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A Balancing Act: p53 Activity from Tumor Suppression to Pathology and Therapeutic Implications

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p53, tumor suppression, transcription factor, cancer, developmental disease, GOF, DNE, therapeutic strategies

Abstract

TP53, encoding the p53 transcription factor, is the most frequently mutated tumor suppressor gene across all human cancer types. While p53 has long been appreciated to induce antiproliferative cell cycle arrest, apoptosis, and senescence programs in response to diverse stress signals, various studies in recent years have revealed additional important functions for p53 that likely also contribute to tumor suppression, including roles in regulating tumor metabolism, ferroptosis, signaling in the tumor microenvironment, and stem cell self-renewal/differentiation. Not only does *p53* loss or mutation cause cancer, but hyperactive p53 also drives various pathologies, including developmental phenotypes, premature aging, neurodegeneration, and side effects of cancer therapies. These findings underscore the importance of balanced p53 activity and influence our thinking of how to best develop cancer therapies based on modulating the p53 pathway.

1. INTRODUCTION

The *TP53* gene, encoding the transcription factor and tumor suppressor protein p53, is the most commonly mutated gene across a wide spectrum of sporadic cancers, with an overall mutation rate of more than 50% (1, 2). Mutant *TP53* allele inheritance also renders individuals prone to developing tumors, as in Li-Fraumeni syndrome (3). These observations highlight the key tumor-suppressive role for p53; this role is further supported by the observed 100% incidence of cancer in *Trp53* null mice (4, 5). p53 functions as a molecular hub that coordinates stress signals and cellular biological responses (6). p53 is normally expressed at low levels in cells, as it is targeted for degradation by the E3 ubiquitin ligase MDM2 (7). In response to various cellular stresses, such as DNA damage, oncogenic signaling, oxidative stress, and nutrient deprivation, p53 is activated, resulting in the induction of specific downstream target genes that are involved in antiproliferative cellular responses, including cell cycle arrest, apoptosis, and senescence (8). In these scenarios, p53 protects cells from potential damage by repairing them or protects tissues by eliminating aberrant cells, thus limiting neoplasia. In addition to these canonical p53 functions, recent studies have revealed additional roles for p53 in regulating tumor metabolism (9), ferroptosis (10), tumor microenvironment signaling (11), and stem cell self-renewal/differentiation (12), any or all of which may contribute to the tumor suppression functions of p53. Despite the fundamental role of p53 in tumor suppression, the transcriptional network of p53 that mediates tumor suppression remains unclear, and strategies to modulate the p53 pathway have not yet become part of the standard of care for cancer therapy (13).

One of the limitations in developing p53-based therapies is that while they are clearly beneficial for organismal health by restraining cancer, inappropriate p53 activation can also cause detrimental effects (**Figure 1**). Accumulating evidence from both mouse models and humans shows that p53 hyperactivity can promote various pathological states, including a host of developmental phenotypes associated with specific syndromes (14), premature aging (15), and neurodegenerative diseases (16). In addition, p53 activation in response to genotoxic cancer therapies can promote

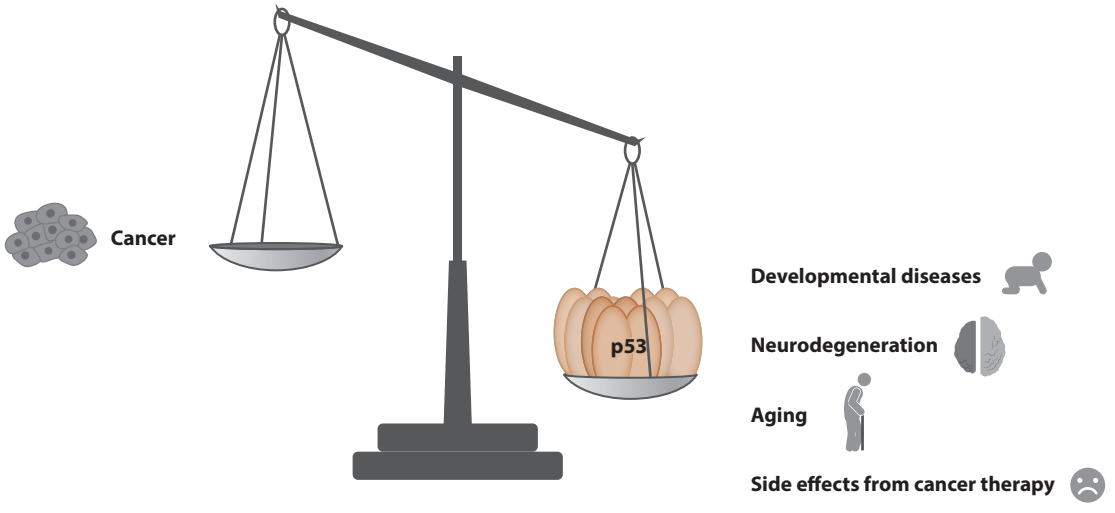

Figure 1

The importance of balanced p53 activity. Too little p53 can promote cancer development, while hyperactive p53 can induce various pathologies, including a host of developmental diseases, neurodegenerative diseases, premature aging, and side effects from cancer therapy.

side effects associated with these DNA-damaging therapies (reviewed in 17). Thus, the deleterious effects of p53 activation in normal tissues pose a challenge for some approaches to developing p53-based cancer therapies. Conversely, strategies to treat p53-driven pathologies through p53 inhibition could help to mitigate phenotypes in these conditions but could come at the cost of promoting cancer.

Advancing p53-based therapies for cancer and for p53-induced pathologies requires an understanding of the molecular underpinnings of p53 action. Mouse models have shown that transcriptional activation potential is critical for the ability of p53 to suppress cancer and to drive pathological phenotypes (14, 18–20). As with other transcription factors, p53 contains discrete domains involved in transactivation, sequence-specific DNA binding, and oligomerization (6, 21) (**Figure 2*a***). Mutations in human tumors occur primarily in the DNA-binding domain (DBD), supporting the importance of p53 transcriptional function for tumor suppression (6). Moreover,

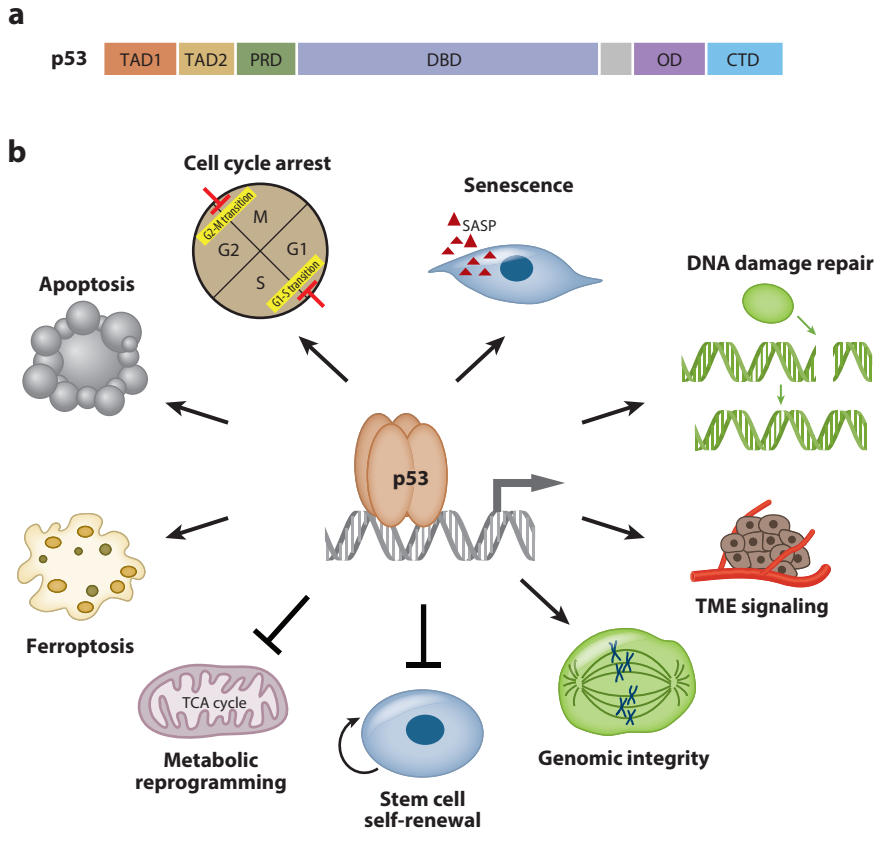

Figure 2

p53 domain structure and proposed p53 functions in tumor suppression. (*a*) The p53 protein contains six major domains: two amino-terminal TADs (TAD1 and TAD2), a PRD, a sequence-specific DBD, an OD, and a CTD. (*b*) Upon activation by various types of cellular stress, p53 activates canonical functions, such as apoptosis, cell cycle arrest, and DNA damage repair, and modulates noncanonical processes including ferroptosis, metabolic reprogramming, anticancer signaling in the tumor microenvironment, and stem cell self-renewal or differentiation. Abbreviations: CTD, carboxy-terminal domain; DBD, DNA-binding domain; OD, oligomerization domain; PRD, proline-rich domain; SASP, senescence-associated secretory phenotype; TAD, transactivation domain; TCA, tricarboxylic acid; TME, tumor microenvironment.

p53 belongs to a family of transcription factors, comprising p53, p63, and p73 (reviewed in 22). These proteins have similar DBDs and can regulate genes through the same DNA sequence motif, but p63 and p73 have primarily developmental functions, in the formation and function of stratified epithelia and multiciliated epithelia, respectively (23, 24).

In this review, we present an overview of the critical importance of balanced p53 activity. We summarize the current knowledge of the cellular and molecular mechanisms of how *p53* loss or mutation promotes cancer development. We also describe evidence from human data and mouse models that reveals how p53 hyperactivation drives a range of pathologies, including in developmental syndromes, aging, and neurodegenerative diseases. Finally, with this perspective on the importance of balanced p53 activity, we discuss therapeutic approaches developed for targeting cancers by modulating the p53 pathway.

2. CANONICAL VERSUS NONCANONICAL p53 FUNCTIONS IN TUMOR SUPPRESSION

2.1. Canonical p53 Functions

The longest-studied p53 cellular responses are cell cycle arrest and apoptosis in response to DNA damage signals, roles that led to p53 being named the guardian of genome (25). In response to DNA damage signals, p53 binds DNA in a sequence-specific manner and transactivates a network of target genes, which in turn induce antiproliferative cellular responses, such as cell cycle arrest, apoptosis, or senescence, depending on the context (6, 8) (**Figure 2*b***).

2.1.1. Cell cycle arrest. p53 induces G1 cell cycle arrest in response to acute DNA damage signals, giving cells a chance to repair their genomes before reentering the cell cycle, preventing potentially oncogenic mutations and maintaining genomic stability. In this capacity, p53 directly transactivates the *Cdkn1a* gene, encoding the cyclin-dependent kinase inhibitor p21, to suppress cell cycle progression (26). Although cells isolated from p21-deficient mice were partially defective in the G1 arrest response to DNA damage, p21-deficient mice were not predisposed to early onset spontaneous tumors as p53-deficient mice were (27, 28). However, mouse genetic experiments have shown that p21 does contribute to tumor suppression in certain contexts, such as in $Trp53^{R172P}$ mice, which express a p53 mutant that can induce cell cycle arrest but not apoptosis (29), supporting the importance of cell cycle regulation for p53-mediated tumor suppression in some settings. Other genes involved in p53-dependent cell cycle arrest, such as *Gadd45a* and *Ptprv*, may also contribute to tumor suppression (30, 31).

2.1.2. Apoptosis. p53 can also trigger apoptosis in response to stressors, including DNA damage, oncogene expression, and nutrient or oxygen deprivation, by transcriptionally activating proapoptotic genes such as the Bcl2 family members *Puma*, *Noxa*, and *Bax* and noncanonical apoptosis genes such as *Perp* (6, 8). p53-induced apoptosis has been shown to be important for suppressing tumorigenesis in some settings. For example, in the *Eμ-myc* transgenic mouse B cell lymphoma model, p53 induced extensive apoptosis in Myc-expressing B cells, while p53-deficient tumors had a defective apoptotic response, resulting in aggressive lymphomas (32). Modulation of the apoptosis machinery via overexpression of Bcl2 or dominant-negative caspase 9 expression in *Eμ-myc* mice with intact *p53* enhanced lymphoma development and relieved the selective pressure to lose *p53*, supporting the importance of apoptosis for tumor suppression in this model. Notably, cells in these tumors retained intact G1 checkpoints and did not display genomic instability, further supporting the importance of p53-driven apoptosis in this model. This notion

was supported by studies of *Eμ-myc;Puma*-deficient mice, which showed accelerated lymphoma development relative to mice with intact Puma (33–35). In addition, tumors in *TgT121* transgenic mice, driven by expression of a truncated SV40 large T antigen in the choroid plexus epithelium, displayed high selective pressure for *p53* inactivation, accompanied by loss of apoptosis (36). *Bax* heterozygosity in *TgT121* transgenic mice with wild-type *p53* was sufficient to reduce apoptosis and decrease the selective pressure for *p53* loss, further suggesting that p53-induced apoptosis is the primary tumor-suppressive p53 function in this model (37).

2.1.3. Dispensability of acute DNA damage responses? Adding to the complexity, studies in recent years have challenged the importance of p53 responses to acute DNA damage in tumor suppression. First, by modulating p53 expression in radiation-induced mouse lymphoma models, it was shown that p53 need not be expressed during irradiation, when it could drive apoptosis, suggesting that the immediate responses to acute DNA damage are dispensable for tumor suppression (38, 39). These initial studies were supported by additional mouse models such as knock-in mice expressing $p53^{25,26}$—a mutant with alterations in p53 transactivation domain 1 (TAD1)—which is unable to activate many classical p53 target genes, including *Cdkn1a*, *Puma*, and *Noxa*. These mice did not retain cell cycle arrest or apoptosis responses to acute DNA damage but were resistant to developing a variety of types of cancer, including medulloblastoma, lung adenocarcinoma, and B and T cell lymphomas (18, 19). These findings suggested that the transcriptional programs underlying acute DNA damage responses are dispensable for tumor suppression. This notion was supported by analyses of the $Trp53^{3KR}$ mouse strain bearing three acetylation site mutations in the p53 DBD (K117R, K161R, and K162R) that render p53 unable to activate *Puma* and *Cdkn1a* or to induce cell cycle arrest and apoptosis in response to DNA damage as well as $Cdkn1a^{-/-};Puma^{-/-};Noxa^{-/-}$ triple knockout mice, which have defective acute DNA damage responses but are nonetheless still resistant to spontaneously arising tumors (40, 41). Collectively, these findings suggest either that there is compensation for these acute DNA damage responses when they are inactivated or, alternatively, that there are other noncanonical p53-regulated functions that are critical for inhibiting tumor development (42).

2.2. Noncanonical p53 Functions

In recent years, additional insight into p53 functions that contribute to suppressing tumor formation has been gained. Novel mechanisms have been proposed for the classical function of p53 in preserving genome stability, including by activating a tetraploidy checkpoint, inhibiting the movement of transposons and repetitive elements, and promoting proper DNA replication fork progression (reviewed in 42). p53 has also been proposed to promote tumor suppression by modulating additional cellular processes, including metabolism, ferroptosis, cross talk in the tumor microenvironment, and stem cell self-renewal and differentiation (9–12) (**Figure 2*b***).

2.2.1. Metabolism. To ensure the production of sufficient energy and to support anabolic needs for rapid growth, cancer cells undergo a metabolic reprogramming known as the Warburg effect, characterized by increased glucose uptake and fermentation of glucose to lactate (43). p53 dampens the Warburg effect by either inhibiting genes involved in glycolysis, such as glucose transporter genes *GLUT1* and *GLUT4*, or activating genes involved in oxidative phosphorylation, such as *GLS2* and *SCO2* (reviewed in 9). Studies of the $Trp53^{3KR}$ mouse strain, which is deficient for canonical p53 functions, indicated that the $p53^{3KR}$ mutant retains the capacity to regulate the expression of some metabolic p53 target genes and to inhibit glycolysis, which may be important for p53 tumor suppressor function (40). In addition to inhibiting the Warburg effect, p53 can also

suppress tumorigenesis by inhibiting the mevalonate pathway (44). The mevalonate pathway is crucial for producing sterols, such as cholesterol, and nonsterol isoprenoids, which are integral to tumor growth. p53 was shown to repress the mevalonate pathway by activating the *Abca1* cholesterol transporter gene, while in p53-deficient cancer cells, the mevalonate pathway was activated, allowing cancer cells to proliferate under low-sterol conditions.

2.2.2. Ferroptosis. Studies of the $p53^{3KR}$ mutant mouse also led to the idea that ferroptosis is important for suppressing tumor formation (10, 45). Ferroptosis is a novel type of programmed cell death characterized by the iron-dependent accumulation of oxidatively damaged phospholipids, and it is initiated by cystine and/or glutathione deprivation. p53 can enhance ferroptosis by repressing the expression of the *SLC7A11* gene, which encodes an important subunit of the cystine-glutamate antiporter system (45). The $p53^{3KR}$ mutant retains the capacity to repress *SLC7A11*, which was proposed to contribute to the tumor suppression function of this mutant. In contrast, knock-in mice expressing the $p53^{4KR}$ (K98R+3KR) mutant, which loses the ability both to suppress *SLC7A11* and to induce ferroptosis, were tumor prone, correlating ferroptosis with tumor suppression (46). Moreover, mice expressing the $TP53^{S47}$ polymorphic variant identified in humans exhibited increased incidence of spontaneous tumors compared with wild-type mice, and cells from the $TP53^{S47}$ mice showed decreased sensitivity to ferroptosis, again linking ferroptosis and tumor suppression (47). However, additional studies have shown that p53 can either inhibit or fail to modulate ferroptosis in other contexts (48–50). Possible explanations for the disparities among studies include context-dependent differences in cell type and ferroptosis-activating stresses and differences in the status of lipoxygenase Alox12, which is critical for p53-induced ferroptosis (51). Future studies need to further interrogate the contexts in which p53 promotes or opposes ferroptosis and the consequences for tumorigenesis.

2.2.3. Signaling in the tumor microenvironment. Studies in recent years have also highlighted important roles for p53 in regulating signaling within the tumor microenvironment, a function of great significance for antitumor immune responses, including both innate and adaptive immune responses. For example, in a mouse liver cancer model, reactivation of p53 induced tumor regression, which was driven by p53 triggering senescence, cytokine secretion, and activation of the innate immune response (52). Another study utilizing 16 distinct genetically engineered mouse breast cancer models revealed that loss of p53 triggered WNT signaling and tumor-associated macrophages to produce IL-1β, which subsequently activated prometastatic inflammation (53). Moreover, in $Kras^{G12D}$-driven pancreatic cancer mouse models, loss of p53 not only increased immunosuppressive myeloid cell infiltration into tumors but also enhanced recruitment of Treg cells, which in turn inhibited T cell antitumor responses (54). Together, these findings suggest that p53 is critical for promoting an antitumor microenvironment.

2.2.4. Stem cell biology. The role of p53 in restricting stem cell self-renewal and promoting differentiation has also been proposed to contribute to blocking tumorigenesis (12). This notion was supported initially by the demonstration that p53 can potently inhibit reprogramming of mature differentiated cells to induced pluripotent stem cells, through activation of the *Cdkn1a* and *miR34a* target genes (55, 56). Activation of *Cdkn1a* and *miR34a* by p53 can also limit cancer cell plasticity and promote the differentiation of human embryonic stem cells (55, 57). In addition, the function of p53 in regulating stem cells is supported by the observation that *TP53* mutations are associated with stem cell signatures in human breast cancer and lung cancer (58). A variety of mouse model studies have also shown that loss of p53 enhances self-renewal of different types of

stem/progenitor cells, such as hematopoietic stem cells, leukemia stem cells, and neural stem cells (reviewed in 8, 59)

2.2.5. Pleiotropy of p53 function. As just described, p53 clearly regulates many important cellular responses. However, these studies did not clearly address whether each specific p53-modulated cellular behavior is fundamentally responsible for suppressing tumorigenesis in a given context, according to the underlying tissue biology, or whether p53 might simultaneously regulate various cellular functions to suppress cancer in a given setting. The ability of p53 to regulate a range of cellular functions in one given context was interrogated in *E1A;Hras*^{G12V} oncogene-expressing primary mouse embryonic fibroblasts (MEFs), a tractable cellular model in which p53 potently suppresses transformation (49). These studies, which were conducted under physiological (5%) oxygen levels to better mimic in vivo conditions than the standard 21% atmospheric oxygen condition, demonstrated that loss of p53 induced pleiotropic effects during transformation, affecting both canonical and noncanonical functions. Notably, modulation of some processes, such as inhibition of invasion, by p53 was only observed in the 5% oxygen condition. These findings suggest that p53 can simultaneously regulate diverse cellular functions in a particular setting to block malignant transformation.

2.2.6. Genetic screens for p53 tumor suppression mediators. Finally, one additional approach that has lent insight into pathways downstream of p53 in tumor suppression relied on unbiased in vivo genetic screens. Building on observations of the p53^{25,26} TAD1 mutant that activates only a small subset of p53 target genes [tumor suppression associated genes (TSAGs)] yet retains full capacity for tumor suppression in multiple mouse models, screens were designed to test the importance of TSAGs in tumor suppression. Short hairpin RNA (shRNA) or single guide RNA (sgRNA) libraries targeting the 87 p53-dependent TSAGs induced by both wild-type p53 and p53^{25,26} were delivered into *E1A;Hras*^{G12V}-expressing MEFs, and the cells were then transplanted subcutaneously into *Scid* mice to allow in vivo tumor growth (60). shRNA or sgRNA elements enriched in tumors, indicative of targeting functional tumor suppressors, were then identified. Importantly, both screens identified the p53 target gene *Zmat3*, encoding an RNA binding protein, as a critical tumor suppressor. The broad importance of *Zmat3* in tumor suppression was demonstrated by studies in *Kras*^{G12D}-driven mouse lung adenocarcinoma and hepatocellular carcinoma models (60), as well as by an shRNA screen for suppressors of lymphomagenesis in mice (61). Zmat3 is thought to inhibit tumor development by directly modulating RNA splicing of diverse transcripts, including *Mdm2* and *Mdm4*, which encode negative regulators of p53, and various other transcripts encoding proteins with diverse cellular functions. This study, along with a recent finding that Zmat3 blocked clonogenicity of human colon cancer cells by modulating CD44 splicing (62), reveals a key link between p53-mediated tumor suppression and splicing. Future analysis of other hits in the aforementioned screens, such as *Ptpn14*, encoding a negative regulator of the Yap oncoprotein, as well as new genetic screens will provide additional perspective on p53 target genes critical for tumor suppression and help to reconstruct the tumor-suppressive programs downstream of p53.

3. UNDERSTANDING THE SIGNIFICANCE OF *TP53* MISSENSE MUTATIONS

Although p53 loss clearly promotes cancer, the vast majority of *TP53* mutations in human cancers—approximately 80%—are missense mutations and can result in the accumulation of mutant p53 proteins with proposed gain-of-function (GOF) activities (13). *TP53* missense mutants

can be categorized into DNA contact mutants, which directly disrupt the interaction between p53 and DNA, and conformational mutants, which alter p53 folding and structure (13). Early studies showed that most p53 missense mutants, which are affected primarily in the DBD, lose the ability to transactivate target genes yet can still tetramerize with wild-type p53, suggesting that dominant-negative effects (DNEs) could account for GOF effects (13). However, mutant p53 molecules were also found to exert GOF effects in the absence of p53.

3.1. Gain-of-Function Activities

While GOF activities for mutant p53 were originally suggested from cell culture experiments (63, 64), the notion was solidified by analysis of *Trp53* knock-in mice. Two mouse models of Li-Fraumeni syndrome were constructed by engineering $Trp53^{R172H}$ and $Trp53^{R270H}$ (corresponding to human tumor hotspot mutants R175H and R273H) into the endogenous *Trp53* locus in mice (65, 66). The tumor latency of the $Trp53^{R270H/+}$ and $Trp53^{R172H/+}$ mice was similar to that of $Trp53^{+/-}$ mice, but these mice displayed increased incidence of carcinomas, and tumors were more invasive and metastatic relative to $Trp53^{+/-}$ mice. Similarly, analysis of patients with Li-Fraumeni syndrome confirmed that patients with the germline $TP53^{R248Q/+}$ genotype had shorter tumor-free survival and increased tumor incidence than patients with a $TP53^{-/+}$ genotype (67). However, it was not clear whether these phenotypes resulted from potential GOF properties or DNEs (68, 69). To address this point, $Trp53^{R270H/-}$ and $Trp53^{R172H/-}$ mice were analyzed (65, 66). These mice were found to develop novel tumor types, including diverse carcinomas, and these tumors were more metastatic, relative to $Trp53^{-/-}$ mice, suggesting that the *Trp53* point mutations conferred GOF properties (65). Moreover, two additional humanized *TP53* knock-in mouse models harboring the $TP53^{R248Q}$ and $TP53^{G245S}$ alleles were constructed to further investigate p53 GOF properties. The $TP53^{R248Q}$ mutation showed GOF potential, as $TP53^{R248Q/-}$ mice displayed earlier onset of all tumor types and significantly shorter survival than either $TP53^{G245S/-}$ or $TP53^{-/-}$ mice (67).

Various mechanisms have been proposed to account for p53 GOF activity (reviewed in 70) (**Figure 3**). First, mutant p53 has been shown to bind and neutralize the function of the p53 family members p63 and p73, to inhibit their transcriptional activity on their target genes, which in turn promotes tumor invasion and metastasis. Second, studies have shown that mutant p53 can bind to DNA elements along with various transcription factors, such as Nrf2, Ets1/2, and Smads, to drive enhanced gene expression from these sites, resulting in protumorigenic consequences, such as the degradation of tumor suppressor proteins or augmented oncogenic signaling.

3.2. Dominant-Negative Effects

Although various lines of evidence support the GOF properties of mutant p53, studies from recent years have challenged this idea and argued that the oncogenic functions of mutant p53 are mainly driven by its DNEs. In one study (71), various *Trp53* missense mutants (ins*G280*, *V170M*, *I192S*, *R270H*, and *R246Q*) were expressed in hematopoietic stem/progenitor cell lines from $Trp53^{-/-}$, $Trp53^{+/-}$, and *Eμ-Myc;*$Trp53^{+/+}$ mice; cells were introduced into lethally irradiated recipient mice; and the development of lymphoma was monitored. None of these mutants accelerated lymphoma development relative to the empty vector control in the $Trp53^{-/-}$ and $Trp53^{+/-}$ genetic backgrounds, suggesting no GOF activity. However, expression of mutants in the *Eμ-Myc;*$Trp53^{+/+}$ background accelerated tumorigenesis, which was attributed to a DNE. RNA sequencing of lymphoma-derived cell lines from these mice suggested that mutant p53 drove tumor growth by repressing the expression of wild-type p53 target genes involved in DNA repair,

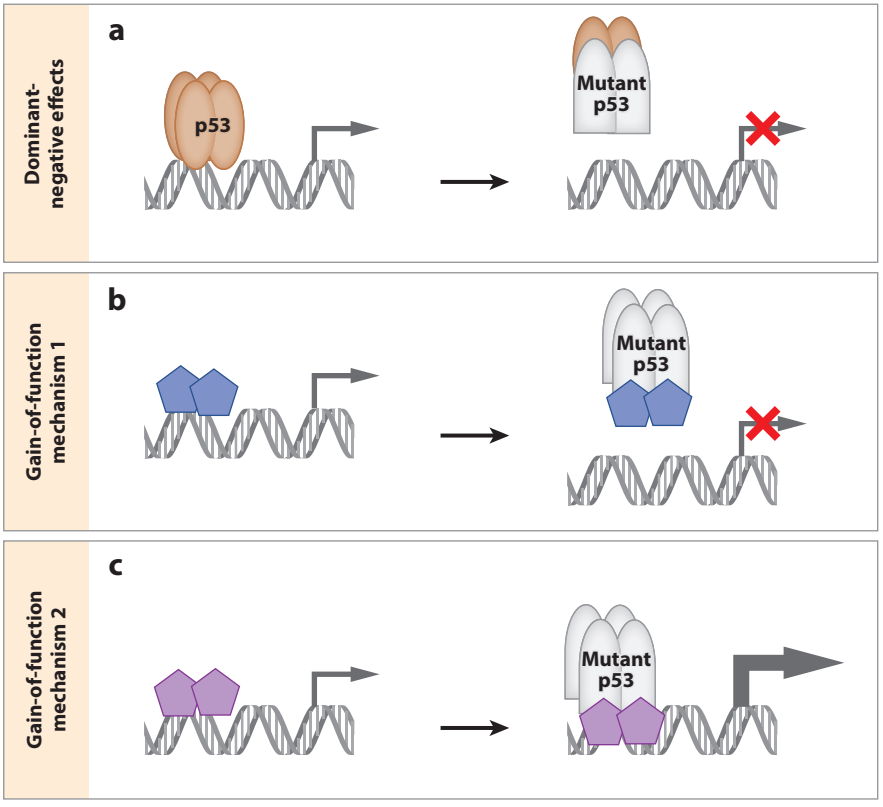

Figure 3

Models for mutant p53 gain-of-function activities. (*a*) p53 mutants can exert dominant-negative effects by tetramerizing with wild-type p53, which in turn reduces the capacity of wild-type p53 to activate target genes and induce cellular responses. (*b*) p53 mutants can bind to p63 and p73, inhibiting their ability to activate their target genes. (*c*) p53 mutants can bind to novel interaction partners, including the Nrf2, Ets1/2, and Smad transcription factors, to drive enhanced expression of genes induced by these partners, resulting in protumorigenic consequences.

proliferation, and metabolism. In another study (72), CRISPR-Cas9 genome editing was used to generate isogenic human acute myeloid leukemia (AML) cell lines expressing the six most frequent *TP53* missense mutations. Assessment of proliferation and apoptosis after DNA damage indicated that $TP53^{missense/-}$ and $TP53^{-/-}$ cells behaved similarly. Furthermore, DNA-binding and transcriptional analyses failed to reveal any GOF transcriptional program common to the mutant p53 variants. In addition, there was no significant difference in tumor-free survival or overall survival between AML patients with *TP53* missense mutation and truncating mutations. These studies suggested that *TP53* missense mutations drive AML through their DNEs. Finally, studies (73) of $Kras^{G12D}$-driven pancreatic cancer development originating from acinar cells in adult mice with $Trp53^{fl/-}$, $Trp53^{fl/LSL\text{-}R270H}$, or $Trp53^{fl/LSL\text{-}R172H}$ genotypes revealed no clear GOF activity for $p53^{R270H}$ or $p53^{R172H}$ in terms of tumor latency or frequency of metastasis. In fact, mice expressing $p53^{R172H}$ showed delayed tumor latency relative to *Trp53* null mice, and there was no absolute selection for expression of mutant protein expression during tumor growth and metastasis.

All in all, it is quite controversial whether *TP53* missense mutations promote tumorigenesis through GOF properties or through DNEs. Several factors could contribute to the differences

between studies. First, there are limitations of the experimental techniques. For example, the data generated from in vitro and mouse xenograft models may fail to mimic the natural cancer microenvironment. Second, it is possible that *TP53* mutations exhibit GOF properties only in specific contexts. Future studies should focus on investigating p53 GOF potential in a variety of cancer contexts; such investigations will be important both for understanding tumor evolution and for developing therapeutic strategies for patients with mutant p53-expressing tumors.

4. p53 HYPERACTIVATION AND PATHOLOGICAL CONSEQUENCES

4.1. p53 Functions in Developmental Syndromes

As discussed above, it is not only *p53* inactivation and point mutation that have pathological consequences, by promoting tumorigenesis; p53 hyperactivation also can induce pathologies ranging from phenotypes characteristic of developmental syndromes to symptoms of neurodegenerative diseases. One of the best-characterized aspects of p53 pathology is its role in developmental syndromes, as p53 can drive a wide range of developmental defects in both mouse models and humans. The deleterious effects of inappropriate p53 activation in vivo were first appreciated through inactivation of the principal negative regulators of p53, Mdm2 and Mdm4, in mouse models. *Mdm2* or *Mdm4* deficiency triggered early embryonic lethality that was rescued by *Trp53* deletion (74–76), while $Mdm2^{+/-}$;$Mdm4^{+/-}$ mice were born but exhibited birth defects, including hematopoietic failure and cerebellar hypoplasia, which were rescued by the deletion of a single *Trp53* allele (77).

The understanding of the role of p53 in developmental defects was expanded by characterizing p53 hyperactivation induced by mutations in *Trp53* itself. For example, the carboxy-terminal domain (CTD) of p53, which functions as a negative regulator of the core DBD, was deleted in $Trp53^{\Delta CTD/\Delta CTD}$ and $Trp53^{\Delta 31/\Delta 31}$ knock-in mice, resulting in p53 hyperactivation (78, 79). $Trp53^{\Delta CTD/\Delta CTD}$ mice, lacking the carboxy-terminal 24 amino acids of p53, displayed severe postnatal developmental defects, including hematopoietic failure and abnormal cerebellum development accompanied by pronounced ataxia, followed by mortality within 2 weeks of birth (78). The hyperactivation of p53 resulted in enhanced senescence in mouse bone marrow cells and was potentially responsible for the hematopoietic failure. Similarly, $Trp53^{\Delta 31/\Delta 31}$ knock-in mice lacking the last 31 residues of the p53 CTD also displayed enhanced p53 activity and developed aplastic anemia, cutaneous hyperpigmentation, and pulmonary fibrosis, symptoms typical of the human dyskeratosis congenita syndrome (79). Mechanistic analyses revealed that p53 hyperactivation promotes downregulation of genes involved in telomere metabolism, including *Dyskerin*, *Rtel1*, and *Tinf2*, as well as telomere shortening. In another study (80), $Trp53^{25,26,53,54/+}$ mice with TAD1/2 domain alterations exhibited late-gestational embryonic lethality along with several phenotypes characteristic of CHARGE (coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and development, genitourinary hypoplasia, and ear abnormalities and deafness) syndrome. This $p53^{25,26,53,54}$ protein is a transcriptionally dead variant, but it can stabilize and hyperactivate wild-type p53, which in turn induces mild hyperactivation of select p53 target genes and increased levels of cell cycle arrest or apoptosis during development. In humans, CHARGE syndrome is typically caused by mutation of the chromatin remodeler *CHD7* (81). Accordingly, analysis of CHARGE patient samples and cell lines revealed p53 hyperactivation, suggesting the important role of p53 in promoting the exquisitely specific set of phenotypes typifying CHARGE syndrome (80).

How p53 drives such specific constellations of phenotypes in different developmental syndromes, however, is still incompletely understood. In one study (82), the mechanisms by which hyperactivated p53 drives distinct sets of developmental defects were investigated by examining

the consequences of modulating the intensity and the spatial pattern of p53 activation during embryogenesis. A panel of mouse models with a series of Cre-regulated conditional alleles to trigger different degrees of p53 activation in distinct cell compartments was used to deconvolute how p53 could drive different phenotypes. For example, mice with mild p53 activation in neural crest cells (NCCs) displayed hypoplastic NCC-derived facial bones and pigmentation defects at postnatal day 21, while moderate p53 activation in NCCs caused embryonic lethality with craniofacial and cardiovascular defects and severe p53 activation in NCCs triggered more dramatic abnormalities such as exencephaly. This study further revealed that p53 hyperactivity in the facial ectoderm, but not in NCCs, could promote ear and eye defects. These analyses helped to deconstruct the tissues in which p53 must be hyperactivated to drive specific developmental phenotypes. In addition, depending on the tissue, these phenotypes were associated with p53-dependent inhibition of proliferation or induction of apoptosis. Interestingly, defects in the neural crest–derived tissues in which p53 drove apoptosis were not dependent on apoptosis, as these defects were still manifested in embryos lacking the apoptotic machinery through deletion of *Caspase 9* or *Puma* (83). Thus, p53 may act through novel cell death pathways to drive developmental defects. The pathways leading to developmental phenotypes were further elaborated through analysis of mice homozygous for $Mdm2^{PND}$, an allele that produces low levels of Mdm2 (84). $Mdm2^{PND/PND}$ mice exhibited elevated levels of p53 and displayed reduced fertility, hematopoietic defects, and hyperpigmented skin. Deletion of *Puma* but not *Cdkn1a* in $Mdm2^{PND/PND}$ mice rescued the fertility and hematopoietic defects. In contrast, deletion of neither *Cdkn1a* nor *Puma* rescued the skin hyperpigmentation phenotype; instead, increased expression of the p53 target gene *Kitl* was found to be responsible for this phenotype through regulation of melanocyte migration. This result is reminiscent of previous studies showing that inappropriate p53 activation in the skin promoted pigmentation through effects on *Kitl* (85). Consistent with these mouse models, two human patients with de novo *TP53* germline variants exhibited bone marrow–failure syndromes associated with hypogammaglobulinemia, growth retardation, and microcephaly (86). Further study showed that these variants displayed loss of 32 residues from the CTD of p53.

p53 is activated in human disease not only by direct mutations in the genes encoding p53 and its regulators but also by stress signals caused by defects in a range of cellular processes, such as ribosome biogenesis, RNA splicing, and DNA repair. Such defects, which can be caused by a variety of gene mutations, have been associated with numerous human developmental syndromes (reviewed in 14). The best characterized of these are the ribosomopathies, in which mutations in ribosomal subunit-encoding genes trigger abnormal ribosome biogenesis and p53-dependent phenotypes. For example, mutations in *Rps19*, encoding ribosomal protein S19, are found in approximately 25% of Diamond-Blackfan anemia patients and cause red blood cell anemia and many developmental phenotypes, including orofacial, hand, limb, and heart defects (87). Missense mutations in *Rps19* and *Rps20* (encoding ribosomal protein S20) in mice induced anemia and skin hyperpigmentation, which were rescued by *Trp53* deletion (85). Moreover, the deletion of chromosome 5q, which includes the gene *RPS14*, is the cause of 5q− syndrome (88, 89). Patients with this syndrome exhibit macrocytic anemia that can be ameliorated by *Trp53* deletion in mouse models of 5q− syndrome. Future studies on these diverse developmental syndromes should focus on investigating the specific cellular and molecular pathways by which p53 promotes these pathological phenotypes; such studies could help with the development of treatment strategies.

4.2. p53 Hyperactivity and Adult Pathologies

Beyond developmental diseases, p53 hyperactivation is also associated with many pathological consequences in adults, including premature aging, various neurodegenerative diseases, and side

effects of genotoxic cancer therapies. The association between hyperactive p53 and premature aging phenotypes was first appreciated through studies in mouse models. *Trp53*$^{+/m}$ mice, expressing the m allele with a deletion of the first six exons of *Trp53*, can produce a carboxy-terminal fragment of p53 that enhances p53 responses (90). Interestingly, these mice displayed reduced life span compared with their *Trp53*$^{+/+}$ littermates, along with a host of early onset aging associated phenotypes, such as lordokyphosis, osteoporosis, organ atrophy, and wound-healing deficiencies. Similarly, mice overexpressing p44, a short isoform of p53, exhibited shortened life span and some aging phenotypes, such as lordokyphosis, diminished reproductive health spans, and reduced bone mineral density (91). Mechanistic analyses indicated the p44 mutant did not affect wild-type p53 levels but induced the hyperactivation of a subset of p53 target genes, resulting in decreased cellular proliferation and increased cellular senescence. Moreover, *Trp53*$^{TSD/-}$ mice expressing a p53 mutant with alterations that mimic constitutive phosphorylation (T21D and S23D), thereby enhancing p53 activity, also displayed aging-related phenotypes, such as severe curvature of the spine, bone marrow hypoplasia, and anemia (92). These findings are consistent with the identification of humans with germline *MDM2* mutations who present with premature aging phenotypes, including hair graying, kidney failure, and short stature, associated with enhanced p53 stability and activity (93).

p53 hyperactivity has also been implicated in various neurodegenerative diseases. Amyotrophic lateral sclerosis and frontotemporal dementia are caused by GGGGCC repeat expansion in the *C9orf72* gene, and a recent study demonstrated that expression of a dipeptide repeat protein produced by this sequence drove p53 stabilization, induction of p53 target gene expression, and p53-dependent apoptosis in neurons (94). Moreover, overexpression of the dipeptide repeat protein in wild-type mice induced neuronal degeneration and decreased mouse survival, and these phenotypes were abated in *Trp53* null mice. These recent findings expand on a set of previous studies showing that p53-induced apoptosis in neurons contributes to pathologies in Alzheimer's, Parkinson's, and Huntington's diseases (reviewed in 16).

p53 activation in normal tissues during treatment with genotoxic cancer therapies contributes to some of the side effects of DNA-damaging cancer therapies and has been modeled in the mouse (17). Specifically, comparison of wild-type and *Trp53* null mice revealed that p53-dependent growth arrest or apoptosis was activated by acute radiation, especially in intensively proliferating tissues, including hair follicles, intestine, thymus, growing bones, spleen, and lachrymal glands, supporting the idea that p53 activation can damage tissues upon cancer therapy (17, 95). Achieving optimal p53 levels in cancer treatments is a critical problem that needs to be addressed in the future.

Understanding the molecular basis for p53-induced pathologies is helpful not only for understanding the pathogenesis of these diseases but also for gaining insights into developing therapeutic strategies for these diseases. While direct pharmacological inhibition of p53 could be helpful for treating such conditions, a caveat to this strategy is that loss of p53 function is associated with increased cancer risk. It may therefore be more desirable to target the specific pathways by which p53 drives corresponding pathological phenotypes.

5. THERAPEUTIC TARGETING OF p53 IN CANCER

As discussed above, *TP53* is the most frequently mutated tumor suppressor gene across all cancer types, and mutant *TP53* drives cancer initiation and progression through DNEs and potential GOF mechanisms. Targeting the mutant p53 state is therefore an appealing strategy for cancer therapy. However, the potential for p53-driven pathologies presents a great challenge. Nonetheless, over decades of study, many strategies have been proposed for targeting the p53 pathway in

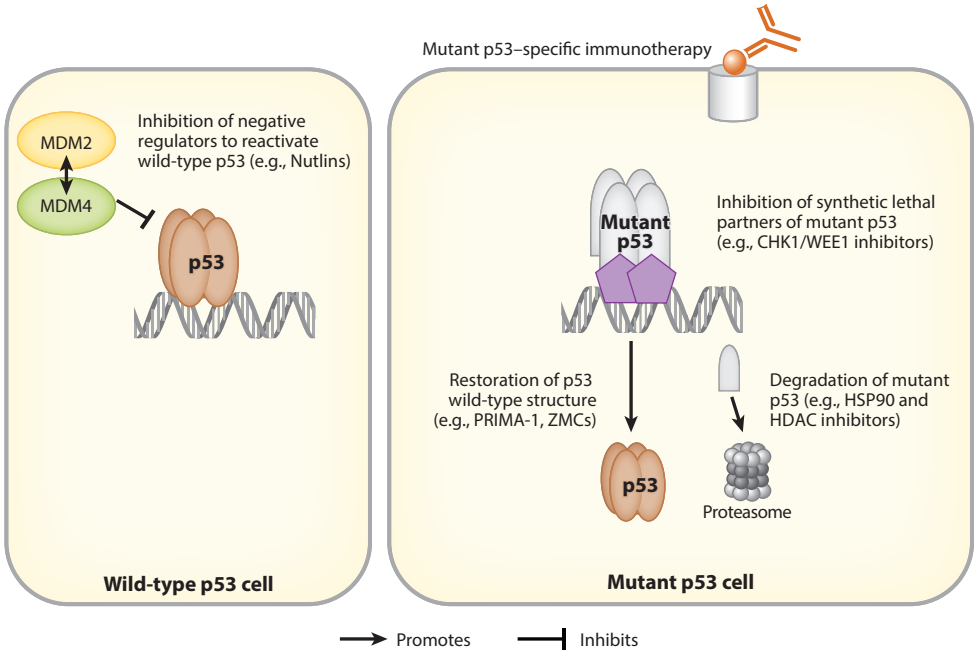

Figure 4

Strategies to restore wild-type p53 function or target mutant p53 in cancer. (*Left*) With cancer cells that retain wild-type p53, reactivation of wild-type p53 functions by displacing MDM2 and MDM4 may be an effective approach. (*Right*) With cancer cells expressing mutant p53, treatment strategies include restoration of wild-type p53 functions, degradation of mutant p53, synthetic lethal targeting of mutant p53, and immunotherapy that targets mutant p53. Abbreviations: HDAC, histone deacetylase; HSP, heat shock protein; ZMC, zinc metallochaperone.

cancer, including activation of wild-type p53 function, restoration of wild-type function to the mutant p53, degradation of mutant p53, synthetic lethal (SL) targeting of mutant p53, and immunotherapy approaches to target mutant p53 (**Figure 4**). It is critical to understand the pros and cons of each strategy, to ensure that the most appropriate approaches are developed and applied clinically.

5.1. Activating Wild-Type p53 Function

For the 50% of cancers that retain wild-type p53, it would be desirable to stimulate wild-type p53 function during cancer therapy. These cancers are sometimes characterized by overexpression of MDM2 and MDM4, negative regulators of p53 (96). MDM2 and MDM4 heterodimers can inhibit p53 transactivation and promote p53 degradation through the E3 ubiquitin ligase activity of MDM2 (96). Thus, disruption of MDM2/4-p53 complexes could reactivate wild-type p53 function. Indeed, small-molecule inhibitors of the MDM2-p53 interaction were developed on the basis of the crystal structure of MDM2 and a 15-residue TAD peptide of p53, revealing that MDM2 has a deep hydrophobic cleft (termed the p53 binding pocket) that is filled by the α-helical region of p53 TAD1 (97). To identify a small molecule that can block the p53-MDM2 interaction by filling the p53 binding pocket, synthetic chemical libraries were screened, leading to the identification of a set of *cis*-imidazoline analogs named Nutlins (98). Nutlins (especially Nutlin-3) promoted p53 protein accumulation, induction of p53-regulated genes, and cell cycle arrest or apoptosis only in

cancer cells with wild-type p53. Nutlin-3 also inhibited tumor growth in mouse xenograft models with intact p53 (98). However, further studies identified drawbacks in the use of Nutlin-3 for cancer therapy, such as low bioavailability, high toxicity, and low efficacy, especially in the treatment of MDM4-overexpressing cancers (99–101). Thus, subsequent efforts were focused on identifying more efficacious MDM2 antagonists or dual MDM2/4 inhibitors, some of which are currently in clinical trials (102). Some of these MDM2 and MDM4 inhibitors act through new mechanisms, including inhibiting the ubiquitin ligase activity of MDM2 (103), disrupting MDM2-MDM4 heterodimerization (104), and blocking the transcription of *MDM2* (105). In addition, as MDM4 and MDM2 have similar structures in their p53-binding domains, a new peptide-based inhibitor has been developed to bind to the p53-binding pocket of both MDM2 and MDM4 (106). A limitation to these strategies is that inhibition of MDM2 in normal tissues can induce hyperactivation of p53, which in turn can cause severe pathologies, as shown in adult $Mdm2^{-/-}$ mice (107). Moreover, although MDM2/MDM4 dual inhibitors are conceptually very promising, these molecules will likely only benefit patients with wild-type p53 tumors. Thus, strategies that restore wild-type functions to mutant p53-expressing tumors are also critically needed.

5.2. Reactivating Mutant p53

The early demonstration that a synthetic peptide derived from the carboxy-terminal negative regulatory domain of p53 can restore wild-type p53 conformation and transactivation function to mutant p53 provided a proof of concept for developing small molecules to reactivate mutant p53 (108, 109). Since this original discovery, a variety of strategies have been employed to identify p53-reactivating molecules. The drug that has advanced furthest in the clinic is PRIMA-1^{MET}, a methylated version of PRIMA-1, which was identified by drug screening assays for reactivators of mutant p53 that could restore sequence-specific DNA binding, transactivation function, and consequent suppression of cancer cell proliferation both in vitro and in vivo (110, 111). PRIMA-1 was found to react covalently with thiols in the central domain of mutant p53 and alter the cellular redox state, contributing to the restoration of wild-type p53 transactivation and apoptotic function in tumor cells (111). The development of PRIMA-1^{MET} is at an advanced stage with several clinical trials ongoing. Results from some clinical trials indicated that PRIMA-1^{MET} was very well tolerated, induced p53-dependent biologic effects in tumor cells, and yielded promising clinical responses (112). However, although PRIMA-1 shows promise as an anticancer drug, several obstacles remain. For example, resistance has been observed in preclinical studies with PRIMA-1, potentially resulting from the overexpression of insulin-like growth receptor and proto-oncogene tyrosine kinases (113). Moreover, mutant p53-independent anticancer properties of PRIMA-1/PRIMA-1^{MET} have been identified in recent years, which make the study of these drugs more complicated (reviewed in 114). Further investigation of the anticancer mechanisms and resistance mechanisms of PRIMA-1 could help to develop combination therapies.

Another group of small-molecule drugs found to restore p53 wild-type structure are the zinc metallochaperones (ZMCs). An in silico screen developed with National Cancer Institute anticancer drug screen data led to the identification of the ZMC compound NSC319726 (ZMC1) (115). ZMC1 can significantly inhibit tumor growth in $TP53^{R175H}$ human tumor xenografts, but not in xenografts with wild-type *TP53*. Mechanistic analyses indicated that misfolding of the p53 conformational mutants was provoked by impaired zinc binding and that ZMCs can refold the p53 protein by donating zinc (116, 117). Beyond p53 refolding, ZMC1 also boosted reactive oxygen species levels, which activated a stress response that contributed to activation of the newly refolded p53 protein through posttranslational modifications (115). Despite being short-lived, short exposure to ZMC1 (as low as 15 min) could achieve optimal efficacy and largely minimized the drug

toxicity, as shown in mouse pancreatic cancer models (118). It is to be hoped that this treatment method will be translated to clinical studies in the near future.

5.3. Degradation of Mutant p53

Another reported strategy for treating patients with tumors expressing mutant *TP53* is to target mutant p53 protein for degradation. It has been demonstrated that heat shock protein 90 (HSP90) and histone deacetylase 6 (HDAC6) play important roles in stabilizing mutant p53 and that HSP90 and HDAC inhibition significantly increased the survival of $Trp53^{R248Q/-}$ and $Trp53^{R172H/R172H}$ mice, whose tumors were potentially addicted to mutant p53 for survival, relative to their $Trp53^{-/-}$ littermates (119, 120). Geldanamycin (GA), a benzoquinone ansamycin antibiotic, was found to reduce the half-life of mutant p53 without affecting wild-type p53 stability either at basal levels or in response to DNA damage (121). GA disrupts the association of HSP90 with various proteins, including mutant p53, thereby restoring ubiquitination and proteasome-mediated degradation to mutant p53 in tumor cells (122, 123). 17AAG, a derivative of GA, can also destroy the mutant p53-HSP90 complex, allowing endogenous MDM2 and the chaperone-dependent E3 ligase CHIP to degrade mutant p53 (124). Additionally, inhibiting the histone deacetylase HDAC6—an obligatory positive regulator of HSP90—with the US Food and Drug Administration (FDA)-approved HDAC inhibitor SAHA killed in vitro cancer cells with *TP53* mutations more effectively than those with wild-type *TP53* or *TP53* loss (119). SAHA destabilized mutant p53 in a similar manner to HSP90 inhibitors and sensitized cancer cells to chemotherapy (119). Moreover, the combination of 17AAG and SAHA synergistically killed breast cancer cells with mutant *TP53* (120). As a result of these preclinical studies, HSP90 inhibitors have entered clinical trials for treating a variety of cancers with *TP53* mutations (125). Since many HDAC inhibitors have been previously approved by the FDA, new molecularly directed clinical trials with patient stratification based on *TP53* mutation types are currently ongoing and might lead to improved treatment effects. The major problem with HSP90 and HDAC inhibitors, however, is that they both regulate the structural and functional integrity of a variety of target proteins beyond mutant p53 (122, 123). Due to the lack of specificity, some HSP90 inhibitors exhibit nonnegligible toxicity and many side effects during treatment. Clinical trials with HSP90 inhibitors have started to involve combination treatment strategies in an attempt to overcome these problems.

5.4. Synthetic Lethal Targeting of Mutant *TP53*

As *TP53* is frequently mutant in cancer cells, while normal cells typically retain intact p53, SL targeting of mutant p53 has been proposed as a cancer therapy that should provide a great therapeutic window. SL describes a situation in which a combination of deficiencies/alterations of two or more genes is lethal, while alteration of only one of these genes is not. Thus, this strategy will allow pharmacological agents that target the SL partners of mutant *TP53* to selectively kill cancer cells but spare normal tissues with wild-type *TP53*. Initial studies using chemical screens or transcriptome analyses identified inactivation of CHK1, WEE1, and PLK1 as SL with p53 deficiency (126–129). This lethality is thought to be due to a deficiency of p53 interrupting the G1/S cell cycle checkpoint and inhibition of CHK1, WEE1, or PLK1 leading to dysregulation of the G2/M checkpoint, which together result ultimately in mitotic catastrophe. While various CHK1, WEE1, and PLK1 inhibitors have been identified and tested in either preclinical studies or clinical trials (128, 130–132), additional investigation is ongoing to increase the selectivity and efficacy of these drugs with combination strategies. With the advancement of technology, researchers have begun to favor genetic RNA interference or CRISPR/Cas9 screens to identify SL partners of mutant *TP53* (133).

5.5. Targeting of Mutant p53 by Immunotherapy

Application of immunotherapy to specifically target mutant p53 molecules is also a promising therapeutic strategy (134). It has been demonstrated that certain p53 mutants are immunogenic; thus, dendritic cell–based vaccines against mutant p53 peptides or T cells recognizing mutant p53 have been developed (135, 136). More recently, the development of an H2-scDb bispecific antibody, which can bind to both the $p53^{R175H}$ peptide–human leukocyte antigen complex and the T cell receptor through different arms and activate antitumor T cell responses, was also described (137). This method theoretically could be used to target cancers with other *TP53* mutations and highlights a new way for developing anticancer immunotherapy. Although promising, these strategies are in very early stages, and additional efforts are needed to overcome side effects and resistance problems, which are a hurdle for every type of treatment strategy.

6. SUMMARY AND FUTURE PERSPECTIVES

The *TP53* gene family has been preserved for more than 600 million years of evolution, underscoring the importance of this gene family for the organism (138). With more than 40 years of study of *TP53*, we have learned a great deal about this gene, but clearly there are additional intriguing questions that need to be answered. First, although many p53 target genes and cellular responses have been identified, it is unclear which pathways are most relevant for tumor suppression in different settings (59). Future studies should consider what is the best approach to characterize the p53 target genes and pathways involved in tumor suppression in different cancer contexts, knowledge that may be important for enhancing therapeutic options based on targeting the p53 pathway. Second, although various classes of *TP53* mutations have been identified and the GOF properties of *TP53* mutations have been widely reported, it is unclear whether tumorigenesis is driven by DNEs or by GOF activities of *TP53* mutations. It is simple to answer this question as context dependent, but it is imperative to uncover the details in different tumor types to decide what therapeutic approaches need to be applied in different contexts. Clearly, not all *TP53* mutations are equal, and thus future research should consider the development of selective modalities that can be used to systematically target different classes of *TP53* mutations. Third, since too little p53 induces tumorigenesis, while too much p53 promotes diverse pathological phenotypes, the question of how to achieve the appropriate balance of p53 levels is a complicated problem to consider in treating both cancer and diseases related to hyperactive p53. Rather than targeting p53 itself, it may be more optimal to target the specific pathways by which p53 drives corresponding diseases. Thus, we are back to our very first question of how to discover the complete transcriptional network of p53 that promotes these diseases in various contexts.

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