Cell Death and Differentiation (2018) 25, 169–179

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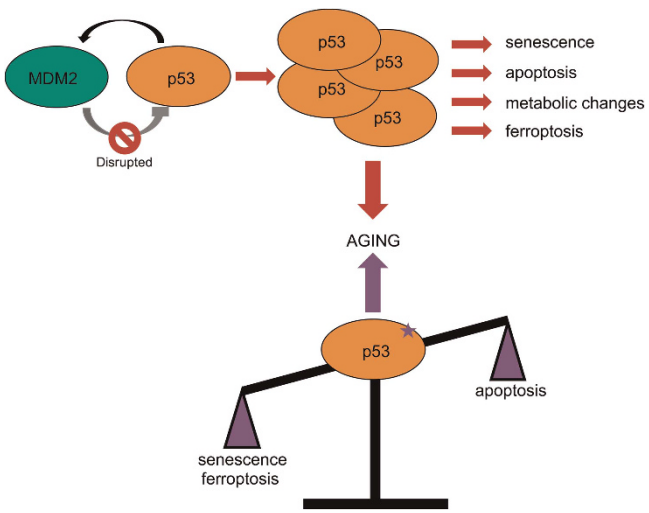
REVIEW

Relevance of the p53–MDM2 axis to aging

Danyi Wu[1] and Carol Prives*,[1]

In response to varying stress signals, the p53 tumor suppressor is able to promote repair, survival, or elimination of damaged cells – processes that have great relevance to organismal aging. Although the link between p53 and cancer is well established, the contribution of p53 to the aging process is less clear. Delineating how p53 regulates distinct aging hallmarks such as cellular senescence, genomic instability, mitochondrial dysfunction, and altered metabolic pathways will be critical. Mouse models have further revealed the centrality and complexity of the p53 network in aging processes. While naturally aged mice have linked longevity with declining p53 function, some accelerated aging mice present with chronic p53 activation, whose phenotypes can be rescued upon p53 deficiency. Further, direct modulation of the p53-MDM2 axis has correlated elevated p53 activity with either early aging or with delayed-onset aging. We speculate that p53-mediated aging phenotypes in these mice must have (1) stably active p53 due to MDM2 dysregulation or chronic stress or (2) shifted p53 outcomes. Pinpointing which p53 stressors, modifications, and outcomes drive aging processes will provide further insights into our understanding of the human aging process and could have implications for both cancer and aging therapeutics.
Cell Death and Differentiation (2018) **25,** 169–179; doi:10.1038/cdd.2017.187; published online 24 November 2017



Graphical Abstract

Facts

- Proper regulation of p53 is essential for normal development and aging processes.
- Cancer and aging are distinct manifestations of the same underlying cause of cumulative genomic changes.
- Genomic damage and stress modulate p53 activity differentially.
- Senescence has a critical role in the aging process.

Open Questions

- Is p53 signaling a driver or downstream effector of aging?
- Which stressors and p53 modifications are responsible for aging phenotypes?
- Can aging effects be reversed and fitness restored at the cellular level?
- Do we need to reconsider the side effects of p53-related therapies?

Aging is the gradual process of cellular deterioration due to cumulative molecular changes over time. At the organismal level, the aging phenomenon is often characterized by outward phenotypes such as shortened stature, decreased lifespan, frailty, skin abnormalities, and graying of the hair.[1,2] The underlying molecular mechanisms of aging feature the depletion of healthy, functional cells leading to tissue and organismal degeneration.[3] This cellular turnover is often attributed to stem cell exhaustion and cellular senescence. Cells undergo senescence upon telomere shortening (replicative senescence) or exposure to genomic damage.[4,5] Instead of continually dividing, senescent cells adopt more flattened morphologies, exhibit declined stress response signaling, and lose homeostatic balance.[6] Over time, the number of senescent cells in an organism can accumulate and develop a senescence-associated secretory phenotype (SASP), producing a chronic inflammatory microenvironment that can accelerate the aging process.[7,8]

Cumulative genomic damage generates chronic stress that can activate stress response pathways. The p53 pathway is one such pathway that strives to maintain genomic integrity and cellular homeostasis by initiating tumor-suppressing cell survival processes such as cell cycle arrest, senescence, and DNA damage repair (Figure 1).[5] p53 can also promote apoptosis and ferroptosis to eliminate damaged cells. p53

[1]Department of Biological Sciences, Columbia University, New York, NY 10027, USA
*Corresponding author: C Prives, Department of Biological Sciences, Columbia University, 1212 Amsterdam Avenue, 816 Fairchild Building, New York, NY 10027, USA. Tel: +1 212 854 2557; E-mail: clp3@columbia.edu
Received 21.7.17; revised 19.9.17; accepted 21.9.17; published online 24.11.17

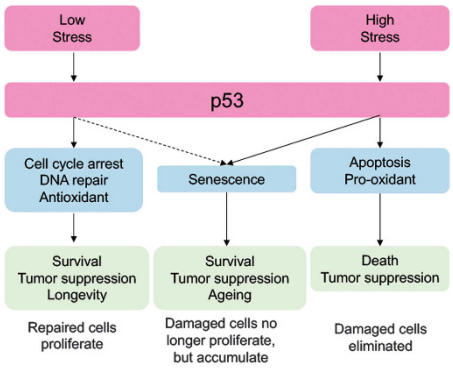


Figure 1 Stress levels regulate alternate p53 responses. The tumor suppressor p53 mediates both survival and killer cellular outcomes in response to stressors. Under low stress conditions, p53 will initiate repair and cell cycle arrest mechanisms that will promote cell survival. Under acute stress conditions, p53 will eliminate the damaged cells from the proliferative pool through apoptosis, senescence, and more

functions as a sequence-specific transcriptional regulator of myriad target genes, some of which are regulated by basal levels of p53, while others require increased levels of p53 most often elicited by numerous endogenous and exogenous stressors.[5] The most well-validated negative regulator of p53 is MDM2.[9,10] MDM2, an E3 ubiquitin ligase, is able to downregulate p53 activity through three mechanisms: (1) poly-ubiquitylation to target p53 for proteasome-mediated degradation; (2) mono-ubiquitylation to export p53 out of the nucleus; and (3) direct binding to p53 to block transactivation of key targets.[11] Though a broad link between the p53–MDM2 axis and aging has been established, the mechanism has not yet been elucidated. In this review, we aim to address the connections between p53–MDM2 axis and human aging disorders, aging-related pathways, aging mouse models, and therapeutic implications.

Aging and Longevity

The study of age-related diseases and accelerated aging disorders (progeroid syndromes) are not only valuable in furthering our understanding of specific pathologies but also for understanding the broader aging phenomenon. In contrast to cancer in which cells gain fitness to proliferate rapidly and uncontrollably, aging features cells that lose fitness.[3,12] How cells respond to DNA damage is usually the determining factor between these two choices. The master regulator p53 determines cellular response to stress and has been linked to human diseases from development through old age.[13] Wild-type p53 function is required for tumor suppression in humans, as evidenced by Li-Fraumeni patients who have germline p53 mutations leading to increased cancer incidence.[14] Cells derived from aged individuals, such as astrocytes, fibroblasts, and retinal pigment epithelial cells, express increased levels of p53 protein,[15–17] despite the intuitive expectation that aged populations may have reduced p53 activity leading to increased tumor incidence. In line with this, individuals with the p53 polymorphism Arg72Pro have increased longevity, though this p53 has reduced apoptotic potential. Dysregulated p53 has also been implicated in neurodegenerative disorders – p53 is upregulated in Alzheimer's disease and is able to interact with mutant huntingtin protein to mediate aberrant transcriptional regulation in Huntington's disease.[18–20] Altogether, the question of whether p53 aids or negates the aging process in humans remains complex, as p53 activity has been associated with both premature aging and longevity.

Progeroid syndromes are often characterized by early onset and rapid progression, with patients displaying accelerated aging phenotypes and shorter lifespans.[21] Although progeroid syndromes only partially phenocopy normal aging, their study provides insights into the mechanisms underlying aging. In many cases, progeroid syndromes are monogenic and driven by defects in DNA repair mechanisms. For example, aberrant nucleotide excision repair has been associated with cerebro-oculo-facio-skeletal syndrome, trichothiodystrophy, and Cockayne syndrome.[22] In addition, deficiencies in non-homologous end joining (NHEJ) have been associated with Werner's syndrome and ataxia telangiectasia.[23] Still other progeroid syndromes such as laminopathies feature disruption of the nuclear architecture via lamin processing, leading to chronic stress and activation of the p53 pathway.[21,24,25] Recently, we reported studies on a patient with accelerated aging symptoms who harbors a homozygous anti-termination mutation in *MDM2* that leads to aberrant regulation of p53.[26] Our data indicate that a functional disturbance of the p53–MDM2 axis can contribute to human aging phenotypes.

Findings in model organisms have further connected p53 with aging, although some appear to contradict progeroid studies. For example, naturally aged mice experience a decline in p53 function at the transcriptional and protein levels, suggesting that their tumor suppressive functions may be compromised.[27] In addition, model systems such as *C. elegans* and *Drosophila* associate loss of wild-type p53 with longevity and extended lifespan, and have implicated p53 activity in autophagy and crosstalk with pathways like the insulin-like growth factor/mTOR pathway.[28–31]

p53 and Aging at the Cellular Level

In their landmark review, Lopez-Otin *et al.*[12] enumerated nine key hallmarks of aging. Notably, the p53 pathway is a master regulator of at least three of these hallmarks: genomic instability; mitochondrial dysfunction; and cellular senescence. p53 is also a central player in regulating pathways involved in metabolic signaling and the immune system, which could contribute to regulating aging mechanisms.

Genome instability. As genomic damage accumulates over time, genomic instability becomes more prevalent. One of the main functions of p53 is to maintain genomic integrity through repair and cell cycle arrest mechanisms to limit proliferation of damaged cells. This potentially explains why many progeroid syndromes with defective repair DNA mechanisms exhibit genomic instability and subsequent elevated p53 activity.[32] Activation of certain p53 target genes is stress- and tissue-dependent and will lead to manifestation of differential phenotypes in mice.[33,34]

Mitochondrial dysfunction. Mitochondria are cellular energy generators that require regular maintenance through mitochondrial DNA (mtDNA) repair and mitophagy, an

organelle-specific clearance of damaged mitochondria.[35] As organisms age, mitochondrial dysfunction increases and respiration efficiency decreases.[36] Thus, defects in proofreading mtDNA enzymes have led to accelerated aging phenotypes in mice.[37] Mitochondrial deterioration can also be partially attributed to increased production of reactive oxygen species (ROS). However, simple elevation of ROS is insufficient to accelerate aging, and has in fact been associated with prolonged lifespan in yeast and *C. elegans*.[38–40] p53 is a key regulator of ROS and is also linked to mitochondrial regulation. For example, the histone deacetylase SIRT1 is involved in mitochondrial biogenesis through cooperation with the transcriptional co-activators PGC-1α and PBC-1β, but can also negatively modulate p53 activity.[41,42] In response to telomere attrition, p53 is able to mediate repression of PGC-1α and PGC1-β, thereby inhibiting mitochondrial biogenesis and function, leading to excess ROS, which can further activate p53 in a feedback loop.[43] Therefore, p53 can be both an upstream mediator and downstream effector of ROS.

The different modes by which the p53 transcriptional program can regulate metabolism and mitochondrial health can be paradoxical such that p53 can induce opposing cellular outcomes. In some cases, this relates to the type or extent of the cellular stress. Normal basal activity of p53 has been implicated in optimizing mitochondrial respiration and reducing ROS through SCO2, TIGAR, and sestrins.[44,45] Upon conditions of mild stress, p53 is able to induce expression of antioxidant genes such as sestrins, *TIGAR*, *GLS2*, *SCO2*, and others.[45] However, upon acute stress p53 can activate prooxidant targets to mediate apoptosis and senescence.[46] It is likely that collective mitochondrial dysfunction due to increased damage and reduced turnover could affect cell signaling, autophagy, and aging.

Crosstalk with the insulin/IGF-1 and mTOR pathways further complicates the p53-metabolism link.[47] Diminished signaling of the insulin/IGF-1 and mTOR pathways has been linked with longevity.[48,49] p53 is able to activate expression of IGF-BP3 and PTEN to downregulate insulin/IGF-1 signaling, as well as TSC-2, AMPK-β1, sestrins, and REDD1 to downregulate mTOR signaling.[46] Moreover, insulin/IGF-1 signaling through IRS-1 can activate PI3K/AKT, which in turn can activate MDM2. MDM2 is able to directly bind to IRS-1 and IGF-1R to target these substrates for degradation.[50,51] Thus, the interplay of p53 and MDM2 with these metabolic pathways are frequently context-dependent and highlight the complexity of p53 in metabolic regulation.

Cellular senescence. Perhaps the most commonly associated phenotype of aging is senescence, a process by which cells undergo a stable, irreversible cell cycle arrest.[52] Although primarily triggered by telomere attrition, exposure to physiological stresses can also lead to senescence. Two key pathways have been linked to senescence: $p16^{INK4a}$/RB and p53/p21. $p16^{INK4a}$ is able to block cell cycle progression by inhibiting cyclin D–CDK4/6 complexes, thereby activating Rb to inhibit E2F transcriptional effects.[53] p53 is able to directly activate *p21*[54–56] and *PAI-1*[57] to mediate cell cycle arrest and senescence. The accumulation of senescent cells, combined with reduced healthy cell turnover and slowed division, is largely thought to drive aging, while clearance of senescent cells has been associated with delayed aging.[58]

Accumulation of senescent cells often leads to chromatin reorganization and the SASP, which includes secretion of proinflammatory chemokines, including cytokines (e.g., interleukin-6 and tumor necrosis factor), growth factors, matrix metalloproteases, and others.[3] SASP can also lead to paracrine signaling to induce further cellular senescence, extracellular matrix deterioration, and disruption of the local microenvironment.[59,60] Although SASP-derived inflammation can attract an immune response and subsequent clearance of senescent scells,[61,62] chronic SASP can also lead to immune evasion through microRNAs and other mechanisms, which may lead to limited clearance and further propagation of senescence and aging phenotypes.[63,64]

The role of p53 in immune regulation, particularly in inflammation and immunosurveillance, may also reinforce SASP and aging processes.[65,66] In fact, p53 loss has been shown to accelerate the aging of the immune system through accumulation of memory T cells, enhanced cytokine production, and limited T-cell proliferation capacity.[67] Wild-type p53 can upregulate targets such as Toll-like receptors, cytokines, and natural killer cell ligands (ULBP1 and ULBP2) that mediate innate immune responses, inflammation, and apoptosis.[68,69] p53 can also induce expression of CCL2 and DD1α to attract natural killer cells and macrophages for senescent and apoptotic cell clearance, respectively.[66,70–72] In addition, p53 can downregulate PDL1 expression via activation of miR-34, thereby effectively inhibiting the PD1 checkpoint.[73] Nevertheless, though p53 activity can engage the innate immune system to facilitate cellular clearance, p53 could potentially promote an inflammatory microenvironment that could aide chronic SASP to drive further tissue deterioration. Therefore, the role of p53 in senescence and immune regulation could be contributing to aging processes.

Aging Mouse Models

Premature aging models. Mouse models are useful in understanding human physiological systems, particularly as genome maintenance genes are conserved across species. Several mouse models have been developed to model progeroid syndromes, including Hutchinson–Gilford progeria syndrome (HGPS) and TTD (Table 1). HGPS is driven primarily by lamin A-processing defects that lead to chronic nuclear architectural stress. *Zmpste24*$^{-/-}$ mice that lack a protease that is required for lamin A processing exhibit HGPS symptoms, upregulated p53 target gene expression, and premature senescence, though basal levels of p53 remain unchanged.[25] The *Zmpste24*$^{-/-}$ aging phenotype is partially rescued by crossing mice into a p53-null background, indicating that p53 is a key driver of the phenotype. Similarly, elimination of *p53* or *p16*^{INK4A} to reduce senescence can rescue the aging phenotype in mice with defects in double strand break repair and spindle assembly checkpoints caused by mutations in *Brca1* and *Bub1b*, respectively.[58,74] *Ku86*$^{-/-}$ mice with defective double-strand break repair also present with aging-related characteristics, including dermal atrophy, hair follicle, and early senescence.[75] However, when

Table 1 Premature aging mouse model

Alteration	Disease model	Phenotype	Reference
Lmna$^{-/-}$	HGPS Defect in lamin processing	Accelerated aging Increased p53 activity, elevated p21 levels	Sullivan *et al.*[132]
Zmpste24$^{-/-}$	HGPS Defect in lamin processing	Accelerated aging Phenotype can be partially rescued by p53-null background p53 target genes upregulated despite normal p53 protein levels Premature senescence	Varela *et al.*[25]
Atr$^{S/S}$	ATR Seckel Defect in single-stranded break repair	Accelerated aging Phenotype aggravated by p53 loss High levels of embryonic replicative stress, genomic instability	Murga *et al.*[78]
Xpd^{R722W}	Trichothiodystrophy (TTD) Defect in NER	Accelerated aging Osteoporosis, early graying, infertility Defect in additional *Xpa* gene accelerates phenotype	de Boer *et al.*[129]
Csb$^{-/-}$	Cockayne's syndrome Defect in NER	Normal aging UV-sensitive	Lu *et al.*[133]
Ercc1^{*292}	Defect in NER	Accelerated aging Reduced lifespan, reduced growth, increased senescence, nuclear abnormalities, accumulation of p53 and p21	Weeda *et al.*[134]
Wrn$^{-/-}$	Werner's syndrome Defect in double-stranded break repair	Normal aging Moderately accelerated senescence Mortality accelerated in p53$^{-/-}$ background	Lebel *et al.*[135] Lombard *et al.*[136]
Ku86$^{-/-}$	Defect in NHEJ	Accelerated aging Early onset senescence, earlier tumorigenesis, hair graying, skin atrophy	Vogel *et al.*[75]
Xrcc4$^{-/-}$	Defect NHEJ	Embryonic lethal Lethality rescued in p53-null background	Gao *et al.*[103]
Brca$^{\Delta 11/\Delta 11}$; *p53*$^{+/-}$	Defect in double-strand break repair and MMR	Accelerated aging Skeletal abnormalities, reduced dermal thickness MEFs exhibit senescence, mediated by p53–p21 axis	Cao *et al.*[74]
Bub1b$^{H/H}$	Defect in spindle assembly checkpoint protein	Accelerated aging Loss of fat, reduced wound healing, cataracts Early onset senescence; accumulation of p53/p21/p16/p19	Baker *et al.*[137]
SnoN$^{m/m}$	Block of antagonist of TGF-β signaling	Accelerated aging Shortened lifespan, osteoperosis, premature senescence, p53 cannot be regulated by Mdm2	Pan *et al.*[77]
mTR$^{-/-}$	Defect in telomerase	Accelerated aging Decreased lifespan, infertility at G6, increased genomic instability Increased senescence	Blasco *et al.*[79] Rudolph *et al.*[80] Chin *et al.*[81]
Sp53/Sp16/SArf/TgTert	Overexpress telomerase Super-p53/p16/ARF	Delayed-onset aging Improved intestinal and skin fitness, increased lifespan	Tomas-Loba *et al.*[83]

List of accelerated aging mouse models associated with defects in lamin processing, ATR signaling, genome maintenance, and telomerase.

generated in a p53-deficient background, *Ku80*$^{-/-}$ mice no longer display early replicative senescence that is p53-dependent, but the mice do experience shortened lifespans due to enhanced tumor incidence.[76] Similarly, *SnoN*$^{m/m}$ mice that can no longer antagonize TGFβ signaling display accelerated aging phenotypes that can be partially rescued by p53 deficiency.[77] Conversely, unlike other accelerated aging models that are rescued upon p53 knockout, ATR-Seckel mice display exacerbated phenotypes when p53 is lost.[78] This could potentially be due to p53 loss overwhelming the mouse with additional genomic instability, as *Atr*$^{S/S}$ mice already exhibit high levels of embryonic replicative stress and shortened lifespans.

Mouse models focusing on the role of telomere shortening have also directly connected senescence to aging. When the RNA component of telomerase (*mTR*) is knocked out, the resulting *mTR*$^{-/-}$ mice have decreased lifespans, shortened telomere lengths, increased senescence, and infertility by the sixth generation.[79,80] In addition, *mTR*$^{-/-}$ mice have more skin lesions, delayed wound healing, and increased genomic instability. Moreover, late-generation *mTR*$^{-/-}$ mice have greater induction of p53 and p21, and experience more extensive apoptosis in certain tissues. This phenotype can be rescued by p53 loss, despite the telomere dysfunction.[81] However, another mouse model featuring telomere dysfunction demonstrates that conditional p53 deletion is unable to rescue the aging phenotype, but rather inhibits the depletion of chromosomally unstable stem cells.[82] On the opposite end of the spectrum, constitutive expression of telomerase reverse transcriptase (*TERT*) in mice with overexpressed p53/p16^{INK4A}6/p19^{ARF} improves fitness and delays aging.[83] Altogether, these mouse models indicate that p53 has somewhat conflicting roles in aging and is context-dependent.

Mdm2–p53 mouse models. Mouse models manipulating the p53 axis directly have also provided insight into how p53 is linked to aging (Table 2). Mounting evidence suggest that simple elevation of p53 levels is insufficient to drive the accelerated aging phenotype in mice. Mice expressing 'super-p53' via introduction of multiple transgenes of wild-type p53 are highly cancer-resistant, but do not display signs of early aging.[84] Furthermore, 'super-ARF/super-p53' mice

Table 2 Mdm2/p53 mouse models

Mouse model	p53 activity	Phenotype	Reference
$p53^{+/m}$ (Δexons 1–6)	Normal basal p53 levels Hyper-stable p53, robust p21 activation	Accelerated aging More tumor resistant	Tyner *et al.*[87]
p44 TG	Normal basal p53 levels, elevated p44 levels Elevated *p21, Mdm2, IGFBP-3*	Accelerated aging More tumor resistant Hyperactive IGF-1 signaling	Maier *et al.*[88]
$p53^{TSD/-}$ (T21D/S23D)	Normal basal p53 levels Hyper-stable p53, constitutive p53 activation	Accelerated aging Phenotype rescued by Puma knockout	Liu *et al.*[93]
$p53^{S18A}$	Normal basal p53 levels Defective *Puma* induction and apoptosis	Accelerated aging in non-tumor-bearing mice Premature senescence	Armata *et al.*[101]
$Mdm2^{\Delta/\Delta}$ (conditional mouse)	Increased p53 levels Increased expression of p53 senescence targets	Accelerated aging Epidermal senescence	Gannon *et al.*[97]
Super p53 (+1 p53 transgene)	Normal basal p53 levels Increased DNA damage response Increased apoptotic response	Normal aging More tumor resistant	Garcia-Cao *et al.*[84]
Super-Arf/p53 (+1 ARF, +1 p53 transgenes)	Normal basal p53 levels Increased expression of *Sesn1/2*	Delayed onset aging More tumor resistant Protects against insulin resistance/glucose intolerance	Matheu *et al.*[85]
$Xrcc4^{-/-}$; $p53^{S18/S23A}$	Normal basal p53 levels Resistant to apoptosis	Accelerated aging Not as tumor prone as *Xrcc4-/- p53-/-* mice	Chao *et al.*[102]
$Xrcc4^{-/-}$; $p53^{3KR3KR}$	Normal basal p53 levels p53 target genes: high Ptgs2, low SLC7A11	Accelerated aging More tumor resistant High genomic instability, ferroptosis	Li *et al.*[104]
$Mdm2^{-/-}$	Unrestricted p53 activity by Mdm2	Embryonic lethal Lethality rescued in p53 null background	Jones *et al.*[94] Montes de Oca Luna *et al.*[95]
$Mdm2^{C462A}$ (disrupted RING domain of MDM2)	p53 cannot be degraded	Embryonic lethal Lethality rescued in p53 null background MDM2 has no E3 ligase activity	Itahana *et al.*[96]
$Mdm2^{Y487A}$	Increased basal p53 levels Low p53 transcriptional activity unless stressed	Normal aging MDM2 has decreased E3 ubiquitin ligase activity, can still bind to MDMX	Tollini *et al.*[138]
$Mdm2^{puro/\Delta 7\text{-}12}$ (*Mdm2* hypomorph)	Normal basal p53 levels More radiosensitive Increased p53-mediated apoptotic response	Normal aging Smaller mice	Mendrysa *et al.*[86]
$Mdm2^{p2/p2}$ (mutated p2 promoter)	Normal basal p53 levels More radiosensitive	Normal aging	Pant *et al.*[100]
$Mdm2^{PND/PND}$ (mutant P2 promoter, neomycin gene, 184 bp deletion in intron 3)	Increased basal p53 levels	Accelerated aging Phenotype rescued in p53 null background Partial rescue with *Puma* deletion	Pant *et al.*[33]

List of aging-related Mdm2–p53 axis mouse models and their associated p53 activities and phenotypes.

exhibit delayed-onset aging.[85] $Mdm2^{puro/\Delta 7-12}$ mice modulate p53 levels through Cre-mediated *Mdm2* ablation.[86] Despite exhibiting 70% ablation of wild-type MDM2 protein, cells from $Mdm2^{puro/\Delta 7-12}$ mice express normal basal p53 protein levels, though they have heightened p53 activity that is correlated with increased tumor resistance. Although these $Mdm2^{puro/\Delta 7-12}$ mice are about 15% smaller in size, radiosensitive, and display hematopoietic deficiencies, the mice do not age prematurely. Combined, these studies indicate that total p53 levels are not directly responsible for aging phenotypes, rather, specific functions of p53 may be contributing factors. In addition, mice that have genetic alterations shifting the balance of p53-dependent cellular outcomes (senescence, ferroptosis, apoptosis, etc.) also appear to display accelerated aging phenotypes. Therefore, we propose that two factors are crucial to driving p53-dependent aging phenotypes: (1) aberrant p53 regulation leading to increased p53 stability; or (2) shifted p53-dependent cellular outcomes (Figure 2).

Mouse models with aberrant p53 regulation. Two studies featuring mice with one functional copy of wild-type p53 and one copy of an N-terminal truncation of p53 display accelerated aging phenotypes.[87,88] The site of the p53 alteration is revealing, as the N terminus of p53 harbors the primary interaction site with MDM2, suggesting that MDM2 is unable to properly regulate p53 in these mice. The Donehower group, generated mice ($p53^{+/m}$) with a heterozygous p53 mutation that deleted the first six exons of p53, leading to hyperactive p53.[87] Although basal p53 protein

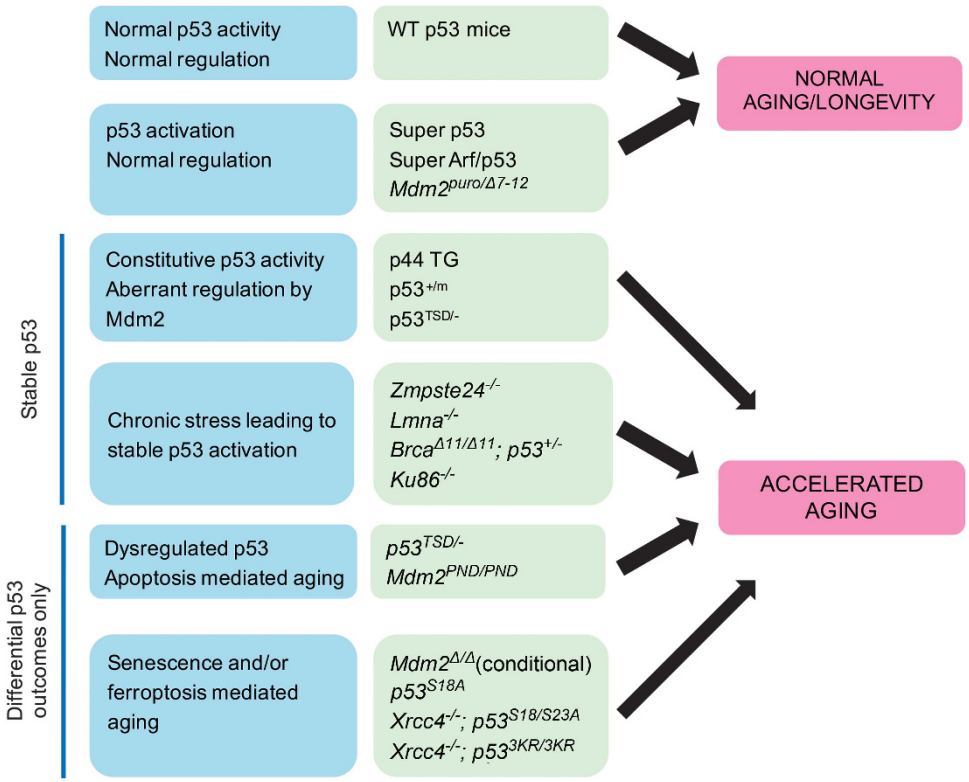


Figure 2 p53-mediated accelerated aging requires stable p53 or differential p53 outcomes. Speculation as to how p53 regulation is linked to aging in mouse models. When p53 is properly regulated, it leads to normal aging phenotypes in mice. However, constitutive p53 activity due to aberrant regulation through deletion or modifications of the N terminus of p53 (the primary interaction site with Mdm2) leads to accelerated aging phenotypes. Mice with defects in double-strand break repair and HGPS mouse models that display chronic nuclear structural stress lead to chronic p53 activation and accelerated aging phenotypes. Engineered mice that lack the entire spectrum of p53 outcomes but can promote differential outcomes (apoptosis, ferroptosis, and senescence) also display accelerated aging phenotypes. This figure is adapted from Serrano and Blasco[130]

levels were similar at least in $p53^{+/m}$ and $p53^{+/+}$ kidney cells, the p53 response in these $p53^{+/m}$ mice were augmented. The $p53^{+/m}$ mice were also highly resistant to cancer, with a sporadic tumor formation of 6% compared to 45% for $p53^{+/+}$ mice. Most notably, the $p53^{+/m}$ mice displayed an unexpected phenotype of accelerated aging, with skeletal defects and organ atrophy. A follow-up study indicated that the m allele produced a p53 protein that was able to interact with, stabilize, and facilitate the nuclear localization of wild-type p53 under basal conditions.[89] A second aging mouse model (p44 TG) from the Scrable group demonstrated that overexpression of a naturally occurring p53 isoform, p44, in the presence of full-length p53 also leads to an accelerated aging phenotype.[88] The p44 protein lacks the N-terminal transactivation domain of p53, as translation is initiated from codon 41 in p53. Interestingly, MEFs from both $p53^{+/m}$ and p44 TG mice express normal p53 protein levels but have constitutively active p53, likely stemming from aberrant regulation of p53 by MDM2 due to the N-terminal truncation. An important clue as to the aging phenotype of the p44 mouse was derived from the finding that these mice have deregulated insulin/insulin-like growth factor signaling. Specifically, cells from these mice displayed increased expression of some p53 targets (*p21, MDM2, and IGFBP-3*) while others (GADD45 and IGF-1-R) were relatively decreased. These observations imply that mice with one N-terminally truncated p53 allele along with one full-length p53 allele display altered, but not across-the-board elevated p53 activity in their cells.

Not only gross deletions, but missense mutations of modification sites within p53 can also predispose mice to premature aging in some cases. Phosphorylation of the N terminus of p53 at key residues (S15, T18, and S20) disrupts the MDM2–p53 interaction and can also lead to aberrant p53 regulation.[90–92] $p53^{TSD/-}$ mice with phosphorylation-mimicking mutations of human T18 and S20 (in mice: T21 and S23, respectively) indicate that this region is critical for mouse development.[93] While homozygous p53 T21D/S23D mice ($p53^{TSD/TSD}$) mice are embryonic lethal, $p53^{TSD/-}$ knock-in mice are born and die by 6 weeks of age, exhibiting accelerated aging phenotypes.[93] Constitutive p53 activation, hematopoietic degeneration, testicular atrophy, and adult stem cell depletion were observed in these heterozygous mice. Elimination of the p53 target *Puma* is able to rescue the segmental progeroid phenotype in $p53^{TSD/-}$ mice, indicating that the aging phenotype in these mice is primarily mediated through p53-dependent apoptosis of the adult stem cell pool.

Direct manipulation of MDM2 adds an additional layer of complexity to understanding the p53 pathway connection to aging (Figure 3). Complete liberation of p53 from the repressive hold of MDM2 leads to massive apoptosis and developmental deficiencies. MDM2 knockout ($Mdm2^{-/-}$) mice are embryonic lethal unless generated in a p53-null

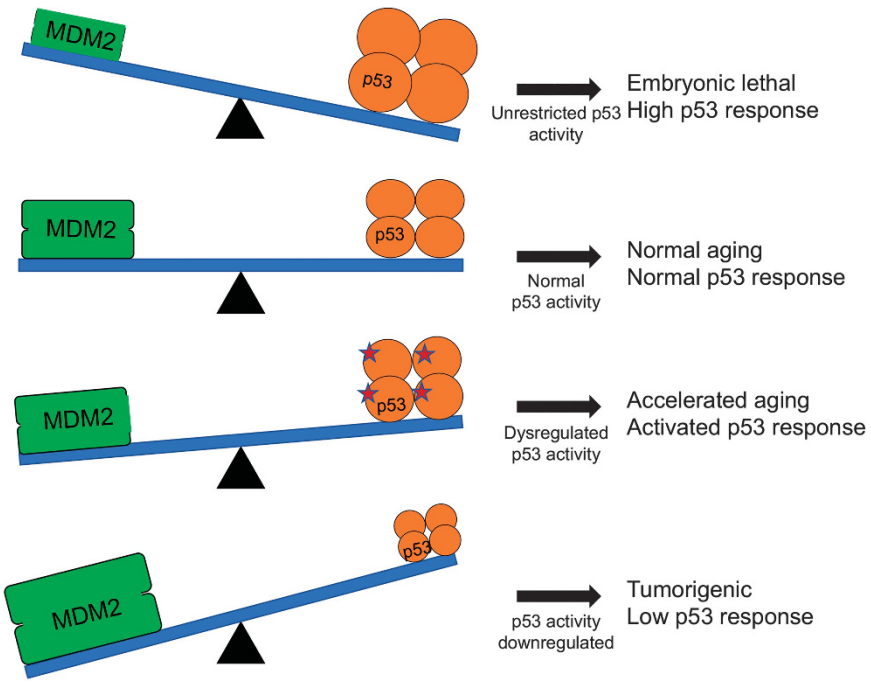


Figure 3 Mdm2–p53 balance is required for normal development. Proper regulation (balance) of p53 by MDM2 is needed for normal aging. Aberrant regulation can lead to tumorigenic, accelerated aging, or lethal phenotypes. Figure is taken and adapted from Poyurovsky and Prives[131]

background, indicating that p53 regulation by MDM2 is essential for development.[94,95] Concordantly, engineered mice that express a key MDM2 RING domain mutation that abrogates E3 ligase activity (*Mdm2*^{C462A}) display an early embryonic lethal phenotype unless present in a p53-null background.[94–96] However, in a conditional mouse model, deletion of *Mdm2* in the epidermis induced p53-mediated senescence and accelerated aging phenotypes in the skin, including decreased wound-healing ability and skin thinning.[97] Notably, p53 activation in the epidermis did not lead to upregulation of pro-apoptotic genes, just senescent targets. As conditional loss of *Mdm2* in several other tissues leads to cell death,[98,99] it is likely that tissue-specific differences account for the senescence outcome observed.

Mouse models with differential p53 outcomes. Activation of specific programs of p53 downstream targets to shift the balance of p53-dependent outcomes could manifest in different phenotypes. Indeed, the *p53*$^{TSD/-}$ accelerated aging phenotype observed is dependent on apoptosis. Nevertheless, altering the transcriptional program of p53 can have unanticipated effects. Engineered homozygous mice that lack the *Mdm2* p2 promoter (*Mdm2*$^{P2/P2}$), which abolishes *Mdm2* as a p53 transcriptional target, age normally, indicating that full activation of *Mdm2* by p53 is not necessary for aging.[100] However, homozygous *Mdm2*$^{PND/PND}$ mice that were serendipitously generated from a mutant P2 promoter harboring a neomycin cassette and partial intron 3 deletion display certain aging-related characteristics, including shorter lifespans, skin hyperpigmentation, and hematopoietic defects.[33,100] Interestingly, *Puma* deficiency rescued some of the reproductive defects, but *p21* loss did not rescue any aging-related abnormalities, indicating that the apoptotic pathway was likely responsible for this particular phenotype as well.

Shifting the p53-dependent response away from apoptosis can also lead to accelerated aging. Knock-in mice p53 mice with an S18A mutation (*p53*^{S18A}) that removes the ATM phosphorylation site that is necessary for Puma-mediated apoptosis have a high cancer incidence. Surprisingly, the non-tumor-bearing mice from this study presented with accelerated aging signs. Cells from the non-tumor-bearing *p53*^{S18A} mice have diminished apoptotic responses and undergo premature senescence.[101] Speculatively, epigenetic variations in the mice may contribute to the differences seen. Similar to the *p53*^{S18A} mice, *p53*$^{S18A/S23A}$ mice are also apoptosis-resistant, and thus highly tumorigenic.[102] However, in another model, the *p53*$^{S18A/S23A}$ background can rescue the lethality of *XRCC4*$^{-/-}$ mice, and the crossed mice (*Xrcc4*$^{-/-}$*; p53*$^{S18A/S23A}$) display accelerated aging phenotypes.[102] XRCC4 is a protein involved in the stabilization of a key ligase in the NHEJ pathway that repairs double-strand breaks. XRCC4 knockout mice are embryonic lethal, unless rescued via co-committal knockout of p53.[103] These models suggest that the apoptotic functions of p53 are not required for genomic instability-driven aging phenotypes. This idea is further supported by recent evidence demonstrating that *Xrcc4*$^{-/-}$ lethality can also be rescued in a *p53*$^{3KR/3KR}$ background in mice (*Xrcc4*$^{-/-}$*;p53*$^{3KR/3KR}$).[104] p53 3KR is an acetylation-defective mutant that is unable to induce cell cycle arrest, senescence, and apoptosis, but can still undergo ferroptosis, an iron-dependent form of cell death that results from lipid peroxidation.[105] The authors speculate that the high levels of ferroptosis could be a contributing factor to the aging process.

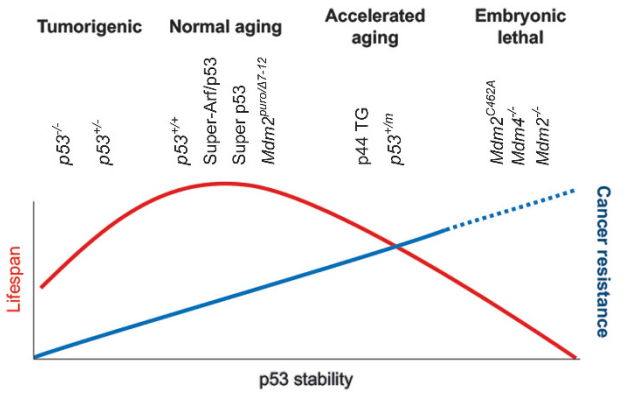


Figure 4 Lifespan and cancer resistance are related to p53 stability. Model for proposed relationship between lifespan and cancer resistance and their links to p53 protein stability. Mice with low p53 or no p53 due to deletion of one or two *TP53* alleles are tumor prone and have shortened lifespans due to cancer. Mice with normal p53 levels or stability have normal lifespans, while super-p53/super-ARF mice have delayed-onset aging. Mice with increased p53 stability can lead to hyper-activation and accelerated aging phenotypes (dip in lifespan), but greater cancer resistance. Finally, mice with likely super-stable p53 due to Mdm2 or MdmX deletion leads to embryonic lethal phenotypes. Dotted line refers to inability to assess cancer resistance due to embryonic lethality. Lifespan and cancer resistance curves are diagrammatic rather than quantitative in terms of actual lifespan and resistance of mice

Altogether, these p53 mouse models implicate a significant role for p53 in accelerated aging processes. However, as mentioned before, simple excess of p53 is insufficient, perhaps because the MDM2–p53 circuitry is intact. Instead, p53 stability may be the critical factor in the lifespan of these mice (Figure 4). We speculate that for p53-mediated aging, p53 must (1) be stably active due to MDM2 dysregulation or chronic stress or (2) shift the balance of its transcriptional program to facilitate specific outcomes. These stress- and modification-dependent responses must vary across tissue types and could account for variable accelerated aging abnormalities. There are also conflicting lines of evidence relating apoptosis to aging phenotypes. Though depletion of stem progenitor cells could be a driver, experimental contexts must be carefully considered to gain further understanding of the roles of programmed cell death in aging. Finally, we propose that the lack of exposure to stressors is another factor that needs to be considered when analyzing these mouse models. Specifically, mice living under laboratory conditions are not exposed to everyday physiological stresses such as UV radiation. Super-p53 and super-ARF/p53 mice have augmented DNA damage responses, which if chronically stimulated, could speculatively lead to altered aging-related phenotypes.

Therapeutic Implications

Side effects of cancer therapeutics? The MDM2–p53 axis presents an attractive target for cancer therapeutics, particularly those that wish to restore wild-type p53 function.[9] Stapled peptides, small-molecule inhibitors, and p53 vaccines have all undergone clinical trials to test the efficacy of targeting this pathway. Compounds such as Nutlin-3 and RITA activate p53 by disrupting the MDM2–p53 interaction and can induce p53-mediated cell cycle arrest and senescence in some cases.[106–109] However, while activation of p53 through pharmacological release from MDM2 has been actively sought for cancer therapy, there may be unanticipated aging consequences due to aberrant p53 activation and altered pathway signaling. Both Nutlin-3 and RITA may induce MDM2-mediated downregulation of IGF-1R signaling through ubiquitylation, which could have potential effects on regulation of the IGF-1 pathway and aging.[110,111] Furthermore, patients with *MDM2* amplifications hyper-progressed upon immunotherapy treatment, suggesting that the p53–MDM2 axis could have a role in immune evasion and limit cellular clearance.[112] As several mouse models have showed that non-aberrant regulation of p53 by MDM2 can restrain p53 activity and promote tumor resistance without the damaging lifespan effects[113] we can remain cautiously optimistic about aging-related deleterious effects of short-term cancer therapies.

Aging therapeutics. Two general questions frame the search for aging therapeutics: can we curtail the aging process and can we reverse the effects of aging? Studies using caloric restriction and mTOR inhibitors have investigated how remodeling metabolic pathways can be used to extend longevity.[114,115] In addition, proof-of-principle studies have demonstrated that aging effects could be reversed in HGPS by a modified oligonucleotide that corrects the aberrant splice site in *LMNA*[116] and by a rho-associated protein kinase inhibitor (Y-27362) that dampens ROS-associated mitochondrial dysfunction.[117]

Other studies have explored direct rejuvenation in mice. Specifically, researchers have attempted to determine whether 'young environments' can produce factors or stimuli that can restore youthfulness by using heterochronic parabioses to connect the circulatory systems of old and young mice.[118] In particular, growth differentiation factor 11 (GDF11) expression was discovered to reverse skeletal and cardiac aging-related dysfunction in mice,[119–121] though other studies imply that GDF11 inhibits muscular regeneration instead.[122,123] The discrepancies seem to stem from antibody reactivity, technical differences, and model organisms.[124] Recently, an HGPS mouse model (*Zmpste24*$^{-/-}$) was used to test *in vivo* GDF11 therapy, revealing that while circulating GDF11 declined in aged mice, daily GDF11 intraperitoneal injections were unable to prolong lifespans.[125] However, combination therapy with GDF11 injection and p53 depletion could potentially be an avenue of exploration to attempt to fully rescue aging symptoms.

Clearance of senescent cells is another therapeutic approach to reverse aging.[58] Multiple compounds have been identified to preferentially target senescence. For example, the compound KU-60019 was discovered as an effective inhibitor of ATM-driven senescence that yielded decreased levels of p53,[126] while the small molecule ABT233 selectively eliminates senescent cells and leads to rejuvenated hematopoietic stem cells.[127] More recently, a FOXO4–p53 interfering peptide was used to selectively clear senescent cells from an aged mouse.[128] By perturbing the interaction between FOXO4 and p53, the peptide was able to induce p53 nuclear exclusion and p53-dependent apoptosis in senescent cells. Intriguingly, the

peptide did not raise total p53 protein levels, but did induce accumulation of serine-15-phosphorylated p53. Along with diminished senescence, a reduction in p21 levels was observed. The resulting clearance of senescent cells led to restoration of fitness in both naturally aged and *XPD*$^{TTD/TTD}$-accelerated aged mice, effectively demonstrating that p53 could be a promising target for aging therapeutics.[128,129]

Taken together, aging mouse models have revealed the complexity of the p53–Mdm2 axis and have solidly placed the p53 network as being key to many aspects of both pathological aging conditions and normal aging. Further research is necessary to determine which p53 stressors, modifications, and outcomes drive aging processes. The p53–MDM2 axis poses new aging-related considerations for p53-activating cancer therapeutics, but has exciting implications for next-generation aging therapeutics.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. This review was prepared under the aegis of a grant from the National Cancer Institute, CA58316.

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