion complexes and metastasis. In human cancer cell lines, p53 can indirectly downregulate key EMT transcription factors such as Snail1, Slug and Zeb1 by inducing their negative regulators.[109–111]

Despite this *in vitro* evidence, understanding how p53 loss contributes to invasion and metastasis *in vivo* has proven challenging. Sarcomas and carcinomas that arise in *Trp53*$^{-/-}$ and *Trp53*$^{+/-}$ mice rarely metastasize, suggesting that *Trp53* loss alone is not sufficient to induce metastasis. Supporting this conclusion are data from a *Kras*^{G12D}-driven lung adenocarcinoma GEMM: although *Trp53* deletion promotes tumor cell dissemination and metastasis, only some mice display these phenotypes, suggesting that *Trp53* loss is not sufficient to initiate dissemination and metastasis but rather allows for stochastic events to initiate metastasis.[9,112] Ultimately, it will be important to understand how the effects of p53 loss on cell motility and invasion *in vitro* contribute to metastasis *in vivo*.

Non-cell autonomous functions of p53. Beyond cell autonomous functions, p53 can stimulate an antitumorigenic microenvironment through non-cell autonomous mechanisms. For example, p53 can oppose angiogenesis by transactivating *Tsp1*, which encodes a secreted angiogenesis inhibitor.[113] Moreover, p53 can affect the immune response by triggering expression of genes involved in immune cell recruitment and surveillance. p53 reactivation in liver carcinomas elicits transcription of inflammatory cytokines such as *Csf1* and *Il15*, which promote recruitment of macrophages, neutrophils and natural killer cells and ultimately tumor clearance.[114] p53 also promotes an anti-tumor microenvironment in hepatocellular carcinoma by inducing senescence of hepatic stellate cells, which subsequently release factors that trigger an antitumorigenic, M1 macrophage phenotype that enhances tumor cell clearance.[115] p53-deficient hepatic stellate cells, meanwhile, skew macrophages to a tumor-promoting M2 phenotype. Finally, p53 also induces *DD1α* to promote macrophage-mediated clearance of apoptotic cells, thereby limiting inflammation in the tumor microenvironment.[116]

p53 activation in the tumor stroma can also engage tumor-suppressive responses. *TP53* mutations are frequently detected in the stroma of human tumors, suggesting that a selective pressure to inactivate *TP53* in stromal cells exists.[117,118] Indeed, inactivating the retinoblastoma family of tumor suppressors in the murine prostate epithelium triggers a paracrine p53-mediated proliferative arrest in the surrounding stromal fibroblasts and selects for *Trp53* mutations.[119] The advantage of p53 deficiency in the stroma is illustrated by experiments in which MCF7 breast cancer cells generate tumors at a faster rate after injection into *Trp53*$^{-/-}$ mice than in *Trp53*$^{+/+}$ mice.[120] These data argue the importance of non-cell autonomous functions of p53 in suppressing tumor development.

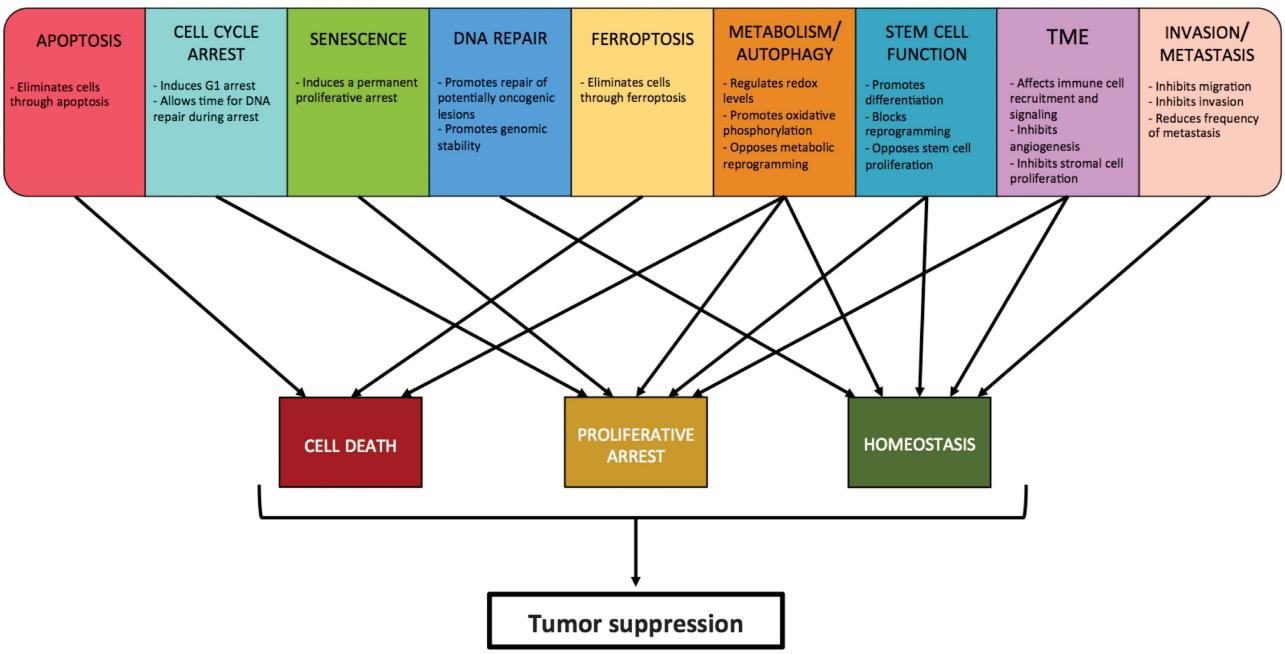

Figure 4 Diverse p53-regulated processes lead to common outcomes. Although p53 regulates many cellular processes (top), they all likely impinge upon the common outcomes of limiting cellular proliferation, through effects on both cell survival and cell division, and maintaining homeostasis. Regulating proliferation and promoting cellular homeostasis can lead to tumor suppression. Multiple p53 functions can potentially contribute to each of these tumor-suppressive outcomes, as indicated by arrows

Reconstructing p53-mediated tumor suppression

In its capacity as a tumor suppressor, p53 must inhibit the propagation of cells with cancer-promoting alterations. Thus, the ability of p53 to impede proliferation by directly inducing cell cycle arrest and promoting cell death provides clear mechanisms by which it could suppress tumorigenesis. Indeed, numerous *in vivo* studies have unequivocally shown that abrogating p53-mediated cell cycle arrest or apoptosis in specific tumor models accelerates tumorigenesis. However, we now appreciate that p53 regulates additional aspects of cellular behavior, including metabolism, migration, differentiation and DNA repair.

Importantly, these diverse responses ultimately lead to common outcomes of affecting cell division, cell viability and cellular homeostasis, which all contribute to blocking tumorigenesis (Figure 4). For instance, by triggering metabolic changes and promoting DNA repair, p53 can indirectly affect cell division and cell survival. Canonical and non-canonical p53 responses thus integrate to produce these common outcomes to quell tumor development. p53-mediated tumor suppression thus likely reflects the combined effects of activation of numerous transcriptional programs and consequent regulation of various aspects of cellular behavior. The prevalence of *TP53* mutations in cancer supports this hypothesis; as a *TP53* mutation has profound effects on the cell, it allows many tumor-suppressive mechanisms to be simultaneously eliminated. In contrast, individual p53 target genes are not commonly found mutated, presumably because their mutation has more minimal effects than a *TP53* mutation.

It remains a challenge to clearly elucidate the set of p53 target genes and downstream responses that contribute to tumor suppression. The list of such genes and responses is still growing, and the importance of only a small fraction has been interrogated *in vivo*. Furthermore, no single or combinatorial deletion of p53 target genes has recapitulated loss of p53 itself, and therefore strategies to assess the collaborative actions of many target genes and responses must be developed. Adding to the challenge is the fact that mechanisms critical for tumor suppression may be context dependent. Studying p53 responses in relevant cancer models, and in particular epithelial models that comprise the majority of human cancers, will be critical in future to highlight mechanisms fundamental for tumor suppression.

Unraveling the details of how p53 suppresses cancer is critical both for understanding tumor development and improving cancer treatment. By delineating pathways downstream of p53 that suppress cancer, we will establish more options and targets for therapeutic interventions. Restoring p53 function in human tumors has proven to be challenging, and targeting downstream pathways may be a more feasible option for attacking p53-mutant tumors. Further interrogation of p53 target genes and cellular responses in mouse models will illuminate how this powerful protein thwarts tumor development and ultimately lead to therapeutic advances.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. We thank Kathryn Bieging-Rolett and Brittany Flowers for critical reading of the manuscript. We apologize to those whose work we could not cite due to spatial constraints.

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