Cellular senescence and the tumour microenvironment
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1. Introduction

Cellular senescence is a phenotype of irreversible cell cycle arrest, and was originally discovered by Hayflick and Moorhead [1] in somatic cells that reached a finite lifespan following several passages. Cellular senescence was initially shown to function as a key mechanism of tumour suppression [2,3]. More recently, however, the chronic and persistent influence of senescent cells on tissue homeostasis has attracted attention with the discovery of the senescence-associated secretory phenotype (SASP) [4-6]. It has become apparent that SASP is often induced when the innate immune system activates NF-κB-associated and/or IFN-associated signalling, both of which induce the expression of genes that encode a series of inflammatory factors [3,7].

In this review, we provide an overview of cellular senescence and the SASP, particularly in relation to the potential clinical investigations of cancer therapies that utilise senescent cells. We also review the recently emerging role of the SASP in stromal as well as tumour cells in remodelling the tumour microenvironment, with

Abbreviations
- AA, arachidonic acid; BETd, BET family protein degrader; C/EBPβ, CCAAT/enhancer-binding protein beta; CAF, cancer-associated fibroblast; CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; CDKI, cyclin-dependent kinase inhibitor; cGAS, cyclic GMP-AMP synthase; CKIα, casein kinase 1 α; CXCL1, C-X-C motif chemokine ligand 1; DAMP, danger-associated molecular pattern; DCA, deoxycholic acid; EMT, epithelial–mesenchymal transition; GATA4, GATA-binding protein 4; H3K27, histone 3 Lys27; HFD, high-fat diet; HSCs, hepatic stellate cells; IFN-I, type-I interferon; IL6, interleukin-6; JAK, Janus kinase; LTA, lipoteichoic acid; MAMP, microbe-associated molecular pattern; MDSCs, myeloid-derived suppressor cells; Mø, macrophage; NHEJ, nonhomologous end-joining; NK, natural killer cells; OIS, oncogene-induced senescence; PAMP, pathogen-associated molecular pattern; PGE2, prostaglandin E2; SASP, senescence-associated secretory phenotype; SC, senescent cells; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; T, T cells; TGF-β, transforming growth factor-β; TIS, therapy-induced senescence; Treg, regulatory T cells; VEGF, vascular endothelial growth factor.
emphasize on their context-dependency that determines whether they promote or suppress cancer development.

2. Regulation of cellular senescence and the SASP

Cellular senescence is a state of permanent cell proliferation arrest [1] that can be induced by a variety of cellular stresses, including persistent DNA damage caused by, for example, oxidative stress and DNA replication errors (Box 1). When irreparable DNA damage is caused by, for example, oxidative stress and DNA cellular stresses, including persistent DNA damage persists, the expression of various p53-target genes are induced, including cyclin-dependent kinase inhibitor (CDKI), p21 (Cip/Kip family) and the INK4 family CDKI p16, which is upregulated by Ets family of transcription factors [8]. These CDKIs collaboratively and persistently arrest the cell cycle, which is the fundamental mechanism that induces cellular senescence (Fig. 1).

The p53 and p16 genes are also frequently inactivated in various human cancers [2], highlighting the importance of these senescence-inducing pathways for tumour suppression. p53 [9] and INK4a (p16) gene loci [10] are frequently mutated in human cancers. In addition, hypermethylation of the p16 gene promoter that silences p16 gene expression is often detected in human cancers [11], illustrating that these cancer cells have lost the cellular senescence-inducing mechanism [12]. Senescent cells are not simply dormant, nonproliferating cells but are metabolically active and secrete a variety of proteins [13]. This phenotype is called the SASP, and is a very important trait of mature senescent cells, which are characterised by their increased secretion of numerous bioactive factors, including cytokines, chemokines, proteases and growth factors [14,15]. More recently, bioactive lipid mediators, such as prostaglandins and exosomes, have been recognised as also being SASP-associated factors [15-17]. The SASP can influence many types of cells that reside in the vicinity of senescent cells, including fibroblasts, immune cells, vascular endothelial cells and tumour cells, in a paracrine manner, activating signalling pathways in the tissue microenvironment [18]. SASP factors can also induce a variety of physiological outcomes, including beneficial effects such as tissue repair [19] and detrimental effects such as tumorigenesis [14], depending on the cell type, stimulus and biological context. Some of this diversity in cultured cells is summarised in the SASP atlas showing that distinct SASP factors are produced depending on cell types [20]. However, even more complex SASP outcomes might occur in vivo in the contexts of disease or ageing. Thus, targeting the SASP in vivo is an emerging but important aspect of senescence research, since it

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**Box 1. Types of cellular senescence.**

Senescence can be classified at least into three subgroups; replicative senescence, oncogene-induced senescence (OIS), and stress-induced premature senescence (SIPS). Although these share many key regulatory mechanisms of senescence-associated cell cycle arrest and all result in permanent cell cycle arrest, other senescence-associated phenotype, such as the SASP, can be greatly different in these three different types of senescent cells. Cellular senescence that is induced after finite number of cell division in normal cells under normal condition is called replicative senescence. This is the most classical form of cellular senescence and is the one discovered by Hayflick and Moorhead [1]. At least in human fibroblasts in vitro, the major driver of replicative senescence is telomere shortening. Mouse fibroblasts and most epithelial cells in vitro undergo replicative senescence without critical telomere shortening, probably due to the activation of stress signals under nonoptimal culture condition [140,141]. Especially, oxidative stress that is caused by ambient oxygen, which is higher than in vivo, can be the major driver of replicative senescence in mouse cells. High levels of oxidative stress or other genotoxic stress can trigger acute cellular senescence response that is called SIPS. Cellular senescence observed in normal ageing often harbours persistent DNA damage foci at telomere region [142]. It seems that this is because DNA damage formed at the telomere region is more difficult to repair and it is not because of telomere shortening. Surprisingly, it is still not clear what type of stress is responsible for the age-related accumulation of senescent cells. OIS is a form of cellular senescence that is triggered by oncogene activation [143] and is a major anti-tumour mechanism which also explains why cellular senescence machinery has evolved. Oncogene-induced senescent cells are found in many precancerous lesions [144-146] and have significant effects on the tumour microenvironment and tumour development, as discussed in detail in this review. Cellular senescence induced by cancer therapy is called therapy-induced senescence (TIS), which at least in some cases is identical to SIPS. Therapy-induced senescent cells also have significant effects on the tumour microenvironment and tumour development. TIS/SIPS are a promising target for future cancer treatment.
could lead to therapies for diseases including cancer [7]. The basic features of senescent cells are summarised in Box 2 and Fig. 2 as markers of senescent cells. However, it should be noted that none of these markers is solely specific to senescent cells. Therefore, it is important to combine several markers in order to precisely identify senescent cells.

The induction of SASP factors, which include many inflammatory cytokines and chemokines, is regulated transcriptionally and post-transcriptionally and requires the activation of NF-κB, thereby eliciting an inflammatory cascade [6,21]. IL-1α plays a primary role in promoting NF-κB signalling by upregulating many SASP factor genes [22]. The inflammasome,
through which IL-1β is activated, has also been shown to induce SASP factor genes [15,23]. Other factors that can induce the expression of SASP factor genes include the transcription factors CCAAT/enhancer-binding protein beta (C/EBPβ) [4,24] and GATA binding protein 4 (GATA4) [25], particularly in oncogene-induced senescent cells. For example, GATA4, whose levels increase during ageing or upon total body irradiation, is stabilised by the DNA damage response and has been shown to collaborate with NF-κB to induce the expression of SASP factors at least in vitro [25]. C/EBPβ and c-Myc also collaborate with NF-κB to induce the expression of SASP factors [4]. In contrast, NOTCH1 can repress C/EBPβ activity and inhibit pro-inflammatory SASP factor secretion in fibroblasts in vitro and in vivo [26]. Suppression of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, that promotes cytokine expression, has been shown to alleviate the SASP in vitro and frailty in vivo in aged mice [27]. SASP factors are also regulated post-transcriptionally, for example, by the RNA-binding protein ZFP36L1, which stabilises SASP mRNA transcripts following the mTOR-mediated activation of MK2 at least in cultured fibroblasts [28]. mTOR and p38MAPK [23] signalling have also been shown to play a crucial role in the regulation of SASP factor expression in cultured fibroblasts. Accordingly, the inhibition of the mTOR pathway by Rapamycin represses SASP factor expression [28].

The SASP is also epigenetically regulated [3,29]. For example, our group has demonstrated that DNA damage response-driven reduction of histone H3K9 dimethylation in the promoter regions of key SASP factor-encoding genes contributes to SASP factor de-repression in cultured fibroblasts as well as in mouse skin papilloma in vivo [30]. Another example showed that pro-inflammatory cytokines in a mouse model of gastric cancer downregulate EZH2, a histone H3K27 methyltransferase in senescent cancer-associated fibroblasts (CAFs). This downregulation maintains SASP factor expression by demethylating H3K27me3 to enhance peritoneal tumour formation in gastric cancer through JAK/STAT3 signalling [31]. Moreover, the histone variants macro-H2A.1 [32] and H2A.J, which accumulate in senescent cells, are also associated with SASP factor induction [33]. Furthermore, the chromatin reader BRD4 has been shown to remodel the enhancer landscape to activate the expression of key SASP factor genes in senescent cells [34]. Understanding how these factors and their upstream signals regulate the SASP might enable cancer treatment that targets the SASP, thereby remodelling the tumour microenvironment (Fig. 3).

3. SASP induction mediated by the innate immune response

Innate immunity has recently emerged as an important mechanism for inducing the expression of SASP factors. The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway plays an important role in the activation of type-I interferon signalling in senescent cells, which is associated with subsequent SASP factor expression. cGAS-STING pathway is triggered by DNA damage-associated double-strand DNA fragment, which has been identified as an important intrinsic mechanism for induction of a variety of SASP factors. However, in vivo, there should be many potential extrinsic triggers such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) including lipoteichoic acid (LTA) a cell wall component of gram-positive bacteria, for SASP factor induction [15]. Moreover, as discussed below, innate immunity can be activated by ageing or obesity, which are both major risk factors for cancer. The prolonged inflammation in ageing and obesity is now understood as a concept of ‘inflammaging’, which is also triggered by ageing-associated innate immune response. We discuss it in the last part of this section (Fig. 4).
3.1. Intrinsic mechanism: cGAS-STING-mediated regulation

Originally, cGAS was identified as a sensor of cytosolic DNA, which is recognised as being a potential indicator of an invading pathogen (since DNA typically exists only in the nucleus or mitochondria). The cytosolic DNA, from whatever origin, can be recognised as DAMP that triggers the innate immune response [35]. cGAS is a sensor that binds cytosolic DNA independently of DNA sequence to induce a conformational change in the catalytic unit of the cGAS enzyme that creates cyclic dinucleotides, such as cyclic GMP-AMP (cGAMP) [36]. These cyclic dinucleotides act on STING, thereby inducing type-I interferon signalling [36]. In senescent cells, DNA fragments are continuously released into the cytoplasm because of the fragility of the nuclear membrane and reduced lamin B1 expression [37]. At least part of these DNA fragments exists in the form of large cytoplasmic chromatin fragments (CCFs). CCFs are lamin A/C-negative and strongly γH2AX-positive, and are generated by a nucleus-to-cytoplasm blebbing of chromatin [38-40]. Normally, cytosolic DNA is degraded by DNases, such as DNase2 and TREX1, or by autophagy/lysosomal pathways [36,41]. However, these DNases are downregulated in senescent cells, leading to the accumulation of cytosolic DNA fragments and the subsequent abnormal activation of the cGAS-STING pathway [41]. Micronuclei also act as stimulators of the cGAS-STING pathway. Micronuclei are small DNA aggregates often produced by chromosomal damage after genotoxic stress, and are observed in senescent cells [38,42,43].

In addition to these cytoplasmic factors, cDNA transcribed from the mRNA of LINE-1 (LINE-1 are retrotransposable elements that contain reverse transcriptase genes) also accumulates in the cytoplasm of senescent cells [44]. LINE-1 is transcriptionally de-repressed in senescent cells and activates a type-I interferon (IFN-I) response, contributing to the persistence of the SASP through the cGAS-STING pathway [44]. These events are observed in many tissues of aged wild-type mice [45]. However, when aged mice are treated with an inhibitor of nucleoside reverse transcriptase, IFN-I activation and age-associated inflammation (inflammaging) are both downregulated in various tissues [45]. Increased LINE-1 cDNA has also been observed in SIRT6-deficient mice, which show accelerated ageing [45]. Together, these studies suggest that the abnormal accumulation of cytosolic DNA stimulates the cGAS-STING pathway, thus contributing to the induction of the SASP. Other innate immune receptors, such as TLR1 [46] and TLR2 [47] are also reportedly associated with cellular senescence and the SASP via their ligand-mediated signalling and can be important SASP regulators in tumour microenvironment as discussed in detail below.

3.2. Extrinsic SASP induction via gut-derived MAMPs and PAMPs

Microbial-associated molecular patterns (MAMPs) and PAMPs are innate immune-activating molecules...
that derive from microbiota and pathogen, respectively, and are linked to senescence and SASP because senescent cells are highly sensitive to innate immune responses. Both intrinsic (e.g. host-derived cytoplasmic DNA) and extrinsic ligands (e.g. gut microbiota-derived lipoteichoic acid) that stimulate innate immunity can promote the SASP. Our group has previously shown that deoxycholic acid (DCA), an obesity-associated gut microbial metabolite, induces cellular senescence and the tumour-promoting SASP in mouse hepatic stellate cells (HSCs) by persistent DNA damage due to reactive oxygen species (ROS) production in the obesity-associated liver tumour microenvironment [48]. In high-fat diet (HFD)-fed mice, gram-positive gut microbiota abundance is greatly increased, and lipoteichoic acid (LTA), a cell wall component of gram-positive bacteria, accumulates in their livers, triggering the SASP, especially in the liver tumour microenvironment. HFD-fed mice lacking TLR2, a receptor that recognises LTA, develop significantly fewer liver tumours than HFD-fed wild-type mice. LTA-TLR2 signal activates COX2 in DCA-induced senescent HSCs and thereby enhances prostaglandin E2 production, which then suppresses the anti-tumour function of CD8+ T cells. Thus, LTA, derived from gram-positive gut microbiota in HFD-fed mice, acts as an extrinsic factor for SASP induction. PGE2, as a SASP factor generated by senescent HSCs, is crucial for suppressing anti-tumour immunity mediated by CD8 T lymphocytes [15]. COX-2 upregulation and the overproduction of PGE2 have also been observed in human nonalcoholic steatohepatitis-associated liver cancer [15]. Moreover, TLR2 [47,49] and TLR4 [50] are also reportedly activated in senescent cells, indicating their potential involvement in the extrinsic control of SASP induction [50].

3.3. Inflammaging

Chronic inflammation, caused by ageing and obesity, can promote cancer progression in many different ways [51], for example, by altering cell proliferation, cell survival, cell renewal, epithelial-mesenchymal transition (EMT), angiogenesis, migration and...
immunosuppression, similarly to the SASP. Low-grade, chronic and sterile systemic inflammation, called inflammaging, is a feature of ageing and obesity [52] and is associated with the SASP in the senescent cells of aged organisms. The age-related activation of inflammatory genes has been observed in many tissues in both mice and humans [53-56]. For example, NF-κB, a pro-inflammatory transcription factor that plays a key role in the SASP, reportedly drives age-related gene expression changes in numerous mouse and human tissues [57], implicating the role of senescent cells in age-related, inflammatory gene activation. Some inflammatory factors can also be inhibited with senolytic drugs that specifically kill senescent cells. For example, when the senolytic drugs, dasatinib and quercetin, were given to mice in combination, they suppressed the age-associated upregulation of the cytokine IL-6 in adipose tissue [58]. It should be noted that age-related increases in DAMPs, MAMPs and PAMPs can also trigger the systemic activation of NF-κB and inflammation [59]. Ageing also diminishes apoptotic cell clearance [60], and immunogenic mtDNA increases in the blood with age, both of which produce DAMPs to trigger innate immunity [61]. Increased levels of lipopolysaccharide (LPS)-binding protein, a surrogate marker for LPS and other bacterial products, are also found in the blood of the elderly, and indicate gut barrier dysfunction [62]. All these factors can activate NF-κB and inflammation. Age-related immune dysfunction, namely immunosenescence, is also likely to play a role in inflamming as discussed later in this review [63]. In conclusion, inflammatory status of the

| Type of cancer | Senescent cell | Senescence inducer | Major role(s) of the SASP | SASP factor(s) |
|---------------|----------------|--------------------|---------------------------|---------------|
| Anti-tumorigenic SASP | Hepatocyte | Hepatocyte | OIS (N-Ras) | Immune-mediated senescent cell clearance | IL-1α [71] |
| Lymphocyte | Lymphocyte | TIS (cyclophosphamide) | Reinforce cellular senescence | ND [21] |
| Melanocyte | Melanocyte | TIS (AURKA or CDK4/6 inhibitor) | Lymphocyte recruitment | CCL5 [150] |
| Melanocyte | Melanocyte | TIS (Aurora inhibitor) | Reinforce cellular senescence | ND [151] |
| Osteoblast | Osteoblast | TIS (Irradiation) | Increased vascularity and improved drug delivery efficacy | IL-6 [152] |
| Pancreatic ductal cell | Pancreatic ductal cell | TIS (MEK and CDK4/6 inhibitors) | Endothelial cell activation followed by accumulation of CD8+ T cells | VEGF [15] |
| Hepatocyte | Hepatocyte | OIS (N-Ras) | Myeloid cell recruitment followed by macrophage differentiation under precancerous environment | CCL5, CXCL1, IL-6 [15] |
| Pro-tumorigenic SASP | Hepatocyte | Hepatocyte | OIS (N-Ras) | Myeloid cell recruitment followed by MDSC differentiation under cancerous environment | CCL2 [70] |
| Hepatocyte | Hepatic stellate cell | HFD-induced senescence | ND | IL-1β [48] |
| Lymphocyte | Lymphocyte | TIS (doxorubicin) | Stemness induction | ND [153] |
| Mammary epithelial cell | Mammary epithelial cell | TIS (doxorubicin) | Mitogenic support | Eotaxin, CXCL5, Rantes [154] |
| Mammary epithelial cell | Fibroblast | DNA damage (bleomycin) | Promotion of cancer invasion | MMPs [155] |
| Mammary epithelial cell | Mammary epithelial cell | OIS (HER2) | Metastasis support | ND [156] |
| Melanocyte | Fibroblast | TIS (CDK4/6 inhibitor) | Myeloid cell recruitment | ND [110] |
| Mesothelial cell | Mesothelial cell | TIS (pemetrexed) | EMT induction and chemoresistance | ND [157] |
| Prostate epithelial cell | Prostate epithelial cell | TIS (PTEN loss) | Myeloid cell recruitment followed by reinforcement of senescence | CXCL1, CXCL2 [158] |
| Prostate epithelial cell | Prostate epithelial cell | TIS (PTEN loss) | MDSC recruitment | ND [121] |
| Thyroid follicular cell | Thyroid follicular cell | OIS (BRAF) | Anoikis resistance | CXCL12 [159] |
microenvironment, which is highly context-dependent, is a crucial upstream regulator of the SASP.

4. Context-dependent effects in the tumour microenvironment

The impact of SASP on the tissue microenvironment in vivo varies depending on the types of cells undergoing senescence (e.g. any of the stromal or epithelial cells, and normal or cancer cells can be senescent), and the cause of senescence (see Box 1), or the trigger of the innate immune pathway for SASP (e.g. cGAS-STING, TLRs, etc.). The SASP not only reinforces cellular senescence in an autocrine manner [4] but also mediates its paracrine effects. SASP factors can remodel tissues in a paracrine manner, for example, by altering the ability of proliferation and migration of adjacent cells, such as stromal cells, immune cells and cancer cells [3,14,64]. SASP factors can also promote angiogenesis and enhance the immunosuppressive microenvironment [65,66]. Overall, the complexity of the context-dependent effects of SASP on the tissue microenvironment highlights the importance of identifying cells that produce specific SASP factors for therapeutic targeting [14,29] (Table 1, Fig. 5A,B).

It is important to note that it is not only the constituents of the SASP are context-dependent but that even a single SASP factor can be pro- or anti-tumorigenic depending on the biological context. For example, the most renowned SASP factor, IL6, helps in establishing cellular senescence through an autocrine effect [6], and can also promote EMT and invasion of cancer cells in vitro [5]. SASP induced by CKIα knockout in intestinal epithelial cells also contribute to reinforcing cellular senescence in normal cells; however, it promotes cell proliferation of p53-mutated precancerous cells [67]. VEGF and TGF-β secreted by senescent cells can induce cellular senescence of the surrounding normal cells at least in vitro [18]. In the tumour microenvironment, however, VEGF secreted by senescent cells may promote angiogenesis [68] and TGF-β secreted by senescent cells may promote extracellular matrix (ECM) deposition, cell migration, and metastasis [69]. CCL2 secreted by senescent hepatocytes recruits CCR2+ myeloid cells, which then become mature and eliminate senescent cells and thereby suppress cancer development. However, this maturation is blocked under the presence of abundant HCC cells and in this case CCR2+ myeloid cells promote cancer progression by suppressing NK cells [70].

4.1. Senescence surveillance in precancerous cells

Cellular senescence is an important tumour suppression mechanism (see Fig. 5A), and when the senescence-inducing machinery in epithelial cells is disrupted, it can lead to the onset of cancer. Another
important tumour prevention mechanism mediated by senescence is the clearance of premalignant cells when they undergo cellular senescence [71,72]. This clearance system for senescent cells is called ‘senescence surveillance’ (Fig. 6A). Premalignant, senescent hepatocytes, which are transduced with oncogenic N-ras in vivo have been shown to be cleared by an antigen-specific immune response, which limits the development of liver cancer [70,71]. This suggests that precancerous cells with senescent cell features tend to be eradicated by immune cells recruited by SASP factors. Indeed, a recent study has shown that senescent cells can be cleared by CAR-T cells, which recognise a senescent cell-specific cell-surface marker [73].

5. Senescent cancer-associated fibroblasts in the tumour microenvironment

Tumour tissues consist of cancer cells and many other cell types, such as immune cells, stromal cells and vascular epithelial cells. Among these, CAFs have recently emerged as a cell type that promotes cancer cell expansion in the tumour microenvironment. CAFs are often senescent and are associated with the inflammatory SASP (Fig. 6B) [74,75]. Inflammatory CAFs in the tumour microenvironment have recently been designated iCAFs [75], and are similar to senescent CAFs exerting SASP [76-78]; myofibroblastic CAFs have been designated myCAFs [76,78]. MyCAF populations display ligand-receptor interactions that are distinct from those of iCAFs. iCAFs appear to be more tumour-promoting than myCAFs by producing chemokines and cytokines [79] showing a higher malignancy in pancreatic tumorigenesis [80]. On the other hand, myCAFs may produce ECM extensively to impede drug delivery despite being less cancer-promoting [81]. iCAFs and myCAFs seem to exclusively act on the surrounding cells.

Senescent CAFs in the tumour microenvironment often play a role in tumour progression. For example, in HFD-fed mice, the entero-hepatic circulation of DCA elicits DNA-damage-associated cellular senescence and the SASP in HSCs [15,48]. In HFD-fed mice that lack IL-1β, an upstream regulator of the cytokine cascade, HSCs in the liver developed a senescence phenotype but show reduced expression of SASP factors [48]. These mice also showed a decline in liver tumour formation, suggesting that the IL-1β-mediated pathway in HSCs plays a role in obesity-associated liver tumour progression. These results suggest that senescent HSCs function as senescent CAFs and play a key role in obesity-associated liver cancer development through the secretion of SASP factors.

Radiotherapy and chemotherapy can both cause therapy-induced senescence (TIS) (see Box 1) due to the DNA damage induction in tissue-resident fibroblasts, which then become senescent CAFs via the DNA damage response [75,82]. These senescent CAFs are often resistant to chemoradiotherapy and have a SASP phenotype that induces the production of pro-tumorigenic factors, such as IL-6 and IL-8, which are associated with immunosuppression and with stroma-mediated therapeutic resistance [83-88]. TIS in stromal cells has been reported to cause undesirable roles such as breast cancer metastasis and therapy resistance in mouse models [83,84]. In addition, senescent CAFs have been observed in the microenvironment of colon [89] and pancreatic tumours in mice, with p38 MAPK signalling inducing the pro-tumorigenic SASP [90]. The upregulation of IL-8, a well-known SASP factor,
in senescent CAFs permits pancreatic ductal adenocarcinoma (PDAC) cells to invade or metastasise [91,92]. The presence of senescent CAFs have also been associated with reduced survival in patients with early stage of pancreatic tumours, showing their effect on cancer prognosis [92], and senescent CAFs also promote EMT and pancreatic cancer progression in mice [93]. As previously mentioned, inflammation (e.g. persistent cytokine secretion) itself can induce cellular senescence and the SASP via paracrine manner. Intriguingly, a recent study suggested that such sequence and persistence of SASP has also been associated with long Covid-19 syndrome [94].

6. CDK4/6 inhibitors and SASP

The activation of cyclin D and CDK4/6, which play pivotal roles in the transition from G1 to S phase, is a feature of many cancer cell types, particularly breast cancer cells [95-97], and so both have been targeted therapeutically. For example, CDK4/6 inhibitors, such as palbociclib, abemaciclib, and ribociclib, are used clinically to treat oestrogen receptor-positive (ER⁺) and human epidermal growth factor receptor 2-negative (HER2⁻) breast cancer [98]. Since CDK4/6 inhibitors mimic the function of p16 [99], the induction of cellular senescence is a likely outcome of this treatment (Fig. 7A). Indeed, these CDK4/6 inhibitors have been shown to induce cellular senescence in many cancer types such as breast cancer (in vitro and in vivo), Ewing sarcoma (in vitro), and neuroblastoma (in vitro) [100-106], although in these studies senescence induction has mainly been judged by SA-β-galactosidase positivity, which can be an indicator of cellular senescence but is not an absolute marker solely because it is also known to be positive in some quiescent cells [107].

CDK4/6 inhibitors not only induce tumour cell cycle arrest but also promote anti-tumour immunity, as they upregulate the expression of endogenous retroviral elements in tumour cells, producing increased intracellular levels of double-stranded RNA, thereby stimulating the production of type III interferons, which in turn enhance tumour antigen presentation [100]. In addition, CDK4/6 inhibitors strongly suppress the proliferation of Treg cells. Eventually, these phenotypic changes promote the cytotoxic T-cell-mediated clearance of tumour cells, enhances the efficacy of cancer therapy by adding the treatment using immune checkpoint blockade. Other reports also suggest that premalignant senescent cells induced by CDK4/6 inhibition do not acquire pro-tumorigenic and detrimental properties in patients with breast cancer patients, suggesting that these effects could be beneficial for senescence surveillance [100]. Senescence-inducing CDK4/6 inhibitors when combined with other types of therapies have also been shown to be effective in a mouse model of PDAC. In this mouse model, CDK4/6 inhibitors in this mouse model induced the SASP in PDAC cells, increasing vascularity and the immune response in the PDAC tumour microenvironment. This initial microenvironmental remodelling improved the efficacy of VEGF inhibitors and immune checkpoint blockade [64]. SASP-mediated improvement of immune checkpoint blockade efficacy was also observed in a topoisomerase I inhibitor- and cisplatin-treated senescent ovarian cancer model [108,109]. These findings suggest that TIS can promote immunotherapy. It should also

![Fig. 7. SASP in cancer therapy. (A) Schematic illustration of the effect of CDK4/6 inhibitor. (B) Schematic illustration of how the combination of chemotherapy and senolysis contribute to cancer therapy.](image-url)
be noted, however, that senescence of fibroblasts caused by prolonged exposure to CDK4/6 inhibitor can accompany the SASP that increases and decreases the population of Gr-1-positive immune cells and CD3-positive cells in the tumour microenvironment, respectively, and promotes melanoma growth in mice due to the suppression of anti-tumour immunity [110]. Therefore, although CDK4/6 inhibitors are promising agents for the future senescence-based cancer therapy, further investigation is warranted to establish their effective usage.

7. Senolysis and senomorphics for cancer therapies

Overall, senescent cells exert detrimental effects on the tumour microenvironment, particularly SASP-induced senescent cells in advanced cancers (Fig. 7B) [14]. Given this, senolytic drugs are currently being developed to target senescent cells to eliminate their deleterious effects [111,112]. Senescent cells cease proliferating, exhibit DNA damage response signals, but remain alive by avoiding apoptosis [112]. This is a key trait of cellular senescence, which can be targeted for therapeutic purposes. Dasatinib and quercetin were the first-identified combination of senolytic drugs that could reactivate suppressed apoptotic signals in senescent cells [113]. Inhibitors of B-cell lymphoma-2 (BCL-2)/BCL-XL have also been developed as senolytic drugs [114]. Another recently identified senolytic drug is the BET family protein degrader (BETd), which induces senolysis via two independent pathways: through the inhibition of nonhomologous end-joining (NHEJ) and the upregulation of autophagic gene expression [115]. In senescent cells, only NHEJ is available as a double-stranded DNA break repair system due to the cell cycle arrest that occurs in cellular senescence. Autophagy is also suppressed in senescent cells and is derepressed by BETd to induce cell death by autophagic gene upregulation [115]. BETd treatment has been shown to effectively suppress liver cancer in mice [115]. Another recently reported and effective senolytic drug is an inhibitor of glutaminase 1 (GLS1), which targets the altered metabolic fragility of senescent cells [116]. Immune-based strategies for eliminating senescent cells have also been reported, such as targeting the senescent cell-specific surface marker, uPA receptor, by generating chimeric antigen receptor re-directed (CAR) T cells [73], or by creating a senolytic vaccine that targets both CD153 on senescent T cells [117] or glycoprotein nonmetastatic melanoma protein B (GPNMB) in senescent endothelial cells [118].

A two-step approach has been proposed for using senolysis to target cancer cells: first, cellular senescence is induced in cancer cells, and then senescent cancer cells are eliminated via senolysis. The senolytic drug, ABT263, has been used to successfully treat chemotherapy-induced senescence in cancer cells in a breast and lung cancer xenograft model. Its administration after treatment, together with either etoposide or doxorubicin (DNA-damaging chemotherapeutic agents), resulted in prolonged tumour suppression in tumour-bearing animals [119]. Similar success was achieved by using a senolytic drug, ABT263, in a xenograft model to eliminate senescent malignant meningioma cells induced by gemcitabine treatment followed by ionising radiation [120]. Senolytic therapy following conventional cancer therapy could thus improve therapeutic outcomes and effectively delay disease recurrence.

Since the SASP seems to be mediating many deleterious effects of senescent cells, suppression or modulation of the SASP instead of killing senescent cells could be another promising approach for targeting senescence-associated diseases. Compared to senolytic drugs, drugs targeting the SASP, namely ‘senomorphics’, may only have transient effects but will also have lower cytotoxicity. Moreover, senomorphic drug can even convert ‘bad senescent cells’ into ‘good senescent cells’. For example, JAK2 inhibitor can reprogram immunosuppressive pro-tumorigenic SASP into inflammatory anti-tumorigenic SASP [121]. Drugs that have been shown to extend animal lifespan, such as rapamycin [122] and metformin [123], can also suppress SASP. These drugs are also known as anti-cancer drugs, suggesting that senomorphic effects might contribute to anti-cancer effects.

8. Immunosenescence is associated with impaired anti-tumour immunity

Cellular senescence occurs in human T-cells, causing the immune system to become dysregulated during normal ageing [124]. This is known as immunosenescence and it plays an important role in tumour development [125,126]. In humans, the thymic generation of new naive T cells dramatically decreases by middle age [127,128] and together with the age-associated clonal expansion of T cells, diminishes the T-cell receptor repertoire [128]. Notably, the incidence and prevalence of cancer also increase with age, possibly due to an increase in senescent T-cell population in aged people [129,130].

Senescent CD8+ T-cell population increase in aged people, in younger individuals with chronic viral
infections, and in patients with certain types of cancers [124,131]. Moreover, Treg cells (natural Tregs or nTregs, and tumour-derived Tregs), as well as tumour cells, suppress the function of naive and effector T cells by inducing T-cell senescence [126,132,133,134]. These senescent T cells exhibit an altered phenotype that exerts suppressive activity on anti-tumour immunity in the tumour microenvironment. A recent study has found that T-cell senescence, induced by Tregs and tumour cells, is mediated by the dysregulation of lipid metabolism and can be reversed pharmacologically. This study also showed that normalising lipid metabolism and reversing senescence reduced tumour progression and extended survival in mouse models of melanoma and breast cancer [135].

Senescent T cells are metabolically active and have a unique SASP, which influences both immune cells and tumour cells in the tumour microenvironment. Recent studies indicate that senescent T cells induced by Treg and tumour cells can secrete proinflammatory cytokines such as IL-6, IL-8 and TNFα [133,134,136]. These cytokines can induce the premature senescence of the surrounding cells via paracrine mechanisms [4,6,137,138], which can induce more senescent T cells in the tumour microenvironment that provide suppressive anti-tumour immunity. Importantly, high levels of senescent CD8+ T cells predict poor prognosis in several types of cancer, such as lung cancer, gastric cancer, renal cell carcinoma, glioblastoma, non-Hodgkin lymphoma, chronic lymphocytic leukaemia and acute myeloid leukaemia [139].

9. Conclusion and perspectives

The molecular mechanisms of cellular senescence and SASP have long been investigated using cultured cells. However, increasing evidence suggests that the phenotypes of senescent cells, including the SASP, might significantly differ in vitro and in vivo. Clarifying the role of cellular senescence in vivo, particularly in the tumour microenvironment, is crucial for a better understanding of cancer development and cancer therapy. As mentioned, the role of the SASP is highly context-dependent; therefore, elucidating the precise mechanism of cellular senescence and SASP in vivo and the development of appropriate senolytics and senomorphics is of great importance. In this regard, it is particularly important not to forget that removing the SASP is not necessarily harmful in the context of cancer treatment. In such a situation, it could be better to alter the SASP factors rather than just suppress it, to promote immune surveillance, for example. Future studies should enable an appropriate usage of senolytics and senomorphics depending on cancer types. Regarding senolytics, it would be important to investigate its long-term impact on tissue turnover. It might possibly occur that the removal of senescent cells increases the burden on the rest of the cells, which could lead to tissue dysfunction eventually. Further investigation of the effects of senolytics and senomorphics together with a better understanding of context-dependent effects of the SASP in vivo would lead to the development of effective therapy for age-related diseases including cancer.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

NO contributed to conceptualisation; MT, YY and MT contributed to writing – original draft; NO and MT contributed to writing – review and editing and funding acquisition.

Data accessibility

Original data is not provided in this review paper.

References

1 Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25:585–621. https://doi.org/10.1016/0014-4827(61)90192-6
2 Chandler H, Peters G. Stressing the cell cycle in senescence and aging. Curr Opin Cell Biol. 2013;25:765–71. https://doi.org/10.1016/j.cceb.2013.07.005
3 Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. Cellular senescence:
Cellular senescence and the tumour microenvironment

12 Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor p16. *Int J Cancer*. 2012;130:1715–25. https://doi.org/10.1002/ijc.27316

13 Wiley CD, Campisi J. The metabolic roots of senescence: mechanisms and opportunities for intervention. *Nat Metab*. 2021;3:1290–301. https://doi.org/10.1038/s42255-021-00483-8

14 Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer*. 2019;19:439–53. https://doi.org/10.1038/s41568-019-0156-2

15 Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, et al. Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. *Cancer Discov*. 2017;7:522–38. https://doi.org/10.1158/2159-8290.CD-16-0932

16 Takahashi A, Okada R, Nagao K, Kawamata Y, Hanyu A, Yoshimoto S, et al. Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nat Commun*. 2017;8:15287. https://doi.org/10.1038/ncomms15287

17 Wiley CD, Sharma R, Davis SS, Lopez-Dominguez JA, Mitchell KP, Wiley S, et al. Oxylipin biosynthesis reinforces cellular senescence and allows detection of senolysis. *Cell Metab*. 2021;33:1124–36.e1125. https://doi.org/10.1016/j.cmet.2021.03.008

18 Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol*. 2013;15:978–80. https://doi.org/10.1038/ncb2784

19 Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell*. 2014;31:722–33. https://doi.org/10.1016/j.devcel.2014.11.012

20 Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol*. 2020;18:e3000599. https://doi.org/10.1371/journal.pbio.3000599

21 Chien Y, Scuoppo C, Wang X, Fang X, Balgley B, Bolden JE, et al. Control of the senescence-associated secretory phenotype by NF-xB promotes senescence and enhances chemosensitivity. *Genes Dev*. 2011;25:2125–36. https://doi.org/10.1101/gad.17276711

22 Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J. Cell surface-bound IL-lalp is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc Natl Acad Sci USA*. 2009;106:17031–6. https://doi.org/10.1073/pnas.0905299106

23 Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J*. 2011;30:1536–48. https://doi.org/10.1038/emboj.2011.69

24 Flanagan KC, Alspach E, Pazolli E, Parajuli S, Ren Q, Arthur LL, et al. c-Myb and C/EBPbeta regulate the DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science*. 2015;349:aaa5612. https://doi.org/10.1126/science.aaa5612
Cellular senescence and the tumor microenvironment

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26 Hoare M, Ito Y, Kang TW, Weekes MP, Matheson NJ, Patten DA, et al. NOTCH1 mediates a switch between two distinct secretomes during senescence. Nat Cell Biol. 2016;18:979–92. https://doi.org/10.1038/ncb3397

27 Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. Proc Natl Acad Sci USA. 2015;112: E6301–10. https://doi.org/10.1073/pnas.1515386112

28 Herranz N, Gallage S, Mellone M, Wuestefeld T, Klotz S, Hanley CJ, et al. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol. 2015;17:1205–17. https://doi.org/10.1038/ncb3225

29 Birch J, Gil J. Senescence and the SASP: many therapeutic avenues. Genes Dev. 2020;34:1565–76. https://doi.org/10.1101/gad.343129.120

30 Takahashi A, Imai Y, Yamakoshi K, Kuninaka S, Ohtani N, Yoshimoto S, et al. DNA damage signaling triggers degradation of histone methyltransferases through APC/C(Cdh1) in senescent cells. Mol Cell. 2012;45:123–31. https://doi.org/10.1016/j.molcel.2011.10.018

31 Yasuda T, Koiwa M, Yonemura A, Kariya T, Hoare M, Ito Y, Kang TW, et al. LINE1 derepression in aged wild-type and SIRT6-deficient mice drives micronuclei links genome instability to innate immunity. Nature. 2017;548:466–70. https://doi.org/10.1038/nature23470

32 Chen H, Ruiz PD, McKimpson WM, Novikov L, Kitisis RN, Gamble MJ. MacroH2A1 and ATM play opposing roles in paracrine senescence and the senescence-associated secretory phenotype. Mol Cell. 2015;59:719–31. https://doi.org/10.1016/j.molcel.2015.07.011

33 Contrepois K, Coudereau C, Benayoun BA, Schuler N, Roux PF, Bischof O, et al. Histone variant H2A.J accumulates in senescent cells and promotes inflammatory gene expression. Nat Commun. 2017;8:14995. https://doi.org/10.1038/ncomms14995

34 Tasdemir N, Banito A, Roe JS, Alonso-Curbelo D, Camilo M, Tschaharganeh DF, et al. BRD4 connects enhancer remodeling to senescence immune surveillance. Cancer Discov. 2016;6:612–29. https://doi.org/10.1158/2159-8290.CD-16-0217

35 Kumar V. A STING to inflammation and autoimmunity. J Leukoc Biol. 2019;106:171–85. https://doi.org/10.1002/JLB.4MIR1018-397RR

36 Hopfner KP, Hornung V. Molecular mechanisms and cellular functions of cGAS-STING signalling. Nat Rev Mol Cell Biol. 2020;21:501–21. https://doi.org/10.1038/s41580-020-0244-x

37 Freund A, Laberge RM, Demaria M, Campisi J. Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell. 2012;23:2066–75. https://doi.org/10.1091/mbc.E11-10-0884

38 Gluck S, Guey B, Guleen MF, Wolter K, Kang TW, Schmacke NA, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. Nat Cell Biol. 2017;19:1061–70. https://doi.org/10.1038/ncb3586

39 Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, et al. Lysosome-mediated processing of chromatin in senescence. J Cell Biol. 2013;202:129–43. https://doi.org/10.1083/jcb.201211210

40 Miller KN, Dasgupta N, Liu T, Adams PD, Vizioli MG. Cytoplasmic chromatin fragments-from mechanisms to therapeutic potential. Elife. 2021;10: e63728. https://doi.org/10.7554/elife.63728

41 Takahashi A, Loo TM, Okada R, Kamachi F, Watanabe Y, Wakita M, et al. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat Commun. 2018;9:1249. https://doi.org/10.1038/s41467-018-03555-8

42 Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. Nature. 2017;548:466–70. https://doi.org/10.1038/nature23470

43 Mackenzie KJ, Carroll P, Martin CA, Murina O, Fluteau A, Simpson DJ, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature. 2017;548:461–5. https://doi.org/10.1038/nature23449

44 De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NJ, Criscione SW, et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature. 2019;566:73–8. https://doi.org/10.1038/s41586-018-0784-9

45 Simon M, Van Meter M, Ablaeva J, Ke Z, Gonzalez RS, Taguchi T, et al. LINE1 derepression in aged wild-type and SIRT6-deficient mice drives inflammation. Cell Metab. 2019;29:871–85.e875. https://doi.org/10.1016/j.cmet.2019.02.014

46 Jin H, Zhang Y, Ding Q, Wang SS, Rastogi P, Dai DF, et al. Epithelial innate immunity mediates tubular cell senescence after kidney injury. JCI Insight. 2019;4: e125490. https://doi.org/10.1172/jci.insight.125490

47 Hari P, Millar FR, Tarrats N, Birch J, Quintanilla A, Rink CJ, et al. The innate immune sensor toll-like receptor 2 controls the senescence-associated secretory phenotype. Sci Adv. 2019;5:eaaw0254. https://doi.org/10.1126/sciadv.aaw0254

48 Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499:97–101. https://doi.org/10.1038/nature12347
49 Mannarino M, Cherif H, Li L, Sheng K, Rabau O, Jarzem P, et al. Toll-like receptor 2 induced senescence in intervertebral disc cells of patients with back pain can be attenuated by o-vanillin. *Arthritis Res Ther.* 2021;23:117. https://doi.org/10.1186/s13075-021-02504-z

50 Davalos AR, Kawahara M, Malhotra GK, Schaum N, Huang J, Ved U, et al. p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. *J Cell Biol.* 2013;201:613–29. https://doi.org/10.1083/jcb.201206006

51 Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008;454:436–44. https://doi.org/10.1038/nature07205

52 Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000;908:244–54. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

53 Berchtold NC, Cribbs DH, Coleman PD, Rogers J, Head E, Kim R, et al. Gene expression changes in the course of normal brain aging are sexually dimorphic. *Proc Natl Acad Sci USA.* 2008;105:15605–10. https://doi.org/10.1073/pnas.0806883105

54 Peters MJ, Joehanes R, Pilling LC, Schurmann C, Davalos AR, Kawahara M, Malhotra GK, Schaum N, Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Hartikainen AL, et al. A transcriptional profile of aging in the normal brain. *PLoS One.* 2013;8:e54600. https://doi.org/10.1371/journal.pone.0054600

55 Rodwell GE, Sonu R, Zahn JM, Lund J, Wilhelmy J, Wang L, et al. A transcriptional profile of aging in the human kidney. *PLoS Biol.* 2004;2:e427. https://doi.org/10.1371/journal.pbio.0020427

56 Schaum N, Lehallier B, Hahn O, Pálovics R, Hosseinzadeh S, Lee SE, et al. Ageing hallmarks exhibit organ-specific temporal signatures. *Nature.* 2020;583:596–602. https://doi.org/10.1038/s41586-020-2499-y

57 Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Chang HY. Motif module map reveals enforcement of aging by continual NF-kappaB activity. *Genes Dev.* 2007;21:3244–57. https://doi.org/10.1101/gad.1588507

58 Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weívoda MM, et al. Senolytics improve physical function and increase lifespan in old age. *Nat Med.* 2018;24:1246–56. https://doi.org/10.1038/s41591-018-0092-9

59 Huang J, Xie Y, Sun X, Zeh HJ 3rd, Kang R, Lotze MT, et al. DAMPs, ageing, and cancer: the ‘DAMP hypothesis’. *Ageing Res Rev.* 2015;24:3–16. https://doi.org/10.1016/j.arr.2014.10.004

60 Aprahamian T, Takemura Y, Goukassian D, Walsh K. Ageing is associated with diminished apoptotic cell clearance in vivo. *Clin Exp Immunol.* 2008;152:448–55. https://doi.org/10.1111/j.1365-2249.2008.03658.x

61 Pinti M, Cevenini E, Nasi M, De Biasi S, Salvioli S, Monti D, et al. Circulating mitochondrial DNA increases with age and is a familiar trait: implications for “inflamm-aging”. *Eur J Immunol.* 2014;44:1552–62. https://doi.org/10.1002/eji.201343921

62 Gonzalez-Quintela A, Alonso M, Campos J, Vizcaíno L, Loidi L, Gude F. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. *PLoS One.* 2013;8:e54600. https://doi.org/10.1371/journal.pone.0054600

63 Santoro A, Bientinesi E, Monti D. Immunosenescence and inflamming in the aging process: age-related diseases or longevity? *Ageing Res Rev.* 2021;71:101422. https://doi.org/10.1016/j.arr.2021.101422

64 Ruscetti M, JP T, Mezzadra R, Russell J, Leibold J, Romesser PB, et al. Senescence-induced vascular remodeling creates therapeutic vulnerabilities in pancreatic cancer. *Cell.* 2020;181:424–41.e21. https://doi.org/10.1016/j.cell.2020.03.008

65 Lee S, Schmitt CA. The dynamic nature of senescence in cancer. *Nat Cell Biol.* 2019;21:94–101. https://doi.org/10.1038/s41556-018-0249-2

66 Ruhland MK, Coussens LM, Stewart SA. Senescence and cancer: an evolving inflammatory paradox. *Biochim Biophys Acta.* 2016;1865:14–22. https://doi.org/10.1016/j.bjba.2015.10.001

67 Pribulda A, Elyada E, Wiener Z, Hamza H, Goldstein RE, Biton M, et al. A senescence-inflammatory switch from cancer-inhibitory to cancer-promoting mechanism. *Cancer Cell.* 2013;24:242–56. https://doi.org/10.1016/j.ccr.2013.06.005

68 Coppe JP, Kauser K, Campisi J, Beauchesne CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. *J Biol Chem.* 2006;281:29568–74. https://doi.org/10.1074/jbc.M603307200

69 Roberts AB, Wakefield LM. The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci USA.* 2003;100:8621–3. https://doi.org/10.1073/pnas.1633291100

70 Eggert T, Wolter K, Ji J, Ma C, Yevsa T, Klotz S, et al. Distinct functions of senescence-associated immune responses in liver tumor surveillance and tumor progression. *Cancer Cell.* 2016;30:533–47. https://doi.org/10.1016/j.ccell.2016.09.003

71 Wang L, et al. A transcriptional profile of aging in the normal brain are sexually dimorphic. *Proc Natl Acad Sci USA.* 2000;97:1244–57. https://doi.org/10.1073/pnas.97.24.1244

72 Xue W, Zender L, Miething C, Dickins RA, Hernandez E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine
liver carcinomas. Nature. 2007;445:656–60. https://doi.org/10.1038/nature05529

73 Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, et al. Senolytic CAR T cells reverse senescence-associated pathologies. Nature. 2020;583:127–32. https://doi.org/10.1038/s41586-020-2403-9

74 Hassona Y, Cirillo N, Heesom K, Parkinson EK, Prime SS. Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte discohesion and invasion. Br J Cancer. 2014;111:1230–7. https://doi.org/10.1038/bjc.2014.438

75 Ragunathan K, Ufold NLE, Oksenhuy V. Interaction between fibroblasts and immune cells following DNA damage induced by ionizing radiation. Int J Mol Sci. 2020;21:8635. https://doi.org/10.3390/ijms21228635

76 Affo S, Nair A, Brundu F, Ravichandra A, Bhattacharjee S, Matsuda M, et al. Promotion of cholangiocarcinoma growth by diverse cancer-associated fibroblast subpopulations. Cancer Cell. 2021;39:883. https://doi.org/10.1016/j.ccell.2021.05.010

77 Nicolas AM, Pesic M, Engel E, Ziegler PK, Diefenhardt M, Kennel KB, et al. Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. Cancer Cell. 2022;40:168–84.e13. https://doi.org/10.1016/j.ccell.2021.01.004

78 Steele NG, Biffi G, Kemp SB, Zhang Y, Drouillard D, Syu L, et al. Inhibition of hedgehog signaling alters fibroblast composition in pancreatic cancer. Clin Cancer Res. 2021;27:2023–37. https://doi.org/10.1158/1078-0432.CCR-20-3715

79 Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med. 2017;214:579–96. https://doi.org/10.1084/jem.20162024

80 Bernard V, Semaan A, Huang J, San Lucas FA, Mula FC, Stephens BM, et al. Single-cell transcriptomics of pancreatic cancer precursors demonstrates epithelial and microenvironmental heterogeneity as an early event in neoplastic progression. Clin Cancer Res. 2019;25:2194–205. https://doi.org/10.1158/1078-0432.CCR-18-1955

81 Biffi G, Oni TE, Spielman B, Hao Y, Elyada E, Park Y, et al. IL1-induced JAK/STAT signaling is antagonized by TGFbeta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. Cancer Discov. 2019;9:282–301. https://doi.org/10.1158/2159-8290.CD-18-0710

82 Demaria M, O’Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov. 2017;7:165–76. https://doi.org/10.1158/2159-8290.CD-16-0241

83 Bent EH, Gilbert LA, Hemann MT. A senescence secretary switch mediated by PI3K/AKT/mTOR activation controls chemoprotective endothelial secretory responses. Genes Dev. 2016;30:1811–21. https://doi.org/10.1101/gad.284851.116

84 Gilbert LA, Hemann MT. DNA damage-mediated induction of a chemoresistant niche. Cell. 2010;143:355–66. https://doi.org/10.1016/j.cell.2010.09.043

85 Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013;501:346–54. https://doi.org/10.1038/nature12626

86 Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16:582–98. https://doi.org/10.1038/nrc.2016.73

87 Krisnawan VE, Stanley JA, Schwarz JK, DeNardo DG. Tumor microenvironment as a regulator of radiation therapy: new insights into stromal-mediated radioresistance. Cancers (Basel). 2020;12:2916. https://doi.org/10.3390/cancers12102916

88 Pazolli E, Alspach E, Milczarek A, Prior J, Piwcnicka Worms D, Stewart SA. Chromatin remodeling underlies the senescence-associated secretory phenotype of tumor stromal fibroblasts that supports cancer progression. Cancer Res. 2012;72:2251–61. https://doi.org/10.1158/0008-5472.CAN-11-3386

89 Guo Y, Ayers JL, Carter KT, Wang T, Maden SK, Edmond D, et al. Senescence-associated tissue microenvironment promotes colon cancer formation through the secretory factor GDF15. Aging Cell. 2019;18:e13013. https://doi.org/10.1111/acel.13013

90 Alspach E, Flanagan KC, Luo X, Ruhland MK, Huang H, Pazolli E, et al. p38MAPK plays a crucial role in stromal-mediated tumorigenesis. Cancer Discov. 2014;4:716–29. https://doi.org/10.1158/2159-8290.CD-13-0743

91 Ansems M, Span PN. The tumor microenvironment and radiotherapy response; a central role for cancer-associated fibroblasts. Clin Transl Radiat Oncol. 2020;22:90–7. https://doi.org/10.1016/j.ctro.2020.04.001

92 Wang T, Notta F, Navab R, Joseph J, Ibrhimov E, Xu J, et al. Senescent carcinoma-associated fibroblasts upregulate IL8 to enhance Prometastatic phenotypes. Mol Cancer Res. 2017;15:3–14. https://doi.org/10.1158/1541-7786.MCR-16-0192

93 Li D, Qu C, Ning Z, Wang H, Zang K, Zhuang L, et al. Radiation promotes epithelial-to-mesenchymal transition and invasion of pancreatic cancer cell by activating carcinoma-associated fibroblasts. Am J Cancer Res. 2016;6:2192–206.

94 Tsuji S, Minami S, Hashimoto R, Konishi Y, Suzuki T, Kondo T, et al. SARS-CoV-2 infection triggers paracrine senescence and leads to a sustained senescence-associated inflammatory response. Nat Aging. 2022;2:115–24. https://doi.org/10.1038/s43587-022-00170-7
Cellular senescence and the tumour microenvironment

95 Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer. 2009;9:153–66. https://doi.org/10.1038/nrc2602

96 Murphy CG. The role of CDK4/6 inhibitors in breast cancer. Curr Treat Options Oncol. 2019;20:52. https://doi.org/10.1007/s11864-019-0651-4

97 Yu Q, Geng Y, Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. Nature. 2001;411:1017–21. https://doi.org/10.1038/35082500

98 Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW, Jager A. Inhibiting CDK4/6 in breast cancer with Palbociclib, Ribociclib, and Abemaciclib: similarities and differences. Drugs. 2021;81:317–31. https://doi.org/10.1007/s40265-020-01461-2

99 Coppe JP, Rodier F, Patil CK, Freund A, Desprez PY, Campisi J. Tumor suppressor and aging biomarker p16(INK4a) induces cellular senescence without the associated inflammatory secretory phenotype. J Biol Chem. 2011;286:36396–403. https://doi.org/10.1074/jbc.M111.257071

100 Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, et al. CDK4/6 inhibition triggers anti-tumour immunity. Nature. 2017;548:471–5. https://doi.org/10.1038/nature23465

101 Goel S, Wang Q, Watt AC, Tolaney SM, Dillon DA, Li W, et al. Overcoming therapeutic resistance in HER2-positive breast cancers with CDK4/6 inhibitors. Cancer Cell. 2016;29:255–69. https://doi.org/10.1016/j.ccell.2016.02.006

102 Gong X, Litchfield LM, Webster Y, Chio LC, Wong SS, Stewart TR, et al. Genomic aberrations that activate D-type cyclins are associated with enhanced sensitivity to the CDK4 and CDK6 inhibitor Abemaciclib. Cancer Cell. 2017;32:761–76.e766. https://doi.org/10.1016/j.ccell.2017.11.006

103 Guenther LM, Dharia NV, Ross L, Conway A, Robichaud AL, Catlett JL, et al. A combination CDK4/6 and IGF1R inhibitor strategy for Ewing sarcoma. Clin Cancer Res. 2019;25:1343–57. https://doi.org/10.1158/1078-0432.CCR-18-0372

104 Rader J, Russell MR, Hart LS, Nakazawa MS, Belcastro LT, Martinez D, et al. Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma. Clin Cancer Res. 2013;19:6173–82. https://doi.org/10.1158/1078-0432.CCR-13-1675

105 Torres-Guzman R, Calsina B, Hermoso A, Baquero C, Alvarez B, Amat J, et al. Preclinical characterization of abemaciclib in hormone receptor positive breast cancer. Oncotarget. 2017;8:69493–507. https://doi.org/10.18632/oncotarget.17778

106 Wagner V, Gil J. Senescence as a therapeutically relevant response to CDK4/6 inhibitors. Oncogene. 2020;39:5165–76. https://doi.org/10.1038/s41388-020-1354-9

107 Yang NC, Hu ML. The limitations and validities of senescence associated-beta-galactosidase activity as an aging marker for human foreskin fibroblast Hs68 cells. Exp Gerontol. 2005;40:813–9. https://doi.org/10.1016/j.exger.2005.07.011

108 Hao X, Zhao B, Zhou W, Liu H, Fukumoto T, Gabrilovich D, et al. Sensitization of ovarian tumor to immune checkpoint blockade by boosting senescence-associated secretory phenotype. iScience. 2021;24:102016. https://doi.org/10.1016/j.isci.2020.102016

109 Paffenholz SV, Salvagno C, Ho YJ, Limjoco M, Baslan T, Tian S, et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. Proc Natl Acad Sci USA. 2022;119:e2117754119. https://doi.org/10.1073/pnas.2117754119

110 Guan X, Lapak KM, Hennessey RC, Yu CY, Shakya R, Zhang J, et al. Stromal senescence by prolonged CDK4/6 inhibition potentiates tumor growth. Mol Cancer Res. 2017;15:237–49. https://doi.org/10.1158/1541-7786.MCR-16-0319

111 Carpenter VJ, Saleh T, Gewirtz DA. Senolytics for cancer therapy: is all that glitters really gold? Cancers (Basel). 2021;13:723. https://doi.org/10.3390/cancers13040723

112 Di Meco R, Krizhanovsky V, Baker D, d’Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021;22:75–95. https://doi.org/10.1038/s41580-020-00314-w

113 Zhu Y, Tchkonia T, Pirskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015;14:644–58. https://doi.org/10.1111/acel.12344

114 Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med. 2016;22:78–83. https://doi.org/10.1038/nm.4010

115 Watkina M, Takahashi A, Sano O, Loo TM, Imay Y, Narukawa M, et al. A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells. Nat Commun. 2020;11:1935. https://doi.org/10.1038/s41467-020-15719-6

116 Johmura Y, Yamanaka T, Omori S, Wang TW, Sugiuira Y, Matsumoto M, et al. Senolysis by glutaminolysis inhibition ameliorates various age-associated disorders. Science. 2021;371:265–70. https://doi.org/10.1126/science.abb5916

117 Yoshida S, Nakagami H, Hayashi H, Ikeda Y, Sun J, Tenma A, et al. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. Nat
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M. Takasugi et al.
141 Ramirez RD, Herbert BS, Vaughan MB, Zou Y, Gandia K, Morales CP, et al. Bypass of telomere-dependent replicative senescence (M1) upon overexpression of Cdk4 in normal human epithelial cells. Oncogene. 2003;22:433–44. https://doi.org/10.1080/0261543030020026

142 Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, Kaplanov JM, et al. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat Cell Biol. 2012;14:355–65. https://doi.org/10.1038/nclb2466

143 Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell. 1997;88:593–602. https://doi.org/10.1016/s0092-8674(00)81902-9

144 Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature. 2005;436:725–30. https://doi.org/10.1038/nature03918

145 Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, et al. Tumour biology: senescence in premalignant tumours. Nature. 2005;436:642. https://doi.org/10.1038/436642a

146 Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuijman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005;436:720–4. https://doi.org/10.1038/nature03890

147 Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA. 1999;92:9363–7. https://doi.org/10.1073/pnas.92.20.9363

148 Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell. 2006;5:187–95. https://doi.org/10.1111/j.1474-9726.2006.00199.x

149 Romagosa C, Simonetti S, Lopez-Vicente L, Mazo A, Lleonart ME, Castelvi J, et al. p16(Ink4a) overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors. Oncogene. 2011;30:2087–97. https://doi.org/10.1038/onc.2010.614

150 Vilgelm AE, Johnson CA, Prasad N, Yang J, Chen SC, Ayers GD, et al. Connecting the dots: therapy-induced senescence and a tumor-suppressive immune microenvironment. J Natl Cancer Inst. 2016;108: djv406. https://doi.org/10.1093/jnci/djv406

151 Liu Y, Hawkins OE, Su Y, Vilgelm AE, Sobolik T, Thu YM, et al. Targeting aurora kinases AE, Sobolik T, Thu YM, et al. Targeting aurora kinases limits tumour growth through DNA damage-mediated senescence and blockade of NF-kB impairs this drug-induced senescence. EMBO Mol Med. 2013;5:149–66. https://doi.org/10.1002/emmm.201201378

152 Kansara M, Leong HS, Lin DM, Popkiss S, Pang P, Garsed DW, et al. Immune response to RB1-regulated senescence limits radiation-induced osteosarcoma formation. J Clin Invest. 2013;123:5351–60. https://doi.org/10.1172/JCI70559

153 Milanovic M, Fan DNY, Belenki D, Dabritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stenness. Nature. 2018;553:96–100. https://doi.org/10.1038/nature25167

154 Jackson JG, Pant V, Li Q, Chang LL, Quinta-Cardama A, Garza D, et al. p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. Cancer Cell. 2012;21:793–806. https://doi.org/10.1016/j.ccr.2012.04.027

155 Liu D, Hornsby PJ. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. Cancer Res. 2007;67:3117–26. https://doi.org/10.1158/0008-5472.Can-06-3452

156 Angelini PD, Zacarias Fluck MF, Pedersen K, Parra-Can-06-3452. https://doi.org/10.1038/onc.2011.485

157 Canino C, Mori F, Cambria A, Diamantini A, Germoni S, Alessandrini G, et al. SASP mediates chemoresistance and tumor-initiating-activity of mesothelioma cells. Oncogene. 2012;31:3148–63. https://doi.org/10.1038/onc.2011.485

158 Di Mitri D, Toso A, Chen JJ, Parra-Can-06-3452. https://doi.org/10.1038/onc.2011.485

159 Kim YH, Choi YW, Lee J, Soh EY, Kim JH, Park TJ. Senescent tumor cells lead the collective invasion in thyroid cancer. Nat Commun. 2017;8:15208. https://doi.org/10.1038/ncomms15208

160 Fukami J, Anno K, Ueda K, Takahashi T, Ide T. Enhanced expression of cyclin D1 in senescent human fibroblasts. Mech Ageing Dev. 1995;81:139–57. https://doi.org/10.1016/0047-6374(95)93703-6