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Senescence Regulation by the p53 Protein Family

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Abstract

p53, a guardian of the genome, exerts its tumor suppression activity by regulating a large number of downstream targets involved in cell cycle arrest, DNA repair, apoptosis, and cellular senescence. Although p53-mediated apoptosis is able to kill cancer cells, a role for cellular senescence in p53-dependent tumor suppression is becoming clear. Mouse studies showed that activation of p53-induced premature senescence promotes tumor regression in vivo. However, p53-mediated cellular senescence also leads to aging-related phenotypes, such as tissue atrophy, stem cell depletion, and impaired wound healing. In addition, several p53 isoforms and two p53 homologs, p63 and p73, have been shown to play a role in cellular senescence and/or aging. Importantly, p53, p63, and p73 are necessary for the maintenance of adult stem cells. Therefore, understanding the dual role the p53 protein family in cancer and aging is critical to solve cancer and longevity in the future. In this chapter, we provide an overview on how p53, p63, p73, and their isoforms regulate cellular senescence and aging.

Keywords

Aging; p53; p63; p73; Senescence

1. Introduction

p53, originally identified as a protein associated with the simian virus 40 large T antigen in 1979 (1–4), guards genomic integrity via regulating numerous cellular processes, including cell cycle arrest, DNA repair, apoptosis, and cellular senescence, in response to various stress signals (5, 6). The p53 pathway is commonly lost in human cancers due to either inactivation of p53 protein or mutations in the *TP53* gene, leading to accumulation of damaged cells and cancer progression, (7). Consistent with this, mice deficient in the *Tp53* gene are highly prone to spontaneous malignancy at a young age (8, 9). In addition, Li–Fraumeni syndrome patients carrying a *TP53* mutation display increased risk of early onset of several types of cancer (10, 11).

Clearance of tumor cells by induction of programmed cell death is well documented as a tumor suppression activity of p53. Recent studies demonstrated that induction of p53-dependent senescence plays a pivotal role in limiting tumor progression in vivo (12–14). Thus, further understanding the mechanism by which p53 is implicated in regulating cellular senescence is of great interest for developing new cancer therapies. Cellular senescence was originally described by Hayflick and Moorhead in the 1960s (15, 16). They found that normal human diploid fibroblasts have a limited lifespan in culture and eventually enter a state of permanent cell cycle arrest called replicative senescence. Senescent cells exhibit enlarged cell size, flattened morphology, inability to synthesize DNA, metabolic active, and expression of the senescence-associated β-galactosidase (SA-β-gal), the latter of which can be detected at pH 6.0 (17, 18). Further studies showed that this life timing is controlled by repeats of telomere, a nucleoprotein structure at chromosome tips, which is undergoing progressive loss during cell divisions (19–21). Due to insufficient telomere after a number of cell doublings exposed or fused chromosome ends trigger DNA damage signals (telomeric

stress signals), leading to senescence. As a major mediator of the DNA damage pathway, p53 has been shown to be critical for telomeric stress-induced cellular senescence (22, 23). In addition, multiple stress signals, including aberrant oncogene activation (24–26) and cancer chemotherapeutic drugs (22, 23, 27), are able to induce senescence-like phenotypes (premature senescence) in both primary and tumor cells via activating the p53 and/or p16 pathways (28). In this chapter, we provide an overview of the role of p53, its isoforms, and its family members, p63 and p73, in regulating cellular senescence and aging.

2. The Role of p53 in Cellular Senescence in Response to Different Stress Signals

2.1. The p53 Protein

The wild-type p53 protein is composed of 393 amino acids and contains several functional domains (Fig. 1). These are the N-terminal activation domain 1 (AD1) and 2 (AD2), the proline-rich domain (PRD), the central core DNA-binding domain (DBD), the nuclear localization signal region (NLS), the tetramerization domain (TD), and the C-terminal regulatory basic domain (BD) (29). As a sequence-specific transcription factor, p53 regulates gene expression by directly binding to a p53-responsive element (p53-RE) in the target gene as a tetramer. The consensus p53-RE is composed of two half sites ($RRRC^{A}/_{T}{}^{A}/_{T}GYYY$, where R represents purine and Y pyrimidine) separated by up to 13 nucleotides (30). Hundreds of p53 targets have been identified and shown to be involved in a variety of cellular responses, such as cell cycle arrest, DNA repair, apoptosis, and cellular senescence, attributing to the tumor suppressor activity of p53.

The level and activity of p53 protein are primarily regulated through posttranslational modifications (Fig. 2). In normal cells, p53 is a short-lived protein which is continuously undergoing ubiquitination and subject to proteasomal degradation. Mouse double minute 2 (MDM2, HDM2 in human), one of the first characterized p53 targets, serves as a major E3 ubiquitin ligase for p53 degradation and thus forms a negative autoregulatory loop to maintain the low level of p53 expression in unstressed conditions (31–34). Upon genotoxic stresses, rapid phosphorylation of p53 at Ser-15 by the Ataxia telangiectasia mutated (ATM), a serine/threonine protein kinase, and at Ser-20 by the checkpoint kinase 2 (Chk2) results in dissociation of p53 from MDM2, leading to p53 stabilization and activation of its downstream processes (35–37). Likewise, phosphorylation of MDM2 at Ser-395 by ATM attenuates the capability of MDM2 in exporting nuclear p53 to cytoplasm for subsequent p53 degradation, thereby enabling p53 accumulation (38, 39). In addition to ubiquitination and phosphorylation, p53 activity is modulated by acetylation (35, 40). Acetylation by histone acetyltransferases (HATs) and deacetylation by histone deacetylases (HDACs) were initially discovered to regulate the extent of histone acetylation and play a critical role in gene transcription (41). p53 was identified as the first non-histone substrate of HATs and HDACs (42, 43). In response to DNA damage, CBP/p300 acetylates p53 on six C-terminal lysine (K) residues (K370, K372, K373, K381, K382, and K386), the same target sites of MDM2-mediated ubiquitination, and hence leads to enhanced stability and DNA binding activity of p53 (44). In addition, acetylation of p53 on K320 by PCAF preferentially directs p53 to activate target genes involved in cell cycle arrest (45), whereas acetylation of p53 on K120 by Tip60/hMOF promotes p53-mediated cell death (46–48). Moreover, acetylation of p53 on K164 by CBP/p300 is required for p53-induced cell cycle arrest and apoptosis (49). Interestingly, emerging evidence showed that *TP53* mRNA stability and translation are regulated by multiple RNA binding proteins including HuR (50), ribosomal protein L26 (RPL26) (51, 52), nucleolin (51), and RNPC1 (53), and microRNAs including miR-125b (54), miR-125a (55), and miR-504 (56).

2.2. The Role of p53 in Replicative Senescence

Detection of γH2AX foci, a sensitive marker for DNA double-strand break (DSB), on dysfunctional telomeres (shortened telomeres or altered telomere state) in senescent cells indicates that telomere-dependent replicative senescence is stress-dependent (57–59) (Fig. 2). These foci contain multiple DNA damage responsive proteins, such as 53BP1 and MRN complex (MRE11, RAD51, and NBS1), and are indistinguishable from the γH2AX foci originated from ionizing radiation-induced DNA DSB (60, 61). Activation of ATM at sites of DNA DSBs elicits phosphorylation of Chk2 and upregulation of p53 (62, 63). ATM is a primary mediator for telomere dysfunction-induced damage signaling. However, in the absence of ATM, ATR (ATM and Rad3-related) substitutes the role of ATM to activate p53 through Chk1-induced p53 phosphorylation at Ser-15 (63). Consistently, enhanced expression of p53 in mice deficient in Terc, the RNA component of telomerase, leads to activation of senescence and reduced tumor formation (64). In contrast, mice deficient in both Terc and p53 bypass senescence and develop tumors (65). These indicate that p53 plays a pivotal role in maintaining telomere dysfunction-initiated senescence.

2.3. The Role of p53 in Oncogene-Induced Senescence

In addition to spontaneous senescence, OIS was discovered as a cancer prevention mechanism in cells exposed to oncogenic Ras. It has been shown that expression of oncogenic Ras promotes acute senescence-like G1 arrest in primary human or rodent cells containing wild-type p53 (24), but initiates cellular transformation in those lacking p53 (66). This indicates that p53 plays a critical role in OIS. There are two underlying mechanisms by which Ras activates the p53-dependent senescence pathway in OIS (67–69) (Fig. 2). First, Ras regulates p53 through the MAPK pathways. It has been shown that Ras utilizes the MAPK signal-transduction pathway, including Raf-1, MEKs (MEK1/2), and MAPKs (ERK1/2), to promote cell cycle arrest in primary cells but malignant transformation in immortal cells (70–72). However, the cell cycle arrest induced by expression of Ras or MEK is abolished in cells lacking p53 (70). In addition, activation of the p38/MAPK pathway, especially its downstream kinase PRAK, leads to p53-dependent senescence by phosphorylating and activating p53 (73, 74). Indeed, ARF can be activated by the Ras/Raf signaling cascade via E2F1 or Dmp1 (75, 76). E2F1 and Dmp1 are capable of directly binding to, and activating, the promoter of the ARF gene (77, 78). $p19^{ARF}$ ($p14^{ARF}$ in human) is encoded from an alternative reading frame within the INK4a/ARF locus which also encodes p16 (79, 80). ARF blocks MDM2-mediated degradation of p53 by repressing MDM2 E3 ligase activity and nuclear export as well as promoting MDM2 degradation (81–84). Interestingly, ARF mediates Ras signaling to p53 in mouse cells but is not required for OIS in human cells (85). Second, Ras regulates p53 by activation of DNA damage response (DDR). It has been shown that ectopic expression of HRasV12 in human diploid fibroblasts induces DNA hyper-replication resulting in prematurely terminated DNA replication and collapse of DNA replication forks, which in turn lead to accumulation of DSBs and subsequent activation of the ATM/ATR-p53 pathway to induce senescence (86–89). In addition, oncogene activation leads to accumulation of reactive oxygen species (ROS), which induces DNA damage (89, 90). Consistent with this, human diploid fibroblasts grown in a restrictive culture condition with low or no ROS production bypass oncogene-induced senescence (91).

2.4. The Role of p53 in Genotoxic Stress-Induced Senescence

Given the importance of the DDR-p53 pathway in both replicative senescence and OIS, it is not surprising that numerous stress signals, such as DNA damage, oxidative stress, insufficient nutrients/growth factors, and improper cell contacts, induce premature senescence in normal human and murine cells (27, 92). Since induction of permanent cell cycle arrest is a potential cancer prevention mechanism by eliminating damaged pre-cancer

cells at the initial stage of transformation, it was postulated that evasion of senescence might be a prerequisite for pre-cancer cells to complete transformation into cancer cells (93–95). Surprisingly, senescence-like growth arrest is induced by ectopic expression of a tumor suppressor gene or treatment with chemotherapeutic drugs in a broad range of human cancer cells, including those derived from colon, ovarian, lung, prostate, and cervical carcinomas (25). Importantly, reports showed that restoration of p53-mediated premature senescence is able to promote cancer regression in mice with hepatocarcinoma and sarcomas (12, 96). Indeed, ectopic expression of wild-type p53 triggers premature senescence in tumor cells lacking endogenous p53 (97, 98). In addition, p53-dependent premature senescence is induced by a variety of chemotherapeutic drugs, including camptothecin, doxorubicin, etoposide, cisplatin, and resveratrol (99). However, treatment with therapeutic agents also induces p53-independent premature senescence. For instance, doxorubicin induces senescence in a number of cancer cell lines deficient in p53 (25, 100, 101).

3. Characteristics of p53-Mediated Cellular Senescence

3.1. Cell Cycle Arrest

Since senescent cells must initially undergo cell cycle arrest, DNA histogram analysis is expected to show decreased number of cells in S phase (cell uptake of BrdU) and increased number of cells in G1 or sometimes also G2/M phase, but no change in the number of cells in sub-G1 phase (dead cells) (101, 102). However, the cell cycle profile for senescent cells is indistinguishable from that for quiescent cells and terminally differentiated cells.

3.2. SA-β-Gal

SA- gal is a commonly used senescence biomarker, which can be detected at pH 6.0 (18). This phenomenon is due to the fact that senescent cells express a high level of lysosomal gal (103–105). It is important to note that overexpression of lysosomal gal itself is not able to initiate senescence (103). Nevertheless, SA- gal staining is a convenient and accepted method to detect cellular senescence in vitro and even in vivo (18, 26). However, gal positive alone is not sufficient to judge whether a cell is in the senescent state. For example, TGF- stimulation induces SA- gal independent of senescence in both cultured human prostate basal cells and epithelial cells in benign prostatic hyperplasia (106). In addition, expression of lysosomal galactosidase beta-1 (GLB1), which accounts for the SA- gal activity in some cancer cells, is not correlated with senescence (103). Moreover, in order to measure cellular senescence in vivo, fresh or frozen tissues are required for this staining assay.

3.3. Senescence-Associated Heterochromatic Foci

Cellular senescence is accompanied by formation of facultative heterochromatin, also called senescence-associated heterochromatic foci (SAHF), wherein DAPI staining would show spot-staining patterns (107). In contrast, DAPI staining would show homogenous staining patterns in non-senescent cells (107). SAHF contains histone H3K9 methylation whereas euchromatin contains histone H3K9 acetylation and K4 methylation (107). Meanwhile, the increased incorporation of heterochromatin protein 1 (HP1) into SAHF is distinct from the pericentric heterochromatin (107). Furthermore, H2AX colocalizes with SAHF (108). Importantly, a combination of positive SAHF and H2AX with negative Ki67 (a proliferation index) is a reliable indicator of senescence for cells in culture and in tissues (109, 110). However, SAHF formation is often detected but not a prerequisite for p53-dependent senescence (107, 111).

3.4. p53 Targets Genes

As mentioned above, activation of the p53 pathway is a common cellular response leading to cellular senescence upon exposure to stress signals (Fig. 2). As a result, senescent cells are characterized by enhanced expression of some p53 targets, such as p21 (30), PML (112), plasminogen activator inhibitor (PAI-1) (113), and DEC1 (101), all of which are recognized as senescence markers, and in turn are able to induce senescence themselves (69, 95, 114–117). p21, a cyclin-dependent kinase inhibitor, plays a critical role in inducing G1 cell cycle arrest by inhibiting the activity of cyclin-CDK2/4 complexes, E2F, and PCNA (118–121). Promyelocytic leukemia protein PML, an essential component of PML nuclear bodies (PML-NBs) that accumulates in senescent cells, is induced by several factors, including oncogenic stress and p53 (112, 116). In turn, PML recruits p16, p53, and pRb/E2F complex to the PML-NBs and hence modulates the expression of downstream target genes of these factors, leading to senescence (116, 122). Importantly, PML forms a positive feedback loop with p53 to trigger cellular senescence by promoting p53 acetylation and phosphorylation or inhibiting p53 degradation by MDM2 (115, 116, 123–125). By contrast, inhibition of p53 acetylation by SIRT1 (a NAD+-dependent class III histone deacetylase) blocks PML-mediated premature senescence (126). PAI-1 induces cellular senescence by inhibiting the activity of uPA, a secreted protein which is capable of activating the MAPK pathway, and subsequently promoting G1/S transition (127). DEC1, a basic helix-loop-helix transcription factor, is capable of inducing DNA damage-induced premature senescence via inhibiting ID1, an oncogene which is found to be downregulated in arrested or senescent cells (128, 129). A recent report showed that Yippee-like-3 (YPEL3), a member of the putative zinc finger motif coding gene family, is regulated by p53 and triggers premature senescence in normal and tumor cells (130). In addition, BTG2, a member of the antiproliferative BTG gene family, is a p53-responsive gene and plays an essential role in replicative senescence (131). Moreover, the miR-34 family is a p53 target and elicits cellular senescence in both primary and tumor cells in response to DNA damage and oncogenic stress (132, 133). It is important to note that p130 is the major pocket protein associated with induction of premature senescence via the DNA damage-p53 signaling cascade in both normal and tumor cells (134–136). However, the mechanism by which p53 increases p130 activity during senescence is still not clear.

Detection of p53 and its downstream effectors can be used to measure the state of senescence for cells both in vitro and in vivo. However, these genes are not always induced in senescent cells by p53 in response to a specific stress signal. Therefore, a combination of multiple senescence markers discussed above together with one or more p53 targets is required to implicate the state of p53-mediated cellular senescence.

4. The Role of p53 Isoforms in Cellular Senescence

The *TP53* gene encodes at least nine different isoforms due to the use of two promoters, two translation initiation sites, and alternative splicing (137) (Fig. 1). The *TP53* gene contains 11 exons. p53, also called α isoform, is a full-length p53 generated from the P1 promoter. An internal promoter in intron 4 leads to production of an N-terminal truncated p53 starting from residue 133, named as Δ133p53 (138). In addition, internal initiation of translation at an AUG codon at position 40 or alternative splice of intron 2 leads to expression of a p53 protein with deletion of the first 39 amino acids, named as Δ40p53 (also called ΔNp53, p47, p53/47, and p44) (138–142). Moreover, alternative splicing of intron 9 produces β and γ isoforms (138) , which lack C-terminal oligomerization domain but contain 10 and 15 unique amino acids at C-termini, respectively (Fig. 1). As a result, nine p53 isoforms, p53 α (full-length p53, referred to as p53 in this chapter), p53 β (also called p53I9 (143)), p53 γ, Δ40p53 α, Δ40p53 β, Δ40p53 γ, Δ133p53 α, Δ133p53 β, and Δ133p53 γ are expressed. These p53 isoforms are found to be expressed in normal human tissues in a tissue-dependent

manner as well as in some tumor tissues (137). This suggests that the expression pattern of p53 isoforms may play a role in tumor formation and affect the outcomes of cancer therapy.

Under an unstressed condition, p53 itself preferentially binds to the p21 and Bax (a pro-apoptotic gene) promoters rather than to the MDM2 promoter, but has no effect on p53 activity (138). However, in response to a stress signal, p53 specifically enhances the Bax gene transcription through physical interaction with p53 and slightly increases p53-mediated apoptosis (138). In contrast, 133p53, which lacks the entire N-terminal AD, is dominant-negative over p53 and diminishes p53-mediated apoptosis (138). Interestingly, it has been shown that induction of p53 and suppression of 133p53 lead to induction of p21 and miR-34, resulting in senescence in cultured cells and colon adenomas (144). This indicates that the biological activity of full-length p53 is likely modulated by its isoforms in normal and tumor tissues. However, other groups have reported that p53 , which lacks the C-terminal BD and most of the TD, is deficient in DNA binding activity and unable to modulate p53-dependent stress responses (143, 145). Furthermore, Np53 is implicated in G2 cell cycle arrest (146). Therefore, further studies are needed to clarify the mechanism by which p53 isoforms affect each other's functions.

5. The Role of p53 Protein Isoforms in Aging

It has been proposed that aging is characterized by decreased capability to maintain and repair somatic cells (147). One of the causes for aging is associated with senescence-mediated decline in the number and/or proliferative capacity of adult stem cells (148, 149). Indeed, elevated expression of senescence-associated markers, SA- gal and p16, is detected in aging tissues (18, 150). As a major mediator of cellular senescence, activation of p53 not only inhibits neoplasia but also promotes organismal aging. Because *Tp53* homozygous knockout mice die from tumors at a young age (8), several mouse models with altered p53 activity have been generated to analyze the role of p53 in aging. These are $Tp53^{+/m}$, 40p53-knockin, pL53, and super p53 mouse models (9, 151).

$Tp53^{+/m}$ mice contain a mutant p53 *m* allele with a deletion mutation in the first six exons of the *Tp53* gene and express an N-terminal truncated p53 protein, called M protein (152). $Tp53^{+/m}$ mice display reduced tumor formation and shortened longevity compared with wild-type littermates (152). However, $Tp53^{m/m}$ mice die before birth and $Tp53^{-/m}$ mice develop tumors similarly to $Tp53^{-/-}$ mice. This suggests that M protein-induced aging phenotypes in $Tp53^{+/m}$ are p53-dependent. Indeed, it has been shown that M protein interacts with and stabilizes p53 by facilitating p53 nuclear localization in the absence of a stress (153). Therefore, it is possible that the resulting phenotypes observed in $Tp53^{+/m}$ mice are due to increased p53 activity. However, this mutant allele also contains a deletion of 24 genes, including *Aloxe3*, *Alox12b*, *Alox15b*, *Chd3*, *Aurkb*, and *Per1*, adjacent to the *Tp53* gene (154). Thus, it is necessary to further analyze whether haploinsufficiency of these 24 genes contributes to altered p53 activity and decreased tumor formation but accelerated aging phenotypes.

Similar to $Tp53^{+/m}$ mice, 40p53-knockin mice, that express a naturally occurring N-terminal truncated p53 isoform, exhibit decreased lifespan and several premature aging phenotypes, including shortened reproductive span, lordokyphosis, and a reduced tumor incidence (155). These phenotypes are likely due to enhanced wild-type p53 activity and hyperactivation of insulin-like growth factor 1 (IGF-1) signaling cascade (155), the latter of which is associated with lifespan in many species (156, 157). The elevated IGF-1 signaling in 40p53 mice leads to sustained induction of p21 through the MAPK pathway and consequently causes cellular senescence and premature aging phenotypes (155). Similar premature aging phenomena were observed in another line of transgenic mice containing

multiple copies of a temperature-sensitive mutant allele p53V135A derived from BALB/c liver DNA, named as pL53 (158). Together, these results indicate that enhanced p53 activity contributes to aging. This hypothesis is supported by several other accelerated aging mouse models, such as mice deficient in Terc (64), Ku80 (also called Ku86, a regulatory unit of DNA-PK) (159), BRCA1 (a tumor suppressor) (160), and Zmpste24 (also called FACE-1, a metalloproteinase involved in the maturation of Lamin A) (161). Conversely, mice deficient in p66shc, a cytoplasmic signal transducer from activated receptors to Ras, live longer along with decreased p53 activity than wild-type controls (162). However, this hypothesis is challenged by another mouse model, called the "super p53" mice.

The super p53 mice carry extra copies of a complete p53 gene, including the p53 promoter and upstream regulatory region (163). As expected, these mice display augmented p53 activity in response to DNA damage and are tumor-resistant. Interestingly, the super p53 mice have normal lifespan without showing any sign of accelerated aging. Similarly, a "super Ink4a/ARF" mouse strain, which carries a transgenic copy of the entire Ink4a/ARF locus (164), and a mouse strain, which carries a hypomorphic allele of MDM2 (165), were generated, both of which have decreased MDM2 activity and hence enhanced p53 activity. Like the super p53 mice, MDM2 hypomorphic and super Ink4a/ARF mice are resistant to spontaneous and carcinogene-induced tumor formation without premature aging. Moreover, the super p53/p19^{ARF} mice, carrying additional copies of both *Tp53* and ARF alleles, were generated by cross-breeding the super p53 mice with the super Ink4a/ARF mice and hence p53 can be further activated (166). Surprisingly, the super p53/p19^{ARF} mice are not only cancer free but also have a significantly extended lifespan. These findings suggest that there is no causal relationship between increased p53-mediated tumor suppression and decreased longevity. Instead, p53 activation in certain circumstances exerts benefits on longevity. There are several potential explanations. First, aging is a consequence of accumulation of DNA damage in tissues, and therefore, by modulating the DDR, p53 is able to reduce age-associated DNA damage and accumulation of damaged cells. Second, p53 activation plays a role in maintaining the stem-cell pool in adult tissues. Third, p53 regulates aging process by modulating other signaling pathways affecting longevity, such as the insulin/IGF-1 and mTOR signaling (167–169). Fourth, p53 may have pro-longevity effects by restraining secretion of the senescence-associated pro-inflammatory cytokines (170, 171), which are known to contribute to chronic age-related inflammation. However, the precise mechanism by which p53 controls the balance between anti-aging and pro-aging needs to be further explored.

6. The Role of p63 and p73 in Cellular Senescence and Aging

p63 and p73, two p53-ancestral genes (172, 173), share highly amino-acid identity with p53 within the AD, DBD, and TD (174, 175) (Fig. 3). The highest degree of homology among the three members is observed within the DBD (>60% amino-acid identity between p53 and p63/p73, and ~85% amino-acid identity between p63 and p73). Unlike p53, the alpha isoforms of p63 and p73 contain a C-terminal sterile alpha motif (SAM), a protein interaction domain involved in developmental regulation. In addition, multiple protein products are generated from both p63 and p73 genes due to the usage of two promoters and alternative RNA splicing (Fig. 3). Therefore, p63 and p73 isoforms possess one of two N termini, transcriptional active (TA) and N-terminally deleted (ΔN), each with five different C termini for p63 (α–ε) (173, 176) and at least ten different C termini for p73 (α–ζ, δ1, δ2, and ε) (172, 177–180). Furthermore, alternative splicing of p73 exon 2 and 3 gives rise to two additional p73 isoforms, ΔNΔp73 and ΔTAp73 (ΔEx2 and ΔEx2/3) (172, 179, 181).

Unlike *Tp53* knockout mice that are prone to tumor formation, *Tp63* knockout mice show severe defect in limb formation and epidermal development (182, 183) and p73-null mice

exhibit developmental defects in central nervous system and inflammatory response (184). These suggest that p63 and p73 play a key role in development. However, due to the high sequence similarity among the p53 family, p63 and p73 act synergistically or antagonistically with p53 in regulating p53-mediated biological activities. Indeed, p63 and p73, especially their TA isoforms, are able to bind to p53-responsive elements and transactivate a set of p53 targets, such as p21, MDM2, Bax, and PUMA, and regulate cell cycle arrest and cell death (174, 185, 186). Recent reports showed that p63 and p73 are also involved in regulating cellular senescence (Fig. 4).

Results obtained from *Tp63* knockout mice suggest that p63 is an essential regulator for epidermal homeostasis (182, 183). Indeed, ΔNp63 plays a predominant role in epidermal commitment during embryogenesis (187, 188). In addition, ΔNp63 is highly expressed in the basal layer of stratified epithelia to maintain proliferative potential and direct proper differentiation of progenitor cells (189, 190). Moreover, *TAp63*-specific knockout mice do not display the same impaired skin development as p63-null mice (191), whereas ΔNp63-null mice develop lesions that resemble those seen in AEC (ankyloblepharon ectodermal dysplasia and clefting) patients, an ectodermal dysplasia characterized by skin fragility (192). This further confirms the critical role of ΔNp63 in epidermal morphogenesis. However, as a p53 homolog, mice deficient in TAp63 develop metastatic tumors (193). In addition, loss of p63 or TAp63 increases the number of metastatic tumors in $Tp53^{+/-}$ and $Tp53^{-/-}$ mice (193, 194). Surprisingly, although *Tp53* heterozygous mice develop tumors, *Tp63* heterozygous mice are tumor-resistant or develop a few tumors along with a decreased lifespan and accelerated aging phenotypes, similar to $Tp53^{+/m}$ mice (195). Consistently, ablation of p63 induces cellular senescence in primary kerotinocytes (195). In addition, germline- or tissue-specific p63 deficiency also leads to enhanced expression of senescence markers, including SA-βgal and PML, concomitant with premature aging features (195). Importantly, a report showed that four females from a family with Rapp–Hodgkin syndrome (RHS), a disease associated with *TP63* mutations, present not only typical RHS (anhidrotic ectodermal dysplasia with cleft lip and palate) but also ophthalmic anomalies such as corneal dystrophy and premature menopause (around 30 years) (196). These data suggest that p63 is implicated in aging progression. It has been shown that overexpression of ΔNp63 inhibits OIS and promotes tumorigenesis in vivo (197), whereas ectopic expression of TAp63 induces senescence and inhibits progression of established tumors in vivo (198). However, the $TAp63^{-/-}$ mice exhibit signs of premature aging, including hair loss, impaired wound healing, kyphosis, and a shortened lifespan (191). It is postulated that TAp63 is necessary for the survival of adult skin stem cells and thus prevents premature aging (191). In addition, TAp63 was shown to regulate senescence and aging via modulating the p53, p16, and IGF pathways (199, 200). These together indicate that TA and ΔN isoforms of p63 play a dual role in cellular senescence and tissue aging in cell-type and stress-specific manners.

Like p53, p73 is activated in response to various stress signals, including DNA damage and oncogenic activation, and regulates a set of p53-dependent and -independent genes involved in cell cycle arrest and apoptosis (185, 201, 202). In the absence of p53, p73 can compensate for p53 function to induce apoptosis in cancer cells and the status of the *TP73* gene is correlated with the survival rate for cancer patients (201, 203–207). Likewise, mice heterozygous of both *Tp53* and *Tp73* exhibit increased tumor burden and metastasis compared to mice heterozygous of *Tp53* alone (194). Mice specifically deficient in TAp73 isoform develop spontaneous and carcinogen-induced tumors and cells from TAp73-null mice display genomic instability associated with enhanced aneuploidy (208). By contrast, cells from ΔNp73-null mice are more sensitive to DNA damage-induced apoptosis in a p53-dependent manner and high levels of ΔNp73 expression associates with poor prognosis in human cancers (209). These results demonstrate that TAp73 is a tumor suppressor whereas

ΔNp73 acts as an oncogene. However, to date, knowledge on the role of p73 in senescence is limited. Similar to p53 and p63, p73 plays a function in the maintenance of stem cells (Fig. 4). Specifically, it has been shown that ΔNp73 is a potent prosurvival factor for neurons (210–212) and TAp73 is required for the long-term maintenance of neural stem cells to elicit neurogenesis in adults (213). As a result, mice heterozygous of TA or ΔNp73 isoforms display age-related neurodegeneration (214). Similar to p53 and p63, p73 has been shown to inhibit expression of IGF-1 receptor (200, 215, 216). This indicates that the IGF-1 signaling is a common target of the p53 family to modulate aging (Fig. 4). Importantly, a recent report provided evidence for the role of TAp73 in aging. It has been shown that tumor-free TAp73-null mice develop premature aging phenotypes with impaired ROS scavenging compared to wild-type littermates (217). Indeed, TAp73 is crucial to maintain proper mitochondrial functions by directly regulating the expression of mitochondrial complex IV subunit Cox4i1 (cytochrome C oxidase subunit 4 isoform 1) (217). However, the molecular basis for p73 in aging-associated disorders needs to be further uncovered.

7. Summary and Remarks

Proliferating cells can exit from the cell cycle and enter a state of permanent cell cycle arrest when they encounter stress signals originated from telomere shortening, aberrant oncogenic activities, and other DNA damage signals. Senescent cells are distinct from quiescent cells by expression of senescence-associated markers, including SA-βgal, SAHF, and activation of the p53 or p16 pathway followed by altered gene expression profile. Emerging evidence showed that cellular senescence associates with multiple pathological disorders, including cancer and aging. Importantly, it has been demonstrated in vivo that induction of cellular senescence is a tumor suppression mechanism by blocking malignant transformation at the initial step of tumorigenesis and by clearance of cancer cells at the late stage of tumor progression. However, the benefit of the cellular senescence-mediated tumor suppression is countered by the cost of accelerated aging. Therefore, a future challenge to develop senescence-based cancer therapy is to reveal the target and pathways that control the balance between cancer and longevity.

As we discussed in this chapter, the p53 family plays a pivotal role in cellular senescence and thus affect cancer progression and aging (Fig. 4). Although induction of cellular senescence by activation of the p53 family contributes to tumor suppression and accelerated aging, reports also showed that the p53 family promotes longevity and maintains tissue homeostasis through protecting renewable stem cells. In addition, studies with the “super p53” mouse models indicate that cancer resistance could be achieved by modulation of p53 activity without causing undesirable effects. Importantly, a combination of increased p53 and ARF activities not only inhibits tumor formation but also increases longevity. Therefore, the p53 pathway is a promising target for senescence-based cancer treatment. However, there are still unsolved questions. For instance, how are p53 isoforms, p63 and p73, involved in making a balance between cancer and aging? What is the molecular basis for p63/p73-mediated cellular senescence? How does p53 family integrate with other signaling pathways related to aging? Therefore, further exploration of these puzzles in vitro and in vivo will enhance our knowledge about the p53 family in senescence-associated diseases and may provide applicable clues for clinical use.

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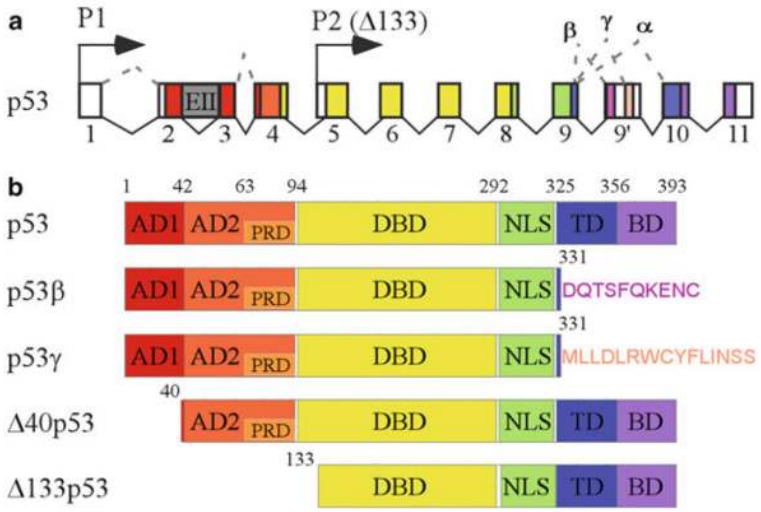

Fig. 1.

The p53 gene and protein structure. (**a**) *TP53* locus, location of the P1 and P2 promoters, and patterns of alternative splicing. p53, also called isoform, is a full-length p53 generated from the P1 promoter. Transcripts initiated from an internal promoter (P2) in intron 4 leads to production of an N-terminal truncated p53 starting from residue 133, named as 133p53. Alternative translation initiation at an AUG codon at position 40 leads to production of a p53 protein with a deletion of the first 39 amino acids (40p53). Although alternative splicing of intron 2 gives rise to a transcript containing intron 2 sequence, the presence of a stop codon in intron 2 also leads to production of 40p53. Compared to the isoforms, the and isoforms have 10 and 15 unique amino acids at C-termini, respectively. (**b**) p53 isoforms and functional domains. *AD* activation domain, *PRD* proline-rich domain, *DBD* DNA binding domain, *NLS* nuclear localization domain, *TD* teramerization domain, *BD* basic domain.

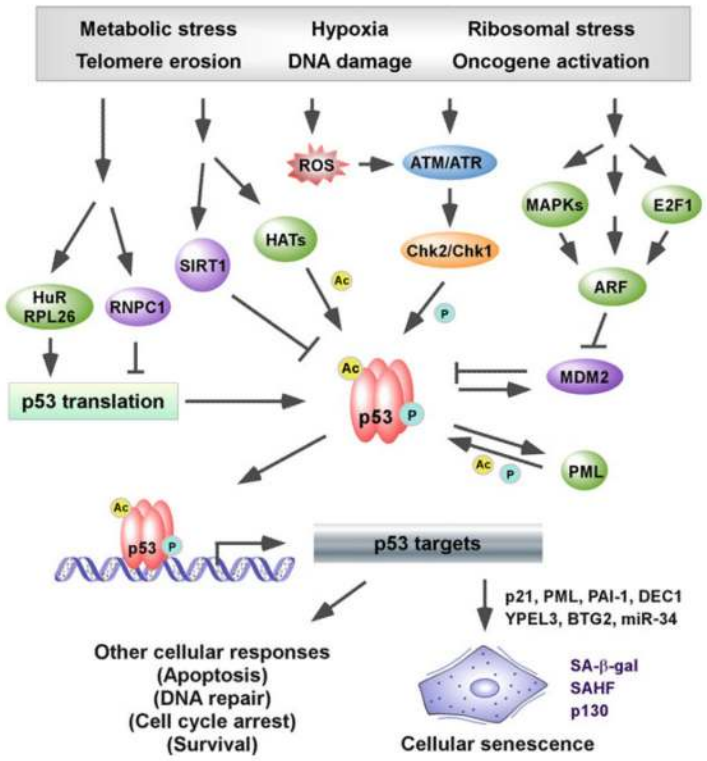

Fig. 2.

The role of p53 in cellular senescence. Upon exposure to numerous stress signals, p53 is regulated by phosphorylation (ATM/ATR-Chk2/Chk1), by acetylation (p300/CBP, PCAF, Tip60/hMOF, and SIRT1), by increased protein stability (ARF-MDM2), and by increased translation rates (RNPC1, HuR, and RPL26). Once activated, p53 regulates a set of downstream targets leading to cellular senescence and other cellular responses. Several senescence markers, including p21, PML, PAI-1, and DEC1, are identi fied as p53 target genes and promote cellular senescence. In addition, PML positively feeds back to promote p53 phosphorylation and acetylation. As a result, senescent cells induced by p53 activation exhibit flattened morphology, enlarged cell size, expression of SA- gal, formation of SAHF, and deregulated expression of p53 targets. In some cases, increased hypophosphorylated p130 is detected in cellular senescence induced by p53 and/or DNA damage.

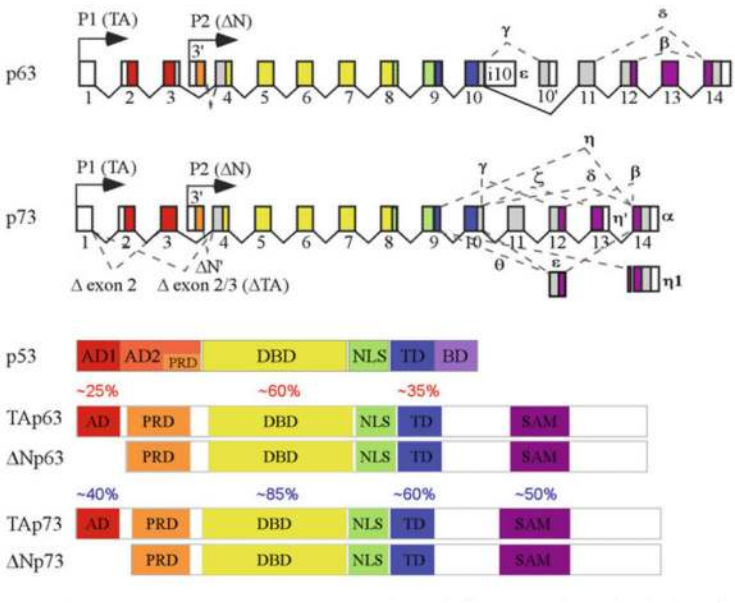

Fig. 3.

The p63 and p73 genes and protein structures. (**a**) *TP63* and *TP73* genes. Transcripts initiated from P1 and P2 promoters give rise to TA and ΔN protein isoforms, respectively. Alternative splicing at C-termini of *TP63* transcripts leads to production of p63 α, β (Δexon 13), γ (Δexons 11–14 and incorporated with additional sequence from exon 10 (i10), δ (Δexons 12 and 13), and ε (generated by alternative termination in exon 10). Alternative splicing at C-termini of *TP73* transcripts leads to production of p73 α, β (Δexon 13), g (Δexon 11), δ (Δexons 11–13), ε (Δexons 11 and 12), ζ (Δexons 11 and 13), η (Δexons 10, 11, and 13), θ (Δexons 10–13), η1 (Δ2 nucleotides at 3′-end of exon 9 and contains 18 nucleotides from exon 13, compared with h), and η′ (generated by alternative termination in exon 13). Alternative splicing at N-termini of *TP73* transcripts leads to production of ΔN-p73 and ΔTAp73 (ΔEx2 and ΔEx2/3). Δ, deleted. (**b**) Homology among the p53 family members. Compared to p53, the a isoforms of p63 and p73 have a unique C-terminal SAM (sterile alpha motif) domain. A high degree of sequence identity is seen between p53 and p63/p73 (on *top*) and between p63 and p73 (at *bottom*).

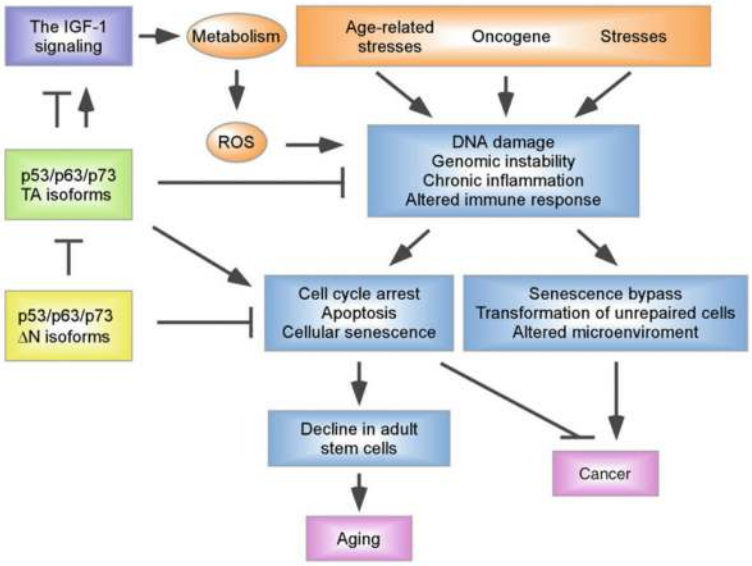

Fig. 4.

The role of p53 family proteins in cancer and aging. Accumulation of abnormal cells caused by aging-associated damage, aberrant oncogenic activity, and other stress signals, contributes to both aging and cancer. Activation of the p53 pathway in response to these stress signals is able to protect adult stem cells to promote longevity and prevent malignant transformation by repairing or eliminating damaged cells. However, accumulation of senescent cells in tissues due to activation of TA isoforms of p53 family members, leads to decline in adult stem cells and accelerated aging. In addition, p53 family members are capable of targeting the IGF-1 signaling cascade, a pathway known to be involved in organismal aging, to regulate longevity. In contrast, ΔN isoforms of p53 family are found to be required for maintenance of stem cell proliferation and act as dominate negative inhibitors of TA counterparts.