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Illuminating p53 function in cancer with genetically engineered mouse models

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Abstract

The key role of the p53 protein in tumor suppression is highlighted by its frequent mutation in human cancers and by the completely penetrant cancer predisposition of *p53* null mice. Beyond providing definitive evidence for the critical function of p53 in tumor suppression, genetically engineered mouse models have offered numerous additional insights into p53 function. *p53* knock-in mice expressing tumor-derived p53 mutants have revealed that these mutants display gain-of-function activities that actively promote carcinogenesis. The generation of *p53* knock-in mutants with alterations in different domains of p53 has helped further elucidate the cellular and biochemical activities of p53 that are most fundamental for tumor suppression. In addition, modulation of p53 post-translational modification (PTM) status by generating *p53* knock-in mouse strains with mutations in p53 PTM sites has revealed a subtlety and complexity to p53 regulation. Analyses of mouse models perturbing upstream regulators of p53 have solidified the notion that the p53 pathway can be compromised by means other than direct *p53* mutation. Finally, switchable p53 models that allow p53 reactivation in tumors have helped evaluate the potential of p53 restoration therapy for cancer treatment. Collectively, mouse models have greatly enhanced our understanding of physiological p53 function and will continue to provide new biological and clinical insights in future investigations.

Keywords

p53; Tumor suppression; Mouse models

1. Introduction

The p53 protein plays a fundamental role in tumor suppression, a notion underscored by the observation that *p53* is mutated in over half of all human cancers of a wide variety of types [1,2]. Further support for the pivotal function of p53 in tumor suppression comes from individuals with Li–Fraumeni Syndrome who inherits a mutant *p53* allele and are highly cancer-prone, developing a characteristic spectrum of tumors including sarcomas, brain

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cancers, breast cancers, and adrenocortical carcinomas [3]. Finally, as described below, definitive evidence for p53's critical role in tumor suppression came from the generation of *p53* knockout mice, as these mice were found to develop cancers at 100% frequency[4–6].

p53 is a sensor for cellular stresses such as DNA damage, hypoxia, or oncogenic signaling [1]. In the presence of such stress signals, p53 is post-translationally modified, resulting in displacement of Mdm2 and Mdm4, negative regulators of p53, and consequent p53 stabilization and activation [7–10]. Upon activation, p53 can trigger specific anti-proliferative responses, including transient cell-cycle arrest, permanent arrest known as cellular senescence, or apoptosis [1]. The temporary cell-cycle arrest response is particularly well studied in response to DNA damage and allows DNA repair before progression through the cell cycle, thus minimizing the propagation of potentially deleterious mutations – a role that led p53 to be named the "guardian of the genome" [11]. In contrast, apoptosis and cellular senescence, the latter of which triggers an innate immune response, are terminal cell fates that cause complete elimination of damaged or premalignant cells [12]. p53 drives these responses primarily by serving as a transcriptional activator that induces programs of gene expression important for each p53 response, although p53 also has some non-transcriptional activities [1,13]. Additionally, typical of a protein that drives different responses upon exposure to diverse stimuli, the p53 protein is regulated by a variety of post-translational modifications in response to stress signals [14,15].

While numerous insights into p53 function and regulation have come from *in vitro* studies, mouse models have been necessary to delineate the *in vivo* significance of these observations in physiological contexts. Given that p53 displays cell type-dependent and context-specific mechanisms of action, analyses in the mouse are useful for revealing the full complexity of its action in different settings. Moreover, the mouse affords the possibility to examine the process of tumorigenesis in the proper tissue microenvironment. In this review, we detail a variety of *p53* mouse models that have advanced our understanding of various aspects of p53 biology, including how p53 tumor mutants not only promote loss-of-function effects but also gain-of-function phenotypes to fuel carcinogenesis, how p53 acts mechanistically as a tumor suppressor, and how post-translational modifications fine-tune p53 activities. Investigations into p53 function using mouse models have greatly illuminated our understanding of this critical tumor suppressor.

2. Knockout models: when the guardian lets its guard down

While the frequent mutations of *p53* in human cancers suggested that p53 inactivation may be causal for tumorigenesis, unequivocal evidence for the importance of p53 in tumor suppression came through the generation of *p53* null mice. *p53* null mice were generated by three different laboratories, in each case through disruption of the p53 sequence-specific DNA binding domain [4–6]. Surprisingly, these studies demonstrated that these mice, for the most part, do not display embryonic lethality. However, $p53^{-/-}$ mice displayed decreased survival compared to wild-type mice, with the majority of mice succumbing to tumors between 2 and 9 months of age, primarily $CD4^{+}CD8^{+}$ T-cell lymphomas and some sarcomas. Moreover, $p53^{+/-}$ mice displayed decreased survival and accelerated tumor development compared to wild-type mice – although not to the extent observed in $p53^{-/-}$

mice – with spontaneous tumors developing with a median latency of ~18 months [16]. $p53^{+/-}$ mice displayed a different tumor spectrum from $p53^{-/-}$ mice, with a preponderance of diverse sarcomas (greater than 50% of all tumors), including fibrosarcomas, osteosarcomas, rhabdomyosarcomas, hemangiosarcomas, anaplastic sarcomas, and lyomyosarcomas, some lymphomas (25–35% of cases), and some adenocarcinomas (~9–11% of cases) [4,5]. The reduced incidence of thymic lymphomas in the $p53^{+/-}$ mice is thought to relate to a limited developmental window for these tumors to arise, before thymic involution, and inadequate time for Loss of Heterozygosity (LOH) to occur during this period [16]. While some tumors developing in $p53^{+/-}$ mice underwent LOH, some did not, indicating that p53 can be haploinsufficient for tumor suppression [17]. Importantly, the propensity of $p53^{+/-}$ mice to develop cancer, and particularly to develop sarcomas, is broadly similar to that of Li–Fraumeni Syndrome patients, suggesting that $p53^{+/-}$ mice can serve as a model for this syndrome. Notably, these studies also revealed some variability in precise phenotypes between different models, which is likely related to the different genetic backgrounds examined, with different enrichment for 129/Sv or C57BL/6 backgrounds. Indeed, the importance of genetic background in modifying the phenotype of p53 loss was underscored by studies of $p53^{+/-}$ mice on a BALB/c background, which revealed a dramatic predisposition to breast cancer [18]. In addition, tissue-specific ablation of p53, using *p53* conditional knockout mice to circumvent the early lethality from lymphomas and sarcomas typical of constitutive *p53* knockout mice, has supported the idea that p53 loss can be sufficient to promote other cancer types, such as epithelial cancers. For example, targeted inactivation of p53 in the mammary gland results in mammary tumors [19,20], and ablation of p53 in the cervical epithelium, in combination with estrogen treatment, promotes cervical cancer [20]. In addition, somatic inactivation of p53 in mature B cells results in IgM+ mature peripheral B-cell lymphomas [21]. Collectively, these studies demonstrated conclusively that p53 deficiency in numerous tissues causes cancer. Moreover, beyond the spontaneous cancer predisposition characterizing $p53^{+/-}$ mice and $p53^{-/-}$ mice, crossing nearly any other tumor-prone strain onto a *p53*-deficient background enhances tumor predisposition in that strain, underscoring how generally p53 impedes tumorigenesis in many contexts [22].

3. Can a *p53* mutation be worse than no p53 at all?

Interestingly, unlike the scenario with *p53* knockout mice where no p53 protein is produced, approximately 75% of *p53* mutations found in human tumors are missense mutations, suggesting that there may be some selective advantage for retaining the mutant allele rather than losing p53 completely [23,24]. p53 is typically mutated in the DNA binding domain (DBD), most commonly at various hotspot sites (R175, G245, R248, R249, R273, R282) [23,25]. Depending on the consequence of the mutations, p53 DBD mutants can be divided into two categories: contact mutants, which harbor mutations in p53 residues that directly contact the DNA duplex, or structural mutants, which carry mutations that disrupt p53 protein conformation, both of which interfere with p53 binding to its response element [25,26]. From cell culture assays, such p53 point mutants were proposed to promote tumorigenesis by exerting dominant-negative, inhibitory effects on the wild-type p53 protein, as well as by manifesting novel, oncogenic gain-of-function (GOF) activities. To

examine the mechanisms by which the point mutants might enhance tumor development, and to re-create models that more accurately recapitulate human cancers, initial experiments focused on generating transgenic mice overexpressing *p53* tumor-derived mutants in various mouse tissues [27–29]. These transgenic strains exhibited an enhanced cancer predisposition, and provided support for dominant-negative action of p53 point mutants, as A135V promoted cancer in combination with a wild-type *p53* allele but not with a *p53* null allele [29]. In contrast, initial evidence for p53 mutant GOF activity came primarily from cell culture and xenograft studies [30].

Ultimately, more physiological *p53* knock-in mutant models were generated, in which the endogenous *p53* mouse allele was replaced with orthologs of specific human *p53* hotspot mutants, allowing expression of the mutant alleles at physiological levels and with proper spatial and temporal regulation (Fig. 1). In the first of these studies, mutant knock-in mice expressing $p53^{R172H}$ (corresponding to the human $p53^{R175H}$ conformation mutant) were generated. It was found that $p53^{R172H/-}$, $p53^{R172H/R172H}$, and $P53^{-/-}$ cohorts displayed identical survival curves and developed similar tumor spectra [31]. $p53^{+/-}$ and $p53^{R172H/+}$ mice also exhibited similar survival curves and tumor spectra, but the $p53^{R172H/+}$ mice developed tumors that metastasized frequently, suggesting potential GOF activity of the $p53^{R172H}$ mutant. In another study analyzing both the $p53^{R172H}$ and $p53^{R270H}$ mutants (corresponding to human $p53^{R175H}$ and the $p53^{R273H}$ contact mutant), $p53^{R270H/+}$ and $p53^{R172H/+}$ mice exhibited similar survival curves to $p53^{+/-}$ mice, but developed different tumor types from the $p53^{+/-}$ mice [32]. Specifically, $p53^{R270H/+}$ mice exhibited an increase in metastatic carcinomas and B-cell lymphomas relative to $p53^{+/-}$ mice, and $p53^{R172H/+}$ mice developed osteosarcomas that were much more metastatic than in $p53^{+/-}$ mice. In addition, $p53^{R270H/-}$ and $p53^{R172H/-}$ mice displayed similar survival curves to $p53^{-/-}$ mice but also developed some novel tumor types. $p53^{R270H/-}$ mice developed an increased incidence of invasive, metastatic carcinomas relative to $p53^{-/-}$ mice, while $p53^{R172H/-}$ mice displayed an enhanced predisposition to both invasive, metastatic carcinomas and hemangiosarcomas relative to $p53^{-/-}$ mice. Collectively, these studies support a GOF mechanism for p53 mutants, as they induced either a broadened tumor spectrum or conferred increased metastatic potential to tumors relative to *p53* nullizygosity. These results were supported by accompanying cell culture assays in which $p53^{R172H/R172H}$ mouse embryonic fibroblasts (MEFs) displayed enhanced proliferation compared to $p53^{-/-}$ MEFs [31]. Additionally, *HrasV12;*$p53^{R172H/R172H}$ MEFs formed more colonies in soft-agar transformation assays than *Hras-V12;*$p53^{-/-}$ MEFs, bolstering the notion that p53 mutants display GOF activity. The mechanism for GOF activity in these contexts appears to be through mutant p53 binding and inhibiting the p53-related transcription factors p63 and p73, which can have tumor suppressor activity themselves. Overall, these were landmark studies for substantiating the significance of the GOF idea *in vivo*.

To more accurately study the human *p53* hotspot mutations, HUPKI (*humanized p53 k*nock-*i*n) mouse models were generated [33]. In these mice, murine *p53* exons 4–9 were replaced with the corresponding human sequences, including specific human tumor mutations. For example, a mouse strain expressing the $p53^{R175H}$ structural mutant, corresponding to murine $p53^{R172H}$, was generated and assessed for GOF activity [34]. *p53hupki*$^{R175H/R175H}$ mice

displayed similar survival rates to *p53*$^{-/-}$ mice, but exhibited a broader tumor spectrum, with the development of peripheral lymphomas and germ cell tumors. These data further support the notion that *p53* hotspot mutations, and in this case the human R175H hotspot mutation, confer GOF activity by enhancing the development of new tumor types. Another p53 hotspot HUPKI mouse model, expressing the p53^{R248W} contact mutant, has supported this idea [35]. Specifically, *p53hupki*$^{R248W/-}$ mice displayed a similar survival curve to *p53*$^{-/-}$ mice, but succumbed to a broader spectrum of tumors, with the development of peripheral lymphomas. The GOF activity of these p53 mutants was attributed to their ability to interact with Mre11, blocking the binding of the Mre11-Rad50-Nbs1 complex to double-strand breaks and preventing proper activation of the Atm cascade. This interference with DNA damage response signaling in turn resulted in defective cell-cycle checkpoints and genetic instability.

It has been proposed that the different *p53* tumor-derived mutants may have varying degrees of GOF activity [36]. Indeed, additional insight into p53 GOF properties and their unique capacities has come to light with the development of additional HUPKI hotspot mouse models, expressing the contact mutant p53^{R248Q} or the conformation mutant, p53^{G245S} [37]. The *p53hupki*$^{G245S/-}$ mice exhibited similar tumor latency and survival rates to *p53*$^{-/-}$ mice. In contrast, *p53hupki*$^{R248Q/-}$ mice displayed diminished survival and decreased tumor latency relative to *p53*$^{-/-}$ mice, indicating a more dramatic GOF than any previously studied *p53* hotspot mouse model. Additionally, both *p53hupki*$^{R248Q/-}$ and *p53hupki*$^{G245S/-}$ mice developed a slightly broader spectrum of tumors, with the appearance of more sarcoma subtypes, carcinomas, and germ cell tumors. Moreover, lymphoma cells from both *p53hupki*$^{R248Q/-}$ and *p53hupki*$^{G245S/-}$ mice displayed enhanced Akt signaling relative to *p53*$^{-/-}$ cells, suggesting a potential mechanism underlying GOF. Interestingly, thymic lymphoma cells from *p53hupki*$^{R248Q/-}$ mice proliferated faster than those from *p53*$^{-/-}$ mice, while those from *p53hupki*$^{G245S/-}$ mice did not, demonstrating a potentially contact mutant-specific GOF phenotype. The *p53hupki*$^{R248Q/-}$ mice also displayed an expansion of hematopoietic and mesenchymal stem cell populations relative to *p53hupki*$^{G245S/-}$ or *p53*$^{-/-}$ counterparts, which may contribute to the rapid tumor onset observed. Consistent with this notion, a similar expansion of mammary stem cells was coupled with a propensity for tumor development in a *p53hupki*^{R175H} mammary mouse model [38]. Notably, the p53^{R248Q} is the most potent GOF mutant reported to date, as it is the first hotspot mutant shown to promote decreased survival relative to p53 deficiency. This side-by-side comparison of *p53hupki*^{R248Q} and *p53hupki*^{G245S} mice, along with previous studies of *p53hupki*^{R175H} and *p53hupki*^{R248W} mice, support the assertion that the different *p53* hotspot mutations can promote varying degrees of oncogenic GOF activity [34,35,37].

Together, these studies demonstrate that the p53 tumor-derived mutants both lose wild-type p53 tumor suppressor activity and acquire oncogenic GOF properties *in vivo*. Particularly compelling for establishing a GOF phenotype has been the comparison between *p53*$^{M/-}$ or *p53*$^{M/M}$ mice and *p53*$^{-/-}$ mice (where M denotes p53 mutant), which has indeed shown that the M alleles enhance tumorigenicity *in vivo*, in the absence of a wild-type *p53* allele. Furthermore, it is becoming clear that different p53 hotspot mutants promote different GOF phenotypes. As additional *p53* hotspot mutant mouse models are generated, we hope to

better elaborate the mutation-specific GOF phenotypes, as well as broader, category-specific (*i.e.* contact mutant *versus* conformation mutant) GOF phenotypes and the molecular underpinnings of these phenotypes. Acquiring a better understanding of how each mutant acts to promote cancer could lead to more effective therapeutic targeting of p53 mutants, depending on the specific mutations and the associated conformation of the encoded proteins.

4. Unveiling p53 domain functions

The p53 protein is a transcriptional activator that induces a plethora of diverse target genes involved in different responses [2]. Typical of transcriptional activators, the protein comprises multiple functional domains, including two transcriptional activation domains (TADs), a so-called proline-rich domain (PRD), a sequence-specific DNA-binding domain (DBD), and an oligomerization domain (OD) through which p53 monomers interact to form tetramers. To better understand the contribution of each domain to p53 functions *in vivo*, a variety of *p53* knock-in mouse strains expressing mutants altered in different p53 domains have been engineered (Fig. 2). These studies have helped delineate mechanisms of p53 action in cell-cycle arrest, senescence, apoptosis, and ultimately tumor suppression.

4.1. Tinkering with the transactivation domains

Although p53 has well-characterized activity as a transcriptional activator, *in vitro* studies have suggested that p53 has additional biochemical activities, such as the ability to repress transcription and to induce apoptosis through direct action at the mitochondria [1,2]. Thus, to directly assess the contribution of transcriptional activation to p53 functions *in vivo*, transcriptional activation-deficient mutant mice were generated. Initially, knock-in mice were generated that expressed $p53^{25,26}$, with mutations L25Q and W26S in the first TAD [39], as this mutant had been reported to be transcriptionally inactive in reporter assays [40]. Analysis of $p53^{25,26/25,26}$ cells showed that $p53^{25,26}$ is indeed severely compromised for inducing most classical p53 target genes such as *p21*, *Noxa*, *Puma*, and *Perp*, both in response to DNA damage and oncogenic signals, indicating that the first TAD is essential for the transactivation of most known p53 target genes [39,41]. However, $p53^{25,26}$ retained the capacity to activate a small set of p53 target genes, such as *Bax*. In terms of biological activity, $p53^{25,26}$ was unable to induce G_1 cell-cycle arrest or apoptosis in response to acute DNA damage signals in both MEFs *in vitro* and radiosensitive tissues *in vivo*. These observations indicate that TAD1 and efficient transcriptional activation of p53 target genes are important for mounting responses to acute DNA damage, consistent with the known requirements for *p21* and *Noxa*, *Puma*, and *Perp* for DNA damage-induced cell-cycle arrest and apoptosis, respectively [42]. Interestingly, however, $p53^{25,26}$ can promote apoptosis in oncogene-expressing MEFs in response to serum deprivation or hypoxia, indicating that apoptosis in response to non-genotoxic stresses occurs without full p53 transactivation [39]. Intriguingly, upon examination of the $p53^{25,26}$ mutant in tumor suppression, it was discovered that it efficiently suppresses the development of tumors derived from multiple different lineages and driven by different oncogenic events, such as Kras-driven non-small-cell lung cancer and Eμ-*myc* triggered lymphomas [41,43]. Thus despite the inability to efficiently activate most canonical p53 target genes, $p53^{25,26}$ suppresses cancer effectively,

suggesting that either its residual transcriptional activation function or an alternate p53 biochemical activity accounts for its ability to suppress cancer.

To distinguish these possibilities, mice expressing a quadruple mutant, $p53^{25,26,53,54}$, with alterations in both the first and second p53 TADs (L25Q; W26S; F53Q; F54S) were generated [41], as this mutant was reported to be transcriptionally inactive *in vitro* [44–46]. To control for the effect of the mutations in TAD2 alone, knock-in mice expressing the $p53^{53,54}$ mutant, with F53Q and F54S alterations, were also generated. Microarray studies of homozygous mutant cells derived from these mice revealed that $p53^{25,26,53,54}$ is incapable of transactivating p53 target genes, displaying a gene expression profile indistinguishable from that of $p53^{-/-}$ cells, while the $p53^{53,54}$ displayed no compromise in transcriptional activity compared to wild-type p53 [41]. The activity of the $p53^{25,26,53,54}$ mutant in tumor suppression was examined in multiple mouse cancer models, and it was found to be completely unable to suppress carcinogenesis. These findings indicate that transcriptional activation is indeed essential for tumor suppression in a variety of different tumor types such as lung cancer and lymphomas [41,43,47]. Not surprisingly, given its intact transcriptional potential, the $p53^{53,54}$ mutant retained full tumor suppression activity [41]. Importantly, since the $p53^{25,26}$ mutant retains tumor suppression activity, yet only robustly transactivates a limited set of p53 targets, analysis of genes efficiently induced by both wild-type p53 and $p53^{25,26}$ has helped uncover a set of novel direct p53 target genes whose expression is tightly associated with tumor suppression. Indeed, knockdown of these genes in allograft assays have demonstrated that some of these targets display tumor suppressor activity. Thus, these findings together suggest that these tumor suppression-associated target genes may represent new key components of the p53 tumor suppression network. In addition, the finding that TAD1 is essential for p53 responses to acute DNA damage, but not for tumor suppression indicates that the ability of p53 to promote responses to acute DNA damage is dispensable for tumor suppression, a conclusion supported by several independent studies [48–50].

Another study also examined whether transcriptional activation is sufficient for p53 function [51]. Knock-in mice were generated expressing a chimeric p53 protein, in which the first 80 amino acids of the amino-terminus comprising the two p53 TADs were replaced with the TAD of the Herpes Simplex Virus VP16 protein. This $p53^{VP16}$ chimera was proficient for DNA binding and transcriptional activation on all p53 target genes tested due to the heterologous TAD, but lacked other potential amino-terminal p53 functions. Studies in MEFs revealed that $p53^{VP16}$ can efficiently induce cell-cycle arrest and cellular senescence, but not apoptosis in response to different DNA damaging agents. These findings suggest that while transcriptional activity may suffice for p53 to trigger arrest responses, eliciting an apoptotic response may require functions other than transcriptional activation, such as the ability of p53 to act at the mitochondria.

4.2. Dissecting DNA binding function: parsing roles of p53-induced cell-cycle arrest and apoptosis in tumor suppression

The majority of mutations found within the p53 DBD abolish sequence-specific DNA binding. However, several different mutations within the p53 DBD have been reported to

selectively affect DNA binding to specific p53 target genes, but not others, helping to further illuminate mechanisms of p53 action. In the first of these studies, knock-in mice expressing $p53^{R172P}$ (also known as $p53^{515G\rightarrow C}$; [52]), the ortholog of the human tumor-derived mutant $p53^{R175P}$, were generated. Previous studies had shown that the $p53^{R175P}$ mutant can bind and activate the cell-cycle arrest target gene *p21* and drive cell-cycle arrest, but it cannot induce the apoptotic target gene *Bax* or apoptosis [53]. Similarly, studies of cells derived from the $p53^{R172P}$ knock-in mice revealed that $p53^{R172P}$ retains partial cell-cycle arrest function, associated with some *p21* induction, but that it cannot induce apoptosis in response to DNA damaging agent treatment [52]. Interestingly, despite a defective apoptotic response, $p53^{R172P/R172P}$ mice exhibited delayed spontaneous tumorigenesis compared to $p53^{-/-}$ mice, with a reduction of the T-cell lymphomas typically seen in $p53^{-/-}$ mice. Subsequently, however, these mice developed other types of lymphomas and sarcomas. These $p53^{R172P/R172P}$ tumors were genomically stable, unlike the aneuploid tumors that develop in $p53^{-/-}$ mice, suggesting that $p53^{R172P}$ may suppress tumorigenesis through the induction of cell-cycle arrest and the inhibition of genomic instability. Additional studies of the $p53^{R172P}$ knock-in mice on a background deficient for the p21 cyclin dependent kinase inhibitor, to more specifically query the importance of cell-cycle arrest for the observed tumor suppression activity, showed that *p21* loss accelerated tumorigenesis [54]. Importantly, this observation suggests that $p53^{R172P}$ indeed suppresses tumor development by inducing cell-cycle arrest and maintaining genome stability. Together, these data strongly implicate cell-cycle arrest as a critical facet of p53-mediated tumor suppression, in at least some tumor types. The inability of $p53^{R172P}$ to suppress the development of late-onset tumors, however, also supports a role for p53 apoptotic function in tumor suppression.

Cooperative DNA binding by p53 can be regulated by the charged residues E180 and R181 within the human p53 DBD [55]. The importance of these residues and of cooperative binding to certain p53 response elements for tumor suppression is suggested by the mutation of these residues in sporadic human tumors and in Li–Fraumeni patients. Moreover, *in vitro* studies demonstrated that the E180R mutation reduced cooperative p53 binding to DNA, specifically affecting binding to p53 response elements in pro-apoptotic genes. To define the physiological effect of reduced cooperative p53 DNA binding, knock-in mice were generated in which the mouse p53 E177 residue (corresponding to human p53 E180) was changed to arginine (E177R; termed $p53^{RR}$) [56]. While the $p53^{RR}$ mutant can activate *p21* and cell-cycle arrest in response to DNA damage signals, the $p53^{RR}$ mutant is defective in inducing pro-apoptotic target genes and apoptosis in DNA-damage-treated or serum-deprived cells. Aging $p53^{RR/RR}$ mice manifested decreased survival relative to wild-type littermates, and were highly cancer-prone, indicating a deficiency in $p53^{RR}$ tumor suppression function, although not to the extent observed with $p53^{-/-}$ mice. Indeed, early-onset T-cell lymphomas typical of $p53^{-/-}$ mice were significantly inhibited in $p53^{RR/RR}$ mice, indicating that $p53^{RR}$ retains tumor suppression function in some settings, like $p53^{R172P}$. Interestingly, $p53^{RR}$ can activate p53 metabolic target genes such as *Gls2* and *Dram* upon DNA damage and can limit glycolysis and ROS accumulation. Thus, despite a deficit in activating apoptotic target genes and apoptosis, this cooperativity mutant still retains the capacity to suppress tumorigenesis in some tissues, potentially attributable to its

ability to induce cell-cycle arrest and/or regulate metabolism. Again, the fact that these mutant mice ultimately succumb to tumors suggests that apoptosis is an important component of p53-mediated tumor suppression in some tissues.

Recently, *p53* knock-in mice carrying acetylation site mutations that selectively affect expression of some p53 target genes were generated. p53 modification by acetylation occurs in response to various stresses, including DNA damage and oxidative stress, and the murine p53 DBD is acetylated at three sites (K117, K161, K162), including two that are conserved in human p53 (K120, K164)[57,58]. Human cell culture studies implicated p53 K120 in the induction of apoptosis target genes, but not cell-cycle arrest target genes [59,60]. Combined mutation of K120 and K164 compromised the upregulation of apoptosis and cell-cycle arrest target genes as well as both p53-mediated apoptosis and cell-cycle arrest [61]. To clarify the *in vivo* role of acetylation of the p53 DBD for different p53 functions, two p53 acetylation site mutant knock-in mouse strains were generated, $p53^{K117R}$ and $p53^{3KR}$ [62]. Analysis of cells derived from $p53^{K117R}$ mice, carrying the acetylation site mutation that renders p53 defective for triggering apoptosis in cultured cells *in vitro*, confirmed that $p53^{K117R}$ could not induce *Puma* or apoptosis in response to DNA damage, suggesting that acetylation at K117 is critical for p53-dependent apoptosis. In contrast, $p53^{K117R}$ is still competent to induce *p21* and cell-cycle arrest in DNA damage-treated cells, as well as to trigger cellular senescence. Analysis of aging $p53^{K117R/K117R}$ cohorts indicated that these mice are resistant to early-onset spontaneous tumor development, again supporting the role of p53 cell-cycle arrest function in tumor suppression. To completely compromise apoptosis and cell-cycle arrest, $p53^{3KR}$ knock-in mice were generated, in which three acetylation sites in the DBD were mutated (K117R, K161R, K162R) [62]. Cells derived from the $p53^{3KR/3KR}$ mice indeed failed to undergo cell-cycle arrest and apoptosis in response to DNA damage, or cellular senescence in response to oncogenic HRasV12. Intriguingly, the $p53^{3KR/3KR}$ mice were also resistant to early-onset spontaneous tumor formation, indicating that this mutant still retains tumor suppressor function despite lacking apoptosis, cell-cycle arrest, and senescence capabilities. Of note, the $p53^{3KR}$ mutant maintains the capacity to activate the metabolic p53 target genes *Gls2* and *Tigar* as well as to restrain glucose uptake, glycolysis, and ROS accumulation. This study therefore suggests that p53's ability to regulate metabolism may play a role in p53-mediated tumor suppression.

4.3. Pulling apart the proline-rich domain

The p53 proline-rich domain (PRD) is found between residues 61-94 or 55-88 of the human and mouse proteins, respectively [63]. The human PRD contains numerous prolines including 5 PXXP repeats, where P represents proline and XX represents any amino acid. However, the precise sequence of the domain is not highly conserved between species, with the mouse PRD containing only two PXXP repeats and the guinea pig containing none, questioning the precise functional role of the PRD [64]. *In vitro* cell culture studies indicated that activation of p53-mediated apoptosis is dependent on the PRD, while induction of *p21* and cell-cycle arrest is not[65–68]. Furthermore, the PRD has been implicated in p53 protein stabilization, which is thought to be regulated through the prolyl isomerase, Pin1 [69]. *In vitro* cell culture studies had shown that p53 becomes phosphorylated in response to stress signals, allowing more avid interaction with Pin1, which in turn engenders a conformational

change in p53 that decreases Mdm2 accessibility and stabilizes p53 [69–71]. To understand the role of the PRD in apoptosis and p53 protein stabilization in a physiological setting, $p53^{\Delta PRD}$ knock-in mice were generated expressing a p53 protein lacking amino acids 75–91 of the PRD, which comprise the two PXXP motifs plus two additional prolines [72]. Surprisingly, in contrast to the previous *in vitro* studies, $p53^{\Delta PRD}$ was completely deficient in inducing cell-cycle arrest in response to DNA damage, possibly due to weak transactivation of *p21*, but remained competent to induce apoptosis in oncogene-expressing MEFs. Analysis of aging $p53^{\Delta PRD/\Delta PRD}$ mice revealed that they were relatively resistant to early-onset tumorigenesis, although they eventually succumbed to some tumors. These findings were supported by the generation of another PRD-deficient mouse strain, expressing $p53^{m\Delta Pro}$, which lacks amino acids 58–88 [73]. These aging $p53^{m\Delta Pro/m\Delta Pro}$ mice were found to develop very few T-cell lymphomas but ultimately did succumb to late-onset B cell lymphomas [73]. Much like the previous study, these experiments suggested that $p53^{m\Delta Pro}$ is compromised for inducing p53-mediated cell-cycle arrest in response to DNA damage signals but showed that $p53^{m\Delta Pro}$ is also defective in inducing apoptotic target genes and triggering apoptosis in response to genotoxic stress [74]. Thus, these observations suggest that the PRD has a context-dependent role in tumor suppression in suppressing early-onset spontaneous cancers.

To refine our understanding of the PRD, additional mouse strains were generated. First, to examine the contribution of Pin1 binding to p53-mediated tumor suppression, $p53^{TTAA}$ knock-in mice were generated, in which the Pin1 binding site was compromised by mutating threonines 76 and 86 to alanines, rendering these residues unphosphorylatable, an essential step for Pin1 binding. Second, to query the importance of the proline motifs, $p53^{AXXA}$ mice were engineered with alterations in the two PXXP motifs (P79A, P82A, P84A and P87A) in the PRD [64]. Mice from both strains were born at Mendelian ratios and had no developmental abnormalities. Moreover, allograft tumor assays performed with oncogene-expressing mutant MEFs derived from these mice demonstrated that both mutants still display intact tumor suppression function, indicating that the PXXP motifs and the Pin1 binding site within the PRD are dispensable for p53 tumor suppressor function. Thus, the PRD plays a role in tumor suppression in some settings, but independently of the PXXP motifs and Pin1 binding.

Within the human p53 PRD, codon 72 is polymorphic, encoding either proline or arginine. Cell culture experiments on these polymorphic p53 variants suggest that they can have different biological activities in terms of their abilities to activate p53 target genes, promote cell-cycle arrest, and localize to mitochondria [75–77]. To understand the contributions of each of these polymorphisms to p53 function, $p53hupki^{P72}$ and $p53hupki^{R72}$ mice were generated [78,79]. In response to DNA damage, $p53^{P72}$ and $p53^{R72}$ protein stabilization [78] and transactivation of the p53 targets *Mdm2* and *Puma* were equivalent. However, $p53^{P72}$ more efficiently induced p21 expression, cell-cycle arrest, and senescence in response to oncogenic signals and DNA-damage than $p53^{R72}$. $p53hupki^{P72/P72}$ thymocytes also displayed increased apoptosis relative to $p53hupki^{R72/R72}$ thymocytes upon DNA-damage as well as increased transactivation of select p53 targets, indicating a functional difference between polymorphic variants. Interestingly, most genes preferentially transactivated by

p53^{P72} include those with roles in inflammation, and these were found to be coordinately regulated by *NF-kB*. Surprisingly, *p53hupki*$^{P72/-}$ and *p53hupki*$^{R72/-}$ mice displayed similar survival profiles and spontaneous tumor spectra. In another set of experiments, *p53hupki*$^{72P/72P}$ and *p53hupki*$^{72R/72R}$ mice were made by simply humanizing exon 4 mouse *p53*. Interestingly, in this model, p53 apoptotic target genes, including *Perp, Puma*, and *Noxa* (but not the cell-cycle arrest target gene *p21*), were all induced to a greater extent by DNA damage in *p53hupki*$^{72R/72R}$ cells than in *p53hupki*$^{72P/72P}$ cells. Accordingly, DNA-damage-treated *p53hupki*$^{72R/72R}$ keratinocytes and intestinal cells displayed increased apoptosis relative to *p53hupki*$^{72P/72P}$ counterparts. Nonetheless, UVB-treated *p53hupki*$^{72P/72P}$ and *p53hupki*$^{72R/72R}$ mice developed skin cancer with similar latencies and multiplicities [79]. Collectively, the data from these two studies indicate that the p53^{P72} and p53^{R72} mutants display similar tumor suppressor activity despite each mutant exhibiting augmented p53 function in certain cellular assays.

The aforementioned studies interrogating the functional significance of different p53 domains using knock-in mouse model studies have provided key new insights into p53 and illustrate the importance of performing functional studies in an *in vivo* setting. Through analysis of the p53 TADs, studies have indicated that transcriptional activation by p53 is important for acute DNA-damage-induced cell-cycle arrest and apoptosis, as well as for tumor suppression. However, the TAD requirements are different in these contexts: TAD1 is essential for responses to acute DNA damage, while dispensable for tumor suppression. These findings in turn have suggested quite surprisingly that p53 responses to acute DNA damage, which have been a major focus of studies on p53 over the years, are dispensable for tumor suppression. Dissecting the DNA binding domain through analysis of mice expressing mutants that selectively compromise p53-mediated apoptosis but not cell-cycle arrest has revealed that cell-cycle arrest activity is associated with suppression of early-onset spontaneous tumors while apoptosis suppresses the development of late-onset tumors. Moreover, evidence suggests that p53 can suppress cancer in some contexts without any activity in acute stress-induced cell-cycle arrest and apoptosis, highlighting the potential role for additional, emerging functions of p53, such as regulating metabolism, in tumor suppression. Finally, the proline-rich domain plays a context-specific role in tumor suppression but not through Pin1 binding or through the PXXP motifs. Thus, while *in vitro* studies can provide an initial hint of the function of a particular p53 domain, defining the precise role of a domain relies on examination in an *in vivo* context, in the proper tissue microenvironment, and where cell-type-specific roles can be uncovered. Continued examination of mouse models will further decipher the many mysteries regarding the mechanisms of p53 action in tumor suppression.

5. Post-translational modifications – modifying the p53 function du jour

p53 responds to diverse cellular stresses by triggering various responses, and therefore must be tightly regulated by post-translational modifications (PTMs) [15]. p53 is regulated by a number of different PTMs, including phosphorylation, acetylation, ubiquitination, sumoylation, neddylation, and methylation. These modifications regulate different aspects of p53 activity, including protein stabilization and target gene activation. Here we describe

knock-in models with alterations in p53 post-translational modification sites to better understand their contribution to p53 function *in vivo* (Fig. 3).

In vitro studies showed that serine 15 in human p53 (serine 18 in mouse p53) becomes phosphorylated by the ATM kinase in response to DNA damage, and that this phosphorylation plays a critical role in stabilizing p53 in response to DNA damage, through the displacement of the negative regulator Mdm2 [8,14]. To interrogate the role of serine 18 phosphorylation *in vivo*, $p53^{S18A}$ knock-in mice were generated in which serine 18 was mutated to a non-phosphorylatable alanine [80,81]. Surprisingly, analysis of MEFs and thymocytes from $p53^{S18A/S18A}$ mice revealed that $p53^{S18A}$ protein stabilization in response to DNA damage is not significantly compromised, indicating that serine 18 phosphorylation is not essential for disruption of the p53-Mdm2 interaction. Instead, the ability of $p53^{S18A}$ to activate various target genes was impaired. Moreover, while DNA-damage-induced cell-cycle arrest was intact in MEFs, there were partial defects in DNA-damage-induced apoptosis. In addition, one study found that unlike $p53^{-/-}$ mice, $p53^{S18A/S18A}$ mice were not prone to tumorigenesis [80], although another study observed that these mice succumbed to enhanced late-onset tumor development relative wild-type mice [82]. Together, these data suggest that p53 serine 18 phosphorylation does not contribute to p53 protein stability, but rather contributes to p53 target gene activation and, as a consequence, p53-dependent apoptosis and late-onset tumor suppression.

In vitro studies showed that phosphorylation of human p53 at serine 20 (mouse p53 serine 23) also plays a key part in p53 stabilization in response to DNA damage signals, again through displacement of Mdm2. To define the role of S23 modification in a physiological context, $p53^{S23A}$ knock-in mice were generated [83,84]. While one study did not observe any compromise in DNA-damage-induced p53 stability or activity, another reported various phenotypes. Whereas $p53^{S23A}$ protein levels and apoptosis-inducing activity were diminished in thymocytes upon irradiation, $p53^{S23A}$ displayed normal stabilization and cell-cycle arrest activity in MEFs upon irradiation. In addition, $p53^{S23A/S23A}$ mice displayed decreased survival relative to wild-type mice, but not to the extent observed in $p53^{-/-}$ mice. Moreover, these mice developed B-cell lymphomas rather than the T-cell lymphomas typifying $p53^{-/-}$ mice. These data show that serine 23 phosphorylation in response to DNA-damage contributes to p53 protein stabilization and p53-dependent apoptosis in a cell-type dependent manner as well as to suppression of B-cell lymphomagenesis. Finally, given that phosphorylation of serines 18 and 23 is thought to coordinately lead to p53 stabilization, a $p53^{S18A\text{-}S23A}$ mouse knock-in strain harboring mutations in both serines was generated [85]. The responses were very cell-type-dependent. In MEFs, $p53^{S18A\text{-}S23A}$ showed normal protein stabilization as well as somewhat impaired transcriptional activity and mildly compromised cell-cycle arrest function upon DNA damage, similar to the $p53^{S18A}$ single mutant, indicating no cooperativity between phosphorylation sites in this context. However, in thymocytes, $p53^{S18A\text{-}S23A}$ was inefficiently stabilized, did not transactivate target genes effectively, and was severely impaired in driving apoptosis in response to DNA damage, indicating that these phosphorylation sites are required to promote apoptosis in thymocytes. Additionally, $p53^{S18A\text{-}S23A/S18A\text{-}S23A}$ mice displayed decreased survival compared to wild-type mice due to succumbing to late-onset spontaneous tumors, a more dramatic phenotype

than that observed by the same group upon analysis of $p53^{S18A/S18A}$ mice. Together, these results suggest that serines 18 and 23 act coordinately to promote apoptosis and tumor suppression in some tissues. Moreover, these findings further highlight the idea that retaining cell-cycle arrest is sufficient to inhibit early-onset spontaneous tumors but lack of apoptosis activity can drive later-onset tumors.

Another p53 protein phosphorylation site linked specifically to p53 apoptotic function *in vitro* is serine 46 of human p53, which is phosphorylated in response to DNA damage, helping to elicit an apoptotic response through the induction of the p53 target gene *p53AIP1* [86]. As there is no conserved residue in murine p53 [87], a HUPKI mouse strain with serine 46 mutation ($p53hupki^{S46A}$) was generated to characterize the *in vivo* role of serine 46 phosphorylation in p53-dependent DNA-damage responses [88]. $p53hupki^{S46A/S46A}$ cells treated with DNA damaging agents displayed decreased induction of apoptotic targets, including *Noxa*, *Puma*, and *Perp*, as well as partially impaired apoptosis compared to counterparts expressing wild-type p53. $p53^{S46A}$ was also slightly compromised in inducing senescence. Thus, this residue is primarily important for p53-mediated apoptosis, but its role in tumor suppression *in vivo* remains to be determined.

While the aforementioned studies suggest a role for p53 phosphorylation in regulating p53 function upon exposure to DNA double-strand break inducers like gamma-irradiation, *in vitro* studies indicate that phosphorylation of serine 389 of murine p53 occurs specifically in response to UV-irradiation, but not gamma-irradiation. To examine the significance of this modification *in vivo*, $p53^{S389A}$ knock-in mice were generated [89]. Relative to wild-type p53, $p53^{S389A}$ displayed decreased DNA-binding and compromised transactivation of such targets as *p21*, *Mdm2*, *Noxa*, and *Bax* as well as impaired apoptosis in response to UV-irradiation but not in response to double-strand break inducers. In keeping with this specific role in responding to UV-irradiation, chronic UV-irradiation of $p53^{S389A/S389A}$ mice increased skin tumor development relative to wild-type mice. These studies thus demonstrate that serine 389 phosphorylation selectively promotes apoptotic and tumor suppressive programs in response to UV-irradiation.

Human p53 can be phosphorylated at serine 315 (serine 312 in mouse) upon irradiation as well as upon endoplasmic-reticulum stress [90,91]. *In vitro* data suggested that phosphorylation at this site is involved in target gene activation or cytoplasmic localization. To clarify the role of phosphorylation at this site in a physiological setting, $p53^{S312A}$ knock-in mice were generated[92,93]. However, analysis of cells from these mice revealed that p53 protein stability, transactivation capacity, cell-cycle arrest function, and apoptotic activity were all intact. Furthermore, survival of $p53^{S312A/S312A}$ mice was comparable to $p53^{+/+}$ mice. However, p53 S312A mutation partially rescues the early embryonic lethality of $Mdm4^{-/-}$ embryos, which is caused by active p53, suggesting that this mutation inactivates p53 function during development [93]. Moreover, $p53^{S312A/S312A}$ mice are more susceptible to radiation-induced tumorigenesis, indicating that phosphorylation at S312 is important for tumor suppression. In addition, p53 phosphorylation at this site has been implicated as critical in ES cells for *Nanog* repression and ES cell differentiation [94], suggesting a role for serine 315 phosphorylation in stem cells as well.

Beyond the key role of phosphorylation in regulating p53, *in vitro* studies have demonstrated that p53 is acetylated in response to stress signals such as DNA damage and oxidative stress [57,58]. The murine p53 C-terminus contains multiple lysine residues (K367, K369, K370, K378, K379, K383, and K384) that can be post-translationally modified by ubiquitination, acetylation, neddylation, sumoylation, or methylation. Two knock-in mouse strains sought to address the importance of these residues by mutating all C-terminal lysines to arginines to block any type of modification at those residues. The first knock-in mouse strain, expressing "$p53^{6KR}$" carried six C-terminal lysine mutations (K367R, K369R, K370R, K378R, K379R, K383R) [95], while the second mouse strain, termed "$p53^{7KR}$" had seven mutations, including the aforementioned ones and one at lysine 384 (K384R), which is not conserved in humans [96]. Surprisingly, homozygous $p53^{6KR}$ and $p53^{7KR}$ mutant cells exhibited rather subtle phenotypes. p53 protein accumulation under basal conditions was similar to that of wild-type p53, an unexpected finding given the envisaged need for these lysines for ubiquitination and destabilization of p53 in unstressed cells. Neither $p53^{6KR}$ nor $p53^{7KR}$ showed dramatic phenotypes in various p53-dependent assays: $p53^{6KR}$ showed a slight compromise in inducing certain p53 target genes and apoptosis in some contexts, while $p53^{7KR}$ engaged DNA-damage-induced cell-cycle arrest and apoptotic programs normally. The only phenotypes evident for $p53^{7KR}$ were increased stabilization and activation of target genes in response to DNA damage, and a slightly enhanced ability to induce senescence relative to wild-type controls, suggesting the mutant is mildly hyperactive [96]. Consistent with this notion, $p53^{7KR}$ impedes hematopoietic stem/progenitor cell proliferation in response to irradiation, rendering $p53^{7KR}$ mice highly radiosensitive [97]. However, both $p53^{7KR}$ and $p53^{6KR}$ were fully competent as tumor suppressors. Thus, together, these data indicate that the modifications at p53's C-terminus may play a role in fine-tuning the p53 response to DNA damage.

The concurrent mutation of many lysines simultaneously in these strains may have masked the specific roles of PTMs on individual residues. To address the role of single lysines, some studies have examined the consequences of altering individual lysines, such as in the knock-in mouse strain expressing $p53^{K117R}$, as described above. In addition, $p53^{K317R}$ knock-in mice were made to pursue the *in vitro* finding that acetylation of human p53 at K320 (corresponding to mouse K317) correlated with enhanced sequence-specific DNA binding and transactivation of p53 targets [98]. However, the $p53^{K317R}$ knock-in mice, defective for acetylation at this residue, surprisingly indicated the opposite. Despite normal p53 protein stabilization in DNA-damage-treated $p53^{K317R/K317R}$ cells, target genes such as *Noxa* and *Pidd* were induced to higher levels than in wild-type cells in response to DNA damage and irradiated $p53^{K317R/K317R}$ mice showed increased apoptosis in the small intestine relative to $p53^{+/+}$ mice. Taken together, the augmentation of both pro-apoptotic gene expression and apoptosis indicates that acetylation at lysine 317 negatively regulates p53 transcriptional activity. This illustration that acetylation on one specific residue can negatively regulate p53 activity, while other acetylation events can positively regulate p53 function, highlights the possibility that the analysis of compound mutants with numerous alterations may obscure the role of a PTM on an individual residue.

Analysis of p53 PTM sites using mutant mouse models has revealed that many of these modifications are required for maximal p53 responses to cellular stresses. In addition, there are context-dependent roles for individual p53 PTMs, in specific cell types or in response to particular stress signals. While some studies have confirmed an expected function for a given PTM based on *in vitro* studies, there have been some surprises, such as the relatively normal stability of $p53^{7KR}$ and $p53^{6KR}$ under basal conditions and the efficient stabilization of $p53^{S18A/S23A}$ in some cell types after DNA damage. In addition, we have learned that stress signals can promote both “activating” and “repressing” PTMs that can fine-tune p53 activity in a particular setting. Importantly, the phenotypes of compound PTM site mutant mice may thus represent the net effect of altering both positively- and negatively-regulated p53 PTMs. Future investigations will provide further insight into the specific role of each PTM and how modifications can be manipulated as therapeutic targets for cancer.

6. Looking upstream of p53

In addition to mutations in p53 itself, alterations in other components of the p53 pathway may contribute to tumorigenesis[99,100]. The significance of perturbations in specific upstream regulators of p53 for carcinogenesis has been unequivocally demonstrated using mouse models. As described above, p53 is normally restrained by negative regulators, the best known of which are Mdm2 and Mdm4 [9,10]. Mdm2 binds the p53 transcriptional activation domains and serves as a key negative regulator both by masking the p53 transactivation domains and by serving as an E3 ubiquitin ligase to destabilize p53. The importance of the Mdm2-p53 interaction in restricting p53 activity was initially shown through the generation of $Mdm2^{-/-}$ mice, which displayed early embryonic lethality that was rescued in the backdrop of p53 nullizygosity [9]. Moreover, Mdm2 transgenic mice demonstrated that Mdm2 promotes the development of multiple tumor types[101]. In further support of a role for p53 pathway alterations in human carcinogenesis, Mdm2 is frequently amplified in 30–40% of human sarcomas of various types [99,101,102] as well as in other tumor types [101]. Akin to Mdm2, the Mdm4 protein also interacts with p53 transactivation domains to block their function. Moreover, although not an E3 ubiqutin ligase itself, Mdm4 can hetero-oligomerize with Mdm2 to enhance Mdm2 stability and ligase activity. As with Mdm2 deficiency, $Mdm4^{-/-}$ mice exhibit early embryonic lethality due to unrestrained p53 activity, and viability is restored upon *p53* loss [103–105]. Mdm4 transgenic mice have bolstered the idea that modulating p53 pathway components can promote cancer, as these mice more readily developed sarcomas than non-transgenic counterparts [106]. Mdm4 is also amplified in human cancers such as melanomas and retinoblastomas, illustrating the significance of this event in human tumorigenesis [107,108].

Oncogenic signals activate p53 through induction of the $p19^{ARF}$ tumor suppressor, which positively regulates p53 by binding and inhibiting Mdm2 [109–111]. The importance of $p19^{ARF}$ in activating p53 tumor suppressor function is underscored by the observation that $p19^{ARF}$ null mice are predisposed to early-onset spontaneous tumors, including fibrosarcomas and lymphomas [112]. Moreover, frequent mutations or deletions of the *Ink4a* locus, which comprises both p16 and $p19^{ARF}$, are found in human cancers. Together, these results are consistent with the important role of $p19^{ARF}$ in activating p53 in response to oncogenic signals as a safeguard against tumorigenesis. Collectively, these studies highlight

the fact that the p53 pathway can be compromised not only through *p53* mutation but also through disruption of other pathway components.

7. Therapeutic promise of p53 activation

As described above, complete loss of p53 function results in a dramatic predisposition to tumor development. An important follow-up question was whether sustained p53 loss is necessary for tumor maintenance, as has been noted with oncogenes, where tumors display “oncogene addiction” [113]. Given the genomic instability that can ensue after p53 loss, it was plausible that reactivation of p53 would no longer be able to dampen tumor growth – a question of paramount importance for evaluating the therapeutic potential of p53 restoration. Thus, several studies in mouse models have investigated this question by directly evaluating the consequence of p53 reactivation in tumors. In initial studies, three different approaches to p53 restoration were taken. In one case, mice homozygous for a $p53^{LSL\text{-}wt}$ allele, in which a floxed transcriptional stop cassette silences the *p53* locus, creating a *p53* null allele, were aged to allow spontaneous tumor development [114]. Upon activation of a tamoxifen-inducible, ubiquitously expressed Cre recombinase to induce widespread p53 expression, the majority of spontaneous lymphomas and sarcomas were found to regress. p53-driven regression of lymphomas was associated with apoptosis, while regression of sarcomas was associated with cellular senescence. Another study leveraged knock-in mice expressing a fusion protein of p53 to the hormone-binding domain of the estrogen receptor ($p53^{ERTAM}$, Fig. 2), which remains inactive until the administration of tamoxifen [50,115]. After allowing Eμ-*myc;*$p53^{ERTAM/-}$ B-cell lymphomas to develop, tamoxifen was used to activate p53, and it was found that tumors underwent efficient regression by p53-mediated apoptosis [116]. Additionally, in a model of hepatocellular carcinoma driven by HRasV12 and expression of an shRNA directed against p53, reactivating p53 by extinguishing the *p53* shRNA also caused tumor regression, associated with cellular senescence and tumor clearance through activation of the innate immune system [12]. All of these studies highlighted the therapeutic potential of p53 reactivation in tumors. However, in contrast to these findings, p53 reactivation in a KrasG12D-driven model of non-small-cell lung cancer showed more limited activity in tumor regression [117,118]. Specifically, p53 restoration led to tumor cell elimination only in malignant adenocarcinomas but not adenomas, and this difference was attributable to lack of a sufficiently potent oncogenic signal to activate p53 in the less malignant lesions. These studies therefore provide a cautionary note in terms of the efficacy of tumor eradication by p53 restoration, and indicate that the potential of reactivating wild-type p53 as a therapeutic avenue for treating p53-deficient tumors may be limited to select contexts.

8. Summary

The development of genetically engineered mice expressing p53 mutants has greatly contributed to our understanding of p53 function during tumorigenesis. These studies have established the importance not only of p53 loss-of-function but also p53 gain-of-function properties for promoting carcinogenesis, setting the stage for more detailed unraveling of the pathways underlying these activities. Structure–function analyses have provided new insight into the molecular and cellular mechanisms underlying p53 action *in vivo*, prompting some

revision of models for how p53 acts. Investigation of the roles of p53 post-translational modification in regulating p53 has yielded an enhanced understanding of how stress signals impinge upon p53. Finally, reactivation of p53 in mouse models has shown the potential of p53 restoration in limiting tumorigenesis in some but not all contexts. Although studies of each mouse model have revealed new insight into p53, they also have raised new questions about p53 function that will require additional investigation both *in vitro* and *in vivo*. Importantly, having a complete and accurate understanding of p53 biology *in vivo* will ultimately pave the way for translating our knowledge into improved cancer therapy.

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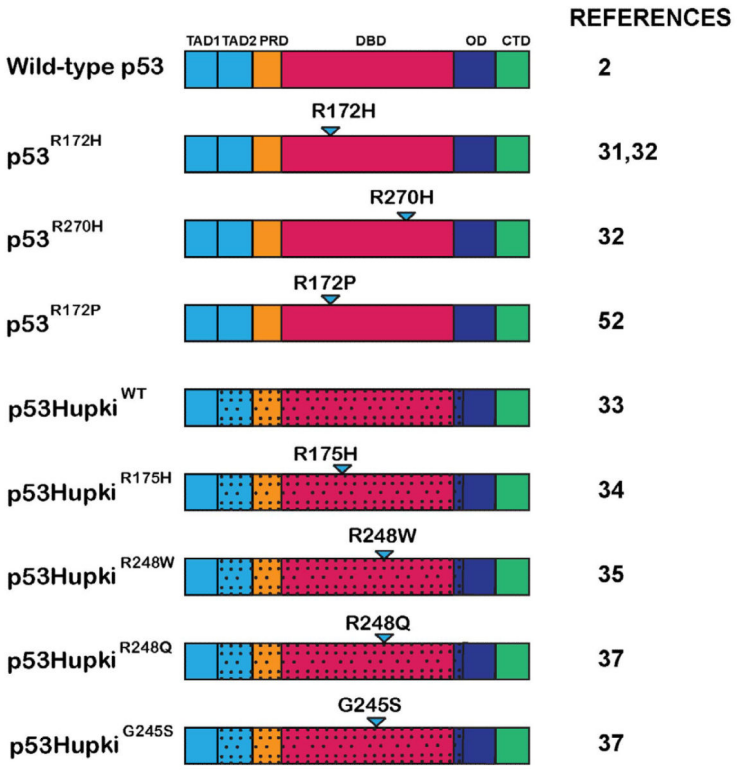

Fig. 1.

p53 tumor-derived mutants examined in knock-in mouse models. The p53 tumor-derived mutants studied in mouse models and described in this review. Wild-type p53 is shown for reference. p53 functional domains: TAD1 = transactivation domain 1; TAD2 = transactivation domain 2; PRD = proline rich domain; DBD = DNA-binding domain; OD = oligomerization domain; CTD = C-terminal domain. Triangles denote mutated amino acid. Stippling indicates regions of p53 replaced with human sequences in the HUPKI mouse models.

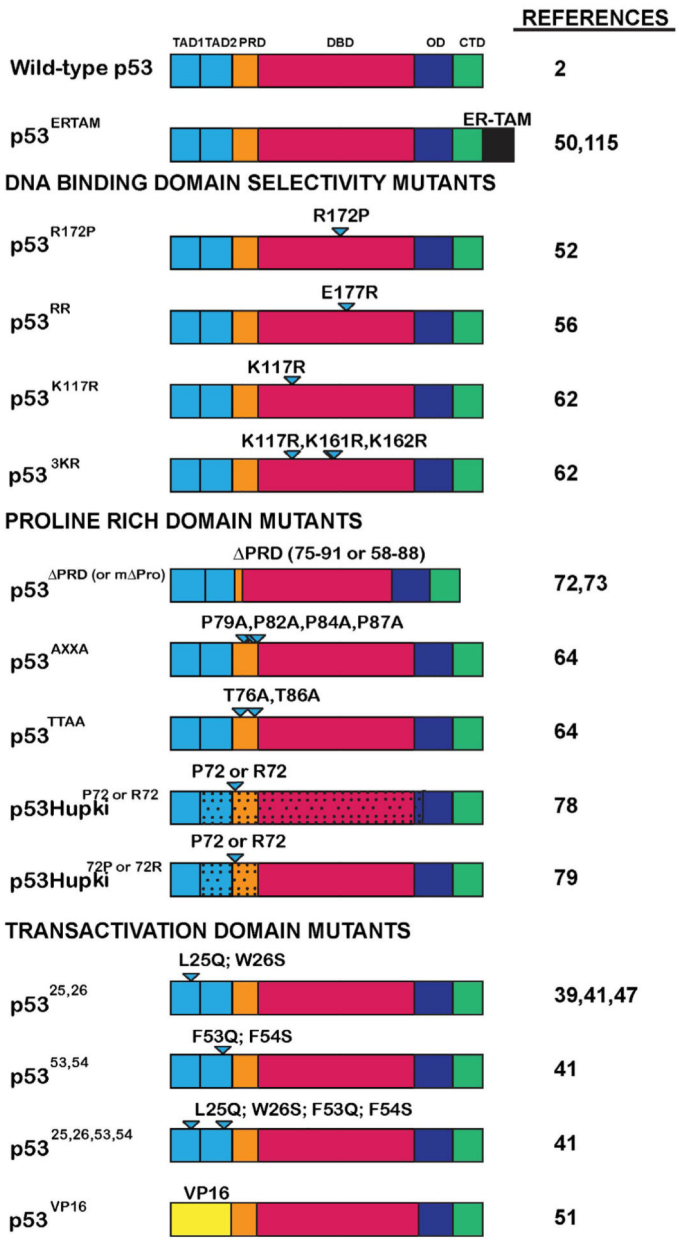

Fig. 2.

p53 domain mutants investigated in knock-in mouse models. The p53 domain mutants described in this review are categorized according to the functional domains targeted. Wild-type p53 is shown for reference. p53 functional domains: TAD1 = transactivation domain 1; TAD2 = transactivation domain 2; PRD = proline rich domain; DBD = DNA-binding domain; OD = oligomerization domain; CTD = C-terminal domain. Triangles denote mutated amino acids. ERTAM = tamoxifen-inducible estrogen receptor ligand-binding domain; VP16-herpes simplex viral protein 16 transactivation domain. RR is so-called because of the E180R mutation adjacent to the unmutated residue R181. Even though it is a tumor-derived mutant, $p53^{R172P}$ is listed here again because it has been used for structure–

function analysis of p53. Stippling indicates regions of p53 replaced with human sequences in the HUPKI mouse models.

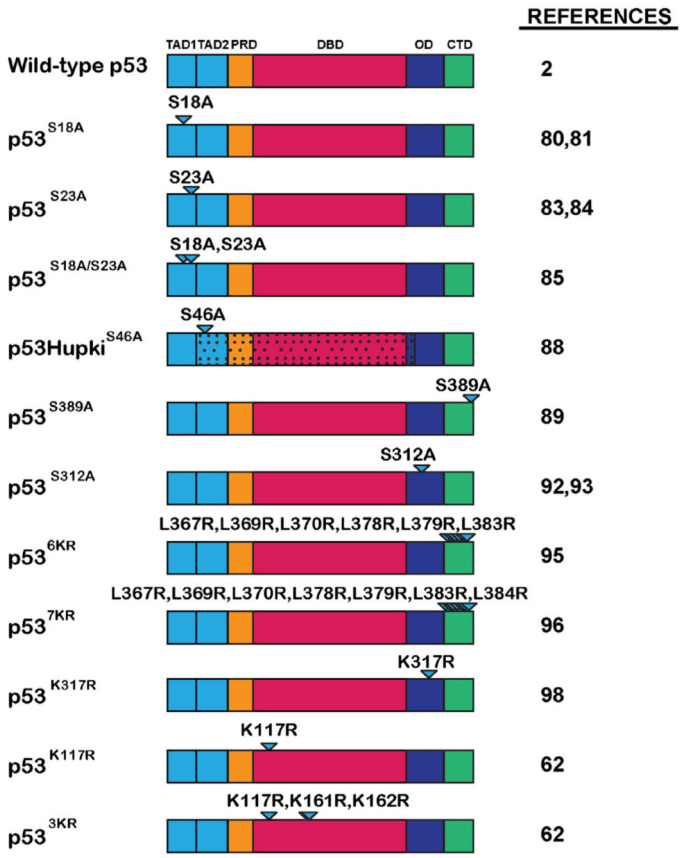

Fig. 3.

p53 post-translational modification site mutants investigated in knock-in mouse models. The p53 post-translational modification site mutants described in this review. Wild-type p53 is shown for reference. p53 functional domains: TAD1 = transactivation domain 1; TAD2 = transactivation domain 2; PRD = proline rich domain; DBD = DNA-binding domain; OD = oligomerization domain; CTD = C-terminal domain. Triangles denote mutated amino acids. Stippling indicates regions of p53 replaced with human sequences in the HUPKI mouse models.