Heterogeneity of senescence cancer cells and their pleiotropic non-cell autonomous effects

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Abstract

Cellular senescence, a response to various stress signals, involves a stable exit from the cell cycle along with significant changes in cell function and physiology. Although many studies have focused on senescence in non-cancerous cells, it is evident that cancer cells can also enter a senescent state. Senescent cancer cells exhibit considerable variability due to both intrinsic and extrinsic factors. In this review, we explore the heterogenous autonomous and non-cell autonomous characteristics of senescent cancer cells and their diverse relationship to tumor progression. In addition, we discuss the potential and challenges of using senotherapies as novel anti-cancer treatments. By understanding the context-specific effects of senescent cancer cells, we may be able to harness their tumor-suppressive benefits while minimizing their harmful consequences, leading to new therapeutic strategies for cancer.

Layman's summary

Cellular senescence is a process where cells permanently stop dividing in response to stress. This has been well-studied in normal cells, but cancer cells can also enter this state. When cancer cells become senescent, they behave in different ways due to both internal and external influences. In response to these changes, senescent cancer cells can communicate differently with cells surrounding them such as immune or endothelial cells. This review focuses on the complex behavior of these senescent cancer cells and how they can either help or block tumor growth. We also discuss the therapeutic potential of "senotherapies"—treatments that target senescent cells—as a new approach to cancer therapy. By better understanding how senescent cancer cells work, we may be able to use their tumor-repressing abilities, which could open up new ways to treat cancer.

Introduction

Cell states integrate both physiological and stress signals to shape tissue equilibrium and homeostasis. The effects on the cell's phenotype depend on the nature of the signals, including their type, intensity, and duration, as well as the cell's capacity to respond. When exposed to potentially damaging stress, damage is reversed, and the cell's structural and functional integrity is restored. Alternatively, the damage might be irreversible, and cells use death mechanisms to limit tissue degeneration. Between these two extremes, cells can enter other states commonly associated with survival that can result in morphological and functional alterations. One of these states is cellular senescence (Kumari & Jat, 2021; Muñoz-Espín et al., 2013).

Cellular senescence describes a non-proliferative but viable cellular state of durable growth arrest that was initially defined as one of the key processes regulating cellular and organismal ageing (Collado et al., 2007). Classically, senescent cells are effectively arrested in either the G1 or G2/M phases of the cell cycle, losing the ability to divide further, although they remain alive and metabolically active for extended periods (Campisi & d'Adda di Fagagna, 2007). However, senescent states are heterogenous, and recent evidence has demonstrated that some cells can escape the senescent state and re-enter the cell cycle. (Galanos et al., 2016; Li et al., 2018; Patel et al., 2016).

In addition to growth arrest, cellular senescence is marked by several characteristic changes in morphology, resistance to apoptosis, and the activation of a distinct hypersecretory profile termed as the senescence-associated secretory phenotype (SASP) (Kumari & Jat, 2021). The SASP

is a distinguishing feature of senescent cells, defined by the secretion of a wide range of factors such as pro-inflammatory cytokines, chemokines, growth and angiogenic factors, matrix metalloproteins (MMPs), and proteases. These released factors can significantly impact the surrounding microenvironment, propagating the stress response to neighboring cells (Campisi & d'Adda di Fagagna, 2007; Collado et al., 2007; Kumari & Jat, 2021).

Cellular senescence has been recognized as an emerging hallmark of cancer (Hanahan, 2022), and it is triggered by several factors, such as oncogene activation, telomere shortening, DNA damage, and oxidative stress (Campisi & d'Adda di Fagagna, 2007; Di Micco et al., 2006; Faraonio, 2022; Victorelli & Passos, 2017). These different stimuli shape the heterogeneity of senescent tumor cells which can also reflect differences in their cellular origins and functional roles within the tumor microenvironment (TME) (Kirschner et al., 2020). Therefore, senescent cancer cells have diverse phenotypic and molecular profiles, influencing their secretory functions and interactions with surrounding cells. As a result of this heterogeneity, senescent cells can exhibit opposite functions in tumor initiation and progression, depending on the context.

On the one hand, acute senescence plays beneficial roles in tissue repair, and regeneration by inhibiting cell growth and stimulating immune surveillance (Jun & Lau, 2010; van Deursen, 2014). However, when cellular stress or macromolecular damage persists for an extended period, it can lead to a process termed chronic senescence (Fang et al., 2020; Kirschner et al., 2020; van Deursen, 2014). This type of senescence is detrimental as it promotes chronic inflammation, impairs tissue regeneration, and can potentially initiate tumorigenesis. The heterogeneous effects of senescent cells, driven by both cell-autonomous and non-cell-autonomous mechanisms, plays a crucial role in influencing tumorigenesis and cancer progression.

This review delves into the complexity of senescent cancer cells and their pleiotropic non-cell-autonomous effects on the tumor microenvironment (TME). In the first chapter, we delve into the intrinsic factors that drive the heterogeneity of senescent cancer cells. The second chapter shifts focus to the extrinsic mechanisms shaping the diversity of senescent cancer cells. Finally, in the third chapter, we examine the therapeutic potential of targeting senescence, highlighting how this knowledge can be leveraged to develop innovative strategies for cancer treatment.

Main

Chapter I: Cancer cell-intrinsic mechanisms underlying the heterogeneity of senescent cancer cells

The variation of cancer senescence exists in several aspects, including differences within senescent cell populations and among senescent cells induced by various stimuli. The umbrella of heterogeneity of senescent cells encompasses diverse factors, including epigenetics, tissue of origin, metabolic pathways, and environmental influences. In this chapter, we will focus specifically on cell-intrinsic factors that can affect the phenotype of cancer senescence.

The role of genetics and epigenetics in senescent cancer cell heterogeneity

Human cancers often exhibit significant transcriptional heterogeneity, characterized by diverse tumor cell populations with distinct transcriptional programs, which contribute to functional variability within the TME (Dawson, 2017). This heterogeneity arises from underlying genetic and epigenetic alterations. It is paramount to mention that this transcriptional diversity may form the foundation for the variability observed in senescent tumor cell phenotypes within and across tumors. While the phenotype of senescent cells is notably heterogeneous, the mechanisms driving this variability are only beginning to be understood. Indeed, Hernandez-Segura et al. identified

senescence transcriptome signatures that are strongly associated with specific stresses and cell types and show that the gene expression profiles of various senescence programs are highly dynamic over time (Hernandez-Segura et al., 2017). They identified 1,311 genes uniquely differentially regulated in senescent fibroblasts. Analysis of the temporal dynamics of these genes revealed an added layer of complexity, showing that the senescence signature varies significantly depending on whether senescence is in its early, intermediate, or late stages.

Replication stress, caused by the high proliferation rate of cancer cells, places a heavy burden on their DNA replication machinery, often leading to DNA damage and stalled replication forks, which can trigger cells to arrest in a senescent state (Herr et al., 2024). This process is genetically regulated by key factors such as telomere shortening, oncogene activation, and genes related to the DNA damage response and cell cycle control, like p53, Rb, p21, and p16, which drive senescence to prevent further genomic instability (Roger et al., 2021; Venkatachalam et al., 2017).

The heterogeneity of senescent cells in cancer is significantly influenced by the underlying genetic mutations. This variability results in diverse senescent phenotypes within tumors, affecting how cells respond to stress and influencing cancer progression and therapeutic outcomes. For example, mutations in tumor suppressors such as the tumor suppressors p53 or p16 alter the molecular pathways through which senescence is triggered or bypassed, with cells lacking functional p53 often evading senescence and contributing to tumor heterogeneity (Torres et al., 2024). For example, in a zebrafish model, a gain-of-function mutation in TP53 drives primary tumorigenesis by secreting the SASP and influencing neighboring cells to become senescent-like and similarly contributing to the SASP, amplifying the protumorigenic capacity of senescence (Haraoka et al., 2022). On the other hand, tumor cells with a loss of p53 show increased sensitivity to therapy-induced senescence due to their incapacity to induce DNA repair (C. Wang et al., 2019). This, combined with their intrinsic resistance to apoptosis, renders them more inclined to become senescent compared to wild type p53 cancer cells.

Telomere status also influences senescence heterogeneity (Tang et al., 2019). Cancer cells experiencing telomere shortening upon replicative stress may enter senescence, but mutations in telomere maintenance genes such as TERT or ALT pathway regulators allow some cells to bypass this mechanism, resulting in a mixture of senescent and proliferating cells within the tumor (Laud et al., 2005; M. Liu et al., 2024).

Oncogene-induced senescence (OIS) is a complex mechanism, in which induction of oncogenic stimuli during early tumorigenesis leads to high levels of DNA replication stress (Seoane et al., 2017). Oncogenes like RAS and MYC can induce OIS, creating distinct molecular profiles of senescent cells depending on the specific oncogene (X. Liu et al., 2018). Cells with different oncogenic drivers show varied senescence markers and secretory profiles, influencing the TME. For example, cells with activated MYC or p53 mutations produce different SASP factors, which can either promote or suppress tumor progression depending on the context (Sodir et al., 2022; C. Wang et al., 2019; Wellenstein et al., 2019). Recent studies have shown that the epigenetic landscape of senescent cells also shapes how oncogene-activated cancer cells either undergo OIS or escape from it (Martínez-Zamudio et al., 2023).

The transition to a senescent state is a complex process that frequently requires the transcriptional regulation of different genes. Given the significant role of chromatin structure in cellular regulation, epigenetic modifications—such as DNA methylation, histone modifications, and chromatin remodeling—are essential for initiating and maintaining senescence (X. Zhu et al., 2021). Even though the transcriptional heterogeneity of the senescent phenotypes remains not fully understood, advanced sequencing techniques are beginning to unravel the diverse

molecular pathways that contribute to this complexity. DNA methylation, specifically, has been observed to alter during cellular senescence in a context-dependent manner. For instance, during replicative senescence, specific regions of the genome can undergo local hypermethylation (Hänzelmann et al., 2015) . In contrast, OIS does not exhibit these DNA methylation changes (Xie et al., 2018). The stimuli that drive senescence can therefore lead to distinct epigenetic modifications, potentially contributing to the transcriptional diversity of senescent phenotypes (Hernandez-Segura et al., 2017; Kwiatkowska et al., 2023; Sakaki et al., 2017). For instance, whole genome DNA methylation patterns were compared between different senescence inducers across different cell lines(Kwiatkowska et al., 2023). The results showed that replicative senescence induced more pronounced epigenetic changes than senescence triggered by ionizing radiation or doxorubicin exposure. Thus, the epigenetic programs of senescent cells depended on the senescence-inducer and the cell type. Conclusively, cancer cells undergoing different types of senescence may exhibit distinct epigenetic modifications and potentially impact their ability to contribute to tumorigenesis. Further research is essential to fully understand these mechanisms and their role in cancer progression.

Even though the mechanisms that regulate tumor cell heterogeneity are still under investigation, current research is focused on characterizing senescence heterogeneity. Recent studies have highlighted how senescent cancer cells can exhibit diverse transcriptomes across different tumor types and even within the same tumor(Burnaevskiy et al., 2023). In these investigations, researchers used large-scale single-cell RNA-sequencing (scRNA-seq) analysis to understand how cell-to-cell variation in gene expression develops among senescent cells. The results from scalable and multiplexed scRNA-seq showed that cells after senescence-inducing stress fall into two major transcriptional clusters; one associated with stress response and the other with tissue remodeling. Interestingly, these findings come in accordance with recent reports of two distinct senescent profiles in the OIS model (Hoare et al., 2016).

Although senescence was originally considered an irreversible state of cell cycle arrest, recent studies indicate that cancer cells can escape from senescence (Duy et al., 2021; Saleh et al., 2019). In the context of therapy-induced senescence (TIS), this ability allows cancer cells to survive drug treatment, potentially leading to tumor regrowth and patient relapse. Interestingly, recent studies have shown that senescent-induced leukemia cells that escape senescence resume proliferation, showing significant heterogeneity (Miller et al., 2023). These cells exhibit asynchronous release from senescence and varying proliferation rates. Most importantly, RNA-seq analysis revealed variability in gene expression, with some of the escaped cells retaining senescence-associatedgenes. These findings suggest that transcriptional heterogeneity may influence which cells escape senescence, which could help to explain why in some cases, senescent cancer cells contribute to tumor regrowth and aggressiveness, while in others, they do not.

The role of tissue of origin in senescent cancer cell heterogeneity

The role of cancer senescence exhibits a dual nature, with the outcomes varying between positive and negative responses. This variation is greatly influenced by specific factors, including the cell type origin. Two key studies highlighted this variability using different cancer models of the same genotype driven by mutant RAS and p53 alterations (Ruscetti et al., 2018a, 2020). In the first study, investigating pancreatic ductal adenocarcinoma (PDAC), the most prominent effects of senescence were SASP-dependent vascular remodeling and T-cell surveillance (Ruscetti et al., 2020). Conversely, in the second study, focusing on lung cancer models, the outcome of senescence involved a SASP-induced NK cell-mediated senescence surveillance (Ruscetti et al., 2018a). Despite the same genetic drivers, these studies indicate the possibility that the tissue-specific reprogramming of the senescent cancer cells' SASP profiles can, in turn, lead to distinct TME responses.

Among the tissue-specific variations in SASP profiles, research has shown that the overall levels of SASP factors can also differ significantly among various tissue types. *In vitro,* screening of senescent human endothelial cells, fibroblasts, preadipocytes, epithelial cells, and myoblasts has revealed significant differences in SASP expression (Schafer et al., 2020). Endothelial cells and preadipocytes exhibited higher levels of SASP factors expression compared to the other cell types. Thus, higher concentrations of SASP might explain why some senescent cells contribute to chronic inflammation. This heightened inflammation could accelerate tumor progression by recruiting immune cells that support tumorigenesis and creating a feedback loop that sustains chronic inflammation and senescence in the TME. Therefore, in the context of cancer, this variability suggests that senescent tumor cells may behave differently depending on their origin. Senescent tumor cells from tissues that produce high levels of SASP factors might exacerbate tumor progression more than those from tissues with lower SASP levels (Palmer et al., 2019; Victorelli et al., 2019).While this concept helps explain the heterogeneity observed in cancer senescence, the specific pathways responsible for inducing senescence in tumor cells remain elusive.

Moreover, the tissue of origin plays a critical role in shaping the behavior of senescent cancer cells due to the unique characteristics of the TME in different organs. It has been increasingly clear that there is a specific TME composition in different tissues as well as within distinct areas within the same tissue. For instance, researchers found that the tumor's location, rather than just the tumor itself, plays a key role in regulating the recruitment of immune cells (Hensel et al., 2017),with significant differences in the proportions of macrophages, DCs, $CD8^+$ T cells, and $CD4^+$ T cells between the same tumor located either in the tibia or subcutaneously. In addition, macrophages located in the liver show distinct transcriptional and physiological differences from the macrophages in the lungs or the microglia in the brain (Lee & Ginhoux, 2022; Ballesteros et al., 2020; Kalucka et al., 2020; Krausgruber et al., 2020; Szabo et al., 2019; Vijay et al., 2019). Therefore, the tissue of origin significantly influences the TME, which in turn affects how senescent cancer cells behave, survive, and interact with surrounding cells. This variability in the TME across different tissues likely contributes to the observed heterogeneity in cancer senescence and its effects on tumor progression

The role of metabolism and nutrient availability in senescent cancer cell heterogeneity

A growing body of literature suggests that, although senescent cells were classically considered inactive due to their lack of proliferative capacity, they undergo significant metabolic alterations. These changes include increased oxidative stress, disrupted proteostasis, impaired metabolic pathways, and the accumulation of oxidized proteins (Hamon et al., 2020). A recent study focused on clear cell renal cell carcinoma (ccRCC) sought to investigate the correlation between senescent cancer cell-related alterations in metabolic processes and ccRCC patient overall survival (OS) (Zhou et al., 2024). The study classified ccRCC patients into two clusters marked by a low or high senescence-metabolism-related risk score (SeMRS), indicating distinct metabolic adaptations based on the senescence status. Of note, depending on the metabolic diversity of senescent cells in ccRCC tumors, low and high SeMRS patients displayed contrasting OS, immune infiltration, and immune escape ability.

One crucial metabolic pathway in senescence is mitochondrial metabolism. Although numerous metabolic alterations have been documented, there remains a lack of consensus regarding the role and functionality of mitochondria. Marsimolle et al. aimed to compare mitochondrial catabolism of human lung fibroblasts subjected to different senescence-inducing stimuli, revealing that mitochondrial catabolic processes vary significantly depending on the type of senescence stimulus (Marmisolle et al., 2023). Their results revealed that only cells that were oxidant hydrogen peroxide (H_2O_2)-senescence or doxorubicin-induced showed reduced

respiratory control ratio and coupling efficiency without AMPK activation or high secretory activity, whereas OIS cells exhibited increased respiration rates, AMPK activation, and elevated SASP markers. Additionally, Ras-induced senescence leads to enhanced fatty acid catabolism and distinct acetyl-CoA metabolism, highlighting the heterogeneity of mitochondrial metabolism based on the senescence stimulus.

Moreover, senescent cancer cells undergo changes in their lipid metabolism. Increased fatty acid oxidation (FAO) supports the energy requirements of senescent cells, and changes in lipid storage and signaling can influence the secretory phenotype of these cells (Wiley & Campisi, 2021). For example, senescent hepatocytes, fibroblasts, and T-cells exhibit upregulation of lipid droplets. This accumulation predominantly leads to an increase in free fatty acids and free cholesterol (Cadenas et al., 2012; Inoue et al., 2017; X. Liu et al., 2021; Maeda et al., 2009; Ogrodnik et al., 2017). Current studies highlight the mevalonate (MVA) pathway, an anabolic route for endogenous cholesterol biosynthesis, as a positive regulator of senescence in human cells (Ziegler et al., 2024). This pathway enhances the transcriptional activity of ERRα, potentially causing mitochondrial dysfunction, ROS production, DNA damage, and p53-dependent senescence. Many studies have investigated the effect of the MVA pathway on cellular senescence by using statins and aminobisphosphonates as inhibitors, yet the findings have been inconsistent. Blocking the MVA pathway with such inhibitors has varying effects on cellular senescence depending on the cell type. For example, inhibiting this pathway has been shown to delay senescence in human umbilical vein endothelial cells (HUVECs), while promoting senescence in oral keratinocytes and having little to no effect on oral fibroblasts (Assmus et al., 2003; R. H. Kim et al., 2011). In line with these studies, research by Roh et al. explored the dynamics of cholesterol localization during senescence and their *in vivo* consequences (Roh et al., 2023). Their work describes a different mechanism, in which senescent cells accumulate cholesterol in their lysosomes, which is crucial for SASP maintenance. Specifically, senescence is linked to the upregulation of the cholesterol exporter protein ABCA1, which functions as a cholesterol importer in lysosomes, to in turn, support mTORC1 activity—a key factor in SASP regulation. Therefore, while the role of cholesterol metabolism in senescence is still not fully understood, these findings suggest that tumor senescent cells may employ diverse mechanisms, further contributing to the complexity of senescence heterogeneity.

In addition, metabolite levels can be different among senescent cancer cells. Although senescent human oral fibroblasts preserve NAD^+ and nicotinamide levels, NAD^+ biosynthesis is often upregulated in tumor cells deriving from human hepatocellular carcinoma (Lv et al., 2021). The NAMPT-regulated NAD^+ salvage pathway has been implicated in modifying the strength of the pro-inflammatory SASP, with replicative senescence IMR90 fibroblasts having lower NAD^+ and pro-inflammatory SASP than OIS fibroblasts (Nacarelli et al., 2019). Therefore, the senescence trigger is key in shaping the metabolic programming of senescent cells, which in turn influences the intensity of the pro-inflammatory SASP. This metabolic rewiring helps explain why senescent cancer cells can exhibit varying pro-inflammatory and tumor-suppressive effects across different tumor types.

While metabolic changes during senescence are extensively documented, their identification as hallmarks of senescence remain challenging. The difficulty lies in the question of whether these alterations are causative factors or consequences of other molecular changes in senescent cells. Recently, Terao et al. demonstrated that cholesterol-mediated NAD^+ depletion activates macrophage senescence, which promotes age-related macular degeneration (AMD) phenotypes such as subretinal lipid accumulation and neurodegeneration (Terao et al., 2024). Moreover, recent studies support that TNF receptor-associated factor 3 interacting protein 2 (TRAF3IP2) accelerated senescence in mesenchymal stem cells via downregulation of NAMPT-mediated NAD^+

biosynthesis(Huang et al., 2023). Yet, the relationship between metabolism and cellular senescence is bidirectional, where metabolic changes can cause cellular senescence in some contexts, while the transcriptional adaptations of the senescent cell might result in a different metabolic reprogramming. In addition, in the context of a tumor where cells are under different types of metabolic stresses, senescent cancer cells might need to constantly adapt their metabolism. However, the magnitude and regulation of this metabolic plasticity are still poorly understood. To this end, comprehensive research on the senescent cancer cell metabolome and its dynamic shaping is needed. Consequently, future efforts are crucial to characterize metabolites as potential markers of senescence and part of the SASP, as well as evaluate their effects on the TME.

The role of early and late senescence in senescent cancer cell heterogeneity

Cellular senescence is a dynamic process. Senescent cells undergo gradual changes over time, meaning they can exist in different states throughout their reprogramming. This progression allows for the distinction between early and late senescent states. For instance, research has demonstrated that early senescent cells release SASP factors that include various growth factors and cytokines, contributing to anti-tumorigenic effects, wound healing, and tissue generation (Oguma et al., 2023). Over time, however, senescent cells enter late senescence stages and modify the composition of the SASP, leading to an enrichment of pro-inflammatory factors that might promote cancer development. This concept of dynamic reprogramming also ties into the distinction between acute and chronic senescence. Acute senescence describes the early induction of the senescence program for a limited duration, which is generally considered tumor-suppressive and plays a role in physiological processes (Burton & Stolzing, 2018). However, if the senescence-inducing stimuli persist or the immune system fails to clear senescent cells, they can enter a chronic state. This state is associated with ongoing inflammation and an increased risk of promoting tumorigenesis.

Interestingly, senescence appears to act in a bimodal pattern, with SASP representing the critical modulating factor (Evangelou et al., 2023). The SASP is known to play a crucial role in enabling senescent cells to carry out their primary functions. Since senescence is a dynamic process, it cannot be assessed as a static cellular state. the dual role of SASP between early and late senescence has led to a hypothesis that SASP initially promotes anti-tumor effects by driving senescence or apoptosis autonomously. However, over time, SASP composition shifts to include factors that suppress immune responses, facilitating tumorigenesis in surrounding cells (Alessio et al., 2023). A meta-analysis of the protein composition of SASP was conducted in order to compare the trends between early (elapsed time since stress 4–7 days) and late (elapsed time since stress over 30 days) OIS (Oguma et al., 2023). Interestingly, the absence of pathways found in over 75% of early-stage datasets suggests that the early period is the most heterogeneous phase of senescence progression. Therefore, it is plausible that this early heterogeneity can significantly influence tumorigenesis, as the diverse cellular states and responses during early senescence may either suppress tumor formation or, conversely, create a microenvironment that favors cancer development, immunosuppression, metastasis, and therapy resistance (Ruhland et al., 2016; Vindrieux et al., 2014; Wang et al., 2022). Indeed, radioresistance in head-and-neck squamous cell carcinoma was correlated with early induction of cellular senescence and NF-κB-dependent production of specific cytokines, such as CXCR2 ligands (Schoetz et al., 2021). Thus, the complexity of these differences between early and late senescence underscores the need for further research to fully understand their mechanisms.

Heterogeneity in early and late senescence is indeed linked to cancer progression, and various studies have highlighted this connection. During senescence, the expression of p16 and p21 is

dynamically regulated (Safwan-Zaiter, Wagner, Michiels, et al., 2022). Mouse models of oncogenic Ras-driven lung cancer showed that early senescent cells, marked by p16 expression, contribute to initial tumor suppression (Walters, 2023). However, over time these cells adopt a SASP profile that promotes tumor growth by inducing a more immunosuppressive TME. However, other findings indicate that p21 expression is mainly associated with early senescence, while p16 expression is linked to more established and irreversible senescence (Dulić et al., 2000). These two regulators have distinct roles in cancer progression (Domen et al., 2022). For instance, Sturmlechner et al. discovered that the cell cycle regulator p21 drives a specialized early form of the SASP known as the p21-activated secretory phenotype (PASP) (Sturmlechner et al., 2021). The PASP includes the chemokine CXCL14, which attracts macrophages to senescent cells with high p21 levels. If these cells restore p21 levels within 4 days, they are not cleared by macrophages. However, if the cells do not normalize p21, macrophages will recruit cytotoxic T cells to eliminate the senescent cells. Unlike other regulators such as p16, p21 uniquely facilitates this "timer" mechanism for immune surveillance and removal of senescent cells. Therefore, in the context of tumorigenesis, early and late senescence are regulated by different mechanisms and co-evolve with the TME. Mechanistic understanding of the transition from early to late-stage senescence might pave the way to a new class of therapeutic interventions in cancer.

Chapter II: Heterogenous non-cell autonomous (extrinsic) effects of senescent cancer cells

During tumorigenesis, senescent cells are part of the tumor ecosystem affecting tumor progression. Within the TME, senescent cells can communicate with surrounding cells to for example recruit immune cells and alter the tumor stroma (Kumari & Jat, 2021b). Since the phenotype of senescent cancer cells is heterogeneously shaped by cancer cell-intrinsic factors, their non-cell-autonomous effects within the TME can vary. This contributes to the complex, pleiotropic effects of senescent cells, which can lead to different outcomes in tumor development and progression.

The dynamic interaction between senescent cancer cells and immune cells

Impact of the senescent cancer cell secretome on shaping immune cell responses

Extensive research has established a strong link between senescent cells and the immune system, highlighting the critical role of innate and adaptive immune cells—such as NK cells, T cells, macrophages, and neutrophils—in mediating the clearance of senescent cells in both healthy and diseased conditions (Arora et al., 2021; Binet et al., 2020; Krizhanovsky et al., 2008; Muñoz-Espín et al., 2013). Under normal conditions, senescent cells chemo-attract immune cells for their clearance, thereby playing a crucial role in maintaining tissue homeostasis and preventing tumorigenesis. This immune attraction also occurs in the context of cancer, where senescent tumor cells elicit a robust antitumor response primarily driven by DCs and $CD8^+$ T cells (Marin et al., 2023). This recognition is largely mediated by the SASP of senescent cells, composed of unique factors depending on the senescent cell phenotype. For instance, research has demonstrated that oncogene-induced senescence leads to the recruitment of $CD8^+$ T cells that can inhibit tumor growth, with their effectiveness depending on the context and the specific SASP factors involved (Kang et al., 2011). In contrast, other studies have shown that the pharmacological inhibition of the DNA-replication kinase CDC7 *in vivo*, the infiltration of CD4 and CD8 T cells does not lead to the clearance of immune cells (C. Wang et al., 2019). Thus, these suggest that the interplay between senescent cells and immune cells is highly dependent on the context.

The role of senescent cell types in modulating the immune microenvironment is highly context-dependent, with specific signaling molecules influencing immune responses differently across various tissues. For instance, Cyclooxygenase-2 (COX-2) is an enzyme that plays a key role in the inflammatory response and is also associated with various pathological conditions, including cancer (B. Liu et al., 2015). The induction of COX-2 in senescent cells and the subsequent production of its downstream factor, prostaglandin E2 (PGE2), play a crucial role in different contexts of senescence. In the liver, COX-2 induction and PGE2 production are essential for the maturation of macrophages and their surveillance of pre-malignant hepatocytes in response to $NRAS^{G12V}$-induced senescence (Gonçalves et al., 2021). However, in the thyroid, $HRAS^{G12V}$-induced production of PGE2 in senescent thyrocytes leads to the polarization of immune-suppressive macrophages that have protumoral effects (Mazzoni et al., 2019).

The pathways underpinning the dual effect of senescence in tumors remain largely unclear. Heterogeneity in senescent states and cell types might influence the context-dependent effects of senescence in cancer. Macrophages, which are one of the most prevalent and critical immune cells in the TME of solid tumors, can also express senescence markers and adopt phenotypes that can affect their polarization (Kloosterman & Akkari, 2023). Tumor-associated macrophages (TAMs) play a key role in orchestrating angiogenesis, remodeling of the extracellular matrix, cancer cell proliferation, metastasis, and immunosuppression (Mantovani et al., 2022). Due to their plasticity, TAMs exhibit a wide array of profiles, which is crucial for preserving tissue homeostasis across various organs (Nobs & Kopf, 2021). TAMs fulfill a range of tasks in response to varied environmental stimuli and have been shown to have dual functions in cancer. In the early onset of tumor development, macrophages can have antitumoral responses, eliminating tumor cells via phagocytosis, pro-inflammatory signaling, and by triggering innate or adaptive anti-tumoral immune responses (Mantovani et al., 2022). Over time, however, as the tumor microenvironment evolves, most macrophages get re-educated to support tumor progression and metastasis through various mechanisms. These include promoting cancer cell survival and proliferation, as well as suppressing innate and adaptive immune responses (Locati et al., 2020; Mantovani et al., 2022; Murray et al., 2014).

TAMs, have long captivated researchers due to their pivotal involvement in tumor growth (Kloosterman & Akkari, 2023). In cancer, TAMs often contribute to an anti-tumor response. For instance, CXCL14 secretion by RAS-overexpressing senescent hepatocytes attracts macrophages which in turn recruit cytotoxic T cells to eliminate the senescent cells (Sturmlechner et al., 2021). In addition, the SASP released by senescent hepatocytes or stellate cells polarizes these macrophages toward a pro-inflammatory phenotype (Lujambio et al., 2013). This polarization enhances the clearance of senescent cells and supports anti-tumor immunity. Studies have demonstrated an increase in macrophages upon senescence induction via treatment with the CDC7 inhibitor (C. Wang et al., 2019). However, macrophages can be hijacked by the tumor cells to support different pro-tumorigenic processes, such as immunosuppression (Kloosterman & Akkari, 2023). TAMs can directly inhibit the activation of T cells and NK cells within the TME, thereby suppressing the protective anti-tumor immune response (Petty et al., 2019). In addition, current investigations have shown that in an oncogenic Ras-driven mouse model of lung adenoma, alveolar senescent macrophages accumulate early during tumor development (Prieto et al., 2023). These macrophages suppress cytotoxic T-cell responses, weakening the immune system's ability to target and destroy tumor cells, indicating that these senescent macrophages promote tumor growth. Therefore, the phenotypic diversity of TAMs in the TME can significantly influence tumor behavior. While this heterogeneity highlights the dual role of TAMs in response to cellular senescence, the specific mechanisms driving these diverse functions remain largely unknown. Future research focusing on understanding how TAM heterogeneity interacts with the complex landscape of senescent cancer cells is of great importance.

Impact of the senescent cancer cell surfaceome on shaping immune cell responses

Despite the role of senescent cells in attracting and recruiting immune cells via SASP, there is still a lack of knowledge regarding which other mechanisms are involved in the interaction of senescent cells and immune cells. In this context, two recent studies demonstrated that mouse senescent liver cancer cells undergo extensive changes beyond the secretion of SASP (H.-A. Chen et al., 2023). They found that senescence causes significant remodeling of cell-surface proteins, such as IFNγ and MHC-I, and related signaling pathways, fundamentally altering how these cells perceive and react to their environment. Notably, liver senescent cancer cells exhibited increased sensitivity to environmental signals such as type II interferon (IFN), leading to a stronger upregulation of antigen processing and presenting mechanisms. This enhanced immunogenic potential makes senescent tumor cells more recognizable to immune surveillance, shedding light on the role of senescence in cancer physiology. In addition, the study revealed that levels of environmental IFNγ and the integrity of type II IFN signaling in senescent cancer cells determine whether these cells are cleared or persist. This suggests that the ability of senescent cells to attract and respond to IFNγ-secreting immune cells greatly impacts their clearance or persistence in tissue. Consequently, depending on their environment, senescent tumor cells can alter their phenotype to become more visible to the immune system.

In this context, studies have revealed a direct interaction between senescent cancer cells and adaptive immune cells, facilitated by heightened expression of MHC-I and PD-L1/2 on these cells. Although the mechanisms behind these alterations remain unclear, research has revealed how senescent cells evade immune control, such as by increasing the expression of the non-canonical MHC-I molecule HLA-E. Specifically, senescent dermal fibroblasts display HLA-E, which interacts with the inhibitory receptor NKG2A on NK cells and highly differentiated $CD8^+$ T cells, thereby dampening immune responses against senescent cells. While this study focused on aging, the underlying mechanism might also be relevant to tumorigenesis (Pereira et al., 2019).

Spatial and metabolic control of the senescent cancer cell-immune cell interaction

Spatial organization and metabolite availability within the tumor plays a crucial role in shaping the behavior of resident and infiltrating immune cells, influencing both tumor progression and treatment outcomes (X. Qiu et al., 2024; Schürch et al., 2020; S. Zhang et al., 2023)Within the tumor, the localization of tumor cells and immune cells, along with their proximity to one another, can dictate whether immune surveillance or immune evasion is promoted. In a growing tumor, metabolite availability, such as glucose, oxygen, and amino acids, significantly affects both cancer cell metabolism and immune cell function (J. Chen et al., 2024). Tumor regions with low oxygen levels or nutrient deprivation often foster immune resistance and drive tumor aggressiveness (Emami Nejad et al., 2021). As a result, the local ecosystem can therefore significantly impact the interaction between senescent cancer cells and immune cells to shape the fate of tumor progression(Xiao et al., 2023).

Emerging research indicates the heterogeneity of senescent cancer cells within different regions of the same tumor (Y. H. Kim et al., 2017). Specifically, studies on papillary thyroid carcinoma have shown that senescent tumor cells are frequently located at the front area of collective invasion and within lymphatic channels and metastatic foci in lymph nodes (Y. H. Kim et al., 2017). Interestingly, in this model, senescent tumor cells initiated collective invasion by creating a gradient of the CXCL12 chemokine at the front edge. Given the variability in senescent tumor cells and the SASP they secrete, it is plausible to hypothesize that this heterogeneity significantly impacts their interaction with the local immune system, even in different regions of the same

tissue, generating local senescent cell niches. For instance, the levels of CXCL12 secreted by senescent cancer cells can significantly influence the immune landscape; high concentrations may enhance immune clearance, while lower levels can create an immunosuppressive microenvironment that facilitates tumor progression and metastasis (Y. H. Kim et al., 2017). Interestingly, TAM distribution and accordingly function are also variable within the TME (Guo et al., 2022). For example, in colorectal cancer, macrophages expressing CD163 are primarily located at the invasive front of the tumor (IF), whereas macrophages expressing CD80 are predominantly localized in the adjacent normal mucosa surrounding the tumor, which indicates a predominant anti-inflammatory phenotype of TAMs. (Pinto et al., 2019). Senescent cells in tumors often display diverse SASP profiles, which could create diverse niches with unique inflammatory characteristics (Coppé et al., 2010). These varying SASP profiles might be instrumental for the recruitment of different TAM subpopulations within each senescent cancer cell niche, resulting in variable effects on tumor progression.

Among the interactions between tumor cells and the immune system, increased evidence suggests that tumor cells suppress the function of immune cells by competing for and consuming nutrients such as glucose, amino acids, and glutamine (J. Qiu et al., 2019). Moreover, metabolites such as lactate, PGE2, and arginine produced by tumor cells profoundly impact immune cells within the TME, triggering metabolic adaptations that hinder anti-tumor immune responses. Interestingly, senescent cancer cells are known to produce high levels of lactate through glycolysis, which is then secreted into the TME (Dou et al., 2023). This lactate can inhibit cytotoxic T cells and promote the protumorigenic polarization of macrophages (Feng et al., 2023). Therefore, through metabolic alterations, senescent cancer cells can influence immune cells and evade immune surveillance, thereby promoting tumor progression. Recent research by Deng et al. has revealed that senescent tumor cells secrete IL-6, which stimulates TAMs to upregulate CD73 expression via the JAK/STAT3 signaling pathway (Deng, Chen, et al., 2024). This upregulation leads to the accumulation of adenosine in the TME, which suppresses anti-tumor immunity. Conversely, inhibiting CD73 enhances $CD8^+$ T cell-mediated anti-tumor immune responses, suggesting promising therapeutic interventions against cancer. These findings underscore the pivotal role of metabolism and TME interactions in cancer biology and highlight CD73 as a potential therapeutic target for enhancing anti-tumor immune responses in the senescence-rewired TME. Nevertheless, the role of metabolites as part of the SASP and their impact on immune cell responses remains largely unknown.

Interplay between senescent cancer cells and the tumor stroma

It has long been described that the extracellular matrix (ECM) is a crucial element of cancer (Yuan et al., 2023). Notably, the ECM experiences significant alterations during aging, which impact tissue mechanics and structure. While the specific relationship between senescent cancer cells and the ECM remains underexplored, senescent cancer cells release Matrix Metalloproteinases (MMPs) as part of their SASP, which can affect ECM remodeling. In fact, a recent pan-cancer analysis in 33 cancer types revealed a positive correlation between senescence-related ECM components, such as MMP2 expression, and immune infiltration (Yan et al., 2023). Thus, MMPs, by degrading various ECM proteins, including collagen, can enhance immune cell infiltration and facilitate tumor metastasis (Winkler et al., 2020). Additionally, previous studies have demonstrated that as part of the SASP, senescent cells secret ECM proteins such as collagen and tenascin-c, thereby contributing to the increased stiffness of the local tissue microenvironment (Calhoun et al., 2016). The exact mechanism responsible for the secretion of these proteins remains unknown, but evidence suggests that mTORC1 may play a contributing role.

Moreover, the stiffness of the ECM varies among tissues (Piersma et al., 2020). For instance, the aggressiveness of breast cancer and PDAC is associated with a stiffer ECM, with experimental models showing causal links between tissue mechanics and malignancy. This variability in ECM stiffness underscores the importance of understanding the heterogeneity of the SASP in different cancer contexts. The composition and intensity of the SASP can vary widely across cancer types, leading to differences in ECM remodeling, as senescent cells may secrete varying levels and types of MMPs and other SASP factors related to the ECM. In addition, the varying stiffness of the ECM can also affect immune cell infiltration (Mai et al., 2024). For instance, some immune cells may infiltrate stiffer tissue areas less efficiently than other types of immune cells. This could partly also explain the distinct types of immune cells recruited in response to senescent cancer cells in different organs, and potentially within different senescent cell niches, as the ECM's physical properties shape the immune landscape. Nevertheless, our understanding of the relationship between cancer senescence and ECM remodeling remains limited. Future studies elucidating these mechanisms are of great importance.

In addition to its role in ECM remodeling, senescent cancer cells also play a crucial role in the remodeling of the tumor vasculature. Vascular endothelial cells (ECs) play an active role in promoting tumor angiogenesis and are a crucial element of the TME (Yang et al., 2021). Senescent cancer cells, via their SASP, can also inhibit the growth of neighboring cancer cells and enhance the vasculature for more efficient drug delivery (L. Wang, Lankhorst, et al., 2022a). In KRAS mutant PDAC, a combination of MEK and CDK4/6 inhibitors can induce tumor senescence, leading to a SASP profile that promotes tumor vascularization. In this context, the SASP activates endothelial cells to increase $CD8^+$ T cell infiltration (Ruscetti et al., 2020). Thus, the induction of senescence in cancer cells can make PDAC more susceptible to chemo- and immunotherapies through its effects on the vasculature and immune system. Similarly, immune surveillance of aged pre-cancerous liver cells relies on SASP-induced NF-κB activation within endothelial cells (Yin et al., 2022). This process is crucial for enabling the adherence and migration of $CD4^+$ and $CD8^+$ T lymphocytes across endothelial barriers. Here, the communication of senescent cells with endothelial cells through the SASP, plays a vital role in recruiting lymphocytes which is essential for mounting an effective immune response against tumors. However, increased and persistent SASP-induced angiogenesis can be detrimental in the long term, where it can facilitate tumor growth and induce an epithelial-to-mesenchymal transition in neighboring cancer cells (L. Wang, Lankhorst, et al., 2022a). This way, the SASP can enhance tumor cell migration and promote metastasis. However, depending on the context, the senescent phenotype, or specific SASP components, the impact on angiogenesis may vary. For instance, it is possible that the early and late stages of senescence have distinct impacts on the TME, influencing both ECM composition and angiogenesis. In a study by Ding et al., both acute (short-term) and chronic (long-term) liver injury were used to explore their effects in mouse models (Ding et al., 2014). Following acute liver injury, liver sinusoidal endothelial cells (LSECs) upregulate the receptor CXCR7, which in turn, triggers the release of angiocrine factors that promote liver regeneration. In contrast, after chronic liver injury, the signaling balance shifts. LSECs suppress the regenerative CXCR7 pathway, driving fibrosis instead of regeneration. Therefore, in the context of senescence, early and late stages may differentially influence signaling pathways, leading to different outcomes upon tumor progression. However, there is currently no evidence to confirm this hypothesis.

Dual roles of the SASP on tumor progression

Although cellular senescence is well-recognized as a powerful cell-autonomous anti-cancer mechanism, research has demonstrated that the SASP secreted by senescent cancer cells can have context-dependent effects, promoting or inhibiting tumor growth depending on the specific conditions. Initial studies on the impact of the SASP on the immune system showed that several factors promote the clearance of senescent cells, suggesting that the SASP orchestrates the removal of senescent cancer cells to maintain tissue homeostasis (Iannello et al., 2013; L. Wang,

Lankhorst, et al., 2022a; Xue et al., 2007). In addition, depending on the tumor model, the effect of the SASP on the host immune system is diverse. A pivotal study conducted by Kansara et al. demonstrated that in a mouse model of radiation-induced osteosarcoma, IL-6 was essential for IR-induced senescence, which in turn, activated the clearance of senescent cells by NKT cells (Kansara et al., 2013). Notably, mice deficient in IL-6 showed accelerated tumors. In contrast, within the TME of a lymphoma model, IL-6 secretion by senescent tumor cells displays a pro-tumorigenic effect by promoting chemoresistance (Bent et al., 2016). Therefore, these findings underscore that the impact of the SASP on cancer progression is highly dependent on the TME it influences. Moreover, in the context of OIS, such as with NRAS, premalignant hepatocytes enter a senescent state and secrete CCL2 to attract CCR2+ monocytic cells. These monocytes mature into macrophages, which play a crucial role in mediating the clearance of the senescent cells, thereby preventing cancer. Conversely, in established hepatocellular carcinoma (HCC), CCL2 released by NRAS-induced senescent hepatocytes recruits, a monocytic cell population that remains undifferentiated and immunosuppressive, ultimately supporting tumor progression (Eggert et al., 2016). Thus, the outcome can vary significantly, with the SASP promoting anti-tumor immune responses and cell clearance in some contexts while contributing to tumor growth and chemoresistance in others.

As tumors evolve over time, they are subjected to immune editing resulting in the reduction of immune-activating receptors and ligands and the increase of inhibitory checkpoints(Russell et al., 2021). This process hampers the innate and adaptive immune systems' ability to identify and destroy pre-malignant and malignant tumor cells. As a result, the release of anti-inflammatory cytokines and various chemokines by tumor cells can attract suppressive immune cells, particularly regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). In turn, these suppressive cells inhibit the cytotoxic activity of NK and $CD8^+$ T cells, thereby dampening anti-tumor immune responses (Russell et al., 2021). Prolonged senescence and chronic SASP activation may also result in similar mechanisms of immune escape. For example, Pepeira et al. demonstrated that senescent nevi lack the expression of induced NKG2D receptor ligands (Pereira et al., 2019). Additionally, the SASP factors senescent cells release, such as IL-6, contribute to the increased expression of the inhibitory MHC molecule HLA-E. This, in turn, suppresses the clearance of these pre-malignant lesions by NK and T cells. Moreover, current studies demonstrate that p16-positive senescent cells upregulate the immune checkpoint protein programmed death-ligand 1 (PD-L1), which stabilizes in these cells due to the inhibition of cell cycle kinases (CDK4/6)(Majewska et al., 2024). This allows senescent cells to create an immunosuppressive environment, preventing effective immune clearance. While the mechanism by which senescent cells alter the immune response in aging is intriguing, it is essential to investigate this phenomenon in the context of cancer. More recently, Chaib et al. identified the upregulation of PD-L2 as a key factor in the persistence of therapy-induced senescent cancer cells, contributing to immune evasion within the TME (Chaib et al., 2024). Furthermore, in various cancer types, there is an increased expression of PD-L1 in cancer cells, which interacts with PD-1 receptors in immune cells, leading to their inactivation or exhaustion. These mechanisms highlight the complex ways in which senescent cells and tumors evade immune surveillance, ultimately promoting tumor persistence and progression.

Chapter III: Use of pro-senescent therapy for precision anti-cancer therapy

Senescence serves as an important anti-cancer defensive mechanism (L. Wang et al., 2022). The activation of senescence in response to stress results in a stable halt of cellular proliferation. As such, many anticancer treatments cause senescence in cancer cells by producing genotoxic stress

or increasing oxidative stress. Indeed, extensive research is being performed on therapy-induced senescence (TIS), a process where cellular senescence is triggered by treatments such as radiation and certain chemotherapies (Tóth et al., 2024;Gallanis et al., 2023; Lee et al., 2022; Zhang et al., 2023).

While many anticancer treatments induce senescence through TIS, research indicates that the prolonged presence of TIS can be harmful in the long term (Demaria et al., 2017; Ruhland & Alspach, 2021). Since senescence describes a highly heterogeneous state, shaped by a range of intrinsic and extrinsic factors, the effects of therapies designed to induce senescence are likely to be diverse. On the one hand, the SASP can initially inhibit cell growth and aid in the recruitment of the immune system, leading to tumor suppression (Ruscetti et al., 2018b). On the other hand, TIS can also lead to chronic accumulation of senescent cells, leading to cancer relapse and invasion (Demaria et al., 2017b; Schmitt et al., 2002; Song et al., 2023). Both of these outcomes are closely related to the SASP and point out the significance of cellular and tissue context (Cahu et al., 2012; Guillon et al., 2019; Milanovic et al., 2018). These dual outcomes underscore the complexity of TIS and the pivotal role that senescent cancer cell heterogeneity plays in determining the effectiveness of treatment. Therefore, understanding the diverse phenotypes and behaviors of senescent cells, particularly how different SASP profiles influence tumor progression or suppression, is essential for developing more targeted and effective anti-cancer therapies that minimize the risk of relapse and tumor aggressiveness.

In this context, the one-two-punch cancer therapy shows great promise (Bousset & Gil, 2022; C. Wang et al., 2019; L. Wang et al., 2022). In this approach, the first step involves using a drug to induce senescence in cancer cells, halting their ability to proliferate. This senescence can be triggered by various factors, including–CDK4/6 inhibitors, Polo and Aurora kinase inhibitors, histone deacetylases, and other epigenetic modifiers (Gallanis et al., 2023; Y. Lee et al., 2022; X. R. Zhang et al., 2023). The second 'punch' then involves administering a different drug specifically designed to target the senescent cancer cells. The second step can be implemented through three primary strategies: using senolytics to directly target and eliminate senescent cells (pharmacological senolysis) senomorphics, which are a class of agents that modify the behavior of senescent cells by altering the effects of the SASP without inducing cell death, and immunotherapy (immune-related senolysis), aimed at restoring or enhancing the immune system's ability to clear senescent cells, all represent potential strategies in addressing senescence-related pathologies.

The use of pharmacological senolysis in cancer therapy

A key characteristic that makes senescent cells proper candidates for senolytic drugs is their altered chromatin structure, which leads to changes in gene expression and affects processes such as apoptosis regulation (Y. Zhu et al., 2015). These alterations create specific 'flaws', making senescent cells ideal candidates for selective targeting by senolytic drugs. Senescent cells often exhibit upregulated expression of BCL-2 family proteins, making them potential targets for senolytic drugs like navitoclax, a BCL-2 inhibitor (Chang et al., 2016; L. Wang et al., 2017; Zhu et al., 2016). Navitoclax has been shown to effectively eliminate various types of senescent cells, including senescent cancer cells, by reactivating their apoptotic pathway. The effectiveness of navitoclax varies across different cell types because the upregulation of BCL-2 or other anti-apoptotic BCL-2 family proteins is not a consistent vulnerability in all senescent cells (Jochems et al., 2021a). This variability is evident as the senolytic response to navitoclax differs widely among various cancer cell lines in laboratory studies, and there are no clear biomarkers to predict which cells will respond to the treatment (Jochems et al., 2021a). However, cells bearing mutations in pro-apoptotic BCL-2 family genes, such as BAX and BAK, are resistant to navitoclax (Marczyk et al., 2020). Therefore, loss-of-function mutations in these pro-apoptotic genes are likely a common

resistance mechanism against navitoclax and other senolytic drugs of the same family. In addition to its limitations, the use of navitoclax is associated with several side effects, the most commonly reported being diarrhea, nausea, and thrombocytopenia (de Vos et al., 2021). While navitoclax is a well-established senolytic drug, recently, galactose-modified prodrugs have gained significant interest in the field of senolytic treatments. Senescent cells have high levels of SA-β-Galactosidase, suggesting that these cells are able to selectively digest prodrugs with a cytotoxic component connected to a cleavable galactose section (Guerrero et al., 2020). In this context, duocarmycin can target senescent cells and induce apoptosis in OIS and other types of senescence stimuli like genotoxic stresses, irradiation, and replicative exhaustion (Guerrero et al., 2020). In fact, duocarmycin eliminates senescent cells and decreases the expression of senescence-associated genes, in an *in vivo* model of whole-body irradiation. Another senolytic drug that belongs to the same group is gemcitabine which was also found to reduce senescent cell viability in many cell types in murine and human *in vitro* models (Cai et al., 2020). Recent research by Wang et al. identified that combining gemcitabine with a KD4MC inhibitor effectively eliminates tumor cells harboring TP53 mutations (K. Wang et al., 2023). In Tp53-deficient models of liver cancer, combining a CDC7 inhibitor, which induces senescence specifically in cancer cells, with an mTOR inhibitor, significantly reduced tumor growth (C. Wang et al., 2019). Notably, important findings have shown that targeting the death receptor 5 (DR5) signaling and inhibiting the death receptor inhibitor cFLIP, results in the efficient killing of a variety of senescent cancer cells (L. Wang, Jin, et al., 2022). Other studies identified Mcl-1 as the most expressed anti-apoptotic gene in senescent tumor cells, thereby treatment with the Mcl-1 inhibitor S63845 leads to the complete elimination of specific senescent tumor cells and metastases (Troiani et al., 2022). These approaches, used in cancer treatment, highlight the "one-two punch" strategy to enhance therapeutic outcomes.

The varying outcomes of senolytics treatments can be attributed to the context-specific role of cancer senescence, as it may influence treatment responses differently depending on the unique genetic and molecular landscape of each cancer type. Current findings have highlighted that senescent cells are not uniform, even within the same tissue type, underscoring a heterogeneous response to the senolytic drug ABT263 (Francesco Neri, 2024). Using high-content image analysis, scientists examined primary human endothelial cells and fibroblasts, focusing on senescence marker expression including SA-β-Gal, γH2AX, LaminB1, HMGB1, and p21. The authors describe that senescent cells arrested in the G2 phase of the cell cycle exhibit higher levels of these markers compared to those arrested in the G1 phase. This study is particularly significant as it provides the first evidence that different subpopulations of senescent cells respond differently to senolytic treatments, underscoring the importance of considering this variability when developing future senolytic therapies.

The use of senomorphics in cancer therapy

As discussed earlier in this review, the SASP plays a crucial role in recruiting immune cells to clear senescent cells and prevent oncogene-induced tumor formation. However, its prolonged presence can disrupt tissue homeostasis and impact treatment outcomes differently across various cancer types. Given that most of the tumor-promoting effects of senescence cells are related to their SASP, drugs that target the SASP are promising alternatives to senolytics (Short et al., 2019). Senomorphics act on suppressing the function of the SASP via targeting senescence-related signaling pathways, like MAPK, NF-κB, mTOR, and IL-1α (Baar et al., 2017; J. Park & Shin, 2022; Yun et al., 2018). Therefore, their function is to potentially maintain the SASP-dependent anti-tumorigenic functions, specifically in the context of cancer senescence (J. Park & Shin, 2022)

As a dominant transcription factor, NF-κB regulates the expression of a variety of genes including SASP factors. Metformin blocks the activity of the NF-κB, thereby decreasing the expression of SASP factors including IL1B, CXCL5, IL6, and IL8 (Moiseeva et al., 2013). More recent findings demonstrate the use of metformin as senomorphic to modulate the SASP by inhibiting mTOR and STAT3 signaling and enhancing the anticancer effect of CDK4/6 inhibitors in head and neck squamous cell carcinoma (Hu et al., 2020). Also, other studies highlight the role of metformin in alleviating IR-induced senescence phenotypes, such as decreasing cell proliferation and increasing the expression of the DNA repair-associated genes BARD1 and RAD51 (J.-W. Park et al., 2022).

A novel concept that explains the paradoxical effect of senescent cells *in vivo* is the presence of distinct phenotypes of senescent cells on the basis of environmental factors. One crucial environmental variable in different tissues and organs is oxygen levels (Carreau et al., 2011). In fact, oxygen plays an important role in the behavior of senescent cells. In hypoxic tissues, senescent cells exhibit reduced production of pro-inflammatory SASP factors, which activate the AMPK pathway (van Vliet et al., 2021). This activation suppresses the mTOR/NF-κB signaling, a key driver of SASP expression. This mechanism presents a potential therapeutic avenue. Studies have shown that hypoxia-mimetic compounds, such as roxadustat, can interfere with SASP factor expression in senescent cells. In mice treated with chemotherapy or naturally aged, these compounds improved physical strength, suggesting that treatment with hypoxia mimetics may open up new possibilities in cancer therapy to counteract the pro-tumorigenic effects of the SASP.

Senescent cells are highly diverse, with some cells displaying only different levels of inflammatory SASP. For instance, cells that overexpress p16 or p53 enter a state of senescence without inducing NF-κB signaling leading to the absence of pro-inflammatory and NF-κB-dependent SASP factors (Coppé et al., 2011; Wiley et al., 2018). This heterogeneity suggests that it might be possible to develop treatments that encourage the beneficial aspects of senescence while minimizing its negative consequences. In this context, one is the use of CDK4/6 inhibitors, which induce senescence in both breast cancer mouse models and patients (Goel et al., 2017; B. Wang et al., 2022) CDK4/6-induced senescent cells display a partial SASP, characterized by p53-regulated components but lacking the pro-inflammatory factors typically driven by NF-κB. Notably, the number of senescent cancer cells in mice exposed to the CDK4/6i abemaciclib was similar to that in mice treated with doxorubicin, but mice treated with abemaciclib suffered less from detrimental effects due to a decrease in pro-inflammatory SASP factors. Essentially, instead of contributing to tumor progression CDK4/6i-induced senescent cells induced a potent antitumour response to stimulate senescent cell clearance. Nevertheless, CDK4/6 inhibitors can trigger aspects of the senescent cell phenotype that are harmful, such as changes in gene expression that lead to cancer stemness (Milanovic et al., 2018). Therefore, the use of CDK4/6i in this strategy needs further research.

While the use of senomorphics holds great promise, this approach has its limitations. SASP inhibitors aim to suppress the production of various SASP factors, which can have both beneficial and harmful effects. Therefore, by inhibiting SASP, senomorphics could reduce both the growth-promoting and tissue-destructive effects of these secreted factors. Moreover, senomorphics need continuous treatment to sustain inhibition of the SASP, although some compounds, such as rapamycin, may lead to prolonged effects after a short-term treatment (Bitto et al., 2016; Mannick et al., 2018). However, important systemic side-effects have been reported by the use of rapamycin and also other senomorphics such as statins, including metabolic dysregulation, thrombocytopenia, hyperlipidemia, impaired wound healing, muscle problems, increased risk of type 2 diabetes and liver damage (J. Li et al., 2014; Ruscica et al., 2023). Consequently, significant

promise remains for the discovery of new SASP inhibitors that specifically target the pro-tumorigenic effects of senescent cancer cells and have minimal side effects on tissue homeostasis.

The use of immune-related senolysis in cancer therapy

Senescent cells are strongly immunogenic. One key mechanism is the upregulation of antigen presentation, allowing them to present unique antigens (Marin et al., 2023). This involves the release of alarmins, activation of IFN signaling, and the enhancement of MHC class I machinery. Additionally, senescent cells display senescence-associated self-peptides, which can activate $CD8^+$ T cells, further amplifying the immune response. Therefore, immunotherapeutic strategies have also been employed to combat senescent cells. To this end, researchers propose an alternative approach where senolytic chimeric antigen receptor (CAR) T cells can be used to target and clear senescent cells. Notably, Amor et al. identified the urokinase-type plasminogen activator receptor (uPAR) as a cell surface protein widely expressed during senescence (Amor et al., 2020). In both *in vitro* and *in vivo* settings, CAR T cells recognizing uPAR, effectively eliminated senescent cancer cells and improved survival outcomes in a lung adenocarcinoma mouse model. In line with these findings, another study investigated the efficacy of the NKG2D receptor, providing a target for CAR-T cells. Interestingly, NKG2D-CAR T cells exhibited strong cytotoxic activity against senescent cells while showing minimal impact on non-senescent cells, indicating their promise as specific senolytic agents (Deng, Kumar, et al., 2024). In support of combining immunotherapy with pro-senescence strategies, a study identified the retinoic-acid-receptor (RAR) agonist adapalene as an effective inducer of senescence in prostate cancer (PCa) cells (Colucci et al., 2024). Activation of RARs by adapalene initiates a robust senescence response and fosters a tumor-suppressive SASP. In preclinical mouse models of PCa, the combination of adapalene with the chemotherapy drug docetaxel produces a SASP that significantly enhances tumor clearance by natural killer (NK) cells, outperforming the effects of either drug used alone. However, even though these approaches seem to be accurate in limiting senescent cells, their success heavily relies on the identification of surface antigens associated with senescence. Considering the heterogeneity of cancer senescence and SASP, the universal application of immunotherapy as a second 'punch' still has limitations. Therefore, in the context of cancer, future investigations using this approach could identify varying cell-surface molecules that are specific to particular senescence contexts, such as tissue and cancer type.

Emerging research highlights the role of OIS tumor cells in attracting immune cells such as T cells and macrophages, to the tumor site and facilitating the clearance of cancer cells (Iannello et al., 2013; Rentschler et al., 2022; C. Wang et al., 2019). This observation underscores the potential of combining TIS with immunomodulation to enhance TIS antitumor efficacy. Ruscetti et al. used a pancreatic ductal adenocarcinoma model, to combine MEK and CDK4/6 inhibitors to induce senescence (Ruscetti et al., 2020). This TIS approach generates SASP, which promotes vascular remodeling, thereby improving chemotherapy uptake and endothelial cell activation to enhance T-cell recruitment into tumors, ultimately synergizing with anti-PD-1 treatment. Similarly, through *in vivo* multilayer-omics analyses, Morita et al. demonstrated that TAK-931, a specific CDC7 inhibitor, induces replication stress that generates aneuploid cells (Morita et al., 2023). These cells generate SASP that strongly promotes tumor infiltration of immune cells and a robust antitumor immune response. Thus, combination treatment with TAK-931 and immune-checkpoint inhibitors (anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies) is confirmed to enhance antitumor activities in the preclinical mouse model (Morita et al., 2023). These findings suggest that TIS can generate an immunologically activated TME and enhance the effectiveness of immune checkpoint inhibitors when used in combination.

Many elements of the TME are important and are mobilized in response to senescent cancer cells and the SASP. One of the abundant populations of immune cells infiltrating a tumor is TAMs (van

Ravenswaay Claasen et al., 1992). Therefore, integrating TAM reprogramming with TIS might be a potential strategy to enhance the efficacy of cancer treatments, opening new avenues for therapeutic intervention. Strategies specifically aimed at targeting macrophages are now becoming a major focus of cancer research. Due to their plasticity and heterogeneity, certain anticancer drugs have been shown to reprogram TAMs, shifting them from a pro-tumor to an anti-tumor phenotype, which improves treatment outcomes (H. Wang et al., 2024). For instance, drugs that cause TIS, such as gemcitabine in pancreatic cancer, 5-fluorouracil in colorectal cancer, and platinum-based chemotherapy in high-grade ovarian cancer have demonstrated such effects (Di Caro et al., 2016; Heath et al., 2021; Malesci et al., 2017). Another approach for TAM remodeling is using monoclonal antibodies. In fact, antibodies that target TAMs expressing Fcγ receptors have shown promise in activating these macrophages to destroy tumor cells via phagocytosis (DiLillo & Ravetch, 2015; Gül & van Egmond, 2015). For instance, antibodies such as rituximab (targeting CD20), trastuzumab (targeting HER2), cetuximab (targeting EGFR), and daratumumab (targeting CD38 on myeloma cells) are currently in clinical use (DiLillo & Ravetch, 2015; Uchida et al., 2004). However, this approach remains in its early stages, and further research is essential to fully understand its application in clinical settings.

Conclusions

Cancer cell senescence represents a highly complex process that involves multiple molecular pathways. Due to the cell-intrinsic and extrinsic complexity of senescent cancer cells, they display diverse phenotypes that can either suppress or promote tumor growth. While senescence initially acts as an inhibitor of tumorigenesis, the prolonged presence of senescent cells is often linked to supporting malignant cell survival and aiding tumor progression (Z. Chen et al., 2005; G. Wang et al., 2021). Consequently, whether senescence acts as a tumor suppressor or promoter depends on the context, such as the stage and tissue it occurs, the type of SASP elicited and the immune cell response elicited.

Over the past decade, a significant amount of work has been performed to comprehend the molecular processes governing senescent cell heterogeneity. This progress has led to the development of advanced *in vitro* models, providing us with a deeper understanding of the role of senescence in cancer initiation and progression. Recent studies developed the SENCAN classifier, a machine learning-based program to detect senescent cancer cells, as a method to and assess the efficacy of senolytics (Jochems et al., 2021b). While the SENCAN classifier was able to trace and track senescent cancer cells *in vitro*, the classifier was not accurate when applied *in vivo*. Similarly, in efforts to study senescence in tumor cells, a RAS-driven lung ADC mouse model was developed to clarify the identity, characteristics, and biological function of Cdkn2a p16-expressing senescent cells (Haston et al., 2023). However, this approach relies on a single marker, thus limiting its ability to capture the full diversity of senescent cell populations. Therefore, the mission to study tumor senescent cells *in vivo* is far from straightforward, and there is a clear need for more comprehensive tools for senescence detection and characterization.

Moreover, to better understand the mechanisms of cancer cell senescence, it is crucial to characterize senescent cells in different biological environments. However, currently, there is no universal marker, unique for senescent cells. Therefore, the need to develop new technologies to study the heterogeneity of senescent cancer cells is of great importance. Interestingly, current research is now focused on developing a machine learning-based program for senescent cell identification (SenCID) (Tao et al., 2024). This approach categorizes senescent cells into six distinct groups, each with unique senescence signatures, levels of stemness, and varying responses to senolytic treatments. Thus, SenCID enables detailed analysis of senescent cell heterogeneity, offering insights into potential gene networks involved in tumorigenesis.

Moreover, a further challenge that is important to address is how specific tumor niches influence the phenotype of senescent cancer cells and shape their interactions with the local TME. The behavior of senescent cells within the complex and dynamic environments of different tumor niches remains poorly understood. The local conditions within each niche—such as oxygen levels, immune cell composition, extracellular matrix, and signaling molecules—can significantly alter how senescent cells behave, including their SASP, immune evasion mechanisms, and impact on tumor progression or suppression (W. Zhang et al., 2024). Notably, tumor niches can lead to a variety of drug responses and limit the effectiveness of TIS within tumors (Llop-Hernández et al., 2022; Reynolds et al., 2024). Therefore, understanding the complexity of the niche-specific behavior of senescent cells and an important avenue for future research.

Even though senotherapies hold significant promise as a cancer treatment strategy, the heterogeneous nature of senescent cancer cells presents a major challenge. Senescent cells vary widely in their transcriptional, metabolic, and SASP profiles, not only across species and cell types but even at the level of individual tumors (Burnaevskiy et al., 2023; Gong et al., 2023). This diversity leads to differential reliance on various pathways that are activated during senescence. Furthermore, many metabolic changes associated with senescence do not exclusively occur in senescent cells. Cells like neurons, cardiomyocytes, and adipocytes also exhibit senescence-like phenotypes, such as elevated expression of p16 and other CDK inhibitors, which may not indicate true senescence (de Mera-Rodríguez et al., 2021; Ogrodnik et al., 2024; Safwan-Zaiter, Wagner, & Wagner, 2022). Such commonalities complicate the development of senotherapies that selectively target senescent tumor cells without affecting other cell types. The need for definitive biomarkers of the tumor's senescent state is crucial to discriminate between senescence and other growth-arrested phases.

Moreover, an important consideration is whether senescence induction via TIS represents the most effective therapeutic approach. Even though the cellular composition of the TME varies among cancer types, hallmark features include immune cells, CAFs, endothelial cells, and adipocytes (Anderson & Simon, 2020). Senescent tumor cells, through their SASP, can propagate the senescent phenotype to surrounding non-senescent cells within the TME (Nelson et al., 2012). This poses a risk that non-senescent cancer cells will be sensitized to senolytic drugs, potentially complicating treatment outcomes. Thus, there is a great need to design therapies that specifically target tumor senescent cells while minimizing off-target effects. For instance, Wang et al induced senescence via inhibition of the DNA replication kinase CDC7 selectively in *TP53* mutant liver cancer cells (C. Wang et al., 2019). In addition, another strategy combines MEK and CDK4/6 inhibitors to target specific KRAS-driven oncogenic signaling in PDAC (Ruscetti et al., 2020).

It is also important to note that the ability of cancer cells to escape from senescence, represents a significant challenge for effective cancer treatment. Recent studies illustrated that some of the senescence tumor cells induced by chemotherapy may escape cycle rest and recover proliferation *in vivo* and *in vitro*, which indicates that senescent cancer cells may be one form of tumor dormancy and contribute to tumor recurrence (Saleh et al., 2019). In this sense, TIS can be described as a “double-edged sword”. On the one hand, cellular senescence can limit the proliferation of tumor cells, though it might also activate a tumor-promoting microenvironment. Further studies are essential to better comprehend the role of senescent tumor cells in cancer dormancy.

Future directions in research should prioritize overcoming these limitations in understanding the intra- and inter-tumor heterogeneity in response to pro-senescence therapies. To meet clinical needs, this is essential for developing more efficient one-two-punch strategies for harnessing the

beneficial effects of pro-senescence therapy while minimizing the detrimental effects of senescent cells.

Overall, to effectively target senescent cancer cells in the TME, several challenges must be addressed: (1) identifying the distinct types of senescent cancer cells within the TME, (2) understanding the specific functions and roles of these various senescent cell populations, and phenotypes, (3) determining the most effective strategies to selectively target senescent cancer cells. Addressing these objectives will enhance our understanding of the dual role of senescence in cancer progression and aid the development of novel pharmacological approaches to effectively combat cancer.

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