Cellular senescence and senescence-associated secretory phenotype via the cGAS-STING signaling pathway in cancer

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Abstract
Cellular senescence is historically regarded as a tumor suppression mechanism to prevent damaged cells from aberrant proliferation in benign and premalignant tumors. However, recent findings have suggested that senescent cells contribute to tumorigenesis and age-associated pathologies through the senescence-associated secretory phenotype (SASP). Therefore, to control age-associated cancer, it is important to understand the molecular mechanisms of the SASP in the cancer microenvironment.

New findings have suggested that the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway, a critical indicator of innate immune response, triggers the SASP in response to accumulation of cytoplasmic DNA (cytoplasmic chromatin fragments, mtDNA and cDNA) in senescent cells. Notably, the cGAS-STING signaling pathway promotes or inhibits tumorigenesis depending on the biological context in vivo, indicating that it may be a potential therapeutic target for cancer. Herein, we review the regulatory machinery and biological function of the SASP via the cGAS-STING signaling pathway in cancer.

Keywords
Cellular senescence, cGAS-STING, DNA damage, SASP, tumorigenesis

1 FEATURES OF CELLULAR SENESCENCE
Cellular senescence is defined as a state of irreversible cell cycle arrest to prevent proliferation of damaged cells and reduce the risk of cancer. Therefore, cellular senescence is believed to be an essential tumor suppression mechanism in vivo. Hayflick and Moorhead initially mentioned "cellular senescence" after observing that primary human cells have a maximal number of cell proliferations in vitro. This proliferation limit caused by telomere shortening has been termed "replicative senescence." Similar phenotypes can be induced by activation of certain oncogenes, "oncogene-induced senescence (OIS)," and a variety of stressors, such as irradiation and oxidative stress. Furthermore, recent studies have demonstrated that treatment with chemotherapeutic drugs or ionizing radiation provokes "therapy-induced senescence (TIS)" in tumor cells. Persistent DNA damage response causes cellular senescence in normal cells, and induces expression of the cyclin-dependent kinase (CDK) inhibitors p16^{INK4a} and p21^{WAF1/CIP1}. p16^{INK4a} inhibits the cyclin D-CDK4/6 complex, whereas p21^{WAF1/CIP1} blocks cyclin E-CDK2 activity. Both checkpoint proteins block retinoblastoma protein, thereby suppressing expression of E2F target genes and inducing senescent cell cycle arrest (Figure 1). Generally, cellular senescence is a state of essentially irreversible cell cycle arrest; however, several studies have reported that a small population of senescent cells can override the senescent cell cycle arrest and re-enter the...
Recently, the selective CDK4/6 inhibitors abemaciclib, palbociclib and ribociclib have been shown to induce senescence-like cell cycle arrest in some cancer cell lines. These effects led to their approval for estrogen receptor-positive breast cancer treatment.

Senescent cells show some typical morphological features, such as a flattened shape and vacuolization. As persistent DNA damage signals are critical for induction of cellular senescence, it has been suggested that DNA damage foci could be used to identify senescent cells. Potential targets include accumulation of 53BP1, phosphorylation of histone H2A.X, or other DNA repair markers. In addition, chromatin reorganization occurs in senescent cells, termed senescence-associated heterochromatin foci (SAHF), which colocalize with trimethylated histone H3 lysine 9 (H3K9me3) and heterochromatin protein 1 and suppress the transcription of proliferation-related genes. Moreover, downregulation of the nuclear lamina protein Lamin B1 also serves as a marker for senescent cells. Of these, senescence-associated β-galactosidase (SA-β-gal) staining is the most common and popular marker for identification of senescent cells. SA-β-gal activity is based on upregulation of the lysosomal β-galactosidase gene (GLB1) and is detectable at pH 6. However, SA-β-gal activity is not specific for senescent cells. Therefore, a combination of several markers is needed to identify senescent cells.

In 2005, several papers reported that cellular senescence occurs in premalignant tumors and benign tumors to prevent cancer development. Coinciding with these reports, it was demonstrated that double knockout of p16INK4a and p21WAF1/CIP1 genes increased the rate of cancer development. These data strongly indicated that cellular senescence is an essential tumor suppression mechanism in vivo. Mice have also been generated to visualize the dynamics of p16INK4a and p21WAF1/CIP1 expression during the aging process. Real-time imaging analysis revealed that senescent cells accumulated in benign tumors and throughout the body with age, suggesting that senescent cells may also have biological roles in aged tissues of the living body.

In the recent decade, many studies have demonstrated that senescent cells secrete a variety of proteins, such as inflammatory cytokines, chemokines, growth factors and MMP. Collectively, this phenotype was termed senescence-associated secretory phenotype (SASP). There is a myriad of physiological activity associated with SASP factors (Figure 2). At first, SASP factors secreted from senescent cells were regarded as paracrine and autocrine enhancers of tumor suppressive effects in cellular senescence to prevent the growth of damaged cells. In addition to this function, SASP...
factors can recruit immune cells to clear the senescent cells, which is termed "senescence-surveillance." Senescence surveillance results in clearance of the senescent cells and stimulates the local immune reaction to eliminate oncogene-expressing cells. Thus, SASP factors might contribute to a fail-safe mechanism of cellular senescence. Furthermore, it has been reported that some SASP factors can promote tissue repairing under a variety of conditions. Demaria et al demonstrated that platelet-derived factor-AA (PDGF-AA) was secreted from senescent skin fibroblasts in the vicinity of damaged tissues to promote optimal wound healing. In response to liver injury, hepatic stellate cells (HSC) began to proliferate and produce extracellular matrixes for tissue repair before undergoing cellular senescence to prevent fibrosis. Moreover, these senescent HSC secreted growth factors, thereby promoting cell proliferation for tissue regeneration. In addition to tissue repair, SASP factors are involved in embryonic development of the apical ectodermal ridge and neural roof plate.

In contrast to the beneficial effects of SASP in the living body, long-term survival of senescent cells also drives age-related diseases, such as atherosclerosis, neuropsychiatric disorders, chronic nephritis and cancer. During the aging process, declining immune function leads to failures in senescence surveillance, resulting in accumulation of senescent cells. Therefore, prolonged secretion of SASP factors, such as interleukin (IL)-1β and MMP, could drive cancer development. Intriguingly, it has been reported that upregulation of IL-8 in senescent carcinoma-associated fibroblasts promotes invasion and metastasis of pancreatic cancer cells. Our previous studies have established that IL-1β secreted from senescent HSC
in the liver plays an essential role in promoting obesity-associated hepatocellular carcinoma (HCC) in the obese mice model. Increased levels of deoxycholic acid (DCA), a gut bacterial metabolite derived from altered gut microbiota in obesity, induced cellular senescence and production of SASP factors in HSC, thereby promoting development of obesity-associated HCC. Moreover, it was found that expression of COX-2, a rate-limiting enzyme involved in prostaglandin biosynthesis, significantly increased in senescent HSC. COX-2 induces overproduction of prostaglandin $E_2$ (PGE$_2$), resulting in suppression of anti-tumor immunity and progression of obesity-associated HCC. Remarkably, cellular senescence has beneficial and harmful effects on cancer development via SASP.

3 | MOLECULAR MECHANISM OF SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE INDUCTION

According to previous reports, numerous mechanisms have been implicated in the regulation of SASP factors. First, the transcription factors nuclear factor $k$B (NF-$k$B) and CCAAT/enhancer-binding protein (C/EBP-$\beta$) were identified as positive regulators of SASP factor expression. Second, elevated IL-1$\alpha$ in the early stage of cellular senescence initiates the inflammatory signaling cascade and forms a positive feedback system to amplify SASP signaling.

Notably, gene expression of SASP factors is regulated by epigenetic mechanisms. It has been previously reported that persistent DNA damage leads to proteasomal degradation of the major histone H3K9 dimethyltransferases, G9a and GLP, causing increased SASP factor genes in senescent cells. Other epigenetic regulators, such as macroH2A1, high mobility group box 2 (HMGB2), mixed-lineage leukemia 1 (MLL1) and bromodomain-containing protein 4 (BRD4), have been demonstrated as regulators of SASP factor gene expression in response to DNA damage. In addition, the nutrient sensing pathways, mTOR and NAD, are involved in SASP regulation.

Recently, it has become apparent that toll-like receptors (TLR), an innate immune receptor, can trigger SASP induction. TLR are pattern-recognition receptors that can recognize microbe-specific molecular signatures, known as pathogen-associated molecular patterns (PAMP) and self-derived molecules from damaged cells, known as damage-associated molecules patterns (DAMP). A recent study by Hari et al indicated that the acute-phase serum amyloids A1 and A2 function as DAMP and are recognized by TLR2. Thereafter, TLR2 initiates the inflammatory signaling cascade and induces SASP. It has also been reported that TLR2 is stimulated by lipoteichoic acid (LTA), a component of the cell wall of gram-positive bacteria. TLR2 stimulation induces expression of SASP factors through the NF-$k$B signaling pathway to promote development of obesity-associated HCC. In concordance with these findings, HMGB1 was also recognized by TLR4, resulting in increased SASP factor secretion. Taken together, these results indicate that senescent cells secrete pro-inflammatory factors in response to genotoxic stress and this may be associated with pathobiological effects in the cancer microenvironment.

4 | A NOVEL PATHWAY FOR SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE INDUCTION: CGAS-STING SIGNALING

The antiviral cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway has recently been identified as a SASP regulator. cGAS was originally observed as a cytosolic DNA sensor that recognizes pathogenic DNA and induces innate immune responses. Once cGAS detects pathogenic DNA, it generates cyclic GMP-AMP (cGAMP). In turn, cGAMP acts as the second messenger that stimulates STING, leading to recruitment and autophosphorylation of TANK-binding kinase 1 (TBK1). Thereafter, TBK1 phosphorylates interferon-regulatory factor 3 (IRF3) transcription factor, resulting in translocation of IRF3 from the cytosol to the nucleus and stimulating transcription of type-I interferon (IFN). TBK1 also activates NF-$k$B to induce expression of IFN and inflammatory cytokines.

The emerging evidence indicates that the cGAS-STING pathway detects self-derived DNA fragments and activates SASP in senescent cells (Figure 3). There are several possibilities for the candidate ligands that activate the DNA-sensing machinery underlying cellular senescence. Downregulation of Lamin B1 leads to collapse of the nuclear envelope, which can trigger release of chromatin fragments from the nucleus to the cytosol, termed cytoplasmic chromatin fragments (CCF). DNA damage promotes the production of cytosolic DNA, such as CCF, mtDNA, cDNA, micronuclei and nuclear buds. However, the mechanism of cytosolic DNA accumulation in the cytoplasm of senescent cells has remained unclear. Therefore, we focused on this molecular mechanism. In normal cells, the cytoplasmic DNases, DNase2 and TREX1, degrade the double-stranded and single-stranded DNA in the cytoplasm to prevent activation of the innate immune response. However, downregulation of DNase2 and TREX1, which are transcriptional targets of the E2F complex, results in accumulation of cytosolic DNA, promoting SASP in senescent cells.

De Cecco et al reported high transcription levels of the long-interspersed element-1 (LINE-1, also known as L1), a retrotransposable element, in senescent cells. LINE-1 possesses high reverse transcriptase activity, which can transcribe mRNA to cDNA in the cytosol. Therefore, increases in LINE-1 transcription during senescence facilitates accumulation of cDNA in the cytoplasm and triggers cGAS-STING signaling to produce SASP factors.

We propose that senescent cells secrete not only inflammatory proteins but also small extracellular vesicles, such as exosomes. Interestingly, cGAMP is also secreted from cancer cells via SLC19A1, which induces activation of the STING pathway in recipient cells. There is a possibility that senescent cells secrete cGAMP to promote the paracrine innate immune response via SLC19A1.
Chronic inflammation caused by SASP via the cGAS-STING pathway is crucial for the development of HCC in the obesity-induced liver cancer model. Knocking out the STING gene blunted SASP factor production in HSC and attenuated the development of obesity-associated HCC in the mouse model. However, Dou et al. demonstrated that inhibiting the cGAS-STING signaling pathway impaired the immuno-surveillance of senescent hepatocytes and premalignant hepatocytes, resulting in tumorigenesis in the liver. Accordingly, function of the cGAS-STING pathway appears to depend on the biological context (Figure 4). Although short-term exposure to SASP factors may encourage immuno-surveillance and prevent tumorigenesis, persistent exposure to SASP factors may cause tissue damage and chronic inflammation linked to tumor growth.

The importance of STING-associated inflammation has also been reported in lung cancer. Kitajima et al provided evidence that STING expression was silenced in KRAS-LKB1 mutant lung cancer.

**FIGURE 3** The cGAS-STING pathway in senescence-associated secretory phenotype (SASP) regulation. Various stressors such as reactive oxygen species (ROS) or UV irradiation cause accumulation of DNA fragments from nucleic double-strand breaks (DSB), termed cytoplasmic chromatin fragments (CCF), in senescent cells. Damaged mitochondria release mtDNA into the cytoplasm. Long-interspersed element-1 (LINE-1) transcription is upregulated, thereby promoting cDNA production in senescent cells. These DNA fragments are recognized by cGAS to generate 2′3′-cyclic GMP-AMP (2′3′-cGAMP). 2′3′-cGAMP activates both STING and TANK-binding kinase 1 (TBK1), resulting in phosphorylation of IRF3. 2′3′-cGAMP also activates IκBa. These transcription factors enter the nucleus and induce expression of type-I interferon (IFN) and inflammatory cytokines. Both IRF3 and NF-κB induce senescence-associated secretory phenotype (SASP) factors such as IFN-β, IL-6 and IL-8, which are known to induce ROS and maintain cellular senescence.

**5 | THE CGAS-STING PATHWAY AND CANCER**
LKB1 is the upstream activator of AMPK. Double mutation of KRAS and LKB1 genes in lung cancer resulted in failure to respond to immune checkpoint blockade. Reinduction of LKB1 and STING restored the immune checkpoint blockade response and promoted T-cell chemotaxis. Collectively, production of SASP factors via the cGAS-STING signaling pathway plays a prominent role in promoting or inhibiting tumorigenesis depending on the biological context in the cancer microenvironment.

6 | CONCLUSION

In this review, we illustrated that SASP factors can induce immuno-surveillance and act as a tumor suppression mechanism. Conversely, SASP factors may provoke both tumorigenesis and age-related diseases due to exposure to chronic inflammation. Many researchers have attempted to establish senolytic drugs to eliminate harmful senescent cells from aged tissues, thereby preventing cancer and age-related pathologies. Several senolytic drugs, such as ABT-737, BET inhibitors and dasatinib plus quercetin, can selectively induce cell death in senescent cells and effectively improve some symptoms of age-related disease, including cancer, by attenuating SASP factor production. However, the off-target effects of these senolytic molecules are still unknown. In addition, evaluating the efficiency of senolytic therapies in the living body is an important factor to optimize treatment. Thus, we need to carefully consider the clinical application of senolytic drugs for cancer treatment and prevention of age-related disorders. The cGAS-STING pathway may be an alternative potential therapeutic target to control cancer. Based on the role of the cGAS-STING pathway in facilitating anti-tumor immunity and SASP induction, modulation of its activity would be beneficial for cancer immune therapy and to inhibit cancer development. Finally, to extend healthy lifespans via effective therapeutic strategies, significantly more research is necessary to investigate the molecular mechanisms of SASP regulation.

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DISCLOSURE

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