Review Article

Multiple facets of p53 in senescence induction and maintenance

Yoshikazu Johmura[1] and Makoto Nakanishi[1,2]

[1]Department of Cell Biology, Graduate School of Medical Sciences, Nagoya City University, Nagoya; [2]Division of Cancer Cell Biology, Department of Cancer Biology, Instuite of Medical Science, The University of Tokyo, Tokyo, Japan

Cellular senescence is a state of durable cell cycle arrest with metabolic activities distinct from those of the proliferative state. Since senescence was originally reported to be induced by various genotoxic stressors, such as telomere erosion and oncogenic signaling, it has been proposed to play a pivotal role in aging-related changes and as an antitumorigenic barrier *in vivo*. However, the mechanisms underlying its induction and maintenance remain entirely elusive. We have recently found that abrupt activation of p53 at G_2 results in a cell skipping mitosis and subsequently undergoing senescence. Surprisingly, we have also found that downregulation of p53 by SCF^{Fbxo22} is crucial for the induction of a senescence-associated phenotype. In this review, we provide an overview of recent advances in understanding the mechanisms underlying the timing and magnitude of activation of p53 during senescence.

More than half a century ago, cellular senescence was first reported by Hayflick and Moorhead[1] as a durable cell cycle arrest of cultured human fibroblasts after a defined number of population doublings. The number of population doublings of normal cells in culture, known as the Hayflick limit, depends on the species used to establish the cell culture. In addition, this number is related to a positive correlation with the life span of the animals,[2] with higher limits observed in cultures derived from long-lived animals. Taken together, these results suggest a strong link between a limited proliferative capacity in culture and the processes observed in organismal aging. Although the physiological importance of cellular senescence in organismal aging has long been unclear, it should be noted that a recent study successfully identified senescent cells in aging tissues of humans.[3] Senescence biomarkers associated with aging and its related pathology are easily detectable in human tissues *in vivo*.[4–8] For example, the number of cells positive for senescence-associated β-Gal is markedly increased in atherosclerotic lesions of the coronary arteries[9] and in benign prostatic hyperplasia.[10] In addition, the number of senescent fibroblasts exponentially increases with age in skin tissues of baboons.[11] The essential role of senescence in aging and age-related disorders is further supported by the observation that clearance of p16-positive senescent cells delays aging-associated disorders and extends lifespan.[12,13]

In addition to organismal aging, cellular senescence is also regarded as a tumor suppression mechanism, functioning in this role by limiting proliferation of cells with aberrant DNA structures *in vivo*. Precancerous tissues show evidence of senescence, and this is suppressed during cancer progression.[7,14–17] This pattern has been generally observed in multiple tumor types.[7,14,16] A DNA damage response (DDR) to DNA double-strand breaks (DSBs), one of the major causes of senescence, is activated in precancerous lesions but not in cancers, although evidence for the presence of DSBs was found in both precancerous and cancerous lesions.[14,15,18] These observations suggest that senescence functions as a tumorigenesis barrier in human precancerous lesions.

Permanent cessation of cell proliferation is a hallmark of senescent cells, but these cells also show specific characteristics,[19,20] and recent observations have extended the role of senescence to biological processes other than cancer and aging, such as development[21] and tissue repair.[22] Senescent cells secrete a diversity of growth factors and pro-inflammatory cytokines, known as the senescence-associated secretory phenotype (SASP). These factors are pleiotropic and induce various local activities, such as recruitment of inflammatory cells and alterations of the tissue microenvironment and likely act as a double-edged sword either facilitating or inhibiting tumorigenesis (Fig. 1). Considered collectively, although the

Fig. 1. Antagonistic roles of senescent cells in tumorigenesis *in vivo*. In response to various stimuli such as replicative stress, oncogenic activation, DNA damage, and oxidative stress, normal cells undergo senescence. Senescence has long been considered to function as an antitumorigenesis barrier in human precancerous lesions. This barrier is likely erected by inducing durable cell cycle arrest in cells with tumorigenic potential, activation of immune checkpoints, and ECM remodeling. However, it has now become apparent that senescent cells secrete various pro-inflammatory cytokines and growth factors, namely the oncogenic senescence-associated secretory phenotype, which promotes chronic inflammation, angiogenesis, catastrophic tissue homeostasis, and even invasion and proliferation of cancer cells. NK, natural killer.
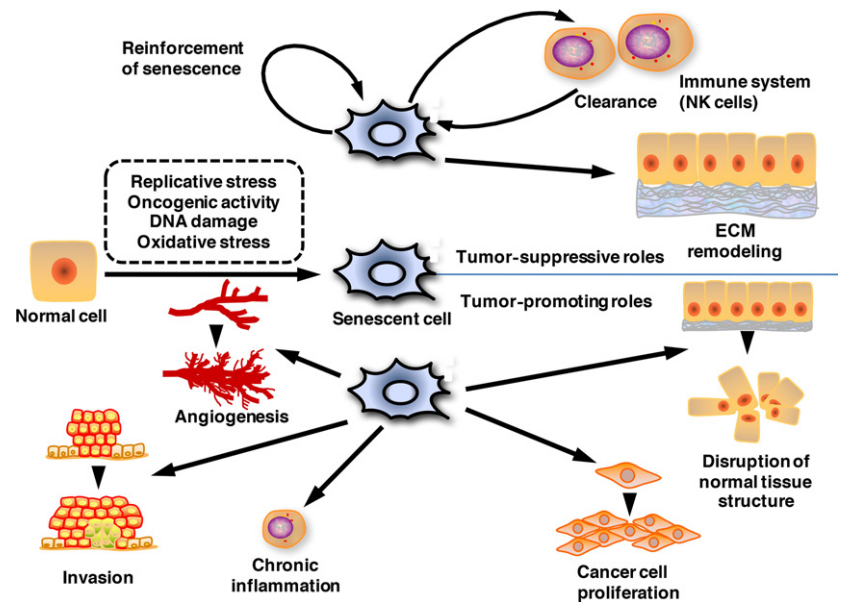
profound role of senescence in diverse biological processes including cancer is evident, the molecular basis underlying the induction of senescence and maintenance of senescent phenotypes has remained elusive. We will discuss this subject in the present review.

Senescence-inducing stimuli

Senescence is now believed to be induced by a diversity of stimuli. The limited proliferative potential of animal cells is generally well-understood to be a consequence of telomere shortening.[23] Progressive telomere shortening ultimately exposes an uncapped DSB that triggers persistent activation of a DDR. An uncapped DSB at a telomere end likely recruits ataxia telangiectasia mutated, which in turn phosphorylates and activates p53.[24] DNA damage across the genome induced by various genotoxic stressors such as ionizing radiation, UV radiation, and oxidative agents as well as chemotherapeutic agents also activates DDR and consequently upregulates p53.[25] The activated p53 then transcriptionally induces pleiotropic factors, such as p21, which collaboratively regulate senescence processes, although induction of some of senescence-associated genes by treatment with doxorubicin appears to be p53-independent.[26]

In addition to canonical DNA damage, oncogene activation or DNA replication stress by overexpression of cyclins also triggers senescence induction. For example, expression of oncogenic mutated Ras (Ras^{v12}) in primary fibroblasts results in p53- and p16-dependent permanent cell cycle arrest, a phenotype morphologically and functionally indistinguishable from replicative senescence.[27] Oncogene activation likely results in reactive oxygen species or in hyperactivation of DNA replication, leading to a DDR, and consequently, p53 activation.

Retinoblastoma 1 (pRb) inactivation mediated by p16 is also known to ensure durable cell cycle arrest, but is unlikely to be regulated by a canonical DNA damage response. Rather, it is stimulated by other stress mechanisms including the p38–MAPK pathway.[28] In contrast, p16 is markedly suppressed by Polycomb-group genes such as *Bmi-1* whose deficiency results in premature senescence and whose overexpression allows for immortalization.[29] Collectively, the p16–pRb pathway can

function in most forms of senescence either alone or in combination with the p53–p21 pathway in a manner dependent on the stressor or cell type.

Induction of senescence independent of p53 and p16 has also been reported. For example, Raf-1-induced growth arrest in human mammary epithelial cells seems to be independent of the presence of functional p16 or the inactivation of p53 and pRb.[30] Senescence induction in the absence of p16 and p53 is of great interest; however, the underlying mechanisms remain elusive.

Mechanisms of senescence induction

A prevailing model suggests that all senescence-inducing stimuli ultimately activate a DNA damage response, which in turn activate both checkpoint kinase–p53 pathways and the p38–MAPK pathway.[31–33] Activated p53 transcriptionally upregulates p21, which in turn suppresses cyclin-dependent kinase 2 (Cdk2)-mediated pRb inactivation, consequently preventing S-phase entry. The p38–MAPK pathway upregulates p16, which prevents Cdk4- or Cdk6-mediated pRb inactivation. This model therefore proposes that in senescent cells, the cell cycle is arrested at G_1 through pRb-mediated inhibition of E2F-dependent transcription. Although the observation that both p53 and pRb family pocket proteins are essential for senescence induction supports this model,[34] the fact that mere activation of p53 and pRb are insufficient for senescence induction argue against it. Therefore, the specific mechanisms and the phase at which senescent cells exit the cell cycle are as yet unclear.

To determine at which phase senescent cells exit the cell cycle, we first used fluorescent ubiquitination-based cell cycle indicator-based live-cell imaging analysis[35] during senescence induction. Interestingly, most human diploid cells treated with several senescence-inducing stimuli showed degradation of the S/G_2 phase indicator, and accumulation of the G_1 phase indicator without entry into mitosis.[36] These results suggest that cells treated with senescence-inducing stimuli undergo mitotic skipping and show tetraploidy at G_1 phase before entering into durable states of proliferative arrest. Similarly, p21-mediated inhibition of Cdk1 and Cdk2 at G_2 phase was proposed to prematurely activate the anaphase-promoting complex/cyclosome

$(APC/C)^{Cdh1}$ to degrade various APC/C substrates, resulting in long-term growth arrest at G_2 phase in the response to DNA damage.[37,38] In addition, the $4N$ genome is also apparent in multiple cell types including fibroblasts passaged extensively *in vitro* as well as in $p16^{Ink4a}$-expressing satellite cells collected from skeletal muscle of aged mice.[39,40] Very interestingly, we also found that human melanocytic nevi with BRAF mutations, a typical feature of senescence *in vivo*,[41] were tetraploid G_1 cells, and these cells presumably resulted from mitotic skipping *in vivo*.[36]

We then attempted to elucidate the underlying mechanism. We first explored changes in the expression of the proteins involved in regulation of G_2/M transition. Surprisingly, all the proteins required for mitotic entry that were tested had almost completely disappeared at a point that coincided with mitotic skipping, suggesting that a loss of mitotic proteins is the major cause of cells evading mitosis. This loss of mitotic regulators as well as mitotic skipping are dependent on functional p53. Most notably, we found that only transient expression of p53 at G_2, but not at G_1 or S phase, is sufficient for a mitosis skip and subsequent induction of senescence. Interestingly, transient induction of p21 at G_2 failed to induce senescence, although it prematurely activated APC^{Cdh1}, which degrades various proteins involved in mitotic initiation at G_2 phase, functioning as a factor in mitotic skipping as reported previously.[37,38]

In addition, p53 suppressed transcription of mitotic regulators through inactivation of pRb family pocket proteins (pRb, p107, and p130).[42] Finally, transient expression of both constitutively active forms of cadherin 1 CDC20 homologue 1 (Cdh1) and pRb at G_2 induced mitotic skipping and senescence independent of p53. Taken together, we conclude

that activation of p53 at G_2 is a critical determinant of senescence induction through its premature activation of APC^{Cdh1} and inactivation of pRb family pocket proteins (Fig. 2).[37] As p53 activation in p53-deficient tumor *in vivo* can produce complete tumor regression through induction of senescence and their clearance by immune responses,[43] collaborative activation of both APC^{Cdh1} and pRb pathways in cancer cells might be a cue to develop an innovative therapeutic method for p53-deficient cancer.

Mechanism of senescence maintenance

A recent body of evidence has led to the proposition that senescence is a dynamic and multistep process.[44] The properties of senescent cells gradually evolve during each process, although durable arrest of cell proliferation is an essential feature. The evolving of senescence involves profound transcriptional changes. Implicated in these changes is a conspicuous subset of genes involved in the SASP. A change in the expression level of p53 during the senescence process is likely a critical event in evolving such properties because p53 has antagonistic effects on the SASP at least in part by suppressing the activity of p38–MAPK, which is required for nuclear factor-κB activation.[45] This notion is supported by the observation that $p53^{+/-}$ mouse embryonic fibroblasts enter into a senescence-like state in spite of loss of the residual wild-type allele during long culture,[46] suggesting that p53 might not be required for the evolving of senescence once cells skip mitosis. These findings suggest that establishment of an SASP requires coordinated suppression of p53 during the senescence process.
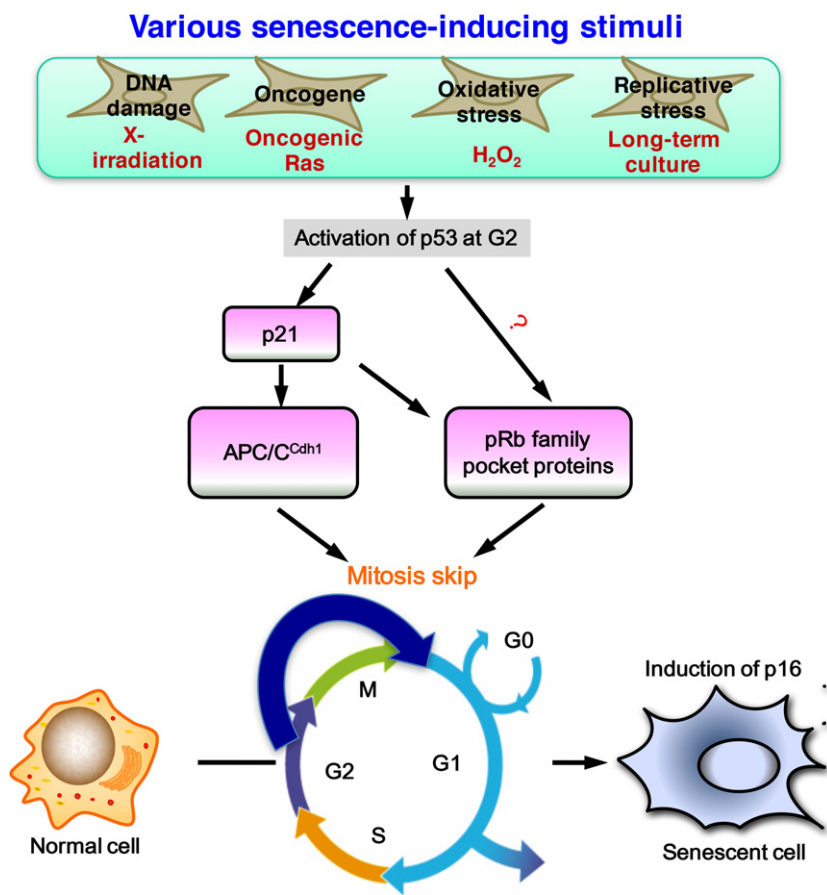

Various senescence-inducing stimuli

Fig. 2. Schematic model for molecular pathways regulating a mitosis skip. Cells exposed to various senescence-inducing stimuli undergo a mitosis skip before entry into a durable arrest of proliferation. This skip is mediated by p53 activation at G_2 that promotes both anaphase-promoting complex/cyclosome $(APC/C)^{Cdh1}$-dependent degradation of mitotic regulators and their retinoblastoma 1 (pRb) family pocket protein-dependent transcriptional suppression. This p53-dependent mitosis skip is necessary and sufficient for senescence induction.
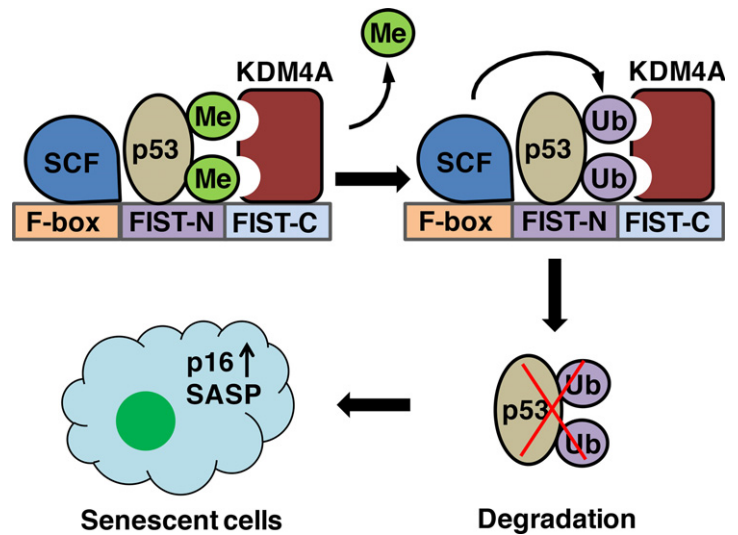
Fig. 3. Schematic representation of SCF^{fbxo22}_lysine-specific demethylase 4A (KDM4A)-dependent degradation for methylated (Me) p53 in senescence. F-box only protein 22 (Fbxo22) is highly expressed in senescent cells in a p53-dependent manner. SCF^{Fbxo22} forms a complex simultaneously with p53 and KDM4A through FIST-N and FIST-C domains on Fbxo22. SCF^{Fbxo22}–KDM4A-mediated ubiquitylation (Ub) and degradation of methylated p53 is essential for the induction of senescence-associated secretory phenotype (SASP) and p16 senescent cells.
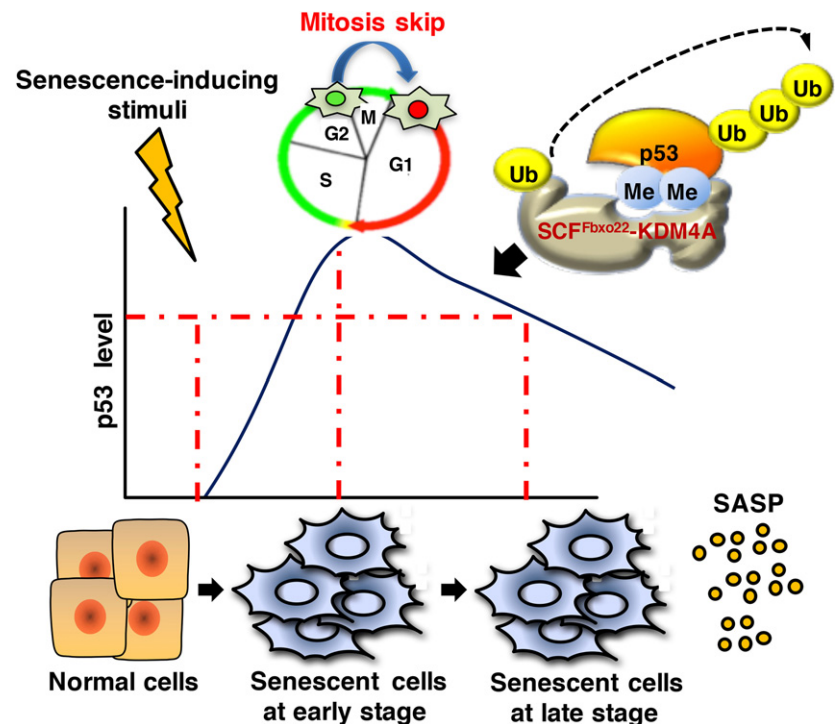

Fig. 4. Proposed model for induction and maintenance of senescence by fine-tuned regulation of p53. On activation of a DNA damage response by a senescence stimulus, the level and activity of p53 abruptly increase at G_2 phase, leading to a mitosis skip and subsequent durable arrest of proliferation (early stage of senescence). Activated p53 then transcriptionally induces F-box only protein 22 (Fbxo22), and the SCF^{Fbxo22}_lysine-specific demethylase 4A (KDM4A) complex ubiquitylates (Ub) methylated (Me) p53, leading to its degradation. Downregulation of p53 is essential for the induction of senescence-associated secretory phenotype (SASP) and p16 in senescent cells (late stage of senescence).

We found that F-box only protein 22 (Fbxo22), a less-characterized F-box protein, is highly expressed in senescent cells based on microarray analysis.[47] The induction of Fbxo22 on exposure of a cell to senescence-inducing stimuli is p53-dependent but occurs at a relatively late phase of senescence. The SCF–Fbxo22 complex was found to ubiquitylate p53 in vitro. In addition, Fbxo22 depletion and overexpression in cells resulted in an increase and decrease in the level of p53, respectively. Although most F-box proteins are reported to interact with substrates through a phosphorylation-dependent degron,[48] Fbxo22 interacts with p53 through a carboxyl-terminal domain (CTD) on the p53 protein[49–51] in a phosphorylation-independent manner. Conversely, Fbxo22 binds to p53 and lysine-specific demethylase 4A (KDM4A) through its FIST-N and FIST-C domains, respectively. These findings prompted us to speculate that Fbxo22 acts in concert with KDM4A to couple the demethylation and ubiquitination of p53 for a selective degradation.

Hence, the depletion of Fbxo22 specifically would increase the level of K370-dimethylated p53 but not that of the acetylated version. Importantly, Fbxo22 knockout mice were smaller with almost half the body weight of their control littermates, reminiscent of the phenotype of knockout mice for Skp2, an E3 ubiquitin ligase for CDK inhibitor p27.[52] Analyses of the p53 protein level of these mice showed that p53 was markedly accumulated in all tissues tested. Considered together, we conclude that the SCF–Fbxo22–KDM4A complex is a novel bona fide E3 ubiquitin ligase for methylated p53. This SCF–Fbxo22–KDM4A-mediated degradation of methylated p53 is necessary for SASP as well as p16 induction in durably arrested cells (Figs 3,4). It should be noted that PHF finger protein 20 (PHF20) binds to doubly K370/K382-dimethylated p53, stabilizing and activating this protein.[53] Depletion of Fbxo22 or KDM4A enhanced the binding of p53 to PHF20 in a methylation-dependent manner. Taken together, SCF–Fbxo22–KDM4A-mediated degradation of

methylated p53 is competed by acetylation of p53 CTD and its interaction with PHF20 (Fig. 3).[47]

Future directions

We identified a "mitosis skip" as an essential part of senescence induction, generating tetraploid G_1 cells with one nucleus as a key event. However, the molecular basis underlying the irreversible cell cycle arrest found in tetraploid G_1 cells remains elusive. Given that p16 is a prerequisite for suppressing DNA replication in tetraploid G_1 cells,[36] elucidation of the regulatory mechanisms underlying the transcriptional induction of p16, especially in the context of the high-order chromatin structure, could provide important clues to understanding the inability of senescent cells to proliferate in response to any form of mitotic signaling.

Several lines of evidence strongly suggest that factors controlling the level and timing of p53 activation at G_2 phase play a principal role in senescence induction. In this respect, most progeroid syndrome patients show a mutation in the genes responsible for specific DNA repair systems[54] and inefficient DNA repair likely increases the duration of G_2 checkpoint activation, leading to an increase in populations of G_2 cells. Therefore, investigation of the relationship between the duration of G_2 checkpoint activation and senescence sensitivity in progeroid patients will provide clues for understanding the rational basis for the pathogenesis of progeroid syndromes. Although the role of senescence in aging-associated phenotypes *in vivo* is still not clear, involvement of p53 function in organismal aging has already been proposed by the observation that augmentation of p53 response in mice showed early aging-associated phenotypes.[55] Thus, fine-tuned regulation of p53 activity likely plays a critical role in organismal aging and lifespan.

Disclosure Statement

The authors declare have no conflict of interest.

References

1 Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961; **25**: 585–621.
2 Röhme D. Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts in vitro and erythrocytes in vivo. *Proc Natl Acad Sci USA* 1981; **78**: 5009–13.
3 de Jesus BB, Blasco MA. Assessing cell and organ senescence biomarkers. *Circ Res* 2012; **111**: 97–109.
4 Paradis V, Youssef N, Dargere D *et al.* Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. *Hum Pathol* 2001; **32**: 327–32.
5 Going JJ, Stuart RC, Downie M, Fletcher-Monaghan AJ, Keith WN. "Senescence-associated" beta-galactosidase activity in the upper gastrointestinal tract. *J Pathol* 2002; **196**: 394–400.
6 Minamino T, Yoshida T, Tateno K *et al.* Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. *Circulation* 2003; **108**: 2264–9.
7 Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005; **120**: 513–22.
8 Jeyapalan JC, Sedivy JM. Cellular senescence and organismal aging. *Mech Ageing Dev* 2008; **129**: 467–74.
9 Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 2002; **105**: 1541–4.
10 Castro P, Giri D, Lamb D, Ittmann M. Cellular senescence in the pathogenesis of benign prostatic hyperplasia. *Prostate* 2003; **55**: 30–8.
11 Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science* 2006; **311**: 1257.
12 Baker DJ, Wijshake T, Tchkonia T *et al.* Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 2011; **479**: 232–6.
13 Baker DJ, Childs BG, Durik M *et al.* Naturally occurring p16 Ink4a -positive cells shorten healthy lifespan. *Nature* 2016; **530**: 184–9.
14 Gorgoulis VG, Vassiliou L-VF, Karakaidos P *et al.* Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* 2005; **434**: 907–13.
15 Bartkova J, Horejsí Z, Koed K *et al.* DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* 2005; **434**: 864–70.
16 Bartkova J, Rezaei N, Liontos M *et al.* Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 2006; **444**: 633–7.
17 Di Micco R, Fumagalli M, Cicalese A *et al.* Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* 2006; **444**: 638–42.
18 DiTullio RA, Mochan TA, Venere M *et al.* 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat Cell Biol* 2002; **4**: 998–1002.
19 Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype : therapeutic opportunities. *J Clin Invest* 2013; **123**: 966–72.
20 Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs Cellular senescence and its effector programs. *Genes Dev* 2014; **28**: 99–114.
21 Muñoz-Espín D, Cañamero M, Maraver A *et al.* Programmed cell senescence during mammalian embryonic development. *Cell* 2013; **155**: 1104–18.
22 Demaria M, Ohtani N, Youssef S *et al.* An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell* 2014; **31**: 722–33.
23 Shay JW, Wright WE. Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis* 2005; **26**: 867–74.
24 Meek DW. Tumour suppression by p53: a role for the DNA damage response? *Nat Rev Cancer* 2009; **9**: 714–23.
25 Rufini A, Tucci P, Celardo I, Melino G. Senescence and aging: the critical roles of p53. *Oncogene* 2013; **32**: 5129–43.
26 Chang B-D, Swift ME, Shen M, Fang J, Broude EV, Roninson IB. Molecular determinants of terminal growth arrest induced in tumor cells by a chemotherapeutic agent. *Proc Natl Acad Sci USA* 2002; **99**: 389–94.
27 Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16(INK4a). *Cell* 1997; **88**: 593–602.
28 Wong ESM, Le Guezennec X, Demidov ON *et al.* p38MAPK controls expression of multiple cell cycle inhibitors and islet proliferation with advancing age. *Dev Cell* 2009; **17**: 142–9.
29 Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999; **397**: 164–8.
30 Olsen CL, Gardie B, Yaswen P, Stampfer MR. Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. *Oncogene* 2002; **21**: 6328–39.
31 Courtois-Cox S, Jones SL, Cichowski K. Many roads lead to oncogene-induced senescence. *Oncogene* 2008; **27**: 2801–9.
32 Adams PD. Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. *Mol Cell* 2009; **36**: 2–14.
33 Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. *Genes Dev* 2010; **24**: 2463–79.
34 Shay JW, Pereira-Smith OM, Wright WE. A role for both RB and p53 in the regulation of human cellular senescence. *Exp Cell Res* 1991; **196**: 33–9.
35 Sakaue-Sawano A, Kurokawa H, Morimura T *et al.* Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* 2008; **132**: 487–98.

36 Johmura Y, Shimada M, Misaki T *et al.* Necessary and sufficient role for a mitosis skip in senescence induction. *Mol Cell* 2014; **55**: 73–84.

37 Gire Â, Baus F, Fisher D, Piette J. Permanent cell cycle exit in G2 phase after DNA damage in normal human fibroblasts. *EMBO J* 2003; **22**: 3992–4002.

38 Wiebusch L, Hagemeier C. p53- and p21-dependent premature APC/C-Cdh1 activation in G2 is part of the long-term response to genotoxic stress. *Oncogene* 2010; **29**: 3477–89.

39 Martínez P, Blasco MA. Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins. *Nat Rev Cancer* 2011; **11**: 161–76.

40 Sousa-Victor P, Gutarra S, García-Prat L *et al.* Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* 2014; **506**: 316–21.

41 Michaloglou C, Vredeveld LCW, Soengas MS *et al.* BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005; **436**: 720–4.

42 Barsotti AM, Prives C. Pro-proliferative FoxM1 is a target of p53-mediated repression. *Oncogene* 2009; **28**: 4295–305.

43 Xue W, Zender L, Miething C *et al.* Senescence and tumor clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007; **445**: 656–60.

44 van Deursen JM. The role of senescent cells in ageing. *Nature* 2014; **509**: 439–46.

45 Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* 2011; **30**: 1536–48.

46 Harvey M, Sands AT, Weiss RS *et al.* In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* 1993; **8**: 2457–67.

47 Johmura Y, Sun J, Kitagawa K *et al.* SCF(Fbxo22)-KDM4A targets methylated p53 for degradation and regulates senescence. *Nat Commun* 2016; **7**: 10574.

48 Cardozo T, Pagano M. The SCF ubiquitin ligase: insights into a molecular machine. *Nat Rev Mol Cell Biol* 2004; **5**: 739–51.

49 Tang Y, Zhao W, Chen Y, Zhao Y, Gu W. Acetylation is indispensable for p53 activation. *Cell* 2008; **133**: 612–26.

50 Huang J, Perez-Burgos L, Placek BJ *et al.* Repression of p53 activity by Smyd2-mediated methylation. *Nature* 2006; **444**: 629–32.

51 Marouco D, Garabadgiu A V, Melino G, Barlev NA. Lysine-specific modifications of p53: a matter of life and death? *Oncotarget* 2013; **4**: 1556–71.

52 Nakayama K, Nagahama H, Minamishima YA *et al.* Targeted disruption of Skp2 results in accumulation of cyclin E and p27(Kip1), polyploidy and centrosome overduplication. *EMBO J* 2000; **19**: 2069–81.

53 Cui G, Park S, Badeaux AI *et al.* PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53. *Nat Struct Mol Biol* 2012; **19**: 916–24.

54 Freitas AA, De Magalhães JP. A review and appraisal of the DNA damage theory of ageing. *Mutat Res* 2011; **728**: 12–22.

55 Tyner SD, Venkatachalam S, Choi J *et al.* p53 mutant mice that display early ageing-associated phenotypes. *Nature* 2002; **415**: 45–53.