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STATE-OF-THE-ART REVIEW

Effects of senescence on the tumour microenvironment and response to therapy

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Cellular senescence is a state of durable cell arrest that has been identified both *in vitro* and *in vivo*. It is associated with profound changes in gene expression and a specific secretory profile that includes pro-inflammatory cytokines, growth factors and matrix-remodelling enzymes, referred to as the senescence-associated secretory phenotype (SASP). In cancer, senescence can have anti- or pro-tumour effects. On one hand, it can inhibit tumour progression in a cell autonomous manner. On the other hand, senescence can also promote tumour initiation, progression, metastatic dissemination and resistance to therapy in a paracrine manner. Therefore, despite efforts to target senescence as a potential strategy to inhibit tumour growth, senescent cancer and microenvironmental cells can eventually lead to uncontrolled proliferation and aggressive tumour phenotypes. This can happen either through overcoming senescence growth arrest or through SASP-mediated effects in adjacent tumour cells. This review will discuss how senescence affects the tumour microenvironment, including extracellular matrix remodelling, the immune system and the vascular compartment, to promote tumourigenesis, metastasis and resistance to DNA-damaging therapies. It will also discuss current approaches used in the field to target senescence: senolytics, improving the immune clearance of senescent cells and targeting the SASP.

Introduction

Cellular senescence was first identified as a highly stable cell cycle arrest caused by the proliferative exhaustion of cultured human fibroblasts [1]. Senescence is a cellular response to stress signals [2,3] preventing damaged cells from proliferating further [4] and acquiring resistance to apoptosis [5] (Fig. 1). The accumulation of senescent cells is part of the natural ageing process, contributing to many age-related diseases including osteoarthritis, pulmonary fibrosis and cancer [6].

Abbreviations
ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; CAF, cancer-associated fibroblast; CDK, cyclin-dependent kinase; EC, endothelial cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; GMB, glioblastoma; GM-CSF, granulocyte macrophage colony-stimulating factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; IL-1α, interleukin-1α; IL-6, interleukin-6; IL-8, interleukin-8; IR, ionising radiation; MMP, matrix metalloproteinase; NSCLC, non-small-cell lung cancer; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor-β; TIS, therapy-induced senescence; TME, tumour microenvironment.

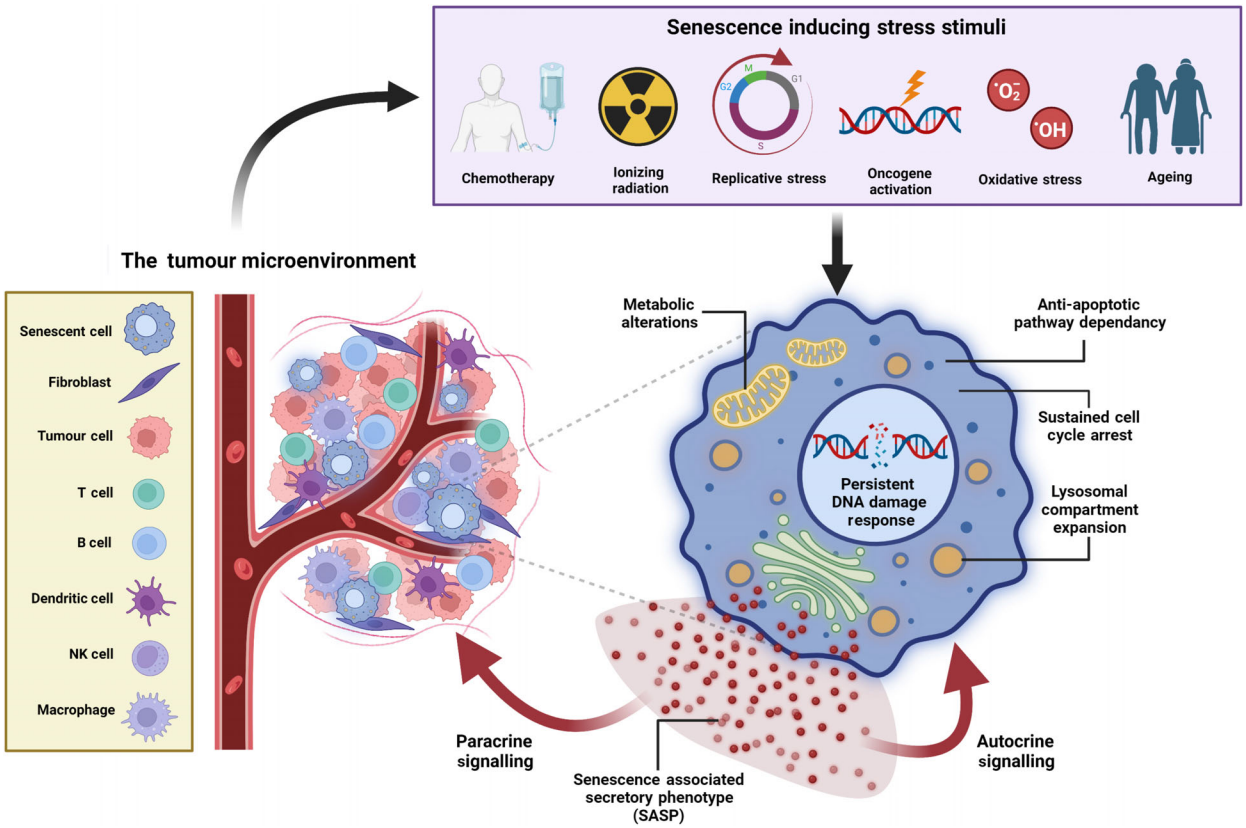

Fig. 1. Overview of the relationship between the tumour microenvironment, senescence inducing stimuli and the hallmarks of the senescent state. Cellular senescence is induced by a variety of stress inducing stimuli, such as chemotherapy, ionising radiation, replicative stress (due to telomere attrition or deficiencies in maintaining appropriate cell cycle checkpoints), activation of oncogenic signalling pathways, oxidative stress and the ageing process. Stress inducing stimuli drive cells into senescence by inducing a persistent DNA damage response. This results in the acquisition of the hallmarks of the senescent state, which include metabolic alterations, dependency on anti-apoptotic pathways, lysosomal compartment expansion and sustained cell cycle arrest. The tumour microenvironment, both pre- and especially post-cancer treatment, is a rich source of senescence inducing stress stimuli and is frequently populated with senescent cell populations. Critically, senescent cells activate the senescence-associated secretory phenotype (SASP), which is comprised of a myriad of pro-inflammatory chemokines and cytokines as well as growth factors and extracellular matrix remodelling proteins. Through the SASP, senescent cells can reinforce their senescent state in an autocrine manner and can influence the tumour microenvironment, promoting tumour progression, in a paracrine manner. Created using BioRender.com.

Senescent cells are heterogeneous and do not show a single phenotype. However, some characteristics are often presented. For example, they are flattened *in vitro*, larger in size with an irregular shape and have an increased nuclear size compared to normal cells. Moreover, the nuclear envelope is compromised due to the loss of lamin B1. Cytoplasmic chromatin fragments and DNA damage are common hallmarks of senescence. Lysosomes also accumulate in the cytoplasm, leading to an increased expression of β-galactosidase (the product of the gene *GLB1*). The nature and chemical composition of the plasma membrane change, including upregulating caveolin-1. Additionally, large dysfunctional mitochondria produce a high level of reactive oxygen species (ROS) [7]. Senescent cells are in a persistently hypo-replicative condition, which is maintained through the expression of cell cycle regulators (e.g. p53, RB, p21, p16), but are metabolically active [4].

One of the main characteristics associated with senescent cells is a marked change in secretory protein expression, termed SASP. Although the SASP composition can vary—for instance, depending on the cell type and the senescence stimulus—typically it includes a range of chemokines, pro-inflammatory cytokines, such as interleukin 8 (IL-8), interleukin 6 (IL-6) and interleukin 1 alpha (IL-1α), growth factors such as TGF-beta (TGFβ), hepatocyte growth factor (HGF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and matrix-remodelling enzymes (MMPs) [7–9].

A functional role for SASP in tumourigenesis has been identified. The presence of senescence during the early stages of tumour development has a beneficial effect in inhibiting progression. This growth arrest is seen as a potential approach to inhibit tumour growth, and indeed, chemotherapy, radiotherapy and some targeted therapies rely on senescence induction of tumour cells. Systemically administered anti-cancer treatments can also induce senescence in distant healthy tissues [10]. The SASP can stimulate immunosurveillance mechanisms by directing the immune system to enhance anti-cancer responses [8,11]. Although cellular senescence can have an onco-suppressive role [12,13], it can also have detrimental effects, contributing to tumour initiation, progression, resistance to therapy and metastatic dissemination [14]. For instance, the SASP can protect tumour cells from the immune system, provide growth factors that promote cancer cell proliferation and stimulate angiogenesis. The excessive build-up of therapy-induced senescent cells can have negative side effects and contribute to progression of chronic conditions, such as neurodegenerative or cardiovascular diseases, which are frequently observed in cancer-surviving patients [15]. Furthermore, senescent cancer cells can eventually lead to uncontrolled proliferation and aggressive tumours, either through overcoming senescence growth arrest (i.e. senescence escape) or through SASP-mediated effects in adjacent tumour and microenvironment cells.

This review will discuss how senescent cells, through SASP, promote cancer by altering the tumour microenvironment (TME), with a focus on senescent fibroblasts, immune cells and endothelial cells. These senescent cells accumulate in the tumour bed upon anti-cancer treatments, and we outline evidence supporting a pro-tumourigenic role of chemo- and radiotherapy. Finally, we discuss approaches that are being tested to eliminate senescent cells or reduce their impact on the TME, such as senolytics, senomorphics and enhancement of the immune system. For further details on senescence mechanisms and pathways, effects on human disease and ageing, we refer the reader to other reviews [1,14,16–21].

Senescence and fibroblasts

The TME comprises multiple cell types and the extracellular matrix (ECM). Tumour formation is a complex and dynamic process in which the interaction between cancer cells and the TME determines whether the initial tumour is eradicated or metastasises [22,23]. Furthermore, the TME plays a crucial part in responses to tumour therapy, contributing to several mechanisms of resistance [24,25]. Senescence in the TME, especially in stromal cells, is a primary cause of therapy resistance and cancer relapse [26,27].

Fibroblasts synthesise the majority of cancer-related ECM and secrete soluble factors, including cytokines, growth factors, enzymes and chemokines into the microenvironment [28,29]. Cancer-associated fibroblasts (CAFs) represent a sub-population of fibroblasts that can be induced to become senescent by anti-cancer therapies. Senescent CAFs are critically involved in tumour progression through the secretion of TGFβ1 and SDF-1, which promote enhanced proliferation of neoplastic epithelial cells and increased cell cycle progression, migration and invasion of cancer cells. Senescent fibroblasts also upregulate the expression of various ECM proteins, MMPs [30] and osteopontin [31] to stimulate preneoplastic cellular proliferation via the activation of the MAPK signalling pathway [32]. *In vitro*, senescent dermal fibroblasts adopt a secretory phenotype resulting in enhanced cancer cell growth and invasion. These fibroblasts promote the growth of non-tumourigenic melanoma cells and enhance the invasion of advanced melanoma cells. *In vivo*, senescent fibroblasts modify the skin microenvironment, altering the skin tissue architecture by enhancing the growth and invasion of normal melanocytes. This interaction leads to melanoma initiation and progression [33]. One of the most important factors that senescent fibroblasts secrete to promote melanoma development is proteases. Dermal fibroblasts that undergo replicative senescence during the ageing process secrete MMP-1 and MMP-2, which impact the early development of tumour metastasis [34]. Furthermore, age-related accumulation of senescent skin fibroblasts leads to the loss of hyaluronan and proteoglycan link protein-1 (HAPLN1), enhancing ECM alignment and driving the promotion of metastasis by melanoma cells [30,32,35,36].

Senescence and immune cells

Senescence-associated secretory phenotype can also create an immunosuppressive environment that promotes tumorigenesis [37]. In patients with hepatocellular carcinoma (HCC), the senescent stroma is associated with poorer survival rates. *In vivo* models of oncogenic-induced hepatocyte senescence reveal that the growth of tumour cells is more aggressive in the presence of senescent cells compared with non-senescent cells, due to activation of the CCL2-CCR2 pathway. This promotes the accumulation of immunosuppressive myeloid cells, preventing natural killer (NK) cells from clearing the tumour cells [38].

Additionally, the loss of p53 leads to uncontrollable cell proliferation, and, in certain settings, an increased chance of genomic instability and resistance to therapy. Interestingly, the re-activation of endogenous p53 caused tumour regression by inducing cellular senescence and triggering the innate immune response to directly target the tumour [39].

Overexpression of tumour suppressor genes, *CDKN2A* (encoding $p16^{Ink4a}$) and *CDKN1A* (encoding $p21^{Cip1/Waf1}$) can have a pro-tumour effect, stimulating the expression of CX3C chemokine receptor 1 (CX3CR1) and leading to resistance to anti-tumour immune response. A high level of $p16^{Ink4a}$ and $p21^{Cip1/Waf1}$ expression is found in myeloid-derived suppressive cells driving enhanced CX3CR1 expression. This prevents cyclin-dependent kinases (CDKs) phosphorylation, thus inhibiting activation of SMAD3 [40]. Deletion of $p16^{Ink4a}$ and $p21^{Cip1/Waf1}$ reduces the expression of CX3CR1, preventing myeloid-derived suppressive cells from accumulating in the tissue, increasing the lifespan of mice and alleviating age-related diseases such as cancer [41]. Furthermore, the expression of $p16^{Ink4a}$ in tumour stromal cells leads to a high risk of reappearance of tumour cells and a poor survival rate in various cancer types. Expression of $p16^{Ink4a}$ in myeloid cells specifically induces macrophages towards a more inflammatory state, promoting tumorigenesis. These data support the idea of clearing $p16^{Ink4a}$ expressing senescent cells to delay the formation of tumours [41], suggesting that senescence drives several mechanisms involved in promotion of tumourigenesis and aggressiveness. In agreement, two recent publications have shown that senescent macrophages can promote tumour initiation and progression in mouse models of KRAS-driven lung adenocarcinoma [42,43]. Interestingly, the tumourigenic senescent macrophages were found to share a SASP signature with senescent macrophages found in the normal lungs of aged mice. These similarities support the notion that ageing creates tumour-permissive environments that can fuel tumour growth in cells carrying oncogenic hits.

Senescent cells can influence tumour progression by releasing pro-inflammatory SASP factors, such as interleukin 6 (IL-6) which recruits myeloid suppressive cells, preventing the CD8+ T-cells from attacking the invasive tumour cells and promoting tumour growth [36]. This indicates that stromal senescent cells have a major role in inducing immunosuppression that favours tumour progression. SASP-mediated immunosuppressive mechanisms in obesity-related liver carcinoma are thought to drive tumour progression. Using mouse models, it was demonstrated that the hepatic translocation of obesity-induced lipoteichoic acid (LTA), enhanced senescence and SASP (including the expression of PGE2) and supressed the anti-tumour immunity effect via PTGER4 receptor [44] thereby, increasing the progression and metastasis of hepatocellular carcinoma. Furthermore, epithelial cells in *Pten*-null prostate tumours undergo senescence and drive immunosuppressive SASP factors in the TME, which is dependent on the activation of Jak2/Stat3 signalling pathways. Genetic or pharmacological inactivation of this pathway reduced levels of SASP factors (cytokines) including macrophage colony-stimulating factor (M-CSF), interleukin-10 (IL10), interleukin-13 (IL13), CXCL1/CXCL2 and GM-CSF. This enhanced anti-tumour immune responses and reduced tumour progression and metastasis. Concomitantly, SASP factors also increase the progression of tumours by recruiting and activating myeloid-derived suppressor cells, which protect the tumour cells from senescence [45]. Together, these studies support the notion that both malignant and stromal cell senescence have a regulatory immune function promoting tumour growth and metastasis.

Senescence and vascular endothelial cells

Vascular endothelial cells (ECs) actively participate in angiogenesis, the formation of new blood vessels, and are an important component of the TME. Angiogenesis is essential for tumour growth and metastasis [46]. Tumour blood vessels structurally and functionally differ from vessels in normal tissue. ECs of neovessels exhibit altered gene expression profiles that can have direct effects on angiogenesis or angiocrine functions (EC-derived paracrine/juxtacrine signalling) [47–52]. Tumour ECs also have properties of senescent cells [53]. DNA-damaging therapy can induce ECs to undergo senescence with a SASP phenotype, which in turn affects other cells within the TME. This promotes an aggressive cancer cell phenotype, in part by secretion of CXCL11 [54]. The increased and sustained activation of the Notch1 receptor on senescent endothelial cells enhances the expression of several SASP chemokines and VCAM1 expression in multiple cancers. Additionally, actin stress fibre formation increases pulling forces in ECs, along with a decrease in cell–cell junctions, facilitating the entry of tumour cells into the circulation, thereby promoting metastasis [55]. Increased VCAM1 expression has been shown to promote neutrophil recruitment in the pre-metastatic niche.

Melanoma cells producing SPARC (secreted protein acidic and rich in cysteine) affect EC VCAM1 in

distant lung tissue, promoting melanoma metastasis and colonisation [56,57]. Moreover, after doxorubicin treatment, senescent ECs in the thymus of a mouse model of B-cell lymphoma released IL-6, creating a peri-vascular 'chemo-resistant niche'. This suggests that tumour cells close to blood vessels survive and continue to grow with a minimal residual burden, serving as a reservoir for tumour relapse [58,59]. Researchers also found that acute doxorubicin treatment induced EC senescence, but not the SASP phenotype. Instead, ECs activate a secretory acute response via ROS-induced p38 signalling pathway. This secretory acute response occurs in the context of PI3K/AKT/mTOR pathway downregulation. This characterises a mechanism that acutely activates a pro-survival paracrine signalling without chronic inflammation or tissue dysfunction that is usually associated with activated canonical SASP [60]. These studies point to the potential role of endothelial cell senescence in pre-metastatic niche formation, promoting tumour cell seeding, colonisation and thus metastasis development.

Senescence and extracellular matrix

The ECM constitutes a highly dynamic and complex environment critical for maintaining tissue homeostasis. It undergoes continuous remodelling and degradation by matrix metalloproteinases (MMP) and A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS). Disease arises when ECM remodelling becomes excessive, ultimately contributing to changes in cell behaviour. During cancer, changes in ECM composition affect cell phenotype and increased deposition of ECM can lead to cellular senescence [61], mediating tumour progression [62]. Increased ECM crosslinking has been implicated in tumour growth and metastasis, mediated by lysyl oxidases (LO). Dysregulation of the LO family of enzymes, secreted by fibroblasts, modifies newly deposited ECM proteins, increasing its stiffness. This stiffness contributes to metastasis and therapeutic resistance [63] and has been linked to lung and liver cancer. Stiffening of the ECM enables the release of stored growth factors, thus contributing further to tumour metastasis and resistance. ECM stiffness leads to increased ROS production, linked to senescence through the activation of STAT3 [64,65]. In breast, and other cancers, increased ECM stiffness induces a malignant phenotype in epithelial cells triggering the secretion of MMPs by senescent cells [34,66–68]. Conversely, a decrease in ECM integrity, caused by decreased secretion of ECM proteins, can increase the chance of melanoma cell invasion and progression occurring [69].

Matricellular proteins, secreted in the extracellular environment, have also been implicated in senescence and cancer, particularly CCN1 and CCN2 family, which includes thrombospondin and tenascin-c. These proteins regulate cell function by binding to a large variety of cell-surface receptors, cytokines and ECM proteins to induce cell senescence. For instance, CCN1 can induce myofibroblast senescence by binding to the integrin α6β1 [70] and mediates endocrine resistance in breast cancer cells [71].

Cancer therapy-induced senescence

Despite the benefits of cancer therapy, such treatments can induce senescence both in malignant and non-malignant cells (known as therapy-induced senescence (TIS)) (Fig. 2). These often can have pro-tumour effects through SASP, favouring tumour progression when resistance to treatment occurs.

The effect of chemotherapy

Doxorubicin is used to treat various cancer types, including acute lymphocytic leukaemia, lymphoma, lung and breast cancer. Despite its efficacy, doxorubicin can induce senescence in non-malignant cells including smooth muscle cells, dermal fibroblasts and other stromal cells [72]. Cisplatin and temozolomide, both alkylating agents, also trigger senescence. Treatment with paclitaxel, a microtubule inhibitor, induces senescence in MCF7 breast cancer, as well as other non-malignant cells, including ECs [73], mononuclear cells in the bone marrow [74] and fibroblasts [26]. Inducing senescence in these cells can provide chemo-resistant reservoirs or niches to tumour cells, facilitated by their SASP factors.

Chemotherapy can also induce senescence in cancer cells, offering a potential mechanism of resistance to therapy, possibly due to 'stemness'. Comparing gene expression analysis between senescent and non-senescent B-cell lymphomas from Eμ-Myc transgenic mice treated with doxorubicin, in H3K9me3 or p53 genetically switchable models, reveals that doxorubicin induces senescence and generates a 'stem-like' phenotype. Tumour cells evade senescence by re-entering the cell cycle, acquiring tumour-initiating capabilities distinct from similar populations of cells treated with chemotherapy [27]. This phenomenon is known as senescence-associated stemness (SAS). Furthermore, the SASP can also drive stem-like epithelial-to-mesenchymal (EMT) phenotypes. Pemetrexed-treated

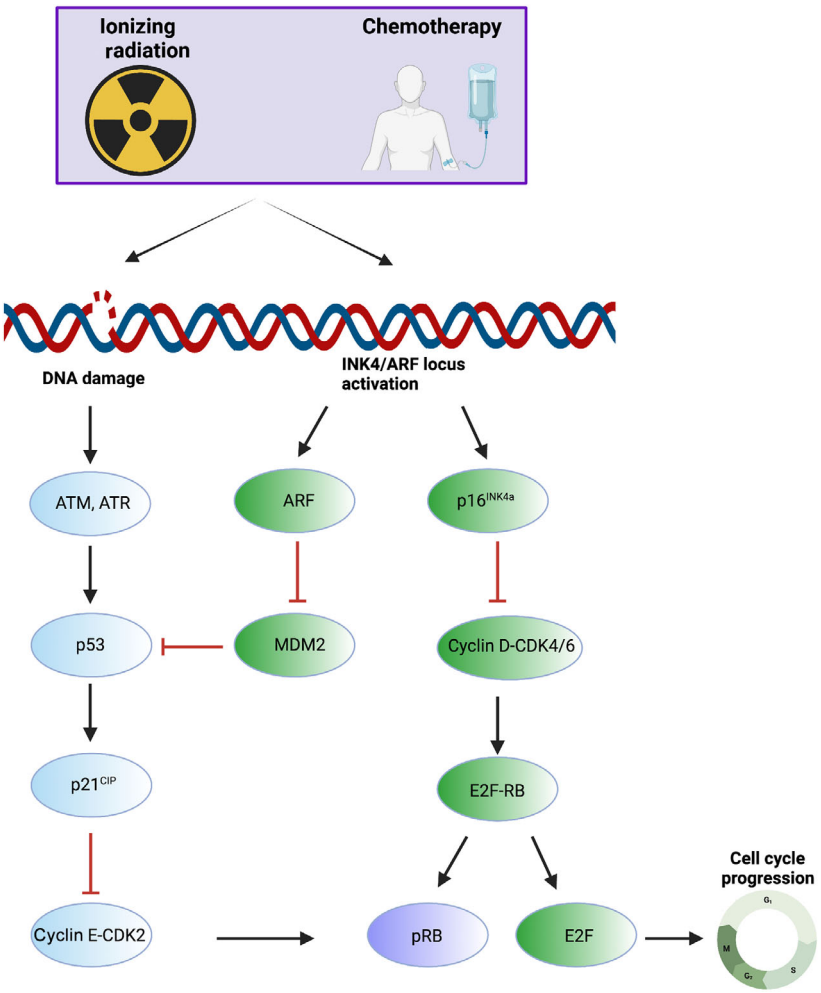

Fig. 2. Growth arrest during senescence and the molecular pathways involved. Multiple triggers, including ionising radiation and chemotherapy give rise to senescence-associated cell cycle arrest. Exit from the cell cycle is mediated by the induction of the $p16^{INK4a}$/Rb and p53/$p21^{CIP1}$ pathway. Created using BioRender.com.

mesothelioma cells induce senescent malignant pleural mesothelioma cells to express SASP factors that amplify EMT-like clonogenic capacities and elevate levels of tumour-initiating capabilities [75].

The effect of targeted therapies

Specific pathway inhibitors have also been shown to be capable of inducing TIS. CDK 4 and 6 play essential roles in the cell cycle transition from G1 to S phase. Active CDK4/6 is present in cancer cells, particularly in breast cancer. Inhibiting CDKs may induce senescence in cancer cells. CDK4/6 inhibitors such as palbociclib, ribociclib and abemaciclib have been approved for use in trials [76–78] and reduce tumour growth by activating a senescence-like programme in cancer cells. Interestingly, exposure to these inhibitors has been shown to induce senescence in non-malignant cells dependent on p53 [79].

Recently, Ruscetti *et al.* [80] demonstrated that the MEK inhibitor, trametinib, in combination with the CDK4/6 inhibitor, Palbociclib, induced senescence and successfully inhibited the proliferation of KRAS mutant lung cancer cells while stimulating natural killer (NK) cells to promote tumour cell death. This drug combination was also found to enhance CD31-positive blood vessels in mouse models of pancreatic ductal adenocarcinoma (PDAC). This enhancement was mediated by an increase in pro-angiogenic SASP factors including VEGF, PDGF and MMPs which promoted endothelial cell activation. Ultimately, this led to enhanced gemcitabine drug delivery and efficacy, resulting in reduced tumour metastasis [81]. These findings highlight the potential of improving the efficacy of conventional chemotherapies by eliminating senescent-like cancer cells through senolytic interventions and improving vascularisation in hypovascularised tissue to enhance drug delivery.

The effect of radiotherapy

Radiotherapy is one of the most common treatments for cancer. Ionising radiation (IR) induces senescence to transiently arrest in an attempt to repair damage. However, this can lead to the accumulation of senescent cells facilitating tumour growth, metastasis and resistance to therapy [82–84]. Radiation-induced senescence in normal tissue can result in tissue fibrosis and organ dysfunction. In NSCLC, following radiotherapy, CAFs exhibit senescence-like characteristics that drive NSCLC cell proliferation and radioresistance through the JAK/STAT pathway. This radioresistance is a major contributor to reduced curability in NSCLC patients [85]. In a mouse model of glioblastoma (GMB), a cancer routinely treated with high doses of radiation in the clinic, radiation-induced senescence in stromal cells promoted the growth of surrounding tumour cells through SASP secretion, leading to therapy resistance and recurrence. Specifically, irradiation induced senescence in astrocytes, resulting in upregulation of p21 and the SASP factor HGF [86]. Other studies have demonstrated that ionising radiation leads to increased expression of senescence-associated β-galactosidase (SA-β-gal) expression in breast, colon cancer, neuroblastoma and fibrosarcoma cells [87,88].

In conclusion, although senescence induction restricts cancer progression and underlies anti-cancer therapy success, the presence of senescent cells in the tumour bed can promote tumour re-growth, relapse and metastasis. To overcome this effect, the 'one-two punch' approach is emerging as a novel strategy to fight cancer and improve the efficacy of standard cancer treatments. It involves the combination of a senescence inducer, either through chemo-, radio- or targeted therapy, followed by treatment with a senolytic to ablate the lingering senescent cells [89].

Targeting senescence

In recent years, targeting cellular senescence has become a central focus in treating age-related diseases, including cancer. Currently, three main therapies are aimed at eliminating cellular senescence: targeting senescent cells (senolytics), targeting the SASP and utilising the immune system to clear out the senescent cells, known as immunosurveillance.

Senolytics

Senolytic drugs are designed to target and eliminate active senescent cells. The primary target proteins of senolytics are involved in apoptosis, that is, p53, p21, PI3K, AKT and BCL-2. For instance, the senolytics ABT-737 and ABT-263 target BCL-W and BCL-XL [90]. Treating senescent adipocytes with dasatinib and quercetin (D + Q) enhances the elimination of senescent cells, reducing the number of naturally occurring senescent cells along with a reduction of the pro-inflammatory secreted cytokines [91]. Since senescent cells are typically resistant to apoptosis [92], targeting senescent cells with FOXO4-D-Retro Inverso (FOXO4-DRI) disrupts FOXO4-p53 interactions, resulting in the selective apoptosis of senescent cells. This approach nullifies doxorubicin-induced toxicity and restores homeostasis in the livers of normal and ageing mice [93]. FOXO4-DRI has also been used to target senescent cells in other diseases [94]. HSP90 inhibitors like 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) and geldanamycin induce apoptosis in oxidative stress, radiation or replication-induced senescent human and murine cells *in vitro*. HSP90 inhibitors eliminate $p16^{Ink4a}$-positive cells, thereby delaying any age-related symptoms or diseases, including cancer [95]. More recently cardiac glycosides have been identified as potent senolytics in a variety of cell contexts both *in vitro* and *in vivo* [96,97].

Despite these encouraging results, the elimination of senescent cells can also be associated with detrimental effects [98] and senolytics can lack specificity and have been associated with toxicity. Consequently, an advanced approach involving nanoparticles containing senolytics, is being explored. These nanoparticles specifically target senescent cells, exploiting the increased lysosome β-galactosidase activity found in senescent cells. Nanoparticles loaded with Palbociclib, and coated with galacto-oligosaccharides are internalised, enabling β-galactosidase to digest the galacto-oligosaccharides and release the nanoparticle contents within the senescent cells [99].

Senomorphics

An alternative approach to inhibit senescence is through targeting and silencing SASP factors [100,101]. Senomorphics aim to modulate the characteristics of senescent cells by impacting the signalling pathways related to seno-inflammation/inflammation and SASP, without inducing apoptosis in senescent cells [102,103]. Senomorphics achieve this by inhibiting SASP pathways through the targeting of p38 MAPK, NF-κB, mTOR and PI3K/AKT processing at the transcriptional or translational level, or mRNA stabilisation. In both *in vivo* and *in vitro* contexts, a (NEMO)-binding domain (NBD) peptide inhibitor targeting the IKK/NF-kB pathway reduced cellular

senescence and delayed age-related symptoms in Zmpste24-deficient mice [104]. The JAK/STAT pathway also regulate SASP factors. Suppression of pro-inflammation and alleviation of age-related tissue dysfunction was achieved by targeting the JAK/STAT pathway using JAK inhibitors or RNAi in senescent cells, resulting in improved physical function in mice [105]. Antibodies targeting individual SASP factors, that is, IL-6, IL-8 and IL-1a/b have also shown potential as senomorphics [7]. Canakinumab, targeting IL-1b, exhibited promise in trials for patients with non-small-cell lung cancer. Bermekimab (MABp1), a neutralising anti-human IL-1α monoclonal antibody, demonstrated potential in treating diseases associated with senescence [106–108]. IL-8, upregulated in SASP, contributes to maintaining cancer cells in EMT and promoting angiogenesis. A humanised monoclonal antibody, ABX-IL-8, reduced the growth of certain tumours in murine models [109]. Additionally, Siltuximab, a neutralising IL-6 antibody, is currently in phase Ib/II trials in combination with Spartalizumab (monoclonal antibody directed against the human programmed death-1 (PD-1) receptor) for treating patients with pancreatic cancer and secondary metastases.

Immune system and clearance of senescent cells

To maintain homeostasis, senescent cells must be cleared by the immune system (anti-senescence function), preventing their accumulation and development of age-associated diseases including cancer. NK cells, macrophages, CD8+ T cells and other immune cells [110,111] are involved in the anti-senescence function to prevent tumour progression [112]. As we age, clearance of senescent cells diminishes, partially due to changes in the function of organs that produce immune cells leading to a decline in immune cell numbers [113].

NK cells possess anti-senescent functions and can recognise receptors and ligands present on the surface of senescent cells [114] and induce their death. For example, the NK cell receptor NKG2D can recognise and bind the ligands MHC class I polypeptide-related sequence A (MICA) and UL16 binding protein 2 (ULBP2) which are expressed on senescent cells [115]. This has been observed in liver fibrosis with senescent hepatic stellate cells (HSCs), whereby the NK receptor NKG2D detects ligands expressed on HSCs and induce their death through perforin exocytosis [116].

CD8+ T cells and macrophages also play an important role in clearing senescent cells and reducing cancer development, progression and metastasis [112]. Two recent publications have highlighted the immune stimulatory properties of senescent cancer cells [117,118]. When senescent cancer cells were used to immunised mice, there was a strong anti-tumour protective response, which was mediated by DCs and CD8+ T cells. In comparison with proliferative cancer cells, senescent cancer cells upregulate the IFNγ receptor and show an exacerbated response to IFNγ present in the TME. Together, these responses result in increased tumour immunosurveillance.

These, and other studies, strengthen the notion that immune cells play a crucial role in postponing the development of diseases associated with the build-up of senescent cells and that therapies, including immunotherapy, that can enhance immune-mediated cell clearance, is an important strategy for treating age-related diseases.

Discussion

While senescence induction offers clear benefits when targeting tumour cells, the accumulation of senescence in the microenvironment due to tumour development or cancer therapies can have pro-tumour effects. For example, senescent cells can lead to tumour cell resistance, maintenance of residual disease, generation of pre-metastatic niches by inducing senescence in healthy distant organs and promoting metastasis formation. Therefore, the use of senotherapeutics to target senescence is a promising approach against cancer.

It is important to note that more research is required to identify specific regulators of senescence, particularly in distinct cell-types and tissues. This involves determining the sites and timing of senescence occurrence. To enhance our understanding of the underlying pathways of senescence, the use of specific inhibitors and cell-type-specific transgenic models, with a focus on cell lineage tracing, would be highly informative [119,120]. Such approaches would allow for a deeper understanding of the pathways involved at a single-cell level and the impact of senescence in different organs, as well as its relation to tumorigenesis. However, it is worth noting that limitations exist with the use of mouse models of senescence. Most of these rely on the expression of $p16^{Ink4a}$ or $p21^{Cip1/Waf1}$, however, the expression of these cell cycle inhibitors may not be sufficient to ascertain senescence. For example, the senescent cell biomarker $p16^{INK4a}$, has been reported in macrophages of adipose tissue from aged mice and peritoneal cavity of young mice [121], suggesting that $p16^{INK4a}$ may not be an exclusive marker for senescent cells. Moreover, other senescent markers including $p21^{Cip1/Waf1}$ are expressed by non-senescent macrophages [122]. However, in two recent papers, which analysed the expression profiles of

macrophages in the contexts of KRAS-driven lung adenocarcinoma and aged lungs, a subpopulation of $p16^{INK4a}$-expressing macrophages with immunosuppressive phenotype were found to exhibit many hallmarks of senescence including the activation of a SASP [42,43]. Future work is required to reveal the similarities/differences between immunosuppressive and senescent macrophages.

Inducing senescence holds potential for targeting tumour cells, but its accumulation due to cancer therapies can have negative consequences. Therefore, a promising approach is to combine TIS (e.g. radiation, chemotherapy) with anti-senescence treatments like senolytics, senomorphics and improved immune function ('One-two punch' approach). This combination could promote tissue renewal and clearance, restoring tissue homeostasis. This approach could also potentially decrease the effect of DNA damaging therapy-induced senescence in distant normal healthy organs, as elimination of senescent cells in the microenvironment of these tissues could potentially reduce metastasis formation, by avoiding the SASP-mediated effects in pre-metastatic niche formation [42,43]. However, further studies are required to explore the effects of TIS in other organs and distant sites, taking into consideration tissue heterogeneity and cell-type-dependent specific effects.

Concerns exist about the consequences of targeting senescence. Senolytics' effects on dead senescent cell accumulation and potential toxicity needs careful consideration. Build-up of senescent cells has detrimental effects but what about accumulation of apoptotic cells? These could also lead to undesirable side effects that could potentially be toxic to the patient. Therefore, it is crucial that the senolytics have a short-term effect while still being effective. It is also important to analyse the location where senescent cells are being targeted and cleared. Highly specific senolytics targeting the particular cell type(s) are required to limit toxicity, in particular when using senotherapies in aged individuals, which are likely to have accumulated abundant senescent cells throughout the organs and tissues.

When targeting SASP with the use of senomorphics, the senescent cells are 'silenced', which then poses the question as to how they will be cleared after being silenced? Since the SASP is involved in recruitment of immune cells, silencing of senescent cells may make them undetectable by the immune system, leading to their accumulation in the tissue. The use of senomorphics also lacks specificity, so utilising conjugated cell-type-specific antibodies with this treatment might lead to improved outcomes. Lastly, using the immune system to clear out the senescent cells can be difficult, particular in older (immunesenescence) or immunosuppressed patients. The immune system can 'rejuvenate' but the use of CAR systems is more like a personalised treatment, it is very costly and takes time to develop. These methods also require a more universal senescence receptor, which, at present, provides limited options. It is crucial, therefore, to use a combination of biomarkers, for example, targeting two senescence markers at least in these personalised treatments, which could resolve this issue. Finally, it is important to note that enhancing the immune system could also activate other autoimmune diseases and, in some cases, could even promote haematopoietic malignancies [123,124].

In conclusion, strategies that preserve the anti-tumour effects of senescence by targeting both malignant and stromal compartments hold promise for innovative anti-cancer therapies.

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Conflict of interest

KMH-D is a consultant for Ellipses, Vasodynamics and RGD-Science. The other authors declare no conflict of interest.

Author contributions

All authors listed have made a significant intellectual contribution to the work and have approved it for publication.

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