Stress-induced Cellular Senescence Contributes to Chronic Inflammation and Cancer Progression

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Abstract: Cellular senescence has long been considered to act as a tumor suppressor or tumor suppression mechanism and described as a phenomenon of irreversible cell cycle arrest. Cellular senescence, however, is now considered to have physiological functions other than tumor suppression; it has been found to be involved in embryogenesis, tissue/organ aging, and wound healing. Surprisingly, cellular senescence is also demonstrated to have a tumor progressive role in certain situations. Senescent cells exhibit secretory phenotypes called senescence-associated secretory phenotype (SASP), which secrete a variety of SASP factors including inflammatory cytokines, chemokines, and growth factors, as well as matrix remodeling factors that promote the alteration of neighboring tissue microenvironments. Such SASP factors have been known to drive the mechanisms underlying the pleiotropic features of cellular senescence. In this review, we examine current knowledge of cellular senescence at molecular and cellular levels, with a focus on chronic inflammation and tumor progression.

Key Words: senescence, senescence-associated secretory phenotype (SASP), reactive oxygen species (ROS), ionizing radiation, heat shock response (HSR)

1. Introduction

Hayflick and Moorhead1) were the first to describe the limited divisions of cells and term this irreversible cell cycle arrest as cellular senescence. Senescent cells remain metabolically active, but their growth is irreversibly halted and their morphological characteristics altered as large, flat, and refractile2,3). Irreversible cell cycle arrest is now thought to be dependent upon the shortening of telomeres; an expression of the catalytic subunit of the telomerase holo-enzyme (hTERT) is enough to bypass cellular senescence4-6). Telomeres are folded d-loop/t-loop structures located at the ends of chromosomes that serve to mask DNA ends from being recognized as DNA double-strand-breaks (DSBs)7,8). Telomerase activity is absent from most normal human somatic cells. After serial divisions, telomeres become too short to have sufficient binding sites for shelterin proteins (the latter being protein complexes known to protect telomeres) and are remodeled with a “capped” structure9). Telomere shortening-related senescence is called replicative senescence. Nonetheless, mouse
embryonic fibroblasts (MEFs) undergo cellular senescence despite telomerase activity\(^5\). Senescence in vitro can be bypassed by culturing in serum-free media or low oxygen conditions in MEFs\(^5,10,11\). Cellular senescence is hence, induced not only by telomere shortening, but also by certain culture conditions\(^5\). Several studies found that irreversible cell cycle arrest can be induced when normal cells are exposed to a variety of stressors, including telomere erosion, oxidative stress, ionizing radiation, and the activation of oncogenes such as Ras\(^12-17\). Such cellular senescence is called premature senescence, and is also known as stress-induced senescence (SIS) or oncogene-induced senescence (OIS). Senescence induced by oncogenic stimuli is recognized as a tumor suppression mechanism due to its irreversible arrest of proliferation\(^18,19\).

However, findings from recent studies suggest that the secretion of inflammatory cytokines, chemokines, and the induction of matrix remodeling factors in senescent cells lead to inflammation and cancer progression\(^5,20\). This phenomenon is known as senescence-associated secretory phenotype (SASP) and as summarized in Tables I and II, SASP factors are associated with multiple functions, either in the suppression or progression of tumorigenesis. The role of this process is dependent on p53-induced cellular senescence; p53 null/mutant cells bypass senescence, and accelerates tumor growth and invasiveness after SASP factor

<table>
<thead>
<tr>
<th>Function</th>
<th>Activation stimuli</th>
<th>SASP factors</th>
<th>Possible mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryogenesis</td>
<td>Development</td>
<td>TGFβ/SMAD, CEBPβ, CSF1</td>
<td>TGFβ/SMAD and PDJ3/FOXO-p21-senescence-tissue remodeling (ref. 22)</td>
</tr>
<tr>
<td>Aging</td>
<td>BubR1(^{{\text{H}1}})</td>
<td>(-)</td>
<td>p16-senescence- age related dysfunction (ref. 29)</td>
</tr>
<tr>
<td></td>
<td>Naturally-occurring (RS?) senescence</td>
<td>(-)</td>
<td>p16-senescence- age related dysfunction (ref. 30)</td>
</tr>
<tr>
<td></td>
<td>IFNa, IL6, TNFa, MX1</td>
<td></td>
<td>telomere instability-cytosolic DNA- cGAS/STING-SASP- HP1β and p16-senescence (ref. 114)</td>
</tr>
<tr>
<td>Wound healing</td>
<td>CCl4</td>
<td>IL11, IL8, IL6</td>
<td>CCl4-p53/16 dependent senescence-SASP-immune system-reduced liver fibrosis (ref. 31)</td>
</tr>
<tr>
<td></td>
<td>Bacterial artificial</td>
<td>PDGF-AA</td>
<td>injury-senescent fibroblasts and endothelial cells- SASP- wound healing (ref. 32)</td>
</tr>
<tr>
<td></td>
<td>(chromosome (BAC), IR (X-ray))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor suppression</td>
<td>CKI(^{{\text{α}0}}) knockout</td>
<td>IxB, TNF, factors suppressed by NSAID</td>
<td>CKI(^{{\text{α}0}})-DNA damage response-SASP factor-p53 dependent senescence (ref. 21)</td>
</tr>
<tr>
<td>Ras</td>
<td>TGFβ family ligands, VEGF, CCL2, CCL20</td>
<td></td>
<td>Ras-IL1 signaling-TGFβ family, VEGF, CCL2 and CCL20-p15 and p21-senescence (ref. 101)</td>
</tr>
<tr>
<td>Tumor promotion</td>
<td>CKI(^{{\text{α}0}}) knockout</td>
<td>IxB, TNF, Factors suppressed by NSAID</td>
<td>CKI(^{{\text{α}0}})-DNA damage response-SASP (p53 mutation/ null) proinvasive gene expression signature (ref. 21)</td>
</tr>
<tr>
<td>Obesity or DMBA</td>
<td>IL6, Grot, CXCL9</td>
<td></td>
<td>obesity-deoxycholic acid-DNA damage-SASP factors-promote hepatocellular carcinoma (ref. 94)</td>
</tr>
<tr>
<td>Bleomycin, RS</td>
<td>IL6, IL8, GM-CSF</td>
<td></td>
<td>p38-AUF1-SASP factors-tumor promotion (ref. 119)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>RS, H-Ras(^{V12}), etoposide</td>
<td>IL1α, IL8</td>
<td>cytoplasmic chromatin-cGAS/STING-NFκB-SASP (ref. 110)</td>
</tr>
<tr>
<td>Drug resistance</td>
<td>doxorubicin</td>
<td>IL6, Timp1</td>
<td>doxysolbicin-p38 activation-IL6 from the thymic stroma-lymphoma cell surviving (ref. 109)</td>
</tr>
</tbody>
</table>
signaling\(^{21}\)). It remains unclear why cells become senescent, yet are not removed from the tissue. This suggests that cellular senescence may have a role other than the suppression of tumorigenesis. Muñoz-Espín \textit{et al.}\(^ {22}\) and Storer \textit{et al.}\(^ {23}\) proposed that the cells become senescent as part of embryogenesis fine-tuning. Both studies found that senescent cells contribute to macrophage infiltration and tissue formation through SASP\(^ {22,23}\).

Cellular senescence is associated with a number of other functions, including organismal aging (Table I). The accumulation of senescent cells is typically observed in aging tissues\(^ {24-28}\). Studies have found that the removal of senescent cells from the tissue prolongs mice lifespan and prevents age-dependent changes in several organs\(^ {29,30}\). Yet, cellular senescence has also been shown to limit the extent of fibrosis following liver damage, and underscore the interplay between senescent cells and the tissue microenvironment\(^ {31}\). This finding demonstrates the contribution of cellular senescence in an additional role of wound-healing and repair in tissue\(^ {31,32}\).

Due to its multiple roles, researchers argue that there may be different types of cellular senescence (Tables I and II). For example, the expression of telomerase has been found to prevent cellular senescence, but not epigenetic aging or DNA methylation-based aging; Kabacik and colleagues\(^ {33}\) suggest that each senescent cell and paracrine cell may have different epigenetic aging backgrounds. In another study, senescent cells during development are removed by immune system such as macrophage and thought not to be associated with DNA damage response\(^ {22,34}\).

Current knowledge of molecular and cellular biology of cellular senescence \textit{S. Kobashigawa et al.}\(^ {43}\) ɾ

### Table II. A summary of the functions and mechanisms of SASP factors and multiple roles of cellular senescence \textit{in vitro} study.

SASP factors are shown to have multiple functions including the establishment of cellular senescence \textit{in vitro}.

<table>
<thead>
<tr>
<th>Function</th>
<th>SASP factors</th>
<th>Possible mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senescence</td>
<td>IL6, IL8, IL1α, IL1β</td>
<td>Ras-ROS-DNA damage-decrement of histone methylation-SASP factors (ref. 12)</td>
</tr>
<tr>
<td></td>
<td>Factors bind to CXCR2</td>
<td>NFκB and C/EBPβ-chemokines-CXCR2 (IL8RB)-p53 dependent senescence (ref. 13)</td>
</tr>
<tr>
<td></td>
<td>IL1α, IL6, TGFβ</td>
<td>IFNγ and IL1 signaling pathways-NOx4-ROS-DNA damage-paracrine senescence (ref. 104)</td>
</tr>
<tr>
<td>Tumor promotion</td>
<td>IL6, IL8, IL1β, GROα, GM-CSF, IFNγ</td>
<td>senescence-SASP factors (ref. 50)</td>
</tr>
<tr>
<td></td>
<td>IL1α, IL6, NFκB, CXCL1, CXCL2</td>
<td>mTOR-SASP factors-prostate tumour growth (ref. 102)</td>
</tr>
<tr>
<td></td>
<td>IL1α, IL6, NFκB, IL8</td>
<td>IL1α-NFκB and C/EBPβ-IL6, IL8-invasiveness of metastatic cancer cells (ref. 103)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>IL6, IL8, osteopontin</td>
<td>1) DNA damage-ATM, NFκB(IL6, IL8) 2) HDAC inhibition-osteopontin-invasive breast cancers (ref. 107)</td>
</tr>
<tr>
<td>Radioresistance</td>
<td>IL6, IL8, IL1β, GROα, GM-CSF, IFNγ</td>
<td>Ras-ROS-DNA damage-decrement of histone methylation-SASP factors (ref. 12)</td>
</tr>
<tr>
<td></td>
<td>NFκB and C/EBPβ-chemokines-CXCR2 (IL8RB)-p53 dependent senescence (ref. 13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/EBPβ, IL6, IL8</td>
<td>SASP factors-p15-positive feed back loop-senescence (ref. 17)</td>
</tr>
<tr>
<td></td>
<td>IL6, IL8, IL1β</td>
<td>BRAF(^ {V600E})-MEK-ERK-IFNγ-down regulation of MEK and ERK (ref. 54)</td>
</tr>
<tr>
<td></td>
<td>GROα, IFNγ</td>
<td>lipopolysaccharides-p21-NFκB-iNOS and SASP factors-NO and neuroinflammatory diseases (ref. 96)</td>
</tr>
<tr>
<td></td>
<td>IL6, GRO, IL8, ICAM1, TNF, IL1</td>
<td>persistent DNA damage- p53- SASP factors (ref. 105)</td>
</tr>
</tbody>
</table>

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\(43\)
2. How is cellular senescence induced?

In replicative senescence, telomere erosion activates DNA damage sensor proteins, such as ataxia telangiectasia-mutated protein kinase (ATM) or ATM and RAD3-related protein kinase (ATR). They, in turn, activate p53, a cell cycle check point protein, and induce irreversible cell cycle arrest\(^7,8\). SIS is also initiated by DNA damage response. Essentially, quiescent cells are not activated in DNA damage response pathways that include p53. A primary difference between quiescent cells and senescent cells is, while quiescent cells are in G0 cell cycle phase, senescent cells are in the G1 phase. The cell cycle arrest and cellular senescence have the same step, such as activation of p53\(^5\). Arrested cells enter the next cell cycle phase when damage is repaired, and where the damage is irreparable, persistent G1 cell cycle arrest undergo cellular senescence. It is important to note that senescent cells are still metabolically active in the G1 phase of the cell cycle.

Senescent cells are arrested in G1 phase due to the constant activation of cyclin dependent kinase inhibitors (CDKI) in the cells\(^13\). There are two types of CDKI: the KIP/CIP family and INK4 family. KIP/CIP family proteins, including p21\(^{Cip1}\) and p27\(^{kip1}\), inhibit a broad range of CDK. INK4 family proteins, including p16\(^{INK4a}\), p15\(^{INK4b}\) and p19\(^{INK4d}\), inhibit CDK4\(^35\). Both p53 and p16\(^{INK4a}\)–Rb pathways play critical roles in the induction of senescence\(^6\). When telomere shortening or DNA damage occurs, p53 is activated by ATM or ATR. P53 then transcriptionally activates p21\(^{Cip1}\) \(^36\). Unlike p21\(^{Cip1}\), p16\(^{INK4a}\) is upregulated from p53 independently\(^37-39\). Although telomeric DNA damage induces p53 activation and not p16\(^{INK4a}\) \(^40\), cellular senescence is dependent on p16\(^{INK4a}\) in some human cells\(^36,41\). In vivo imaging has demonstrated that p16\(^{INK4a}\) positive senescent cells, accumulated with age, cross talk with p53 through DNA damage response-dependent reduction of DNA (cytosine-5)-methyltransferase 1 (DNMT1)\(^24\). All CDKs that phosphorylate Rb proteins is cooperatively inactivated by p21\(^{Cip1}\) and p16\(^{INK4a}\), thereby triggering full Rb activation and premature senescence cell cycle arrest at G1 phase\(^42,43\). The inactivation of Rb and p53 in senescent MEFs results in the reversal of senescent phenotype, leading to cell cycle re-entry. This suggests that Rb and p53 are not only required for the onset of cellular senescence, but also for the maintenance of senescence in MEFs\(^44,45\).

Over-expression of an oncogene, Ras, induces the upregulation of E2F transcription factor. Over-activated E2F transcription factor binds to the p19\(^{INK4d}\) promoter, which in turn suppresses MDM2, a p53 ubiquitination enzyme, and induces p53 accumulation\(^46\). Recent findings revealed alternative functions of p19\(^{INK4d}\) in halting cell proliferation independent from p53\(^37-49\). For example, Webber and colleagues\(^48\) showed that an over-expression of p19\(^{INK4d}\) in p53/ MDM2/ p19\(^{INK4d}\) triple knockout cells stopped cell proliferation. Tago et al.\(^49\) found that the p53-independent tumor-suppressive effects of p19\(^{INK4d}\) may be mediated by its ability to enhance sumoylation of a diverse group of protein targets.

It is still debatable whether cellular senescence is strictly irreversible\(^5\). There have been instances where the inactivation of interleukins bypasses senescence\(^17,45,50\). This may be accounted by the multi-phased establishment of cellular senescence via: phase 1 — triggering events; phase 2 — initiation of the senescence response; phase 3 — entry into senescence; and phase 4 — further deepening of senescence phenotypes\(^51\). DNA damage response occurs in phase 2 and is reversed when DNA damage is repaired. P16\(^{INK4a}\) is expressed in phase 3 and likely contains irreversible cell cycle arrest. Phase 4 contains an altered chromatin structure, called senescence-associated heterochromatic foci (SAHF)\(^52\). As cells undergo phases 1 to 4, the probability of proliferation decreases, and they are led towards irreversible arrest\(^51\). While CDKI is a key player in the initiation of cellular senescence, the process remains a complicated one and the precise mechanisms for the
establishment of cellular senescence is still unknown. We summarized the induction of cellular senescence in Table III via means other than the CDKI pathway, with a focus on SASP factors and reactive oxygen species. Studies have demonstrated the roles of SASP factors such as IL-8, IL-6, IL-1α, IL-1β, PAI-1, IGFBP-5, and IGFBP-7, in the establishment of both replicative senescence and OIS (Tables I and II)\(^{12,13,21,53-55}\). Such SASP factors are downstream targets of C/EBPβ and NF-κB transcription factors, and typically associated with inflammation\(^5\). Another study by Acosta et al.\(^{13}\) demonstrated that the downregulation of a chemokine receptor, CXCR2, inhibits replicative senescence and OIS. Since CXCR2 is activated not only by IL-8 but also by other cytokines, this finding suggests that multiple SASP factors simultaneously contribute to the establishment of senescence. Interestingly, all the SASP factors found to be associated with replicative senescence (IL-8, IL-6, PAI-1, IGFBP-5) are factors that induce p53-dependent senescence\(^{13,53,55}\). Hence, it is likely that SASP-mediated establishment of cellular senescence is closely linked with DNA damage responses that involve p53. Additionally, it suggests that persistent DNA damage is required for the induction of these SASP factors\(^5\).

Table III. List of mechanisms underlying the establishment of senescent cells, other than CDKI pathways. The SASP factors and ROS are likely to be involved in the establishment of cellular senescence.

Abbreviations: replicative senescence (RS), ionizing radiation (IR), normal human fibroblasts (HFs), mouse embryonic fibroblasts (MEFs).

<table>
<thead>
<tr>
<th>Factors for senescence</th>
<th>Activation stimuli</th>
<th>Possible mechanism</th>
<th>Types of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-) p19</td>
<td></td>
<td>Senp3-SUMOylated protein (Npm/B23)-cell cycle arrest act independently of the Mdm2-p53 axis, G1 phase arrest sumoylation of endogenous Mdm2 and NPM proteins-tumor suppression</td>
<td>mouse fibroblasts (NIH 3T3), 293T cell, MEFs (ref. 47)</td>
</tr>
<tr>
<td>Ras</td>
<td>paracrine activation of the SASPs</td>
<td></td>
<td>HFs (Embryo and breast), Epithelial cells (ref. 50)</td>
</tr>
<tr>
<td>BRAF(^{V600E})</td>
<td>BRAF(^{V600E})-synthesis and secretion of IGFBP7-autocrine/paracrine pathways to inhibit BRAF-MEK-ERK signaling-senescence and apoptosis</td>
<td></td>
<td>HFs (BJ), human melanoma cell, human primary melanocyte (ref. 54)</td>
</tr>
<tr>
<td>RS, H-Ras(^{V12E})</td>
<td>NFXs and C/EBPβ-chemokines-CXCR2 (IL8RB)-p53-dependent senescence</td>
<td></td>
<td>HFs (IMR-90) (ref. 13)</td>
</tr>
<tr>
<td>SASP factors</td>
<td></td>
<td>p53-PAI1-PKB β - GSK3β-cytosolic CyclinD1-senescence-IGFBP5-p53-senescence telomere instability-cytosolic DNA-cGAS-STING-autophagosome-senescence telomere instability-cytosolic DNA-cGAS-STING-SASP-HF1β and p16-senescence</td>
<td>HFs and HFs (BJ) (ref. 53)</td>
</tr>
<tr>
<td>CKI(^{αΔgut}) KO</td>
<td>1) CKI(^{αΔgut})-DNA damage response-SASP factor-p53-dependent senescence 2)p53 mutation/null-proinvasive gene expression signature</td>
<td></td>
<td>human umbilical endothelial cells (HUVECs) (ref. 55)</td>
</tr>
<tr>
<td>Ras and ROS</td>
<td>Ras-ROS-DNA damage-decrement of histone methylation-SASP factors RS, H-Ras(^{V12E}), etoposide</td>
<td></td>
<td>HFs (IMR-90), Human mammary epithelial cells (HMECs) (ref. 113)</td>
</tr>
<tr>
<td>20% and 40% O(_2)</td>
<td>TGFβ and IL1 signaling pathways-Nos4-ROS-DNA damage-paracrine senescence</td>
<td></td>
<td>HFs (W138), mouse (vivo) (ref. 114)</td>
</tr>
<tr>
<td>ROS</td>
<td></td>
<td>ROS-chromatin fragments in cytosol-cGAS-SASP factors</td>
<td>mouse (vivo) and mouse epithelial organoids (ref. 21)</td>
</tr>
</tbody>
</table>

**Notes:**
- **Senp3-SUMOylated protein (Npm/B23)**: Cell cycle arrest
- **Act independently of the Mdm2-p53 axis, G1 phase arrest**: Tumor suppression
- **Senp3-SUMOylated protein (Npm/B23)-cell cycle arrest act independently of the Mdm2-p53 axis, G1 phase arrest sumoylation of endogenous Mdm2 and NPM proteins-tumor suppression**: 
- **BRAF\(^{V600E}\)-synthesis and secretion of IGFBP7-autocrine/paracrine pathways to inhibit BRAF-MEK-ERK signaling-senescence and apoptosis**: 
- **NFXs and C/EBPβ-chemokines-CXCR2 (IL8RB)-p53-dependent senescence**: 
- **p53-PAI1-PKB β - GSK3β-cytosolic CyclinD1-senescence-IGFBP5-p53-senescence telomere instability-cytosolic DNA-cGAS-STING-autophagosome-senescence telomere instability-cytosolic DNA-cGAS-STING-SASP-HF1β and p16-senescence**: 
- **1) CKI\(^{αΔgut}\)-DNA damage response-SASP factor-p53-dependent senescence 2)p53 mutation/null-proinvasive gene expression signature**: 
- **Ras-ROS-DNA damage-decrement of histone methylation-SASP factors**: 
- **TGFβ and IL1 signaling pathways-Nos4-ROS-DNA damage-paracrine senescence**: 
- **ROS-chromatin fragments in cytosol-cGAS-SASP factors**: 
- **HFs (vivo) and mouse epithelial organoids (ref. 21)**

**References:**
- Acosta et al. (2013)
- Kobashigawa et al. (2021)
Reactive oxygen species (ROS) are involved in the establishment and stabilization of senescence. Studies have reported that hydrogen peroxide treatment or cell culturing under hyperoxic conditions may lead to premature cellular senescence\(^{56,57}\), and the relief of oxidative stress can retard this process\(^{58-60}\). ROS accelerate telomere shortening, damage DNA directly, and induce DNA damage response\(^{61-64}\). Conversely, the activation of major downstream effectors of the DNA damage response can induce ROS production\(^{65-67}\). The DNA damage response triggers mitochondrial dysfunction, leading to enhanced ROS production through p53, p21\(^{Cip1}\), GADD45A, p38, GRB2, and TGF\(\beta\)\(^{68}\). Takahashi et al.\(^{69}\) observed a cooperation between the p16\(^{ink4d}/Rb\) pathway and mitogenic signals to induce ROS, which thereby activates protein kinase C delta (PKC\(\delta\)) in human senescent cells. In addition, once activated, PKC\(\delta\) promotes the further generation of ROS, thus establishing a positive feedback loop to sustain ROS-PKC\(\delta\) signaling\(^{69}\). This sustained activation of ROS-PKC\(\delta\) signaling results in a blocking of cytokinesis via the reduction of WARTS (a mitotic exit network kinase also referred to as LATS1) in human senescent cells. Another study by Jun et al.\(^{70}\) reported that the matricellular protein CCN1, which is dramatically expressed at the site of wound repair, induces DNA damage response pathways, and activates p53 and ROS-generating RAC1-NOX1 complex. This results in the ROS-dependent activation of the p16\(^{ink4d}/Rb\) pathway, leading to fibroblast senescence.

3. Radiation-induced cellular senescence

Radiation-induced senescent cells have similar morphologies as replicative senescent cells. They have enlarged and flattened shapes, and are stained positive by senescence-associated beta-galactosidase (SA-\(\beta\)-gal), a marker of cellular senescence\(^{71}\). Radiation induces DSBs that activate ATM and p53 (Fig. 1), demonstrating that as with replicative senescence, radiation-induced senescence requires p53 activation\(^{72}\). The dependence of cell radio-sensitivities on induced DNA damage and the ability of cells to repair DNA damage has been well-established. Cell cycles are arrested to allow for DNA damage repair after exposure to radiation, and resume after DNA damage repair is complete. Where irreparable DNA damage occurs, cells undergo either apoptosis or senescence. The definitive factor for either apoptosis or senescence remains unknown, but research suggests the types of cells and damage to be important determinants\(^5\). For example, ionizing radiation induces senescence in fibroblasts as well as apoptosis in lymphocytes.

The generation of radicals during irradiation is thought to be one of the most damaging factors for nucleic DNA. The generation of radicals during irradiation is strongly related to the induction of cellular fate, including the induction of radiation-induced cellular senescence. However, treatment with ascorbic acid during irradiation (pre-treatment) was not effective for suppressing radiation-induced cellular senescence\(^{55}\). A number of studies also consistently found delays in the increase of ROS

![Fig. 1. A scheme of radiation-induced cellular senescence. Both p53-related G1 cell cycle arrest and secretion of SASP factors (NF-\(\kappa\)B, IL-6, IL-8) are required for the establishment of senescence.](image-url)
with peaks on the third day post irradiation\(^{15,68,73-75}\).

Interestingly, delayed ROS may be involved in radiation-induced cellular senescence; treatment of ascorbic acid after irradiation (post-treatment) was found to decrease the number of SA-β-gal positive cells and phosphorylated p38\(^{15}\). Passos \textit{et al.}\(^{68}\) also observed that p21\(^{Cip1}\) induces mitochondrial ROS through serial signaling through p53, p21\(^{Cip1}\), GADD45A, p38, GRB2, and TGFβ. The pathway is necessary and sufficient for the stability of growth arrest during the establishment of the senescent phenotype\(^{68}\). Another study found that mitochondria depletion abrogates the secretion of SASP factors (e.g. IL-6 and IL-8) and the development of cellular senescence in irradiated cells\(^{76}\). In addition, Acosta \textit{et al.}\(^{13}\) established the necessity of chemokine receptor CXCR2 in the establishment of cellular senescence. We present a model of cellular senescence induction by radiation based on existing observations in Fig. 1, where DSBs have been observed to activate ATM through auto-phosphorylation. While the downstream of ATM is important for cell cycle arrest, this alone is insufficient for senescence. Positive feedback of p38 and the mitochondrial ROS pathway may induce SASP factors, such as NF-κB, IL-6, and IL-8. As presented, both G1 arrest and SASP factor signaling are required for radiation-induced cellular senescence.

4. Heat stress and cellular senescence

Heat stress has been applied as a form of cancer therapy. Heat stress over 42.5°C affects cancer cells more selectively than normal cells and is likely to lead cancer cells to death. In many studies, temperatures ranging from 38°C to 45°C is typically applied. Heat stress causes a myriad of DNA damage, including double-strand-breaks, single-strand-breaks, partial DNA re-replication, and centrosome over-duplication\(^{77,79}\). Additionally, cell cycle arrest such as G1/S arrest and G2/M arrest is induced to enable DNA repair\(^{80}\). Heat stress stimulates heat shock factor 1 (HSF1), which in turn promotes the expression of heat shock proteins (HSPs) by recognising simple binding sites in the HSPs promotor region, heat shock elements (HSEs). HSPs function as molecular chaperones by unfolding and correctly folding misfolded proteins under stress conditions\(^{81,82}\). Both HSF1 and HSPs play important roles in heat shock response (HSR), the protective response to various cellular stresses. It is evident that through these responses, heat stress leads cells to apoptosis, proliferation, quiescence, and cellular senescence (Fig. 2)\(^{83,84}\). Nonetheless, the mechanism driving cellular senescence by heat shock has yet to be identified.

![Fig. 2](image)

\textbf{Fig. 2.} Both cellular senescence and HSR inhibit each other. Depending on the extent of stress, cells go into ① proliferation and quiescence, ② cellular senescence, or ③ apoptosis. The induction of cells to cellular senescence or growth depends on the balance between heat stress and HSR. However, HSR suppresses cellular senescence through inhibition of p38 in mild heat stress.
The sensitivity of heat stress depends on the cell cycle phase. Cycling cells are found to be more sensitive to heat stress than quiescent cells. Only cells in the early S phase are induced to prolonged G2/M arrest and cellular senescence in response to heat stress, suggesting that heat-stress induced cellular senescence is initiated by DSBs accompanying DNA replication. Activation of p53 leads to the next G2/M arrest. The degradation and transcriptional suppression of mitotic regulators (Cdt1-switching) may result in mitosis skip and then irreversible G1/S arrest. This mitosis skip is necessary and sufficient for senescence induction.

Surprisingly, the activation of HSR is known to suppress cellular senescence. The difference between the induction and the suppression of cellular senescence lies in the trigger; they are caused by different HSR conditions. HSF1 inhibits p38 activation and consequently, the SASP pathway. HSF1 is adversely suppressed by p38 activation in senescent cells, which show weakened HSR, resulting in senescent cells that are susceptible to elimination from the tissue given heat stress. In addition, some SASP factors are reduced through inhibition of p38 after mild heat stress. Through the elimination of senescent cells, stressless and stressful cells are distinguished in order to keep tissue homeostasis. Our hypothesis is that the balance between heat shock and HSR affects cellular fate including cellular senescence, apoptosis, proliferation, and quiescence (Fig. 2). Lethal heat shock is likely to induce apoptosis. Adequate HSR to heat stress may lead cells to proliferation rather than cellular senescence, while weak HSR may lead cells to cellular senescence rather than proliferation. In fact, repeated heat stress has an anti-aging effect through the elimination of senescent cells.

5. Senescence-associated secretory phenotype (SASP)

As discussed above, SASP factors demonstrate a wide range of functions, such as cellular senescence, inflammation, aging, tumor suppression, tumor progression, embryogenesis, wound healing, and radioresistance. While SASP factors, such as NF-κB, IL-8 and IL-6, are known to be radiation-induced bystander factors, recent studies demonstrate a contrary side to SASP factors in tumor suppression and progression. Some in vivo examinations have shown that IL-1α, IL-6 and IL-8 induce cellular senescence and suppress tumor growth, yet, these SASP factors have also been found to contribute to tumor promotion (Table I). It is now known that SASP induced senescence requires p53 activity, and SASP factors promote cell growth in p53-null cells. In vivo changes to SASP factors, such as matrix metalloproteases, in its insoluble protein/extracellular matrix components, including decreases in a number of collagens and proteoglycans, can be observed. Consequently, senescent cells affect neighboring cells through paracrine signaling, and alter the tissue microenvironments. Another in vitro study demonstrated that SASP factors induce an epithelial-mesenchymal transition, which marks an important step in cancer progression and metastasis.

The cell contextual difference of responses for SASP factors where pre-malignant cells tend to promote proliferation, contrary to normal cells, remains unclear. Mechanisms for SASP induction are still being investigated. SASP is recently viewed as a dynamic process that can be divided into several phases. The first phase of secretion occurs within a few hours after the induction of DNA damage. However, the onset of this phase is not sufficient to initiate cellular senescence, since it does not preclude complete DNA repair or apoptosis. Early self-amplifying SASP is initiated in the next phase within a few days after DNA damage.
damage. Important SASP factors, such as IL-1α, are secreted during this phase\(^{101-103}\). Over the subsequent 4-10 days, the autocrine effect of SASP intensifies secretions of most SASP factors, ultimately leading to the formation of mature SASP. Paracrine cell senescence is promoted by SASP\(^{104}\), and the development of cellular senescence and the secretion of SASP factors form positive feedback loops\(^{50}\).

SASP is initiated by genotoxic stress such as telomere shortening, radiation, oncogene activation and oxidative stress\(^{50,105}\). Notably, the initiation of cellular senescence requires irreparable DNA lesions and sustained DNA damage response\(^{7,40,105,106}\). DNA damage response has been found to induce the degradation of H3K9 histone methyltransferases through APC/C\(^{\text{Cdh1}}\) ubiquitin ligase, and expression of SASP factors such as IL-6 and IL-8\(^{12}\). The depletion of DNA damage response including ATM, Chk2, NBS1, and H2AX is sufficient to prevent the secretion of SASP factors\(^{20,105,107}\). While a defect of ATM suppresses SASP, the administration of ATM inhibitors does not\(^{105,108,109}\). DNA damage response is important for the induction of SASP, and development of mature SASP over a short duration. Taken together, ATM-dependent DNA damage response is clearly involved in the regulation of SASP, but the direct relationship between ATM-dependent DNA damage response and SASP, and its functional elements remain to be elucidated.

The innate immunity cytosolic DNA-sensing cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) pathway has been reported as another SASP pathway in recent researches\(^{110-115}\). The accumulation of cytoplasmic chromatin like micro-nuclei, activates cGAS-STING cytoplasmic DNA sensors, provoking SASP through the activation of NF-κB\(^{110-112}\). Nassour et al.\(^{113}\) reported the activation of autophagy by telomere erosion through the cGAS-STING pathway, and the bypassing of replicative senescence by autophagy inhibition.

The mitogen activated protein kinase p38 is another important pathway in response to genotoxic stress. Although p38 is well-known for its role in the regulation of several cytokines such as IL-1, IL-6, IL-8 and TNF\(\alpha\)\(^{116-118}\), its direct role in the regulation of SASP was only recently identified\(^{108,119}\). Unlike ATM-dependent DNA damage response, p38 is not immediately activated following genotoxic stress; activation only occurs a few days after genotoxic stress\(^{10}\). P38 activates downstream targets, MSK1 and MSK2, which phosphorylate p65, a transactivation subunit of NF-κB, and initiates the expression of many SASP factors\(^{100,120-122}\).

6. Cancer therapy and cellular senescence

As outlined in Table I, cellular senescence contributes to chronic inflammation and cancer progression through secretion of SASP factors in vivo\(^{21,94}\). Moreover, the accumulation of senescent cells predisposes one to age-related diseases and shortens one’s lifespan\(^{30}\). We believe that the prevention of cellular senescence or elimination of senescent cells in normal tissue is an important issue to address in radiation and hyperthermia therapy. The inhibition of mitochondrial ROS is one of the effective tools in preventing cellular senescence after radiation exposure\(^{15,76}\). In addition, a recent study demonstrated the efficacy of senolytic drugs in the selective elimination of senescent cells\(^{23}\); a combined treatment of dasatinib and quercetin (D + Q) was found to reduce the number of senescent cells in aged tissue and improve health span\(^{24}\). Another study found ABT-263 and ABT-737, inhibitors of BCL-2 family, to reduce senescent stem cells in radiation-exposed and aged mice\(^{225}\). Baker and colleagues\(^{29,30}\) showed the selective elimination of p16\(^{\text{NK4a}}\)-positive senescent cells by apoptosis using AP20187. AP20187 is a synthetic drug that induces dimerization of the membrane-bound
myristoylated FK506-binding-protein-caspase 8 (FKBP-Casp8) fusion protein, expressed downstream of p16INK4a promoter. Further developments are needed given that currently, this method requires the genetic manipulation of FKBP-Casp8 insertion downstream of p16INK4a promoter. Rapamycin is another senolytic drug that targets SASP through the inhibition of mTOR pathway. Rapamycin treatment applied to different organisms has been observed to extend their lifespan. We suggest further investigations into SASP and the identification of time at which senescent cells should be removed after radiotherapy and chemotherapy, since SASP factors are also involved in positive functions such as wound healing and repair. By extension, replicative senescence, SIS, and OIS are important processes to be elucidated.

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Summary of Abbreviations
- ATM; ataxia telangiectasia-mutated protein kinase
- ATR; ATM and RAD3-related protein kinase
- CDK1; cyclin dependent kinase inhibitors
- cGAS-STING; cyclic GMP-AMP synthase-stimulator of interferon genes
- DNMT1; DNA (cytosine-5)-methyltransferase 1
- DSBs; DNA double-strand-breaks
- HSEs; heat shock elements
- HSF1; heat shock factor 1
- HSPs; heat shock proteins
- HSR; heat shock response
- MEFs; mouse embryonic fibroblasts
- OIS; oncogene-induced senescence
- PAI-1; plasminogen activator inhibitor-1
- PKCδ; protein kinase C delta
ROS; reactive oxygen species
SASP; senescence-associated secretory phenotype
SA-β-gal; senescence-associated beta-galactosidase
SIS; stress-induced senescence

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ストレス誘発細胞老化は
炎症・がん細胞増殖促進に関与する

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要　旨：細胞老化は細胞増殖を停止させることで、がん抑制機構として長らく考えられてきた。しかしながら近年では、細胞老化はがん抑制の他に、発生、組織の老化、損傷修復など、様々な機能があることがわかってきている。驚くべきことに、p53変異、欠失などのある一定の条件下では、細胞老化は分泌因子を介してがん促進に寄与することもわかってきている。老化細胞は炎症性サイトカイン、ケモカイン、増殖因子、マトリックスリモデリング因子などの分泌（senescence-associated secretory phenotype; SASP）を亢進させ、周辺環境を変化させる。そのようなSASP因子は、細胞老化が多面的機能を持つ要因となっている。本総説では、慢性炎症、がん促進に着目した、細胞老化に関する分子、細胞レベルで得られている知見を紹介する。