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REVIEW ARTICLE

The emerging role of senescent cells in tissue homeostasis and pathophysiology

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Cellular senescence is a state of permanent growth arrest and is thought to play a pivotal role in tumor suppression. Cellular senescence may play an important role in tumor suppression, wound healing, and protection against tissue fibrosis in physiological conditions *in vivo*. However, accumulating evidence that senescent cells may have harmful effects *in vivo* and may contribute to tissue remodeling, organismal aging, and many age-related diseases also exists. Cellular senescence can be induced by various intrinsic and extrinsic factors. Both p53/p21 and p16/RB pathways are important for irreversible growth arrest in senescent cells. Senescent cells secret numerous biologically active factors. This specific secretion phenotype by senescent cells may largely contribute to physiological and pathological consequences in organisms. Here I review the molecular basis of cell cycle arrest and the specific secretion phenotype in cellular senescence. I also summarize the current knowledge of the role of cellular senescence *in vivo* in physiological and pathological settings.

Keywords: *cellular senescence*; *cell proliferation*; *senescence-associated secretory phenotype*; *inflammation*; *immortalization*; *age-associated diseases*

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After undergoing a certain number of divisions, normal human diploid fibroblasts enter an irreversible non-dividing state, termed replicative senescence. Hayflick et al. reported that normal human diploid fibroblasts can divide 50–60 times but after that, they stop dividing irreversibly (1,2). The number of divisions that cells completely reach at the end of the replicative lifespan has been termed as the Hayflick limit. Senescent cells show enlarged and flattened morphology and the formation of a TOR-autophagy spatial coupling compartment (TASCC) in the cytoplasm (3,4) and senescence-associated heterochromatin foci (SAHF) in the nucleus (5–10). Active staining of senescence-associated β-galactosidase (SA-β-gal) is commonly used as a marker for cellular senescence (11). Senescence has been reported to occur in a number of other cell types such as keratinocytes (12), melanocytes (13), endothelial cells (14), epithelial cells (15), glial cells (16), adrenocortical cells (17), T lymphocytes (18), and even tissue stem cells (19). Replicative senescence is not dependent on chronological time in culture but rather depends on the number of divisions that cells undergo in culture (20–23). It is thought that telomere shortening, which occurs at each cell division because of incomplete replication, is the counting mechanism for the induction of replicative senescence (22,24). Telomeres become critically short after extensive division, and telomere ends are recognized as DNA double-strand breaks (25–27). This activates a DNA damage response (DDR), and cell division is then arrested by this activated DDR, mainly through p53 tumor suppressor activity. The expression of cyclin-dependent kinase (CDK) inhibitors, p21 and p16, is upregulated in senescent cells (28–35). p21 and p16 inhibit CDK2 (36–38) and CDK4/6 activities (39), respectively, and result in the activation of the tumor suppressor Rb, which is inactivated by CDK2 and CDK4/6 through phosphorylation. Activated Rb forms a complex with the E2F transcription factor, which is important for DNA synthesis and S phase progression of the cell cycle, and inhibits the E2F activity in senescent cells. Therefore, senescent cells cannot enter the S phase of the cell cycle and are basically maintained at the G1 phase of the cell cycle (40–42). p21 expression is transcriptionally regulated by p53, another important tumor suppressor (43). The senescence arrest is established

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and maintained through two major tumor suppressor pathways, p53/p21 and p16/RB (44–47), and it is now thought to be a barrier to malignant transformation (Fig. 1a).

A similar irreversible, non-dividing state, called cellular senescence, can be induced by the exposure of cells to excessive extrinsic stressors. These stimuli include strong mitogenic signals such as overexpression of activated oncogenes, DNA-damaging agents, oxidative stress, disruption of epigenetic regulation, and ectopic expression of tumor suppressors (48–55). This is called stress-induced premature senescence (SIPS) (51). SIPS occurs independently of telomere shortening. Because there are many similarities when cells senesce in both cases, replicative senescence and SIPS, the term cellular senescence is commonly used to indicate both states of non-division.

In general, it has been thought that senescent cells were arrested in the G1 phase, although there was a report that replicatively senescent cells were arrested in both G1 and G2 phases (56). Recently, some groups have reported that p53 activation in the G2 phase in response to various senescence-inducing stimuli induces cellular senescence through mitosis skip (57–59). They elegantly used the FUCCI system and time-lapse live cell imaging and showed that these senescent cells are tetraploid (4N) but stay in the G1 phase of the cell cycle. This mitosis skip and senescence induction were mediated by p53-dependent premature activation of the anaphase-promoting complex/cyclosome and its coactivator Cdh1 (APC/C^{Cdh1}). Activation of p53 at the G2 phase in response to senescence-inducing stimuli resulted in the induction of p21 that inhibited CDK1 and CDK2 activities. This inhibition by p21 led to premature activation of APC/C^{Cdh1} and degradation of various mitotic regulators to skip mitosis (Fig. 1b). p16 was required for the maintenance of the senescent state but not for the induction of the mitosis skip. These results suggest that the p53-dependent cell fate is determined by the cell cycle stage in which p53 is activated.

Senescence-associated secretory phenotype

Another important characteristic of senescent cells is that the expression of many genes largely changes during

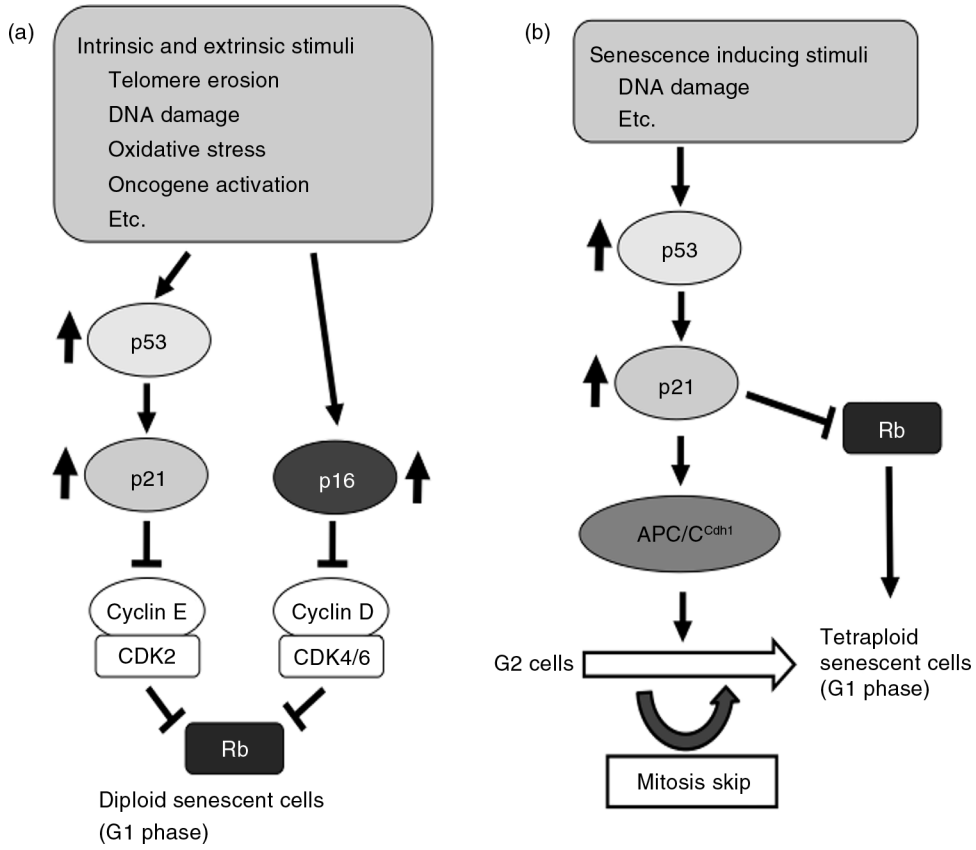

Fig. 1. Schematic diagram of cell cycle arrest in senescent cells. (a) Diploid senescent cells. In response to various intrinsic and extrinsic stimuli such as telomere erosion, DNA damage, oxidative stress, and activated oncogene overexpression, cells enter a senescent state. In senescent cells, CDK inhibitors, p21 and p16, are upregulated and the Rb protein is maintained in the active state. Active Rb inhibits the transition from the G1 to S phase of the cell cycle. (b) Tetraploid senescent cells. At the G2 phase of cell cycle, the p53/p21 pathway is activated in the cells exposed to senescence, inducing stimuli. APC/C^{Cdh1} is prematurely activated *via* the accumulation of p21, and mitosis skip occurs in these cells. The Rb family of proteins is also important for the induction and maintenance of senescence.

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senescence. Senescent cells secrete numerous biologically active factors, including the proinflammatory cytokines interleukin (IL)-6 and IL-8, chemokines [monocyte chemoattractant proteins (MCPs), macrophage inflammatory proteins, growth-regulated protein alpha (GROα)], growth factors [vascular endothelial growth factor (VEGF), granulocyte/macrophage-colony stimulating factor, transforming growth factor-beta (TGF-β)], and proteases [matrix metalloproteinases (MMPs)] (60,61). Because these factors act in autocrine and paracrine manners and have pleiotropic effects for surrounding cells, they may affect the surrounding microenvironment as well as the senescent cell itself and may be involved in tissue remodeling in organisms. This is called senescence-associated secretory phenotype (SASP) (62) or senescence messaging secretome (SMS) (63). Because SASP has complex and divergent effects, this may explain the role of cellular senescence in organismal aging and the incidence of age-related diseases and pathologies. There are now many reports that indicate senescent cells accumulate in aged and disease-related tissues (64–67). This suggests that cellular senescence actively contributes to the aging process and progression of some diseases at the organismal level. It has been suggested that the low-level chronic inflammation often observed during aging in tissues without obvious infection is due to senescent cells and SASP (68–70).

It is now evident that senescence can be transmitted to normal cells by SASP in a paracrine or autocrine manner (71). Acosta et al. showed that SASP induced by oncogene-induced senescence (OIS) can induce paracrine senescence in normal cells using both coculture systems *in vitro* and human and mouse models of OIS. TGF-β, VEGF, CCL2, and CCL20 are among SASP components that were identified as the factors that mediate paracrine senescence. TGF-β was a major player for the induction of p21 and $p15^{INK4b}$ that contributed to cell growth arrest in paracrine senescent cells. The secretion of mature forms of IL-1 is increased during OIS, suggesting that the inflammasome is activated in oncogene-induced senescent cells (72). In fact, components of the inflammasome such as caspase-1, ASC, and NLRP3 were increased at the protein level during OIS. Finally, they showed that inhibitors for caspase-1 or IL-1 receptor were *downregulated SASP* components during OIS. This suggested that the activation of the inflammasome is directly involved in the expression of SASP. Inflammasome and IL-1 signaling are activated in senescent cells, and IL-1 that is induced from senescent cells by SASP is also involved in paracrine senescence.

SASP is mainly linked to DDR or epigenomic disruption (60,73,74). SASP is not recognized in normal senescent cells that have ectopically overexpressed p21 or p16, although these cells undergo a senescence growth arrest and show several other features of senescent cells (75). It has been reported that glucocorticoid treatment of senescent cells suppresses the secretion of several SASP components, including some proinflammatory cytokines, without affecting tumor suppressive growth arrest (76). This finding indicates that growth suppression and SASP in senescent cells are segregated processes. ATM, Chk2, and NBS1, which are involved in DDR, are important for the initiation and maintenance of SASP. Importantly, these proteins contribute to SASP after the establishment of persistent DNA damage signaling (73). The rapid robust DDR that is activated immediately after DNA damage does not induce SASP. On the other hand, p53, which is located downstream of ATM and Chk2, suppresses SASP and knockdown of p53, resulting in augmented expression of IL-6.

SASP is mainly mediated by transcription factors such as nuclear factor-kappa B (NF-kB) (77–79) and CCAAT/enhancer binding protein beta (C/EBPβ) (72). An initial step in SASP involves the transcriptional activation of IL-1α in response to senescence-inducing stimuli. A cell surface-bound isoform of this cytokine binds to its plasma membrane-associated IL-1 receptor, which in turn activates a downstream signaling cascade to stimulate NF-kB and C/EBPβ transcription factors (80). These transcription factors in turn activate the transcription of genes that encode various SASP proteins such as IL-6 and IL-8.

Epigenetic regulation of SASP induction has been described (81). This involves a decrease in the expression of DNA methyltransferase 1 (DNMT1), which is observed during senescence (82). The authors showed that IL-6 and IL8 expression related to SASP was induced by the knockdown of DNMT1 in normal human fibroblasts. The ubiquitination of G9a/GLP, H3K9 methyltransferases, by APC/C^{Cdh1} ubiquitin ligase was induced in response to decreased expression of DNMT1, followed by proteasomal degradation of G9a/GLP. Consequently, H3K9me2 levels in transcriptional regulatory regions of IL-6 and IL-8 genes decreased and their expression was activated. It was also confirmed that the expression of G9a/GLP decreases and the expression of IL-6 and GROα mRNA is reduced in the lung, spleen, and intestine of aged mice. More recently, it has been reported that SIRT1, an NAD^+-dependent histone deacetylase, is involved in suppressing the expression of SASP components such as IL-6 and IL-8 through deacetylating histones around the promoter regions of these genes (83).

Cellular senescence: beneficial effects

Tumor suppression

Although tumor-derived or virally transformed cells proliferate indefinitely in culture, normal cells enter senescence after reaching a typical number of divisions. Genetic studies using cell fusion technology by which normal human cells were fused with various immortal cell lines demonstrated that the resulting hybrids could not

proliferate indefinitely (84–87). This result indicated that the senescent phenotype is dominant and suggested that immortal cells appear by defects in genes or pathways involved in growth arrest to escape cellular senescence. This was the first evidence for a role of cell senescence in tumor suppression (84).

In 1997, Serrano et al. reported that the overexpression of oncogenic H-Ras (H-RasG12V) in normal human, mouse, and rat fibroblasts induces growth arrest along with the accumulation of p53 and p16, similar to cellular senescence (48). They also showed that p53/p21 and Rb/p16 pathways are important for OIS because the inactivation of either p53 or p16 prevents Ras-induced growth arrest. Because OIS cannot be bypassed by the ectopic expression of hTERT (88), it is obvious that OIS is independent of telomere erosion. There is accumulating evidence that cellular senescence functions as a barrier against transformation and prevents the expansion of precancerous cells *in vivo*. Senescent cells can be identified in premalignant tumors *in vivo* because they are positive for SA-β-gal and express p16 (82, 89–91). Braig et al. showed that the methylation of lysine 9 of histone H3 by Suv39h1 is important for the induction of cellular senescence in a T-cell lymphoma model using Eμ-N-Ras transgenic mice. The incidence of T-cell lymphomas strikingly increased by a defect in Suv39h1 in this model. Michaloglou et al. showed that mutant $BRAF^{E600}$, which has an oncogenic mutation from valine to glutamic acid, induces cellular senescence in normal melanocytes along with an accumulation of SA-β-gal-positive senescent cells, which express mutant $BRAF^{E600}$. Inactivation of senescence pathways by the deletion or mutation of tumor suppressor genes such as p53 or Rb as well as oncogene expression is required for the progression to malignant tumors (92,93). Although cells that have defects in the tumor suppressors PTEN and NF1 can still senesce, these cells easily transform into malignant tumors by the inactivation of other genes such as p53 (94,95). Interestingly, it has been reported that the reactivation of functional p53 in some mouse tumor models causes the induction of cellular senescence and tumor regression (96,97).

Wound healing

Cellular senescence is also important for wound healing in the skin. Fibroblasts are recruited into injury sites and differentiate into myofibroblasts, specialized contractile fibroblasts, which deposit extracellular matrix for repair. At the end of wound healing, the matricellular protein CCN1, which is highly expressed in affected areas, binds to its receptor, integrin α6β1, and activates the production of oxidative stress in myofibroblasts (98). Increased oxidative stress causes myofibroblast senescence during wound healing in the skin, which protects against progression to excessive fibrosis. Indeed, in mice expressing a mutant CCN1 that cannot bind to integrins, the wounds had fewer senescent cells and resulted in significantly more fibrosis.

Recently, Demaria et al. reported on a beneficial role of SASP by senescent cells in wound healing using a new transgenic mouse model (99). They generated a transgenic mouse line expressing the 3MR (trimodality reporter) fusion protein using a p16 promoter. This fusion protein contains functional domains of a synthetic *Renilla* luciferase (LUC) to identify senescent cells *in vivo*, monomeric red fluorescent protein (mRFP) to isolate senescent cells by fluorescence activated cell sorting, and truncated herpes simplex virus 1 thymidine kinase (HSV-TK) to selectively kill senescent cells by adding ganciclovir (GCV). Using this mouse model, they showed that senescent cells are transiently induced at the injury site during cutaneous wound healing and the effective elimination of senescent cells by GCV results in a significant delay in wound healing. This result indicated that senescent cells that are transiently induced at the injury site accelerate skin repair. They also found that platelet-derived growth factor AA (PDGF-AA), which is secreted as SASP from senescent cells at the injury site, is a key factor for wound closure. PDGF-AA secreted from senescent cells was involved in the differentiation of non-senescent fibroblasts into myofibroblasts, which plays a critical role in wound contraction during wound healing. Topically applied recombinant PDGF-AA to wounds on senescent cell-eliminated p16-3MR mice significantly increased the percentage of myofibroblasts and restored wound closure, although this was not significant for the reduction of collagen deposition (fibrosis). They speculated that other SASP factors such as proteases may contribute to the reduced fibrosis. This clearly indicates that SASP of senescent cells has a beneficial effect in physiological situations.

Liver fibrosis

Liver fibrosis occurs as a result of excessive accumulation of extracellular matrix proteins, including collagen. Advanced liver fibrosis can result in cirrhosis and liver failure. The role of the senescence program in acute liver injury induced by a liver-damaging agent (CCl_4) *in vivo* has been reported (100). Hepatic stellate cells are activated by damage and begin to produce the components of the extracellular matrix for repairing the damage. The stellate cells subsequently become senescent and secrete SASP factors, including MMPs, to repair the fibrotic scar. SASP associated with stellate cell senescence attracts immune cells and the senescent stellate cells are cleared by attracted natural killer cells. The clearance of senescent cells by immune cells attracted by SASP factors, which are secreted by senescent cells, also seems to be an important step in halting tissue repair when the process is completed. In mice deficient in the p53/p21 or p16/Rb pathways, stellate cells continue to proliferate and do not enter senescence, and fibrosis in the liver is markedly increased.

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Therefore, it is thought that cellular senescence is important for controlling tissue repair and the maintenance of the integrity of the organ.

Cardiac fibrosis

Cellular senescence also plays a pivotal role in the regulation of cardiac fibrosis after myocardial infarction (MI) in a mouse model (101). Senescent cardiac fibroblasts accumulated in infarcted hearts 1 week after MI in wild-type mice. This was accompanied by upregulation of the senescence markers p53, p16, and p21 as well as SA-β-gal activity. Importantly, in p53-deficient mice, the accumulation of senescent fibroblasts, macrophage infiltration, and MMPs such as MMP2 and MMP9 were significantly reduced; however, collagen deposition was enhanced after MI. This result indicated that p53-mediated cellular senescence is important for limiting cardiac collagen deposition and cardiac fibrosis.

Developmentally programmed senescence

More recently, an interesting finding that cellular senescence occurs during development, and senescent cells are most likely to be involved in promoting tissue remodeling during development has been reported (102,103). The senescent cells were identified throughout the mouse embryo, including the mesonephros, apical ectodermal ridge, neural roof plate, and endolymphatic sac of the inner ear. The authors suggested that embryonic senescent cells are important for tissue growth and organ formation during development. Embryonic senescent cells were highly dependent on p21 but not p53 and DNA damage. Indeed, the expression of p21 was positively regulated by TGF-β/SMAD and PI3K/FOXO pathways. p21-deficient mice had defects in embryonic senescence, although apoptosis partially compensated outcomes by loss of senescence because of p21 deficiency. Developmental senescent cells also shared an expression profile with OIS, including SASP factors. Because developmental senescent cells were cleared by infiltrating macrophages during tissue remodeling, SASP in these senescent cells plays a pivotal role in tissue remodeling during development.

Apart from embryonic development, it is known that cellular senescence occurs in a physiologically programmed manner. Physiological senescence is induced in normal megakaryocytes (104) and placental syncytiotrophoblasts (105) during their maturation.

Cellular senescence: detrimental effects

Tumor promotion

Although cellular senescence suppresses malignant transformation, secreted factors by SASP such as inflammatory cytokines (IL-1, IL-6, IL-8, MCP2, and others), proteases (MMPs and others), and growth factors (VEGF and others) (60,72) may facilitate the progression of surrounding tumor cells and accelerate metastasis by affecting the tissue microenvironment (106–109). Coinjection of senescent fibroblasts with mouse or human epithelial tumor cells into immunocompromised mice significantly stimulated tumor growth (106,107,110). SASP factors secreted from senescent cells can also stimulate precancerous cells to obtain more malignant phenotypes, including epithelial to mesenchymal transition (EMT), which accelerates the invasion and migration of tumor cells into tissues (111). There are reports that IL-6 and IL-8 secreted by senescent cells contribute to EMT in premalignant epithelial cells (60,111,112). Some proteases such as MMPs, which are secreted by senescent cells by SASP, may also contribute to tissue remodeling and tumor cell migration. Similar upregulation of proteases is also observed in some tumor cells. Secreted proteases from senescent cells in the microenvironment of cancer tissues may accelerate tumor migration in a coordinated manner with tumor-derived proteases.

Age-related degenerative phenotypes

It is thought that senescent cells are implicated in many age-associated degenerative diseases in both normal and pathological situations. Senescent cells in tissues are most likely to affect the normal tissue structure and tissue integrity through SASP. To elucidate whether senescent cells can drive age-associated degenerative pathology, Baker et al. (113) have produced a transgenic mouse line in which senescent cells during the progression of age-related disorders could be eliminated by the administration of a drug. In this model, termed INK-ATTC (apoptosis through targeted activation of caspase), a transgene expresses the p16 promoter-driving ATTC fusion protein (caspase 8 fused to the FK506-binding protein). Upon the administration of the inducer AP20187, the fusion protein dimerizes, thereby activating caspase 8 activity, and p16-positive senescent cells are specifically killed by apoptosis. INK-ATTC mice were crossed with the hypomorphic BubR1 ($BubR1^{H/H}$) progeroid mice because these mice have a markedly shortened lifespan in comparison with most mice. Although the elimination of p16-positive senescent cells by the administration of the drug did not cause extension of lifespan in this progeroid model, it significantly delayed the onset of age-related phenotypes such as sarcopenia, cataracts, and loss of subcutaneous fat. More importantly, this improvement was able to be achieved even in late-life clearance of the p16-positive senescent cells. This study provided the first direct evidence that senescent cells contribute to the progression of tissue pathology during aging.

Adipocyte senescence, obesity, and diabetes

Obesity is a condition in which excess fat is accumulated in the body and has a negative effect on health. Obesity is most commonly caused by excessive food intake and/or low physical activity, leading to the accumulation of

white adipose tissues. Excess accumulation of fat in adipocytes triggers an inflammatory response in adipose tissue and results in the initiation of systemic pathological processes (114). Undesired adipokines such as TNF-α are produced by this inflammatory response and lead to insulin resistance and type 2 diabetes (115–117). SA-β-gal-positive senescent cells are accumulated in the adipose tissues obtained from obese mice or humans, and this is accompanied by the accumulation of p53 and p21, upregulation of SASP factors, and infiltration of inflammatory cells (118). Interestingly, the deletion of the p53 gene in mice reduced senescent cells in adipose tissue, reduced the production of inflammatory cytokines, and improved insulin resistance induced by a high-fat diet (HFD). These results suggested that p53 is activated in adipose tissues in obesity, induces senescence in adipocytes, and these adipocytes produce inflammatory cytokines because of SASP. Therefore, adipocyte senescence is associated with obesity and is tightly linked to pathological consequence led by obesity.

It is known that insulin resistance initially causes compensatory proliferation of β cells during the pathogenesis of type 2 diabetes. It is generally thought that this compensatory proliferation eventually leads to proliferative exhaustion of β cells and diabetes. The number of β cells and their proliferating rate were significantly increased in C57BL/6J mice 4 months after feeding HFD. However, at 12 months after feeding HFD, proliferative β cells were reduced, oxidative stress was increased, and SA-β-gal-positive senescent β-cells were significantly increased in β-cell islets (119). This strongly supports the idea that the cellular senescence of β cells contributes to the pathogenesis of diet-induced diabetes.

Atherosclerosis

Cellular senescence has been implicated in the development of vascular pathologies, particularly atherosclerosis. SA-β-gal-positive senescent endothelial cells are recognized in advanced atherogenic plaques on human coronary arteries (120). It is also recognized that the expression of endothelial nitric oxide synthase (eNOS) decreases and the expression of proinflammatory factors as well as p53, p21, and p16 increases in these pathological blood vessels. The induction of premature senescence through the p53/p21-dependent pathway has been observed in human vascular smooth muscle cells (VSMCs) by angiotensin II (Ang II) treatment in a mouse model of atherosclerosis (121). This was accompanied by the production of proinflammatory factors from senescent VSMCs *via* SASP by Ang II treatment. The deficiency of p21 markedly reduced the production of proinflammatory factors by Ang II treatment and prevented the development of atherosclerosis in this mouse model.

However, the beneficial effect of cellular senescence on atherosclerosis has also been reported. Mouse models deficient in cell cycle regulators such as p53 (122), p21 (123), p27 (124), and ARF (125) show augmented susceptibility to the development of atherosclerosis, whereas the overexpression of p53 in mice protects them from mechanically induced neointimal thickening in femoral arteries but not native atherosclerosis (126). Further investigation is required to resolve this discrepancy.

Other diseases associated with cellular senescence

It is thought that other human diseases such as sarcopenia (127–130), osteoarthritis (131,132), and pulmonary fibrosis (133,134) are associated with cellular senescence. Astrocyte senescence has also been proposed to be involved in the pathogenesis of Alzheimer's disease (135) and Parkinson's disease (136). Reports that indicate cellular senescence associated with human diseases has been increasing. Cellular senescence may contribute to more pathologies in many age-associated diseases under both beneficial and detrimental circumstances.

Concluding remarks

Cellular senescence is a state of essentially irreversible growth arrest, and it has been proposed that this has developed as an antitumor mechanism. In addition to growth arrest, senescent cells secret numerous proinflammatory factors through SASP. There is now much evidence that SASP of senescent cells contributes to the pathogenesis of age-associated diseases. It has been speculated that the accumulation of senescent cells in tissues accelerates tissue remodeling triggered by SASP factors, reduces tissue integrity and function, and contributes to organismal aging. In fact, senescent cells are found in many tissues under pathological conditions or advanced aging. It has also been shown that senescent cells have a positive impact *in vivo*. For example, cellular senescence coordinates the process of tissue remodeling in some physiological situations. In the case of embryonic development, cellular senescence occurs throughout the embryo and functions to promote tissue remodeling. There is some evidence that transiently induced senescent cells protect the progression of pathogenesis in some diseases and, in fact, may have more beneficial functions *in vivo* than what is thought.

Further investigation of the molecular mechanism of cellular senescence, particularly *in vivo*, promises to open a new avenue for achieving healthy aging and establishing a new strategy to prevent age-associated diseases.

Conflict of interest and funding

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