

Review

Senescence and SASP in the Stromal Microenvironment of Breast Cancer: Dual Role in Progression and Therapeutic Options

Maria Bogachek¹, David Sergeevichev^{1,3}, Anastasia Leonteva^{1,2}, Alevtina Pinova¹, Vladimir Richter², Anna Nushtaeva^{1,2*}

¹Scientific Center of Genetics and Life Sciences, Sirius University of Science and Technology, Krasnodar 354340, Russia. ²Laboratory of Biotechnology, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk 630090, Russia. ³Meshalkin National Medical Research Center, Novosibirsk, 630090, Russia

[Received July 7, 2026; Revised September 10, 2026; Accepted September 12, 2026]

ABSTRACT: Cellular senescence, a process characterized by the typically persistent cell-cycle arrest that occurs in response to DNA damage, plays a dual role in carcinogenesis. This complexity is predominantly influenced by the presence of senescent cancer-associated fibroblasts within the tumor microenvironment. While the induction of cellular senescence in early-stage tumor cells has been shown to inhibit proliferation, the action of senCAFs has been demonstrated to result in adverse consequences. It has been demonstrated that senescent cells are significant producers of the senescence-associated secretory phenotype. The SASP creates an immunosuppressive, therapy-resistant tumor microenvironment (TME) that promotes cancer progression. The current review is dedicated to the analysis of the contradictory role of senescence with focus on senCAFs in breast cancer progression. The review is devoted to the possible mechanisms of senescence induction in CAFs, their functional heterogeneity, and the key effector molecules of SASP (TGF- β , IL-6, MMPs, CXCL12, OPN) that trigger epithelial-mesenchymal transition and suppress antitumor immunity, including NK cell functionality. A particular focus has been placed on paracrine and autocrine regulatory loops, such as the OPN and CXCL12 axis, which have been shown to enhance the aggressive phenotype of both tumor cells and cancer-associated fibroblasts. The ensuing discourse will delve into the innovative strategies of the "one-two punch approach," which are designed to target senCAFs with the objective of counteracting BC progression and enhancing the prognosis for breast cancer patients

Keywords: senescence, cancer-associated fibroblasts, SASP, stromal tumor microenvironment, breast cancer

Introduction

Despite the advancements in therapeutic interventions and early detection strategies, breast cancer (BC) persists as an emerging global health concern, representing the most prevalent form of cancer among the female population. In 2022, 2.3 million new cases of BC were documented, resulting in 670,000 mortalities due to the disease. A thorough analysis of epidemiological big data on mortality and incidence suggests a 38% increase in

incidence and a 68% increase in mortality by the year 2050 [1].

The development of new therapeutic approaches requires comprehensive understanding of the heterogeneity and intricate interactions of cells within the tumor microenvironment (TME). These cells play a pivotal role in tumor progression and the development of resistance to therapy. The present review focuses on a critical component of the TME, namely, cancer-associated fibroblasts (CAFs). These cells strongly

***Correspondence should be addressed to:** Dr. Anna Nushtaeva, Scientific Center of Genetics and Life Sciences, Sirius University of Science and Technology, Sirius, Krasnodar Region, 354340, Russia. Email: nushtaeva.aa@talantiuspeh.ru or nushtaeva.anna@gmail.com

Copyright: © 2026 Bogachek M. et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

influence tumor progression by expressing extracellular matrix (ECM) proteins that create a rigid stromal barrier around tumor cells [2].

In the context of BC, a taxonomic classification system has been developed to categorize CAFs within the TME. This system comprises four distinct subsets, designated as CAF-S1 through CAF-S4. Subtypes CAF-S1 and CAF-S4 have been identified as highly active myofibroblasts that promote tumor progression, while CAF-S2 and CAF-S3 have been shown to resemble normal, quiescent fibroblasts [3,4]. CAFs comprise a heterogeneous population that includes senescent CAFs (senCAF), a subpopulation of significant importance for the progression of cancer. The induction of senescence in these cells can be induced by both oncogenic stress and anticancer therapy [5]. The hypothesis posits that this fibroblast may be considered one of the subtypes in future classifications as senCAF-SX (Fig. 1). SenCAF-SX is primarily conceptualized as a proposed senescent state or phenotype superimposed on existing CAF populations.

Initially, senescence was regarded as an irreversible cell cycle arrest that functioned to suppress tumors. However, recent studies have demonstrated the duality of its impact on tumor development [6]. It has been established that senCAF exacerbate fibrosis and inflammation in the TME by secreting large amounts of extracellular matrix components and pro-inflammatory factors. This process, therefore, promotes malignant tumor transformation [2]. Furthermore, studies have demonstrated that senCAF have the capacity to induce tumor cell senescence through paracrine signaling, thereby establishing a positive feedback loop that "promotes cancer senescence." For instance, the role of senescent stroma in maintaining immunosuppression in the TME is widely recognized [7]. A critical factor in their pro-oncogenic effect is the ability of senCAF to secrete pro-inflammatory factors that form the senescence-associated secretory phenotype (SASP). SASP proteins include interleukins and cytokines, chemokines, growth factors, and extracellular matrix proteases [8, 9]. Examples of key SASP factors that maintain aggressive tumor characteristics and suppress the effector functions of immune cells are transforming growth factor beta (TGF- β), interleukin-6 (IL-6), matrix metalloproteinases (MMPs), and osteopontin (OPN) [10, 11]. A critical component of paracrine and autocrine regulation is the Osteopontin/Twist-related protein 1 (OPN/Twist1) and C-X-C motif chemokine ligand 12/C-X-C motif chemokine receptor 4 (CXCL12/CXCR4) axes. These axes promote the transition of CAFs to an aggressive phenotype and the activation of EMT. This process results in the enrichment of the tumor with an aggressive pool of cancer stem cells (CSCs) [12-14].

SASP factors may serve as markers of aging-related activity and as therapeutic targets. The employment of senescence inhibitors, encompassing senolytics that selectively clear senescent cells and senomorphics, which impede the functional activities of SASP components, including those expressed by senCAF, emerges as a potentially efficacious approach for combination therapy. This approach is poised to enhance the efficacy of treatment regimens and ameliorate the prognoses of cancer patients [14].

The etiology and biological consequences of senescence vary substantially by context. In the field of oncology, it is imperative to differentiate between two forms of senescence: age-associated (chronological) senescence and therapy-induced senescence (TIS). The process of age-associated senescence can be defined as the progressive accumulation of senescent cells in tissues during the natural aging of the body [5]. The primary catalysts in this instance are telomere shortening, the accumulation of DNA damage and chronic oxidative stress [15]. These cells secrete pro-inflammatory factors, forming a SASP phenotype that contributes to the development of age-dependent pathologies, chronic inflammation and fibrosis. In the context of oncology, the age-related accumulation of senescent cells in the stromal and immune compartments engenders a favourable environment for tumor progression by modulating the immune response and remodelling the extracellular matrix [16].

In contrast, TIS is characterised by premature cellular senescence induced by medical interventions, predominantly chemo- and radiotherapy [17]. The fundamental distinction between TIS and other forms of injury is the former's capacity to develop rapidly in response to acute, high-intensity damage, as opposed to the more gradual and chronological progression associated with aging. TIS can be induced in both malignant and normal cells, resulting in a dual clinical effect: on the one hand, it is the desired antitumor mechanism that halts the growth of cancer cells. The induction of cellular senescence in precancerous epithelial cells constitutes an established tumor-suppressive barrier. This barrier operates through the p53/p21 and p16/Rb pathways to enforce stable proliferative arrest. In addition, it has been demonstrated that the secretion of chemoattractants by the aforementioned cells facilitates immune surveillance, thereby recruiting macrophages and NK cells for the purpose of clearing senescent cells [16]. An illustration of the antitumor function of SASP is provided by the chemokine CXCL14. This is a significant mediator of immune surveillance, which, through the recruitment of cytotoxic T lymphocytes and the restriction of immunosuppressive populations, has been shown to impede the progression of triple-negative BC in vivo [18].

Conversely, this may result in adverse effects, including systemic accelerated aging of the body and damage to healthy tissues [19].

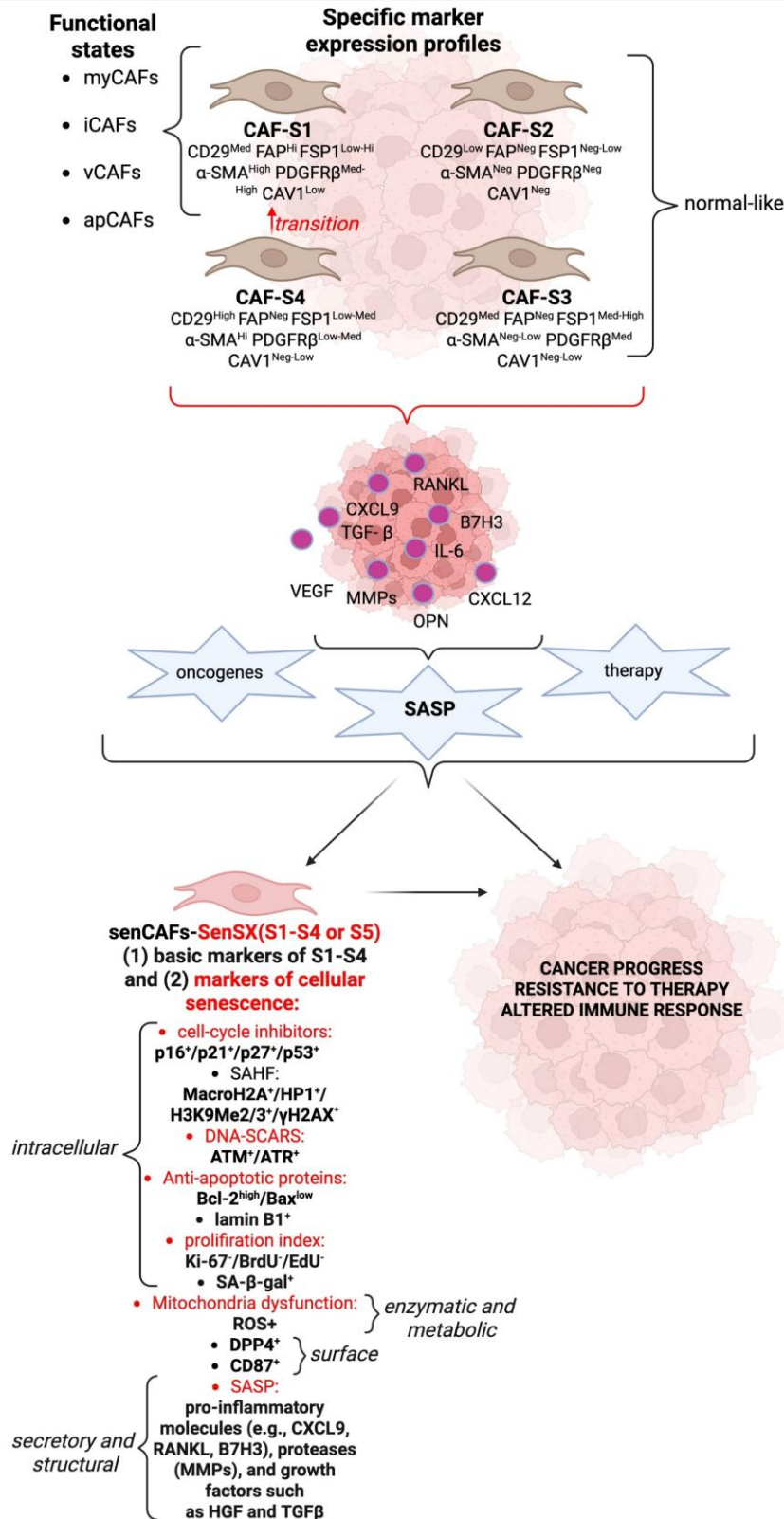


Figure 1. Heterogeneity of CAFs subpopulations including S1-S4 types. Identified by a panel of senescence markers, SenCAFs-SX subpopulation has been demonstrated to be induced by oncogenes, therapeutic interventions, and the SASP derived from tumor cells, thereby exerting a modulatory effect on cancer progression. The illustration presented herein delineates the theoretical plasticity of CAF subtypes: Furthermore, S1-S4 have been observed to transform into S5 (senCAF-SX) during treatment. The question of whether S5 is a distinct, stable subtype or a transient state remains unresolved and requires further investigation. SenCAF-SX is primarily defined as a senescent state or phenotype superimposed on existing CAF populations.

Therefore, the fundamental differences between these two phenomena are manifest on several levels. Firstly, the heterogeneity of senescent cells in TIS is significantly higher because they arise in genetically unstable tumor cells with multiple mutations already overcoming classical senescence barriers on the way to immortalization. Secondly, the clinical consequences of TIS are more controversial. Despite the initial antitumor effect, persistent senescent cancer cells can form a reservoir for disease recurrence and acquire aggressive properties after escaping from the senescent state. Conversely, the process of age-associated senescence is predominantly associated with the chronic pro-carcinogenic effect of SASP on the microenvironment.

The objective of this review is to underscore the intricate and contentious function of SASP and senescence within the tumor microenvironment. The review will emphasize the role of senescent stromal cells and the potential for novel therapeutic approaches to counteract the deleterious effects of senescent cells in BC development.

1. Role of senescence in the stromal microenvironment of breast cancer

1.1. Senescence markers

Cellular senescence is defined as a state of stable terminal proliferation arrest [20]. Cells undergo cellular senescence in response to various DNA-damaging stress factors. As is well-documented in the relevant literature, the process of aging has undergone various forms throughout history, each distinguished by its specific inducer [8]. Consequently, it is feasible to differentiate between multiple terms.

The term "replicative aging" refers to the characteristics of cells that are cultured until the irreversible cessation of proliferation. The initial observations of cellular senescence were made *in vitro* on primary cells. In this setting, the depletion of proliferative potential led to irreversible senescence, which was associated with telomere shortening [21]. Shortened telomeres are recognized as DNA double-strand breaks, which in turn trigger the DNA damage response (DDR).

The term "oncogene-induced aging" (also known as "oncogene-induced senescence," OIS) is described in the context of cells that overexpress an oncogene. The process of oncogenic signaling pathway activation has been demonstrated to induce cellular senescence, which functions as an innate immune defense system that prevents malignant transformation in the early stages of oncogenesis [22].

The subsequent term was "cell aging under the influence of therapy such as chemotherapy and radiation therapy". In such cases, tumor cells may undergo apoptosis or enter a state of TIS. Such cells are often eliminated by the immune system; however, some can persist in a dormant state, which can subsequently contribute to the development of resistance [23]. In contrast to replicative and oncogene-induced senescence, TIS occurs directly as a result of therapeutic intervention and can be considered a desirable target of therapy, allowing for sustained suppression of tumor growth.

The other terms were "aging induced by mitochondrial dysfunction in cells" [24] and "immunologically induced aging for cells exposed to excess amounts of pro-inflammatory factors" [25]. A close examination of these conditions reveals shared characteristics, yet each exhibits distinctive features, underscoring the substantial heterogeneity of aging phenotypes within living organisms. The underlying mechanisms of cellular senescence have been extensively researched in the scientific community and have been thoroughly documented in numerous publications, including the seminal works of [26-28].

The process of senescence is marked by the decline of cell proliferation capacity and the subsequent elimination of senescent cells, a process that involves the participation of various immune cells, including NK cells, macrophages, and neutrophils. In instances where the process of senescent cell clearance is compromised, the persistence of these cells accompanied by SASP secretion becomes a possibility [29].

In light of the established evidence for the reversibility of cellular senescence, this process is now comprehended not merely as a permanent cessation of cell division [30]. Instead, it is regarded as an intricate network of alterations that give rise to a multifaceted, complex phenotype. The conventional approach to the identification of senescent cells in tissues and cell cultures is based on a combination of morphological, biochemical and molecular criteria [31]. The identification of senescent cells within a tumor and the TME involves the evaluation of distinct morphological alterations that are characteristic of senescence, including an increase in cell size, a flattened shape, the presence of multiple or enlarged nuclei, a prominent Golgi apparatus, and vacuolization of the cytoplasm. Furthermore, it is imperative to consider the modulation of signalling pathways that inhibit the transcription of genes responsible for proliferation. In addition, the expression of anti-apoptotic proteins that support the persistence of senescent cells and prevent apoptosis must be taken into account [32]. The morphological and molecular characteristics of senescence are presented in Figure 2.

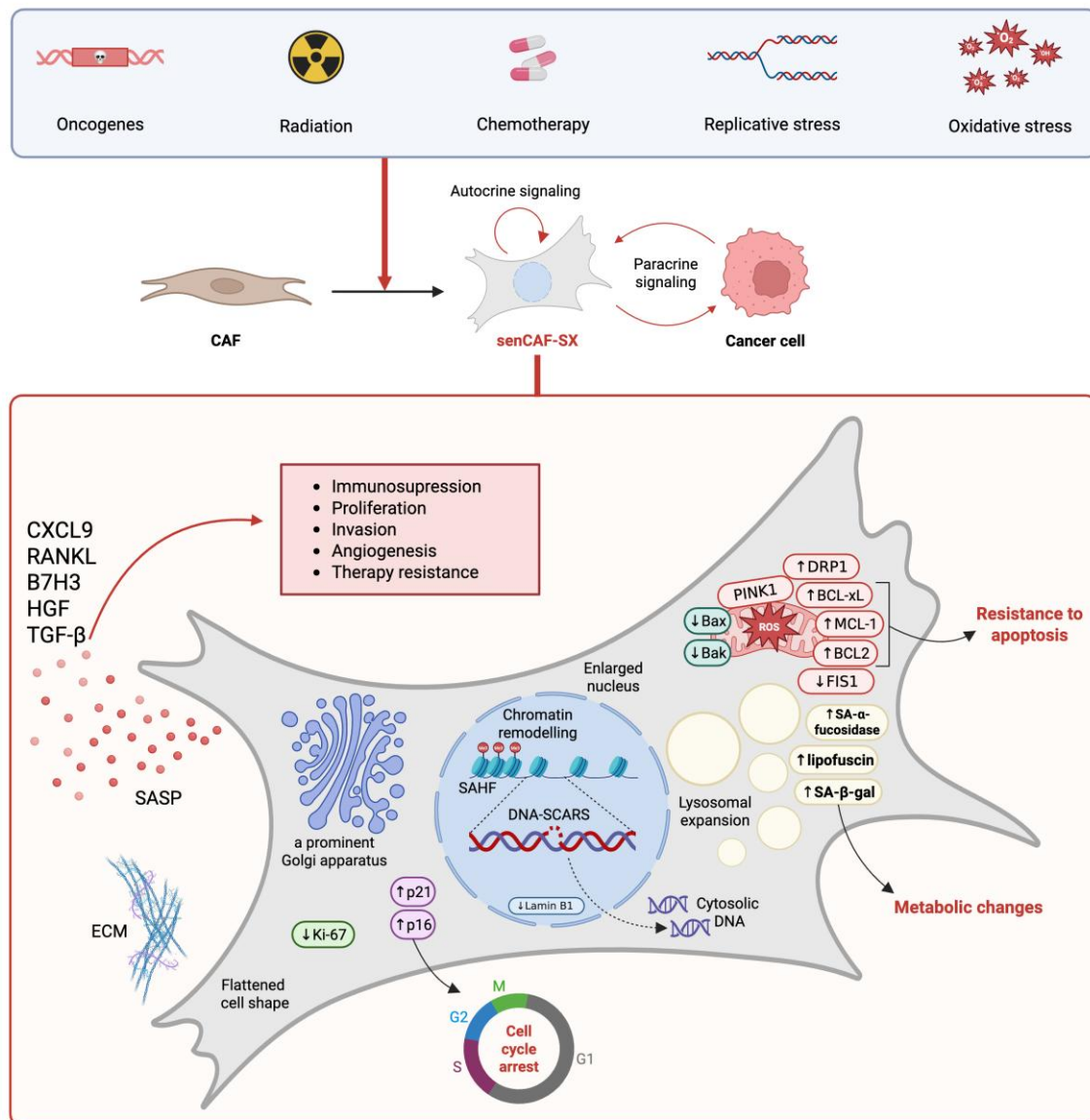


Figure 2. The morphological and molecular characteristics of senescence. Senescent CAFs result from the action of various inducers. SenCAFs-SX are significant producers of the SASP maintaining an immunosuppressive TME, resistance to therapy and progression of cancer. Senescence markers are presented including SA- β -Gal, CDK inhibitor proteins p16INK4a and p21WAF/Cip1, chromatin remodeling markers, expression of anti-apoptotic and pro-apoptotic proteins, metabolic markers along with morphologic changes.

SA- β -Gal. The enzyme known as senescence-associated β -galactosidase (SA- β -Gal) has been identified as a key player in the process of cellular aging and deterioration. SA- β -Gal has been the most widely used histo- and cytochemical marker of senescence for two decades. However, it is imperative to acknowledge that SA- β -Gal activity should be regarded exclusively as a surrogate marker of heightened lysosomal content and autolysosomal activity. It should not be interpreted as a direct indication of a senescence-specific enzymatic function. Its increased activity at pH 6.0 reflects the

increased number of lysosomes and elevated lysosomal activity characteristic of senescent cells. The elevated expression of the lysosomal enzyme galactosidase beta 1 has been identified as the causative agent of SA- β -Gal activity. This augmented expression can be attributed to an effort to counteract the buildup of damaged, non-degradable macromolecules and organelles within secondary lysosomes [33, 34]. The detection of SA- β -Gal allows for the visualization of senescence foci in tumor tissue in situ.

Notwithstanding its operational utility, SA- β -Gal is not exclusively specific for senescence. Indeed, it can be detected in confluent cell monolayers, melanocytes, cell cultures under serum withdrawal conditions, or in the presence of certain compounds (TGF- β , heparin, TPA) [33, 35]. The activity of SA- β -galactosidase was studied in a series of breast tumors including fibroadenoma (a non-invasive, benign mesenchymal tumor), proliferative fibrocystic mastopathy (a pre-cancerous condition) and infiltrating breast carcinoma. The study revealed normal levels of SA- β -galactosidase activity in fibroadenoma cells, elevated levels in proliferating ductal cells in fibrocystic mastopathy, and the highest levels in invasive breast carcinoma cells [36]. Wang et al. (2024) established the senescent fibroblast model by continuously passaging the BJ fibroblasts until they reached senescence [37]. In comparison to young cells, fibroblast BJ cells exhibited an enlarged, flattened morphology and were positive for SA- β -gal marker.

In addition to SA- β -Gal, other lysosomal markers include lipofuscin and SA- α -fucosidase. Lipofuscin, a pigment accumulated by both senescent epidermal cells and dermal fibroblasts, is an aggregate of oxidized proteins and lipids, and it is detected by Sudan black B staining. SA- α -fucosidase, a lysosomal marker, exhibits augmented activity during the process of senescence.

Cell proliferation. A salient feature of senescent cells is their incapacity to proliferate in conditions that would typically stimulate cell division [31, 38]. In contrast to quiescent (G0) cells, which possess the capacity to re-enter the cell cycle in response to mitogenic signals, senescent cells have lost this ability. Ki-67, BrdU (5-Bromo-2'-deoxyuridine) and EdU (5-Ethynyl-2'-deoxyuridine) are standard markers of cell proliferation (division), which decrease sharply or disappear completely during cellular senescence, as senescent cells halt their cell cycle [39]. However, it should be noted that the absence of these markers is also characteristic of quiescent cells (G0), meaning that their combination with other markers is required for the reliable identification of senescence.

Cyclin-dependent kinase (CDK) inhibitors: p16INK4a and p21WAF/Cip1

Prior to a detailed examination of specific molecular mechanisms, it is imperative to acknowledge a general caveat that pertains to the entire field: none of the classical markers discussed below exhibit absolute specificity for cellular senescence. The expression of these genes is highly dependent on the specific cell type, the nature of the stimulus that induces senescence, and the spatiotemporal stage of the process. In the ensuing sections, the functional roles of these markers as

employed in the cited studies are presented. The fundamental limitations of their standalone diagnostic value are analyzed systematically in the Limitations.

Researchers frequently employ CDK inhibitor proteins p16INK4a and p21WAF/Cip1 as markers of cellular senescence. These proteins effectively induce cell cycle arrest in response to DNA damage. p21WAF/Cip1, also known as CDK inhibitor 1A, serves as the primary mediator of p53-dependent senescence via the p53/p21WAF/Cip1 signaling pathway in response to DNA damage [40]. It must be noted, however, that p21 predominantly functions as a canonical effector of transient, reparative arrest, and its expression alone does not constitute proof of a terminal senescence state. p21WAF/Cip1, which belongs to the Cip/Kip family, is capable of blocking various phases of the cell cycle. The arrest of the G1 phase (suppression of cyclin D-CDK4/6), the G1/S transition (suppression of cyclin E/CDK2 and cyclin A/CDK2), as well as the G2 and G2/M phases (inhibition of cyclin B/CDK1) are of particular interest [41]. The activation of DDR signaling, including ATM (ataxia telangiectasia mutated), ataxia-telangiectasia, and Rad3-related (ATR) kinases, occurs as a result of persistent stress stimuli. These kinases subsequently phosphorylate Checkpoint Kinase 2 (CHK2). CHK2 phosphorylates p53, which in turn activates the p53/p21WAF/Cip1 axis. Consequently, p21WAF/Cip1 is able to restrict the function of CDK2, which is capable of carrying out the phosphorylation of retinoblastoma protein (RB). RB functions as a potent repressor of genes required for DNA replication [32, 42]. Hypophosphorylation of RB results in the disruption of E2F transcription, a process that is imperative for the progression of the cell cycle to the S phase. This disruption consequently leads to cell cycle arrest [43].

p16INK4a, a tumor suppressor, is known to be activated in response to oncogenic stress and other stimuli. Its mechanism of action involves the inhibition of CDK4/6, thereby maintaining a hypo-phosphorylated form of the retinoblastoma protein Rb. This, in turn, has been shown to cause cellular senescence [32]. It is important to note that p16INK4a expression does not necessarily indicate senescence; its levels are known to increase with age in the absence of other senescence hallmarks, and certain stress stimuli can induce p16 without establishing a full senescence program.

Silencing p16 INK4A with small interfering RNA (siRNA) activated breast fibroblasts and increased their secretion of stromal cell-derived factor 1 (SDF-1 α) and matrix metalloproteinase (MMP)-2. Consequently, the culture medium from these cells stimulated the proliferation of epithelial cells. In addition, the migration and invasion of BC cells were enhanced in a SDF-1 α -dependent manner. The observed effect was found to be

mediated by the induction of EMT. Conversely, increasing p16 INK4A levels through ectopic expression or decreasing AUF1 levels resulted in reduced secretion of SDF-1 α and MMP-2, as well as the suppression of the pro-carcinogenic effects of CAFs. Furthermore, p16 INK4A-deficient fibroblasts promoted the formation and growth rate of breast tumor xenografts in mice [44].

The predictive value of p16INK4a and p21WAF/Cip1 as senescence markers is limited because neither is strictly required for the induction or maintenance of senescence. Additionally, the expression of these markers can vary depending on the cell type and the stimulus that induces senescence. For instance, lapatinib therapy has been observed to induce p15- and p27-dependent senescence in HER2-positive BC cells without increasing p16INK4a and p21WAF/Cip1 [45]. Another example is androgen-induced cellular senescence in prostate cancer cells, which is mediated through the androgen receptor/the cell cycle inhibitor p15INK4b signaling axis [46]. The complexity of detecting senescence is also determined by the fact that the signaling pathway mediating it can change. For example, in the early phase, glyoxal-induced senescence is realized through the protein kinase B-FOXO3a-p27KIP1 pathway, which mediates cell cycle arrest. In contrast, late-stage senescence is realized through the p16INK4a/pRb pathway [47].

Senescence-associated heterochromatin foci (SAHFs). A hallmark of the epigenetic profile of senescent cells is the presence of condensed SAHFs, which hinder the transcription of genes involved in proliferation. SAHFs have been found to be enriched in the histone mark H3K9me3 (tri-methylation of histone H3 at lysine 9), its binding partner heterochromatin protein 1 γ (HP-1 γ), and the non-histone chromatin protein HMGA2. The presence of SAHFs in tumor cells indicates profound repression of E2F-dependent genes and the stability of the senescent phenotype [48]. Recent findings have indicated that primary human fibroblasts (e.g. BJ, MRC-5) undergoing replicative or premature senescence induced by oncogenic H-Ras, chemotherapeutics, or bacterial cytolethal distending toxin exhibit stimulus and cell-type-dependent SAHF formation. Oncogenic H-Ras induced SAHF in all cell types, whereas etoposide, doxorubicin, hydroxyurea, bacterial toxin, and telomere attrition triggered SAHF only in MRC-5 fibroblasts, not in BJ fibroblasts or keratinocytes. DAPI-defined SAHF was observed in paraffin sections of Ras-transformed cultured fibroblasts; however, it was not detected in human lesions across tumorigenesis stages. Consequently, in contrast to the pervasive γ H2AX damage marker, SAHF does not serve as a universal senescence hallmark. These genes are generally inducible by oncogenic stress; however, they are cell-type-restricted under genotoxic and replicative

senescence. Furthermore, while culture SAHF correlates with p16INK4a upregulation, such prototypic structures are undetectable in tissues, including premalignant lesions, despite elevated p16INK4a and other senescence features [49].

DNA-SCARS. Genotoxic stress, such as that induced by chemotherapy, has been shown to create so-named "DNA segments with chromatin alterations reinforcing senescence" (DNA-SCARS), which are persistent foci of DNA damage. It has been established that DNA-SCARS are not amenable to repair and exhibit several hallmarks, including association with promyelocytic leukemia nuclear bodies, deficiency of the DNA repair proteins replication protein A and RAD51, and accumulation of activated forms of the DDR protein mediators CHK2 and p53, as well as histone γ H2AX and p53-binding protein 1 (53BP1) [50]. Exposure to ionizing radiation has been demonstrated to induce irreparable DNA double-strand breaks in human fibroblasts, thereby triggering a persistent DNA damage response and premature cellular senescence. It has been established that profound chromatin remodeling, which ultimately results in SAHF, serves as a pivotal epigenetic mechanism that controls SASP. It has been demonstrated that persistent 53BP1 foci evolve into DNA-SCARS, which localize at the periphery of SAHFs, with the histone variant H2A.J progressively incorporated into these structures during senescence progression. It is noteworthy that shRNA-mediated knockdown of H2A.J disrupts senescence-associated chromatin reorganization and completely abrogates SASP, thereby suppressing the production of inflammatory mediators without affecting cell cycle arrest. These findings provide mechanistic insights into SASP regulation and identify H2A.J as a potential target for selective ablation of the pro-inflammatory senescent phenotype [51].

Cell senescence, including that of fibroblasts, is characterized by specific changes in the expression of nuclear proteins. These changes include the depletion of the nuclear lamina protein Lamin B1 and the release of the nuclear high mobility group box 1 (HMGB1) into the extracellular space. HMGB1 is subsequently recognized by immune cells as a stress signal [52-55]. A correlation has also been observed between chromatin changes, such as H3K27me3 loss, and upregulation of key senescence genes [52].

The administration of bleomycin chemotherapy has been demonstrated to induce alterations in the nuclear structure of cells, manifesting in a reduction of the nuclear membrane component lamin B1 and the leakage of nuclear DNA with γ H2AX into the cytoplasm. As a result, the presence of cytosolic chromatin fragments is detected by the cytosolic sensor cyclic GMP-AMP synthase (cGAS), which functions as a stimulator of the interferon

gene (STING) pathway. Activation of the cGAS-STING pathway instigates the NF- κ B cascade, which promotes the production of pro-inflammatory SASP factors and links nuclear damage to the inflammatory phenotype of the microenvironment [56-58].

Anti-apoptotic proteins overexpression, such as B-cell lymphoma-2 (BCL-2), BCL-2-like 1 (BCL-2L1), myeloid cell leukemia-1 (MCL-1), and SURVIVIN, has been observed to support the persistence of senescent cells. This phenomenon may be considered a hallmark of cell senescence, along with a concurrent decrease in pro-apoptotic proteins, including Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist/killer (Bak) [59, 60]. Furthermore, the study revealed that fibroblasts from patients with scleroderma rely on BCL-2L1 or MCL-1/BCL-2L1 for survival [61]. Bonneaud et al. (2022) established that MCL-1 mRNA expression positively correlates with stromal score in publicly available expression datasets from luminal breast cancers. CAFs derived from breast cancers, irrespective of their subtypes, exhibit elevated levels of MCL-1 expression in comparison to normal fibroblasts. This finding was established using CAFs grown *ex vivo* in culture conditions that favor their myofibroblastic phenotype [62]. For instance, in human diploid fibroblasts undergoing replicative senescence, elevated constitutive levels of the anti-apoptotic gene, Bcl-2, and diminished expression of the pro-apoptotic gene, Bax, were observed, thereby determining resistance to oxidative stress [63]. *In vitro* studies have demonstrated that fibroblasts undergoing chemotherapeutic treatment exhibit a pro-tumorigenic stromal-derived signal response, and that their eradication can be accomplished by targeting the anti-apoptotic protein BCL-2L1 [64].

The expression of SASP proteins is not an exclusive characteristic of senescence, as many of these proteins are involved in inflammatory processes. Recent studies have demonstrated the heterogeneity of senescent cells and identified the Integrin-associated protein (IAP, CD47) receptor as a potential marker for identifying subpopulations capable of escaping DNA damage-induced senescence. CD47 is one of the receptors for the SASP component thrombospondin 1 (TSP1), which has been demonstrated to play a role in the maintenance of cellular senescence. *In vitro* models, including MCF7 BC cells, LS 174T colorectal cancer cells, and primary lung fibroblasts, were utilized to ascertain that cells entering complete division arrest retain CD47^{high}, while CD47^{low} cells are not recognized by TSP1 and avoid senescence [30].

In addition to the widely used senescence markers previously enumerated, novel markers are currently being nominated. These include plasma membrane proteins such as DPP4 (CD26), Tumor Necrosis Factor Receptor

Superfamily Member 10D (TNFRSF10D, CD264), Urokinase Plasminogen Activator Receptor (uPAR, CD87), Intercellular Adhesion Molecule-1 (ICAM1, CD54), Neurogenic Locus Notch Homolog Proteins NOTCH1 and NOTCH3 (CD36), as well as Programmed Death-Ligand-1 (PD-L1, CD274) and major histocompatibility complex (MHC I) molecules [65-68]. Thus, the mass spectrometry analysis demonstrated the selective expression of DPP4 on the surface of senescent human diploid fibroblasts. This renders the fibroblasts susceptible to the action of NK cells and resulting in their sensitization to cell death [69].

Metabolic markers. Mitochondrial dysfunction is a hallmark of cellular senescence, along with DNA damage and cell cycle disruption. The prevailing definition of mitochondrial dysfunction, as delineated in the extant literature, is a reduction in mitochondrial membrane potential and a concomitant loss in respiratory capacity per mitochondrion when at a steady state [70]. The presence of dysfunctional mitochondria decreased efficiency of oxidative phosphorylation, and increased production of ROS has been observed in senescent cells. This phenomenon can lead to permanent cell cycle arrest, as evidenced by studies [71, 72].

During the process of senescence, there is a decrease in mitochondrial membrane potential ($\Delta\Psi_m$). This decrease is the result of calcium accumulation from the endoplasmic reticulum through mitochondria-ER contact sites (MERCS). There is also an increase in proton leak. These changes, in conjunction with an imbalance in NAD⁺/NADH metabolites, result in a reduction in the efficiency of the electron transport chain, leading to increased ROS production and decreased ATP synthesis [73, 74]. A decrease in mitochondrial membrane potential is an early indicator of mitochondrial dysfunction and is widely used as a biomarker of senescence.

Disruption of mitochondrial dynamics (the balance between fusion and fission) is another significant mechanism of senescence. The division process is governed by two primary regulators: DRP1 (dynamin-related protein 1) and Fis1 (Mitochondrial Fission 1 Protein). In senescent cells, a shift in the balance towards mitochondrial fragmentation is observed, and excessive activation of DRP1 is associated with increased ROS production and accelerated senescence [75]. Conversely, in certain types of senescent cells, such as fibroblasts, the presence of enlarged or giant mitochondria has been observed, a phenomenon that is also associated with the aging process. Consequently, in both old human diploid fibroblasts and Mv1Lu cells undergoing senescence in response to TGF- β 1 or H₂O₂ exposure, the presence of significantly elongated giant mitochondria has been observed [76]. This phenomenon was found to be

associated with a decrease in the expression of the protein Fis1, which functions as a modulator of fission.

Mitophagy is a selective mechanism for the removal of mitochondria damaged by the lysosome. The PINK1/Parkin pathway has been the most thoroughly characterized mechanism regulating mitophagy in mammals [77].

The disruption of this process has been linked to the accumulation of dysfunctional organelles and the development of senescence. With age, this system for the disposal of damaged organelles deteriorates, a process that is exacerbated by increased ROS and decreased NAD⁺ levels.

A significant ancillary mechanism has been identified that links mitochondrial dysfunction to SASP activation. Apoptosis is accompanied by widespread mitochondrial outer membrane permeabilization (MOMP), leading to cell death [78]. Conversely, the named minority MOMP manifests exclusively in a subset of mitochondria, culminating in senescence by engaging BAX and BAK, forming macropores. This process facilitates the release of mitochondrial DNA into the cytosol, thereby triggering the activation of the cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS-STING) pathway and SASP [79-81]. A study of the response of stromal cells to therapeutic interventions in the context of the TME revealed similar patterns of dynamic mitochondrial changes. These alterations are contingent on autophagy, as evidenced by elevated levels of mitochondrial respiration and fatty acid oxidation, and SASP production that fosters the aggressive characteristics of TNBC. This finding underscores the pivotal function of mitochondrial metabolism in the paracrine regulation of the TME [82].

Consequently, markers such as DRP1, Parkin, PINK1, along with indicators of membrane potential and mitochondrial respiration, are considered potential biomarkers of senescence, reflecting the state of mitochondrial dynamics and quality.

Mitochondrial dysfunction in senescent fibroblasts exerts profound paracrine effects on adjacent tumor cells through metabolic coupling. During the autophagy-senescence transition, these fibroblasts undergo metabolic reprogramming toward aerobic glycolysis, accompanied by increased secretion of L-lactate and ketone bodies. These substances are subsequently utilized by cancer cells for oxidative phosphorylation. The experimental ablation of mitochondrial transcription factor A (TFAM), a master regulator of mitochondrial respiratory chain integrity, in fibroblasts recapitulated this dysfunctional phenotype, inducing oxidative stress, loss of respiratory chain components, and hyperproduction of H₂O₂ and L-lactate. In the context of the study, the research team observed that the absence of TFAM in fibroblasts led to a shift in their

metabolic profile, with a notable increase in glycolysis. This alteration, it was found, had a substantial impact on the growth of a BC MDA-MB-231 xenograft model, with the growth promotion being independent of tumor angiogenesis [83].

SASP phenotype. IL-6. The prominent source of IL-6 is CAFs, which promote the formation of inflammatory TME, the growth and radioresistance of BC cells [84, 85]. It is imperative to underscore the pivotal function of IL-6 in modulating inflammatory processes across diverse tumor types, notably BC, while concurrently acknowledging its substantial impact on the induction of EMT, a process facilitated by the IL-6-JAK-STAT3 signaling pathway [86, 87]. IL-6 interacts with the IL-6 receptor α (IL-6R α), which subsequently binds to glycoprotein 130 (gp130) to form a hexameric receptor complex that transmits the signal. Janus kinases (JAKs) are then activated; these phosphorylate signal transducer and activator of transcription 3 (STAT3) to activate it, which leads to the regulation of gene expression, promoting the proliferation and invasiveness of cancer cells whilst inhibiting apoptosis [87]. Consequently, Nagasaki et al. discovered that CAFs extracted from colorectal cancer specimens secrete IL-6, which in turn potentiates the expression of VEGF by fibroblasts, thereby stimulating angiogenesis. A notable finding that bears significant implications for the development of effective therapeutic interventions is the observation that the blockade of the IL-6 receptor within the stromal component of the tumor milieu resulted in a substantial decrease in tumor growth in vivo [88]. Wu et al. detected IL-6 secretion by CAFs isolated from gastric cancer samples, which enhances cancer cell migration in vitro and leads to EMT via the JAK2/STAT3 signaling pathway. The employment of JAK2/STAT inhibitors or anti-IL-6 antibodies hindered the reduction of E-cadherin and the increase of N-cadherin or ZEB2 in gastric cancer cells, consequently maintaining their epithelial phenotype. A notable observation is the impact of suppressing IL-6 expression in CAFs on the reduction of gastric adenocarcinoma metastasis in vivo. Consequently, the suppression of the IL-6-JAK2-STAT3 axis has been shown to impede the impact of CAFs on the migration and metastasis of gastric cancer cells [89]. The report by Guo et al. suggested a role for the paracrine effect of IL6 secreted by BC CAFs in radioresistance and the potential for blocking the IL6/STAT3 axis to reduce tumor growth and improve sensitivity to therapy [84]. A notable observation regarding the role of IL-6 in the function of BC senCAF is the finding by Ruhland et al. that senCAF induce an immunosuppressive BC tumor microenvironment by secreting IL-6, thus promoting tumorigenesis [7].

IL-8. Interleukin-8 (CXCL8) has emerged as a critical driver of BC aggressiveness, functioning both as a key component of the SASP and as a central mediator of pro-tumorigenic remodeling within the TME. Through C-X-C chemokine receptor type CXCR1/2 receptor-mediated signaling, IL-8 stimulates proliferative capacity, invasive potential, angiogenic activity, and recruitment of pro-tumorigenic immune and stromal cells, thereby promoting malignant progression across major solid malignancies [90]. Clinically, elevated IL-8 levels, whether measured systemically or within tumor tissues, are consistently correlated with poor prognosis, diminished therapeutic response, and resistance to immune checkpoint inhibitor therapy [90]. Large-scale bioinformatics analyses have confirmed that IL-8 expression correlates with an immunosuppressive TME, marked by increased infiltration of M0 macrophages and activated mast cells alongside reduced CD8⁺ T cells and resting mast cells. Furthermore, IL-8 is linked to established indicators of immune evasion, including EMT, checkpoint molecule expression, high mutational burden, and an unfavorable immunophenoscore [91]. In BC patients, high IL-8 mRNA levels are specifically associated with aggressive, poorly differentiated features and worse overall survival [92].

Within the context of senescent fibroblasts, IL-8 plays a particularly critical role. In the context of senescent stromal fibroblasts, the role of IL-8 as a component of the SASP becomes evident. The expression of IL-8 is dependent on persistent DDR signalling, which is mediated by ATM kinase activation and NF- κ B transcriptional activity. This regulatory feature differentiates IL-8 from other SASP factors, such as osteopontin, which have different expression patterns. [93]. Functionally, senescent primary normal breast luminal cells can activate stromal fibroblasts in an IL-8-dependent manner through the STAT3 signaling pathway. These activated fibroblasts, adopting a myofibroblastic phenotype, then promote EMT transition and enhance stemness properties of BC cells through paracrine mechanisms both *in vitro* and *in vivo* [94]. The pro-tumorigenic impact of IL-8 on BC cells is profound: exposure of MCF-7 cells to IL-8 triggers the emergence of cells with fibroblastoid morphology, elevated CD44 expression, enhanced migratory capacity, self-renewal ability, and multilineage differentiation potential - hallmarks consistent with both EMT and stemness acquisition. These phenotypic alterations closely parallel those induced by senescent cell-conditioned medium. Importantly, such treatment further augments IL-6 and IL-8 secretion by MCF-7 cells, establishing an autocrine regulatory loop that perpetuates the inflammatory milieu. Neutralizing antibodies against IL-6 and IL-8 abrogate these effects, confirming these cytokines as principal

mediators of EMT and stemness-related changes evoked by the senescent microenvironment. Sustained exposure to IL-8 has been demonstrated to promote the emergence of secondary senescent cells, thereby indicating a self-perpetuating mechanism in which senescence and inflammation mutually reinforce one another. This ultimately fosters the acquisition of EMT and stem-like traits at the population level, thus enhancing tumour aggressiveness [95]. Recent studies have further identified a specific subpopulation of myofibroblastic cancer-associated fibroblasts positive for tetraspanin 8 (TSPAN8⁺ myCAFs), which exhibit a senescence-like phenotype. These cells secrete SASP factors including IL-8, thereby enhancing cancer cell stemness and promoting chemoresistance in breast cancer [96]. Collectively, these findings establish IL-8 as a central node linking senescence, fibroblast activation, and breast cancer aggressiveness, positioning the IL-8/CXCR1/2 axis as a compelling therapeutic target for disrupting the tumor-promoting features of the senescent stromal compartment.

VEGF Vascular endothelial growth factor plays a pivotal role in the process of angiogenesis and is a significant component of the SASP. VEGF is frequently overexpressed by both BC cells and cells of the TME, including fibroblasts and immune cells. Elevated levels of VEGF have been demonstrated to be associated with poor prognostic factors in BC, including high tumor grade, increased metastatic potential, and a poor prognosis for patients. Its expression is considered a negative prognostic marker, with a concomitant decrease in overall survival [97].

The family of pro-angiogenic factors VEGF consists of several secreted proteins, including: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF-A has been identified as the predominant factor that induces pathological angiogenesis in malignant tumors. The binding of VEGF-A to VEGFR-1 and VEGFR-2 receptors on the endothelial surface has been demonstrated to support the proliferation and migration of endothelial cells, vascular permeability, and the activation of macrophages and granulocytes. VEGFR3 plays a pivotal role in lymphangiogenesis and formation of distant metastases by the lymphatic route [97, 98].

The CAFs-dependent pro-angiogenic potential can be elevated by the upregulation of VEGF-A and IL-8, along with their upstream effectors mTOR and HIF-1 α , by the influence of DNA methyltransferase 1 on their expression/secretion [99, 100].

The influence of VEGF on the fibrous component of the TME is particularly significant. VEGF activates fibroblasts, induces MMP expressions, and ECM remodeling. VEGF has been observed to interact with TGF- β , a process that has been shown to enhance ECM

remodeling and promote the formation of a desmoplastic reaction. These processes generate a TME that is mechanically and biochemically altered, thereby promoting tumor progression [100-102].

At the cellular level, VEGF has been observed to stimulate endothelial cells, promote abnormal pericyte recruitment, and attract mesenchymal stromal cells [101].

In TNBC patients who carry a mutation in the BRCA1 gene, it has been demonstrated that VEGF signaling mediates interaction between CAFs and tumor endothelial cells promoting angiogenesis and endothelial cell migration [103].

The VEGF/VEGFR signaling has the ability to recruit myeloid immune cells, such as tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSC) regulating the immune response during the tumor promotion and progression [104, 105]. TAMs effector molecule VEGF promotes angiogenesis, ECM remodeling, and T-cell anergy [106]. VEGF has also been shown to inhibit dendritic cell maturation and promote the expansion of regulatory T cells, thereby enhancing immunosuppression.

In the context of BC progression, VEGF has been identified as a critical mediator of EMT, a process that involves the activation of transcription factors such as Snail, the suppression of E-cadherin expression, and the activation of PI3K/AKT- and MAPK-dependent cascades. These processes contribute to the increased invasive potential of tumor cells and metastasis [107].

From an architectural perspective, VEGF instigates the formation of aberrant vasculature, characterized by the uncoordinated proliferation of vessels, their compromised integrity, and the absence of pericytes, resulting in the formation of hypoxic niches and the facilitation of tumor cell intravasation. These features not only contribute to tumor progression but also determine resistance to therapy [108, 109].

MMPs. Matrix metalloproteinase (MMP) and membrane-type MMPs (MT-MMPs) are zinc-dependent endopeptidases that have proteolytic activity against components of the extracellular matrix. The critical function of MMPs in cancer invasion and metastasis has been well-documented in previous studies. When a tumor spreads to neighboring tissues, cancer cells overcome the basement membrane using invadopodia rich in MMPs, whose proteolytic activity causes degradation of the ECM. The expression of MMP has been observed in both cancer cells and CAFs. The metalloproteases that have been identified include MMP2, MMP9, MMP14 along with MMP10 and MMP13 [110, 111].

MMP10. As has been demonstrated in previous studies, there is a notable increase in the expression levels observed in tumors that have metastasized to the lymph nodes when compared to patients with tumors that are

localized. MMP10 has been identified higher in lymph node-positives compared with lymph node-negative BC [112]. Plasma MMP10 concentrations were elevated in BC patient samples in comparison to control groups, comprising healthy patients and patients with benign tumors [113]. It is evident that a significant function of MMP10 is the promotion of tumor angiogenesis [114].

MMP13. MMP13 expression has been detected in the TME of tumors, and its role in the development of the tumor process is significant [115]. As demonstrated by Zigrino et al. (2009), the requirement for MMP13 expression by stromal microenvironment cells in the development of melanoma invasion and metastasis was previously established. B16F1 melanoma cells were grafted into the flank of wild-type and MMP13^{-/-} mice. The results demonstrated a reduction in tumor growth and metastasis in the absence of MMP13. Notably, the absence of MMP13 led to a decrease in melanoma vascularization, indicating a critical function for MMP-13 in the process of angiogenesis [115].

The absence of MMP13 also had an impact on the growth of squamous cell carcinomas grafted into the flank of MMP13^{-/-} subjects, as evidenced by a slowing of tumor growth and a reduction in vascularization. Concurrently, a decline in VEGF expression was detected, potentially attributable to the absence of ECM-bound VEGF release resulting from MMP13-mediated ECM degradation [116].

The mechanisms of MMP13 action in BC include regulation of the physical properties of the microenvironment. MMP13 has been demonstrated to function as a dynamic modulator of the physical characteristics of the extracellular matrix through dual mechanoregulatory mechanisms. On the one hand, MMP13-mediated collagen degradation promotes "softening" of the TME, facilitating tumor cell invasion. Conversely, due to crosstalk with TAMs and CAFs, MMP13 promotes pathological stromal compaction (stromal fibrosis) through aberrant matrix deposition and cross-linking. The role of MMP13 in the BC TME is particularly evident in the context of senescent CAFs. As previously reported, senCAF^s secrete MMP13, which promotes immunosuppression and tumor growth [110, 117].

Despite considerable efforts, attempts to target MMPs have encountered significant challenges in clinical trials, resulting in the failure to achieve the established targets. Recent studies have illuminated a potential MMP-independent mechanism of tumor cell invasion that may underlie the efficacy of clinical trials of MMP inhibitors [118].

In addition to proteolytic damage to the extracellular matrix, the remodeling of basement membranes by tumor cells in the presence of fibroblasts, including tension and

stretching, has been described. The authors propose that an alternative mechanism of basement membrane damage by mechanical forces should be considered when developing new therapeutic agents.

Transforming growth factor beta (TGF- β). TGF- β is a pleiotropic cytokine that functions as a pivotal regulator of the TME. It has been demonstrated that this process coordinates the differentiation and secretory activity of immune cells by influencing multiple signaling pathways, including the canonical SMAD protein cascade. The culmination of this cascade is the nuclear translocation of the cells and the subsequent transcriptional regulation [119, 120]. In the context of pancreatic ductal adenocarcinoma, TGF- β has been shown to contribute to the maintenance of heterogeneity within the CAF population. The inflammatory phenotype of CAFs is induced by IL-1-mediated Leukemia Inhibitory Factor expression and Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) activation, while TGF- β suppresses IL-1R1 receptor expression and promotes CAF differentiation into myofibroblasts [121]. The impact of TGF- β on the TME frequently results in immunosuppression. Utilizing *in vivo* and *in vitro* models, it has been demonstrated that TGF- β maintains FOXP3 expression and the immunosuppressive function of Treg, thereby initiating the transition of conventional effector T cells to induced Tregs [122, 123].

A further suppressive mechanism has been demonstrated to be associated with the effect of TGF- β on the cytotoxic activity of NK cells. Specifically, TGF- β has been shown to diminish the expression of the C-type lectin receptor NKG2D on tumor cells, which is essential for NK cell recognition [124]. Furthermore, the combination of TGF- β and IL-15 has been shown to induce the conversion of NK cells to an Innate Lymphoid Cell type 1 - like phenotype with loss of cytotoxic activity. It is important to note that this process is mediated by different intracellular pathways: TGF- β exerts its influence through the activation of Transforming growth factor- β -activated kinase 1 (TAK1), which in turn activates p38 mitogen-activated protein kinase p38MAPK that is responsible for modulation of the inflammation. IL-15, on the other hand, exerts its influence through the Ras/Raf/MEK/ERK cascade. Canonical Smad-dependent signaling has been demonstrated to be non-instrumental in this particular conversion [125].

Furthermore, TGF- β has been shown to impede IL-15-mediated activation of mTOR (mammalian target of rapamycin), a constituent of the pro-inflammatory cytokine signaling pathway, leading to a reduction in NK cell cytotoxic activity. The deletion of the TGF- β RII receptor subunit has been demonstrated to restore IL-15-dependent mTOR signaling and enhance NK cell activity, including the eradication of metastasis *in vivo* [126]. The

transfer of genetically modified NK cells that express the dominant-negative TGF- β receptor type II (DNT β RII) has been shown to reduce lung cancer growth and metastasis *in vivo*, thereby confirming the critical role of TGF- β in suppressing NK cell activity and indicating a potential new direction in NK cell therapy [127]. An additional immunosuppressive pathway has been identified that is mediated by TGF- β 1. This pathway is characterized by the combined action of TGF- β 1 and IL-15 on peripheral CD56⁺ NK cells, leading to a decrease in CD25 expression on CD103⁺CD56⁺ NK-like cells. These cells have been demonstrated to possess inhibitory activity [128].

It is imperative to underscore the complexity of the effects of TGF- β . The activation of NK cells during IL-2 stimulation and TGF β imprinting induces NK cell hypersecretion of interferon-gamma (IFN γ) and tumor necrosis factor-alpha (TNF α) in response to tumor targets [129]. Consequently, the presented data constitute a coherent yet multifaceted depiction of TGF- β 's involvement in cellular senescence and tumor immunosuppression, with each phenomenon exhibiting a discernible molecular basis.

Chemokines (CXCL and CCL)

Recent studies have demonstrated the importance of the CXC chemokine receptor type 4/signal transducer and activator of transcription 3 (CXCR4/STAT3) axis in the activation of CAFs. These findings are in parallel with the TGF- β signaling pathways. This signaling axis has been demonstrated to facilitate the conversion of NFs to CAF-like cells within the TME, thereby enhancing their migratory capacity and metastatic potential in lung cancer *in vivo*, and inducing the expression of α -SMA and vimentin. Consequently, CXCR4/STAT3 is a primary target for the development of therapeutic interventions aimed at impeding the effects of CAFs within the TME, including the transformation of NFs into CAF-like cells [12]. In addition, the study demonstrated that the secretion of a chemokine, designated as CXC Chemokine Receptor type 4 (CXCR3), by the CAF-S1 subtype fosters the accumulation of CD4⁺CD25⁺ cells and their subsequent differentiation into Tregs, thereby sustaining immunosuppression within the TME [3].

The strategic targeting of the CXCR4 receptor has emerged as a promising therapeutic approach. The mechanism of action of the chemokine receptor pathway, specifically the CXCL12/CXCR4 axis, has been demonstrated to regulate monocyte recruitment and differentiation into M2-phenotype macrophages within the TME. Consequently, this results in elevated migration of carcinoma cells and augmented expression of EMT markers, including Sox2, Oct4, and Nanog. An increase

in the number of CD44⁺ and CD105⁺ tumor stem cells was also noted, which contributes to the acquisition of chemoresistance [13].

Elevated levels of MMP-1 and CXCR4 were observed in CAFs obtained from primary breast cancers, which were associated with invasive and migratory phenotypes [130]. The transformation of human mammary fibroblasts into CAF myofibroblast is a critical step in the development of tumors. This process is initiated by the formation of a self-sustaining autocrine signaling pathway via TGF- β and SDF-1 α . This pathway leads to the transition of resident human mammary fibroblasts into CAF myofibroblast, which in turn promote tumor growth [131]. A comprehensive review of the pivotal role of the CXCL12/CXCR4 axis in EMT, metastasis, and BC progression can be found in [132].

An additional mechanism of action of the chemokine, CXCL12, produced by CAFs is to block p62-mediated autophagy degradation of PD-L1. This effect is mediated through the JAK2/STAT3 axis, with the expression of CYLD deubiquitinase promoting p62 accumulation in bladder cancer cells. Consequently, a combination of receptor blockade of the CXCR4 receptor and inhibitors of the PD1/PD-L1 axis is required for a more effective transition from a "cold" to a "hot" (immunogenic) TME [133]. Together, the CXCR4/STAT3 and TGF- β pathways provide a coherent framework for understanding TME function. Through these distinct yet overlapping signaling systems, the TME sustains immunosuppression, activates CAFs, and drives tumor progression.

CXCL9 (C-X-C motif chemokine ligand 9) is one of the main ligands of the CXCR3 receptor and is one of the SASP chemokines associated with the antitumor effects of cellular senescence. In the context of TIS, it has been demonstrated that the inhibition of CDK4/6 with palbociclib leads to the induction of a SASP that is enriched in chemokines such as CXCL9, CXCL10, CXCL11, and MHC class I polypeptide-related sequence B. These chemokines, in turn, promote the recruitment of NK- and T- cells to the TME [134]. In murine models of BC, CDK4/6 inhibitors have been shown to boost the effectiveness of T-cell-based immunotherapies. This potentiation is attributed to the upregulation of chemokines such as CCL5, CXCL9, and CXCL10 within tumor cells following CDK4/6 inhibitors treatment. The released chemokines preferentially draw activated CD8⁺ T lymphocytes into the TME, while largely sparing Tregs [135]. CXCL9 is highly expressed in the ER^{neg} subgroup and positively correlated with the immune-related factors Lymphocyte Activation Gene 3 (LAG3), PD-1, PD-L1 and CTLA4. Thus, CXCL9 is a biomarker for predicting the status of immune infiltration in ER-negative BC [136]. However, the antitumor effects of the chemokine CXCL9

may be epigenetically suppressed. It was reported that enhancer of zeste homolog 2 (EZH2)-driven epigenetic repression of inflammatory SASP components within the pancreatic TME curtails the antitumor activity of NK- and T- cells after TIS. However, pharmacological blockade of EZH2 reverses this effect, boosting the release of chemokines including CCL2, CXCL9, and CXCL10, thereby facilitating robust immune cell infiltration and regression of cancer in mice [137]. These findings suggest the presence of a comparable mechanism within the BC TME.

Osteopontin (OPN). In 2009, Pazolli et al. utilized whole genome transcriptional profiling to compare senescent fibroblasts with their younger counterparts. OPN has been identified as one of the most highly expressed transcripts in senescent fibroblasts [138]. This analysis identified OPN as one of the elevated transcripts, determining its significant functional role in paracrine stimulation of preneoplastic cell growth. Consequently, OPN inhibition emerges as a promising therapeutic strategy for mitigating the role of senescent fibroblasts in promoting tumor growth in vitro and in vivo. Furthermore, OPN has been shown to promote the transition of resident fibroblasts to myofibroblasts through the activation of Twist1, thereby contributing to tumor progression via EMT transition [139].

OPN/CXCL12. Butti et al. emphasized the function of OPN as a pivotal paracrine regulator within the tumor microenvironment, promoting EMT through the secretion of OPN-driven CAFs, as well as through the processes of angiogenesis and the secretion of CXCL12 [139]. OPN, a byproduct of the tumor, facilitates this transition by interacting with the integrins CD44 and $\alpha\beta3$ on the surface of fibroblasts. These integrins subsequently function as mediators of signal transduction via Akt and ERK, thereby inducing Twist1-dependent gene expression. Subsequent to this, OPN-activated CAFs secrete the chemokine, CXCL12, which in turn triggers the EMT in tumor cells. The maintenance of this interaction is contingent upon the presence of OPN, which is produced by cancer cells, and of CXCR4, which is expressed by activated fibroblasts. The study by Liu et al. demonstrates that OPN induces the production of CXCR4 in CAFs via the activation of β -catenin/hypoxia-inducible factor-1 α (HIF-1 α)-mediated transcription [140].

Elevated levels of OPN and CXCL12 have been demonstrated to correlate with resistance to immunotherapy in patients diagnosed with colorectal cancer [141]. STAT3 has been identified as a critical mediator of inflammatory signaling. Research has revealed that it is frequently found to be hyperactivated in senescent tissues, thereby establishing an additional regulatory layer between OPN and CXCR4 [142, 143]. NF- κ B has been identified as a pivotal regulator of both

OPN and pro-inflammatory chemokines, including CXCL12 [144]. Furthermore, the pro-inflammatory effects of OPN are amplified by the activation of intracellular signaling pathways, such as NF- κ B and mitogen-activated protein kinases (MAPKs), including p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) [145]. The persistent DDR, which is mediated by ATM and NF- κ B, underlies the activation of canonical SASP members [93]. Consequently, further research could concentrate on identifying the structural determinants of the interaction between OPN and CXCL12, as well as on the clinical validation of therapeutic strategies targeting this key regulatory hub of the senescent microenvironment.

Tumor necrosis factor α (TNF- α). A pivotal inflammatory mediator within the BC TME is TNF- α , which, in conjunction with IL-1 β , exerts a significant influence on the phenotype of CAFs and MSCs, thereby promoting the production of pro-inflammatory chemokines, including CCL2 (MCP-1), CXCL8 (IL-8), and CCL5 (RANTES). The CCL2 signaling pathway has been demonstrated to be associated with the activation of the transcription factor NF- κ B, which has been shown to promote the development of aggressive invasive BC by increasing the migration of monocytic cells into the tumor site and the formation of myeloid infiltrates in the tumor tissue. The targeting of inflammatory-stromal interactions by means of NF- κ B inhibition has emerged as a promising therapeutic approach [146].

RANKL (Receptor Activator of Nuclear Factor κ B Ligand, encoded by the TNFSF11 gene), a member of the TNF superfamily, has emerged as a potential component of SASP, a term denoting a series of secreted molecules expressed by senescent cells within the TME. In a model of chemotherapy-induced bone loss, it was demonstrated that the senescence of CXCL12-rich reticular cells (CAR cells) and bone marrow adipocytes is accompanied by enhanced RANKL production and activation of osteoclastogenesis. Conversely, the inhibition of the p38MAPK-MK2 signaling pathway has been shown to reduce SASP-associated RANKL expression and prevent bone loss [147]. The activation of the RANK signaling pathway has been associated with the development of a senescence-like phenotype in luminal epithelial cells of the mammary gland. In the early stages, this process limits tumor transformation; however, it can later contribute to tumor progression and the development of metastases [148]. Furthermore, RANKL has been demonstrated to play a pivotal role in the establishment of an immunosuppressive TME by increasing the number of Foxp3⁺ Tregs and influencing the differentiation of CD4⁺ T lymphocytes, thereby contributing to a weakening of the antitumor immune response. In the context of BC, elevated RANKL expression within the primary tumor

has been demonstrated to be a significant predictor of an increased risk of developing bone metastases. RANKL has been shown to function as a chemoattractant for RANK-positive tumor cells and to interact with the CXCR4/CXCL12 axis, thereby facilitating bone tissue colonization [148, 149]. The RANKL-inhibiting monoclonal antibody denosumab is already employed to prevent skeletal complications. A mounting body of evidence indicates RANKL blockade may also reduce local immunosuppression in the TME, thereby expanding the potential applications of this therapy.

B7-H3 (also known as CD276), a member of the B7 family of immune checkpoint molecules, has been found to be overexpressed in numerous malignancies, including BC. While B7-H3 does not constitute a conventional soluble component of SASP, its expression exhibits a functional correlation with the senescent microenvironment. Pro-inflammatory SASP cytokines, particularly IL-6 and TNF- α , activate NF- κ B and STAT3 signaling pathways, which can induce B7-H3 expression on tumor and stromal cells, enhancing the immunosuppressive phenotype of the TME [150]. B7-H3 has been shown to attenuate the antitumor immune response by suppressing the activity of CD8⁺ T and NK cells, and promotes the accumulation of Tregs cells and TAMs. In the context of BC, B7-H3 has been observed to activate the Raf/MEK/ERK cascade through its interaction with the major vault protein (MVP). This activation has been implicated in the induction of EMT and the escalation of tumor metastatic potential [151]. B7-H3 expression has been detected in CAFs of various tumor types, where it is associated with the formation of T-cell exhaustion in the stromal compartment [152, 153]. In TNBC, elevated B7-H3 expression is associated with an “immune-cold” phenotype, while targeting of B7-H3 with monoclonal antibodies has been shown to reduce immunosuppression and enhance the efficacy of anti-PD-1 immunotherapy [154, 155].

1.2. Senescence Cell Identification.

Analysis of advantages and limitations of key approaches. The identification of senescent cells remains a methodological challenge, particularly in complex tissues where single-marker approaches are insufficient. Machine learning methods are currently employed extensively to facilitate more precise identification of senescent cells, as outlined in a recent review [156]. These tools differ substantially in their input data requirements, underlying algorithms, and the biological questions they are designed to address. In the following section, an examination of the predominant approaches is conducted, with the classification of these approaches based on the type of

data utilized. A comparative summary is provided in Table S1.

Gene signature-based classifiers. A recent development in this field is the Senescence Identification Tool (SIT), which identifies senescent cells based on the analysis of gene signatures associated with cell cycle arrest and senescence pathways. In vivo studies employing SIT have elucidated the substantial heterogeneity of these cells, whereby distinct clusters of senescent cells manifest unique SASP profiles and survival mechanisms. This heterogeneity is directly relevant to the development of targeted therapy, as it implies that different senescent subpopulations may respond differently to senolytic intervention [157].

However, SIT is subject to two notable limitations. Firstly, its classification is constrained by a predetermined set of reference genes, which can result in the exclusion of cells exhibiting unconventional or atypical senescence pathways. Secondly, SIT operates exclusively at the transcriptomic level, disregarding protein expression profiles and cell morphology, both of which may contain functionally relevant information.

The SenMayo signature embodies an alternative gene-based approach that was meticulously developed to detect tissue- and species-specific senescence. Its validation has included bone biopsies from elderly humans, supporting its applicability to human tissue [158]. The primary advantage of SenMayo is its capacity for cross-tissue mobility, which is derived from a curated consensus of senescence-associated genes. The primary constraint of this approach is analogous to that of all transcriptomic signatures: it reflects steady-state mRNA levels, which may not correspond to active protein expression, and it does not capture post-transcriptional regulation or spatial context.

Single-cell and spatial transcriptomic frameworks. The DeepScience framework operates with single-cell and spatial transcriptomics data, thereby enabling the identification of senescent cells while preserving information about their tissue context [159]. This spatial dimension confers a distinct advantage over bulk or dissociated-cell approaches, as it enables the assessment of whether senescent cells reside in specific microenvironmental niches. The associated limitation is practical in nature: spatial transcriptomics datasets remain costly and technically demanding, which constrains throughput and accessibility.

The SenPred pipeline was developed using single-cell RNA sequencing of two- and three-dimensional cellular models of deeply senescent fibroblasts, with the aim of improving the detection of senescent cells in vivo [160]. The program's primary strength stems from its training on a well-defined senescence phenotype across both two-dimensional (2D) and three-dimensional (3D) culture

models. This training may enhance the program's generalizability to tissue contexts. Nevertheless, SenPred was trained exclusively on fibroblasts. Its applicability to other stromal or epithelial cell types remains to be validated.

The SenSCOUT framework was developed to delineate functional senescence subtypes employing a primary dermal fibroblast model [161]. The approach entails a multifaceted strategy encompassing single-cell imaging, machine learning methodologies, a range of induced senescence conditions, and the utilization of several protein biomarkers. The integration of imaging and protein-level data provides a significant advantage over purely transcriptomic classifiers, as it captures functional features of senescence rather than solely transcriptional signatures. The trade-off, however, is an increase in the complexity of the experiment. SenSCOUT necessitates the collection of multi-parametric input data, which may present challenges in terms of standardization across diverse research laboratories.

Cross-platform and imaging-based approaches. The scCamAge model represents a context-sensitive approach trained on yeast and shown to predict chemically induced and replicative senescence in human fibroblasts based on single-cell images, shape, and bioactivity data [162]. Its principal strength is the use of morphological features, which are independent of transcriptomic noise and species-specific gene annotation. However, the cross-species transfer from yeast to human fibroblasts, while demonstrated in proof-of-concept, raises questions about how broadly the model can be applied to other cell types and tissue environments without retraining.

The SAMP-Score is a machine learning classification method that utilizes morphology as a feature extractor. It was developed for the purpose of screening pro-senescence compounds. Its utility has been demonstrated by the identification of QM5928, a novel pro-senescence compound [163]. The merits of this approach are evident in its aptitude for high-throughput screening, a process where the implementation of transcriptomic or proteomic profiling would be impractical. The limitation is intrinsic to its design: it is optimized for compound screening in vitro and is not intended as a general-purpose classifier for senescent cell detection in complex tissues.

Integrative initiatives. The SENCAN classifier is a more recent development, currently in the early stages of in vitro validation, designed for the detection of therapy-induced senescence in cancer cell cultures. It is associated with data available on the Cancer SENCopedia platform [164]. Its advantage is its specific focus on therapy-induced senescence in cancer cells, a context of direct translational relevance. However, it has not yet been validated beyond in vitro settings, and its current

scope is limited to the datasets represented in the SENESclopedia resource.

The SenNet program, coordinated by the NIH, adopts an organ-based approach, taking into account health status and life stage to enable the discernment of senescence hallmarks across tissues [65]. Rather than being a single classifier, SenNet represents a consortium-driven framework for generating reference data. Its strength lies in the breadth and standardization of its data resources. Its limitation is that the utility of the framework depends on the completion and accessibility of the underlying datasets, which are still being accumulated.

Taken together, the available computational tools reflect a trade-off between specificity, data requirements, and generalizability. Transcriptomic signatures such as SIT and SenMayo are easy to deploy but remain confined to the gene expression level. Single-cell and spatial frameworks such as DeepScience, SenPred, and SenSCOUT provide deeper biological resolution but demand richer and more expensive datasets. Imaging-based methods such as scCamAge and SAMP-Score offer independence from transcriptomic assumptions but are currently narrow in their intended application. No single tool in the current literature satisfies all requirements simultaneously. The choice of method must therefore be dictated by the specific biological question, the available data type, and the required throughput.

1.3. Senescence inducers:

1.3.1. Oncogene-induced cellular senescence in both tumor cells and the microenvironmental cells.

The DDR is initiated by the occurrence of DNA damage, which is induced by persistent oncogenic stress. This process is accompanied by the activation of ATM (Ataxia-telangiectasia mutated)/ATR (Ataxia telangiectasia and Rad3-related) kinases, which results in the phosphorylation and stabilization of p53 and the subsequent transcriptional activation of p21WAF/Cip1. The function of p21WAF/Cip1 is to impede the activity of cyclin-CDK complexes, leading to hypophosphorylation of pRB and ultimately resulting in irreversible cell cycle arrest. Another distinctive feature of OIC is the persistent activation of the NF- κ B and p38MAPK pathways, which support senescence and the production of SASP components independent of the canonical DDR [5].

1.3.2. Chemotherapy-induced senescence.

Cytotoxic therapy has been demonstrated to induce DNA damage in tumor cells; however, in some cases, senescence can be induced due to the use of low doses of chemotherapy and prolonged administration [165].

Presently, the majority of research is centered on the study of tumor cell senescence and its impact on stemness, drug resistance, and its relationship with apoptosis. A modest body of research has been dedicated to the senescence of fibroblasts and CAFs, which exhibit analogous patterns of SASP secretion by senescent cells.

Alkylating agents (e.g., cisplatin and its derivatives) have been shown to induce alkylation and covalently bind to DNA, forming cross-links. This process has the potential to cause DNA breakage during DNA replication and to activate DDR [165]. In 2004, Zhao et al. revealed that treatment of primary fibroblast cultures with cisplatin induced cellular senescence, marked by cell growth cycle arrest at G1, accompanied by a high frequency of apoptosis, elevated p53 expression, and loss of membranous gap junctions [166].

In 2019, Gomes et al. found that DNA damage induces cellular senescence in 3D-cultured MCF7 cells. The acquired senescence phenotype exhibited a positive correlation with elevated ATR-Chk1 signaling, which in turn mediated cisplatin sensitivity [167]. Upregulated mRNA K-Ras, VEGF-A, and STAT3 were detected in cisplatin-induced TNBC MDA-MB-231 cells [168]. In 2022, Sriratanasak et al. demonstrated that cisplatin treatment induced cellular senescence in lung cancer cells, leading to stemness induction via GRP78/Akt signal transduction with an increase in stemness markers, including CD44, CD133, Nanog, Oct4, and Sox2 [169].

Topoisomerase inhibitors (doxorubicin or etoposide) prevent the repair of DNA breaks required to reduce the tension of the unwound DNA, a process that is essential for reducing the tension of the unwound DNA strand during replication. This results in the activation of p53/p21WAF/Cip1 or p16, leading to cell cycle arrest and subsequent senescence [165].

Comprehensive research has been dedicated to investigating the effects of doxorubicin in inducing cellular senescence in both in vitro and in vivo models. This research has been further substantiated by the availability of clinical data [165].

The pro-tumor effects of conditioned medium containing SASP factors from doxorubicin-induced senescent human normal WI-38 fibroblasts on osteosarcoma cells have been delineated. This finding lends support to the hypothesis that targeting senescent cells could emerge as an effective strategy for cancer treatment, particularly in elderly patients, who often exhibit high levels of these cells in multiple tissues and organs [170]. The critical role of SASP-induced senescence was also confirmed in vivo: plasma from Doxorubicin mice induced aortic stiffening ex vivo with implication of glycation stress that can be inhibited with the senolytic ABT263 [171].

A series of studies on doxorubicin-induced senescence in MCF-7 cells have identified hallmarks of senescence, including telomere dysfunction, the pivotal role of RB family proteins in initiating and maintaining a protracted growth-arrested state, and elevated p21WAF/Cip1 expression [172-176]. It is important to note that doxorubicin-induced senescence is reversible. The capacity to reestablish proliferative capacity in MCF7 cells following treatment with doxorubicin and administration to senescent cells has been substantiated, attributable to their capacity to impede downregulation of CDC-2 and preserve an intact p53 checkpoint [177].

Taxanes (paclitaxel, docetaxel, and vinca alkaloids) induce microtubule dysfunction by disrupting mitosis. Paclitaxel has been observed to induce cellular senescence in BC cells, as well as in melanoma and neuroblastoma cells. In contrast, docetaxel has been found to induce senescence in prostate cells [165]. In 2009, Prencipe et al. investigated the function of mitotic arrest deficiency protein 2 (MAD2) in the process of cellular senescence. Their findings revealed that the reduction of MAD2 expression in MCF7 BC cells led to premature senescence that was accompanied by elevated levels of IL-6 and IL-8 secretion. These cytokines, which are key components of the SASP, have the capacity to influence neighboring cells. Consequently, the authors hypothesize that the presence of compromised MAD2 levels in tumor cells may be associated with the mechanism of resistance to paclitaxel [178].

In 2016, Khongkow et al. discovered that the sensitivity of BC cells and mouse embryonic fibroblasts to cellular senescence induction by paclitaxel depends on the Forkhead box protein M1-Kinesin family member 20A (FOXM1-KIF20A) signaling pathway. Suppression of this pathway led to increased paclitaxel-induced senescence, with a loss of clonogenic capacity and the induction of senescence-associated β -galactosidase activity. The authors of this study propose a novel approach to overcoming chemoresistance, namely, the strategic targeting of the FOXM1-KIF20A axis [179].

A comprehensive description of the impact of chemotherapy on BC cell senescence is provided in a review by Milczarek et al. (2020) [180].

In 2025, a detailed study of therapy-induced senescence by Bajtai et al. screened the toxicity of a diverse panel of FDA-approved anticancer drugs and revealed that the taxanes paclitaxel and docetaxel were ineffective against the TIS phenotype. Bulk and single-cell RNA sequencing, in conjunction with surface proteome analysis, substantiated the transient nature of TIS and the universality of escape from TIS across BC cells, implicating a KRAS-driven escape mechanism. A parallel phenomenon of resistance in tumor cells with a

TIS phenotype has been documented in response to the antimetabolite Gemcitabine [181].

Palbociclib (PD-0332991) has been designated as the first selective CDK4/6 inhibitor to be approved for the treatment of ER⁺/HER2- metastatic BC. The process of senescence induction is associated with the inhibition of CDK4/6 kinases. Upon activation by D-type cyclins, these kinases can induce phosphorylation-mediated inactivation of the retinoblastoma protein (pRB). This inactivation is imperative for initiating the G1-to-S cell cycle transition [182].

A number of researchers have demonstrated the capacity of palbociclib to induce cellular senescence in BC cells. In 2016, Hu et al. demonstrated that MCF-7 cells underwent senescence following treatment with palbociclib or an antiestrogen in vitro, with the combination treatment exhibiting an additive effect. The anti-estrogenic effect of Palbociclib on MCF-7 cells was further confirmed by the sustained cell cycle arrest observed in the presence of the antiestrogen [183].

Another CDK4/6 inhibitor, abemaciclib, has been shown to induce a similar senescence effect by inhibiting the phosphorylation of the retinoblastoma tumor suppressor, thereby inducing G1 cell cycle arrest in BC cells [184].

In 2021, Jost et al. investigated the radiosensitizing effects of Palbociclib, revealing the induction of senescence not only in the luminal subtype of BC cells (MCF-7), but also in TNBC cells (MDA-MB-231) and normal fibroblasts (SBLF7, SBLF9). This finding was accompanied by the identification of elevated lysosomal β -galactosidase activity. The downregulation of the FOXM1 transcription factor, which is associated with senescence suppression, has been implicated as a mechanism involved in senescence. The combination of palbociclib and radiotherapy exhibited an additive effect on cell cycle arrest, suggesting that this combination may be a promising approach for combination therapy [185]. In a recent 2024 study, Favaretto et al. demonstrated the reversibility of palbociclib-induced senescence and the influence of pro-inflammatory secretome components, such as IL-8 and acute-phase serum amyloid A1, on neutrophil recruitment and activation. This phenomenon may present a therapeutic opportunity for the elimination of senescent cells, with the involvement of neutrophils [186].

The antimetabolites gemcitabine and methotrexate, which disrupt the incorporation of purines and pyrimidines into DNA during the S phase, are capable of inducing senescence via a p21-mediated mechanism. Methotrexate has been shown to induce p53-mediated senescence in the absence of DNA damage. Consequently, methotrexate-induced senescence in MCF-

7 cells was facilitated by the activation of p53 and p21WAF/Cip1 [187].

Bleomycin, a cytotoxic antibiotic, has also been observed to induce p21-mediated senescence. A primary series of studies on the mechanisms of bleomycin-mediated cell senescence have been conducted in models of lung pathologies, including inflammatory diseases and cancer. The mechanism of action in question has been linked to DNA damage, which in turn causes single- and double-strand breaks in cellular DNA. These DNA breaks lead to genomic instability in the damaged cells [188, 189]. In 2009, Kasper et al. demonstrated that Caveolin-1 plays a role in bleomycin-induced apoptosis and senescence of normal and lung cancer cells [189].

Preliminary investigations have indicated that the mechanism of action of bleomycin inducing DNA breaks is related to the formation of a complex between bleomycin and Fe²⁺ via the generation of ROS, which induces the DDR and ER stress [190-192]. While bleomycin is recognized for its propensity to induce fibrosis, the underlying mechanism is intricate and contingent on the specific disease entity and the presence of inflammatory changes.

In 2000, Yamamoto et al. demonstrated that bleomycin increased the expression of alpha(I) collagen, fibronectin, and decorin in human dermal fibroblasts in a dose-dependent manner in vitro. This process was associated with a slight increase in the expression of TGF-beta and connective tissue growth factor [193].

In 2025, Miura et al. demonstrated that bleomycin promotes cellular senescence by activating the cGAS-STING pathway without a direct effect on fibrosis in a model of induced interstitial pneumonia. Consequently, depending on the inflammatory microenvironment, bleomycin-mediated ROS generation may accelerate senescent collapse and induce cell death, such as necrosis [56].

With regard to the underlying mechanisms of bleomycin-induced senescence in lung cancer cells, studies conducted in 2020 by Sai et al. have demonstrated that this process is associated with the suppression of autophagy via the PI3K/Akt/mTOR pathway, resulting from the downregulation of PTEN [194].

The exposure of T47D human BC cells to conditioned medium from bleomycin-induced senescent lung fibroblasts resulted in a transition to a more aggressive motility phenotype [195].

PARP inhibitors. The mechanism of antitumor action is associated with the inhibition of Poly(ADP-ribose) polymerase (PARP), which disrupts DNA repair. This defect is characteristic of BRCA1 or BRCA2 mutant tumors. In the context of the MCF-7 cell model, veliparib has been observed to induce cellular senescence in irradiated BC cells and tumors [196].

Tamoxifen. Notwithstanding the estrogen receptor blockade, the administration of tamoxifen has been observed to inhibit the mitochondrial membrane-stabilizing enzyme CK2, resulting in the accumulation of ROS within the cell and the subsequent development of DDR. This process is accompanied by the activation of p53/p21WAF/Cip1/CDK, leading to the inhibition of the senescence pathway in BC cells [197].

1.3.3. Senescence induced by radiation therapy

The induction of senescence by radiation therapy, such as γ -irradiation or X-ray exposure, is facilitated through p53 activation and the subsequent induction of DDR. The critical role of p53 has been demonstrated using MCF-7 cells [198]. The fibroblast senescence within the TME during radiotherapy has been demonstrated to be associated with the development of fibrotic side effects, including radiation-induced lung fibrosis [199]. A paracrine effect of such senescent fibroblasts on cell fate in adjacent cell types, such as epithelial cells, has been described [200].

A study of the process of cellular senescence induction in BC CAFs revealed the upregulation of the tumor-promoting cell surface proteoglycan syndecan 1. This upregulation is induced by the autocrine action of the TGF- β /Smad/Sp1 signaling pathway, as well as via a paracrine action of TGF- β expressed by TNBC MDA-MB-231 [201].

1.3.4. The potential for the senescence to be reversed.

The phenomenon of reversibility of senescence was thoroughly examined in the review. A multitude of observations has led to the report that cells that have quantitatively entered a state of full-fledged senescent growth arrest may either maintain this arrest over an extended period or subsequently escape from it [202]. To illustrate this, the proliferation of Braf-V600E-mutant melanoma cells, following the induction of oncogene-induced senescence (OIS) by the withdrawal of oncogenic signals, was observed to resume in the absence of the Braf-V600E inhibitor Vemurafenib [203]. Additional evidence for this claim can be found in TIS models, where the transient expression of Suv39h1 or p53 allowed cells to escape growth arrest and reinitiate proliferation once these key senescence-maintaining proteins dropped below a functional threshold [26, 204].

A variety of mechanisms that have been described provide a foundation for the process of exit from the senescence-associated growth arrest. These mechanisms are distinguished by the abrogation of cell-cycle control, which is mediated by multiple regulators, including p16INK4a, p21Cip1, and c-Myc [205-208]. A notable

mechanism involves the capacity of senescent tumor cells, frequently distinguished by elevated autophagy levels, to circumvent metabolic distress and revitalize their population. This process frequently entails a propensity for cannibalism toward neighboring cells [209, 210]. A comprehensive analysis reveals that cellular senescence is not an inevitable consequence. Rather, cells have the capacity to evade this state through various mechanisms, including the transient expression of senescence-maintaining factors, the dysregulation of cell-cycle checkpoints, and the utilization of autophagy-driven survival strategies. These findings bear significant consequences for cancer therapy and the development of senolytic interventions.

1.3.5. Antitumor effect of SASP and senescence in the early stages of oncogenesis.

Two mechanisms that mediate the antitumor role of senescence at the early stages of oncogenesis include cell cycle arrest and activation of immune surveillance. These mechanisms are triggered by OIS, which results from the activation of proto-oncogenes (Ras, BRAF, ErbB2/HER2) in precancerous epithelial cells. High levels of oncogene Ras activation, corresponding to tumor levels bearing endogenous Kras2 mutations, have been shown to induce Ink4a-Arf-dependent cellular senescence. This process prevents increased proliferation due to activation of p16/INK4 (by blocking CDK4 and CDK6, which are necessary for retinoblastoma protein activation) and p14/ARF (by activating p53 by blocking its main inhibitor, MDM2) [211]. In vivo models have demonstrated that diminished senescence associated with ARF deletion is associated with the progression of ErbB2-induced carcinomas [212].

Furthermore, a significant mechanism of tumor suppression that is facilitated by the p53-p21WAF/Cip1 axis involves epithelial cell senescence, which is induced by the loss of the PTEN suppressor.

The elimination of senescent cells is contingent upon the recruitment of immune system cells (e.g., NK-, T-cells, macrophages) via SASP components such as chemokines (e.g., CCL2, CXCL10/11) and interleukins (e.g., IL-1 α / β). Specifically, the activation of the Kv11.1 potassium channel in ER⁺ BC cells has been shown to induce the SASP, which in turn activates CD4⁺ T-helper 1 (Th1) and memory T cell phenotypes. This series of events culminates in the release of TNF α , resulting in the demise of senescent BC cells [213]. Conversely, the suppression of STAT3 constitutive activation in TNBC has been observed to trigger senescence and, through the SASP, stimulate CD4⁺ T and NK cells [214].

1.4. The tumor microenvironment: diversity, plasticity, and identification of senescent CAFs.

CAFs are the predominant stromal cell population in BC and other solid tumors. Along with other TME components, such as vascular endothelial cells, pericytes, and immune cells, CAFs significantly influence tumor progression [3, 215]. CAFs have been identified as originating from various cell types within the TME, including resident fibroblasts, mesenchymal stem cells, endothelial cells, and adipocytes [216-218]. CAFs have also been proposed to originate from tumor cells via EMT [219]. The initial identification of CAFs revealed the following markers: alpha-smooth muscle actin (α -SMA, ACTA2), CD90 (Thy1), platelet-derived growth factor receptors α/β (PDGFR α/β), integrin beta-1 (ITGB1, CD29), podoplanin (PDPN), osteonectin (SPARC), fibroblast activation protein (FAP), fibroblast-specific protein 1 (FSP1, S100A4), caveolin 1 (CAV1), and vimentin (VIM) [3, 220, 221].

The CAF population is heterogeneous, and numerous researchers have identified distinct CAF subsets with different functional significance [222]. Preliminary investigations into heterogeneity were undertaken employing mouse models. For instance, in 2018, Bartoschek et al. analyzed 768 transcriptomes of mesenchymal cells from a genetically engineered mouse model of BC in 2018. The researchers identified three distinct subpopulations and their precursors: matrisome CAFs (mCAFs), which are descendants of resident fibroblasts; vascular CAFs (vCAFs), which originate from a perivascular location; and developmental CAFs (dCAFs), which share expression patterns with the tumor epithelium [216]. Furthermore, myCAFs have been observed to localise in proximity to cancer cells, thereby forming a barrier. In contrast, iCAFs have been found to be situated further out in the stroma, where they have been shown to regulate inflammation. vCAFs have been identified in association with blood vessels, while antigen-presenting (apCAFs) have been associated with areas where immune cells congregate [223, 224].

In 2020, Sebastian et al. used a syngeneic mouse model and scRNA-seq to identify CAF subpopulations, including myofibroblastic CAFs (myCAFs) that express α -SMA and other contractile proteins, iCAFs that express inflammatory cytokines, and CAFs that express major histocompatibility complex (MHC) class II proteins, which are generally found in antigen-presenting cells. A similar finding was reported in the study of pancreatic cancer CAFs [225-227]. In 2017, Öhlund et al. identified a CAF subpopulation with elevated α -SMA expression located immediately adjacent to neoplastic cells in mice, as well as a distally located subpopulation that secretes inflammatory mediators [226].

In 2020, Wu et al. performed a study on TNBCs, distinguishing two subpopulations: CAFs with myofibroblast features and two perivascular-like subpopulations characterized by high expression of growth factors and immunomodulatory molecules [228].

Another salient feature of CAFs is their capacity for plasticity, which is primarily governed by the TGF- β signaling pathway. In 2022, Krishnamurthy et al. discovered that TGF- β receptor type 2 signaling is crucial for pancreatic cancer fibroblast lineage diversity and the development of cancer-associated LRRC15⁺ myofibroblasts. The prevention of this signaling results in the transition of CAFs toward universal fibroblasts [229]. In a previous study, Biffi et al. employed organoid and mouse models to identify TGF- β and IL-1 as tumor-secreted ligands that promote CAF heterogeneity. The researchers demonstrated that IL-1 can induce LIF expression and subsequent JAK/STAT activation, thereby contributing to the generation of inflammatory CAFs. Furthermore, evidence was presented demonstrating that TGF- β antagonizes this process by downregulating IL-1R1 expression and promoting differentiation into myofibroblasts.

In 2021, Wu et al. presented a single-cell, spatially resolved transcriptomics atlas of BC, identifying CAF states, including s1, which has features of mesenchymal stem cells (MSCs), inflammatory-like fibroblasts (iCAFs), with high expression of stem-cell markers (ALDH1A1, KLF4, and LEPR) and pathways related to chemo-attraction and complement cascades (CXCL12 and C3), as well as differentiated states s4 and s5, which resemble myofibroblast-like (myCAF) states through the increased expression of ACTA2, TAGLN, FAP, and COL1A115 and the enrichment of ECM-related pathways. The plasticity of these cells was confirmed by the finding of transitioning CAFs in s3 that shared features of both MSC/iCAFs and myCAFs [230].

In 2018, Costa et al. identified four subgroups of CAFs (S1-S4) using FACS based on different expression levels of known CAF markers such as VIM, α SMA, FAP, FSP1/S100A4, PDGFR α and CAV1 [3, 231]. Consequently, a FAP^{high} CAF-S1 subgroup was identified, which is functionally associated with immunosuppression [232]. In addition, a FAP^{low}/ α SMA^{high} CAF-S4 profile was identified, and it was found to be associated with aggressive tumor characteristics such as invasion and metastasis. Furthermore, subgroups of putatively inactivated fibroblasts were identified, including a CAF-S2 subgroup that was negative for the expression of all markers and a CAF-S3 subgroup that was FSP1⁺/PDGFR β ⁺ [233, 234]. The functions of the latter subgroup have yet to be elucidated. The heterogeneity of CAF subpopulations is illustrated in Figure 1. Subsequent

examination of the heterogeneity of senescent fibroblasts by Ilyina et al. in 2025 corroborated the plasticity of senescent fibroblasts, thereby unveiling the divergent functional characteristics of BC primary cell cultures [4].

In 2023, Cords et al. defined the phenotypic categories of vascular CAFs, matrix CAFs, immune CAFs, tumor-like CAFs, interferon-response CAFs, antigen-presenting CAFs, dividing CAFs, and reticular-like CAFs. These researchers used a scRNA-seq dataset of stromal cells from tumors of 14 BC patients to define these categories [235].

In 2024, Croizer et al. concentrated on two subsets of FAP⁺ CAFs: iCAFs and myCAFs. They created an integrative map of BC using single-cell spatial transcriptomics and functional assays. The researchers identified ten spatially organized cellular niches associated with FAP⁺ CAFs, which they designated as EcoCellTypes. The transition from detoxifying iCAFs (Detox-iCAFs) to immunosuppressive myCAFs with ECM production (ECM-myCAFs) was found to be mediated by DPP4 and Yap. The formation of immunosuppressive EcoCellTypes has been associated with the ability of ECM-myCAFs to polarize TREM2⁺ macrophages, as well as regulatory NK and T cells. In contrast, the formation of immunoprotective EcoCellTypes has been associated with FOLR2⁺ macrophages [236].

The issue of senescent fibroblast typing and their role in contemporary BC remains unresolved, and the identification of answers is the focus of ongoing research. The process of cellular senescence in CAFs can be induced by ROS, DNA damage, or chemotherapeutic stress. The subsequent release of a series of inflammatory molecules, termed the SASP, has been demonstrated to promote tumor growth and metastasis [156]. In their seminal 2011 study, Witkiewicz et al. made the groundbreaking discovery that the localization of senescent cells in the stroma of ductal carcinoma in situ (DCIS) was associated with disease recurrence and progression, including the development of metastasis. This finding, which was subsequently corroborated in additional studies, emphasizes the pivotal role of senescent cells in the development of this deadly cancer [237]. Research by Bartholomew et al. has indicated that CAV1 can serve as a marker for senCAFs, with experimental evidence showing that overexpression of CAV1 can induce CAF senescence [238].

In 2024, Ye and colleagues identified a cluster of senescent myofibroblasts in murine and human tumors, including HER2⁺, ER⁺, and TNBC and DCIS. The researchers demonstrated a link between senescent adipose tissue-derived mesenchymal stem cells (senCAFs) and tumor recurrence. The presence of elevated levels of senescence markers such as p16, whose

increased expression was limited to myCAFs, p15, and BCL-W, was observed in senescent CAFs. The authors identified ECM-related factors, including collagen type I, collagen type XII, MMP10, and MMP13, as well as SASP factors with immune-modulating capacity CXCL9, RANKL (coded by TNFSF11), B7H3 (coded by CD276), and TGF- β . Utilizing an *in vivo* model, it was observed that the secretion of ECM components by senCAF resulted in the inhibition of NK cell functionality [110].

During the process of intracellular signaling, TSPAN8 recruits MAPK11 kinase to phosphorylate RBBP6 ubiquitin ligase, thereby inducing the degradation of SIRT6. This results in an enhancement of the activity of GLS1 and PYCR1 enzymes. This phenomenon has been observed to trigger the secretion of aspartate and proline by TSPAN8-positive tumor-associated myofibroblasts (myCAFs), thereby establishing a metabolic niche conducive to the proliferation of tumor cells. Furthermore, these same myCAFs enhance the stemness of adjacent cancer cells by releasing SASP factors IL-6 and IL-8, reducing their sensitivity to chemotherapy.

A study of p16⁺ CAFs in the stroma of pancreatic ductal adenocarcinomas revealed the presence of these cells, and elevated levels of immune-regulatory genes were observed in premalignant lesions in both mice and humans [239].

It is noteworthy that certain similarities exist between the SASP components secreted by senCAFs, which often include TGF- β , matrix-remodeling enzymes (MMPs), and CXC chemokines, and the mechanisms of their regulation, regardless of the type of senescence and the fibroblasts' affiliation with the TME. Consequently, a pivotal function was identified for p38 (p38MAPK) in regulating the occupancy of AU-rich element RNA-binding protein 1 on SASP mRNAs, thereby modulating their stability. This, in turn, facilitates the tumor-promoting activities of CAFs and senescent fibroblasts [240].

The SenPred ML model was employed to ascertain the proportion of senescent cells within the four fibroblast subtypes: secretory reticular, pro-inflammatory, secretory papillary, and mesenchymal. The analysis revealed that secretory papillary fibroblasts exhibited the highest percentage of senescence prediction (40.25%) [160].

A study of the expression of a panel of known senescence markers and SASP factors (CXCL12, CDKN1A (p21WAF/Cip1), CDKN2A (p16/Arf), IGF1, IGFBP3, IL-6, MMP3, SERPINE1, TP53) was conducted, and the highest positive correlation coefficient was found to be for IGFBP3 and CXCL12 [160]. Comparative analysis with the expression profiles of old fibroblasts revealed an age-related loss of their identities [241].

The role of TGF β 1 and SDF-1 α secretion by senCAFs in maintaining aggressive tumor characteristics such as proliferation, invasion, and migration is well-documented [242]. The overexpression of MMPs and OPN has been demonstrated to result in MAPK activation. This, in turn, induces pre-neoplastic cellular proliferation [138, 196, 243, 244].

The functions of the aforementioned SASP components have been thoroughly studied and described in detail in terms of their impact on the TME, leading to the development of immunosuppression and drug resistance. Subsequent research endeavors will facilitate the mapping of subpopulations of senescent fibroblasts and their association with particular sets of SASP proteins and their functional significance. This topic will be addressed in the subsequent chapters.

The transition of CAFs to a senCAF is driven by both internal factors, such as replicative exhaustion, and external signals from the tumor microenvironment and clinical interventions, such as chemotherapy and radiotherapy [245] (Figure 1). Fridman and Tainsky (2008) identified universal genes regulating senescence/immortalization and found that the key regulator genes represented six pathways: the cell cycle pRB/p53, cytoskeletal, interferon-related, insulin growth factor-related, MAP kinase and oxidative stress pathway [246]. The presence of characteristics associated with senescent cells has been observed in senCAF, including cell cycle arrest, which is facilitated by the p53/p21 and p16 INK4a pathways, persistent DNA repair, and SASP. A study by Bartholomew et al. demonstrated that CAV1, a marker of CAFs, can also serve as a marker for senCAFs. Moreover, experimental data has indicated that the over-expression of CAV1 can induce ageing in CAFs [238]. Harada et al. (2026) demonstrated a distinct spatial distribution of myCAFs and iCAFs throughout the tumor and showed that senCAFs were predominantly located at the visible tumor margin in pancreatic ductal adenocarcinoma [224]. As indicated by Ye et al., the p16⁺ population expresses markers that are predominantly restricted to myCAFs. Consequently, these p16⁺ myCAFs also exhibited augmented levels of other markers of cellular senescence that are frequently observed, including p15 (encoded by CDKN2B) and various SASP factors supporting senescence [110, 247]. It has been demonstrated that SenCAFs maintain the molecular characteristics of CAFs. Moreover, numerous studies have indicated that they manifest specific features observed in myCAFs, including elevated levels of α -SMA expression [96, 110, 248]. Despite the implementation of a variety of methodological frameworks, contemporary multi-omic studies (scRNA-seq and mass cytometry) have elucidated a significant degree of overlap in the classifications obtained. scRNA-seq analysis of breast

cancer has revealed the heterogeneity of the CAF-S1 population. The population under study consists of subclusters that correspond to myCAF-like cells, which are characterized by elevated expression of α -SMA and matrix genes, and iCAF-like cells, which exhibit inflammatory cytokine expression [249, 250]. Conversely, the CAF-S4 subtype is characterized by an accumulation of genes linked to myofibroblast differentiation, suggesting its classification as a variant of myCAF with augmented invasive potential (Figure 1). The S1-S4 classification functions as a marker-based classification that also encompasses less well-characterized groups of normal-like fibroblasts, whilst myCAF/iCAF/apCAF/vCAFs provides a functional interpretation within these populations. While the CAF-S1-S4 classification offers a more detailed breakdown, this is achieved at the expense of reduced functional specificity. A limitation of these classifications is that they do not encompass the hallmarks of cellular senescence, which include cell cycle arrest, DNA damage and the SASP. The prevailing hypothesis is that senCAF-SX may arise from any of S1-S4, though it is most commonly hypothesised that it originates from myCAF-containing S1 or S4 (Figure 1). However, upon acquisition of the senescent phenotype, the characteristics of SenCAF-SX are rendered qualitatively divergent. SenCAF-SX primarily as a proposed senescent state or phenotype superimposed on existing CAF populations, unless stronger lineage-resolved evidence supports classification as a distinct fifth subtype. The senCAF SASP is distinguished from the secretome of other CAF subtypes by its unique composition, which includes a specific set of immunomodulatory and ECM-remodeling factors [8, 245]. These factors determine the unique role of senCAF SASP in the TME. The hypothesis is that, in contrast to S1 (pro-inflammatory) and S4 (immunosuppressive) cells, senCAFs generate a hyper-inflammatory stromal-derived SASP, which is linked to irreversible cell cycle arrest and the capacity to induce paracrine aging. It is acknowledged that there is currently no single “gold standard” for identifying senCAF, and the development of multi-parameter approaches incorporating transcriptomic and proteomic profiles is recommended. In regard to this matter, for the practical identification of senCAF-SX, a multi-level system employing a panel of markers at each tier is required:

CAF identity: CAFs markers and negative markers for E-cadherin, EpCAM and CD31 [251, 252].

Process of ageing: p16⁺/p21⁺, SA- β -gal⁺(pH=6), γ H2AX⁺, Ki-67⁻

SASP phenotype (recommended): SASP signature that has been specifically identified for senCAFs in a defined cancer type (for example CXCL9, RANKL, B7H3, HGF, and TGF- β for BC senCAFs [110])

2. Directions of the senescence targeting therapy.

The development of therapeutic approaches that utilize inhibitors of senescence must consider the complex and contradictory effects of senescence on cells of the microenvironment and tumor cell plasticity [14]

The initiation of tumor cell senescence by radiotherapy and chemotherapy has been shown to terminate tumor cell proliferation and contribute to a decrease in tumor growth and metastasis [253, 254].

Concurrently, surviving metabolically active tumor cells and senescent cells in the microenvironment exert a pro-carcinogenic effect through the action of SASPs, causing tumor phenotype reprogramming with EMT and immunosuppression of the TME, stimulation of angiogenesis, inflammation, and potentiation of tumor cell migration and invasion.

In this regard, a "one-two punch approach" is proposed as a promising experimental strategy. This approach involves the initial utilisation of therapy that induces the senescence of tumour cells, followed by the subsequent elimination of senescent cells. The elimination is affected through the administration of senolytics (e.g., BCL-2 inhibitors), which selectively eliminate senescent cells, and senomorphic agents (e.g., senostatic PI3K/mTOR inhibitors and anti-inflammatory agents), which modulate or suppress SASP activity. The objective of this approach is to eradicate tumour cells that have survived therapy and aggressive CAFs. This will impede the effects of the secretome. [253, 255]. The treatment regimen employing senolytics and senomorphics, as well as the status of ongoing clinical trials, are presented in Figure 3 and Table S2.

Senolytics.

ABT-199 (Venetoclax). It has been demonstrated that the process through which ER⁺ BC cells evade death is facilitated by BCL-2. Consequently, clinical trials of combination therapy with Venetoclax and CDK4/6 inhibitors (palbociclib, aromatase inhibitor letrozole [PALVEN] have been undertaken in ER⁺, BCL-2⁺ BC. The results of these trials were encouraging: a total of 60% of patients demonstrated a partial response to the therapeutic regimen, while 33% exhibited disease stabilization [256]. Data has also demonstrated the potential of venetoclax to enhance the suppression of doxorubicin-exposed TNBC cells [257]. The elimination of senescent cells from the TME holds considerable promise in terms of reducing immunosuppression. For instance, in an in vivo pancreatic adenocarcinoma model, the elimination of senCAFs with venetoclax resulted in an

augmented proportion of activated CD8⁺ T cells and an enhanced response to immunotherapy in vivo [239].

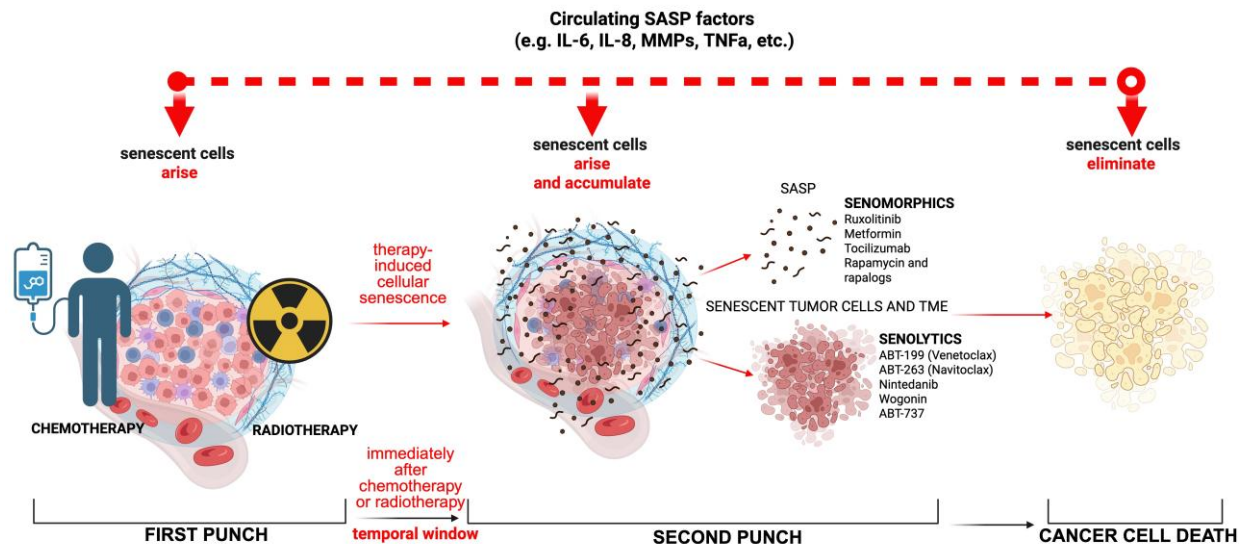


Figure 3. The concept of one-two punch therapy: the initial prescription of a standard chemotherapy and radiotherapy regimen to induce cell arrest of tumor cells, followed by senolytic administration to kill senescent cells and senomorphics to block the effects of SASP factors.

ABT-263 (Navitoclax), a BH3 mimetic, has been shown to inhibit Bcl-2, Bcl-w, and Bcl-xL. It is currently being evaluated in clinical trials for its effectiveness in treating leukemia [258].

There are ongoing efforts to treat patients with TNBC who have mutations in BRCA1/BRCA2, or Partner and Localizer of BRCA2 (PALB2) genes in conjunction with a PARP inhibitor (olaparib) [259]. In vivo models of TNBC demonstrate the efficacy of the "senescence induction and senolysis" strategy, which integrates the CDK4/6 inhibitor palbociclib, which induces cellular senescence, and navitoclax as a senolytic [260]. Preclinical studies of TNBC have demonstrated the efficacy of navitoclax in increasing the efficacy of EGFR-targeted Antibody-Drug Conjugate ADCs [261]. In combination with the chemotherapy drug doxorubicin, navitoclax and venetoclax enhance the antitumor effect of doxorubicin [257].

Navitoclax has demonstrated the capacity to eliminate BC cells that are capable of surviving chemotherapy. Experimental evidence from gene editing experiments has confirmed that the survival of BC cells in senescence is critically determined by BCL-xL or MCL1. The present study has identified low expression of NOXA, a specific antagonist of MCL1, as a factor contributing to resistance to ABT-263. This finding necessitates the combination of ABT-263 with MCL1 inhibition [262].

A salient observation is the selectivity of navitoclax as an apoptosis inducer for CAFs, with diminished effect on quiescent fibroblasts or tumor cells, as evidenced in an in vitro and in vivo cholangiocarcinoma model with tumor growth suppression and survival enhancement effects [263].

Nintedanib. The senolytic effect of the multikinase inhibitor is mediated through STAT3 inhibition, contributing to a decrease in the number of senescent cells and collagen deposition in a vivo model of bleomycin-induced lung fibrosis [264].

Extensive in vitro studies in a TNBC cell model demonstrated reduced cell proliferation and suppressed stem cell-like properties via activated tumor protein p73, which disrupted the p53-peroxisome proliferator-activated receptor α (PPAR α)/PI3K-Akt signaling axis. A decline in EMT markers, including Snail and zinc finger E-box-binding homeobox protein 1 (ZEB1), was also observed, contributing to reduced tumor invasiveness. In vivo, nintedanib treatment led to a reduction in tumor growth, angiogenesis, and stiffness [265]. The efficacy of nintedanib in combating BC has been substantiated by the positive outcomes of Phase II studies. These studies involved patients diagnosed with BC who received nintedanib in conjunction with antifibrotic therapy and chemotherapy. The findings indicated a decrease in the gene signature known as the mechanical conditioning score. This score serves as a marker for ECM stiffness and has been associated with a reduction in the risk of relapse

[266]. In 2024, a promising FAP-CAR-CM@PLGA-AB biomimetic platform was created. The platform under consideration consists of vesicles that have been chimerized with a FAP single-chain fragment and PLGA nanoparticles that have been co-encapsulated with nintedanib and ABT-263. The platform was developed for the purpose of eliminating and senescent CAFs. This approach would effectively counteract radiation resistance and immunosuppressive TME characteristic of BC [267].

Wogonin. Another promising compound capable of suppressing tumor growth in vivo and the proliferation of ER⁺ and TNBC cells in vitro is wogonin, a natural flavonoid from *Scutellaria baicalensis*. Phase I toxicity studies were conducted in patients with metastatic BC [268].

In 2020, it was demonstrated that wogonin induces senescence in TNBC cells MDA-MB-231 by inactivating the antioxidant enzyme TXNRD2. Consequently, this led to the occurrence of oxidative stress and cell cycle arrest, accompanied by increased P16 expression, SA- β -gal activity, SAHF, and a suppression of the SASP. This, in turn, resulted in the suppression of tumor progression [269]. A comprehensive review of preclinical observations pertaining to wogonin's antitumor activity and the underlying mechanisms, frequently involving MMP-9 suppression, is available [270].

Wogonin has been shown to exert a distinct effect on senescent fibroblast, exhibiting the capacity to suppress cellular senescence by impeding the activity of CDK9. This suppression leads to a reduction in p53 phosphorylation, a hallmark of cellular senescence, and a concomitant decrease in p21WAF/Cip1 levels, which are crucial for initiating senescence. Consequently, wogonin has been demonstrated to decrease the synthesis of ECM components in a model of pulmonary fibrosis [271]. Wogonin's capacity trigger cellular senescence in cancerous cells while concurrently repressing fibrosis is a distinctive property of this agent. Further study of wogonin's effects on senescent fibroblasts is necessary to enable its clinical implementation.

ABT-737. A notable antitumor effect against BC has been documented for another small-molecule BH3 mimetic, ABT-737. In the context of BC, the process of cellular senescence in CAF cells has been observed to impede the function of NK cells, which are responsible for antitumor surveillance. The administration of the senolytic ABT-737 has been shown to diminish the growth of tumors, indicating that the elimination of senescent CAF cells may have a direct impact on the proliferation and recurrence of cancer cells [110]. This compound functions as a selective inhibitor of anti-apoptotic proteins belonging to the BCL-2 family, including BCL-2, BCL-xL, and BCL-W. Notably, ABT-

737 has been shown to have the capacity to specifically induce apoptosis in senescent cells [28]. Consequently, ABT-737 enhances the sensitivity of BC cells to the chemotherapy drug docetaxel, thereby improving the tumor response to chemotherapy and overall survival in vivo [272]. The combination of ABT-737 with the mTOR pathway inhibitor MLN0128 (sapanisertib/INK128) has been shown to exhibit a robust synergistic effect in suppressing renal cell carcinoma growth [273]. ABT-737 functions by inducing apoptosis; however, its efficacy is often limited by MCL-1, a pro-survival protein. It has been demonstrated that MLN0128/INK128 is capable of reducing MCL-1 levels, thereby overcoming resistance and synergizing with BH3 mimetics such as ABT-737 to trigger cell death.

uPAR-targeting therapeutics. Urokinase-type plasminogen activator receptor (uPAR, CD87) is a frequently induced marker of senescence. It has been demonstrated to be a viable target for the selective elimination of senescent cells [274]. A multitude of uPAR-targeted antitumor therapeutic agents have been developed, including nanoplatforms that carry therapeutic agents, photodynamic therapy/photothermal therapy platforms, oncolytic virotherapy, gene therapy technologies, monoclonal antibody therapy, and tumor immunotherapy. These agents aim to suppress tumor growth, metastatic processes, and drug resistance [275]. Preliminary investigations revealed that the administration of chimeric antigen receptor (CAR) T cells targeting uPAR led to the effective reversal of fibrotic processes in murine models. Subsequent integrative profiling has demonstrated that uPAR is frequently expressed across a broad spectrum of solid tumors, with a notable prevalence in those harboring TP53 alterations and RAS pathway hyperactivation. In such conditions, malignant cells adopt a progenitor-like transcriptional state, which is sustained by a specialized stromal niche that is both uPAR-positive and exhibits hallmarks of senescence. The administration of human uPAR CAR-T cells has been demonstrated to effectively eliminate both the tumor parenchyma and their stromal infrastructure. This has led to the induction of prolonged remissions in a variety of preclinical models, the eradication of widespread metastatic outgrowths, and the enhancement of activity when utilized in conjunction with senescence-inducing pharmacological agents. Most critically, this robust therapeutic index does not translate into persistent myelotoxicity, even in humanized murine hosts reconstituted with a functional human immune repertoire [276]. A method of adaptive therapy involving the use of senolytic CAR-T cells that express uPAR-specific CAR has been successfully applied to target senescent cells and ameliorate the progression of lung cancer in vivo [277].

Senomorphics

Ruxolitinib. The JAK2 inhibitor ruxolitinib has been shown to suppress the JAK2/STAT3 signaling pathway, exhibiting a selective effect that reduces the immunosuppressive component of SASP induced by the Aurora kinase inhibitor alisertib (Ali), while preserving the immunogenic potential of senescent cells. The investigation yielded promising results, with the use of Ali-based nanocrystals in conjunction with the JAK2 inhibitor ruxolitinib (Rux) (Ali-Rux) demonstrating a notable efficacy. Immunization with Ali-Rux-orchestrated senescent cells induced TME activation, characterized by the activation of antigen-presenting cells, an increase in the recruitment and activation of CD8⁺ T and NK cells, and a decrease in MDSCs. When combined with PD-L1 blockade, Ali-Rux induced a robust antitumor immune response, a promising development for the field of immunotherapy [278].

NF- κ B inhibitors. NF- κ B inhibitors have a similar effect: metformin (a widely used antidiabetic drug) can weaken IKK-NF- κ B activation, reducing the secretion of IL-6 and IL-8, and glucocorticoids cortisol and corticosterone, which can inhibit NF- κ B by reducing IL-6 and IL-1 α secretion [279, 280].

An interesting observation is metformin's ability to modulate SASP factors induced by the CDK4/6 inhibitor LY2835219 by suppressing the mTOR and STAT3 signaling cascades. LY2835219-induced SASP enhanced the stemness of head and neck squamous cell carcinoma cells; however, this phenomenon was attenuated when the drug was combined with metformin [281].

Metformin's anti-proliferative activity against TNBC has previously been demonstrated; its effect on TNBC stemness remains an open question and may be the subject of further study [282]. Metformin has been shown to inhibit the proliferation of cancer cells that overexpress MYC and exhibit a senescent phenotype. This finding has the potential to inform the development of new targeted therapies for patients with triple-negative BC [283].

Tocilizumab. The use of the monoclonal antibody tocilizumab has been demonstrated to result in the inhibition of IL-6, as evidenced by the notable outcomes observed in clinical trials conducted on patients diagnosed with BC. Tocilizumab suppresses the pro-carcinogenic effects of BC-associated fibroblasts through inhibition of the STAT3/AUF1 pathway [284]. The administration of tocilizumab followed by cisplatin and docetaxel for patients with locally advanced TNBC resulted in a complete response (pCR) rate of 100% in patients with tumors bearing BRCA1/2 mutations [285].

Rapamycin. An additional target is the mTOR pathway. It has been demonstrated that rapamycin and other gerostatics do not induce cell death in senescent

cells; rather, these compounds have been shown to retard cell growth, gerogenesis and oncogenesis. Gerostatics primarily act on non-senescent cells, reducing their hyperfunction and slowing their geroconversion to senescence [286]. As demonstrated by Lewis et al., the reversal of the phenotypic changes in the senescent stroma with rapamycin prevents the initiation of carcinoma [287]. In addition, it has been shown that rapamycin impedes the capacity of senescent fibroblasts to promote tumor proliferation in murine models [288]. It has been demonstrated that rapamycin (sirolimus) and its analogs (rapalogs) modulate the SASP by suppressing protein synthesis and reducing the expression of NF- κ B components. Recent studies have demonstrated the potential of rapamycin to mitigate the pro-oncogenic effects of SASP, which includes the stimulation of precancerous cell proliferation and angiogenesis. This finding suggests that rapamycin may have a role in combination therapy with antitumor agents [289, 290].

Vaccines. SenoVaxTM is the most advanced candidate. The vaccine is of the autologous type, and it is generated by loading the patient's dendritic cells with a lysate derived from senescent fibroblasts. In preclinical studies, SenoVaxTM demonstrated the capacity to impede the proliferation of lung, breast, pancreatic, and glioma tumors in murine models. The mechanism of action of this agent involves the activation of CD8⁺ T cells, which in turn eliminate senescent cells within the TME. In July 2024, Immorta Bio submitted an Investigational New Drug application (IND #30745) to initiate clinical trials for the treatment of non-small cell lung cancer. The vaccine has also demonstrated a synergistic effect with immune checkpoint inhibitors (anti-PD-L1, anti-CTLA-4) [291].

Discussion

A thorough review confirms that cellular senescence has a significant duality in the breast TME. This duality fundamentally challenges conventional therapies. [14, 292]. On the one hand, it is well established that senescence induction acts as a tumor-suppressive defense. Conversely, it has been elucidated how senCAFs entirely circumvent this regulatory process, thereby engendering an inflammatory, immunosuppressive, and chemoresistant niche that actively promotes tumor progression [110, 239, 293-295]. This ambivalence presents a central therapeutic conundrum that the field has only recently begun to appreciate: standard-of-care genotoxic chemotherapy and radiotherapy, while effectively targeting proliferating tumor cells, invariably generate a substantial pool of therapy-induced senCAFs in the stromal compartment. Consequently, the TME undergoes a paradoxical shift toward a pro-tumorigenic

state following initial treatment. This provides the mechanistic rationale for targeting therapy-induced senescence sequentially rather than perceiving it as an inert bystander effect.

A critical layer of complexity, one that has been largely overlooked in BC research until recently, stems from the profound phenotypic heterogeneity of senCAFs. SenCAFs are not a uniform population defined only by growth arrest and SA- β -Gal. Instead, they form a dynamic spectrum of functional states that partly overlap with, but sometimes diverge from, known CAF subtypes. Recent high-parameter single-cell studies have identified a distinct subpopulation of senescence-like myofibroblasts characterized by TSPAN8 expression (TSPAN8⁺ myCAFs) in BC. The clinical significance of this population is noteworthy, as their prevalence is associated with chemotherapy resistance and diminished patient survival. Mechanistically, these cells operate through a dual-axis system. The secretion of SASP factors, particularly IL-6 and IL-8, maintains tumor cell stemness via JAK/STAT3 signaling. Concurrently, the deposition of a dense, fibronectin-rich ECM network physically impedes drug penetration and provides integrin-mediated survival signals that confer chemoresistance [96]. This functional duality within a single senescent subpopulation demonstrates the limitations of the simplistic view of SASP as solely pro-inflammatory. Concurrently, Ye et al. (2024) corroborated these findings by identifying senescent myofibroblastic CAFs in both mouse and human mammary tumors. These CAFs were found to be capable of suppressing NK cell activity through the secretion of ECM proteins. Consequently, the presence of senescent myCAFs has been associated with the development of an immunosuppressive TME and a poor disease course [110].

The findings from gastrointestinal tumors offer additional evidence that reinforces the tissue-specific characteristics of senCAF biology. This highlights the significant implications for the extrapolation of findings from other tumor types to BC. In the context of gastric cancer, senCAFs have been shown to promote peritoneal tumor growth through an epigenetic mechanism involving H3K27me3 demethylation and subsequent activation of the JAK/STAT3 pathway. This finding underscores the pivotal role of chromatin remodeling in sustaining a pro-tumorigenic SASP program [296]. In the context of colorectal cancer, a distinct mechanism becomes evident: IL-1 α , secreted following irradiation, triggers a DNA damage response in adjacent fibroblasts, polarizing them toward a pro-inflammatory, senescent-like phenotype that paradoxically confers resistance to subsequent therapy [297]. In a recent study, Meguro et al. expanded the anatomical scope of this phenomenon by identifying p16^{high}-senCAFs in the aged bladder. These cells forge a

cancer-permissive niche through CXCL12 secretion, suggesting that age-associated accumulation of senescent fibroblasts may pre-condition tissues for aggressive tumor behavior [298]. For BC specifically, a key future task is to map specific senCAF phenotypes to existing BC CAF taxonomies, such as the widely used Costa classification (S1-S4). This task will provide a foundation for the entire field. In addition, it will be essential to decode how their distinct SASP repertoires differentially orchestrate immunosuppression, metabolic reprogramming, and drug resistance within the distinct microenvironmental niches of the breast.

Defining the senolytic window: a provisional, testable framework

As previously mentioned, a biological rationale underlies the "one-two punch" strategy in breast cancer. In brief, the state of cellular senescence in a fraction of tumor and stromal cells is induced by chemotherapy or targeted therapy. The subsequent senolytic treatment aims to eliminate these cells to prevent SASP-mediated tumor-promoting effects, including EMT induction, extracellular matrix remodeling, and metastatic niche formation. A critical yet unresolved parameter in this strategy pertains to the temporal relationship between senolytic administration and the induction of senescence [14, 253, 254]. The requirements are conceptually straightforward: the senolytic would ideally be administered after a sufficient senescent cell burden has accumulated, but before irreversible SASP-mediated remodeling of the tumor microenvironment has consolidated. The challenge lies in translating this conceptual requirement into measurable, testable criteria [15, 57, 299].

To the best of our knowledge, no published studies have systematically compared different post-induction time points for senolytic administration in immunocompetent models of residual breast cancer. Furthermore, there is a need to establish validated biomarker thresholds that define the opening and closing of the therapeutic window. The absence of such data elucidates the rationale behind initially conceptualizing the timing problem as a knowledge gap rather than a readily solvable parameter, as delineated in the Limitations section. Nevertheless, a provisional framework can be articulated based on available kinetic data from related models, with the explicit understanding that each element would need to be tested prospectively before any clinical application.

Temporal criterion

The most fundamental parameter is the interval between the senescence-inducing regimen and the initial senolytic dose. Preclinical studies employing DNA-damaging

agents in various epithelial tumor models have indicated that senescence-associated markers, including SA- β -Gal activity, p21 and p16 expression, and SASP component secretion, can become detectable within approximately 48 to 72 hours after treatment. However, in many models, these markers do not reach a plateau until approximately one week post-exposure [164, 172, 300]. Concurrently, the initial phase following treatment is characterized by a predominance of apoptotic cell death and acute DNA damage signaling. In this context, the administration of a senolytic during the acute phase is predicted to target a limited number of senescent cells while potentially interfering with p53-dependent apoptotic clearance or normal tissue repair processes. Consequently, the lower boundary of the window appears to be delineated by the transition from acute damage response to established senescence. This transition, based on the kinetics mentioned above, is unlikely to occur before day 3 to 5 post-chemotherapy in most models.

The upper boundary is less easily defined and likely depends on the rate of SASP-mediated remodeling. In models in which senescent fibroblasts or tumor cells persist, measurable changes in the microenvironment have been reported to emerge within approximately two to four weeks of senescence induction. These changes include collagen deposition, fibroblast activation, or inflammatory infiltration [7, 56, 138]. This suggests that the window for intervention may be narrow, on the order of one to three weeks after the last chemotherapy dose. However, this estimate is derived from heterogeneous models and should not be mistaken for a validated parameter. The central point is that the temporal window would need to be mapped empirically for each specific chemotherapy-senolytic combination and for each tumor subtype, since senescence induction efficiency and SASP composition can vary substantially across contexts.

This finding indicates that the timeframe for intervention may be constrained, with a window of opportunity ranging from one to three weeks following the final chemotherapy administration. However, it is important to note that this estimate is derived from heterogeneous models and should not be mistaken for a validated parameter. The central point of this study is that the temporal window would need to be mapped empirically for each specific chemotherapy-senolytic combination and for each tumor subtype, since senescence induction efficiency and SASP composition can vary substantially across contexts.

Biomarker logic

The dearth of a reliable method to measure senescent cell burden in real time is widely regarded as a principal barrier to operationalizing the window. Tissue biopsy

remains the most direct approach; however, it is an invasive procedure that is subject to sampling error and is not well suited to repeated monitoring. A potentially more pragmatic approach involves the utilization of the secretome of senescent cells. The release of SASP factors, such as IL-6 and IL-8, into the extracellular space can be measured in serum. [301]. However, serum levels of SASP factors are frequently associated with underlying inflammatory conditions and thus cannot be considered a reliable indicator of senescence per se [302]. Consequently, the development of plasma assays for SASP signatures linked to senCAFs in a given tumor type, such as the panel comprising CXCL9, RANKL, B7H3, HGF, and TGF- β for BC senCAFs, alongside the assessment of their dynamic alterations with disease progression, represents a promising avenue.

The rationale underpinning this approach is uncomplicated; however, it would necessitate meticulous validation. The anticipated pattern is a transient decline in circulating SASP factors immediately following chemotherapy due to tumor cytoreduction, followed by a secondary rise reflecting the emergence of the senescent compartment. The inflection point of this secondary rise has the potential to define the opening of the window. Conversely, the sustained decline of the same panel after senolytic treatment would indicate successful elimination of the target population and closure of the window.

The hypothesis that circulating senescence-associated microRNAs, particularly miR-34a and miR-146a, can serve as complementary markers has been proposed [303-306]. Their potential advantage lies in their relative stability in circulation and the possibility of multiplexed detection. However, neither the SASP protein panel nor the miRNA approach has been validated as a specific indicator of senescent burden in the post-chemotherapy setting. Both are subject to confounding by non-senescent inflammatory processes, and their kinetics may lag behind the cellular events they are intended to monitor. Any clinical use of these biomarkers would therefore depend on prior demonstration of a quantitative correlation between blood levels and tissue senescent cell burden in appropriate preclinical models.

Imaging approaches

Non-invasive imaging of senescent cells is an area of active investigation, though, to our knowledge, no tracer has yet entered routine clinical use. Several groups have developed small-molecule probes activated by lysosomal β -galactosidase, capitalizing on the elevated lysosomal activity observed in senescent cells [307-309]. Other approaches target BCL-2 family proteins, which can be upregulated in senescent cells and are thought to contribute to their resistance to apoptosis [28, 59; 262;

310]. The utilization of positron emission tomography with tracers has the potential to yield spatial and temporal data concerning the distribution of senescent cells. However, it should be noted that the majority of these agents have been evaluated in proof-of-concept settings. The extent of their sensitivity, specificity, and safety profile in the context of chemotherapy-treated patients remains to be elucidated. Diffusion-weighted magnetic resonance imaging offers a less specific but more readily available alternative. Changes in tissue cellularity and stromal inflammation associated with SASP may be detectable as alterations in apparent diffusion coefficient values. To our knowledge, this possibility has not been systematically explored in the context of therapy-induced senescence. Rather, it should be considered an open question.

Stop criteria

The definition of the therapeutic window necessitates not only an opening criterion but also a criterion for closure. In a preclinical setting, this phenomenon could be defined empirically by the absence of senescent cells in tissue sections after treatment. In a clinical context where tissue access is limited, a decline in the same circulating SASP panel below a pre-specified threshold would serve as a pragmatic surrogate. Furthermore, standard toxicity parameters (liver function tests, renal function, hematological recovery) must be incorporated into any decision rule, given that current senolytics are not fully selective for tumor-associated senescent cells and could also affect senescent cells in normal tissues. The safety margin is expected to depend on the senolytic agent used and the tissue reservoir of senescent cells at the time of dosing.

This safety consideration is not merely hypothetical. A number of senolytic agents are currently under investigation. These include the BCL-2 family inhibitor navitoclax, which has been associated with dose-limiting thrombocytopenia. In addition, combinations such as dasatinib plus quercetin carry a documented risk of hepatotoxicity [311]. These toxicities are of particular concern in the post-chemotherapy setting, where hepatic and hematopoietic reserves may have already been compromised by prior cytotoxic treatment. Any proposal to administer senolytics in this context, especially when such use would fall outside approved indications, must therefore be preceded by a rigorous assessment of risk versus benefit. The rationale for off-label use cannot be based solely on biological plausibility. The necessity of this approach is predicated on the requirement of substantiating that the anticipated enhancement in tumor control or the prevention of metastasis would be of greater benefit than the potential for organ injury induced by the

pharmaceutical agent, when considered within a patient population that is already exhibiting vulnerability.

The aforementioned framework is not intended to function as a clinical protocol; rather, it is designed to serve as a structured set of hypotheses for preclinical testing. The temporal criterion would need to be mapped empirically for each combination therapy. The validation of biomarkers must precede any effort at clinical translation. While imaging methods hold great promise, they are still in the investigational stage. It is imperative that the criteria for cessation encompass both efficacy surrogates and safety parameters. The framework under discussion provides a language and a set of experimental questions. The fundamental unresolved issue remains unchanged: the reliable *in vivo* detection of the post-chemotherapy accumulation of senescent cells in real time, and the evaluation of whether interventions scheduled to coincide with this detection yield superior outcomes in comparison to fixed-schedule administration. Until these questions are addressed in suitable models, the clinical applicability of the "one-two punch" strategy remains a hypothesis rather than a recommendation.

Limitations

However, these efforts are hampered by methodological limitations that are widespread in the senescence literature and underlie the field's reproducibility concerns. The fundamental problem is the absence of a universal, specific, and context-independent senescence marker. The classical triad of p16INK4a, p21WAF1/Cip1, and SA- β -Gal activity, while operationally useful, is highly contingent on cell type, senescence inducer, and temporal stage. It is noteworthy that each of these markers can be expressed in non-senescent contexts. For instance, p16 is overexpressed during normal aging and in response to certain stress stimuli without full senescence commitment. In addition, p21 functions as a canonical p53 target in transient cell cycle arrest, and SA- β -Gal reflects lysosomal activity rather than senescence *per se* [25, 176, 299]. This ambiguity gives rise to systematic misidentification and likely explains some of the contradictory findings regarding the abundance and role of senCAFs across different BC subtypes. Overcoming this challenge necessitates a transition from single-marker histology to comprehensive, multi-omics approaches that capture the multidimensional nature of the senescent state. A multifaceted approach has been proposed, integrating single-cell transcriptomics, spatially resolved multiplexed immunohistochemistry, quantitative SASPome proteomic profiling, and machine learning-based senescence classifiers. These classifiers, including SenPred, which predicts senescence from transcriptomic data; DeepScence, which uses deep learning on morphological

features; and scCamAge, which estimates senescence burden at single-cell resolution, are critical to establish a consensus "senescence signature" for both tumor and stromal cells, including immune cells. A signature of this nature would serve as the foundation for reliable patient stratification, enabling clinicians to distinguish between patients whose tumors harbor "therapeutic" senescent epithelial cells versus "pathogenic" senCAFs - a distinction that currently cannot be made in clinical practice [160, 162, 260].

From a therapeutic standpoint, the "one-two punch" concept remains the most mechanistically sound and clinically promising strategy for translating senescence biology into oncology practice. The initial therapeutic approach generally consists of standard chemotherapy, radiotherapy, or targeted cell cycle inhibitors, such as CDK4/6 inhibitors (e.g., palbociclib, ribociclib), which have been shown to induce robust senescence in tumor cells and adjacent fibroblasts. For the second strike, candidates include BCL-2 family inhibitors, which target the anti-apoptotic dependency acquired by senescent cells [313-315]. ABT-199 (venetoclax), a BCL-2-selective inhibitor, has demonstrated the capacity to selectively eradicate senCAFs in preclinical BC models. This results in the reactivation of CD8⁺ T-cell-mediated tumor immunity and the reversal of the immunosuppressive milieu established during the first phase of treatment. ABT-263 (navitoclax), a more broadly effective BCL-2/BCL-xL/BCL-W inhibitor, demonstrated antitumor efficacy in models of triple-negative BC when administered sequentially with the CDK4/6 inhibitor palbociclib [260]. A critical factor influencing the efficacy of this approach is the temporal window of senolytic administration: delivery must be timed for the post-induction phase, after tumor cyto-reduction but before irreversible TME remodeling by senCAFs. A provisional framework has been delineated for the potential definition of this window, employing temporal, biomarker, and imaging criteria. However, the validity of these criteria remains unconfirmed through experimental validation. The central unresolved issue remains the absence of pharmacokinetic-pharmacodynamic models that quantitatively relate senescent cell burden, SASP kinetics, and the timing of senolytic intervention. Absent such models, even the most meticulously designed provisional framework remains speculative, and its translation to clinical practice is not currently substantiated [316].

However, the clinical translation of this strategy is constrained by several limitations and controversies that require further investigation before widespread clinical adoption. First-generation senolytics that target a single SCAP (senescent cell anti-apoptotic pathway) node, such as BCL-2 family inhibitors, demonstrate a restricted range

of action against the heterogeneous landscape of senescent cell types within a tumor, and their significant off-target toxicities are well-documented. The thrombocytopenia and neutropenia caused by navitoclax, even upon brief exposure, result from its on-target BCL-xL inhibition in platelets and neutrophils, respectively [258, 317]. This toxicity is mechanism-based and unlikely to be fully resolved by dosing schedule modifications alone. Therefore, it was proposed that a poly-pharmacology approach - lightly impacting multiple SCAP nodes simultaneously with lower, sub-toxic doses of combined agents - may achieve broader and more effective senolysis with significantly improved tolerability [255]. This principle has proven successful in antimicrobial combination therapy, where multi-target engagement prevents the emergence of resistance. Secondly, and more concerning from a clinical safety perspective, the pharmacological effects of senolytics can be paradoxical and profoundly context-dependent. In a colorectal cancer model, the dasatinib/quercetin senolytic combination elevated IL-6 levels and promoted both migration and EMT, an effect opposite to that of navitoclax in the same conditions [318]. This disturbing finding suggests that certain senolytic regimens may inadvertently convert a relatively contained tumor into a more invasive and metastatic phenotype. The mechanistic basis for this observation remains obscure but may involve differential targeting of senescent subpopulations or off-target kinase inhibition by dasatinib. This underscores the absolute necessity of a profound mechanistic understanding prior to clinical application and highlights the potential of bioinformatics and mathematical modeling to prospectively predict such adverse interaction networks, thereby informing the rational design of safer senolytic combinations.

As a therapeutic alternative or complement to senolysis, senomorphics - small molecules that suppress the deleterious SASP without physically eliminating senescent cells - offer a conceptually distinct approach with a different risk-benefit profile. Key senomorphics under investigation include JAK2 inhibitors such as ruxolitinib, which attenuate STAT3-mediated SASP transcription, and NF-κB inhibitors such as metformin, which reduce the expression of pro-inflammatory cytokines including IL-6 and IL-8 that are central to the pro-oncogenic effects of senCAFs [253, 319]. By circumventing the induction of apoptosis, senomorphics effectively evade the theoretical risk of overwhelming tissue clearance capacity in the event of massive stromal cell death. This outcome, which could potentially occur in tumors with an exceptionally high senescent cell burden, is averted by the aforementioned mechanism. However, this advantage is accompanied by a significant limitation: because senomorphics suppress the immune-surveillance

signals that typically alert NK cells and macrophages to identify and eliminate senescent cells, their prolonged use could theoretically result in the buildup of senescent cell that evade immune recognition yet remain metabolically active [320]. These persistent cells may potentially evade SASP suppression or undergo secondary mutations that restore their pro-tumorigenic activity. This concern renders the combination of senomorphics with immunotherapy a rational, and arguably essential, strategy to ensure eventual immune-mediated clearance of the suppressed senescent population. Indeed, recent preclinical research has validated the concept of this approach, demonstrating that nanoparticle-mediated co-delivery of the Aurora kinase inhibitor alisertib (to induce senescence) and ruxolitinib (to suppress SASP) not only induces tumor senescence but also, crucially, enhances the response to anti-PD-L1 immune checkpoint blockade [278, 321]. This suggests a three-component therapeutic axis: senescence induction, SASP modulation, and immune reactivation.

A primary constraint of senolytic and senomorphic therapies is the recognition that senescence is not exclusively a pathological process. It plays critical physiological roles in embryonic development, wound healing, tissue regeneration, and tumor suppression. SASP-mediated intercellular communication has been demonstrated to play a pivotal role in tissue repair, regeneration, and development. Transient SASP has been demonstrated to play a role in wound healing, while its chronic activation has been associated with inflammation, fibrosis, and tumorigenesis [322-324].

A multitude of pivotal SASP factors, including IL-6, TGF- β , and MMPs, have been shown to fulfill indispensable physiological functions that extend beyond the process of senescence. IL-6 has been demonstrated to play a multifaceted role in biological processes, including the maintenance of cell cycle arrest during senescence, the regulation of normal immune responses, and the promotion of tissue homeostasis. TGF- β and MMPs, which are components of the SASP, regulate extracellular matrix remodeling, which is necessary for both normal tissue homeostasis and reparative processes. This creates serious limitations for senomorphics that modulate the SASP. The majority of senomorphic compounds exhibit pleiotropic effects, acting on multiple pathways beyond the SASP. The suppression of the SASP may inadvertently disrupt beneficial immune responses or cellular stress responses necessary for maintaining tissue integrity.

A similar set of problems has been identified in the context of senolytics, a class of compounds that are designed to eliminate senescent cells. These agents have the potential to eliminate not only pathological SASP-secreting cells but also beneficial senescent cells that play

a role in tissue repair, regeneration, or tumor suppression. In the skin or liver, transient senescence coordinates reparative processes through SASP-mediated recruitment of immune and progenitor cells. Premature removal of such cells may impair healing or lead to excessive fibrosis. The indiscriminate elimination of senescent cells has the potential to disrupt regenerative responses, tissue homeostasis, or even increase the risk of carcinogenesis in certain contexts.

Consequently, the translation of senolytic and senomorphic therapies into clinical practice necessitates a sophisticated comprehension of the context-dependent functions of SASP factors. Therapeutic strategies must aim to selectively modulate pathological SASP components while preserving their essential physiological roles in endocrine regulation, immune function, and extracellular matrix homeostasis.

In the future, therapeutic development must prioritize precision targeting strategies to overcome the systemic toxicity that currently limits the clinical utility of senolytic agents. Promising innovations include second-generation senolytics that exploit the characteristic increase in lysosomal mass and β -galactosidase activity observed in many, though not all, senescent cells. Galacto-oligosaccharide-coated nanoparticles loaded with cytotoxic payloads and β -galactosidase-activated prodrugs - exemplified by nav-Gal, a galactose-conjugated derivative of navitoclax that is preferentially cleaved and activated within SA- β -Gal^{high} senescent cells - offer a means of achieving tumor-localized senolysis while sparing normal tissues [325, 326]. An alternative strategy that has shown equal promise involves the exploitation of the heightened lysosomal ATPase dependency of certain senescent cell types [300]. Furthermore, some senescent cells including fibroblasts exhibit a profound dependency on glutamine metabolism as a compensatory pH-buffering system to neutralize the acidity generated by their hyperactive lysosomes. Pharmacological inhibition of glutaminase renders these cells acutely vulnerable to apoptosis [300]. An alternative approach, characterized by its distinctiveness and the sophistication of its immunological mechanisms, involves the use of immune-mediated clearance through the employment of chimeric antigen receptor CAR-T- or NK cells that are directed against specific surface markers associated with senescence [327]. The most prominent candidates include uPAR, which is extensively upregulated on senescent cells of various lineages, and DPP4, which identifies a subset of senescent fibroblasts. A notable conceptual convergence merits highlighting: senCAFs directly secrete factors, including ECM proteins and soluble ligands, that actively suppress NK cell toxicity and promote NK cell exhaustion [328]. Consequently, the targeted elimination of these cells via

CAR-NK or CAR-T therapy possesses the dual capacity of eliminating a source of immunosuppressive SASP and physically eradicating the cells responsible for NK cell inhibition [329, 330]. This process disrupts a pivotal mechanism of resistance to immune checkpoint inhibitors. The observed synergy in preclinical models signifies the necessity for meticulous, mechanism-guided preclinical and clinical investigation. The integration of senescence-modulating therapy into standard BC treatment regimens, encompassing appropriate combinations of chemotherapy, senolytics, senomorphics, and immunotherapy, represents a new and largely unexplored therapeutic frontier.

Beyond adoptive cell therapies, vaccine-based approaches constitute a conceptually distinct immunological strategy. The same senescence-associated surface antigens that have been explored as CAR targets, most notably uPAR, have the potential to function as immunogens, thereby stimulating endogenous T-cell responses against senCAFs. Recent findings have provided direct proof-of-principle for a uPAR-targeted peptide vaccine developed by SenoVax. This vaccine has been shown to reduce senescent cell burden, SASP-associated inflammation, and age-related fibrosis [291]. Earlier research on fibroblast activation protein (FAP)-targeted vaccination had already demonstrated that the stromal compartment, despite lacking classical tumor neoantigens, can be subjected to vaccine-induced T-cell attack [277, 331]. However, extrapolating these findings to senCAFs is hindered by significant obstacles. To date, no antigen has been identified that is exclusively expressed in senescent cells. The markers currently in use are also expressed in non-senescent activated fibroblasts and other cell types. Furthermore, senCAFs have been shown to actively suppress antigen presentation and T-cell function through SASP-derived factors. This suggests that a vaccine-induced response would need to overcome the very immunosuppressive environment it seeks to eliminate. The potential for off-target immune reactivity against non-senescent stromal cells remains an unresolved issue. The potential for addressing these barriers through antigen engineering, combinatorial immunomodulation, or the identification of more specific senCAF markers remains an open question that requires dedicated investigation.

From the perspective of evaluating combined therapeutic approaches, it is essential to develop and utilize heterotypic 3D breast cancer models comprising tumor cells and fibroblasts. These models facilitate the reflection of the regulatory function of CAFs in shaping the response of the tumor microenvironment to immunotherapeutic interventions [332].

Conclusion

Therefore, the role of cellular senescence in breast tumor progression is a subject of controversy. On the one hand, senescence impedes early carcinogenesis. On the other hand, senescence drives later progression via senCAFs, which establish an immunosuppressive niche through SASP factors and ECM proteins. The transition to a senCAF state is induced by both intrinsic stimuli (replicative exhaustion) and extrinsic challenges, including chemotherapy and radiotherapy. Despite the growth arrest exhibited by these subpopulations, TSPAN8+ myCAFs have been observed to promote tumor progression, thus providing novel therapeutic targets for investigation.

The identification of senescent cells remains methodologically complex. Canonical markers such as SA- β -Gal, p16, and p21, along with SASP components IL-6 and CXCL8 vary with cell type, senescence inducer, and stage, and are also expressed in some non-senescent contexts [333, 334]. This variability extends to in vivo settings, where single-cell transcriptomic analyses of senescent murine and human tissues revealed substantial divergence in p16Ink4a and p21Cip1 expression across cell types and anatomical locations, suggesting that senescence is a multifaceted process with distinct molecular characteristics rather than a single uniform state [335]. Distinct transcriptional features of primary and secondary senescence have also been demonstrated in mouse models, regulated by ERK and p38 MAPK pathway activation, respectively [336]. Reliable identification therefore requires a combined assessment of morphology, critical CDK inhibitors, SA- β -Gal activity, SAHF, and SASP components.

Cellular senescence plays a controversial role in breast tumor progression: it impedes early carcinogenesis yet drives later progression via senCAFs, which establish an immunosuppressive niche through SASP factors and ECM proteins. The transition to a senCAF state is induced by both intrinsic stimuli (replicative exhaustion) and extrinsic challenges, including chemotherapy and radiotherapy. Despite their growth arrest, subpopulations such as TSPAN8+ myCAFs actively promote tumor progression, offering new therapeutic targets.

In light of this duality, a promising experimental strategy is the "one-two punch" approach, which involves therapy-induced senescence followed by selective senolysis using agents such as venetoclax or navitoclax. Alternatively, senomorphics (e.g., ruxolitinib, metformin) have been demonstrated to suppress the SASP without eliminating senescent cells; however, impaired immune surveillance may permit the accumulation of uncleared senescent cells. This limitation provides a rationale for the combination of senomorphics with immunotherapy.

Notably, the secretion of factors by senCAFs that directly suppress NK cell activity suggests a potential for overcoming resistance to immune checkpoint inhibitors through their elimination. Future work must overcome systemic toxicity. Tumor-specific senolytic prodrugs and immune-mediated clearance (e.g., CAR-T/NK cells against uPAR or DPP4) are promising approaches.

Acknowledgements

The schematic figures were created using [BioRender.com](https://www.biorender.com).

Funding

The sections: Introduction, Role of senescence in the stromal microenvironment of breast cancer, Senescence inducers, The tumor microenvironment: diversity, plasticity, and identification of senescent CAFs, Directions of the senescence targeting therapy, Discussion and Conclusion, (authors: Maria Bogachek, David Sergeevichev, Anastasia Leonteva, Alevtina Pinova and Anna Nushtaeva) were written using funds from the project implemented within the framework of the state program of the “Sirius” federal territory, Scientific and technological development of the «“Sirius” Federal Territory» (Agreement No. 27-03 dated 27 September 2024); writing of the sections: Classification of senCAFs (authors: Vladimir Richter) was supported by the Russian state-funded project for ICBFM SB RAS (grant number 125012900932-4). Financial support for publications is divided between the Scientific Centre of Genetics and Life Sciences at Sirius University of Science and Technology (90% under the state programme of the ‘Sirius’ federal territory, Agreement No. 27-03, dated 27 September 2024) and the Institute of Chemical Biology and Fundamental Medicine at the Siberian Branch of the Russian Academy of Sciences (10% under RSFP 125012900932-4).

Author contributions

Conceptualization, M.B. and A.N.; writing—original draft preparation, M.B., A.L. and A.P.; writing—review and editing, M.B., A.N., and D.S.; supervision, V.R. and A.N.; project administration, V.R. and A.N.; funding acquisition, V.R. and A.N. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2026.0869

References

- [1] Kim J, Harper A, McCormack V, Sung H, Houssami N, Morgan E, et al. (2025). Global patterns and trends in breast cancer incidence and mortality across 185 countries. *Nat Med*, 31:1154-1162.
- [2] Ruan G, Wang X, Ou H, Guo D (2025). Cancer-associated fibroblasts: dual roles from senescence sentinels to death regulators and new dimensions in therapy. *Front Immunol*, 16:1635771.
- [3] Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, et al. (2018). Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell*, 33:463-479.e10.
- [4] Ilyina A, Leonteva A, Berezutska E, Abdurakhmanova M, Ermakov M, Mishinov S, et al. (2025). Exploring the heterogeneity of cancer-associated fibroblasts via development of patient-derived cell culture of breast cancer. *Int J Mol Sci*, 26:7789.
- [5] Ajoalabady A, Pratico D, Bahijri S, Eldakhakhny B, Tuomilehto J, Wu F, et al. (2025). Hallmarks and mechanisms of cellular senescence in aging and disease. *Cell Death Discov*, 11:364.
- [6] Demirci D, Dayanc B, Mazi FA, Senturk S (2021). The Jekyll and Hyde of cellular senescence in cancer. *Cells*, 10:208.
- [7] Ruhland MK, Loza AJ, Capietto AH, Luo X, Knolhoff BL, Flanagan KC, et al. (2016). Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. *Nat Commun*, 7:11762.
- [8] Wang B, Han J, Elisseeff JH, Demaria M (2024). The senescence-associated secretory phenotype and its physiological and pathological implications. *Nat Rev Mol Cell Biol*, 25:958-978.
- [9] Sahu P, Satapathy T (2025). Immunopharmacology of senescence: targeting the senescence-associated secretory phenotype (SASP)—a mechanism-based review. *Inflammopharmacology*, 33:4291-4310.
- [10] Chambers CR, Ritchie S, Pereira BA, Timpson P (2021). Overcoming the senescence-associated secretory phenotype (SASP): a complex mechanism of resistance in the treatment of cancer. *Mol Oncol*, 15:3242-3255.
- [11] Flanagan KC, Alspach E, Pazolli E, Parajuli S, Ren Q, Arthur LL, et al. (2017). c-Myb and C/EBP β regulate OPN and other senescence-associated secretory phenotype factors. *Oncotarget*, 9:21-36.
- [12] Ma Z, Yu D, Tan S, Li H, Zhou F, Qiu L, et al. (2025). CXCL12 alone is enough to reprogram normal fibroblasts into cancer-associated fibroblasts. *Cell Death Discov*, 11:156.
- [13] Li X, Bu W, Meng L, Liu X, Wang S, Jiang L, et al. (2019). CXCL12/CXCR4 pathway orchestrates CSC-like properties by CAF recruited tumor associated macrophage in OSCC. *Exp Cell Res*, 378:131-138.

- [14] Schmitt CA, Wang B, Demaria M (2022). Senescence and cancer — role and therapeutic opportunities. *Nat Rev Clin Oncol*, 19:619-636.
- [15] Bektas A, Schurman SH, Sen R, Ferrucci L (2018). Aging, inflammation and the environment. *Exp Gerontol*, 105:10-18.
- [16] Zhou L, Ma B, Ruscetti M (2025). Cellular senescence offers distinct immunological vulnerabilities in cancer. *Trends Cancer*, 11:334-350.
- [17] Ness KK, Kirkland JL, Gramatges MM, Wang Z, Kundu M, McCastlain K, et al. (2018). Premature physiologic aging as a paradigm for understanding increased risk of adverse health across the lifespan of survivors of childhood cancer. *J Clin Oncol*, 36:2206-2215.
- [18] Gibbs C, So JY, Ahad A, Michalowski AM, Son DS, Li Y (2022). CXCL14 attenuates triple-negative breast cancer progression by regulating immune profiles of the tumor microenvironment in a T cell-dependent manner. *Int J Mol Sci*, 23:9314.
- [19] Sutanto H, Ahkami A, Fetarayani D, Romadhon PZ (2025). A narrative review of therapy-induced senescence in cancer: mechanisms, immune interplay, and therapeutic opportunities. *J Egypt Natl Canc Inst*, 37:78.
- [20] Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. (2019). Cellular senescence: defining a path forward. *Cell*, 179:813-827.
- [21] Hayflick L (1965). The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res*, 37:614-636.
- [22] Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*, 88:593-602.
- [23] Zhang Y, Yang J (2011). The impact of cellular senescence in cancer therapy: is it true or not? *Acta Pharmacol Sin*, 32:1199-1207.
- [24] Wiley CD, Velarde MC, Lecot P, Liu S, Sarnoski EA, Freund A, et al. (2016). Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab*, 23:303-314.
- [25] Faust HJ, Zhang H, Han J, Wolf MT, Jeon OH, Sadtler K, et al. (2020). IL-17 and immunologically induced senescence regulate response to injury in osteoarthritis. *J Clin Invest*, 130:5493-5507.
- [26] Milanovic M, Fan DNY, Belenki D, Däbritz JHM, Zhao Z, Yu Y, et al. (2018). Senescence-associated reprogramming promotes cancer stemness. *Nature*, 553:96-100.
- [27] Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM (2014). Senescence and apoptosis: dueling or complementary cell fates? *EMBO Rep*, 15:1139-1153.
- [28] Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, et al. (2016). Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun*, 7:11190.
- [29] Dong Z, Luo Y, Yuan Z, Tian Y, Jin T, Xu F (2024). Cellular senescence and SASP in tumor progression and therapeutic opportunities. *Mol Cancer*, 23:181.
- [30] Guillon J, Petit C, Moreau M, Toutain B, Henry C, Roché H, et al. (2019). Regulation of senescence escape by TSP1 and CD47 following chemotherapy treatment. *Cell Death Dis*, 10:199.
- [31] Biran A, Zada L, Abou Karam P, Vadai E, Roitman L, Ovadya Y, et al. (2017). Quantitative identification of senescent cells in aging and disease. *Aging Cell*, 16:661-671.
- [32] Becker T, Haferkamp S (2013). Molecular mechanisms of cellular senescence. In: *Senescence and Senescence-Related Disorders*.
- [33] Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O (2009). Protocols to detect senescence-associated beta-galactosidase (SA- β -gal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc*, 4:1798-1806.
- [34] Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, et al. (2006). Senescence-associated β -galactosidase is lysosomal β -galactosidase. *Aging Cell*, 5:187-195.
- [35] Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA*, 92:9363-9367.
- [36] Bosmann HB, Hall TC (1974). Enzyme activity in invasive tumors of human breast and colon. *Proc Natl Acad Sci USA*, 71:1833-1837.
- [37] Wang N, Fang Y, Hou Y, Cheng D, Dressler EV, Wang H, et al. (2024). Senescent cells promote breast cancer cells motility by secreting GM-CSF and bFGF that activate the JNK signaling pathway. *Cell Commun Signal*, 22:478.
- [38] Gluchowska A, Bojakowski K, Janiszewska D, Staniak K, Dudkowska M, Mosieniak G (2025). A signature of senescence based on an analysis of selected markers in primary vascular smooth muscle cells cultured in vitro and derived from atherosclerotic plaque. *Aging Dis*. Advance online publication.
- [39] Kudlova N, De Sanctis JB, Hajduch M (2022). Cellular senescence: molecular targets, biomarkers, and senolytic drugs. *Int J Mol Sci*, 23:4168.
- [40] Wang G, Cheng X, Zhang J, Liao Y, Jia Y, Qing C (2021). Possibility of inducing tumor cell senescence during therapy (Review). *Oncol Lett*, 22:496.
- [41] Yan J, Chen S, Yi Z, Zhao R, Zhu J, Ding S, et al. (2024). The role of p21 in cellular senescence and aging-related diseases. *Mol Cells*, 47:100113.
- [42] Maheshwari M, Harioudh MK (2026). Subcellular compartmentalization dependent dichotomy of p21 in cancer. *Mol Biol Rep*, 53:848.
- [43] Abbas T, Dutta A (2009). p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer*, 9:400-414.
- [44] Al-Ansari MM, Hendrayani SF, Shehata AI, Aboussekhra A (2012). p16INK4A represses the paracrine tumor-promoting effects of breast stromal fibroblasts. *Oncogene*, 32:2356-2364.
- [45] McDermott MSJ, Conlon N, Browne BC, Szabo A, Synnott NC, O'Brien NA, et al. (2019). HER2-targeted tyrosine kinase inhibitors cause therapy-induced senescence in breast cancer cells. *Cancers*, 11:197.

- [46] Mirzakhani K, Kallenbach J, Rasa SMM, Ribaud F, Ungelenk M, Ehsani M, et al. (2021). The androgen receptor - lncRNASAT1-AKT-p15 axis mediates androgen-induced cellular senescence in prostate cancer cells. *Oncogene*, 41:943-959.
- [47] Halkoum R, Salnot V, Capallere C, Plaza C, L'honoré A, Pays K, et al. (2022). Glyoxal induces senescence in human keratinocytes through oxidative stress and activation of the protein kinase B/FOXO3a/p27KIP1 pathway. *J Invest Dermatol*, 142:2068-2078.e7.
- [48] Narita M, Nuñez S, Heard E, Narita M, Lin AW, Hearn SA, et al. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*, 113:703-716.
- [49] Kosar M, Bartkova J, Hubackova S, Hodny Z, Lukas J, Bartek J (2011). Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16INK4a. *Cell Cycle*, 10:457-468.
- [50] Rodier F, Muñoz DP, Teachenor R, Chu V, Le O, Bhaumik D, et al. (2011). DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. *J Cell Sci*, 124:68-81.
- [51] Isermann A, Mann C, Rübe CE. (2020). Histone variant H2A.J marks persistent DNA damage and triggers the secretory phenotype in radiation-induced senescence. *Int J Mol Sci*, 21:9130.
- [52] Shah PP, Donahue G, Otte GL, Capell BC, Nelson DM, Cao K, et al. (2013). Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. *Genes Dev*, 27:1787-1799.
- [53] Matias I, Diniz LP, Damico IV, Araujo APB, Neves LDS, Vargas G, et al. (2022). Loss of lamin-B1 and defective nuclear morphology are hallmarks of astrocyte senescence in vitro and in the aging human hippocampus. *Aging Cell*, 21:e13521.
- [54] Wang AS, Ong PF, Chojnowski A, Clavel C, Dreesen O (2017). Loss of lamin B1 is a biomarker to quantify cellular senescence in photoaged skin. *Sci Rep*, 7:15678.
- [55] Rouillard ME, Hu J, Sutter PA, Kim HW, Huang JK, Crocker SJ (2022). The cellular senescence factor extracellular HMGB1 directly inhibits oligodendrocyte progenitor cell differentiation and impairs CNS remyelination. *Front Cell Neurosci*, 16:833186.
- [56] Miura Y, Milad N, Takata A, Kanazawa S (2025). Bleomycin promotes cellular senescence and activation of the cGAS-STING pathway without direct effect on fibrosis in an idiopathic pulmonary fibrosis model. *Aging (Albany NY)*, 17:2189-2211.
- [57] Miller KN, Dasgupta N, Liu T, Adams PD, Vizioli MG (2021). Cytoplasmic chromatin fragments—from mechanisms to therapeutic potential. *Elife*, 10.
- [58] Xiang W, Wang S, Pan Y, Cong X, Lu J, Zhang Y (2024). Cytoplasmic chromatin fragments: divergent roles in senescence and cancer. *Ageing Cancer Res Treat*, 3:202523.
- [59] Basu A (2022). The interplay between apoptosis and cellular senescence: Bcl-2 family proteins as targets for cancer therapy. *Pharmacol Ther*, 230:107943.
- [60] He S, Sharpless NE (2017). Senescence in health and disease. *Cell*, 169:1000-1011.
- [61] Lagares D, Santos A, Grasberger PE, Liu F, Probst CK, Rahimi RA, et al. (2017). Targeted apoptosis of myofibroblasts with the BH3 mimetic ABT-263 reverses established fibrosis. *Sci Transl Med*, 9:eal3765.
- [62] Bonneaud TL, Lefebvre CC, Nocquet L, Basseville A, Roul J, Weber H, et al. (2022). Targeting of MCL-1 in breast cancer-associated fibroblasts reverses their myofibroblastic phenotype and pro-invasive properties. *Cell Death Dis*, 13:787.
- [63] Sanders YY, Liu H, Zhang X, Hecker L, Bernard K, Desai L, et al. (2013). Histone modifications in senescence-associated resistance to apoptosis by oxidative stress. *Redox Biol*, 1:8-16.
- [64] Perkins DW, Steiner I, Haider S, Robertson D, Buus R, O'Leary L, et al. (2024). Therapy-induced normal tissue damage promotes breast cancer metastasis. *iScience*, 27:108503.
- [65] Suryadevara V, Hudgins AD, Rajesh A, Pappalardo A, Karpova A, Dey AK, et al. (2024). SenNet recommendations for detecting senescent cells in different tissues. *Nat Rev Mol Cell Biol*, 25:1001-1023.
- [66] Rossi M, Abdelmohsen K (2021). The emergence of senescent surface biomarkers as senotherapeutic targets. *Cells*, 10:1740.
- [67] Marin I, Boix O, Garcia-Garijo A, Sirois I, Caballe A, Zarzuela E, et al. (2023). Cellular senescence is immunogenic and promotes antitumor immunity. *Cancer Discov*, 13:410-431.
- [68] Chen HA, Ho YJ, Mezzadra R, Adrover JM, Smolkin R, Zhu C, et al. (2023). Senescence rewires microenvironment sensing to facilitate antitumor immunity. *Cancer Discov*, 13:432-453.
- [69] Kim KM, Noh JH, Bodogai M, Martindale JL, Yang X, Indig FE, et al. (2017). Identification of senescent cell surface targetable protein DPP4. *Genes Dev*, 31:1529-1534.
- [70] Hruby AJ, Higuchi-Sanabria R (2025). Mitochondrial dysfunction in cellular senescence: a bridge to neurodegenerative disease. *NPJ Aging*, 11:99.
- [71] Correia-Melo C, Marques FD, Anderson R, Hewitt G, Hewitt R, Cole J, et al. (2016). Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J*, 35:724-742.
- [72] Guo Y, Guan T, Shafiq K, Yu Q, Jiao X, Na D, et al. (2023). Mitochondrial dysfunction in aging. *Ageing Res Rev*, 88:101955.
- [73] Moiseeva O, Bourdeau V, Roux A, Deschênes-Simard X, Ferbeyre G (2009). Mitochondrial dysfunction contributes to oncogene-induced senescence. *Mol Cell Biol*, 29:4495-4507.
- [74] Shivani AP, Milee M, Rajakumara E (2026). Allosteric activation of PARP2 self-PARylation by SUMO constrains DNA-break-dependent catalytic functions. *FEBS J*. Advance online publication.
- [75] Mai S, Klinkenberg M, Auburger G, Bereiter-Hahn J, Jendrach M (2010). Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent

- cells and enhances resistance to oxidative stress through PINK1. *J Cell Sci*, 123:917-926.
- [76] Yoon YS, Yoon DS, Lim IK, Yoon SH, Chung HY, Rojo M, et al. (2006). Formation of elongated giant mitochondria in DFO-induced cellular senescence: involvement of enhanced fusion process through modulation of Fis1. *J Cell Physiol*, 209:468-480.
- [77] Wang S, Long H, Hou L, Feng B, Ma Z, Wu Y, et al. (2023). The mitophagy pathway and its implications in human diseases. *Signal Transduct Target Ther*, 8:304.
- [78] Bock FJ, Tait SWG (2019). Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol*, 21:85-100.
- [79] Ichim G, Lopez J, Ahmed SU, Muthalagu N, Giampazolias E, Delgado ME, et al. (2015). Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. *Mol Cell*, 57:860-872.
- [80] Lai P, Liu L, Bancaro N, Troiani M, Cali B, Li Y, et al. (2025). Mitochondrial DNA released by senescent tumor cells enhances PMN-MDSC-driven immunosuppression through the cGAS-STING pathway. *Immunity*, 58:811-825.e7.
- [81] Dou Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangenstein KJ, et al. (2017). Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature*, 550:402-406.
- [82] Corn KC, Martello SE, Menon VK, Britto LS, Simmons KM, Mohamed YK, et al. (2026). Radiation-induced autophagy regulates fibroblast mitochondrial metabolism and crosstalk with triple-negative breast cancer cells. *Cell Rep*, 45:117096.
- [83] Balliet RM, Capparelli C, Guido C, Pestell TG, Martinez-Outschoorn UE, Lin Z, et al. (2011). Mitochondrial oxidative stress in cancer-associated fibroblasts drives lactate production, promoting breast cancer tumor growth: understanding the aging and cancer connection. *Cell Cycle*, 10:4065-4073.
- [84] Guo Z, Zhang H, Fu Y, Kuang J, Zhao B, Zhang L, et al. (2023). Cancer-associated fibroblasts induce growth and radioresistance of breast cancer cells through paracrine IL-6. *Cell Death Discov*, 9:6.
- [85] Chen J, Wei Y, Yang W, Huang Q, Chen Y, Zeng K, et al. (2022). IL-6: the link between inflammation, immunity and breast cancer. *Front Oncol*, 12:903800.
- [86] Grivennikov SI, Greten FR, Karin M (2010). Immunity, inflammation, and cancer. *Cell*, 140:883-899.
- [87] Manore SG, Doheny DL, Wong GL, Lo HW (2022). IL-6/JAK/STAT3 signaling in breast cancer metastasis: biology and treatment. *Front Oncol*, 12:866014.
- [88] Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H (2013). Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumor angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumor-stroma interaction. *Br J Cancer*, 110:469-478.
- [89] Wu X, Tao P, Zhou Q, Li J, Yu Z, Wang X, et al. (2017). IL-6 secreted by cancer-associated fibroblasts promotes epithelial-mesenchymal transition and metastasis of gastric cancer via JAK2/STAT3 signaling pathway. *Oncotarget*, 8:20741-20750.
- [90] Campbell LM, Maxwell PJ, Waugh DJ (2013). Rationale and means to target pro-inflammatory interleukin-8 (CXCL8) signaling in cancer. *Pharmaceuticals (Basel)*, 6:929-959.
- [91] Liao H, Li H, Song J, Chen H, Si H, Dong J, et al. (2023). Expression of the prognostic marker IL-8 correlates with the immune signature and epithelial-mesenchymal transition in breast cancer. *J Clin Lab Anal*, 37:e24797.
- [92] Chen E, Qin X, Peng K, Xu X, Li W, Cheng X, et al. (2018). Identification of potential therapeutic targets among CXC chemokines in breast tumor microenvironment using integrative bioinformatics analysis. *Cell Physiol Biochem*, 45:1731-1746.
- [93] Pazolli E, Alspach E, Milczarek A, Prior J, Piwnicka-Worms D, Stewart SA (2012). Chromatin remodeling underlies the senescence-associated secretory phenotype of tumor stromal fibroblasts that supports cancer progression. *Cancer Res*, 72:2251-2261.
- [94] Al-Khalaf HH, Ghebeh H, Inass R, Aboussekhra A (2019). Senescent breast luminal cells promote carcinogenesis through interleukin-8-dependent activation of stromal fibroblasts. *Mol Cell Biol*, 39:e00359-18.
- [95] Ortiz-Montero P, Londoño-Vallejo A, 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.
- [96] Fan G, Yu B, Tang L, Zhu R, Chen J, Zhu Y, et al. (2024). TSPAN8⁺ myofibroblastic cancer-associated fibroblasts promote chemoresistance in patients with breast cancer. *Sci Transl Med*, 16:eadj5705.
- [97] Brogowska KK, Zajkowska M, Mroczko B (2023). Vascular endothelial growth factor ligands and receptors in breast cancer. *J Clin Med*, 12:2412.
- [98] Kang Y, Li H, Liu Y, Li Z (2024). Regulation of VEGF-A expression and VEGF-A-targeted therapy in malignant tumors. *J Cancer Res Clin Oncol*, 150:221.
- [99] Al-Kharashi LA, Tulbah A, Arafah M, Eldali AM, Al-Tweigeri T, Aboussekhra A (2022). High DNMT1 expression in stromal fibroblasts promotes angiogenesis and unfavorable outcome in locally advanced breast cancer patients. *Front Oncol*, 12:877219.
- [100] Chen H, Xu X, Li J, Xue Y, Li X, Zhang K, et al. (2024). Decoding tumor-fibrosis interplay: mechanisms, impact on progression, and innovative therapeutic strategies. *Front Pharmacol*, 15:1491400.
- [101] Xu M, Zhang T, Xia R, Wei Y, Wei X (2022). Targeting the tumor stroma for cancer therapy. *Mol Cancer*, 21:208.
- [102] Oey O, Sunjaya AF, Khan Y, Redfern A (2023). Stromal inflammation, fibrosis and cancer: an old intuition with promising potential. *World J Clin Oncol*, 14:230-246.
- [103] Lee CM, Hwang Y, Jeong JW, Kim M, Lee J, Bae SJ, et al. (2024). BRCA1 mutation promotes sprouting angiogenesis in inflammatory cancer-associated

- fibroblast of triple-negative breast cancer. *Cell Death Discov*, 10:5.
- [104] Albonici L, Labiano Almiñana S, Tanaka T, Bei R (2022). Editorial: the impact of angiogenic growth factors and hypoxia on the antitumor immune response. *Front Immunol*, 13:903105.
- [105] Bourhis M, Palle J, Galy-Fauroux I, Terme M (2021). Direct and indirect modulation of T cells by VEGF-A counteracted by anti-angiogenic treatment. *Front Immunol*, 12:616837.
- [106] Ma C, Teoh HK, Zhao Y, Wang Y, Zhao J, Liu Y, et al. (2026). Reprogramming the immunosuppressive breast cancer microenvironment: integrating cellular, metabolic, and stromal targets for rational immunotherapy. *Front Immunol*, 17:1760782.
- [107] Wanami L, Chen H, Peiro S, Garcíadeherrerros A, Bachelder R (2008). Vascular endothelial growth factor-A stimulates Snail expression in breast tumor cells: implications for tumor progression. *Exp Cell Res*, 314:2448-2453.
- [108] Wang L, Liu W, Broussy S, Han B, Fang H (2024). Recent advances of anti-angiogenic inhibitors targeting VEGF/VEGFR axis. *Front Pharmacol*, 14:1307860.
- [109] Mou J, Li C, Zheng Q, Meng X, Tang H (2024). Research progress in tumor angiogenesis and drug resistance in breast cancer. *Cancer Biol Med*, 21:571–585.
- [110] Ye J, Baer JM, Faget DV, Morikis VA, Ren Q, Melam A, et al. (2024). Senescent CAFs mediate immunosuppression and drive breast cancer progression. *Cancer Discov*, 14:1302-1323.
- [111] Vosseler S, Lederle W, Airola K, Obermueller E, Fusenig NE, et al. (2009). Distinct progression-associated expression of tumor and stromal MMPs in HaCaT skin SCCs correlates with onset of invasion. *Int J Cancer*, 125:2296-2306.
- [112] McGowan P, Duffy M (2008). Matrix metalloproteinase expression and outcome in patients with breast cancer: analysis of a published database. *Ann Oncol*, 19:1566-1572.
- [113] Piskór BM, Przyłipiak A, Dąbrowska E, Sidorkiewicz I, Niczyporuk M, Szmitkowski M, et al. (2020). Plasma level of MMP-10 may be a prognostic marker in early stages of breast cancer. *J Clin Med*, 9:4122.
- [114] Chen Y, Zhou Y, Chen J, Yang J, Yuan Y, Wu W (2024). Exosomal lncRNA SNHG12 promotes angiogenesis and breast cancer progression. *Breast Cancer*, 31:607-620.
- [115] Zigrino P, Kuhn I, Bäuerle T, Zamek J, Fox JW, Neumann S, et al. (2009). Stromal expression of MMP-13 is required for melanoma invasion and metastasis. *J Invest Dermatol*, 129:2686-2693.
- [116] Lederle W, Hartenstein B, Meides A, Kunzelmann H, Werb Z, Angel P, et al. (2009). MMP13 as a stromal mediator in controlling persistent angiogenesis in skin carcinoma. *Carcinogenesis*, 31:1175-1184.
- [117] Sun X, Hu X (2025). Unveiling matrix metalloproteinase 13's dynamic role in breast cancer: a link to physical changes and prognostic modulation. *Int J Mol Sci*, 26:3083.
- [118] Glentis A, Oertle P, Mariani P, Chikina A, El Marjou F, Attieh Y, et al. (2017). Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane. *Nat Commun*, 8:924.
- [119] Calon A, Tauriello D, Batlle E (2014). TGF-beta in CAF-mediated tumor growth and metastasis. *Semin Cancer Biol*, 25:15-22.
- [120] Tie Y, Tang F, Peng D, Zhang Y, Shi H (2022). TGF-beta signal transduction: biology, function and therapy for diseases. *Mol Biomed*, 3:45.
- [121] Biffi G, Oni TE, Spielman B, Hao Y, Elyada E, Park Y, et al. (2019). IL1-induced JAK/STAT signaling is antagonized by TGFβ to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. *Cancer Discov*, 9:282-301.
- [122] Marie JC, Letterio JJ, Gavin M, Rudensky AY (2005). TGF-β1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J Exp Med*, 201:1061-1067.
- [123] Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. (2003) Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med*, 198:1875-1886.
- [124] Lazarova M, Steinle A (2019). Impairment of NKG2D-mediated tumor immunity by TGF-β. *Front Immunol*, 10: 2689.
- [125] Hawke LG, Mitchell BZ, Ormiston ML (2020). TGF-β and IL-15 synergize through MAPK pathways to drive the conversion of human NK cells to an innate lymphoid cell 1-like phenotype. *J Immunol*, 204:3171-3181.
- [126] Viel S, Marçais A, Guimaraes FS, Loftus R, Rabilloud J, Grau M, et al. (2016). TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci Signal*, 9:ra19.
- [127] Yang B, Liu H, Shi W, Wang Z, Sun S, Zhang G, et al. (2013). Blocking transforming growth factor-β signaling pathway augments antitumor effect of adoptive NK-92 cell therapy. *Int Immunopharmacol*, 17:198-204.
- [128] Chung DC, Garcia-Batres CR, Millar DG, Wong SWY, Elford AR, Mathews JA, et al. (2024). Generation of an inhibitory NK cell subset by TGF-β1/IL-15 polarization. *J Immunol*, 212:1904-1912.
- [129] Foltz JA, Moseman JE, Thakkar A, Chakravarti N, Lee DA (2018). TGFβ imprinting during activation promotes natural killer cell cytokine hypersecretion. *Cancers*, 10:423.
- [130] Eck SM, Côté AL, Winkelman WD, Brinckerhoff CE (2009). CXCR4 and matrix metalloproteinase-1 are elevated in breast carcinoma-associated fibroblasts and in normal mammary fibroblasts exposed to factors secreted by breast cancer cells. *Mol Cancer Res*, 7:1033-1044.
- [131] Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, et al. (2010). Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proc Natl Acad Sci USA*, 107:20009-20014.

- [132] Zielińska KA, Katanaev VL (2020). The signaling duo CXCL12 and CXCR4: chemokine fuel for breast cancer tumorigenesis. *Cancers (Basel)*, 12:3071.
- [133] Zhang Z, Yu Y, Zhang Z, Li D, Liang Z, Wang L, et al. (2023). Cancer-associated fibroblasts-derived CXCL12 enhances immune escape of bladder cancer through inhibiting P62-mediated autophagic degradation of PDL1. *J Exp Clin Cancer Res*, 42:316.
- [134] Lee DH, Imran M, Choi JH, Park YJ, Kim YH, Min S, et al. (2024). CDK4/6 inhibitors induce breast cancer senescence with enhanced anti-tumor immunogenic properties compared with DNA-damaging agents. *Mol Oncol*, 18:216-232.
- [135] Uzhachenko RV, Bharti V, Ouyang Z, Blevins A, Mont S, Saleh N, et al. (2021). Metabolic modulation by CDK4/6 inhibitor promotes chemokine-mediated recruitment of T cells into mammary tumors. *Cell Rep*, 35:108944.
- [136] Liang YK, Deng ZK, Chen MT, Qiu SQ, Xiao YS, Qi YZ, et al. (2021). CXCL9 is a potential biomarker of immune infiltration associated with favorable prognosis in ER-negative breast cancer. *Front Oncol*, 11:710286.
- [137] Chibaya L, Murphy KC, DeMarco KD, Gopalan S, Liu H, Parikh CN, et al. (2023). EZH2 inhibition remodels the inflammatory senescence-associated secretory phenotype to potentiate pancreatic cancer immune surveillance. *Nat Cancer*, 4:872-892.
- [138] Pazolli E, Luo X, Brehm S, Carbery K, Chung JJ, Prior JL, et al. (2009). Senescent stromal-derived osteopontin promotes preneoplastic cell growth. *Cancer Res*, 69:1230-1239.
- [139] Butti R, Nimma R, Kundu G, Bulbule A, Kumar TVS, Gunasekaran VP, et al. (2021). Tumor-derived osteopontin drives the resident fibroblast to myofibroblast differentiation through Twist1 to promote breast cancer progression. *Oncogene*, 40:2002-2017.
- [140] Liu S, Zhang Z, Wang Z, Liu C, Liang G, Xu T, et al. (2026). SPP1 drives colorectal cancer liver metastasis and immunotherapy resistance by stimulating CXCL12 production in cancer-associated fibroblasts. *Cancer Res*, 86:58-79.
- [141] Pang B, Sun H, Ouyang M, Hu C (2026). SPP1 (osteopontin): a key regulator orchestrating cancer progression and non-cancerous pathologies - therapeutic opportunities and future directions. *Front Cell Dev Biol*, 14:1912468.
- [142] Shi M, Li H, Liang R, Lin H, Tang Q (2025). The transcription factor STAT3 and aging: an intermediate medium. *Biogerontology*, 26:55.
- [143] Ma Z, Zhou F, Jin H, Wu X (2024). Crosstalk between CXCL12/CXCR4/ACKR3 and the STAT3 pathway. *Cells*, 13:1027.
- [144] Urtasun R, Lopategi A, George J, Leung TM, Lu Y, Wang X, et al. (2012). Osteopontin, an oxidant stress sensitive cytokine, up-regulates collagen-I via integrin $\alpha(V)\beta(3)$ engagement and PI3K/pAkt/NF κ B signaling. *Hepatology*, 55:594-608.
- [145] Lang F, Li Y, Yao R, Jiang M (2025). Osteopontin in chronic inflammatory diseases: mechanisms, biomarker potential, and therapeutic strategies. *Biology*, 14:428.
- [146] Katanov C, Lerrer S, Liubomirski Y, Leider-Trejo L, Meshel T, Bar J, et al. (2015). Regulation of the inflammatory profile of stromal cells in human breast cancer: prominent roles for TNF- α and the NF- κ B pathway. *Stem Cell Res Ther*, 6:87.
- [147] Raut GK, Malachowski T, Melam A, Ramalho-Oliveira R, Holt T, Luo X, et al. (2025). Chemotherapy-induced adipo-lineage cell senescence drives bone loss. *Nat Commun*, 17:1042.
- [148] Pérez-Chacón G, Santamaría PG, Redondo-Pedraza J, González-Suárez E (2025). RANK/RANKL signaling pathway in breast development and cancer. *Adv Exp Med Biol*, 1464:309-345.
- [149] Wu X, Li F, Dang L, Liang C, Lu A, Zhang G (2020). RANKL/RANK system-based mechanism for breast cancer bone metastasis and related therapeutic strategies. *Front Cell Dev Biol*, 8:76.
- [150] Zhou WT, Jin WL (2021). B7-H3/CD276: an emerging cancer immunotherapy. *Front Immunol*, 12:701006.
- [151] Mielcarska S, Kot A, Dawidowicz M, Kula A, Sobków P, Kłaczka D, et al. (2025). B7-H3 in cancer immunotherapy-prospects and challenges: a review of the literature. *Cells*, 14:1209.
- [152] Thiery J (2022). Modulation of the antitumor immune response by cancer-associated fibroblasts: mechanisms and targeting strategies to hamper their immunosuppressive functions. *Explor Target Antitumor Ther*, 3:598-629.
- [153] Ding M, Zhu G, Chen T, Zhu J, Mao S, Tang X, et al. (2025). Integrated analysis identifies CD276 in fibroblasts as a malignancy predictor and regulator of neutrophil infiltration in hepatoblastoma. *Hepatol Commun*, 9:e0826.
- [154] Mei J, Cai Y, Zhu H, Jiang Y, Fu Z, Xu J, et al. (2024). High B7-H3 expression with low PD-L1 expression identifies armored-cold tumors in triple-negative breast cancer. *NPJ Breast Cancer*, 10:11.
- [155] Huang Y, Zhang HL, Li ZL, Du T, Chen YH, Wang Y, et al. (2021). FUT8-mediated aberrant N-glycosylation of B7H3 suppresses the immune response in triple-negative breast cancer. *Nat Commun*, 12:2672.
- [156] Colucci M, Sarill M, Maddalena M, Valdata A, Troiani M, Massarotti M, et al. (2025). Senescence in cancer. *Cancer Cell*, 43:1204-1226.
- [157] Troiani M, Colucci M, D'Ambrosio M, Guccini I, Pasquini E, Varesi A, et al. (2022). Single-cell transcriptomics identifies Mcl-1 as a target for senolytic therapy in cancer. *Nat Commun*, 13 :2177.
- [158] Suresh S, Karthik G, Ouyang JF, Chrysostomou V, Tang SA, Petretto E, et al. (2024). SenMayo transcriptomic senescence panel highlights glial cells in the ageing mouse and human retina. *NPJ Aging*, 10:60.
- [159] Qu Y, Ji B, Dong R, Gu L, Chan C, Xie J, et al. (2025). Single-cell and spatial detection of senescent cells using DeepScience. *Cell Genom*, 5:101035.
- [160] Hughes BK, Davis A, Milligan D, Wallis R, Mossa F, Philpott MP, et al. (2025). SenPred: a single-cell RNA sequencing-based machine learning pipeline to classify deeply senescent dermal fibroblast cells for the detection of an in vivo senescent cell burden. *Genome Med*, 17:2.

- [161] Kamat P, Macaluso N, Li Y, Agrawal A, Winston A, Pan L, et al. (2025). Single-cell morphology encodes functional subtypes of senescence in aging human dermal fibroblasts. *Sci Adv*, 11:eads1875.
- [162] Gautam V, Duari S, Solanki S, Gupta M, Mittal A, Arora S, et al. (2025). scCamAge: a context-aware prediction engine for cellular age, aging-associated bioactivities, and morphometrics. *Cell Rep*, 44:115270.
- [163] Wallis R, Hughes BK, Moore M, O'Sullivan EA, McIlvenna LC, Gammon L, et al. (2025). SAMP-Score: a morphology-based machine learning classification method for screening pro-senescence compounds in p16 positive cancer cells. *Aging (Albany NY)*, 17:2688-2716.
- [164] Jochems F, Thijssen B, De Conti G, Jansen R, Pogacar Z, Groot K, et al. (2021). The Cancer SENESCopedia: a delineation of cancer cell senescence. *Cell Rep*, 36:109441.
- [165] Bousset L, Gil J (2022). Targeting senescence as an anticancer therapy. *Mol Oncol*, 16:3855-3880.
- [166] Zhao W, Lin ZX, Zhang ZQ (2004). Cisplatin-induced premature senescence with concomitant reduction of gap junctions in human fibroblasts. *Cell Res*, 14:60-66.
- [167] Gomes LR, Rocha CRR, Martins DJ, Fiore APZP, Kinker GS, Bruni-Cardoso A, et al. (2019). ATR mediates cisplatin resistance in 3D-cultured breast cancer cells via translesion DNA synthesis modulation. *Cell Death Dis*, 10:459.
- [168] Lajos R, Braicu C, Jurj A, Chira S, Cojocneanu-Petric R, Pileczki V, Berindan-Neagoe I (2018). A miRNAs profile evolution of triple negative breast cancer cells in the presence of a possible adjuvant therapy and senescence inducer. *J BUON*, 23:692-705.
- [169] Sriratanasak N, Chunhacha P, Ei ZZ, Chanvorachote P (2022). Cisplatin induces senescent lung cancer cell-mediated stemness induction via GRP78/Akt-dependent mechanism. *Biomedicines*, 10:2703.
- [170] Bientinesi E, Lulli M, Becatti M, Ristori S, Margheri F, Monti D (2022). Doxorubicin-induced senescence in normal fibroblasts promotes in vitro tumor cell growth and invasiveness: the role of quercetin in modulating these processes. *Mech Ageing Dev*, 206:111689.
- [171] Venkatasubramanian R, Darrah MA, Mahoney SA, Hutton DA, Maurer GS, Ludwig KR, et al. (2025). Cellular senescence mediates doxorubicin chemotherapy-induced aortic stiffening: role of glycation stress. *Hypertension*, 82:1767-1777.
- [172] Elmore LW, Rehder CW, Di X, McChesney PA, Jackson-Cook CK, Gewirtz DA, et al. (2002). Adriamycin-induced senescence in breast tumor cells involves functional p53 and telomere dysfunction. *J Biol Chem*, 277:35509-35515.
- [173] Jackson JG, Pereira-Smith OM (2006). Primary and compensatory roles for RB family members at cell cycle gene promoters that are deacetylated and downregulated in doxorubicin-induced senescence of breast cancer cells. *Mol Cell Biol*, 26:2501-2510.
- [174] Huun J, Lønning PE, Knappskog S (2017). Effects of concomitant inactivation of p53 and pRb on response to doxorubicin treatment in breast cancer cell lines. *Cell Death Discov*, 3:17026.
- [175] Jackson JG, Pant V, Li Q, Chang LL, Quintás-Cardama A, Garza D, et al. (2012). p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. *Cancer Cell*, 21:793-806.
- [176] Inao T, Kotani H, Iida Y, Kartika ID, Okimoto T, Tanino R, et al. (2019). Different sensitivities of senescent breast cancer cells to immune cell-mediated cytotoxicity. *Cancer Sci*, 110:2690-2699.
- [177] Elmore LW, Di X, Dumur C, Holt SE, Gewirtz DA (2005). Evasion of a single-step, chemotherapy-induced senescence in breast cancer cells: implications for treatment response. *Clin Cancer Res*, 11:2637-2643.
- [178] Prencipe M, Fitzpatrick P, Gorman S, Masetto M, Klinger R, Furlong F, et al. (2009). Cellular senescence induced by aberrant MAD2 levels impacts on paclitaxel responsiveness in vitro. *Br J Cancer*, 101:1900-1908.
- [179] Khongkow P, Gomes AR, Gong C, Man EP, Tsang JW, Zhao F, et al. (2016). Paclitaxel targets FOXM1 to regulate KIF20A in mitotic catastrophe and breast cancer paclitaxel resistance. *Oncogene*, 35:990-1002.
- [180] Milczarek M (2020). The premature senescence in breast cancer treatment strategy. *Cancers*, 12:1815.
- [181] Bajtai E, Kiss C, Bakos E, Langó T, Lovrics A, Schád É, et al. (2025). Therapy-induced senescence is a transient drug resistance mechanism in breast cancer. *Mol Cancer*, 24:128.
- [182] Traina T, Cadoo K, Gucalp A (2014). Palbociclib: an evidence-based review of its potential in the treatment of breast cancer. *Breast Cancer (Auckl)*, 8:123-133.
- [183] Hu W, Sung T, Jessen BA, Thibault S, Finkelstein MB, Khan NK, et al. (2016). Mechanistic investigation of bone marrow suppression associated with palbociclib and its differentiation from cytotoxic chemotherapies. *Clin Cancer Res*, 22:2000-2008.
- [184] Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, et al. (2017). CDK4/6 inhibition triggers anti-tumor immunity. *Nature*, 548:471-475.
- [185] Jost T, Heinzerling L, Fietkau R, Hecht M, Distel LV (2021). Palbociclib induces senescence in melanoma and breast cancer cells and leads to additive growth arrest in combination with irradiation. *Front Oncol*, 11:740002.
- [186] Favaretto G, Rossi MN, Cuollo L, Laffranchi M, Cervelli M, Soriani A, et al. (2024). Neutrophil-activating secretome characterizes palbociclib-induced senescence of breast cancer cells. *Cancer Immunol Immunother*, 73:113.
- [187] Hattangadi DK, DeMasters GA, Walker TD, Jones KR, Di X, Newsham IF, et al. (2004). Influence of p53 and caspase 3 activity on cell death and senescence in response to methotrexate in the breast tumor cell. *Biochem Pharmacol*, 68:1699-1708.
- [188] Chen J, Stubbe J (2005). Bleomycins: towards better therapeutics. *Nat Rev Cancer*, 5:102-112.
- [189] Kasper M, Barth K (2009). Bleomycin and its role in inducing apoptosis and senescence in lung cells - modulating effects of caveolin-1. *Curr Cancer Drug Targets*, 9:341-353.

- [190] Perera UE, Organ L, Dewage SNV, Dersch HB, Stent A, Snibson KJ (2021). Increased levels of ER stress and apoptosis in a sheep model for pulmonary fibrosis are alleviated by in vivo blockade of the KCa3.1 ion channel. *Can Respir J*, 2021:1-11.
- [191] Oberley LW, Buettner GR (1979). The production of hydroxyl radical by bleomycin and iron (II). *FEBS Lett*, 97:47-49.
- [192] Moseley PL (1989). Augmentation of bleomycin-induced DNA damage in intact cells. *Am J Physiol Cell Physiol*, 257:C882-C887.
- [193] Yamamoto T, Eckes B, Krieg T (2000). Bleomycin increases steady-state levels of type I collagen, fibronectin and decorin mRNAs in human skin fibroblasts. *Arch Dermatol Res*, 292:556-561.
- [194] Sai X, Qin C, Wu Y, Zhao Y, Bian T (2020). Downregulation of PTEN mediates bleomycin-induced premature senescence in lung cancer cells by suppressing autophagy. *J Int Med Res*, 48 :300060520923522.
- [195] Aifuwa I, Giri A, Longe N, Lee SH, An SS, Wirtz D (2015). Senescent stromal cells induce cancer cell migration via inhibition of RhoA/ROCK/myosin-based cell contractility. *Oncotarget*, 6:30516-30531.
- [196] Efimova EV, Mauceri HJ, Golden DW, Labay E, Bindokas VP, Darga TE, et al. (2010). Poly(ADP-ribose) polymerase inhibitor induces accelerated senescence in irradiated breast cancer cells and tumors. *Cancer Res*, 70:6277-6282.
- [197] Lee Y, Kang BS, Bae Y (2014). Premature senescence in human breast cancer and colon cancer cells by tamoxifen-mediated reactive oxygen species generation. *Life Sci*, 97:116-122.
- [198] Jones KR, Elmore LW, Jackson-Cook C, Demasters G, Povirk LF, Holt SE, et al. (2005). p53-dependent accelerated senescence induced by ionizing radiation in breast tumour cells. *Int J Radiat Biol*, 81:445-458.
- [199] Cogno N, Bauer R, Durante M (2022). An agent-based model of radiation-induced lung fibrosis. *Int J Mol Sci*, 23:13920.
- [200] Buchholzki M, Stasch LM, Budeus B, Ketheeswaranathan V, Sevindik ZF, Jendrosseck V, et al. (2025). Senescent fibroblasts modulate the radiation response of neighboring epithelial cells. *Cell Death Discov*, 11:468.
- [201] Liakou E, Mavrogonatou E, Pratsinis H, Rizou S, Evangelou K, Panagiotou PN, et al. (2016). Ionizing radiation-mediated premature senescence and paracrine interactions with cancer cells enhance the expression of syndecan 1 in human breast stromal fibroblasts: the role of TGF- β . *Aging (Albany NY)*, 8:1650-1669.
- [202] Reimann M, Lee S, Schmitt CA (2024). Cellular senescence: neither irreversible nor reversible. *J Exp Med*, 221:e20232136.
- [203] Haferkamp S, Borst A, Adam C, Becker TM, Motschenbacher S, Windhövel S, et al. (2013). Vemurafenib induces senescence features in melanoma cells. *J Invest Dermatol*, 133:1601-1609.
- [204] Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AHFM, Schlegelberger B, et al. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature*, 436:660-665.
- [205] Saleh T (2024). Therapy-induced senescence is finally escapable, what is next? *Cell Cycle*, 23:713-721.
- [206] Alotaibi M, Sharma K, Saleh T, Povirk LF, Hendrickson EA, Gewirtz DA (2016). Radiosensitization by PARP inhibition in DNA repair proficient and deficient tumor cells: proliferative recovery in senescent cells. *Radiat Res*, 185:229-245.
- [207] Hsu CH, Altschuler SJ, Wu LF (2019). Patterns of early p21 dynamics determine proliferation-senescence cell fate after chemotherapy. *Cell*, 178:361-373.e12.
- [208] Afifi MM, Crncec A, Cornwell JA, Cataisson C, Paul D, Ghorab LM, et al. (2023). Irreversible cell cycle exit associated with senescence is mediated by constitutive MYC degradation. *Cell Rep*, 42:113079.
- [209] Tonnessen-Murray CA, Frey WD, Rao SG, Shahbandi A, Ungerleider NA, Olayiwola JO, et al. (2019). Chemotherapy-induced senescent cancer cells engulf other cells to enhance their survival. *J Cell Biol*, 218:3827-3844.
- [210] Dudkowska M, Staniak K, Bojko A, Sikora E (2021). The role of autophagy in escaping therapy-induced polyploidy/senescence. *Adv Cancer Res*, 150:209-247.
- [211] Sarkisian CJ, Keister BA, Stairs DB, Boxer RB, Moody SE, Chodosh LA (2007). Dose-dependent oncogene-induced senescence in vivo and its evasion during mammary tumorigenesis. *Nat Cell Biol*, 9:493-505.
- [212] Sinha VC, Qin L, Li Y (2015). A p53/ARF-dependent anticancer barrier activates senescence and blocks tumorigenesis without impacting apoptosis. *Mol Cancer Res*, 13:231-238.
- [213] Saatloo MV, Delisi D, Eskandari N, Krieg C, Gentile S (2025). Kv11.1-dependent senescence activates a lethal immune response via tumor necrosis factor alpha. *Neoplasia*, 63:101148.
- [214] De Martino M, Tkach M, Bruni S, Rocha D, Mercogliano MF, Cenciarini ME, et al. (2020). Blockade of Stat3 oncogene addiction induces cellular senescence and reveals a cell-nonautonomous activity suitable for cancer immunotherapy. *Oncoimmunology*, 9:1715767.
- [215] Sidaway P (2018). Breast cancer: fibroblast subtypes alter the microenvironment. *Nat Rev Clin Oncol*, 15:264-265.
- [216] Bartoschek M, Oskolkov N, Bocci M, Lövrot J, Larsson C, Sommarin M, et al. (2018). Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat Commun*, 9:5150.
- [217] Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H (2015). Cancer-associated fibroblasts: their characteristics and their roles in tumor growth. *Cancers*, 7:2443-2458.
- [218] Gascard P, Tlsty TD (2016). Carcinoma-associated fibroblasts: orchestrating the composition of malignancy. *Genes Dev*, 30:1002-1019.
- [219] Cirri P, Chiarugi P (2011). Cancer associated fibroblasts: the dark side of the coin. *Am J Cancer Res*, 1:482-497.

- [220] Musa M (2020). Single-cell analysis on stromal fibroblasts in the microenvironment of solid tumours. *Adv Med Sci*, 65:163-169.
- [221] Bogachek M, Kazakova A, Sergeevichev D, Vladimirov S, Richter V, Nushtaeva A (2025). S100A4/FSP1: a prognostic marker and a promising target for antitumor therapy. *Int J Mol Sci*, 26:9370.
- [222] Ermakov MS, Nushtaeva AA, Richter VA, Koval OA (2022). Cancer-associated fibroblasts and their role in tumor progression. *Vavilovskii Zh Genet Selektcii*, 26:14-21.
- [223] Chen X, Zhou Z, Xie L, Qiao K, Jia Y, Liu S, et al. (2025). Single-cell resolution spatial analysis of antigen-presenting cancer-associated fibroblast niches. *Cancer Cell*, 43:2224-2240.e9.
- [224] Harada Y, Masuda A, Matsuura T, Nagata K, Nanno Y, Masamune A, et al. (2026). Spatial profiling identified senescent cancer-associated fibroblasts localized in the border region of human pancreatic ductal adenocarcinoma. *Cell Mol Gastroenterol Hepatol*, 20:101808.
- [225] Sebastian A, Hum NR, Martin KA, Gilmore SF, Peran I, Byers SW, et al. (2020). Single-cell transcriptomic analysis of tumor-derived fibroblasts and normal tissue-resident fibroblasts reveals fibroblast heterogeneity in breast cancer. *Cancers*, 12:1307.
- [226] Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvisé M, et al. (2017). Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med*, 214:579-596.
- [227] Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al. (2019). Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. *Cancer Discov*, 9:1102-1123.
- [228] Wu SZ, Roden DL, Wang C, Holliday H, Harvey K, Cazet AS, et al. (2020). Stromal cell diversity associated with immune evasion in human triple-negative breast cancer. *EMBO J*, 39:e104063.
- [229] Krishnamurthy AT, Shyer JA, Thai M, Gandham V, Buechler MB, Yang YA, et al. (2022). LRRC15+ myofibroblasts dictate the stromal setpoint to suppress tumor immunity. *Nature*, 611:148-154.
- [230] Wu SZ, Al-Eryani G, Roden DL, Junankar S, Harvey K, et al. (2021). A single-cell and spatially resolved atlas of human breast cancers. *Nat Genet*, 53:1334-1347.
- [231] Kazakova AN, Lukina MM, Anufrieva KS, Bekbaeva IV, Ivanova OM, Shnaider PV, et al. (2024). Exploring the diversity of cancer-associated fibroblasts: insights into mechanisms of drug resistance. *Front Cell Dev Biol*, 12:1403122.
- [232] Zheng J, Hao H (2024). The importance of cancer-associated fibroblasts in targeted therapies and drug resistance in breast cancer. *Front Oncol*, 13:1333839.
- [233] Obradovic A, Graves D, Korrer M, Wang Y, Roy S, Naveed A, et al. (2022). Immunostimulatory cancer-associated fibroblast subpopulations can predict immunotherapy response in head and neck cancer. *Clin Cancer Res*, 28:2094-2109.
- [234] Louault K, Li RR, DeClerck YA (2020). Cancer-associated fibroblasts: understanding their heterogeneity. *Cancers*, 12:3108.
- [235] Cords L, Tietscher S, Anzeneder T, Langwieder C, Rees M, de Souza N, et al. (2023). Cancer-associated fibroblast classification in single-cell and spatial proteomics data. *Nat Commun*, 14: 4294.
- [236] Croizer H, Mhaidly R, Kieffer Y, Gentric G, Djerroudi L, Leclerc R, et al. (2024). Deciphering the spatial landscape and plasticity of immunosuppressive fibroblasts in breast cancer. *Nat Commun*, 15:2806.
- [237] Witkiewicz AK, Rivadeneira DB, Ertel A, Kline J, Hyslop T, Schwartz GF, et al. (2011). Association of RB/p16-pathway perturbations with DCIS recurrence. *Am J Pathol*, 179:1171-1178.
- [238] Bartholomew JN, Volonte D, Galbiati F (2009). Caveolin-1 regulates the antagonistic pleiotropic properties of cellular senescence through a novel Mdm2/p53-mediated pathway. *Cancer Res*, 69:2878-2886.
- [239] Assouline B, Kahn R, Hodali L, Condiotti R, Engel Y, Elyada E, et al. (2024). Senescent cancer-associated fibroblasts in pancreatic adenocarcinoma restrict CD8+ T cell activation and limit responsiveness to immunotherapy in mice. *Nat Commun*, 15:6162.
- [240] Alspach E, Flanagan KC, Luo X, Ruhland MK, Huang H, Pazolli E, et al. (2014). p38MAPK plays a crucial role in stromal-mediated tumorigenesis. *Cancer Discov*, 4:716-729.
- [241] Solé-Boldo L, Raddatz G, Schütz S, Mallm J, Rippe K, Lonsdorf AS, et al. (2020). Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming. *Commun Biol*, 3:188.
- [242] Reynolds LE, Maallin S, Haston S, Martinez-Barbera JP, Hodivala-Dilke KM, Pedrosa A (2023). Effects of senescence on the tumour microenvironment and response to therapy. *FEBS J*, 291:2306-2319.
- [243] Luo X, Ruhland MK, Pazolli E, Lind AC, Stewart SA (2011). Osteopontin stimulates preneoplastic cellular proliferation through activation of the MAPK pathway. *Mol Cancer Res*, 9:1018-1029.
- [244] Ou HL, Hoffmann R, González-López C, Doherty GJ, Korkola JE, Muñoz-Espín D (2021). Cellular senescence in cancer: from mechanisms to detection. *Mol Oncol*, 15:2634-2671.
- [245] Feng Y, Zhi X, Xiao T, Feng L (2026). Senescent cancer-associated fibroblasts in cancer progression: from formation to therapeutic opportunities. *Mech Ageing Dev*, 230:112158.
- [246] Fridman AL, Tainsky MA (2008). Critical pathways in cellular senescence and immortalization revealed by gene expression profiling. *Oncogene*, 27:5975-5987.
- [247] Nan L, Guo P, Hui W, Xia F, Yi C (2025). Recent advances in dermal fibroblast senescence and skin aging: unraveling mechanisms and pioneering therapeutic strategies. *Front Pharmacol*, 16:1592596.
- [248] Belle JI, Sen D, Baer JM, Liu X, Lander VE, Ye J, et al. (2024). Senescence defines a distinct subset of myofibroblasts that orchestrates immunosuppression in pancreatic cancer. *Cancer Discov*, 14:1324-1355.

- [249] Mhaidly R, Mechta-Grigoriou F (2020). Fibroblast heterogeneity in tumor micro-environment: role in immunosuppression and new therapies. *Semin Immunol*, 48:101417.
- [250] Chhabra Y, Weeraratna AT (2023). Fibroblasts in cancer: unity in heterogeneity. *Cell*, 186:1580-1609.
- [251] Han C, Liu T, Yin R (2020). Biomarkers for cancer-associated fibroblasts. *Biomark Res*, 8:64.
- [252] Zhao Z, Li T, Yuan Y, Zhu Y (2023). What is new in cancer-associated fibroblast biomarkers? *Cell Commun Signal*, 21:96.
- [253] McHugh D, Durán I, Gil J (2024). Senescence as a therapeutic target in cancer and age-related diseases. *Nat Rev Drug Discov*, 24:57-71.
- [254] Wang L, Lankhorst L, Bernards R (2022). Exploiting senescence for the treatment of cancer. *Nat Rev Cancer*, 22:340-355.
- [255] Chaib S, Tchkonja T, Kirkland JL (2022). Cellular senescence and senolytics: the path to the clinic. *Nat Med*, 28:1556-1568.
- [256] Lindeman G, Muttiah C, Martin FC, Christie M, Dawson S, Desai J, et al. (2025). Abstract P2-07-18: results from PALVEN: a phase 1b study of palbociclib, letrozole and venetoclax in ER and BCL2-positive metastatic breast cancer. *Clin Cancer Res*, 31:P2-07-18.
- [257] As Sobeai HM, Alanazi AM, Alotaibi F, Alhoshani A, Alhazzani K, Almutairi MM, et al. (2026). Differential responses to the combination of navitoclax and venetoclax with doxorubicin in murine models of triple negative breast cancer. *Front Cell Dev Biol*, 14:1661424.
- [258] Mohamad Anuar NN, Nor Hisam NS, Liew SL, Ugusman A (2020). Clinical review: navitoclax as a pro-apoptotic and anti-fibrotic agent. *Front Pharmacol*, 11:564108.
- [259] Mackay H, Mes-Masson A, Rodier F, Lheureux S, Jerzak KJ, Hassan SN, et al. (2023). Exactis-03: a phase I trial of the combination of olaparib and navitoclax in women with high grade serous ovarian cancer and triple negative breast cancer. *J Clin Oncol*, 41:TPS5623.
- [260] Estepa-Fernández A, García-Fernández A, Lérída-Viso A, Blandez JF, Galiana I, et al. (2023). Combination of palbociclib with navitoclax based-therapies enhances in vivo antitumoral activity in triple-negative breast cancer. *Pharmacol Res*, 187:106628.
- [261] Zoeller JJ, Vagodny A, Daniels VW, Taneja K, Tan BY, DeRose YS, et al. (2020). Navitoclax enhances the effectiveness of EGFR-targeted antibody-drug conjugates in PDX models of EGFR-expressing triple-negative breast cancer. *Breast Cancer Res*, 22:132.
- [262] Shahbandi A, Rao SG, Anderson AY, Frey WD, Olayiwola JO, Ungerleider NA, et al. (2020). BH3 mimetics selectively eliminate chemotherapy-induced senescent cells and improve response in TP53 wild-type breast cancer. *Cell Death Differ*, 27:3097-3116.
- [263] Mertens JC, Fingas CD, Christensen JD, Smoot RL, Bronk SF, Werneburg NW, et al. (2013). Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. *Cancer Res*, 73:897-907.
- [264] Cho H, Hwang J, Yang EJ, Kim E, Kim J, Kim SY, et al. (2022). Nintedanib induces senolytic effect via STAT3 inhibition. *Cell Death Dis*, 13:760.
- [265] Zou X, Li S, Huang S, Niu R, Liu G, Wang Z (2025). Harnessing TP73-targeted nintedanib: a novel strategy to halt triple-negative breast cancer via p53-PPAR α /PI3K-Akt pathway suppression. *Int J Oncol*, 67:1-15.
- [266] Quintela-Fandino M, Bermejo B, Zamora E, Moreno F, García-Saenz JÁ, Pernas S, et al. (2024). High mechanical conditioning by tumor extracellular matrix stiffness is a predictive biomarker for antifibrotic therapy in HER2-negative breast cancer. *Clin Cancer Res*, 30:5094-5104.
- [267] Jian C, Wu T, Wang L, Gao C, Fu Z, Zhang Q, et al. (2024). Biomimetic nanoplatform for dual-targeted clearance of activated and senescent cancer-associated fibroblasts to improve radiation resistance in breast cancer. *Small*, 20:e2309279.
- [268] Patel P, Joshee N, Rimando A, Parajuli P (2013). Anti-cancer scopes and associated mechanisms of Scutellaria extract and flavonoid wogonin. *Curr Cancer Ther Rev*, 9:34-42.
- [269] Yang D, Guo Q, Liang Y, Zhao Y, Tian X, Ye Y, et al. (2020). Wogonin induces cellular senescence in breast cancer via suppressing TXNRD2 expression. *Arch Toxicol*, 94:3433-3447.
- [270] Naeem H, Shahbaz M, Momal U, Irshad N, Imran M, Hussain M, et al. (2025). Anticancer potential of wogonin: a comprehensive treatise. *Food Sci Nutr*, 13:e71128.
- [271] Wang L, Lin F, Liu Y, Li W, Ding Q, Duan X, et al. (2024). Wogonin protects against bleomycin-induced mouse pulmonary fibrosis via the inhibition of CDK9/p53-mediated cell senescence. *Front Pharmacol*, 15:1407891.
- [272] Oakes SR, Vaillant F, Lim E, Lee L, Breslin K, Feleppa F, et al. (2012). Sensitization of BCL-2-expressing breast tumors to chemotherapy by the BH3 mimetic ABT-737. *Proc Natl Acad Sci USA*, 109:2766-2771.
- [273] Nayman AH, Siginc H, Zemheri E, Yencilek F, Yildirim A, Telci D (2019). Dual-inhibition of mTOR and Bcl-2 enhances the anti-tumor effect of everolimus against renal cell carcinoma in vitro and in vivo. *J Cancer*, 10:1466-1478.
- [274] Huang Y, Liu T (2020). Step further towards targeted senolytic therapy: therapeutic potential of uPAR-CAR T cells for senescence-related diseases. *Signal Transduct Target Ther*, 5:155.
- [275] Zhai BT, Tian H, Sun J, Zou JB, Zhang XF, Cheng JX, et al. (2022). Urokinase-type plasminogen activator receptor (uPAR) as a therapeutic target in cancer. *J Transl Med*, 20:135.
- [276] Zhang Z, Ho Y, Fang X, Kim M, Li M, Luan W, et al. (2026). A convergent uPAR-positive tumor ecosystem creates broad vulnerability to CAR T cell therapy. *Cell*, 189:2898-2917.e42.
- [277] Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, et al. (2020). Senolytic CAR T cells reverse senescence-associated pathologies. *Nature*, 583:127-132.

- [278] Wang Z, Chen Y, Fang H, Xiao K, Wu Z, Xie X, et al. (2024). Reprogramming cellular senescence in the tumor microenvironment augments cancer immunotherapy through multifunctional nanocrystals. *Sci Adv*, 10:eadp7022.
- [279] Moiseeva O, Deschênes-Simard X, St-Germain E, Igelmann S, Huot G, Cadar AE, et al. (2013). Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF- κ B activation. *Aging Cell*, 12:489-498.
- [280] Laberge RM, Zhou L, Sarantos MR, Rodier F, Freund A, de Keizer PL, et al. (2012). Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. *Aging Cell*, 11:569-578.
- [281] Hu Q, Peng J, Jiang L, Li W, Su Q, Zhang J, et al. (2020). Metformin as a senostatic drug enhances the anticancer efficacy of CDK4/6 inhibitor in head and neck squamous cell carcinoma. *Cell Death Dis*, 11:925.
- [282] Williams CC, Singleton BA, Llopis SD, Skripnikova EV (2013). Metformin induces a senescence-associated gene signature in breast cancer cells. *J Health Care Poor Underserved*, 24:93-103.
- [283] Savelius M (2020). Metformin induces senescence-like proliferation arrest in MYC-high triple-negative breast cancer cell lines [Master's thesis]. Helsinki: University of Helsinki.
- [284] Al-Jomah N, Al-Mohanna FH, Aboussekhra A (2021). Tocilizumab suppresses the pro-carcinogenic effects of breast cancer-associated fibroblasts through inhibition of the STAT3/AUF1 pathway. *Carcinogenesis*, 42:1439-1448.
- [285] Al-Tweigeri T, Alraouji N, Tulbah A, Akhtar S, Alzahrani M, Najjar S, et al. (2024). Abstract PO3-25-11: unprecedented responses to neoadjuvant sequential administration of tocilizumab followed by cisplatin/docetaxel for locally advanced triple negative breast cancer patients. *Cancer Res*, 84:PO3-25-11.
- [286] Blagosklonny MV (2021). Anti-aging: senolytics or gerostatics (unconventional view). *Oncotarget*, 12:1821-1835.
- [287] Lewis DA, Travers JB, Machado C, Somani AK, Spandau DF (2011). Reversing the aging stromal phenotype prevents carcinoma initiation. *Aging (Albany NY)*, 3:407-416.
- [288] Blagosklonny MV (2023). Cancer prevention with rapamycin. *Oncotarget*, 14:342-350.
- [289] Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, et al. (2015). MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat Cell Biol*, 17:1049-1061.
- [290] Ding F, Yu Y, Zhao J, Wei S, Zhang Y, Han JH, et al. (2025). The interplay of cellular senescence and reprogramming shapes the biological landscape of aging and cancer revealing novel therapeutic avenues. *Front Cell Dev Biol*, 13:1593096.
- [291] Ichim TE, Lopes G, Reznik R, Bykoriz V, Fortunati CA, Pascual KA, et al. (2025). Reduction of solid tumors by senescent cell immunization. *J Transl Med*, 23:1365.
- [292] Luo J, Sun T, Liu Z, Liu Y, Liu J, Wang S, et al. (2025). Persistent accumulation of therapy-induced senescent cells: an obstacle to long-term cancer treatment efficacy. *Int J Oral Sci*, 17:59.
- [293] Zhang Q, Lou Y, Fang H, Sun S, Jin R, Ji Y, et al. (2024). Cancer-associated fibroblasts under therapy-induced senescence in the tumor microenvironment (Review). *Exp Ther Med*, 27:150.
- [294] Park SS, Choi YW, Kim J, Kim HS, Park TJ (2021). Senescent tumor cells: an overlooked adversary in the battle against cancer. *Exp Mol Med*, 53:1834-1841.
- [295] Zhang X, Wang Y, Cui W, Li D, Song J, et al. (2025). The multifaceted contributions of cancer-associated fibroblasts to drug resistance in primary and metastatic tumors. *Drug Resist Updat*, 82:101273.
- [296] Yasuda T, Koiwa M, Yonemura A, Miyake K, Kariya R, Kubota S, et al. (2021). Inflammation-driven senescence-associated secretory phenotype in cancer-associated fibroblasts enhances peritoneal dissemination. *Cell Rep*, 34:108779.
- [297] Nicolas AM, Pesic M, Engel E, Ziegler PK, Diefenhardt M, Kennel KB, et al. (2022). Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. *Cancer Cell*, 40:168-184.e13.
- [298] Meguro S, Johmura Y, Wang T, Kawakami S, Tanimoto S, Omori S, et al. (2024). Preexisting senescent fibroblasts in the aged bladder create a tumor-permissive niche through CXCL12 secretion. *Nat Aging*, 4:1582-1597.
- [299] Muthamil S, Kim H, Jang H, Lyu J, Shin UC, Go Y, et al. (2024). Biomarkers of cellular senescence and aging: current state-of-the-art, challenges and future perspectives. *Adv Biol (Weinh)*, 8:e2400079.
- [300] Dörr JR, Yu Y, Milanovic M, Beuster G, Zasada C, Däbritz JH, et al. (2013). Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature*, 501:421-425.
- [301] Barajas-Gómez BA, Rosas-Carrasco O, Morales-Rosales SL, Pedraza Vázquez G, González-Puertos VY, Juárez-Cedillo T, et al. (2017). Relationship of inflammatory profile of elderly patients serum and senescence-associated secretory phenotype with human breast cancer cells proliferation: role of IL6/IL8 ratio. *Cytokine*, 91:13-29.
- [302] Podkówka R, Wiśniewska J, Korybalska K, Wieczorowska-Tobis K, Knapowski J (2002). Plasma level of interleukin-6 and interleukin-8 in the elderly [in Polish]. *Przegl Lek*, 59:230-233.
- [303] Olivieri F, Rippo MR, Procopio AD, Fazioli F (2013). Circulating inflamma-miRs in aging and age-related diseases. *Front Genet*, 4:121.
- [304] Olivieri F, Lazzarini R, Recchioni R, Marcheselli F, Rippo MR, Di Nuzzo S, et al. (2013). MiR-146a as marker of senescence-associated pro-inflammatory status in cells involved in vascular remodelling. *Age (Dordr)*, 35:1157-1172.
- [305] Fulzele S, Mendhe B, Khayrullin A, Johnson M, Kaiser H, Liu Y, et al. (2019). Muscle-derived miR-34a increases with age in circulating extracellular vesicles

- and induces senescence of bone marrow stem cells. *Aging* (Albany NY), 11:1791-1803.
- [306] Kuiper LM, Mens MMJ, Wu JW, Goudsmit J, Ma Y, Liang L, et al. (2025). Plasma microRNA signatures of aging and their links to health outcomes and mortality: findings from a population-based cohort study. *Genome Med*, 17:70.
- [307] Wu F, Liu J, Tao M, Wang M, Ren X, Hai Z (2023). β -Galactosidase-activatable fluorescent and photoacoustic imaging of tumor senescence. *Anal Chem*, 95:10481-10485.
- [308] Liu J, Ma X, Cui C, Chen Z, Wang Y, Deenik PR, et al. (2021). Noninvasive NIR imaging of senescence via in situ labeling. *J Med Chem*, 64:17969-17978.
- [309] Hu L, Dong C, Wang Z, He S, Yang Y, Zi M, et al. (2023). A rationally designed fluorescence probe achieves highly specific and long-term detection of senescence in vitro and in vivo. *Aging Cell*, 22:e13896.
- [310] Lafontaine J, Cardin GB, Malaquin N, Boisvert JS, Rodier F, Wong P (2021). Senolytic targeting of Bcl-2 anti-apoptotic family increases cell death in irradiated sarcoma cells. *Cancers*, 13:386.
- [311] Hickson LJ, Langhi Prata LGP, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, et al. (2019). Senolytics decrease senescent cells in humans: preliminary report from a clinical trial of dasatinib plus quercetin in individuals with diabetic kidney disease. *EBioMedicine*, 47:446-456.
- [312] Lin R, Un H, Kang Y, Lei J, Chen L, Liu R, et al. (2025). Senescence-related gene signature predicts prostate cancer progression and identifies PCNA as a therapeutic target via multi-omics machine learning integration. *Br J Cancer*, 134:662-675.
- [313] Whittle JR, Vaillant F, Surgenor E, Policheni AN, Giner G, Capaldo BD, et al. (2020). Dual targeting of CDK4/6 and BCL2 pathways augments tumor response in estrogen receptor-positive breast cancer. *Clin Cancer Res*, 26:4120-4134.
- [314] Kalimutho M, Parsons K, Mittal D, López JA, Srihari S, Khanna KK (2015). Targeted therapies for triple-negative breast cancer: combating a stubborn disease. *Trends Pharmacol Sci*, 36:822-846.
- [315] Alcon C, Kovatcheva M, Morales-Sánchez P, López-Polo V, Torres T, Puig S, et al. (2024). HRK downregulation and augmented BCL-xL binding to BAK confer apoptotic protection to therapy-induced senescent melanoma cells. *Cell Death Differ*, 32:646-656.
- [316] Kaefer A, Yang J, Noertersheuser P, Mensing S, Humerickhouse R, Awni W, et al. (2014). Mechanism-based pharmacokinetic/pharmacodynamic meta-analysis of navitoclax (ABT-263) induced thrombocytopenia. *Cancer Chemother Pharmacol*, 74:593-602.
- [317] Josefsson EC, Vainchenker W, James C (2020). Regulation of platelet production and life span: role of Bcl-xL and potential implications for human platelet diseases. *Int J Mol Sci*, 21:7591.
- [318] Bogdanova DA, Kolosova ED, Pukhalskaia TV, Levchuk KA, Demidov ON, Belotserkovskaya EV (2024). The differential effect of senolytics on SASP cytokine secretion and regulation of EMT by CAFs. *Int J Mol Sci*, 25:4031.
- [319] Cao L, Li K, Li Q, Tong Q, Wang Y, Huang L (2025). The controversial role of senescence-associated secretory phenotype (SASP) in cancer therapy. *Mol Cancer*, 24:283.
- [320] Pereira BI, Devine OP, Vukmanovic-Stejic M, Chambers ES, Subramanian P, Patel N, et al. (2019). Senescent cells evade immune clearance via HLA-E-mediated NK and CD8⁺ T cell inhibition. *Nat Commun*, 10:2387.
- [321] Liu N, Wu J, Deng E, Zhong J, Wei B, Cai T, et al. (2025). Immunotherapy and senolytics in head and neck squamous cell carcinoma: phase 2 trial results. *Nat Med*, 31:3047-3061.
- [322] Lavarti R, Alvarez-Diaz T, Marti K, Kar P, Raju RP (2025). The context-dependent effect of cellular senescence: from embryogenesis and wound healing to aging. *Ageing Res Rev*, 109:102760.
- [323] Cuollo L, Antonangeli F, Santoni A, Soriani A (2020). The senescence-associated secretory phenotype (SASP) in the challenging future of cancer therapy and age-related diseases. *Biology*, 9:485.
- [324] Kita A, Yamamoto S, Saito Y, Chikenji TS (2024). Cellular senescence and wound healing in aged and diabetic skin. *Front Physiol*, 15:1344116.
- [325] González-Gualda E, Páez-Ribes M, Lozano-Torres B, Macias D, Wilson JR 3rd, González-López C, et al. (2020). Galacto-conjugation of navitoclax as an efficient strategy to increase senolytic specificity and reduce platelet toxicity. *Aging Cell*, 19:e13142.
- [326] Guerrero A, Guiho R, Herranz N, Uren A, Withers DJ, Martínez-Barbera JP, et al. (2020). Galactose-modified duocarmycin prodrugs as senolytics. *Aging Cell*, 19:e13133.
- [327] Bari R, Kang L, Gosiewska A, Hariri RJ (2026). The therapeutic potential of natural killer (NK) cells to combat aging and age-related diseases. *Discov Immun*, 3:5.
- [328] Gao D, Kan P, He Y, Sun S, Tang L, Yang F (2025). Senescent immune cells in the tumor microenvironment: emerging insights into cancer immunotherapy resistance. *Front Immunol*, 16:1656733.
- [329] Zingoni A, Antonangeli F, Sozzani S, Santoni A, Cippitelli M, Soriani A (2024). The senescence journey in cancer immunoediting. *Mol Cancer*, 23:68.
- [330] Liu Y, Zhang Z, Li T, Xu H, Zhang H (2022). Senescence in osteoarthritis: from mechanism to potential treatment. *Arthritis Res Ther*, 24:174.
- [331] Shahvali S, Rahiman N, Jaafari MR, Arabi L (2023). Targeting fibroblast activation protein (FAP): advances in CAR-T cell, antibody, and vaccine in cancer immunotherapy. *Drug Deliv Transl Res*, 13:2041-2056.
- [332] Leonteva A, Abdurakhmanova M, Bogachek M, Belovezhets T, Yurina A, Troitskaya O, et al. (2025). The activity of human NK cells towards 3D heterotypic cellular tumor model of breast cancer. *Cells*, 14:1039.
- [333] Milligan D, Newman J, Caley M, Wainwright L, Jenkins G, Philpott M, et al. (2017). Development of a 3D living

- skin equivalent to explore the influence of senescence on the skin ageing phenotype. *J Invest Dermatol*, 137:S310.
- [334] Kudo M, Yonezawa R, Mizobata H, Kinoshita S, Asakawa S (2026). Stage-dependent transcriptomic changes in human dermal fibroblast senescence model. *Aging (Albany NY)*, 18:349-368.
- [335] Saul D, Jurk D, Doolittle ML, Kosinsky RL, Han Y, Zhang X, et al. (2025). Distinct senotypes in p16- and p21-positive cells across human and mouse aging tissues. *EMBO J*, 44:7295-7325.
- [336] Sogabe Y, Shibata H, Kabata M, Tanaka A, Mitsunaga K, Sunadome K, et al. (2025). Characterizing primary and secondary senescence in vivo. *Nat Aging*, 5:1568-1588.
- [337] Cao Q, Wu X, Zhang Q, Gong J, Chen Y, You Y, et al. (2023). Mechanisms of action of the BCL-2 inhibitor venetoclax in multiple myeloma: a literature review. *Front Pharmacol*, 14:1291920.
- [338] Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. (2016). Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N Engl J Med*, 374:311-322.
- [339] Royce ME, Osman D. (2015). Everolimus in the treatment of metastatic breast cancer. *Breast Cancer (Auckl)*, 9:1-8.