Review Article

Roles and mechanisms of cellular senescence in regulation of tissue homeostasis

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Cellular senescence is the state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has therefore long been considered to suppress tumorigenesis, acting as a guardian of homeostasis. However, surprisingly, emerging evidence reveals that senescent cells also promote secretion of a series of inflammatory cytokines, chemokines, growth factors and matrix remodeling factors, which alter the local tissue environment and contribute to chronic inflammation and cancer. This newly identified senescence phenotype, termed the senescence-associated secretory phenotype (SASP) or the senescence-messaging secretome (SMS), is induced by DNA damage that promotes the induction of cellular senescence. All of these senescence-associated secreting factors are involved in homeostatic disorders such as cancer. Therefore, it is quite possible that accumulation of senescent cells during the aging process *in vivo* might contribute to age-related increases in homeostatic disorders. In this review, current knowledge of the molecular and cellular biology of cellular senescence is introduced, focusing on its positive and negative roles in controlling tissue homeostasis *in vivo*. (*Cancer Sci* 2013; 104: 525–530)

Higher eukaryotes, including humans, fulfill their life cycles with embryogenesis, birth, growth, maturity and aging. Human life expectancy is very long, since each life stage is expanded, compared with those of other higher eukaryotes. The major cause for longer human life is thought to be the precise mechanisms of cellular and organismal homeostatic control.[1] However, the extended human lifespan could result in a startling rise in the incidence of cancer in later life.[2] To meaningfully impact the healthcare and the well-being of this aging population, there is an urgent need for enhanced understanding of the molecular mechanisms maintaining cellular homeostasis and the consequences of its disruption, which might increase aging-associated cancer. One of the emerging mechanisms for maintaining cellular homeostasis is "cellular senescence". Cellular senescence is the state of essentially irreversible cell cycle arrest induced by a variety of potentially oncogenic stimuli, such as telomere erosion, oxidative stress or activation of certain oncogenes, and is considered to act as an important tumor suppression mechanism.[3–8] Thus, cellular senescence has long been considered a fail-safe mechanism against carcinogenesis by maintaining cellular and organismal homeostasis. However, recently it has been suggested that senescent cells have the potential to secrete various inflammatory cytokines, chemokines and matrix remodelling factors that deleteriously alter tissue homeostasis, leading to chronic inflammation and ⁄ or cancer promotion.[8,9] Therefore, it is quite possible that the accumulation of senescent cells *in vivo* might contribute to age-related increases in cancer and other aging-associated illnesses as well. In this review, the molecular events associated with these two faces of cellular senescence are discussed.

What is cellular senescence?

Most normal human somatic cells can divide only a limited number of times in culture. They permanently stop dividing after a finite number of cell divisions and enter a state of irreversible cell proliferation arrest.[10,11] This phenomenon is called "cellular senescence" or "replicative cellular senescence". Unlike quiescent (G0 phase) cells, which are induced by low serum or contact inhibition conditions, senescent cells are irreversibly arrested predominantly in the G1 phase and are no longer able to divide even with proliferative stimuli, although they remain viable and metabolically active for long periods of time (Fig. 1).[3–8] In contrast, most cancer cells appear to have bypassed this proliferative limit and evaded cellular senescence.[3–8] Thus, cellular senescence has long been considered as a barrier to cancer by playing an important role in preventing the extensive cell divisions required for malignant transformation.[3–8]

In human cells, the mechanism underlying replicative cellular senescence is thought to be telomere shortening. Telomeres are located at the ends of eukaryotic chromosomes and they have special DNA structures with repetitive DNA elements to protect the DNA ends from degradation and ⁄ or chromosomal end-to-end fusion.[12] The telomere length is maintained by a specific enzyme called telomerase, which is expressed in human germ-line cells or stem cells, but not in most normal somatic cells. Due to the nature of the DNA replication process and the lack of telomerase, telomeres become shorter with each round of cell division.[13] Eventually, progressive telomere erosion results in telomere dysfunction and this is thought to initiate DNA damage response signals to activate p53-dependent checkpoints that contribute to cellular senescence.[12,14]

In contrast to human cells, there is no strong evidence that rodent cells undergo replicative cellular senescence by telomere erosion,[15,16] because the telomeres in rodent cells prepared from most laboratory animals are quite long and many somatic rodent cells have telomerase activity. However, the senescence-like proliferative block in rodent cells occurs without detectable telomere shortening. This phenomenon suggests that a mechanism other than telomere shortening can cause cellular senescence in rodent cells. In this regard, it is interesting to note that primary mouse embryonic fibroblasts

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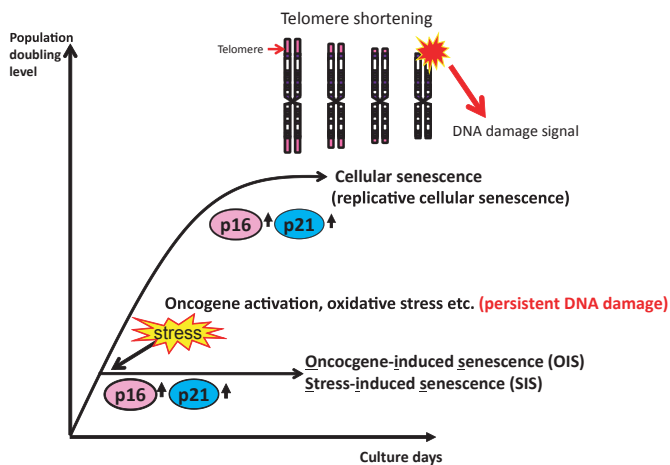E-mail: naoko.ohtani@jfcr.or.jp

Fig. 1. What is cellular senescence? Most normal human somatic cells stop dividing after a finite number of cell divisions and enter a state of irreversible cell proliferation arrest. This phenomenon is called "cellular senescence" or "replicative cellular senescence". In human cells, the mechanism underlying replicative cellular senescence is thought to be telomere shortening. Recent studies in human cells revealed that a similar irreversible proliferation block can be induced quite rapidly when normal cells are exposed to a variety of potentially oncogenic stimuli, such as excessive levels of reactive oxygen species, treatment with DNA damaging agents or activation of certain oncogenes. Because all of these stimuli induce irreparable DNA damage in both human and murine cells, it is plausible that persistent DNA damage signals induce cellular senescence. These types of oncogenic stress-induced senescence are now referred to as "oncogene-induced senescence" or "stress-induced senescence". Both p16 and p21 upregulation contributes to the induction of both types of cellular senescence.

reportedly proliferate indefinitely if the cells are maintained under appropriate culture conditions, such as low oxygen conditions.[17] In addition, rat oligodendrocyte precursor cells and rat Schwann cells do not senesce in serum-free medium, but the presence of serum induces senescence.[18] These findings clearly demonstrate that cellular senescence can be induced without apparent telomere shortening when cells are exposed to non-physiological conditions *in vitro*. Notably, the fact that anti-oxidant treatment delays cellular senescence induction strongly suggests that reactive oxygen species (ROS) can trigger cellular senescence.[1,2,8] Recent studies in human cells revealed that a similar irreversible proliferation block can be induced quite rapidly when normal cells are exposed to a variety of potentially oncogenic stimuli, such as excessive levels of ROS, treatment with DNA damaging agents or activation of certain oncogenes.[3–8,19–21] Because all of these stimuli induce irreparable DNA damage in both human and murine cells, it is plausible that persistent DNA damage signals induce cellular senescence. These types of oncogenic stress-induced senescence are now referred to as "oncogene-induced senescence" (OIS) or "stress-induced senescence" (SIS) (Fig. 1).

Molecular machinery of cellular senescence for tumor suppression

The basic mechanisms for the induction of senescence cell cycle arrest have been well documented recently.[22,23] In mammalian cells, the RB and p53 tumor suppressor proteins play critical roles in the induction of cellular senescence.[22,23] In particular, RB and its family members, p107 and p130, are essential for the onset of senescence cell cycle arrest.[24–27] The activities of RB-family proteins are precisely regulated by phosphorylation, protein–protein interactions and other protein modifications.[28,29] A series of cyclin-dependent kinases

(CDK) including CDK2, CDK4 and CDK6, play key roles in regulating the activities of RB-family proteins.[30] When RB is phosphorylated by these CDK, it loses its ability to bind to and repress the functions of the E2F family of transcription factors, thereby resulting in gene transcription required for the initiation of DNA replication and cell cycle progression (Fig. 2).[28–30]

Senescent cells arrest themselves in the G1 phase of the cell cycle and are no longer able to replicate by synthesizing DNA. In many cases, this is because the CDK are inactivated in senescent cells by CDK inhibitors (CDKI).[30] There are two different classes of CDKI, the KIP/CIP family CDKI (p21^{Cip1}, p27^{Kip1} and p57^{KipII}) and the INK4 family CDKI (p16^{Ink4a}, p15^{Ink4b}, p18^{Ink4c} and p19^{Ink4d}). The KIP/CIP family members are known to inhibit a broad range of CDK, whereas the INK4 family proteins specifically bind and inactivate CDK4 and CDK6.[30] In normal proliferating cells, the expression levels of CDKI are very low.[31–33] However, in response to irreparable DNA damage caused by a variety of oncogenic stimuli, expression levels of the *p21^{Cip1}* and *p16^{Ink4a}* genes are significantly upregulated by p53-dependent and p53-independent mechanisms, respectively.[31–35] In addition, simultaneous induction of p21^{Cip1} and p16^{Ink4a} cooperatively and efficiently inactivates all CDK that phosphorylate RB-family proteins,[36–38] thereby causing senescence cell cycle arrest (Fig. 2). It is noteworthy that the senescence pathway including p16^{Ink4a}, CDK and RB are highly deregulated in nearly all human cancers,[39] illustrating the importance of the p16/RB pathway in tumor suppression through senescence induction.

Once RB is fully activated, particularly by p16^{Ink4a} expression, senescence cell cycle arrest becomes irreversible and is no longer revoked by the subsequent inactivation of RB and p53 in human somatic cells.[40] Interestingly, inactivation of RB and p53 enables human senescent cells to reinitiate DNA synthesis, but they subsequently fail to complete the cell cycle, suggesting that there might be additional block(s) in the G2 and/or M phase.[40–42] We have previously shown that activation of the p16^{Ink4a}–Rb pathway cooperates with mitogenic signals to induce elevated intracellular levels of reactive oxygen species (ROS), thereby causing activation of PKCδ, a critical downstream mediator of the ROS signaling pathway and leading to a cytokinetic block.[43] Moreover, once it is activated by ROS, PKCδ in turn promotes further production of ROS, thus establishing a positive feedback loop to maintain ROS–PKCδ activation in senescent cells.[43] Sustained activation of ROS–PKCδ signaling irreversibly blocks cytokinesis, at least partly by reducing the level of LATS1 (also known as WARTS), a mitotic exit network kinase essential for cytokinesis.[43] Moreover, p21^{Cip1} is also required for autonomous ROS production in senescent cells,[44] indicating that both p21^{Cip1} and p16^{Ink4a} play critical roles in inhibiting not only DNA replication but also cytokinesis in senescent cells. These lines of evidences suggest that the cytokinetic block might provide an additional safeguard against the proliferation of senescent cells, especially if RB and p53 subsequently become inactivated (Fig. 2).

Risk of chromosomal instability in senescent cells

As mentioned above, we have shown that an increased level of ROS and activation of ROS–PKCδ signaling in senescent cells causes the degradation of LATS1. The reduction of LATS1 in senescent cells could contribute to blocking cytokinesis of cells. However, importantly, the excess level of ROS is a well-known DNA damaging agent[45] and LATS1 is reported to have a tumor suppressive role.[46] Moreover, the cytokinetic block is known to cause aneuploidy, which is associated with chromosomal instability, a hallmark of tumorigenesis.[47]

Fig. 2. Molecular mechanisms of cellular senescence. A series of cyclin-dependent kinases (CDK) including CDK2, CDK4 and CDK6 play key roles in regulating the activities of RB-family proteins. When RB is phosphorylated by these CDK, it loses its ability to bind to and repress the functions of the E2F family of transcription factors, thereby resulting in gene transcription required for the initiation of DNA replication and cell cycle progression. In response to irreparable DNA damage caused by a variety of oncogenic stimuli, expression levels of the $p21^{Cip1}$ and $p16^{Ink4a}$ CDK inhibitors are significantly upregulated by p53-dependent and p53-independent mechanisms, respectively. Activation of the $p16^{Ink4a}$–Rb pathway cooperates with mitogenic signals to induce elevated intracellular levels of reactive oxygen species (ROS), thereby causing activation of PKCδ, a critical downstream mediator of the ROS signaling pathway, and leading to a cytokinetic block that might provide an additional safeguard against the proliferation of senescent cells, especially in the case of RB and p53 subsequently become inactivated.
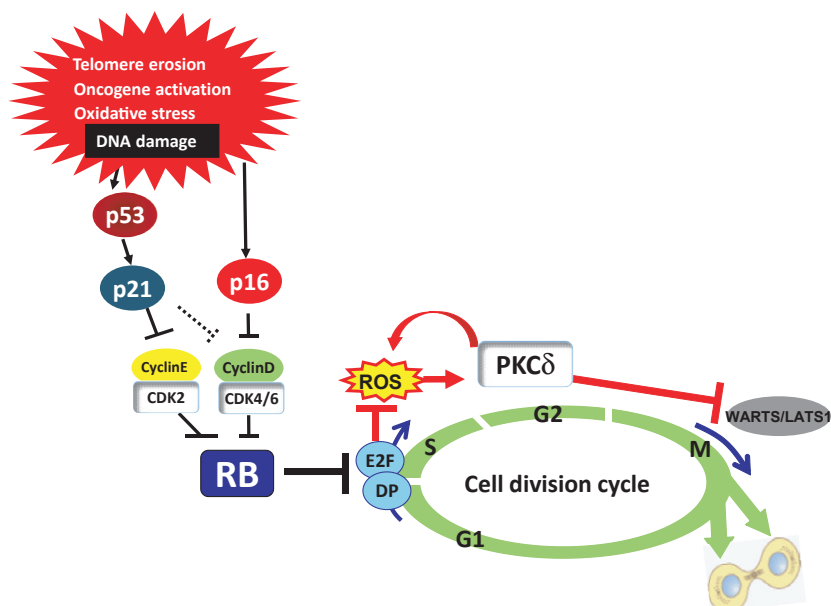
It is also worthwhile emphasizing that another mechanism linking cellular senescence and aneuploidy has recently emerged, which is associated with the accumulation of telomere damage in stress-induced senescence.[48,49] Intriguingly, since DNA damage in the telomeric sequence resists repair, it has been reported that a large fraction of exogenously induced DNA damage tends to be accumulated in telomeres.[48] Moreover, this DNA damage in telomeres is shown to be irrespective of telomere length or telomerase activity both *in vitro* and *in vivo* and is correlated with age.[48,49] In addition, Davoli *et al.*[50] have recently shown that telomere damage has the potential to induce the bypass of mitosis through APC/C^Cdh1-dependent degradation of Geminin, thereby resulting in whole genome reduplication and tetraploidy. Furthermore, they showed that the tetraploidization driven by telomere damage, together with inactivation of RB and/or p53 tumor suppressors, enhanced the tumorigenic transformation in mouse cells that resembled human aneuploid cancer cells.[51] These findings strongly suggest that the "persistent" DNA damage of telomeres observed in senescent cells could cause cancerous aneuploidization.

It is well known that aneuploidy is a major cause of the transformation and progression of most human cancers. It is therefore possible that many of the aneuploid senescent cells could transform into malignant cancer cells. Indeed, it has been reported that tumorigenic cells spontaneously emerged from senescent cells.[52] These notions imply that although the onset of cellular senescence initially acts as a barrier to cancer, accumulation of senescent cells might eventually promote

tumorigenesis in later life, contributing to an age-related increase in cancer.

Senescence-associated secretome

There is no doubt that cellular senescence functions as an important tumor suppression mechanism. However, unlike apoptotic cells, senescent cells remain viable for long periods of time and accumulate with age in various organs and tissues. Indeed, we and others confirmed that senescent cells with high p16 expression accumulate in various aged organs (Fig. 3),[53,54] with accumulation of the DNA damage markers. Of particular biological importance, it has recently become apparent that senescent cells exhibit increased expression of genes encoding a series of secreted proteins, such as inflammatory cytokines, chemokines and matrix remodeling factors, which alter the local tissue environment and/or contribute to chronic inflammation and tumorigenesis.[55–57] This newly identified senescent phenotype, termed the senescence-associated secretory phenotype (SASP) or the senescence-messaging secretome (SMS)[57,58] (hereafter referred to as senescence-associated secretome), is observed in both replicative and oncogene-and/or stress-induced cellular senescence.[57] The senescence-associated secretome is known to be induced by DNA damage[59] and can be beneficial or deleterious, depending on the biological context (Fig. 4).[8,9] Although the mechanisms of senescence-associated secretomes have been investigated mainly using normal fibroblasts,[55–57] a series of papers reported that the secretome by cellular senescence is
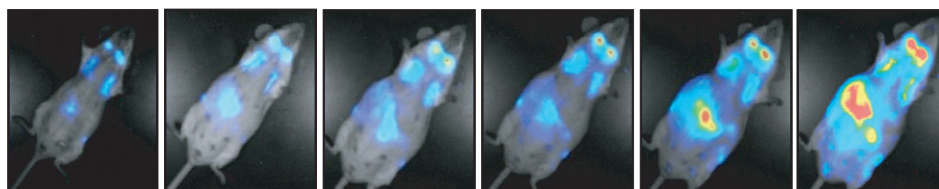

Fig. 3. Dynamics of *p16 Ink4a* gene expression during the aging process *in vivo*. The p16-luc mice, an imaging mice for p16 gene expression *in vivo*, were subjected to non-invasive bioluminescence imaging throughout their entire life span. Accumulation of p16 was confirmed in the aged mouse.
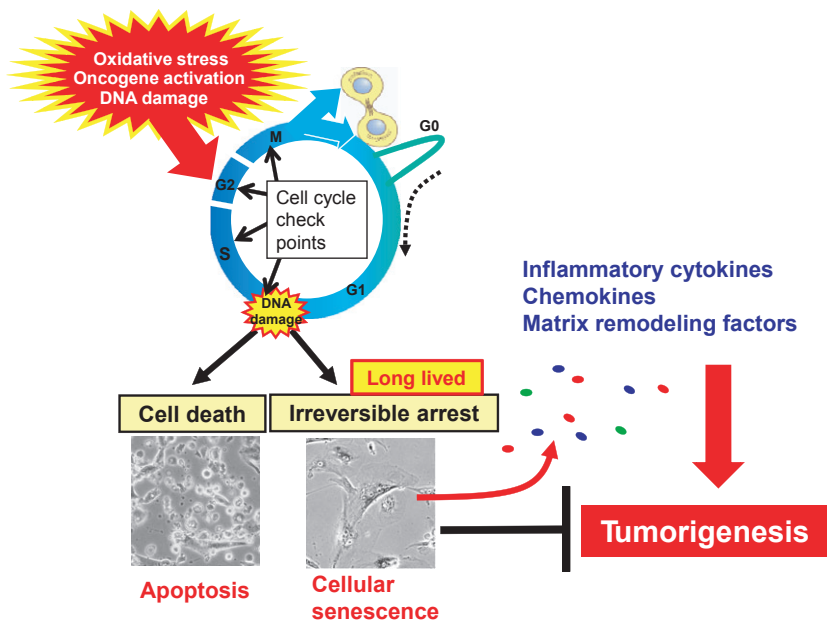
Fig. 4. Senescence-associated secretome. Irreparable DNA damage provokes either apoptosis or cellular senescence depending on the strength of stress and ∕or cellular context. Recently it has become apparent that long-lived senescent cells exhibit increased expression of genes encoding a series of secreted proteins, such as inflammatory cytokines, chemokines and matrix remodeling factors, which alter the local tissue environment and∕or contribute to chronic inflammation and tumorigenesis. Cellular senescence initially prevents proliferation of damaged cells, thereby acting as a fail-safe mechanism. However, in the long term, senescent cells might eventually promote tumorigenesis by accelerating chromosomal instability and∕or production of senescence-associated secretome factors.

observed in other types of cells such as human melanocytes,[56] human endothelial cells[60] and human breast epithelial cells,[57] as well as cancerous cells such as human melanoma cells[61] and mouse lymphoma cells.[62]

Factors such as IL-6, IL-8 and plasminogen activator inhibitor-1 (PAI-1), which are secreted from senescent fibroblasts, can reportedly promote tumor suppression by reinforcing the senescence proliferation block induced by an activated oncogene or oxidative stress.[55,56,63] However, IL-6 and IL-8 are also known to promote malignant transformation in cooperation with certain oncogenes.[64–66] Moreover, secreted factors from senescent fibroblasts have been shown to induce an epithelial–mesenchymal transition, an important step in cancer progression and metastasis.[57] These findings, together with observations that the proteins secreted by senescent cells can promote degenerative hyper-proliferative or metastatic changes in neighboring cells,[57,61] indicate that the release of the senescence-associated secretome factors results in both beneficial and harmful consequences. Thus, these secreted factors can act in an autocrine manner to reinforce the senescence cell cycle arrest,[55,56,62,63] while in contrast they can have deleterious cell non-autonomous side-effects that promote tumorigenesis.[9,57,61,64–66] The senescence-associated secretome might also be involved in the effect of the microenvironment of cancer tissue. Indeed, the stromal fibroblasts of human ovarian cancer tissue were reportedly senescent cells and secreted a tumor-promoting chemokine,[67] implying that the senescence-associated secretome might promote human ovarian cancer. Therefore, considering the accumulation of senescent cells with age, it is possible that the deleterious side-effects of the senescence-associated secretome might also contribute to the age-related increase in cancer (Fig. 4).

Epigenetic regulation of the senescence-associated secretome

Both the DNA damage response (DDR) and the activation of NF-κB and C∕EBPβ transcription factors are reported to play key roles in the onset of the senescence secretome phenotype.[55–58,61,62,68–70] However, it remains unclear how these two events cooperate to establish senescence-associated secretome. Recently, we noted that the levels of histone 3 lysine 9 di-methylation (H3K9me2), an epigenetic mark for euchromatic gene silencing,[53,71–73] were strikingly reduced around the *IL-6* and *IL-8* gene promoters in senescent human diploid fibroblasts (HDF).[71] Moreover, we found that this phenomenon was due to the proteasomal degradation of G9a and GLP, the major H3K9 mono- and di-methyltransferases, through DDR-dependent activation of the APC∕C^{Cdh1} ubiquitin ligase.[71] It is also noteworthy that GLP has been shown to bind to the NF-κB subunit RelA and represses chromatin at RelA-occupied genes, such as the *IL-6* gene, by promoting H3K9 methylation.[74] Intriguingly, G9a has been shown to block the transcriptional activation potential of C∕EBPβ through the methylation of lysine-39 of C∕EBPβ.[75] All of these observations suggest that G9a∕GLP might regulate senescence-associated secretome factor gene expression through multiple mechanisms in response to persistent DNA damage in senescent cells.

Conclusions

It is clear that cellular senescence plays an important role in tumor suppression,[3–9] as illustrated by the increase in tumor incidence in mice with an engineered block in senescence induction.[38] Moreover, several lines of evidence suggest that senescent cells can facilitate tumor clearance in certain settings by promoting immunological responses *in vivo*.[76–78] Thus, with no doubt, cellular senescence has a positive contribution to organismal defences against cancer. However, sustained cellular senescence is also associated with the secretion of pro-inflammatory and pro-proliferative factors, as well as a cytokinesis block that might lead to chromosomal instability and DNA damage, which are phenomena associated with cancer. Although there is the idea that the facilitation of cellular senescence is an attractive method for cancer therapy, the harmful side-effects need to be seriously considered. Greater understanding of the molecular mechanisms linking cellular senescence and cellular homeostatic disorders will provide valuable new insights into their roles in the generation of age-associated cancers, the avoidance of undesirable side-effects and the development of novel therapeutic strategies for aging-associated diseases.

Disclosure statement

The authors have no conflict of interest.

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