Genetics and Molecular Biology, 47(suppl 1), e20230311 (2024)

DOI: https://doi.org/10.1590/1678-4685-GMB-2023-0311

Young Latin American Geneticists – Special Issue
Review Article

Modulation of tumor plasticity by senescent cells: Deciphering basic mechanisms and survival pathways to unravel therapeutic options

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Abstract

Senescence is a cellular state in which the cell loses its proliferative capacity, often irreversibly. Physiologically, it occurs due to a limited capacity of cell division associated with telomere shortening, the so-called replicative senescence. It can also be induced early due to DNA damage, oncogenic activation, oxidative stress, or damage to other cellular components (collectively named induced senescence). Tumor cells acquire the ability to bypass replicative senescence, thus ensuring the replicative immortality, a hallmark of cancer. Many anti-cancer therapies, however, can lead tumor cells to induced senescence. Initially, this response leads to a slowdown in tumor growth. However, the longstanding accumulation of senescent cells (SnCs) in tumors can promote neoplastic progression due to the enrichment of numerous molecules and extracellular vesicles that constitutes the senescence-associated secretory phenotype (SASP). Among other effects, SASP can potentiate or unlock the tumor plasticity and phenotypic transitions, another hallmark of cancer. This review discusses how SnCs can fuel mechanisms that underlie cancer plasticity, like cell differentiation, stemness, reprogramming, and epithelial-mesenchymal transition. We also discuss the main molecular mechanisms that make SnCs resistant to cell death, and potential strategies to target SnCs. At the end, we raise open questions and clinically relevant perspectives in the field.

Keywords: Aging, cancer, tumor microenvironment, plasticity, senotherapy.

Received: November 02, 2023; Accepted: March 21, 2024.

Key concepts about cellular senescence

Senescence is a cellular state of loss of proliferative capacity due to multiple cell divisions or exposure to stresses. Telomere erosion due to the inefficiency of the DNA replication machinery at the ends of chromosomes results in blocking cell proliferation, a phenomenon known as replicative senescence. Physiologically, this mechanism helps to prevent mitotically aged cells, which potentially carry DNA changes, from transmitting genetic alterations to daughter cells. In addition to telomere shortening, stresses such as DNA damage, oncogenes activation, loss of tumor suppressor genes, or damage in cellular components like mitochondria or cytoskeleton can also induce a cell to senesce, the so-called induced (or premature) senescence (Di Micco *et al.*, 2021). Although a few references using genetic silencing of senescence effectors show that these strategies can possibly revert the phenotype (Beauséjour *et al.,* 2003; Afifi *et al.,* 2023), *in vitro* and *in vivo* data support its irreversible nature (Gorgoulis *et al.,* 2019; Afifi *et al.,* 2023). It contrasts with quiescence, characterized by its reversibility and the reacquisition of responsiveness to growth factors (Beauséjour *et al.,* 2003; Blagosklonny, 2011). Senescent cells (SnCs) exhibit distinct morphological features, including enlarged and flattened cell shape, increased cytoplasmic granularity, and altered nuclear structure including chromatin changes with the emergence of heterochromatin foci. Molecular characteristics include the upregulation of cell cycle inhibitors (e.g., $p16^{INK4/Arf}$ and $p21^{CIP1}$, hereafter named only as p16 and p21, respectively), DNA damage response activation (in most cases), increased activity of senescence-associated beta-galactosidase (SA-β-gal), and a secretory program called senescence-associated secretory phenotype or (SASP), consisting of soluble molecules and extracellular vesicles (Hernandez-Segura *et al.,* 2018; Gorgoulis *et al.,* 2019).

Although non-proliferative, SnCs are metabolically active, especially considering their secretory capacity,

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including the release of growth factors, cytokines, chemokines, matrix metalloproteinases, and other molecules. Thus, on the one hand, undergoing senescence helps to prevent the formation of neoplasms by avoiding the replication of cells carrying damages of different natures. On the other hand, the accumulation of SnCs in formed tumors, a feature recently included as a new hallmark of cancer (Hanahan, 2022), could positively contribute to tumor progression.

SnCs can modulate mechanisms in neighbor cells that are not senescent. Among these mechanisms is cellular plasticity, another feature recently listed as a typical feature of cancer. This review discusses the main findings about the impact of SnCs on tumor plasticity and phenotypic transition processes, like epithelial-to-mesenchymal transition (EMT), cancer cell stemness, differentiation, and reprogramming. We also raised the main signaling pathways SnCs use to survive, which ultimately allows their maintenance in the tumor, enabling them to play their pro-tumor role while revealing potential targets to sensitize SnCs to die. At the end, we discuss critical open questions and challenges in the field of senescence, which has become one of the points of most significant translational potential in cancer biology.

It is worth noting that, initially, studies were focused on deciphering senescence features in normal senescent cells until discovering that cancer cells can also undergo this cell fate as well, introducing the new concept of senescent cancer cells (SnCCs – i.e., cancer cells undergoing cellular senescence mainly by oncogene activation or induced by therapies). However, other cell types from the tumor microenvironment (TME) can also undergo senescence, such as stromal cells (Guillon *et al.,* 2019; Pardella *et al.,* 2022; Ye *et al.,* 2023), cancer-associated fibroblasts (CAFs) (Higashiguchi *et al.,* 2023), immune cells (Bruni *et al.,* 2019), and endothelial cells (Abdelgawad *et al.,* 2022; Bloom *et al.,* 2023), highlighting the contribution of both tumor and non-tumor cells to the population of senescent cells in the TME. Since several essential senescent cell characteristics are shared by both cell types, in this review, we use 'SnCs' for general senescent cell behaviors and phenotypes, and 'SnCCs' to refer exclusively to cancer senescent cells.

Senescence in cancer: allies turned adversaries

SnCs are found in all major human organs. Once senescent, the cell loses its proliferative capacity, becoming unresponsive to growth factors (Hinds and Pietruska, 2017). Thus, at first, the senescent barrier acts as an endogenous antitumor mechanism, blocking the proliferative capacity of transformed cells (Vargas *et al.,* 2012), a premise that is reinforced by the abundance of SnCs in many benign tumors (Collado and Serrano, 2010). However, human cells can undergo genetic or epigenetic changes that prevent or attenuate this anti-proliferative response, favoring an immortalized cellular phenotype. In this process, "immortal" tumor subclones successfully evade the senescent barrier, enabling tumor progression and the acquisition of malignant phenotype. (Collado and Serrano, 2010). To acquire this feature, tumor cells must overcome the Hayflick limit, which denotes the finite number of divisions human cells can undergo, attributed to the shortening of telomeres (Shay and Wright, 2000). This unlimited proliferative capacity is acquired primarily through negative modulation or loss of the TP53-p21 pathway or CDKN2A loci, which encodes the CDK inhibitors p16 and $p14^{Arf}$. (Takeuchi *et al.,* 2010; Terzian *et al.,* 2010; Hernandez-Segura *et al.,* 2018). This molecular evasion of senescence was already described at different stages of aggressiveness of skin (melanoma), lung, colon, and breast tumors, among others (Meeker *et al.,* 2004; Bennecke *et al.,* 2010).

The induction of cellular senescence by numerous chemotherapeutics (CT) and radiotherapy began to be a target of attention from the observation that DNA damage generated by these therapies can lead some subclones of tumor cells to Therapy-Induced Senescence (TIS – i.e., senescence induced by radiotherapy or drugs like chemotherapeutics, microtubules inhibitors, targeted therapies, hormone receptor antagonists, among others) (Acosta and Gil, 2012; Wang *et al.,* 2022) (Figure 1A and B). However, many studies evaluating TIS consider entering senescence as a terminal cell state, neglecting that, despite being non-proliferative, these cells remain metabolically active in the cellular composition of the tumor (Collado and Serrano, 2010). In this way, through cellular mechanisms of communication like juxtacrine (i.e., a direct contact between cells) or paracrine (i.e., between nearby cells through soluble molecules) signaling, SnCs could interfere with the function of other non-SnCs in the TME, including non-senescent cancer cells (non-SnCCs), normal cells, immune cells, cancer stem cells (CSCs), endothelial cells, CAFs, and stromal cells (Figure 1A and 1B) (Yousefzadeh *et al.,* 2021). Accordingly, SnCs could modulate several aspects related to tumor progression, such as cell proliferation, cell death, cell migration, angiogenesis, and resistance to therapy. Likewise, SnCs may also stimulate phenotypic transitions and unlock the cellular plasticity of other tumor and non-tumor cells from the TME (Figure 1C) (Wang *et al.,* 2022).

In general, signaling mediated by SnCs in the TME involves both the secretion of soluble molecules and the direct contact with other cells through membrane nanotube formation or cell-cell contact. The myriad of components secreted to the extracellular environment by SnCs constitutes the so-called Senescence-Associated Secretory Phenotype (SASP), which is a chronic and dynamic feature that develops progressively over time from senescence triggering (Coppé *et al.,* 2008; Muñoz-Espín *et al.,* 2013, Demaria *et al.,* 2014; Alessio *et al.,* 2023). SASP contains high levels of growth factors such as Epidermal Growth Factor (EGF) and Vascular Endothelial Growth Factor (VEGF) (Strieter *et al.,* 2006), transforming factors such as Gro-1 (Yang *et al.,* 2006), molecules that stimulate the EMT such as TGF-β (Coppé *et al.,* 2008), enzymes that promote the invasion of adjacent tissues such as matrix metalloproteinases (MMPs) (Liu and Hornsby, 2007; Ruhland *et al.,* 2016), among others (Figure 1B). In addition to these soluble molecules, SnCs can also secrete exosomes and microvesicles. These membrane-delimited extracellular vesicles can transport from small signaling molecules, proteins, and metabolites to messenger RNA, regulatory RNA, and even small pieces of DNA (Wallis *et al.,* 2020). In this way, SnCs (donors) can transfer molecules to other cells (acceptors) from neighbor cells in a paracrine manner to more distant cells through blood circulation (Tkach and Thery, 2016).

Through extracellular vesicles, therefore, SnCs can influence the behavior of other cells, modulate anticancer immunity, induce tumor progression, and facilitate the metastatic process (Hoshino *et al.,* 2015; Yokoi *et al.,* 2017).

Thus, TIS could initially reduce the speed of tumor growth by blocking cell proliferation in some susceptible neoplastic subclones. However, the long-term accumulation of SnCs could lead to an enrichment of SASP in the TME, favoring the proliferation, malignancy, and resistance of non-SnCCs (Figure 1C). Indeed, recent studies suggest that patients with enrichment of senescent signatures have a worse prognosis for liver cancer (Li and Xue, 2023; Zhang S *et al.,* 2023), colorectal cancer (Tan *et al.,* 2023), glioma (Yang *et al.,* 2021), among others (Zhang *et al.,* 2022) with rare exceptions showing the opposite (Zhou *et al.,* 2022).

Therefore, SnCs have two main phenotypic characteristics: 1) an autonomous feature characterized by loss of proliferative capacity and cellular fitness, culminating in the inability to leave descendants; and 2) a non-autonomous feature characterized by the secretion of soluble molecules and extracellular vesicles (SASP) and direct interaction with other cells. These two characteristics have different dominances

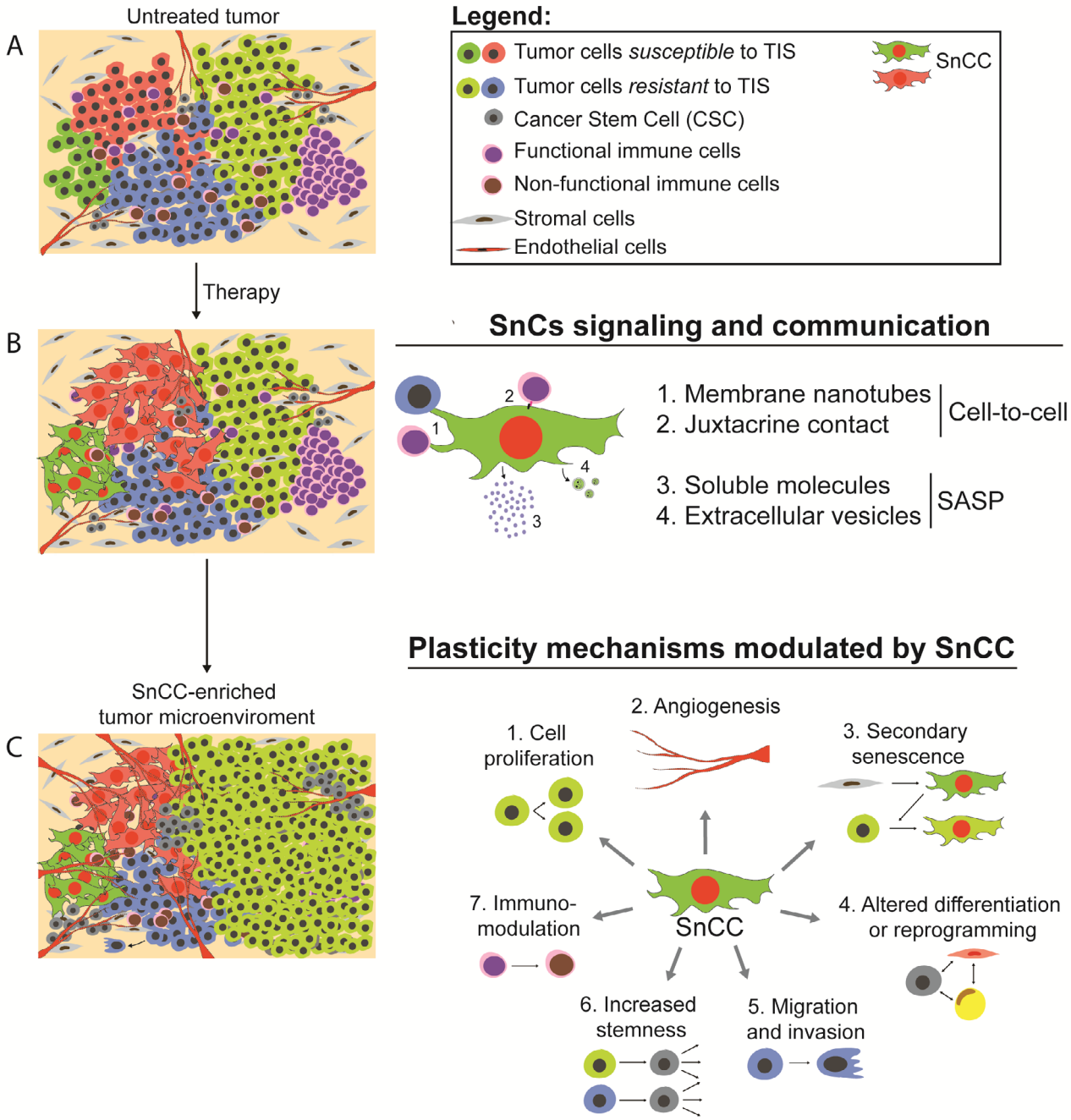

Figure 1 – Tumor microenvironment (TME) modulation by Senescent Cells (SnCs). (A) Tumor heterogeneity is represented by tumor cell subpopulations and other cell subtypes found in the TME. (B) Senescence-inducing therapies give rise to a new cell subtype capable of modulating the phenotype of the other cells that make up the tumor niche (light blue cells); on the right, the most common types of cellular communication of SnCs are shown. (C) The heterogeneous composition of the tumor niche and, on the right, the main mechanisms modulated by the senescent cell. Abbreviations: SASP, senescence-associated secretory phenotype; SnCC, senescent cancer cell; TIS, therapy-induced senescence.

considering carcinogenesis: the loss of proliferative capacity of some tumor cells, mainly due to senescence induced by oncogenes or loss of tumor suppressor genes, should contribute to preventing the proliferation of transformed cells and, therefore, the progression of early tumors. In these contexts, SnCCs should predominate to the detriment of proliferative cells. However, if in the microenvironment of early neoplasms, subclones with proliferative potential emerge, the second phenotypic characteristic of SnCs (i.e., their influence on other cells from the TME) may start to dominate and contribute to a gradual progression of the tumor, with a relative reduction in the number of SnCCs due to the proliferation of non-SnCCs. Thus, considering tumors that have already formed (and, therefore, have a greater chance of being diagnosed), proliferative cells are predominant about SnCs for evolutionary reasons. However, although proliferative cells are naturally resistant to senescence induced by changes in oncogenes and tumor suppressor genes, they can be sensitive to senescence induced by damage-inducing therapies. However, due to the well-established intratumoral heterogeneity, which can encompass dozens to hundreds of different tumor subclones, not all subclones are sensitive to TIS, as illustrated in Figure 1B. Thus, some subclones acquire a senescent phenotype and begin to secrete molecules promoting phenotypes that favor tumor progression, such as growth factors and extracellular matrix remodeling enzymes. Variables such as the number of subclones that will enter senescence, the constitution of the SASP of SnCCs, the phenotype of tumor cells that do not enter senescence, and the constitution of the TME may affect the prognosis of patients. However, to date, evidence suggests that regardless of these and other variables, the presence of SnCs in the TME of already-formed tumors appears to be associated with a worse prognosis, indicating a dominance of the pro-tumor (non-autonomous) effect of the senescent cell phenotype.

Role of SnCs in tumor plasticity

SnCs and epithelial to mesenchymal transition (EMT)

One of the most relevant mechanisms modulated by SASP is EMT, where tumor cells of epithelial origin acquire characteristics of mesenchymal cells, such as resistance to death induced by loss of cell adhesion, cell elongation, and greater migratory capacity (Mittal, 2018). Therefore, despite the obligation of EMT in the process of metastasis has been questionable mainly because EMT is a spectrum of phenotypes rather than a binary event (Mittal, 2018; Lourenco *et al.,* 2020), it may facilitate tumor malignancy and spread (Mittal, 2018). These events are clinically relevant since metastases are responsible for more than 80% of deaths associated with cancer (Kalluri and Weinberg, 2009).

Through the secretion of Hepatocyte Growth Factor (HGF) and the activation of c-Met and MAPK in tumor cells, irradiation-induced fibroblasts promoted the migration and spread of pancreatic cancer cells (Ohuchida *et al.,* 2004). Likewise, in an animal model, the SASP from senescent CAFs promoted the peritoneal spread of gastric cancer by activating the JAK/STAT3 pathway in cancer cells (Yasuda *et al.,* 2021). Finally, senescent fibroblasts can also induce the migration of endothelial cells through the secretion of VEGF (Coppé *et al.,* 2006) and increase their contact with tumor cells, favoring angiogenesis and tumor development (Orr and Wang, 2001). The promotion of EMT by conditioned medium containing SASP from senescent fibroblasts has also been observed in other tumor types such as breast (Ortiz-Montero *et al.,* 2017), prostate (Bavik *et al.,* 2006), bladder (Goulet *et al.,* 2019) and ovary (Lawrenson *et al.,* 2010).

As for the SASP produced by senescent fibroblasts, SASP derived from SnCCs is also able to promote EMT in non-SnCCs from breast and colorectal cancer (Tato-Costa *et al.,* 2016; Ortiz-Montero *et al.,* 2017; Goulet *et al.,* 2019). Furthermore, SASP can also favor EMT and increased cell migration in pre-malignant cells (Coppé *et al.,* 2008; Lawrenson *et al.,* 2010). This observation reinforces the hypothesis that the accumulation of SnCs in benign lesions could favor their malignancy, justifying the elimination of these cells as a mechanism to prevent tumor progression (Choi *et al.,* 2022; Kohli *et al.,* 2022). Effector molecules from SASP involved in EMT promotion include interleukins like IL-6 (Bavik *et al.,* 2006) and IL-8 (Bavik *et al.,* 2006; Tato-Costa *et al.,* 2016; Ortiz-Montero *et al.,* 2017), growth factors like HGF (Ohuchida *et al.,* 2004) and FGF-7 (Bavik, Coleman *et al.,* 2006) and amphiregulin (Bavik *et al.,* 2006). In this context, an essential translational aspect concerns the cell non-autonomous role of mutations in driver genes in the role of SASP. Mutations in the Ras oncogene or in TP53 enhance and accelerate the secretion of promalignant SASP in cells that have such alterations favoring EMT and TME remodeling, characterizing a non-autonomous effect of these mutations on tumor biology (Coppé *et al.,* 2008).

Considering the intratumor heterogeneity, it is plausible to assume that some tumor subclones have differential sensitivity to TIS. In colorectal cancer cells subjected to 5-Fluorouracil (5-FU) treatment, specific subclones experienced apoptosis or underwent senescence, while others resisted to the drug. (Cho *et al.,* 2020; Baldasso-Zanon *et al.,* 2024). In a condition like that, inferring that resistant clones are susceptible to the SASP from TIS cells is plausible. Indeed, in primary rectal cancer samples, the EMT markers are increased close to niches of SnCs compared to regions where SnCs are absent (Tato-Costa *et al.,* 2016). Corroborating that, ascites samples from metastatic gastric cancer patients presented an increase in fibroblasts producing proinflammatory SASP (i.e., IL-6, IL-8, VEGF, and others), compared to patients with no metastasis (Yasuda *et al.,* 2021).

Translationally, another relevant aspect regarding the effect of SnCs on cancer concerns the clinical treatment protocol. For example, neoadjuvant therapies can lead to an enrichment of SnCs in the TME, as in the case of breast cancer treated with Doxorubicin (Achuthan *et al.,* 2011; Febres-Aldana *et al.,* 2020; Saleh *et al.,* 2021) or colorectal cancer treated with chemotherapy (5-FU or Doxorubicin) plus radiotherapy (Tato-Costa *et al.,* 2016). Consequently, although it initially reduces tumor growth rate, this enrichment could lead to a worse prognosis for patients in the long term. On the other hand, considering adjuvant therapy schedules, for most chemotherapy drugs, patients are exposed to multiple cycles

of drug exposition, interspersed with periods of recovery for the patient. Thus, the first treatment cycle may lead to the enrichment of SnCs in the TME. In this context, patients exposed to senescence-inducing therapies could benefit from consecutive chemotherapy treatment followed by senolytic treatment to eliminate SnCs. This approach is promising since it is plausible to infer that SnCCs resist re-exposure to chemotherapy. However, there is no strong evidence on this issue. Finally, alternative chemotherapy protocols have emerged in recent years in which lower doses of chemotherapy drugs are used for more extended periods. However, this strategy may lead to an even more significant enrichment of the senescent population and inflammatory cytokine secretion in the TME (Rodier *et al.,* 2009) since intermediate DNA damage, for example, favors senescence over apoptosis, which requires higher rates of damage to be triggered (Zhang *et al.,* 2010).

In conclusion, multiple pieces of evidence converge to a clear role for SASP in promoting EMT and increasing aggressiveness in non-SnCCs. More than one cell type can undergo senescence in the TME after therapies. Therefore, the SASP origin can be tumor cells, fibroblasts, or other cell types. Consequently, despite the initial effect of therapies in controlling tumor growth through apoptosis and senescence, in the long term, SASP may induce EMT in those tolerant subclones, increasing tumor aggressiveness. This crosstalk may explain, at least partially, the association between high levels of senescence in the TME and worse clinical prognosis. Discovering the exact molecules responsible for the pro-tumor role played by SASP, as well as the tumor signaling pathways responsive to these molecules, is essential to allow the development of therapies that may inhibit both the production of these molecules by SnCs and the responsiveness of tumor cells to these signals.

Cell differentiation and stemness

The balance between maintaining cells in an undifferentiated state and cell differentiation is disturbed in tumor biology. The clinical relevance of this aspect supports the classification of tumor grade, with poorly differentiated or undifferentiated tumors being classified as having a higher grade. The main cellular phenotype associated with stemness is cancer stem cells (CSCs), a subpopulation of cancer cells likely responsible for tumor initiation, growth, and recurrence. CSCs have intrinsic features like greater migratory capacity, more remarkable plasticity, greater capacity for invasiveness, and survival outside the primary focus (Batlle and Clevers, 2017). In addition to these phenotypic hallmarks, CSCs express specific markers, allowing their identification in different tumor types. Considering the abovementioned characteristics, an enrichment of CSCs and stemness markers may suggest heightened aggressiveness and a less favorable prognosis (Yang *et al.,* 2020).

The enrichment of SnCs in the TME led to increased CSCs and stemness markers, mainly through SASP molecules. In colorectal cancer, melanoma, and hematological malignancies, the enrichment of senescence markers was associated with increased classic stemness markers like CD133, CD44, LGR5, and CD34. The effect of SnCs in increasing population stemness may be mediated, at least partially, by the reprogramming of non-CSCs into CSC-like tumor cells, with increased tumor formation *in vivo* (Milanovic *et al.,* 2018). SnCs can also induce the dedifferentiation of tumor cells, thus providing more remarkable plasticity to transformed cells and resulting in the emergence and maintenance of a subpopulation of CSCs (Cahu *et al.,* 2012; Castro-Vega *et al.,* 2015). Likewise, premalignant mammary epithelial cells exposed to senescent fibroblasts lose differentiated features and undergo malignant transformation. Furthermore, the injection of premalignant cells with senescent fibroblasts in mice accelerated tumor formation compared to injecting tumor cells alone (Krtolica *et al.,* 2001; Parrinello *et al.,* 2005). Therefore, the accumulation of therapy-induced SnCs could promote, in the long term, the process of tumor resistance and recurrence, marked by the greater pharmacological tolerance of the CSCs, followed by the reacquisition of the proliferative capacity of the surviving subpopulation. Furthermore, in addition to TIS, OIS also seems to lead to a SASP that stimulates cell stemness and plasticity (Ritschka *et al.,* 2017).

In addition to the nonautonomous effects, cells with senescent features seem to release small aneuploid cells by unknown mechanisms. These small cells can re-enter the cell cycle and acquire a stem-like state through activating the Wnt pathway (Milanovic *et al.,* 2018). Although interesting, it is still not clear how this happens, whether the senescent cell can reprogram itself to acquire a stem state or whether the senescent cell can generate new cells through endoreplication or budding (Zhang, *et al.,* 2014; Czarnecka-Herok *et al.,* 2022). This behavior is commonly observed in polyploid-giant cancer cells (PGCCs), which have features of SnCs and have been associated with tumor recurrence in several cancer types (White-Gilbertson and Voelkel-Johnson, 2020). Complementary, using cellular models that allow SnCs to re-enter the cell cycle through genetic or epigenetic alterations, cells released from senescence showed more significant formation of colonies *in vitro* and tumors *in vivo* compared to those cells that had never undergone a senescent state (Yang *et al.,* 2017). However, it is important to mention that this is an artificial experimental model in which cells are forced to re-enter the cell cycle, with no concrete evidence showing the spontaneous reversibility of senescence in either physiological or pathological contexts.

Molecularly, NF-kβ, a key factor controlling the transcription of proinflammatory molecules, had a central role in the effect of SnCs on increasing the population stemness. Likewise, pro-inflammatory molecules like IL-6 play a positive role in the maintenance of stemness capacity by CSCs (Korkaya *et al.,* 2011), which is closely related to the pro-stemness role induced by SnCs. Indeed, senescence-associated IL-6 and IL-8 increased the expression of CD44, a CSC marker, in breast cancer cells. Interestingly, the exposure to a senescence-conditioned medium also led tumor cells to the expression and secretion of IL-6 and IL-8 and the induction of senescence, thus building an autocrine pro-tumoral signaling (Figure 2). Other soluble molecules from SASP, like TGF-β and VEGF, can also promote secondary senescence (Matsuda *et al.,* 2023). Promisingly, neutralizing these interleukins using specific antibodies can reverse the effects of a senescence-conditioned medium (Ortiz-Montero *et al.,* 2017).

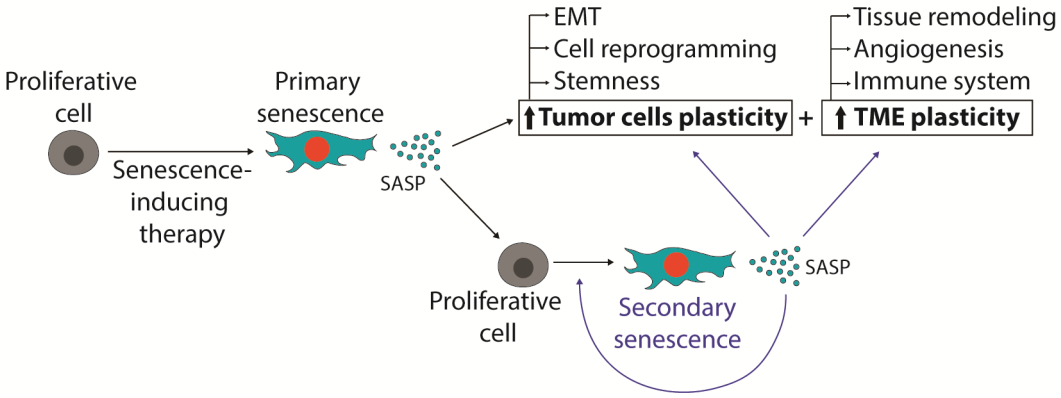

Figure 2 – Positive feedback between senescence induction and increased tumor plasticity. After the induction of senescence by a chemotherapy drug (CT), there is an enrichment of SASP in the TME. Molecules in SASP such as IL-6, IL8, and TNF-α fuel the plasticity of tumor cells (including cell reprogramming, increased stemness, and epithelial-to-mesenchymal transition) and TME. Additionally, SASP molecules can induce more cells to enter a senescent state (secondary senescence), producing even more SASP. This creates a feedback loop that perpetuates increased plasticity and tumor heterogeneity. Abbreviations: EMT, epithelial-to-mesenchymal transition.

Physiologically, maintaining cells in a stem state or cell reprogramming during tissue injury or development is essential for adaptive responses, tissue repair, and homeostasis. As raised, paracrine factors secreted by SnCs may be necessary to enrich these phenotypes. However, upon reacquisition of tissue homeostasis, this undifferentiated cellular phenotype needs to be reverted to a state of differentiation. In the same way that contexts of long-lasting tissue stress such as chronic inflammation predispose to cellular transformation, the presence of SnCs and its secreted molecules like proinflammatory interleukins for long periods could favor the maintenance of cells in an undifferentiated state that is more permissive to cellular transformation (Yasuda *et al.,* 2021), tumor progression or recurrence (Demaria *et al.,* 2017) (Figure 2).

SnCs and cell reprogramming

In addition to controlling cellular differentiation, genomic reprogramming is another central mechanism of cellular plasticity necessary for tissue homeostasis. Chronic disturbances in these two events are involved in tumor initiation, progression, and prognosis (Ohnishi *et al.,* 2014; Xiong *et al.,* 2019). As raised in this section, initial evidence suggests that SnCs could modulate tumor cells' reprogramming through autonomous and non-autonomous mechanisms.

Most evidence of the role of SnCs in cellular reprogramming comes from tissue damage and repair models. The tissue damage and repair microenvironment share many characteristics observed in the TME, including inflammatory cells and molecules, extracellular matrix remodeling, intense paracrine signaling, angiogenesis, cell death, and proliferation (Jin and Jin, 2020). Stress-induced SnCs favor *in vivo* reparative cellular reprogramming in skeletal muscle fibers (Chiche *et al.,* 2017) and gastric, pancreatic, and kidney epithelial cells (Mosteiro *et al.,* 2016). This effect is exerted mainly through molecules present in the SASP, such as IL-6 (Mosteiro *et al.,* 2016; Chiche *et al.,* 2017; Mosteiro *et al.,* 2018) and TNF-α (Mosteiro *et al.,* 2016), whose production is mediated by the INK4/Arf locus. These factors can modulate the expression and activity of effector molecules, mainly transcription factors such as Nanog (Mosteiro *et al.,* 2016; Chiche *et al.,* 2017). However, the effect of SASP on cell differentiation or reprogramming seems to depend, among other variables, on the time of exposure to SASP. In keratinocytes, for example, transient exposure to SASP promoted an increase in the expression of stem cell markers. On the other hand, prolonged exposure to SASP induced senescence in these cells, with a loss of regenerative capacity (Ritschka *et al.,* 2017). Furthermore, the reduction of SnCs by genetic clearance (i.e., the selective killing of cells with specific genetic features using a gene construct, (Baker *et al.,* 2011) of cells expressing high levels of p16 or by using senotherapeutics (i.e., a class of drugs or interventions aimed at targeting and eliminating SnCs, (Zhang L *et al.,* 2023) increased *in vitro* and *in vivo* somatic reprogramming. These strategies also increased the establishment of induced Pluripotent Stem Cells (iPSC), a type of stem cell that is artificially generated from non-pluripotent somatic cells (Takahashi and Yamanaka, 2006), as well as histopathological features of tissue regeneration in the liver (Grigorash *et al.,* 2023). Corroborating this, chronic SASP led to reduced stemness in intestine organoids and impaired crypt formation, an effect that was mediated by secreted PTK7 present in the SASP composition (Yun *et al.,* 2023).

As raised, the transient enrichment of SnCs in human tissues in response to local signals is beneficial and contributes to maintaining or reestablishing tissue homeostasis. On the other hand, its enrichment induced by therapies (i.e., from exogenous stress to the body) in the TME is usually associated with a worse prognosis and tumor progression (Domen *et al.,* 2022; Zhang S *et al.,* 2023). This deleterious effect of SnCs on the TME may be related to a) the presence of SnCs for more extended periods than those observed in contexts of tissue development and repair; b) the accumulation of SnCs, exceeding the beneficial threshold and leading to deleterious effects; c) the composition of SASP produced by SnCCs cells, which may differ from the SASP produced in other pathophysiological responses. In the TME, the presence of SnCs, even for a short time, could fuel the plasticity of some tumor subclones through the modulation of

cellular reprogramming, differentiation, EMT, among others. Therefore, this may lead to increased tumor heterogeneity or capacity to adapt to stresses such as anti-tumor therapies (Castro-Vega *et al.,* 2015). This crosstalk reinforces the senoprevention arm (i.e., preventing cells from entering senescence, directing them to cell death) as the best therapeutic strategy related to the modulation of cellular senescence.

As raised, data from other human physiopathological responses has provided evidence that allows us to extrapolate the results to tumor biology, supporting translationally relevant hypotheses. Multiple factors secreted by SnCs can modulate the phenotypic plasticity of tumor cells. Among the biggest challenges in the area, it is necessary to understand what these factors are, how to modulate its production by SnCs, and how to act specifically on SnCs present in the TME without affecting SnCs involved in physiological responses critical for homeostasis such as tissue repair and remodeling.

Survival mechanisms of senescent tumor cells

The state of cell proliferation arrest observed in SnCs arises from the integration of molecular signals that not only block cell cycle progression but also ensure that these cells remain alive. This phenotype is mediated by a complex network of pro-survival molecular signals known as Senescent-Cell Anti-apoptotic Pathways (SCAPs) (Zhu *et al.,* 2015). Among these pathways, the Bcl-2 family, MDM-2/TP53/p21, and PI3K/AKT/mTOR pathways are the most extensively investigated for their role in governing the survival of SnCCs (Table 1).

Bcl-2 family pathway

Resistance to programmed cell death is a recognized hallmark of cancer, characterized by an altered molecular profile featuring heightened expression or activity of anti-apoptotic proteins, often accompanied by a reduction in the function of pro-apoptotic proteins (Hanahan, 2022). This altered pattern of apoptotic proteins directly contributes to the survival of tumor cells and plays a pivotal role in triggering and maintaining the senescent state in cancer. One of the leading protein families involved in resistance to programmed cell death is the Bcl-2 family, which is constituted by pro-apoptotic and anti-apoptotic members responsible mainly for modulating the triggering of the intrinsic apoptosis pathway. However, each Bcl-2 family member has also distinct influences on non-canonical molecular mechanisms such as cellular senescence (Fan *et al.,* 2020). The expression level of individual Bcl-2 family proteins can affect the initiation of senescence differently. Once cells become senescent, members of the Bcl-2 family can be differently modulated in SnCs, not only enhancing the resistance to programmed cell death but also sustaining their permanent cell cycle arrest state (Basu, 2022).

Bcl-2 family anti-apoptotic proteins

Anti-apoptotic proteins from the Bcl-2 family are often overexpressed in several tumor types, favoring the resistance to programmed cell death (Kaloni *et al.,* 2023). However, despite belonging to the same subgroup, each anti-apoptotic protein from the Bcl-2 family seems to influence both the triggering and the maintenance of the senescent state in tumor cells in a particular way, as depicted below and shown in Table 1.

Bcl-2 and Bcl-X(L): these Bcl-2 anti-apoptotic proteins play a crucial role in governing the delicate balance between cell life and death by avoiding the release of apoptotic factors throughout the mitochondrial outer membrane. In addition to their negative role in programmed cell death, both proteins control the initiation of cell cycle arrest, thus blocking apoptosis and simultaneously triggering TIS or OIS (Drullion *et al.,* 2012; Gayle *et al.,* 2019).

Moreover, the overexpression of Bcl-X(L) in tumor cells before treatment can attenuate apoptosis induction, favoring senescence triggering (Gayle *et al.,* 2019). These pieces of evidence show how important Bcl-2 and Bcl-X(L) are for the senescence initiation process, and their expression levels are definitive in determining the fate of a tumor cell in response to some types of therapy. For this reason, pharmacological inhibitors targeting these proteins, such as ABT263 (Navitoclax), have been using to eliminate SnCs, as discussed in the next section.

On the other hand, despite demonstrating a similar effect on initiating senescence, Bcl-2 and Bcl-X(L) seem to have different relevance in its maintenance. Pharmacological or molecular inhibition of Bcl-X(L) can induce SnCCs to apoptosis (Selt. However, Venetoclax, a specific inhibitor of Bcl-2, did not impact the viability of established SnCs (Selt *et al.,* 2023), suggesting that Bcl-X(L) may be more critical not only for inducing but also for maintaining the senescent state. Controversially, some evidence show that downregulating Bcl-X(L) can also trigger senescence, but accompanied by an increase in the expression of the cell cycle inhibitor p21 (Ikezawa *et al.,* 2017). Therefore, the triggering of the senescent state may be a consequence of p21accumulation rather than the reduction of Bcl-X(L).

Mcl-1: another anti-apoptotic member of the Bcl-2 family, Mcl-1 is also responsible for controlling the mitochondrial permeability, preventing the release of pro-apoptotic molecules from the mitochondrial inner space. However, the interplay between Mcl-1 and senescence in tumor biology is complex and controversial. While Mcl-1 can contribute to the survival of SnCCs and non-SnCCs by preventing apoptosis, its overexpression, unlike the above-discussed anti-apoptotic proteins, may also be associated with the evasion of senescence, allowing cancer cells to persist with their proliferation potential (Bolesta *et al.,* 2012). In the same way, the downregulation of Mcl-1 may allow the triggering of senescence in cancer cells (Bolesta *et al.,* 2012). However, once senescent, the inhibition of this protein does not affect the viability of SnCCs (Selt *et al.,* 2023), showing its importance for initiating the process but not for its maintenance.

Bcl-W: little is known about the involvement of Bcl-W in the induction or maintenance of the senescent state in tumor cells. Similarly to Bcl-X(L), one of the few pieces of evidence showed that Bcl-W overexpression also increases the induction of senescence in cancer cells, and its suppression by miRNA attenuates the permanent arrest in the cell cycle (Choi *et al.,* 2018). Although it is also a protein responsible for negatively controlling the permeability of the mitochondrial outer membrane, thus preventing the triggering of apoptosis, more evidence is needed to define its actual involvement in the triggering and maintenance of senescence.

Table 1 – Survival pathways of SnCs involving Bcl-2 family protein, PI3K/AKT, and the MDM-2/TP53 pathways.

Author	Year	Molecular Marker	Molecular Status	Pre or Post Senescence	Main Outcome	Model
Bcl-2 family pathway						
Drullion C	2012	Bcl-2	Overexpression	Pre	Bcl-2 upregulation blocked apoptosis and increased levels of senescence in response to Imatinib.	Leukemia
Ikezawa K	2017	Bcl-X(L)	Downregulation	Pre	Downregulation of Bcl-X(L) by siRNA triggered oncogene-induced senescence in high-grade tumors, associated with p21 overexpression.	Pancreas tumor
Gayle S	2019	Bcl-X(L)		Pre	Bcl-X(L) was overexpressed in those cell lines that triggered senescence in response to BETi treatment. Bcl-2, Bim, BAX, and Mcl-1 levels were not changed in senescent cells.	Breast Cancer
Gayle S	2019	Bcl-X(L)	Overexpression	Pre	Bcl-X(L) overexpression in BETi-treated cells shifted the response from apoptosis to senescence.	Breast Cancer
Gayle S	2019	Bcl-X(L)	Downregulation	Pre/post	Bcl-X(L) inhibition induced apoptosis in response to BETi even after BETi-induced senescence had already occurred.	Breast Cancer
Selt F	2023	Bcl-X(L)		Pre	Oncogene-induced senescence increased Bcl-X(L) expression. Modulation of other anti-apoptotic Bcl-2 family proteins were not detected.	Pilocytic Astrocitoma
Selt F	2023	Bcl-X(L)	Downregulation	Post	Downregulation of Bcl-X(L) (Navitoclax or A-1331852) reduced the viability of senescent cells by apoptosis triggering. Bcl-2 and Mcl-1 inhibitors (Venetoclax and S63845) did not impact the viability of senescent cells.	Pilocytic Astrocitoma
Choi J	2018	Bcl-W	Overexpressison	Pre	Overexpression of Bcl-W promoted premature senescence by activating the TP53 pathway, increasing TP53, p21, Notch2 and $p16^{INK4A}$.	Glioblastoma and Lung Cancer
Choi J	2018	Bcl-W	Downregulation	Pre	Downregulation of Bcl-W using miR-95-5p decreased premature senescence by suppressing Bcl-W and p21 expression.	Glioblastoma and Lung Cancer
Bolesta E	2012	Mcl-1	Overexpression	Pre	Overexpression of Mcl-1 before treatment abrogates the doxorubicin-induced senescence in TP53+ cells, reducing p21	Human cancers
Bolesta E	2012	Mcl-1	Downregulation	Pre	Downregulation of Mcl-1 before treatment triggered doxorubicin-induced senescence in TP53- cells, increasing p21	Colon cancer
Wu G	2022	Bid and BAX	Release from inhibitor	Post	The BH3 mimetic A-1331852 induced caspase-dependent senescent cell death by releasing Bid and BAX through disrupting Bcl-X(L)/Bid and Bcl-X(L)/BAX complexes.	Human Lung carcinoma
Werner L	2015	BAX		Post	Senescence induced by irradiation plus MDM-2 inhibitor induced TP53 accumulation, followed by increase in p21 and BAX.	Melanoma and Sarcoma
Drullion C	2012	Bim	Downregulation	Post	Blocking apoptosis by Bim downregulation increased senescence levels.	Leukemia
PI3K/AKT pathway						
Xu X	2014	AKT	Allosteric inhibition	Pre	Administration of AKT inhibitor (MK-2206) induces senescence through increasing ROS production and miR-182 expression, accompanied by an increase in TP53, p21 and $p16^{INK4A}$	Leiomyoma
Jung S	2019	PTEN	Downregulation	Pre	PTEN downregulation by RNAi induces senescence through ATK-mTORC1/2 activation, followed by activation of the TP53-p21 axis, independently of DDR and ROS generation.	Breast cancer
MDM-2/TP53 pathway						
Yosef R	2017	p21	Downregulation	Post	Knockdown of p21 by RNAi in DNA damage-induced senescent cells induced DNA lesions, resulting in cell death through ATM and NF-kβ activation.	Non-Small Cell Lung Carcinoma

Although there are other anti-apoptotic proteins in the composition of the Bcl-2 family, there is no evidence about their direct involvement in regulating senescence in cancer. Therefore, it is possible to define that the involvement of Bcl-2 family anti-apoptotic proteins seems to depend not only on their anti-apoptotic properties but also on its influence in modulating the expression and activity of other proteins responsible to modulate the cell cycle arrest, such as p21 protein. Classifying proteins from the Bcl-2 family as anti-apoptotic molecules does not necessarily imply assigning them a role as senescence-inducing proteins, which must be considered in developing senolytic therapies.

Bcl-2 family pro-apoptotic proteins

Pro-apoptotic proteins within the Bcl-2 family also modulate the initiation and maintenance of senescence in tumor cells. These proteins are usually found at reduced levels or in stoichiometric imbalance with anti-apoptotic proteins in tumor cells, hampering apoptosis and potentially influencing senescence. However, there is little evidence correlating and explaining how the expression levels or activity of pro-apoptotic proteins of the Bcl-2 family can modulate the senescent state in cancer.

Bim: the Bcl-2 interacting mediator of cell death (Bim) is a pro-apoptotic protein that initiates apoptosis by binding to and neutralizing anti-apoptotic Bcl-2 proteins, such as Bcl-2, Bcl-X(L), and Mcl-1. Through these interactions, BIM contributes to releasing pro-apoptotic factors from the inner space of mitochondria, leading to the activation of caspases, and ultimately to cell death (Kale *et al.,* 2018). Likewise, reducing the expression level of Bim inhibits apoptosis while increases senescence in human leukemia cells (Drullion *et al.,* 2012). Nonetheless, little evidence explains how Bim can favor senescence.

Bid and BAX: Bid serves as a BH3-only protein and links the intrinsic and extrinsic apoptotic pathways. Upon activation, Bid triggers mitochondrial outer membrane permeabilization, contributing to the release of pro-apoptotic factors. On the other hand, BAX is a multi-domain pro-apoptotic protein that plays a crucial role in regulating mitochondrial integrity. Activated BAX undergoes conformational changes and translocates to the mitochondrial outer membrane, where it promotes permeabilization through the formation of pores, allowing the release of apoptogenic factors from the inner space of this organelle, ultimately leading to cell death. Thus, cell survival depends on a stoichiometric balance between anti- and pro-apoptotic proteins of this family, regulating the apoptosis process through an inhibitory physical interaction of anti-apoptotic protein units with pro-apoptotic proteins. BH3 mimetic molecules like A-1331852, which function as a subgroup of proteins known as pro-apoptotic BH3-only proteins, induce apoptosis in senescent lung carcinoma cells by disrupting the Bcl-X(L)-mediated inhibitory interaction with pro-apoptotic proteins Bid and BAX, (Wu *et al.,* 2022). However, increasing pro-apoptotic proteins like BAX does not prevent the senescent state in tumor cells, whether, at the same time, senescence inducer proteins such as cell cycle inhibitor p21 are being simultaneously increased (Werner *et al.,* 2015), suggesting that apoptosis inducers are not enough to block the triggering of the senescent state.

MDM-2/TP53/p21 pathway

Another extensively studied SCAP in cancer is the MDM-2/TP53/p21 signaling pathway, which is responsible for sensing DNA damage caused by several stress signals and inducing cell cycle arrest for DNA repair. Persistent DNA damage signals activate TP53, a transcription factor for several target genes that control cell cycle arrest, apoptosis, and senescence. Despite the wide variety of molecular targets modulated by TP53, the induction of CDKN1A gene, which encodes the p21 protein, represents the main contribution of TP53 in triggering senescence and preserving cell survival. As a member of the CDK inhibitor family, p21 mediates the expression of several molecular targets that result in cell cycle arrest at either the G1/S or G2/M checkpoints (Rufini *et al.,* 2013; Al Bitar and Gali-Muhtasib, 2019). However, p21 seems to have an even greater relevance as an effector molecule in the dual decision between apoptosis or senescence in response to stress. In addition to inducing cell cycle arrest, p21 can also interact with Bcl-2 proteins and inhibit apoptosis, reinforcing the decision for senescence (Martinez *et al.,* 2002; Yosef *et al.,* 2017). In SnCCs resulting from DNA damage, the downregulation of p21 can trigger cell death through ATM and NF-kβ activation, highlighting the significance of high levels of p21 to maintaining the senescent state (Yosef *et al.,* 2017).

p16 pathway

In addition to the action of p21, which seems to be important in the senescence initiation process (Kuilman *et al.,* 2010), another crucial factor for this cellular outcome in cancer is p16, which seems to be more involved in the senescence maintenance in a protein level-dependent manner (Rayess *et al.,* 2012). Currently, p16 is considered a tumor suppressor protein because of its physiological role as a cell cycle inhibitor and its downregulated expression in many tumors. Intriguingly, overexpression of p16 has also been described in several tumors (Romagosa *et al.,* 2011). The central role of p16 is to inhibit the cyclin D1/CDK4/6 complex, preventing the hyperphosphorylation of the Rb protein. This event allows the release of E2F to mediate the expression of effector molecules controlling the progression of the cell cycle phase G1 for S phase (Peurala *et al.,* 2013). Thus, high levels of p16 seem essential in maintaining the permanent cell cycle arrest present in SnCs. The suppression of p16 in SnCs could reverse the cell cycle arrest only when p53 was also inactivated since p21 can compensate for maintaining the senescent state. Likewise, once p16 is highly expressed, the downregulation of TP53 cannot reverse the cell cycle arrest (Campisi, 2005), suggesting that p16 seems to be as relevant as p21 for the triggering and maintenance of the senescent state. Therefore, although p21 can directly participate in cell survival through the inhibitory modulation of apoptosis, together with p16 it is fundamental for maintaining the senescent state.

PI3K/AKT/mTOR pathway

The PI3K/Akt/mTOR pathway, an intricate signaling cascade, is pivotal in regulating various cellular processes, including cell growth, survival, and senescence. This pathway is tightly regulated and plays a crucial role in normal cellular functions, and its dysregulation is commonly observed in various diseases, particularly cancer. Hyperactivation of this

pathway is associated with uncontrolled cell growth, evasion of apoptosis, and resistance to anti-cancer therapies. However, although much is known about the dysregulation of the PI3K/Akt/mTOR pathway in the context of cancer, there is little evidence directly correlating its modulation with survival, specifically in SnCCs. It is known that it can also regulate p21 expression to initiate senescence in tumor cells, either in a DNA damage response-dependent (Xu *et al.,* 2014) or independent manner (Jung *et al.,* 2019). Thus, more evidence is needed to define the actual involvement of this signaling pathway in modulating the survival of SnCs.

Senotherapies in cancer: From senoprevention to senolysis

Since the persistence of SnCs in the TME may favor tumor growth, increased heterogeneity, and resistance to therapy (Krtolica *et al.,* 2001; Castro-Vega *et al.,* 2015), removing SnCs could reduce these pro-tumoral phenotypes (Figure 3B and 3C), improving the prognosis (Sieben *et al.,* 2018). In recent years, researchers have developed a range of molecules to target SnCs, known as senotherapies. Many of these molecular signaling pathways targeted by senotherapeutics are crucial for the survival of SnCs, resulting

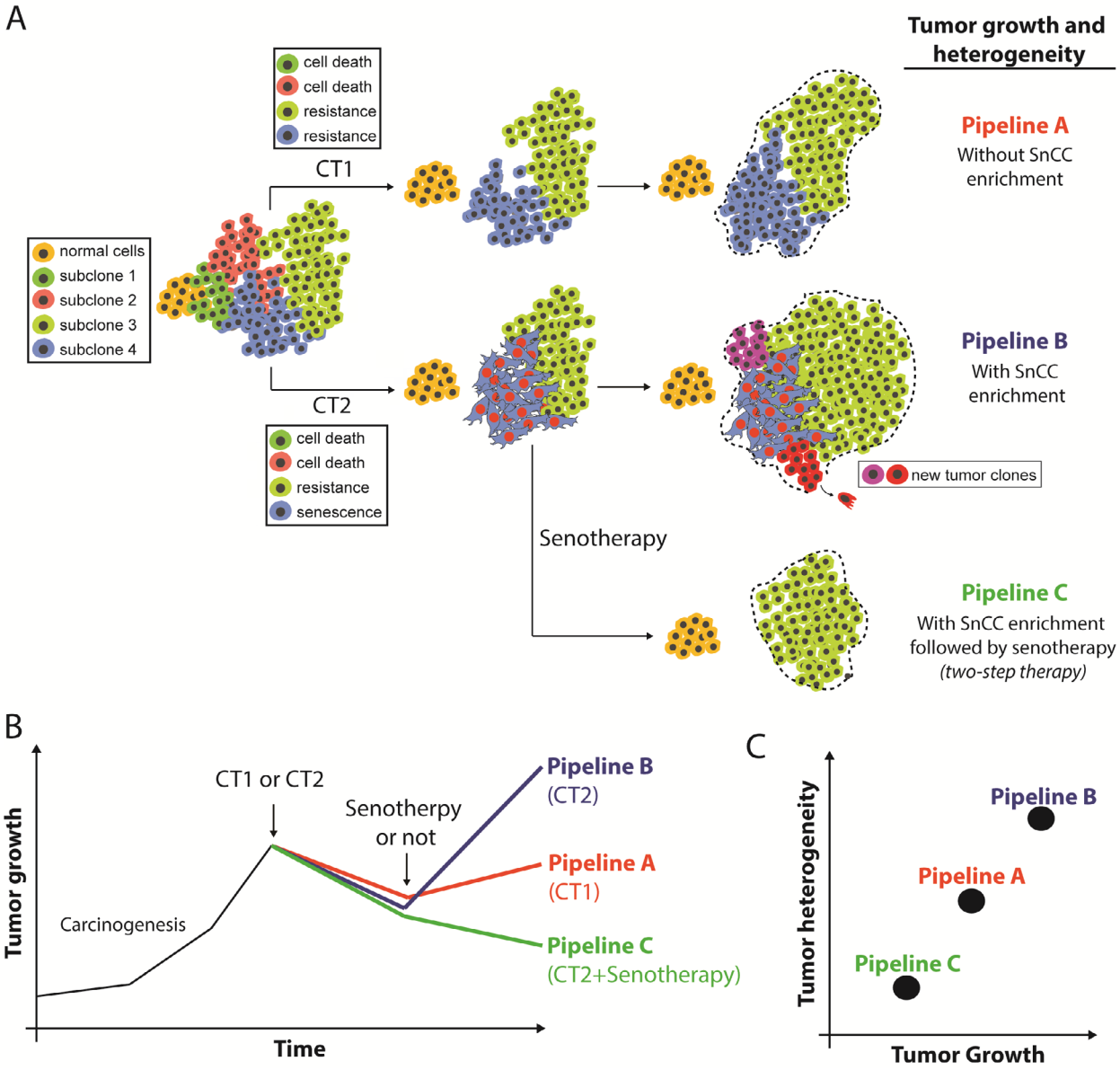

Figure 3 – The impact of Senescent Cells (SnCs) and senotherapies in tumor growth. A). Representative model of the parenchyma of a tumor formed by 4 subclones of tumor cells and 1 subclone of a normal cell. **Top (pipeline A)** – cell response to chemotherapy 1 (CT1) considering 2 tumor clones sensitive to cell death and 2 tumor clones resistant to therapy. Note that there is no induction of senescence in this example. **Bottom (pipeline B)** – response to chemotherapy 2 (CT2) considering 2 tumor clones sensitive to cell death, 1 tumor clone resistant to therapy and 1 clone sensitive to entry into a senescent state. Note that there is an enrichment of SnCs in this example, with increased heterogeneity and greater tumor growth compared to the Pipeline A model. **Pipeline C** represents two-step therapy, in which after the enrichment of SnCs in the TME, there is treatment with a senolytic compound that induces SnCs to cell death. Note that this is the treatment pipeline with the smallest tumor size at the end of the model. **B)** Growth curve simulations for pipelines A, B, and C. **C)** Scatter plot of heterogeneity and tumor growth for pipelines A, B, and C. Abbreviations: CT, chemotherapeutics; SnCC, senescent cancer cell.

in the senolysis. Preliminary evidence indicates that these compounds can eliminate SnCs *in vivo* (Ellison-Hughes, 2020), leading to decreased inflammation, improved organ and tissue function, and ultimately increased survival in animal models (Xu *et al.,* 2018; Lewis-McDougall *et al.,* 2019; Novais *et al.,* 2021).

As raised in previous section, numerous mechanisms contributing to the resistance to apoptosis and the survival of SnCs have been identified (Table 1). Based on these findings, researchers have investigated pharmacological inhibitors of these pathways for their potential to induce senescent cell death. Indeed, compounds that target anti-apoptotic proteins from the Bcl-2 family exhibit senolytic properties. For instance, ABT-263 (Navitoclax), a specific Bcl-2 inhibitor, and ABT-737, an inhibitor of Bcl-W, Bcl-X(L), and Mcl-1, have shown senolytic effects (Yosef *et al.,* 2016; Zhu *et al.,* 2016). Fisetin, a specific Bcl-X(L) inhibitor, has demonstrated similar properties (Yousefzadeh *et al.,* 2018). Quercetin also triggers senescent cell death by interfering with the Bcl-X(L) protein, with more significant results observed when combined with Dasatinib (Islam *et al.,* 2023). Additionally, mTOR inhibitors, such as AZD8055, have shown senolytic effects inducing apoptosis by modulation of Bcl-2 family proteins (Sharma *et al.,* 2014). Cardiac glycosides have also displayed senolytic potential by affecting the pro-apoptotic Bcl-2 family protein NOX activator 1 (Guerrero *et al.,* 2019).

Other senolytics that operate putatively independently of PI3K and mTOR pathways have been explored. For example, ARV825, a hetero-bifunctional proteolysis-targeting chimera, reduces *XRCC4* gene expression, hinders the recruitment of p53-binding protein 1 (53BP1), disrupts non-homologous end-joining DNA repair, and triggers apoptosis (Wang *et al.,* 2022). As an alternative approach, researchers have explored cellular senotherapies using Chimeric Antigen Receptor T-cells (CAR-T) targeting surface proteins expressed by SnCs, such as the PLAUR protein (Amor *et al.,* 2020).

Furthermore, oxidative phosphorylation is another molecular process that significantly influences the survival of SnCs. Its inhibitors (e.g. metformin) could sensitize SnCs while also acting as senomorphics, another class of senotherapeutics capable of altering the composition of the SASP through the modulation of intracellular pathways (Cheng *et al.,* 2022). Molecules with senomorphic properties have become increasingly relevant since signaling mediated by some proteins encoded by oncogenes can also modulate the production of SASP. Some examples are MEK1/2 (Ruscetti *et al.,* 2018, 2020), tyrosine kinase receptors such as EGFR (Alexander *et al.,* 2015; Romaniello *et al.,* 2022), and the mTOR kinase (Garbers *et al.,* 2013; Herranz *et al.,* 2015; Laberge *et al.,* 2015) for which targeted therapies are approved. Indeed, the EGFR inhibitor erlotinib (Alexander *et al.,* 2015), the MEK1/2 inhibitor trametinib (Schick *et al.,* 2015), and mTOR inhibitors (Herranz *et al.,* 2015) change the production and secretion of SASP molecules by SnCs. Thus, the genetic status of a given cancer and the targeted drugs chosen to treat it may affect the constitution of SASP, characterizing them as senomorphic molecules. However, additional evidence is needed to support this evidence in vivo. Finally, although these pathways can affect cell survival in human cells in general, whether the status of these oncogenes affects the sensitivity to analytics is still being determined.

Notably, SnCs may exhibit a reduced response to typical chemotherapy, which targets DNA replication in the S phase. They also frequently present heightened anti-apoptotic proteins and drug resistance, aiding their survival during subsequent cycles of treatments, which is the standard practice in the clinics. Thus, the rational combination of antineoplastic and senolytic therapies can mitigate SnCs' pro-tumor effects. Considering that senescence also plays physiological roles like development and tissue repair, it is essential to understand the peculiarities of each type of senescence induced by different types of stimuli from different origins or contexts to enable the selective elimination of SnCCs from TME without affecting the beneficial roles that the physiological senescence perform in other biological situations.

From an evolutionary perspective, senescence can act as a tumor suppressor mechanism, ensuring successful reproduction in young individuals despite potential drawbacks later in life. However, it may become detrimental in older organisms. This is evident from the accumulation of SnCs in older organisms and their presence at sites of age-related pathologies, including cancer. However, there is a lack of quantitative data stratifying SnCs in age-related cancers before and after therapy-inducing senescence. Notwithstanding, there is a recognized understanding of age-related differences in tumors. For example, in breast cancer, older patients exhibit variations in tumor histology based on age (Schonberg *et al.,* 2010; Lodi *et al.,* 2017; Wang *et al.,* 2018), distinct subtype distributions (Dreyer *et al.,* 2013; Jenkins *et al.,* 2014), and age-specific patterns of tumor mutations in comparison to younger patients (Syed *et al.,* 2014). Likewise, some studies suggest significant changes in tumor-infiltrating immune cells between tumors in older versus younger individuals (Thomas *et al.,* 2013; Chen *et al.,* 2015). However, whether increased SnCs in older people mediate these differences is unknown. Therefore, the distinct TME between young and old patients suggests that anti-cancer therapy inducing senescence may not yield similar outcomes across age groups. Furthermore, it is plausible to infer that cells from older individuals would be more 'primed' for senescence (e.g., with increased basal histone H2AX signal) so that senescence could be induced by lower levels of damage than necessary for induce senescence in cells of younger people. However, it is essential to point out that most human cancers present cells with overexpression of the telomerase, which allows them to evade replicative senescence and attenuate the signals that would make them 'primed' for senescence.

Finally, another clinical characteristic associated with aging that may impact the role of senescence in some tumor types concerns the deregulation of hormone production or signaling (Khosla *et al.,* 2020). In certain hormone-related cancers, like from breast and prostate, a crosstalk between therapies targeting hormone receptors or signaling pathways and senescence has been proposed. By using a combination of drugs inhibiting Human Epidermal growth factor Receptor 2 (HER2) and Rb checkpoint, Viganò *et al.* (2022) showed that breast cancer cells exposed to these drugs undergo senescence

(Viganò *et al.,* 2022). Other studies corroborate the observation of senescence induction in breast and prostate cancer cells exposed to hormone-receptor antagonists tamoxifen (Lee *et al.,* 2014) and bicalutamide (Carpenter *et al.,* 2021), or androgen deprivation (Ewald *et al.,* 2013). Likewise, data from patients with ER+ and HER2+ breast cancer enrolled in a clinical trial showed that exposure to a combination of drugs that target these molecules presented a higher expression of senescence-related genes (Viganò *et al.,* 2022). These data reinforce the complexity of senescence in pathophysiological contexts such as endocrine regulation, which is affected not only in numerous types of cancer but also during aging and other metabolic diseases. Therefore, the dysregulation of the crosstalk between hormonal regulation, aging, and cellular transformation may not only be the target of therapies but also underlie the pathogenesis of tumors of an endocrine nature associated with aging.

Open questions and perspectives

Despite the advances made in recent years, many questions related to SnCCs and their impact on the TME remain open. Considering the biology of these transformed cells, it is necessary to characterize:

a. Differences and similarities between SnCs and SnCCs as well as the heterogeneity of SnCCs, considering SnCCs subtypes (or subpopulations) or states (Figure 4A).

b. Whether the progressive acquisition of the senescent phenotype is unidirectional and irreversible (Figure 4B – top) or if SnCCs can assume different states in a dynamic manner (Figure 4B – bottom).

c. The morphological plasticity of SnCCs (Figure 4C), including mechanisms controlling this process.

d. The SASP of different subtypes or states of SnCCs (Figure 4D), which may influence the impact of these cells in the TME.

In addition to the biology of SnCs, some aspects related to senescence induced by antitumor therapies also need to be better understood, such as:

a. Differences between SnCCs induced by distinct chemotherapeutics (CT) (Figure 4E – left) and whether a pro-senescent CT induces multiple or a specific SnCC subtype (Figure 4E – right). Also, the characteristics of SnCCs induced by targeted or endocrine therapies (Figure 4F).

b. How SnCCs respond to the re-treatment with the CT that induced the phenotype or to another CT (Figure 4G).

c. Differences and similarities between SnCCs from different tumor types (Figure 4H) or between SnCCs from primary and metastatic tumors (Figure 4I).

d. The spatial organization of SnCCs in the TME (e.g. if these cells form niches or if they present a diffuse pattern of distribution) (Figure 4F).

e. What is the best strategy of senotherapy to attenuate the pro-tumor effects played by SnCCs in the TME (Figure 4H).

Although molecular biology tools have advanced enormously, most of the data regarding the role of SnCC in tumor plasticity still come from *in vitro* studies using limited and simplistic models based on cell population data. In contrast, data from primary samples are still limited. Furthermore, much evidence is based on tissue bulk data, making it difficult to determine which cell type in the sample undergoes senescence (e.g., tumor cells or stromal cells) and contributes to the biological effects of SnCs. Finally, much evidence comes from end-point analysis, which hampers conclusions about phenotypic plasticity and dynamics (Begnini *et al.,* 2022). Finding answers to the above questions will require strategies combining cellular and molecular tools, in addition to the development of new models and protocols, especially for live single-cell tracking and *in vivo* models.

The presence of SnCs in the TME and unlocking phenotypic plasticity are the two most recent features included as hallmarks of cancer (Hanahan, 2022). As discussed throughout this article, the first seems to influence the second strongly. The mechanisms of cellular plasticity modulated by SnCs are interconnected and are, to a certain extent, interdependent. Changes in nuclear gene expression, for example, are fundamental not only for stem cell differentiation and cellular reprogramming but also for phenotypic changes observed in EMT or metabolic changes. Furthermore, in some models, especially considering highly heterogeneous tumor populations, SnCCs may affect different cellular plasticity mechanisms depending on the background of the target cell. This explains, at least in part, the multiple phenotypic responses, like increased secondary senescence, stemness, and EMT, observed in the same tumor cell population *in vitro* after exposure to a SnCs-conditioned medium. Furthermore, more than one phenotype associated with cellular plasticity, such as increased stemness, EMT, and greater migratory capacity, can occur in the same cell after exposure to SASP (Parrinello *et al.,* 2005). Finally, it is essential to highlight that SASP can induce more cells to senescence (secondary senescence), promoting the persistence of SASP effects (Figure 2).

As raised throughout the article, SnCs may contribute to TME remodeling during tumor formation and progression through multiple cell communication mechanisms, affecting the spatial organization and the functional status of stromal and immune cells. Most of the effects played by SnCs on mechanisms associated with tumor plasticity are exerted by the SASP. Thus, both the neutralization of SASP molecules and the modulation of intracellular pathways involved in SASP production are potential targets for therapies (Ortiz-Montero *et al.*, 2017) to interrupt the positive feedback established between senescence and tumor plasticity events. However, both SASP molecules and these signaling pathways play critical physiological roles too, so acting on them can be complex and lead to significant side effects. Thus, the

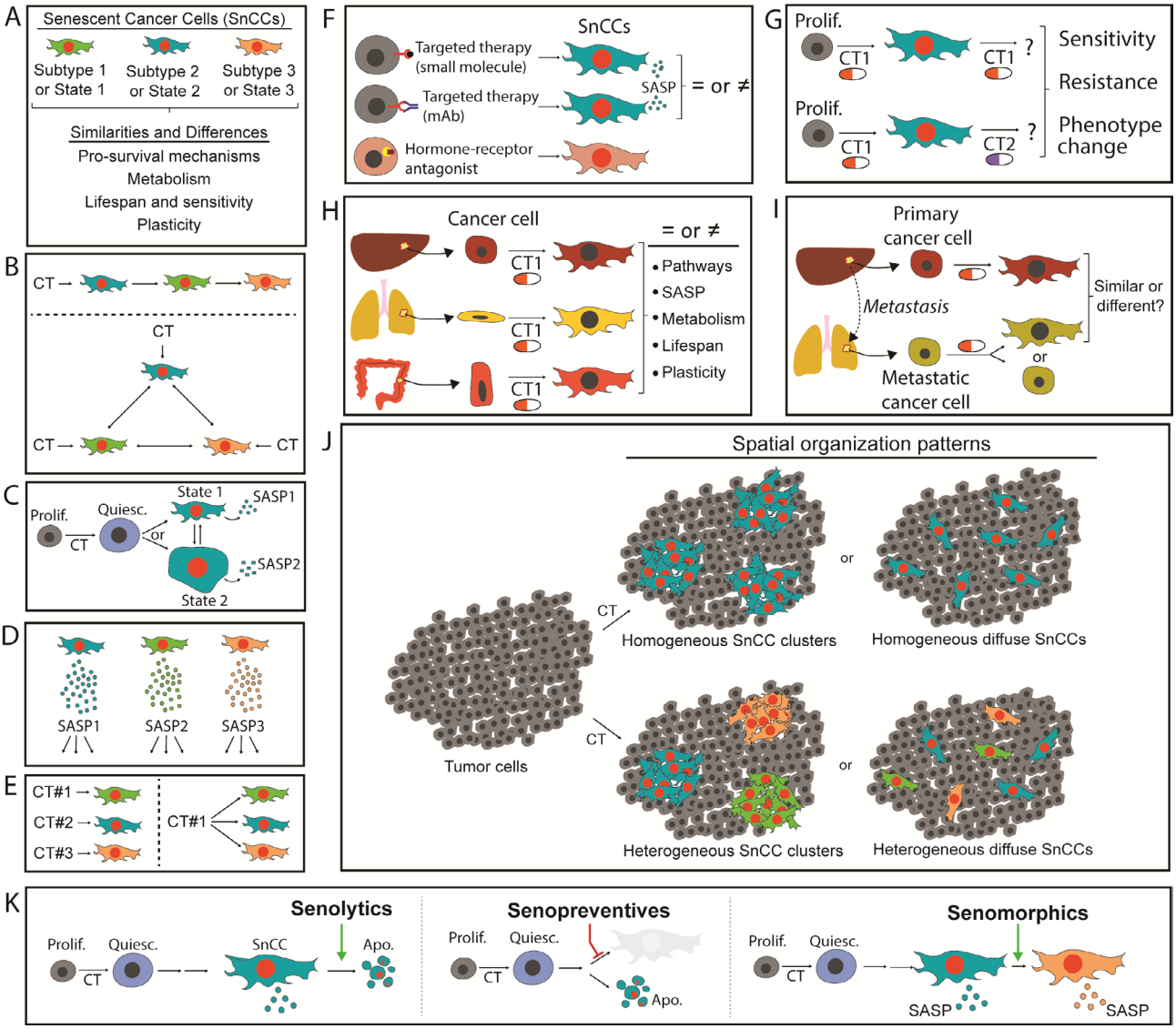

Figure 4 – Open questions and perspectives. This figure summarizes questions that remain open about the biology of senescent tumor cells, especially related to therapy-induced senescence. **A)** It is not known how many senescent subtypes (or states) are induced after therapy, and differences or similarities between them. **B)** Considering initial evidence that points to phenotypic heterogeneity among tumor cells, it is not clear whether the same senescent cell is plastic enough to assume different subtypes (or states), nor whether a chemotherapeutic drug can induce different subtypes or states of senescence (e.g., from tumor cells with different molecular backgrounds). **C)** Although it is possible to observe morphological heterogeneity between SnCs, it is not clear whether the same senescent cell can transition between different subtypes or phenotypic states. **D)** SnCs of different subtypes (or states) may have qualitative and quantitative differences in SASP. **E)** It is not clear whether different chemotherapeutic agents induce SnCs of different subtypes or states, nor whether the same chemotherapy agent can induce SnCs with different subtypes or phenotypic states. **F)** Other classes of anti-cancer drugs, such as targeted therapies and hormone receptor antagonists, can also induce senescence. **G)** The sensitivity of cells induced to senescence by different chemotherapeutic agents is not known, both for the same chemotherapy agent that primarily induced senescence (top) and for other chemotherapy agents (cross-sensitivity, bottom). **H)** It is well established that tumor cells from different organs undergo cellular senescence after therapy. However, similarities and differences in the phenotype of these cells are not known. **I)** Comparison between SnCs derived from related primary and metastatic tumors. Differences and similarities between these cells are not known. **J)** Possible patterns of spatial organization of SnCs in the TME. SnCs, whether homogeneous among themselves or not, appear in clusters or diffuse. **K)** Main strategies targeting SnCs. Top – senolysis (induction of death of SnCs); middle – senoprevention (prevent cells from entering senescence, inducing them to cell death); bottom – senomorphics (modulation of SASP composition). Abbreviations: Apo., apoptotic cell; CT, chemotherapeutics; mAB, monoclonal antibody; Prolif., proliferative cell; Quiesc., quiescent cell.

use of therapies targeting SnCs and their secretome might be rationally defined depending on the tumor type, the primary treatment chosen, and the tissue context where that tumor developed. All these aspects make SnCs a central player that must be better understood in the context of carcinogenesis and response to therapy, having potential as a marker for association with prognosis and targeted therapeutic modulation.

Acknowledgements

We would like to thank the funding agencies, especially CNPq (Edital , FAPERGS, CAPES and FIPE-HCPA.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Author Contributions

AOS reviewed the literature and wrote the manuscript, created Table 1, and formatted the manuscript for submission; TCB reviewed the literature and wrote the manuscript; JEV reviewed the literature and wrote the manuscript; LRF reviewed the literature and wrote the manuscript EFC conceived the review, created the figures, and wrote the manuscript, all authors read and approved the final version of text, figures and table.

References

Abdelgawad IY, Agostinucci K and Zordoky BN (2022) Cardiovascular ramifications of therapy-induced endothelial cell senescence in cancer survivors. Biochim Biophys Acta Mol Basis Dis 1868:166352.

Achuthan S, Santhoshkumar TR, Prabhakar J, Nair SA and Pillai MR (2011) Drug-induced senescence generates chemoresistant stemlike cells with low reactive oxygen species. J Biol Chem 286:37813-37829.

Acosta JC and Gil J (2012) Senescence: A new weapon for cancer therapy. Trends Cell Biol 22:211-219.

Afifi MM, Crncec A, Cornwell JA, Cataisson C, Paul D, Ghorab LM, Hernandez MO, Wong M, Kedei N and Cappell SD (2023) Irreversible cell cycle exit associated with senescence is mediated by constitutive MYC degradation. Cell Rep 42:113079.

Al Bitar S and Gali-Muhtasib H (2019) The role of the cyclin dependent kinase inhibitor $p21^{cip1/waf1}$ in targeting cancer: Molecular mechanisms and novel therapeutics. Cancers (Basel) 11:1475.

Alessio N, Acar MB, Squillaro T, Aprile D, Ayaz-Güner Ş, Di Bernardo G, Peluso G, Özcan S and Galderisi U (2023) Progression of irradiated mesenchymal stromal cells from early to late senescence: Changes in SASP composition and anti-tumour properties. Cell Prolif 56:e13401.

Alexander PB, Yuan L, Yang P, Sun T, Chen R, Xiang H, Chen J, Wu H, Radiloff DR and Wang XF (2015) EGF promotes mammalian cell growth by suppressing cellular senescence. Cell Res 25:135-138.

Amor C, Feucht J, Leibold J, Ho IJ, Zhu C, Alonso-Curbelo D, Mansilla-Soto J, Boyer JA, Li X, Giavridis T, *et al.* (2020) Senolytic CAR T cells reverse senescence-associated pathologies. Nature 583:127-132.

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL and van Deursen JM (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479:232-236.

Baldasso-Zanon A, Silva AO, Franco N, Picon RV, Lenz G, Lopez PL and Filippi-Chiela EC (2024) The rational modulation of autophagy sensitizes colorectal cancer cells to 5-fluouracil and oxaliplatin. J Cell Biochem 125:e30517.

Basu A (2022) The interplay between apoptosis and cellular senescence: Bcl-2 family proteins as targets for cancer therapy. Pharmacol Ther 230:107943.

Batlle E and Clevers H (2017) Cancer stem cells revisited. Nat Med 23:1124-1134.

Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S and Nelson PS (2006) The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. Cancer Res 66:794-802.

Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P and Campisi J (2003) Reversal of human cellular senescence: Roles of the p53 and p16 pathways. EMBO J 22:4212-4222.

Begnini KR, Pereira LC, Faccioni JL, Lenz G and Filippi-Chiela EC (2022) Bioimaging approaches for quantification of individual cell behavior during cell fate decisions. Biochem Soc Trans 50:513-527.

Bennecke M, Krieg Ll, Bajbouj M, Retzlaff K, Robine S, Jung A, Arkan MC, Kirchner T and Greten FR (2010) Ink4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. Cancer Cell 18:135-146.

Blagosklonny MV (2011) Cell cycle arrest is not senescence. Aging (Albany NY) 3:94-101.

Bloom SI, Islam MT, Lesniewski LA and Donato AJ (2023) Mechanisms and consequences of endothelial cell senescence. Nat Rev Cardiol 20:38-51.

Bolesta E, Pfannenstiel LW, Demelash A, Lesniewski ML, Tobin M, Schlanger SE, Nallar SC, Papadimitriou JC, Kalvakolanu DV and Gastman BR (2012) Inhibition of Mcl-1 promotes senescence in cancer cells: Implications for preventing tumor growth and chemotherapy resistance. Mol Cell Biol 32:1879-1892.

Bruni E, Cazzetta V, Donadon M, Cimino M, Torzilli G, Spata G, Leonardi G, Dieli F, Mikulak J and Mavilio D (2019) Chemotherapy accelerates immune-senescence and functional impairments of Vδ2. J Immunother Cancer 7:347.

Cahu J, Bustany S and Sola B (2012) Senescence-associated secretory phenotype favors the emergence of cancer stem-like cells. Cell Death Dis 3:e446.

Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. Cell 120:513-522.

Carpenter V, Saleh T, Min Lee S, Murray G, Reed J, Souers A, Faber AC, Harada J and Gewirtz DA (2021) Androgen-deprivation induced senescence in prostate cancer cells is permissive for the development of castration-resistance but susceptible to senolytic therapy. Biochem Pharmacol 193:114765.

Castro-Vega LJ, Jouravleva K Ortiz-Montero P, Liu WY, Galeano JL, Romero M, Popova T, Bacchetti S, Vernot JP and Londono-Vallejo A (2015) The senescent microenvironment promotes the emergence of heterogeneous cancer stem-like cells. Carcinogenesis 36:1180-1192.

Chen S, Liu H, Su N, Zhang G and Wang L (2015) Myeloid-derived suppressor cells promote age-related increase of lung cancer growth via B7-H1. Exp Gerontol 61:84-91.

Cheng FF, Liu YL, Du J and Lin JT (2022) Metformin's mechanisms in attenuating hallmarks of aging and age-related disease. Aging Dis 13:970-986.

Chiche A, Le Roux I, Joest M, Sakai H, Aguin SB, Cazin C, Salam R, Fiette L, Alegria O, Flamant P *et al.* (2017) Injury-induced senescence enables *in vivo* reprogramming in skeletal muscle cell. Stem Cell 20:407-414.

Cho YH, Ro EJ, Yoon JS, Mizutani T, Kang DW, Park JC, Kim T, Clevers H and Choi KY (2020) 5-FU promotes stemness of colorectal cancer via p53-mediated WNT/beta-catenin pathway activation. Nat Commun 11:5321.

Choi JY, Shin HJ and Bae HI (2018) miR-93-5p suppresses cellular senescence by directly targeting Bcl-w and p21. Biochem Biophys Res Commun 505:1134-1140.

Choi YS, Erlich TH, Franque M, Rachmin I, Flesher JL, Schiferle EB, Zhang Y, Pereira da Silva M, Jiang A, Dobry AS *et al.* (2022) Topical therapy for regression and melanoma prevention of congenital giant nevi. Cell 185:2071-2085.

Collado M and Serrano M (2010) Senescence in tumours: Evidence from mice and humans. Nat Rev Cancer 10:51-57.

Coppé JP, Kauser K, Campisi J and Beausejour CM (2006) Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem 281:29568-29574.

Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, Nelson PS, Desprez PY and Campisi J (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6:2853-2868.

Czarnecka-Herok J, Sliwinska MA, Herok M, Targonska A, Strzeszewska-Potyrala A, Bojko A, Wolny A, Mosieniak G and Sikora E (2022) Therapy-induced senescent/polyploid cancer cells undergo atypical divisions associated with altered expression of meiosis, spermatogenesis and emt genes. Int J Mol Sci 23:8288.

Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dollé ME *et al.* (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 31:722-733.

Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM *et al.* (2017) Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov 7:165-176.

Di Micco R, Krizhanovsky V, Baker D and Fagagna F (2021) Cellular senescence in ageing: From mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol 22:75-95.

Domen A, Deben C, De Pauw I, Hermans C, Lambrechts H, Verswyvel J, Siozopoulou V, Pauwels P, Demaria M, Wiel M *et al.* (2022) Prognostic implications of cellular senescence in resected non-small cell lung cancer. Transl Lung Cancer Res 11:1526-1539.

Dreyer G, Vandorpe T, Smeets A, Forceville K, Brouwers B, Neven P, Janssens H, Deraedt K, Moerman P, Van Calste B *et al.* (2013) Triple negative breast cancer: Clinical characteristics in the different histological subtypes. Breast 22:761-766.

Drullion C, Tregoat C, Lagarde V, Tan S, Gioia R, Priault M, Djavaheri-Mergny M, Brisson A, Auberger P, Mahon FX *et al.* (2012) Apoptosis and autophagy have opposite roles on imatinib-induced K562 leukemia cell senescence. Cell Death Dis 3:e373.

Ellison-Hughes GM (2020) First evidence that senolytics are effective at decreasing senescent cells in humans. EBioMedicine 56:102473.

Ewald JA, Desotelle JA, Church DR, Yang B, Huang W, Laurila TA and Jarrard DF (2013) Androgen deprivation induces senescence characteristics in prostate cancer cells *in vitro* and *in vivo*. Prostate 73:337-345.

Fan Y, Cheng J, Zeng H and Shao L (2020) Senescent cell depletion through targeting BCL-family proteins and mitochondria. Front Physiol 11:593630.

Febres-Aldana CA, Kuritzky N, Krishnamurthy K, Poppiti R and Howard L (2020) Evaluation of the expression of P16INK4A by immunohistochemistry in post-neoadjuvant chemotherapy hormone receptor negative breast cancer specimens. Breast Dis 39:51-59.

Garbers C, Kuck F, Aparicio-Siegmund S, Konzak K, Kessenbrock M, Sommerfeld A, Häussinger D, Lang PA, Brenner D, Mak TW *et al.* (2013) Cellular senescence or EGFR signaling induces Interleukin 6 (IL-6) receptor expression controlled by mammalian target of rapamycin (mTOR). Cell Cycle 12:3421-3432.

Gayle SS, Sahni JM, Webb BM, Weber-Bonk KL, Shively MS, Spina R, Bar EE, Summers MK and Keri RA (2019) Targeting BCL-xL improves the efficacy of bromodomain and extra-terminal protein inhibitors in triple-negative breast cancer by eliciting the death of senescent cells. J Biol Chem 294:875-886.

Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G *et al.* (2019) Cellular senescence: Defining a path forward. Cell 179:813-827.

Goulet CR, Champagne A, Bernard G, Vandal D, Chabaud S, Pouliot F and Bolduc S (2019) Cancer-associated fibroblasts induce epithelial-mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. BMC Cancer 19:137.

Grigorash BB, Essen D, Liang G, Grosse L, Emelyanov A, Kang Z, Korablev A, Kanzler B, Molina C, Lopez E *et al.* (2023) p16(High) senescence restricts cellular plasticity during somatic cell reprogramming. Nat Cell Biol 25:1265-1278.

Guerrero A, Herranz N, Sun B, Wagner V, Gallage S, Guiho R, Wolter K, Pombo J, Irvine E, Innes AJ *et al.* (2019) Cardiac glycosides are broad-spectrum senolytics. Nat Metab 11:1074-1088.

Guillon J, Petit C, Toutain B, Guette C, Lelièvre E and Coqueret O (2019) Chemotherapy-induced senescence, an adaptive mechanism driving resistance and tumor heterogeneity. Cell Cycle 18:2385-2397.

Hanahan D (2022) Hallmarks of cancer: New dimensions. Cancer Discov 12:31-46.

Hernandez-Segura A, Nehme J and Demaria M (2018) Hallmarks of cellular senescence. Trends Cell Biol 28:436-453.

Herranz N, Gallage S, Mellone M, Wuestefeld T, Klotz S, Hanley CJ, Raguz S, Acosta JC, Innes AJ, Banito A *et al.* (2015) mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol 17:1205-1217.

Higashiguchi M, Murakami H, Akita H, Kobayashi S, Takahama S, Iwagami Y, Yamada D, Tomimaru Y, Noda K, Gotoh K *et al.* (2023) The impact of cellular senescence and senescence-associated secretory phenotype in cancer-associated fibroblasts on the malignancy of pancreatic cancer. Oncol Rep 49:98.

Hinds P and Pietruska J (2017) Senescence and tumor suppression. F1000Res 6:2121.

Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S *et al.* (2015) Tumour exosome integrins determine organotropic metastasis. Nature 527:329-335.

Ikezawa K, Hikita H, Shigekawa M, Iwahashi K, Eguchi H, Sakamori R, Tatsumi T and Takehara T (2017) Increased Bcl-xL expression in pancreatic neoplasia promotes carcinogenesis by inhibiting senescence and apoptosis. Cell Mol Gastroenterol Hepatol 4:185-200.

Islam MT, Tuday E, Allen S, Kim J, Trott DW, Holland DW, Donato AJ and Lesniewski LA (2023) Senolytic drugs, dasatinib and quercetin, attenuate adipose tissue inflammation, and ameliorate metabolic function in old age. Aging Cell 22:e13767.

Jenkins EO, Deal EM, Anders CK, Prat M, Perou CM, Carey LA and Muss HB (2014) Age-specific changes in intrinsic breast cancer subtypes: A focus on older women. Oncologist 19:1076-1083.

Jin MZ and Jin W (2020) The updated landscape of tumor microenvironment and drug repurposing. Signal Transduct Target Ther 5:166.

Jung SH, Hwang HJ, Kang D, Park HA, Lee HC, Jeong D, Lee K, Park HJ, Ko YG and Lee JS (2019) mTOR kinase leads to PTEN-loss-induced cellular senescence by phosphorylating p53. Oncogene 38:1639-1650.

Kale J, Osterlund EJ and Andrews DW (2018) BCL-2 family proteins: Changing partners in the dance towards death. Cell Death Differ 25:65-80.

Kalluri R and Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119:1420-1428.

Kaloni D, Diepstraten ST, StrasserA and Kelly GL (2023) BCL-2 protein family: Attractive targets for cancer therapy. Apoptosis 28:20-38.

Khosla S, Farr JN, Tchkonia T and Kirkland JL (2020) The role of cellular senescence in ageing and endocrine disease. Nat Rev Endocrinol 16:263-275.

Kohli J, Ge C, Fitsiou E, Doepner M, Brandenburg SM, Faller WJ, Ridky TW and Demaria M (2022) Targeting anti-apoptotic pathways eliminates senescent melanocytes and leads to nevi regression. Nat Commun 13:7923.

Korkaya H, Liu S and Wicha MS (2011) Regulation of cancer stem cells by cytokine networks: Attacking cancer's inflammatory roots. Clin Cancer Res 17:6125-6129.

Krtolica A, Parrinello S, Lockett S, Desprez PY and Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging. Proc Natl Acad Sci U S A 98:12072-12077.

Kuilman T, Michaloglou C, Mooi WJ and Peeper DS (2010) The essence of senescence. Genes Dev 24:2463-2479.

Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, Curran SC, Davalos AR, Wilson-Edell KA, Liu S *et al.* (2015) MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat Cell Biol 17:1049-1061.

Lawrenson K, Grun B, Benjamin E, Jacobs IJ, Dafou D and Gayther SA (2010) Senescent fibroblasts promote neoplastic transformation of partially transformed ovarian epithelial cells in a three-dimensional model of early stage ovarian cancer. Neoplasia 12:317-325.

Lee YH, Kang BS and Bae YS (2014) Premature senescence in human breast cancer and colon cancer cells by tamoxifen-mediated reactive oxygen species generation. Life Sci 97:116-122.

Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, Shin Teoh T, Prata L, Cottle BJ, Clark JE, Punjabi PP, Awad W, Torella D *et al.* (2019) Aged-senescent cells contribute to impaired heart regeneration. Aging Cell 18:e12931.

Li F and Xue X (2023) Identification and characterization of an ageing-associated 13-lncrna signature that predicts prognosis and immunotherapy in hepatocellular carcinoma. J Oncol 2023:4615297.

Liu D and Hornsby PJ (2007) Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. Cancer Res 67:3117-3126.

Lodi M, Scheer L, Reix N , Heitz D, Carin AJ, Thiébaut N, Neuberger K, Tomasetto C and Mathelin C (2017) Breast cancer in elderly women and altered clinico-pathological characteristics: A systematic review. Breast Cancer Res Treat 166:657-668.

Lourenco AR, Ban Y, Crowley MJ, Lee SB, Ramchandani D, Du W, Elemento O, George JT, Jolly MK, Levine H *et al.* (2020) Differential contributions of Pre- and Post-EMT tumor cells in breast cancer metastasis. Cancer Res 80:163-169.

Martinez LA, Yang J, Vazquez ES, Rodriguez-Vargas Mdel C, Olive M, Hsieh JT, Logothetis CJ and Navone NM (2002) p21 modulates threshold of apoptosis induced by DNA-damage and growth factor withdrawal in prostate cancer cells. Carcinogenesis 23:1289-1296.

Matsuda S, Revandkar A, Dubash TD, Ravi A , Wittner BS, Lin M, Morris R, Burr R, Guo H, Seeger K *et al.* (2023) TGF-beta in the microenvironment induces a physiologically occurring immune-suppressive senescent state. Cell Rep 42:112129.

Meeker AK, Hicks JL, Gabrielson E, Strauss WM, De Marzo AM and Argani P (2004) Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. Am J Pathol 164:925-935.

Milanovic M, Fan DNY, Belenki D, Dabritz JHM, Zhao Z, Yu Y, Dorr JR, Dimitrova L, Lenze D, Monteiro Barbosa IA *et al.* (2018) Senescence-associated reprogramming promotes cancer stemness. Nature 553:96-100.

Mittal V (2018) Epithelial mesenchymal transition in tumor metastasis. Annu Rev Pathol 13:395-412.

Mosteiro L, Pantoja C, Alcazar N, Marion RM, Chondronasiou D, Rovira M, Fernandez-Marcos PJ, Munoz-Martin M, Blanco-Aparicio C, Pastor J *et al.* (2016) Tissue damage and senescence provide critical signals for cellular reprogramming *in vivo*. Science 354:aaf4445.

Mosteiro L, Pantoja C, Martino A and Serrano M (2018) Senescence promotes in vivo reprogramming through p16(INK)(4a) and IL-6. Aging Cell 17:e12711.

Muñoz-Espín D, Cañamero M, Maraver A, Gómez-López G, Contreras J, Murillo-Cuesta S, Rodríguez-Baeza A, Varela-Nieto I, Ruberte J, Collado M *et al.* (2013) Programmed cell senescence during mammalian embryonic development. Cell 155:1104-1118.

Novais EJ, Tran VA, Johnston SN, Darris KR, Roupas AJ, Sessions GA, Shapiro IM, Diekman BO and Risbud MV (2021) Long-term treatment with senolytic drugs Dasatinib and Quercetin ameliorates age-dependent intervertebral disc degeneration in mice. Nat Commun 12:5213.

Ohnishi K, Semi K, Yamamoto T, Shimizu M, Tanaka A, Mitsunaga K, Okita K, Osafune K, Arioka Y, Maeda T *et al.* (2014) Premature termination of reprogramming *in vivo* leads to cancer development through altered epigenetic regulation. Cell 156:663-677.

Ohuchida K, Mizumoto K, Murakami M, Qian LW, Sato N, Nagai E, Matsumoto K, Nakamura T and Tanaka M (2004) Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. Cancer Res 64:3215-3222.

Orr FW and Wang HH (2001) Tumor cell interactions with the microvasculature: A rate-limiting step in metastasis. Surg Oncol Clin N Am 10:357-381.

Ortiz-Montero P, Londono-Vallejo A and Vernot JP (2017) Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line. Cell Commun Signal 15:17.

Pardella E, Pranzini E, Nesi I, Parri M, Spatafora P, Torre E, Muccilli A, Castiglione F, Fambrini M, Sorbi F *et al.* (2022) Therapy-induced stromal senescence promoting aggressiveness of prostate and ovarian cancer. Cells 11:4026.

Parrinello S, Coppe JP, Krtolica A and Campisi J (2005) Stromal-epithelial interactions in aging and cancer: Senescent fibroblasts alter epithelial cell differentiation. J Cell Sci 118:485-496.

Peurala E, Koivunen P, Haapasaari KM, Bloigu R and Jukkola-Vuorinen A (2013) The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer. Breast Cancer Res 15:R5.

Rayess H, Wang MB and Srivatsan ES (2012) Cellular senescence and tumor suppressor gene p16. Int J Cancer 130:1715-1725.

Ritschka B, Storer M, Mas F, Heinzmann A, Ortells MC, Morton JP, Sansom OJ, Zender L and Keyes WM (2017) The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. Genes Dev 31:172-183.

Rodier F, Coppé JP, Patil CK, Hoeijmakers MA, Muñoz DP, Raza SR, Freund A, Campeau E, Davalos AR and Campisi J (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 11:973-979.

Romagosa C, Simonetti S, Lopez-Vicente L, Mazo A, Lleonart ME, Castellvi J and Ramon y Cajal S (2011) p16(Ink4a)

overexpression in cancer: A tumor suppressor gene associated with senescence and high-grade tumors. Oncogene 30:2087-2097.

Romaniello D, Gelfo V, Pagano F, Ferlizza E, Sgarzi M, Mazzeschi M, Morselli A, Miano C, D'Uva G and Lauriola M (2022) Senescence-associated reprogramming induced by interleukin-1 impairs response to EGFR neutralization. Cell Mol Biol Lett 27:20.

Rufini A, Tucci P, Celardo I and Melino G (2013) Senescence and aging: The critical roles of p53. Oncogene 32:5129-143.

Ruhland MK, Coussens LM and Stewart SA (2016) Senescence and cancer: An evolving inflammatory paradox. Biochim Biophys Acta 1865:14-22.

Ruscetti M, Leibold J, Bott MJ, Fennell J, Kulick A, Salgado NR, Chen CC, Ho YJ, Sanchez-Rivera FJ, Feucht J *et al.* (2018) NK cell-mediated cytotoxicity contributes to tumor control by a cytostatic drug combination. Science 362:1416-1422.

Ruscetti M, Morris JP, Mezzadra R, Russell J, Leibold J, Romesser PB, Simon J, Kulick A, Ho IJ, Fennell M *et al.* (2020) Senescence-induced vascular remodeling creates therapeutic vulnerabilities in pancreas cancer. Cell 181:424-441.

Saleh T, Alhesa A, Al-Balas M, Abuelaish O, Mansour A, Awad H, El-Sadoni M, Carpenter VJ and Azab B (2021) Expression of therapy-induced senescence markers in breast cancer samples upon incomplete response to neoadjuvant chemotherapy. Biosci Rep 41:BSR20210079.

Schick U, Kyula J, Barker H, Patel R, Zaidi S, Gregory C, Hafsi H, Roulstone V, Deutsch E, McLaughlin M *et al.* (2015) Trametinib radiosensitises RAS- and BRAF-mutated melanoma by perturbing cell cycle and inducing senescence. Radiother Oncol 117:364-375.

Schonberg MA, Marcantonio ER, Li D, Silliman RA, Ngo L and McCarthy EP (2010) Breast cancer among the oldest old: Tumor characteristics, treatment choices, and survival. J Clin Oncol 28:2038-2045.

Selt F, Sigaud R, Valinciute G, Sievers P, Zaman J, Alcon C, Schmid S, Peterziel H, Tsai JW, Guiho R *et al.* (2023) BH3 mimetics targeting BCL-XL impact the senescent compartment of pilocytic astrocytoma. Neuro Oncol 25:735-747.

Sharma S, Yao HP, Zhou YQ, Zhou J, Zhang R and Wang MH (2014) Prevention of BMS-777607-induced polyploidy/senescence by mTOR inhibitor AZD8055 sensitizes breast cancer cells to cytotoxic chemotherapeutics. Mol Oncol 8:469-482.

Shay JW and Wright WE (2000) Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol 1:72-76.

Sieben CJ, Sturmlechner I, Sluis B and van Deursen JM (2018) Two-Step senescence-focused cancer therapies. Trends Cell Biol 28:723-737.

Strieter RM, Burdick MD, Mestas J, Gomperts B, Keane MP and Belperio JA (2006) Cancer CXC chemokine networks and tumour angiogenesis. Eur J Cancer 42:768-778.

Syed BM, Green AR, Nolan CC, Morgan DA, Ellis IO and Cheung KL (2014) Biological characteristics and clinical outcome of triple negative primary breast cancer in older women – comparison with their younger counterparts. PLoS One 9:e100573.

Takahashi K and Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.

Takeuchi S, Takahashi A, Motoi N, Yoshimoto S, Tajima T, Yamakoshi K, Hirao A, Yanagi S, Fukami K, Ishikawa Y *et al.* (2010) Intrinsic cooperation between p16INK4a and p21Waf1/Cip1 in the onset of cellular senescence and tumor suppression in vivo. Cancer Res 70:9381-9390.

Tan L, Xiaohalati X, Liu F, Liu J, Fu H, Zhang Y, Gao J, Tao K, Wang G, Wang L *et al.* (2023) Identification of aging and young subtypes for predicting colorectal cancer prognosis and immunotherapy responses. Int J Mol Sci 24:1516.

Tato-Costa J, Casimiro S, Pacheco T, Pires R, Fernandes A, Alho I, Pereira P, Costa P, Castelo RB, Ferreira J *et al.* (2016) Therapy-induced cellular senescence induces epithelial-to-mesenchymal transition and increases invasiveness in rectal cancer. Clin Colorectal Cancer 15:170-178.

Terzian T, Torchia EC, Dai D, Robinson SE, Murao K, Stiegmann RA, Gonzalez V, Boyle GM, Powell MB, Pollock PM *et al.* (2010) p53 prevents progression of nevi to melanoma predominantly through cell cycle regulation. Pigment Cell Melanoma Res 23:781-794.

Thomas NE, Busam KJ, From L, Kricker A, Armstrong BK, Anton-Culver H, Gruber SB, Gallagher RP, Zanetti R, Rosso S *et al.* (2013) Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. J Clin Oncol 31:4252-4259.

Tkach M and Thery C (2016) Communication by extracellular vesicles: Where we are and where we need to go. Cell 164:1226-1232.

Vargas J, Feltes BC, Poloni J, Lenz G and Bonatto D (2012) Senescence: An endogenous anticancer mechanism. Front Biosci 17:2616-2643.

Viganò L, Locatelli A, Ulisse A, Galbardi B, Dugo M, Tosi D, Tacchetti C, Daniele T, Győrffy B, Sica L *et al.* (2022) Modulation of the estrogen/erbb2 receptors cross-talk by CDK4/6 inhibition triggers sustained senescence in estrogen receptor- and ErbB2-positive breast cancer. Clin Cancer Res 28:2167-2179.

Wallis R, Mizen H and Bishop CL (2020) The bright and dark side of extracellular vesicles in the senescence-associated secretory phenotype. Mech Ageing Dev 189:111263.

Wang L, Lankhorst L and Bernards R (2022) Exploiting senescence for the treatment of cancer. Nat Rev Cancer 22:340-355.

Wang MX, Ren JT, Tang LY and Ren ZF (2018) Molecular features in young vs elderly breast cancer patients and the impacts on survival disparities by age at diagnosis. Cancer Med 7:3269-3277.

Werner LR, Huang S, Francis DM, Armstrong EA, Ma F, Li C, Iyer G, Canon J and Harari PM (2015) Small molecule inhibition of mdm2-p53 interaction augments radiation response in human tumors. Mol Cancer Ther 14:1994-2003.

White-Gilbertson S and Voelkel-Johnson C (2020) Giants and monsters: Unexpected characters in the story of cancer recurrence. Adv Cancer Res 148:201-232.

Wu G, Li X, Zhan Y, Fan X, Xu L, Chen T and Wang X (2022) BID- and BAX-mediated mitochondrial pathway dominates A-1331852-induced apoptosis in senescent A549 cells. Biochem Biophys Res Commun 627:160-167.

Xiong S, Feng Y and Cheng L (2019) Cellular reprogramming as a therapeutic target in cancer. Trends Cell Biol 29:623-634.

Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, Inman CL, Ogrodnik MB, Hachfeld CM, Fraser DG *et al.* (2018) Senolytics improve physical function and increase lifespan in old age. Nat Med 24:1246-1256.

Xu X, Lu Z, Qiang W, Vidimar V, Kong B, Kim JJ and Wei JJ (2014) Inactivation of AKT induces cellular senescence in uterine leiomyoma. Endocrinology 155:1510-1519.

Yang G, Rosen DG, Zhang Z, Bast RS, Mills GB, Colacino JA, Mercado-Uribe I and Liu J (2006) The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. Proc Natl Acad Sci U S A 103:16472-16477.

Yang L, Fang J and Chen J (2017) Tumor cell senescence response produces aggressive variants. Cell Death Discov 3:17049.

Yang L, Shi P, Zhao G, Xu J, Peng H, Zhang J, Zhang G, Wang X, Dong Z, Chen F *et al.* (2020) Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther 5:8.

Yang Y, Chen X, Sun J, Chen S, Yang Y, Ma Q and Yang J (2021) Cell aging related genes can be used to characterize clinical prognoses and further stratify diffuse gliomas. Sci Rep 11:19493.

Yasuda T, Koiwa M, Yonemura A, Miyake K, Kariya R, Kubota S, Yokomizo-Nakano T, Yasuda-Yoshihara N, Uchihara T, Itoyama R *et al.* (2021) Inflammation-driven senescence-associated secretory phenotype in cancer-associated fibroblasts enhances peritoneal dissemination. Cell Rep 34:108779.

Ye M, Huang X, Wu Q and Liu F (2023) Senescent stromal cells in the tumor microenvironment: Victims or accomplices? Cancers (Basel) 15:1927.

Yokoi A, Yoshioka Y, Yamamoto Y, Ishikawa M, Ikeda SI, Kato T, Kiyono T, Takeshita F, Kajiyama H, Kikkawa F *et al.* (2017) Malignant extracellular vesicles carrying MMP1 mRNA facilitate peritoneal dissemination in ovarian cancer. Nat Commun 8:14470.

Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, Vadai E, Dassa L, Shahar E, Condiotti R *et al.* (2016) Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat Commun 7:11190.

Yosef R, Pilpel N, Papismadov N, Gal H, Ovadya Y, Vadai E, Miller S, Porat Z, Ben-Dor C and Krizhanovsky V (2017) p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. EMBO J 36:2280-2295.

Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, Ling YY, Melos KI, Pirtskhalava T, Inman CL *et al.* (2018) Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine 36:18-28.

Yousefzadeh MJ, Flores RR, Zhu Y, Schmiechen ZC, Brooks RW, Trussoni CE, Cui Y, Angelini L, Lee KA, McGowan SJ *et al.* (2021) An aged immune system drives senescence and ageing of solid organs. Nature 594:100-105.

Yun J, Hansen S, Morris O, Madden DT, Libeu CP, Kumar AJ, Wehrfritz C, Nile AH, Zhang Y, Zhou L *et al.* (2023) Senescent cells perturb intestinal stem cell differentiation through Ptk7 induced noncanonical Wnt and YAP signaling. Nat Commun 14:156.

Zhang G, Dong K, Liu J and Zhou W (2022) Prognosis and tumor immune microenvironment of patients with gastric cancer by a novel senescence-related signature. Medicine (Baltimore) 101:e30927.

Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ and Robbins PD (2023) Targeting cellular senescence with senotherapeutics: Senolytics and senomorphics. FEBS J 290:1362-1383.

Zhang S, Mercado-Uribe I, Xing Z, Sun B, Kuang J and Liu J (2014) Generation of cancer stem-like cells through the formation of polyploid giant cancer cells. Oncogene 33:116-128.

Zhang S, Zheng Y, Li X, Zhang S, Hu H and Kuang W (2023) Cellular senescence-related gene signature as a valuable predictor of prognosis in hepatocellular carcinoma. Aging (Albany NY) 15:3064-3093.

Zhang XP, Liu F and Wang W (2010) Coordination between cell cycle progression and cell fate decision by the p53 and E2F1 pathways in response to DNA damage. J Biol Chem 285:31571-31580.

Zhou L, Niu Z, Wang Y, Zheng Y, Zhu Y, Wang C, Gao X, Gao L, Zhang W, Zhang K *et al.* (2022) Senescence as a dictator of patient outcomes and therapeutic efficacies in human gastric cancer. Cell Death Discov 8:13.

Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M *et al.* (2015) The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. Aging Cell 14:644-658.

Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai M, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB *et al.* (2016) Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell 15:428-435.

Associate Editor: Alberto R. Kornblihtt