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REVIEW

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# Cellular senescence in cancer: clinical detection and prognostic implications

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## Abstract

Cellular senescence is a state of stable cell-cycle arrest with secretory features in response to cellular stress. Historically, it has been considered as an endogenous evolutionary homeostatic mechanism to eliminate damaged cells, including damaged cells which are at risk of malignant transformation, thereby protecting against cancer. However, accumulation of senescent cells can cause long-term detrimental effects, mainly through the senescence-associated secretory phenotype, and paradoxically contribute to age-related diseases including cancer. Besides its role as tumor suppressor, cellular senescence is increasingly being recognized as an in vivo response in cancer patients to various anticancer therapies. Its role in cancer is ambiguous and even controversial, and senescence has recently been promoted as an emerging hallmark of cancer because of its hallmark-promoting capabilities. In addition, the prognostic implications of cellular senescence have been underappreciated due to the challenging detection and sparse *in* and ex vivo evidence of cellular senescence in cancer patients, which is only now catching up. In this review, we highlight the approaches and current challenges of *in* and ex vivo detection of cellular senescence in cancer patients, and we discuss the prognostic implications of cellular senescence based on *in* and ex vivo evidence in cancer patients.

**Keywords:** Senescence, Oncogene-induced senescence, Therapy-induced senescence, SASP, Cancer, Detection, Prognosis

## Background

### Cellular senescence in cancer

Cellular senescence is a cell state characterized by four interdependent hallmarks: (i) a durable and generally irreversible cell-cycle arrest; (ii) a senescence-associated secretory phenotype (SASP); (iii) macromolecular damage; and (iv) an altered metabolism [1]. Apart from the involvement in physiological processes, such as developmentally-programmed senescence [2, 3], tissue repair and wound healing [4, 5], cellular senescence is mainly a cellular stress response designed to eliminate damaged cells [6], and it is induced by numerous damage-inducing triggers, including ageing, DNA damage, reactive oxygen species, activation of oncogenes or inactivation of tumor-suppressor genes and inflammatory cytokines [1, 7].

Senescence was first described in vitro in human fetal diploid cell strains by Hayflick and Moorhead in 1961 to explain the finite lifespan of normal human cells as these do not proliferate indefinitely [8]. This phenomenon was already linked to cancer early on [9], as most cancer cells acquire the potential for unlimited cellular division and gain an infinite lifespan. During the following decades, the hypothesis that cellular senescence is an evolutionary homeostatic mechanism designed to irreversibly limit cell proliferation of damaged cells, which are at risk of malignant transformation, and to protect against cancer became more broadly accepted [10, 11]. However, the beneficial effect of cellular senescence in the context of

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(pre)malignant transformation rather results from the broader biological purpose of senescence, as an important mechanism, next to apoptosis, to eliminate many kinds of damaged cells in physiological and pathological processes, in order to maintain tissue homeostasis [6]. During normal embryogenic development, cellular senescence is a programmed mechanism that plays instructive roles [3], promotes tissue remodeling [2], and is also involved in tissue repair and wound healing [4, 5]. Cellular senescence is also considered as a crucial endogenous tumor suppressor mechanism. In this context, senescent cells have been identified in non-malignant and premalignant tissues in human tumor xenograft models such as lung adenomas [12], human benign melanocytic nevi [13], benign prostatic hyperplasia (BPH) [14], colon adenoma [15–17], precancerous urinary bladder [17] and intraepithelial prostatic neoplasia (PIN) [18] specimens. Oncogene-induced senescence (OIS) (i.e., senescence as a response to the activation of an oncogene or inactivation of a tumor-suppressor gene [19]) in transgenic mice has shown to suppress tumorigenesis of T cell lymphoma [20], prostate cancer [21], melanoma [22], lung adenocarcinoma [23] and pancreatic ductal adenocarcinoma [24]. This clearly marks the benefit of the senescence-associated growth arrest for preventing the expansion of pre- or fully malignant cells.

In fact, the idea that senescence only has a net positive effect on suppressing tumor growth was contradicted by the findings that senescent malignant [25] as well as non-malignant cells [26–29] are capable of driving tumor growth. Senescent cells stay metabolically active and can secrete a plethora of largely pro-inflammatory cytokines, chemokines, growth factors and matrix-remodeling proteases, collectively known as the SASP [30], capable of creating a protumorigenic microenvironment and driving tumorigenesis [31, 32]. Due to their genomic instability and the possibility to acquire additional mutations, cancer cells can also override the senescence-associated cell-cycle arrest and escape from the non-proliferative compartment [33–37]. Hence, the generally irreversible senescence-associated cell-cycle arrest is not necessary terminal for senescent cancer cells [1]. In addition, both non-malignant senescent cells and premalignant cells accumulate with ageing [38] due to an impaired clearing of senescent cells by the immune system over time [6] and accumulating oncogenic mutations acquired throughout life [39, 40], respectively. As such, the possibility of both occurring and interacting in close proximity increases in late life [38]. When this occurs, the SASP of non-malignant senescent cells can drive tumorigenesis of premalignant cells [38] opposing the net beneficial effect of senescent cells as a regulator of tissue homeostasis and tumor suppressor, paradoxically contributing to cancer development [38]. Besides its role as tumor suppressor, cellular senescence is increasingly being recognized as an in vivo response in cancer patients to various anticancer therapies (i.e., therapy-induced senescence (TIS) [41, 42]).

Taken together, the role of cellular senescence in cancer is ambiguous and even controversial, and senescence has recently been promoted as an emerging hallmark of cancer because of its hallmark-promoting capabilities [43]. In addition, the prognostic implications have been underappreciated due to the challenging detection and sparse in vivo and ex vivo evidence in cancer patients, which is only now catching up.

In this review, we first highlight the approaches and current challenges of *ex* and in vivo detection of cellular senescence in cancer patients. Next, we provide a comprehensive overview of available data regarding senescence in cancer patients, and discuss the prognostic implications of both OIS and TIS based on *ex* and in vivo evidence of cancer patients with solid tumors. Finally, we propose a simplified model for the observed differential prognostic outcomes of OIS and TIS in cancer patients.

## Main text

### Detection of cellular senescence in cancer patients

Identification and quantification of senescent cells in cancer patients in a clinical context is a challenging task since there are no specific and universal markers for senescent cells yet [1, 44]. Nonetheless, as an emerging hallmark of cancer [43], *ex* and in vivo evidence for cellular senescence residing in human tissue has gained more attention in the last decade [1] and efforts are made to accurately detect senescent cells in cancer patients. Below, we provide an overview of the different (pre)clinical approaches to detect cellular senescence in human tissue, pointing out the advantages and difficulties to implement these as clinical tools for the diagnosis and follow-up on cellular senescence in the context of a cancer patient.

#### *Ex vivo detection in patient tissue samples*

The best known and most widely used marker of cellular senescence is enhanced activity of acidic lysosomal β-galactosidase in senescent cells [45, 46], as lysosomes increase in number and size when cells become senescent [47]. The senescence-associated beta-galactosidase (SA-β-Gal) activity is often considered the gold standard for identifying senescent cells, despite SA-β-Gal activity was reported as a non-universal marker for cellular senescence [48]. Although absent in most proliferating and quiescent cells [44], SA-β-Gal activity is expressed in certain cell types (i.e., macrophages [49], bone marrow cells [50], melanocytes and sebaceous and eccrine gland cells [48]) and in vitro cells under certain cell culture

conditions (i.e., confluence and serum starvation [51–53]) independent of a senescent cell state. Also, SA-β-Gal is not essential for senescence as cells can become senescent without expressing SA-β-Gal [54]. Of note, SA-β-Gal detection is only possible in fresh snap-frozen tissue samples [45], thus hampering its use in a clinical context.

To overcome the disadvantages of SA-β-Gal as marker for senescence, a biotin-linked Sudan Black B (SBB) analogue was designed to detect lipofuscin accumulation in senescent cells [45]. Lipofuscin is a non-degradable aggregate of oxidized lipids and proteins [55], that accumulate in lysosomes of senescent cells due to senescence-related lysosomal malfunction, and is considered a hallmark of cellular senescence [1, 56]. In contrast to the enzymatic SA-β-Gal activity, lipofuscin is preserved in fixed materials [51]. As such, detection of cellular senescence is feasible in formalin-fixed paraffin-embedded (FFPE) archival tissue samples using the SSB histochemical stain [45]. The interpretation of the assay requires some experience, as lipofuscin aggregates can be very small and background dirt can be wrongly interpreted as positive SBB-positive lipofuscin aggregates, comprising the overall sensitivity [45]. Interestingly, endogenous lipofuscin is linked to chronic liver disease and can be detected by autofluorescence in biopsied samples of human liver tissue [57]. As such, autofluorescence of lipofuscin in the context of cellular senescence could potentially be exploited to detect senescence in patient samples.

Other commonly used markers of cellular senescence are the cell cycle inhibitors $p16^{INK4a}$ and $p21^{WAF1/Cip1}$, as most senescence-inducing triggers lead to the activation of the cell cycle inhibitor pathways $p53/p21^{WAF1/Cip1}$ and/or $p16^{INK4a}$ [7] (Fig. 2). While $p21^{WAF1/Cip1}$ expression occurs early after senescence induction and is reversible upon tumor suppressor protein p53 inactivation, $p16^{INK4A}$ expression is frequently induced late after senescence induction and is irreversible upon p53 inactivation [44, 58, 59]. $p21^{WAF1/Cip1}$ expression is therefore more likely to represent early cellular senescence, whereas $p16^{INK4a}$ expression represents a more established and durable senescence response [44]. However, $p21^{WAF1/Cip1}$ can be expressed by non-senescent cells in case of DNA-damage [60] and the genes encoding for $p21^{WAF1/Cip1}$ and $p16^{INK4a}$ (i.e., *CDKN1A* and *CDKN2A*, respectively) were not identified within the core transcriptome signature of senescent cells [61].

The senescence-associated cell cycle arrest, which occurs in G1 and possibly in G2 phase of the cell cycle [62], is marked by the absence of the proliferation marker Ki67 [1]. However, Ki67 is also absent in other cell states with a temporarily and durable cell-cycle withdrawal in $G_0$, such as quiescence and terminally differentiated cells, respectively. 5-ethynyl-2′-deoxyuridine (EdU) is another proliferation markers and a thymidine analog that, when administered to cells, can incorporate into DNA during replication [44]. Unfortunately, EdU is not applicable for ex vivo tissue samples as active proliferation is required after biopsy.

As SA-β-Gal activity [56], $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ expression [60] and absence of Ki67 expression [63] are neither specific nor universal for cellular senescence, the International Cell Senescence Association [1] and others [44] recommend combining different markers for the detection of cellular senescence with the highest accuracy. However, combining all the markers in the same tissue sample is not yet possible without incurring artifactual false positives or negatives [44]. Therefore, current senescence validation in patient tissue samples can be determined either in snap-frozen samples using a sequential staining for SA-β-Gal and Ki67 on independent sequential and adjacent sections, or in FFPE tissue samples using a double-staining for lipofuscin and/or Ki67, $p21^{WAF1/Cip1}$ or $p16^{INK4a}$ combined with a sole staining of the remaining markers (Ki67, $p21^{WAF1/Cip1}$ or $p16^{INK4a}$) on sequential and adjacent Sects [44]. Additional immunohistochemical confirmation can be achieved by detection of components of senescence-associated heterochromatin foci (SAHF), including histone variant macroH2A [64], di- or trimethylated lysine 9 histone H3 (H3K9me2/3), heterochromatin protein (HP) 1 α, β and γ [64, 65] and high mobility group A (HMGA) proteins [64, 66, 67], and DNA damage foci such as phosphorylated H2AX (γH2AX) [68] (Fig. 1).

Using flow-cytometry, senescent cells can be identified and quantified on single-cell level by combining SA-β-Gal activity with staining of molecular markers for cellular senescence (e.g., γH2AX) and absence of Ki67 and/or high mobility group box 1 (HMGB1) protein [69]. Recently, a preliminary proof of concept method was developed to detect senescent cells with imaging flow cytometry based on measuring autofluorescence and morphological parameters, and on applying recent artificial-intelligence (AI) and machine learning (ML) tools [70], potentially facilitating cellular senescence detection without a multi-marker strategy.

Indirect markers of cellular senescence such as messenger RNA (mRNA) expression of $p16^{INK4a}$, $p21^{WAF1/Cip1}$ and lamin B1 (i.e., a nuclear lamina component and downregulated in case of senescence [71]), and selected senescence core genes can be determined by reverse transcription–polymerase chain reaction (RT-PCR) [44] (Fig. 1). However, both flow-cytometry and RT-PCR techniques require tissue dissociation and case-specific control samples, and do not provide any spatial information on senescent cells residing in the tissue, limiting

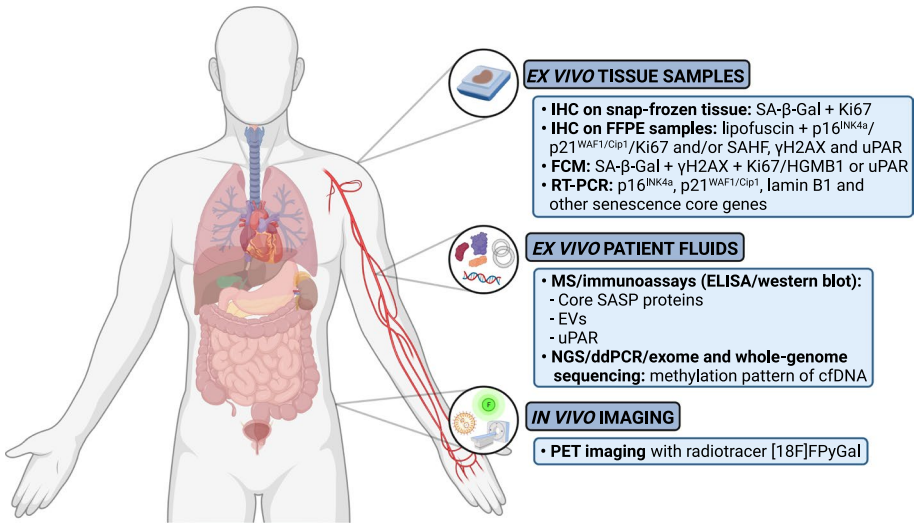


**Fig. 1** Overview of approaches of *ex* and in vivo detection of cellular senescence with corresponding senescence markers in cancer patients. IHC, immunohistochemistry; SA-β-Gal, senescence-associated beta-galactosidase; FFPE, formalin-fixed paraffin-embedded; SAHF, senescence-associated heterochromatin foci; γH2AX, phosphorylated H2AX; FCM, flow-cytometry; HGMB1, high mobility group box 1; RT-PCR; reverse transcription–polymerase chain reaction; MS, mass spectrometry; SASP, senescence-associated secretory phenotype; EVs, extracellular vesicles; NGS, next-generation sequencing; ddPCR, digital droplet polymerase chain reaction; cfDNA, cell-free DNA; PET-imaging, positron emission tomography-imaging

their clinical utility for solid tumors. Although various senescence core genes have been determined [72], the senescent phenotype is dynamic and heterogeneous and depends on the tissue of origin and senescence-inducing trigger [72–77]. It is currently unclear which specific core genes should be included in order to confirm cellular senescence in a disease- and tissue-specific context [44]. Recently, using machine learning, a gene expression classifier (SENCAN classifier) was developed for the detection of senescence in cancer samples. Using transcriptome data as input, the SENCAN classifier was able to classify whether cancer cells are senescent or not. Unfortunately, whereas the SENCAN classifier is able to accurately detect senescence in many cancer cells in vitro, its accuracy to detect senescence in in vivo cancer samples is still unclear [76].

Of note, uPAR was recently identified as a cell surface protein that is broadly and specifically upregulated in senescent cells of mice using RNA-sequencing datasets derived from three independent and robust mouse models of OIS as well as TIS [78]. uPAR is involved in many intracellular signaling pathways that promote cell motility, invasion, proliferation and survival [79] and is expressed by tumor and stromal cells in a wide variety of human cancers where its expression frequently indicates poor prognosis [79]. In this context, uPAR expression and secretion (i.e., soluable uPAR) could be an interesting biomarker of senescence in cancer patients detected by immunohistochemistry, flow-cytometry or enzyme-linked immunosorbent assay (ELISA) [78] (Fig. 1).

### *Ex vivo detection in patient fluids*

Detection of tumor-specific circulating material in patient fluids by means of liquid biopsy is an emerging field in oncology, with important clinical implications for personalized medicine [80]. In contrast to surgical or biopsy tissue samples, liquid biopsies are not subject to sampling bias, tumor heterogeneity and can be obtained repeatedly to monitor the evolution of the molecular profile of the tumor which may cause drug resistance [81]. However, the detection of cellular senescence via liquid biopsy in patient blood or urine requires specific senescence-associated circulating material including proteomes, extracellular vesicles (EVs) and circulating cell-free DNA (cfDNA).

First, SASP proteins can be measured by mass spectrometry or immunoassays (ELISA, western blot) in patient plasma [44, 74]. A proteomic atlas of core SASP secreted proteins originating from multiple senescence inducers and cell types was recently determined [74], enabling senescence detection by the presence of core SASP proteins, such as growth/differentiation factor 15 (GDF15), matrix metalloproteinase-1 (MMP1), stanniocalcin-1 (STC1), tissue inhibitor of metalloproteinases 1 and 2 (TIMP1 and TIMP2) [44] (Fig. 1). However, several of these core soluble SASP proteins have also been identified as biomarkers of human disease [75] and are

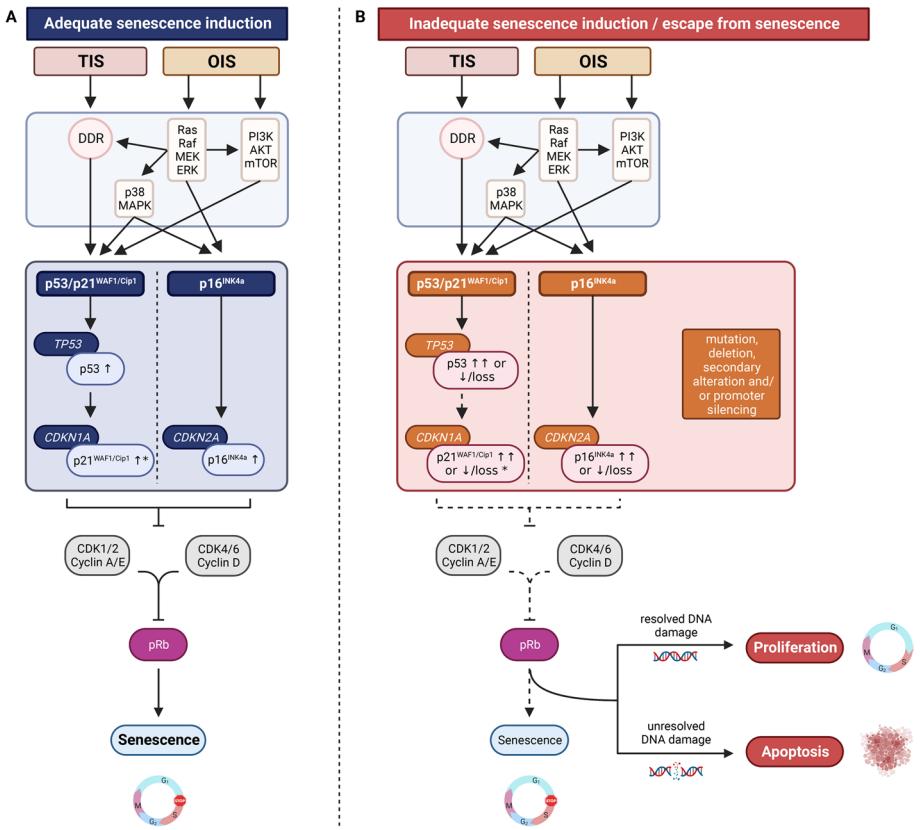


**Fig. 2** Molecular pathways of OIS and TIS. **A** Adequate senescence induction via participation of the DDR and the Ras-Raf-MEK-ERK, PI3K/AKT/mTOR and p38/MAPK signaling pathways resulting in functionally activated cell cycle inhibitor pathways (solid arrows) and upregulation of tumor suppressor proteins p53, $p21^{WAF1/Cip1}$ and $p16^{INK4a}$. Functional $p21^{WAF1/Cip1}$ and/or $p16^{INK4a}$ induce a stable cell cycle arrest by inhibition of CDK (i.e., CDK1, CDK2, CDK4 and CDK6)—cyclin (i.e., cyclin A, E and D) complexes, thereby preventing phosphorylation of the retinoblastoma protein (solid inhibitor lines), which blocks S-phase entry and induces senescence (solid arrow). **B** Inadequate senescence induction or escape from senescence due to (acquired) mutations, deletions, secondary alterations and/or promoter silencing affecting cellular control genes *TP53* (encoding p53), *CDKN1A* (encoding $p21^{WAF1/Cip1}$) *CDKN2A* (encoding $p16^{INK4a}$), resulting in absent or dysfunctional cell cycle inhibitor pathway activation (dotted arrow) and absent or dysfunctional tumor suppressor proteins to induce or maintain senescence (dotted inhibitor lines and arrow). Depending on whether the DNA damage is repaired, the cell may resume proliferation or go into apoptosis. * $p21^{WAF1/Cip1}$ can also be activated by pathways that are independent of p53 [125]. OIS, oncogene-induced senescence; TIS, therapy-induced senescence; DDR, DNA damage response; CDK, cyclin-dependent kinase; pRb, retinoblastoma protein

positively associated with age, frailty and adverse post-surgery outcomes [82]. For example, GDF15, MMP1 and STC1 have been identified as a biomarker for cardiovascular disease [83], several cancers [84] and for Alzheimer's disease [85], respectively. SASP biomarkers currently lack sensitivity to detect and attribute senescence in patient plasma to specific pathologies, including cancer [75]. However, SASP protein profiles differ among cell type, senescence-inducing trigger and interval after senescence induction [62, 74, 75], as well as age category [82] as senescent cells accumulate with increasing age [6]. By determining disease-, tissue-, and inducer-specific SASP factors as well as robust core SASP factors secreted by senescent cells in multiple contexts [75], it will become possible to attribute the secretion of certain SASP factors to the presence of senescent cells in patients in the near future.

Next to SASP proteins, senescent cells of human origin (i.e., foreskin primary [86], normal lung [87] and diploid [88] fibroblasts, prostate [87, 89], hepatocellular [87] and triple negative breast [90] cancer cells, retinal pigment epithelial cells [88] and human chondrocytes [91]) are capable of releasing EVs in patient fluids such as blood and urine [92]. (Fig. 1). EVs are small, lipid-bilayer enclosed, cell-derived particles that bear surface molecules that allow them to target recipient cells and contain transmembrane and enclosing cytosolic proteins and RNA [75, 93]. Once internalized, EVs release

their content into the cytosol modifying the physiological state of the recipient cell [93] and enabling cell communication with neighboring as well as distant cells. As for SASP proteins, EV production and content drastically differ in physiological [94] and pathological [92] conditions, making EVs an additional interesting source of disease biomarkers. EVs of senescent cells are capable of transmitting paracrine senescence to neighboring cells [86, 91], contain chemotherapy and key proteins involved in cell proliferation after chemotherapeutic challenge [90] and can even promote cancer cell proliferation [88]. Interestingly, protein content of EVs secreted by senescent cells differs from secreted SASP proteins [74, 86], suggesting that SASP and EVs do not act as surrogate biomarkers and have different clinical significance and value [75]. However, as for SASP proteins, it is still unclear how EVs secreted by senescent cells exactly behave in physiologic and disease-specific contexts, and to what extend they depend on age, tissue and senescence-inducing trigger [75].

Detection and analysis of cfDNA by means of next-generation sequencing, digital droplet polymerase chain reaction, exome or whole-genome sequencing [95] could be a third appealing strategy to detect and monitor the senescence burden in cancer patients. Senescent cells exhibit a DNA methylation pattern of promoter hypermethylation mainly involving metabolic regulators, whereas transformed cells exhibit a DNA methylation pattern of promoter hypermethylation involving primarily pro-survival and developmental genes [96] (Fig. 1). Also, using a machine learning based approach trained with different early passage and senescent cells, a DNA methylation fingerprint of cellular senescence (DNAmSen) was developed and validated in clinical patient samples, such as whole blood and skin tissue [97]. With this approach, clear and robust correlations were found between the patient's age and DNAmSen present in the corresponding sample. Interestingly, also elevated DNAmSen were observed in lung samples from patients with COPD and lung cancer compared to those of healthy controls [97]. Not surprisingly, the release of cfDNA is affected by type of treatment and timing from treatment exposure, and also heavily depend on the cellular response to treatment. Of note, it is thought that blocks the release of cfDNA whereas apoptosis and necrosis are key contributor of its release [98].

#### *In vivo detection in patients*

Currently, there is no established method to detect cellular senescence in vivo in patients. Detection can be achieved by chromogenic [99, 100] or fluorogenic [101–107] probes, preferentially hydrolyzed by SA-β-Gal, resulting in color- or fluorescence-enhanced senescent cells. Chromogenic [108] or fluorogenic [109] probes hydrolyzed by other lysosomal hydrolases overexpressed in senescent cells, such as α-L-fucosidase [110], can be used as well. Nanoparticles containing fluorescent dyes and probes have been developed that selectively release their content when the cap of the nanoparticle is hydrolyzed by SA-β-Gal after endocytosis [111–113], or by interaction with CD9 receptors [114] or β2 microglobulin [115], both preferentially expressed by senescent cells. Due to low tissue penetrance and autofluorescence the clinical use of these fluorescent probes and nanoparticles may be limited in patients [116]. An alternative method could be the detection of endogenous lipofuscin as endogenous lipofuscin, next to ex vivo in patient samples, can also be monitored in vivo and non-invasively via imaging, as has been shown in mice with chronic liver disease [57].

It should be noted that all these approaches were validated only in vitro or in vivo in mice and its use in patients should be further investigated. Currently, there is one first-in-human trial in cancer patients evaluating the safety and imaging characteristics of a novel senescence-specific radiotracer [18F]FPyGal (i.e., a radioactive form of SA-β-Gal) that can be tracked non-invasively in the body through positron emission tomography (PET) imaging (SenPET; NCT04536454) [117] (Fig. 1). Whether this strategy is sufficient to detect all senescent cells [118] due to the aforementioned limitations of SA-β-Gal as a specific marker of senescence has yet to be determined.

### Prognostic implications of cellular senescence in cancer patients

Senescence is considered to exert beneficial effects by halting cancer development and promoting survival in early life, but it is proposed to have detrimental effects later in life when senescent cells accumulate due to ageing and/or inappropriate removal [32, 38, 119]. Based on preclinical cancer research, these antagonistically pleiotropic effects of senescence are thought to be highly dependent on the type of cancer and senescence trigger [30, 32, 62]. The prognostic implications of cellular senescence in cancer are therefore often unpredictable primarily due to the dual role of the SASP [32].

#### *Senescence burden*

The senescence-associated cell cycle arrest is considered fundamentally tumor-suppressive and the induction occurs through the involvement of different signaling and downstream cell cycle inhibitor pathways. Genotoxic stress induced by anticancer therapies results in a DNA-damage response (DDR) which leads to p53 and $p21^{WAF1/Cip1}$ activation whereas oncogenic signaling and tumor suppressor inactivation results in downstream activation

of both p53/p21$^{WAF1/Cip1}$ and p16$^{INK4a}$ via participation of the DDR and the Ras-Raf-MEK-ERK, PI3K/AKT/mTOR and p38/MAPK signaling pathways [6, 7, 62]. As such, TIS is primarily induced through p53/p21$^{WAF1/Cip1}$ pathway activation whereas OIS is induced through either p53/p21$^{WAF1/Cip1}$ and/or p16$^{INK4a}$ pathway activation (Fig. 2A). Upregulation of functional tumor suppressor proteins p53, p21$^{WAF1/Cip1}$ and p16$^{INK4a}$ inhibit downstream cyclin-dependent kinase (CDK)—cyclin complexes, such as CDK2—cyclin E and CDK CDK4/6—cyclin D, preventing phosphorylation of the retinoblastoma protein [7]. Hyperphosphorylation of this tumor suppressor protein blocks S-phase entry [120] and is responsible for the induction of senescence [6] (Fig. 2A). Of note, despite tumor suppressor proteins p53, p21$^{WAF1/Cip1}$ and/or p16$^{INK4a}$ are primarily involved in TIS and OIS (Table 1), senescence can be induced [121] as well as bypassed [7, 16, 122–124] independent of p53/p21$^{WAF/Cip1}$ and/or p16$^{INK4a}$ pathway activation and inactivation/abrogation, respectively. Also, p21$^{WAF1/Cip1}$ can be activated by pathways that are independent of p53 [125].

There is abundant *ex* and in vivo evidence in several tumor types that OIS acts as a tumor-suppressive mechanism preventing the expansion of pre- or fully malignant cells (Table 1). OIS is found in precursor lesions and in low TNM stage tumors with more favorable clinicopathologic features [13, 202, 209], whereas in full-blown malignant lesions OIS-related markers are often dysregulated or completely lost [173] and correlate with higher TNM stage tumors and poor clinicopathological parameters [136, 164, 211, 212] (Table 1). Tumor suppressor protein p16$^{INK4a}$ often comes forward as the main regulator for maintaining the OIS-associated cell cycle arrest which is considered to be more crucial for maintaining the senescence-associated cell cycle arrest whereas p53/p21$^{WAF1/Cip1}$ pathway activation is more involved in the initiation of senescence [59]. Mutations, deletions, secondary alterations and/or promoter silencing of cellular control genes (i.e., *TP53*, *CDKN1A* and *CDKN2A*) encoding for tumor suppressor proteins p53, p21$^{WAF1/Cip1}$ and p16$^{INK4a}$ may result in inadequate senescence induction or escape from senescence due to absent or dysfunctional cell cycle inhibitor pathway activation and absent or dysfunctional tumor suppressor proteins to induce or maintain OIS. Hence, dysregulated (i.e., decreased or overexpressed) expression or complete loss of tumor suppressor proteins p16$^{INK4a}$, p21$^{WAF1/Cip1}$ and p53 are often correlated with increasing grade of malignancy and tumor progression, and associated with a negative prognostic outcome [139, 140, 158, 170, 187, 205–207, 213, 214, 236] (Table 1) (Fig. 2B).

However, in certain tumor types, the (abundant) presence of OIS or expression of senescence-associated markers is also linked to worse prognosis [132, 197, 221, 237, 238, 248]. Perhaps even more surprisingly, both absence and extensive presence of senescence in CRC was associated with negative prognosis whereas moderate presence was associated with the best prognosis [174], demonstrating that an extensive senescence burden can paradoxically impair clinical outcome in contrast to a moderate senescence burden.

Concerning TIS, evidence demonstrates that TIS is an in vivo relevant outcome of various anticancer therapies in several tumor types (Table 1). For example, in breast, colorectal and prostate cancer TIS was observed after neoadjuvant genotoxic chemotherapy [150, 177, 178] and antihormone therapy [204]. Therapy-induced senescent cells were identified in residual drug-resistant tumors [150] and in samples with partial or incomplete pathological response to neoadjuvant therapy [149], suggesting TIS might persist after neoadjuvant therapy [203] and is responsible for incomplete tumor regression [204]. The presence of TIS is however linked to contradictory clinical outcomes and is associated with worse [135, 137] as well as improved [163, 176, 178] prognosis depending on tumor type. For example, while in non-small cell lung cancer (NSCLC) TIS is associated with worse OS [135], in CRC a higher proportion of therapy-induced senescent cells after chemotherapy treatment was associated with a longer progression-free survival (PFS) compared to when the proportion of senescent tumor cells did not change before and after chemotherapy [178]. Thus, regardless of the type of cancer, the senescence burden of OIS and TIS seems to be an important determinant affecting the outcome in cancer patients.

#### *Secretion, composition and time-dependent impact of SASP*

Whereas the senescence-associated cell cycle arrest acts tumor-suppressive, SASP factors secreted by senescent cells can be both tumor-suppressive and tumor-promoting [255]. The main signaling pathways involved in SASP regulation include NF-κB, p38, mTOR, C/EBPβ and JAK2/STAT3 [16, 256–260]. Interleukin (IL)-1α is secreted by oncogene-induced and therapy-induced senescent cells and initiates the production of key SASP proteins such as IL-6 and IL-8 through activation of NF-κB and C/EBPβ [62, 261]. The senescent phenotype is subsequently enforced autocrinally by IL-6 [16] and IL-8 [122] and transmitted paracrinally to neighboring cells by IL-1α [262], further enhancing the production of these SASP factors. Abundant SASP factors IL-6 and IL-8 have both anti-tumorigenic and pro-tumorigenic effects [263]. For example, both interleukins mediate the recruitment of macrophages, T cells and natural killer (NK) cells supporting immune surveillance and elimination of senescent cancer cells [32] but also create a chronic

**Table 1** Prognostic implications of OIS and TIS based on available *ex* and in vivo evidence of cancer patients with solid tumors according to cancer type and senescence trigger

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| **NSCLC** | Resected NSCLC | Lipofuscin | CS | High expression associated with worse OS | [126] |
| | | $p21^{WAF1/Cip1}$, cyclin E and Ki67 | | | [127] |
| | Resected NSCLC SCC | $p21^{WAF1/Cip1}$ | | Positive expression associated with improved survival | [128] |
| | Resected NSCLC AC / SCC | | | Reduced expression in stage III compared to stage I or II | |
| | NSCLC SCC | $p16^{INK4a}$ | | High expression associated with improved OS | [129] |
| | Resected NSCLC AC / SCC | | | Loss of expression associated with worse OS | [130] |
| | | macroH2A1.1 | | Low expression associated with worse DFS | [131] |
| | Resected NSCLC AC | Tumoral senescence signature (lipofuscin, $p16^{INK4a}$, $p21^{WAF1/Cip1}$ and Ki67[c]) | | Tumoral senescence signature associated with worse DFS and OS | [132] |
| | Resected NSCLC AC / SCC / other histology | | TIS: platinum-based CT with or without RT | Evidence of TIS as treatment outcome | |
| | NSCLC AC | Senescence-related gene signature: *FOXM1*, *HJURP*, *PKM*, *PTTG1* and *TACC3* | CS | High expression associated with:<br>- disease progression and worse OS<br>- immune-suppressive and protumorigenic TME<br>- high expression of immune checkpoint genes and TMB levels | [133] |
| | | Senescence marker genes: *VPS26A* and *LANCL1* | | High expression associated with decreased survival | [134] |
| | NSCLC AC | Senescence marker genes: *VAMP3*, *STX4* and *ARMX3* | | High expression associated with increased survival | |
| | NSCLC AC / SCC | Senescence marker genes: *NTAL*, *DEP1* and *B2MG* | | High expression associated with mixed survival outcomes depending on histology | |
| | Resected NSCLC | SA-β-Gal and CDK1 | TIS: CB/PTX | Expression demonstrates TIS as treatment outcome | [37] |
| | | SA-β-Gal | TIS: CB/PTX or CRT[d] | Expression associated with worse OS | [135] |
| | NSCLC AC | CS gene score | CS | Lower CS gene score in primary tumors compared to adjacent normal solid tissue | [136] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | NSCLC SCC | | | | |
| **Malignant pleural mesothelioma** | Resected MPM | SA-β-Gal, $p21^{WAF1/Cip1}$ and PAI1 and mRNA $p21^{WAF1/Cip1}$ and PAI1 | TIS: platinum-based CT | - Increased expression after neoadjuvant CT<br>- Stable disease associated with PAI1 expression, time-to-progression and worse OS | [137] |
| **Breast cancer** | Breast cancer | Senescence marker gene panel: *DEP1, NTAL, EBP50, STX4, VAMP3, ARMX3, B2MG, LANCL1, VPS26A* and *PLD3* | CS | High expression associated with increased OS | [134] |
| | | CS gene score | | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| | Resected primary invasive ductal carcinoma | $p16^{INK4a}$ and p53 | | Expression associated with worse DFS and OS | [138] |
| | Resected breast cancer | $p16^{INK4a}$ | | Overexpression associated with unfavorable prognostic indicators (high grading, negative estrogen receptor status, inverse progesterone receptor status and high Ki67 expression) and indicative of a more undifferentiated malignant phenotype | [139] |
| | | $p21^{WAF1/Cip1}$ and p53 | | Low $p21^{WAF1/Cip1}$ expression along with p53 overexpression associated with short DFS and OS, suggesting p53 overexpression reflects complete abrogation of p53 function | [140] |
| | | $p21^{WAF1/Cip1}$ | | Expression associated with worse DFS and OS | [141–143] |
| | Early breast cancer | uPA-PAI1 | | - High levels associated with worse DFS<br>- Patients with high levels benefit more strongly from adjuvant CT | [144, 145] |
| | Resected triple negative breast cancer | macroH2A1.1 mRNA ratio | | High ratio associated with worse DFS | [146] |
| | Resected breast cancer | SA-β-Gal | TIS: CP/DOX/5-FU | Expression demonstrated TIS as treatment outcome | [147] |
| | | $p21^{WAF1/Cip1}$, $p27^{Kip1}$, p53 and cyclin D3 | TIS: CP/MTX/5-FU or ECX/DOC | | [148] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | | $p21^{WAF1/Cip1}$, H3K9Me3 and lamin B1[c] | TIS: DOC/DOX/CP, PTX/DOX/CP, DOX/CP, DOC/CP, 5-FU/EPX/CP or 5-FU/EPX/CP followed by DOC | | [149] |
| | | mRNA of $p16^{INK4a}$, $p21^{WAF1/Cip1}$ and CCNA1 | TIS: EPX/CP | - High expression demonstrated TIS as treatment outcome<br>- Persistent senescent cells evaded immune clearance | [150] |
| | | Lipofuscin | CS | Expression present in TME | [151] |
| | | | TIS: CT[d] | | |
| **Cervical, uterine, UCEC and ovarian cancer** | Normal cervical epithelium | $p16^{INK4a}$, $p21^{WAF1/Cip1}$, $p15^{INK4a}$ and $p14^{ARF}$ | OIS | Almost completely negative expression | [152, 153] |
| | Cervical dysplastic and SCC | | | Overexpression | |
| | Normal cervical epithelium, CIN and cervical carcinoma | $p21^{WAF1/Cip1}$ | | Higher expression in cervical carcinoma compared to normal cervical epithelium and CIN | [154] |
| | | | | Expression associated with advanced stage | [155] |
| | Cervical AC | | | Expression associated with favorable prognosis | [156] |
| | Cervical SCC | CS gene score | CS | Positively correlated with PD-L1 protein expression and T cell cytotoxicity | [136] |
| | UCEC | | | Lower score in primary tumors compared to adjacent normal solid tissue | |
| | Resected pEOC | SA-β-Gal | | Expression demonstrated spontaneous CS | [157] |
| | | $p21^{WAF1/Cip1}$ and p53 | | Low $p21^{WAF1/Cip1}$ expression along with p53 overexpression associated with higher recurrence rate, suggesting p53 overexpression reflects complete abrogation of p53 function | [158] |
| | Resected HGSOC | SA-β-Gal and γH2AX | | Higher expression in older patients | [159] |
| | Resected OC | p53, $p16^{INK4a}$ and pRb | | Negative or high p53, high $p16^{INK4a}$ and reduced pRb expression associated with worse OS | [160] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Primary OC | SA-β-Gal, $p16^{INK4a}$, H1-β and Ki67[c] | | Identification of senescent CAFs adjacent to epithelial ovarian cancer cells suggested to promote ovarian cancer tumorigenesis | [161] |
| | Normal ovary, primary OC, metastasis of OC, recurrent OC | $p21^{WAF1/Cip1}$ | | - Expression gradually increased from normal ovary through primary OC, metastasis of OC and recurrent OC<br>- Expression associated with decreased time-to-progression | [162] |
| | Advanced-stage serous OC and suboptimally debulked OC | Senescence marker genes: *VAMP3, ARMCX3* and *B2MG* | CS/TIS[d] | High expression associated with decreased survival | [134] |
| | | Senescence marker genes: *EBP50* and *NTAL* | | High expression associated with increased survival | |
| | Resected HGSOC | $p16^{INK4a}$ and lamin B1[c] | TIS: CB/PTX | High expression associated with improved 5-year OS | [163] |
| **Esophageal cancer** | Precursor and ESCC lesions | Dec1 | OIS | Expression demonstrated OIS as tumor-suppressive mechanism | [164] |
| | Resected ESCC | | | Low expression:<br>- correlated with poor clinicopathological parameters (i.e., T-stage, lymph node metastasis and pathological TNM-stage)<br>- associated with worse DFS | |
| | | $p16^{INK4a}$, $p21^{WAF1/Cip1}$, pRb, Bax protein and cyclin D1 | | Combined high expression and low cyclin D1 expression associated with improved OS | [165] |
| | Normal tissue, precursor lesions and ESCC | $p16^{INK4a}$, $p14^{ARF}$ and $p15^{INK4b}$ | | Increased expression in ESCC and in poorly differentiated specimens with lymph node metastasis, suggesting involvement of CS in cancer progression | [166] |
| | | $p21^{WAF1/Cip1}$ | | - Expression frequently found in precursor lesions and invasive ESCC compared to normal tissue<br>- High expression associated with worse OS in curatively treated ESCC | [167] |
| | EC | Senescence gene signature: *ADH1B*, *IL1A*, *SERPINE1*, *SPARC*, *EZH2* and *TNFAIP2* | | Enriched senescence gene signature in noncancerous cells of TME associated with improved OS | [168] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | ESCC | CS gene score | CS | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| **Gastric cancer** | Resected gastric cancer | Senescence gene signature: *ADH1B*, *IL1A*, *SERPINE1*, *SPARC*, *EZH2* and *TNFAIP2* | CS | Enriched senescence gene signature in noncancerous cells of TME associated with:<br>- improved DFS and OS<br>- related to MSI, higher TMB and improved benefit from immunotherapy | [168] |
| | | Senescore based on proteins ADH1B, IL1A, SERPINE1, SPARC, EZH2 and TNFAIP2 | | High-senescore protein expression associated with improved OS | |
| | Gastric AC | CS gene score | | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| | Gastric cancer | $p21^{WAF1/Cip1}$ and mRNA of $p21^{WAF1/Cip1}$ | | Increased expression associated with improved OS | [169] |
| | Primary gastric adenocarcinoma | $p21^{WAF1/Cip1}$ and p53 | | Low $p21^{WAF1/Cip1}$ expression along with p53 overexpression associated with more aggressive tumoral characteristics, higher recurrence rate and poorer survival, suggesting p53 overexpression reflects complete abrogation of p53 function | [170] |
| **Colorectal cancer** | *KRAS* and *BRAF* mutated benign serrated polyps | SA-β-Gal, $p16^{INK4a}$ and Ki67[c] | OIS | - Expression demonstrated OIS<br>- OIS increased with degree of dysplasia | [171, 172] |
| | Colon adenoma | SA-β-Gal, $p16^{INK4a}$, p53, HP1α, HP1γ, H3K9me3 and Ki67[c] | | Expression demonstrated OIS as tumor-suppressive mechanism | [15–17] |
| | Dysplastic aberrant crypt foci and adenomas | $p21^{WAF1/Cip1}$ | | Lower expression suggesting dysregulated expression of cell-cycle controlling genes in tumorigenesis of CRC | [173] |
| | Early invasive colorectal carcinoma | SA-β-Gal, $p16^{INK4a}$, p53, HP1α, HP1γ, H3K9me3 and Ki67[c] | | Reduced or lost expression demonstrated loss of OIS | [15, 17, 171, 172] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Resected primary CRC | $p21^{WAF1/Cip1}$, NTAL, ARMCX3, EBP50 and γH2AX | CS | - Absent and extensive expression associated with negative prognosis, moderate expression with best prognosis<br>- Distance between senescent cells and $CD8^+$ T cells and a higher % $CD8^+$ T cells near senescent cells linked to increased DSS and PFS, suggesting tumor-suppressive potential of CS is determined by TME and immune cell-mediated elimination of senescent tumor cells | [174] |
| | | $p16^{INK4a}$ and Ki67[c] | | - Intratumoral senescence (high $p16^{INK4a}$ and low Ki67) associated with reduced T cell infiltrates and low-grade inflammatory cell infiltrate<br>- Low $p16^{INK4a}$ expression associated with decreased survival | [175] |
| | CRC | Senescence marker genes: *VPS26A*, *ARMCX3* and *B2MG* | | High expression associated with decreased survival | [134] |
| | | Senescence marker genes: *NTAL* | | High expression associated with increased survival | |
| | | Senescence gene signature: *ADH1B*, *IL1A*, *SERPINE1*, *SPARC*, *EZH2* and *TNFAIP2* | | Enriched senescence gene signature in noncancerous cells of TME associated with improved OS | [168] |
| | Metastasized CRC | p-ERK, HP1γ and PAI1 | TIS: 5-FU/leucovorin | - Expression demonstrated TIS<br>- TIS associated with longer PFS | [176] |
| | Resected CRC | SA-β-Gal and mRNA of $p21^{WAF1/Cip1}$, $p16^{INK4a}$ and IL-8 | TIS: 5-FU and concomitant RT | - Increased expression demonstrated TIS<br>- TIS increased rectal cancer invasiveness by upregulation of EMT related genes | [177] |
| | CRC | $p21^{WAF1/Cip1}$ | TIS: bevacizumab-based CT | - Increased expression demonstrated TIS<br>- TIS associated with longer PFS | [178] |
| | CRC AC | | CS | Decreased expression associated with higher Dukes stage, metastasis and worse survival | [179] |
| | CRC | | | Downregulation and negative expression associated with MSI | [180, 181] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | | | | Decreased expression associated with lymph node and/or liver metastasis and worse survival | [182, 183] |
| | | p53 | | Absent expression associated with MSI | [181] |
| | CRC AC | CS gene score | | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| | Rectal AC | | | | |
| | Rectal cancer | $p21^{WAF1/Cip1}$ | TIS: concurrent CRT (5-FU alone or with OXP) | Increased expression demonstrated TIS of inflammatory CAFs with a pronounced stromal response | [184] |
| | | Senescence gene signature | | | |
| | PC caused by cancer of the appendix, colon, rectosigmoid or rectum | SA-β-Gal, $p16^{INK4a}$, $p21^{WAF1/Cip1}$, H3K9me3 and Ki67[c] | OIS | - Increased expression demonstrated that PC is characterized by senescent tumor cells, and showed features of stemness<br>- Low or absent SA-β-Gal expression in primary tumor and liver metastasis samples compared to high SA-β-Gal expression in PC samples, suggesting that the peritoneal cavity is a metastatic niche that induces senescence, whereas no signs of senescence induction within the metastatic environment of the liver<br>- Absent SA-β-Gal expression in TILs of primary tumor and liver metastasis samples compared to elevated SA-β-Gal expression in TILs of PC samples, suggesting that senescent PC cells induce senescence in TILs | [185] |
| | | Senescence-associated and SASP genes | | Upregulated in PC compared to primary tumor samples, and have a distinct SASP, demonstrating that senescent PC with stem cell-like features express a unique SASP | |
| **Pancreatic cancer** | Resected PanIN and PDAC | $p16^{INK4a}$ and Ki67[c] | OIS | High expression in low-grade PanINs, no expression in PDAC | [186] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | | $p21^{WAF1/Cip1}$ | | Expression increased with increasing grade of malignancy, demonstrating that aberrant cell cycle regulatory genes may be important in early development and progression of PanIN | [187] |
| | Precursor lesions (acinar to ductal metaplasia), PanINs and PDAC | SA-β-Gal | | Expression only in precursor lesions (acinar to ductal metaplasia) and PanINs, no expression in PDAC | [188] |
| | Normal pancreas, pancreatitis and PDAC | SIN3B | | - Absent or low expression in control pancreas and PDAC<br>- Strong expression in pancreatitis and PanINs and correlated with IL-1α | [189] |
| **Hepatocellular carcinoma** | Cirrhosis, dysplasia and HCC | Senescence-related genes: *FAM38D*, *ATAD2*, *TOP2A*, *CCNE2*, *CRNDE*, *EPCAM*, *TMEM27*, *TFPI2*, *FOS*, *NAT2*, *GPR128*, *CYP39A1*, *FAM134B*, *SDCBP2* and *MUM1L1* | CS | Increased expression in liver cirrhosis, dysplasia being a transitional state to HCC and HCC that displayed immortal gene expression phenotypes | [190] |
| | Chronic hepatitis C and cirrhosis | SA-β-Gal | | Expression correlated with fibrosis progression in cirrhosis and chronic hepatitis C, suggesting CS predispose to HCC development | [191, 192] |
| | | $p21^{WAF1/Cip1}$ | | Expression higher in cirrhosis compared to chronic hepatitis and associated with HCC development, suggesting $p21^{WAF1/Cip1}$-related tumorigenesis in HCC | [193] |
| | Biliary cirrhosis | $p21^{WAF1/Cip1}$/$Ki67^{c}$ ratio | | Increased expression ratios | [194] |
| | Cirrhosis and HCC | $p21^{WAF1/Cip1}$ and $p16^{INK4a}$ | | Increased expression in cirrhosis, strongly reduced in HCC | [195] |
| | Normal, chronic hepatitis C and HCC | SA-β-Gal | | - Expression gradually increased from normal through chronic hepatitis C samples and HCC<br>- Expression in non-tumoral liver tissue correlated with HCC in surrounding liver | [192] |
| | HCC | CS gene score | | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Peritumoral HCC tissue | Senescence-related gene signature: *FAM38D*, *ATAD2*, *TOP2A*, *CCNE2*, *CRNDE*, *EPCAM*, *TMEM27*, *TFPI2*, *FOS*, *NAT2*, *GPR128*, *CYP39A1*, *FAM134B*, *SDCBP2* and *MUM1L1* | OIS | Presence associated with:<br>- early recurrence and poor survival<br>- associated with chemokine (CCL2, CCL5 and CXCL11) and myeloid-specific gene expression and depletion in NK cell-specific gene activity | [196] |
| | | $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ | | Expression correlated with increased presence of CCR2 + myeloid cells | |
| | HCC | Senescence-related genes: *LRP4, OPRK1, PRAC2, N4BP3, GAL, CORO2B, FZD7, SEPTIN3, SMOX, EPO, MSC, GLP1R, HOXC6, PAPPA2, STK39, DLGAP5, THEM64, UNC5B, SLC16A11, CDH1, PRR15L, CCDC146, FAM117A, SLC2A4, CD2* and *STAT4* | | High senescence score:<br>- negatively correlated with the infiltration level of immunostimulating cells (plasma cells, CD8 T cells, activated CD4 memory T cells, gamma delta T cells and M1 macrophages) and positively correlated with the infiltration of immune-suppressive cells (memory B cells, naive CD4 T cells, M0 macrophages, M2 macrophages and eosinophils)<br>- negatively correlated with the expression levels of immune checkpoint related genes (i.e., *CD274, LAG3, PDCD1L, SIGLE* and *TIFIT*) and lower response rate to immunotherapy<br>- related to immune dessert subtype of HCC<br>- associated with worse survival | [197] |
| | | Senescence marker protein 30 | | - Decreased expression in adjacent non-tumor tissue, larger tumor size and enhanced TMN-stage<br>- Decreased expression associated with worse OS | [198] |
| **Cholangiocarcinoma** | Premalignant bile duct adenomas, ductular reactions and CCA | $p16^{INK4a}$ | OIS | Expression in most premalignant bile duct adenomas and ductular reactions whereas barely expression in CCA, demonstrating OIS as tumor-suppressive mechanism | [199–201] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | CCA | CS gene score | CS | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| **Prostate cancer** | Prostate IN | SA-β-Gal and CXCR2 | CS | Expression demonstrates CS as tumor-suppressive mechanism | [122] |
| | BPH and prostate IN | SA-β-Gal, HP1α and HP1γ | | | [14, 18] |
| | Primary prostate cancer | GLB1 | | Expression associated with:<br>- favorable clinicopathologic features (T stage and non-metastatic samples)<br>- improved prostate specific antigen-free survival | [202] |
| | Resected prostate cancer | | TIS: ADT[d] | Increased expression in tissues undergoing ADT longer than 5 months and in clinically more favorable intermediate grade cancers, demonstrating TIS as treatment outcome | [203] |
| | | GLB1, HP1γ and Ki67[c] | | Increased expression suggests TIS might be responsible for incomplete tumor regression | [204] |
| | | mRNA of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ | TIS: MIT | Increased expression and expression of a SASP (increased mRNA levels encoding IL-6, IL-8, GM-CSF, GRO-α, IGFBP-2, and IL-1β), demonstrating TIS with SASP expression as treatment outcome | [73] |
| | | mRNA of $p16^{INK4a}$, $p21^{WAF1/Cip1}$ and CCNA1 | TIS: MIT and MIT/DOC | - High expression demonstrated TIS as treatment outcome<br>- Persistent senescent cells evaded immune clearance | [150] |
| | | $p21^{WAF1/Cip1}$ | CS | Increased expression associated with high Gleason score and worse survival | [205, 206] |
| | | | TIS: ADT[d] | - Increased expression associated with p53 accumulation after ADT, suggesting TIS as treatment outcome<br>- Increased expression associated with worse survival | [206] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Prostate AC | CS gene score | CS | - Lower score in primary tumors compared to adjacent normal solid tissue<br>- Positively correlated with PD-L1 protein expression and T cell cytotoxicity<br>- High score associated with improved PFS and OS<br>- Lower score associated with higher Gleason score, T and N stages<br>- Predicted active immune response and better prognosis | [136] |
| **Bladder cancer** | Precancerous and cancerous urinary bladder | $p16^{INK4a}$, HP1α, HP1γ and H3K9me3 | OIS | Expression demonstrated OIS as tumor-suppressive mechanism in precancerous and cancerous lesions | [17] |
| | Radical cystectomy or transurethral resection | $p16^{INK4a}$, $p21^{WAF1/Cip1}$, p53 and pRb | | Aberrant individual and/or combined expression associated with recurrence and worse OS | [207] |
| | Transitional cell carcinoma | $p21^{WAF1/Cip1}$ | | - Expression associated with worse DFS and OS in superficial lesions<br>- Loss of expression associated with worse DFS and OS in invasive lesions when accompanied by p53 accumulation | [208] |
| | Bladder urothelial carcinoma | CS gene score | CS | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| **Skin cancer** | Human benign naevi | SA-β-Gal and $p16^{INK4a}$ | OIS | Increased expression demonstrated $p16^{INK4a}$-dependent OIS as tumor-suppressive mechanism | [13, 209] |
| | Dermal neurofibroma | | | | [210] |
| | Dysplastic naevi and radial early melanoma | $p16^{INK4a}$, p53 and $p21^{WAF1/Cip1}$ | | Less $p16^{INK4a}$ expression and some p53 and $p21^{WAF1/Cip1}$ expression demonstrated p53-dependent OIS as tumor-suppressive mechanism | [209] |
| | Advanced melanoma | | | No $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ expression in advanced melanomas demonstrated escape from $p16^{INK4a}$-dependent and/or p53-dependent OIS | |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Benign melanocytic and dysplastic naevi, in situ, invasive and metastatic melanoma | $p16^{INK4a}$ | | Expression gradually decreased with increasing grade of malignancy | [211, 212] |
| | Primary melanoma | | | No association with DFS or OS | [212] |
| | Cutaneous malignant melanoma | | | Loss of expression correlated with tumor cell proliferation, thicker lesions and invasive stage | [213, 214] |
| | Vertical growth phase melanoma | | | Loss of expression associated increased tumor cell proliferation and poor prognosis | [215] |
| | Aggressive nodular malignant melanoma | | | Loss of expression associated with recurrent disease | [216] |
| | Melanoma | CS gene score | CS | - Positively correlated with PD-L1 protein expression and T cell cytotoxicity<br>- Exhibited higher AUCs than the TIDE score for predicting immunotherapy response | [136] |
| | Basal cell carcinoma | | | CS gene scores of malignant cells from non-responders significantly decreased after treatment whereas posttreatment CS scores significantly increased in ICB responders | |
| | Merkel cell carcinoma | | | | |
| **Thyroid cancer** | Early-stage papillary thyroid microcarcinoma, PTC and anaplastic thyroid carcinoma | $p16^{INK4a}$, $p21^{WAF1/Cip1}$ and IGFBP7 | OIS | Expression gradually decreased with increasing grade of malignancy indicating involvement of OIS in thyroid carcinogenesis | [217] |
| | $^{V600E}$*BRAF* PTC | SA-β-Gal and $p16^{INK4a}$ | | Expression next to proliferating cancer cells demonstrating OIS and cells escaping from OIS co-exist in $^{V600E}$*BRAF* PTC | [218] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | ($^{V600E}$*BRAF*) PTC | SA-β-Gal, $p16^{INK4a}$, and Ki67[c] and mRNA of $p16^{INK4a}$ | | - Senescent tumor cells frequently present at invasive borders with features of collective invasion and high invasive ability with expression of a SASP<br>- Senescent tumor cells existed during lymphovascular invasion and metastasis<br>- Increased expression of CXCL12 in presence of senescent tumor cells in collective invasion area and diffuse CXCR4 expression in all PTC, demonstrating senescent tumor cell involvement in collective invasion and metastasis of PTC | [219] |
| **Bone and soft tissue tumors** | High grade sarcoma | $p16^{INK4a}$ | OIS | Decreased expression | [220] |
| | Dedifferentiated liposarcoma, synovial sarcoma and leiomyosarcoma | | | Decreased expression associated with reduced survival indicating $p16^{INK4a}$-dependent OIS as tumor-suppressive mechanism | |
| | Liposarcoma | Senescence marker genes: *VPS26A* and *VAMP3* | | High expression associated with increased survival | [134] |
| | | Senescence marker genes: *STX4* | | High expression associated with decreased survival | |
| | Osteosarcoma | Aging-/senescence-induced gene risk score | | High score associated with:<br>- worse OS<br>- higher copy number variation score, implying a higher degree of tumor cell malignancy<br>- immune cold tumors: lack of innate immune activation of infiltrating immune cells, less infiltration of antigen presenting cells, high TIDE score and T cell rejection<br>- higher exhaustion and T cell proliferation scores<br>- enriched MIF, CLEC and VEGF signaling pathway which are involved in osteosarcoma growth and metastasis and blood vessel growth | [221] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| **Renal cell carcinoma** | Primary RCC | p400 | OIS | Decreased expression associated with advanced tumor stage, higher grade of malignancy, regional lymph node metastasis and poor prognosis | [222] |
| | RCC | SA-β-Gal, p53, Dec1, Ki67[c] and Raf-1[c] | TIS: sunitinib | Increased expression demonstrates TIS as treatment outcome | [223] |
| | | CS gene score | CS | - Lower score in primary tumors compared to adjacent normal solid tissue<br>- Exhibited higher AUCs than the TIDE score for predicting immunotherapy response | [136] |
| | KIRP | | | | |
| **Brain malignancies** | PA | SA-β-Gal, $p16^{INK4a}$, p53 and Ki67[c] | OIS | Identification of OIS responsible for slow growth pattern, lack of progression to higher-grade tumors and high OS | [224] |
| | | Senescence-associated genes: *CDKN2A, CDKN1A, CEBPB, GADD45A, and IGFBP7* | | | |
| | | SASP factors: FGF2, IL-15, CSF3, VEGFA, IL-17A, CCL2, CXCL8, CSF2, CCL3, IFNγ, IL-6, IL-13, CCL11 and IL-1β | | High expression and upregulation of SASP associated with favorable PFS, demonstrating OIS is regulated by SASP | [225] |
| | High-grade tumors and adult LGG | *CDKN2A* and *TP53* | | Homogeneous deletion of *CDKN2A* and secondary alterations of *CDKN2A* and *TP53* more common | [226–228] |
| | IDH-mutant and lower grade (WHO grade II-III) astrocytoma | | | Absence of mutations associated with increased survival | [229, 230] |
| | ACP | $p21^{WAF1/Cip1}$, p53, GLB1, γH2AX, phosphor-DNA-PKc and Ki67[c] | | Identification of senescent cells harboring molecular signature of OIS and SASP, demonstrating OIS and SASP drive cell transformation and tumor initiation | [231] |
| | | SASP factors: IL-1β, IL-6, IL-8, IL-10, IL-18, TNFα and IFNγ | | Identification of SASP, demonstrating OIS and SASP drive cell transformation and tumor initiation | [232] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Medulloblastoma | *CDKN2A* and p53 pathway | | Frequent *CDKN2A* promoter methylation and p53 pathway mutations, demonstrating OIS escape underlies tumor progression | [233–235] |
| | Low-grade diffuse astrocytoma | $p16^{INK4a}$ and pRb | | Loss of expression associated with shorter survival | [236] |
| | Glioma | Senescence score based on senescence-associated genes: *CCL2, CCL7, CDKN1A, COPG, CSF2RB, CXCL1, ICAM-1, IGFBP-3, IL-6, IL-8, SAA4, TNFRSF-11B, TNFSF-11 and TP53* | CS | Senescence score:<br>- associated with poor prognosis<br>- correlated with older age<br>- increases with WHO histological grade (lowest values for low-grade astrocytomas (WHO II), higher values for anaplastic astrocytomas (WHO III) and highest values for glioblastomas (WHO IV) and gliosarcomas), linking senescence-associated gene signature to disease progression | [237] |
| | | SA-β-Gal and Ki67[c] | | Identification of senescent cells | [238] |
| | | Senescence gene signature: *ANXA5*, *APOE*, *CD151*, *CDKN1A*, *CDKN2A*, *CDKN2B*, *CTSB*, *CTSD*, *CTSL*, *CTSZ*, *EMP3*, *FTH1*, *LFITM3*, *LGFBP2*, *LGFBP3*, *LAMP1*, *LAMP2*, *LGALS1*, *MT1*, *OCIAD2*, *PDLIM4*, *RBP1*, *S100A11*, *SEP11*, *SDC4*, *SPARC* , *TIMP1*, *TM4SF1*, *TMSB4X*, *TNC* and *TNFRSF12A* | | Senescence gene signature associated with shorter survival | |
| | Normal and reactive brain tissue and glioma | $p21^{WAF1/Cip1}$ | | Increased expression in glioma compared to normal and reactive brain tissue, suggesting $p21^{WAF1/Cip1}$-related tumorigenesis in glioma | [239] |
| | Astrocytic glioma | | | Expression associated with worse DFS | [240] |
| | Astrocytic and high-grade glioma | Senescence marker genes: *NTAL* and *STX* | | High expression associated with decreased survival | [134] |
| | Astrocytic and high-grade glioma and glioblastoma | Senescence marker genes: *VPS26A, ARMCX3* and *B2MG* | | High expression associated with increased survival | |
| | Glioblastoma multiforme | CS gene score | CS | Positively correlated with PD-L1 protein expression and T cell cytotoxicity | [136] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| **Head and neck cancer** | Oropharyngeal SCC | $p16^{INK4a}$ | OIS | Expression associated with favorable prognosis regardless of HPV status | [241] |
| | Laryngeal, hypopharyngeal or oral SCC | | | No prognostic value | [242] |
| | Normal, benign hyperplastic skin and oral lesions | | | Negative expression | [243, 244] |
| | dysplastic and carcinoma in situ skin and oral SCC | | | Heterogeneous expression | |
| | Advanced skin and oral SCC | | | Consistent expression at areas of microinvasion and at superficial margins | |
| | deeply invasive skin and oral SCC | | | Near to complete absent expression, demonstrating $p16^{INK4a}$-dependent OIS as tumor-suppressive mechanism | |
| | Oral SCC | $p16^{INK4a}$, p53, pRb and cyclin D1 | | - Loss of $p16^{INK4a}$ expression earliest event in tumorigenesis<br>- Deregulation of pRb and p53 associated with malignant transformation and adverse prognosis | [245] |
| | Premalignant dysplastic and SCC of skin and oral epithelium and HNSCC | *CDKN2A* | | High frequency of gene mutation, deletion and promoter silencing | [243, 246] |
| | HNSCC | H3K9Me | | - Identification of senescent cells in 67.1% of biopsies<br>- More senescent cells in tumor center compared to invasive front<br>- No prognostic impact | [247] |
| | | Senescence score based on senescence-associated genes: *DUSP16*, *EHF*, *ITSN2*, *DUSP3*, *HDAC4*, *TXNIP*, *KL*, *MAP4KI*, *PIK3R5*, *YPEL3*, *CDKN2A*, *MAP2K7*, *PIAS4*, *POU5F1*, *EZH2*, *DGCR8*, *TYK2*, *BTG3*, *SOCS1*, *G6PD*, *TXN*, *DPY30*, *AURKA*, PDCD10, *PSMD14*, *FXR1*, *PCGF2*, *GAPDH*, *PSMB5*, *RSL1D1*, *IL1A*, *CDK6*, *LIMA1*, *CAV1*, *SERPINE1*, *HSPA5*, *NEK6*, *ASPH*, *MAP2K1*, *ACLY*, *TOP1* | | High-senescence score:<br>- associated with worse OS<br>- correlated to poor clinicopathological parameters (histologic grade, TNM-stage, T-stage and lymph node metastasis) | [248] |
| | Pharyngeal and laryngeal HNSCC | $p21^{WAF1/Cip1}$ | | High expression associated with poor OS | [249] |

**Table 1** (continued)

| Malignancy | Sample specification | Senescence marker[a] | Type of senescence[b] | Prognostic implication | Reference |
|---|---|---|---|---|---|
| | Oral SCC | | | | [250] |
| | Tonsillar SCC | | | Expression associated with favorable DSS | [251] |
| | Laryngeal and oral HNSCC | | | Higher expression associated with improved OS in stage III patients | [252] |
| | Minor salivary gland adenoid cystic carcinoma | $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ | CS | Strong and intense $p21^{WAF1/Cip1}$ expression and complete negative $p16^{INK4a}$ expression, suggesting transient senescence insufficient to maintain the senescence-associated cell cycle arrest, avoiding cell death by senescence and favoring tumor growth | [253] |
| | HNSCC | H3K9Me | TIS: RT with or without 5-FU/CP or 5-FU/CB | - Less senescent cells in post-RCT samples<br>- No prognostic impact | [247] |
| | | mRNA of CXCR2 receptor and/or its ligands (CXCL1–3, CXCL5, CXCL7, and CXCL8) | TIS: CRT[d] | Increased expression associated with impaired DSS, demonstrating TIS and SASP production determines radioresistance | [254] |
| | | CS gene score | CS | Lower score in primary tumors compared to adjacent normal solid tissue | [136] |
| **Thymic cancer** | Thymic cancer | CS gene score | CS | Positively correlated with PD-L1 protein expression and T cell cytotoxicity | [136] |

[a] detected by immunohistochemistry unless otherwise specified

[b] defined as CS unless otherwise specified

[c] negative marker of senescence

[d] not further specified

*5-FU* 5-fluorouracil, *AC* Adenocarcinoma, *ACP* Adamantinomatous craniopharyngioma, *ADT* Androgen deprivation therapy, *AUCs* Areas under the curve, *BPH* Benign prostate hypertrophy, *CAFs* Cancer-associated fibroblasts, *CB* Carboplatin, *CCA* Cholangiocarcinoma, *CIN* Cervical intraepithelial neoplasia, *CLEC* C-type lectin-like, *CP* Cisplatin, *CP* Cyclophosphamide, *CRC* Colorectal cancer, *CRT* chemoradiotherapy, *CS* Cellular senescence, *CT* chemotherapy, *DFS* Disease-free survival, *DOC* Docetaxel, *DOX* Doxorubicin, *DSS* Disease-specific survival, *EC* Esophageal cancer, *ECM* Extracellular matrix, *ECX* Epirubicin, *EMT* Epithelial-mesenchymal transition, *ESCC* Esophageal squamous cell cancer, *HCC* Hepatocellular carcinoma, *HGSOC* High-grade serous ovarian cancer, *HNSCC* Head and neck squamous cell carcinoma, *ICB* Immune-checkpoint blockade, *IN* intraepithelial neoplasia, *KIRP* Kidney renal papillary carcinoma, *LLG* Low-grade glioma, *MIF* Macrophage migration inhibitory factor, *MIT* Mitoxantrone, *MPM* Malignant pleural mesothelioma, *MSI* Microsatellite instability, *MTX* Methotrexate, *NSCLC* Non-small cell lung cancer, *OC* Ovarian cancer, *OIS* Oncogene-induced senescence, *OS* Overall survival, *OXP* Oxaliplatin, *PA* Pilocytic astrocytoma, *PanIN* Pancreatic intraepithelial neoplasia, *PC* Peritoneal cancer, *PD-L1* Programmed death-ligand 1, *PDAC* Pancreatic ductal adenocarcinoma, *pEOC* Primary epithelial ovarian cancer, *PFS* Progression-free survival, *pRb* Retinoblastoma protein, *PTC* Papillary thyroid cancer, *PTX* pPaclitaxel, *RCC* Renal cell carcinoma, *RCT* Radiochemotherapy, *RT* Radiotherapy, *SASP* Senescence-associated secretory phenotype, *SCC* Squamous cell carcinoma, *SS* Senescence signature, *TIDE* Tumor immune dysfunction and exclusion, *TILs* Tumor-infiltrating lymphocytes, *TIS* Therapy-induced senescence, *TMB* Tumor mutational burden, *TME* Tumor microenvironment, *UCEC* Uterine corpus endometrial carcinoma, *VEGF* Vascular endothelial growth factor, *WHO* World Health Organization

inflammatory TME driving cancer development [73] and attract myeloid derived suppressor cells that suppress T [264] and NK cells [196] and blocks IL-1α signaling, preventing paracrine senescence in neighboring cancer cells [265]. Next to pro-inflammatory cytokines, the SASP may consist of a variety of chemokines (e.g., CCL2 and CXCL1), angiogenic factors (e.g., VEGF), growth factors (e.g., HGF, PDGF, EGF and TGFα), matrix-remodeling enzymes (e.g., MMP1 and MMP3) and bioactive lipids [62, 263]. However, its composition is highly dynamic [263], complex and variable and depend on the cell type, senescence-inducing trigger and type of senescence [62, 74, 75], resulting in cancer-specific and context-dependent effective SASP levels. Besides its variable composition, the SASP is suggested to have a time-dependent impact [31]. Whereas the short term presence of SASP is suggested to be primarily tumor-suppressive, the long term presence of pro-inflammatory SASP factors can drive cancer [31, 32]. Thus, depending on the secretion, composition and the duration of its presence, the net effect of the SASP may be tumor-suppressive or tumor-promoting, thereby either enhancing or opposing the tumor-suppressive property of the senescence-associated cell cycle arrest.

Evidence from patients with various tumor types show that oncogene-induced senescent cells are capable of secreting a tumor-promoting and immune-suppressive SASP that is linked to impaired clinical outcome. NSCLC patients with an elevated senescence-related gene signature score overexpressed an immune-suppressive SASP and demonstrated decreased infiltration levels of cytotoxic T cells and NK cells and increased levels of immune-suppressive cells (i.e., neutrophils, cancer-associated fibroblasts, regulatory T cells, and resting NK cells), disease progression and worse OS [133]. The importance of the interaction between the SASP and immune surveillance of senescent tumor cells is further emphasized by the finding that, in CRC, both a lower average distance between senescent cells and T cells as well as a higher percentage of T cells near senescent cells were linked to improved survival, suggesting that the tumor-suppressive potential of cellular senescence is determined by the TME and immune cell-mediated elimination of senescent tumor cells [174]. The SASP of senescent cells can also direct neighboring cells and drive cell transformation and tumor initiation [231, 232] and mediate collective invasion and metastasis [219], as evidenced in adamantinomatous craniopharyngioma and papillary thyroid cancer. Also in precursor lesions of pancreatic ductal adenocarcinoma (PDAC) (i.e., pancreatitis and pancreatic intraepithelial neoplasias), a senescence-associated inflammatory SASP was linked to PDAC progression [189].

Increasing evidence demonstrates that also therapy-induced senescent cells can produce a tumor-promoting and immune-suppressive SASP that might impair clinical outcome. For example, in response to genotoxic chemotherapy, TIS and a protumorigenic SASP were observed in prostate cancer resection samples [73], and overexpression of SASP factor were associated with impaired outcome in head and neck squamous cell carcinoma patients [254] as early TIS and SASP production upon radiotherapy was demonstrated in a preclinical model. In an elucidative study, therapy-induced senescent cells of breast and prostate cancer patients were found to evade immune clearance by shedding of natural killer group 2D (NKG2D) ligands and paracrine suppression of NKG2D-receptor-mediated immunosurveillance [150]. Of importance, since TIS depends on p53/p21$^{WAF1/Cip1}$ pathway activation (Fig. 2), the tumoral p53 status indirectly determines SASP production and outcome after treatment with senescence-inducing anticancer therapies. This was illustrated in an in vivo p53 wild-type breast cancer model where TIS was induced instead of cell death after chemotherapy treatment and resulted in minimal regression of the tumor and early relapse through the secretion of protumorigenic SASP [266]. Accordingly, breast cancer patients harboring a *TP53* mutation showed an improved response to anthracycline-based chemotherapy [267, 268].

In contrast, abundant evidence links both OIS [165, 202] and TIS [163, 176, 178] to improved outcome (Table 1). In this scenario, it is conceivable that oncogene-induced and therapy-induced senescent cells secrete moderate to low effective SASP levels and/or secrete a SASP with a net tumor-suppressive and immune-promoting effect. For example, in pilocytic astrocytoma (PA), a low grade glioma and most common brain tumor in children, SASP factors were upregulated, and high levels of IL-1β and SASP expression were associated with favorable PFS [224]. The SASP was therefore suggested to regulate OIS in PA [225] and held responsible for the slow growth pattern, the lack of progression to higher-grade astrocytomas and the high OS of affected patients [224]. In addition, no oncogene-induced or therapy-induced senescent cells were identified in chemotherapy-naïve and neoadjuvant chemotherapy treated breast cancer samples, suggesting tumoral senescent cells either were already cleared by the immune system or bypassed senescence [151].

#### *Senescence in the TME*

There is mounting evidence that senescence also occurs in the TME and has prognostic implications. In gastric cancer, CRC and esophageal cancer patients, an enriched senescence gene signature in noncancerous

cells, but not in cancerous cells, of the TME (e.g., endothelial cells, enteroendocrine cells, macrophages and fibroblasts) resulted in a longer disease-free survival and OS [168]. In contrast, identification of senescent cancer-associated fibroblasts (CAFs) adjacent to epithelial ovarian cancer cells in ovarian cancer specimens were suggested to promote ovarian cancer tumorigenesis [161]. The presence of a senescence-associated gene signature in peritumoral tissue of hepatocellular carcinoma (HCC) patients was also associated with early recurrence and poor survival as peritumoral OIS induced an accumulation of C–C chemokine receptor $2^+$ myeloid cells through secretion of C–C motif chemokine ligand 2, resulting in NK cell inhibition and enhanced HCC growth [196]. Interestingly, in a murine rectal cancer and patient-derived tumor organoids model, IL-1α was found to predispose inflammatory CAFs to p53-mediated TIS upon irradiation, which in turn resulted in chemoradiotherapy resistance and disease progression through the secretion of cytokines and extracellular matrix constituents supporting the invasion and metastasis of cancer cells and counteracting the irradiation-induced tumor cell death [184]. Consistently, the presence of inflammatory CAFs in pre-therapeutic patient biopsies resulted in poor chemoradiotherapy response and low IL-1α receptor antagonist serum levels, which enhances IL-1 signaling and predisposes inflammatory CAFs to TIS, correlated with poor prognosis in rectal cancer patients [184].

Hence, cellular senescence is not solely limited to cancerous cells but also occurs in cells of the TME as well as of the immune system [269].

#### *Model for differential prognostic outcomes of OIS and TIS in cancer patients.*

Evidence of several cancer types (presented in Table 1) suggests that the prognostic implications of OIS and TIS are highly context-dependent and primarily depend on the (i) the senescence burden; (ii) the secretion; and (iii) the composition of the SASP and/or duration of SASP presence. Therefore, in a simplified schematic model, we present different scenarios that could provide a rationale for the differential outcomes of cellular senescence observed in cancer patients, based on the interplay between these three factors, i.e., (i) the senescence burden (i.e., low, moderate or high); (ii) the secretion (i.e., low or high); and (iii) the composition of the SASP (i.e., net tumor-promoting and immune-suppressive or net tumor-suppressive and immune-promoting) and/or duration of SASP presence (i.e., short term or long term) (Fig. 3).

The senescence-associated cell cycle arrest is considered tumor-suppressive whereas the composition of the SASP and/or duration of SASP presence determines whether the senescence-associated cell cycle arrest is reinforced or opposed. The degree of SASP secretion and levels, which depend on the senescence burden, determines to which extent the senescence-associated cell

(See figure on next page.)

**Fig. 3** Model for differential prognostic outcomes of cellular senescence (OIS and TIS) in cancer patients. Tumor-suppressive and immune-promoting SASP—Short term beneficial SASP effect. In case of a net tumor-suppressive and immune-promoting SASP or short term presence of SASP, immune recruitment will result in immune clearance of senescent cancer cells as well as non-senescent cancer cells, thereby reinforcing cellular senescence to provide adequate tumor suppression. However, in case of a (**A**) low senescence burden, the effects of the SASP are expected to be less profound as the overall SASP levels secreted by the low number of tumoral senescent cells are lower compared to SASP levels in case of a moderate or high senescence burden. Therefore, the senescence-associated cell cycle arrest as well as the SASP levels are expected to be insufficient to provide an adequate tumor suppression. In case of a (**B**) moderate senescence burden, the senescence-associated cell cycle arrest can increasingly be reinforced in case of low and high SASP secretion, respectively, to provide adequate tumor suppression. In case of a (**C**) high senescence burden, the senescence-associated cell-cycle arrest can be reinforced by the tumor-suppressive and immune-promoting SASP in case of high as well as low SASP secretion due to the large number of tumoral senescent cells. As such, in case of a net tumor-suppressive and immune-promoting SASP, a high senescence burden result in improved outcome. Tumor-promoting and immune-suppressive SASP—Long term detrimental SASP effect. In case of a net tumor-promoting and immune-suppressive SASP or long term presence of SASP, the senescence-associated cell cycle arrest can be opposed by the SASP by molding an immune-suppressive and protumorigenic TME and stimulating immune evasion. However, in case of a **(D)** low senescence burden, the effects of the SASP are expected to be less profound as the overall SASP levels secreted by the low number of tumoral senescent cells are lower compared to SASP levels in case of a moderate or high senescence burden. Therefore, the senescence-associated cell cycle arrest in case of a low senescence burden is not opposed by the SASP, however, the senescence-associated cell cycle arrest is insufficient to prevent tumor proliferation. In case of a **(E)** moderate senescence burden, the senescence-associated cell cycle arrest can be opposed by the SASP in case of high SASP secretion, whereas in case of low SASP secretion the senescence-associated cell cycle arrest overrules the lower SASP levels, resulting in differential tumor-promoting and tumor-suppressive effects, respectively. In case of a **(F)** high senescence burden, the senescence-associated cell cycle arrest can be opposed and overruled by the protumorigenic effects of the SASP in case of high as well as low SASP secretion, as the overall SASP levels produced by the large number of tumoral senescent cells are elevated, even in case of low SASP secretion. As such, in case of a net tumor-promoting and immune-suppressive SASP, a high senescence burden can paradoxically result in worse outcome. ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; VEGF, vascular endothelial growth factor; NK cell, natural killer cell; SASP, senescence-associated secretory phenotype; ↑, high; =, moderate; ↓, low; >, greater-than; <, less-than; ⧖, time

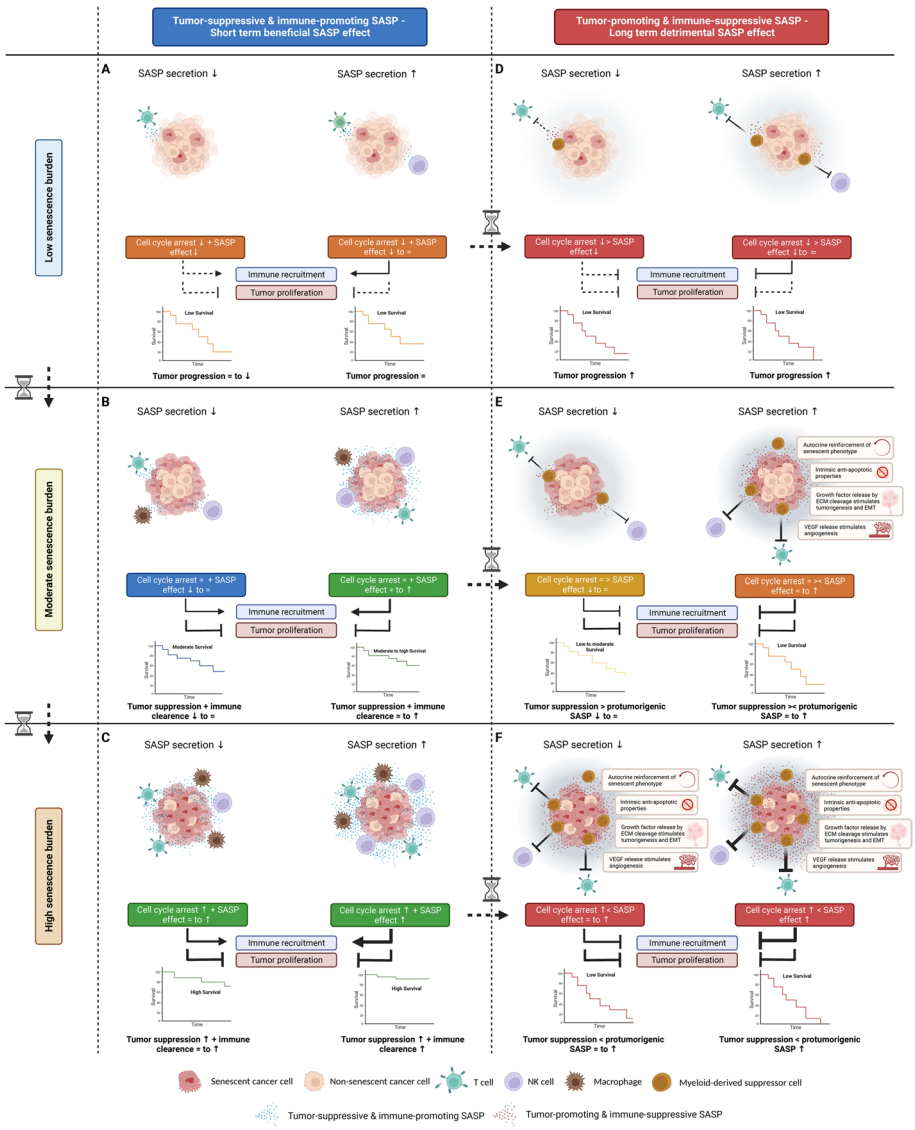


**Fig. 3** (See legend on previous page.)

cycle arrest is reinforced or opposed. As such, OIS and TIS can have tumor-suppressive and tumor-promoting properties (Fig. 3A-F).

The proposed model provides a rationale for the differential outcomes of OIS and TIS observed within the same cancer type, such as in [174], as well as between different types of cancer (Table 1). Accordingly, cancer cells originating from urinary systems, glands and soft tissues (e.g., prostate cancer, adenoid cystic carcinoma, RCC and melanoma) exhibited relatively higher cellular senescence gene scores than tumors originating from reproductive organs (e.g., breast cancer, cervical SCC and OC) [136], and were correlated with SASP factors and immune related genes, suggesting SASP-induced immune infiltration. The infiltration of immune cells varied however in a cancer-specific pattern [136], contributing to the context-dependency of the proposed model and the interplay between the senescence burden, the secretion and composition of the SASP.

Of note, as senescent cells can reinforce their senescent phenotype in an autocrine fashion [16, 122] and paracrinally transmit the senescent phenotype to adjacent malignant and non-malignant cells [262, 270], the tumoral senescence burden can increase over time resulting in altered tumoral repercussions (Fig. 3, vertical arrow hourglasses) in addition to the differential time-dependent impact of SASP (Fig. 3, horizontal arrow hourglasses). Thus, cellular senescence in cancer should

be considered as a dynamic, rather than an irreversible, static condition [271], with antitumorigenic and protumorigenic features that can change over time.

## Conclusion

### Conclusions and future perspectives

Clinical evidence of cellular senescence in cancer patients has long been underestimated, in part due to the difficult detection, since currently no specific and universal markers for senescent cells exist. Historically, cellular senescence was primarily considered as an endogenous tumor suppressor mechanism halting the proliferation of damaged cells which are at risk of malignant transformation, thereby protecting against cancer. However, during the last two decades, a more nuanced view on the involvement of cellular senescence in tumorigenesis and response to therapy has emerged. Here, we provided a comprehensive overview on the prognostic implications of cellular senescence in cancer patients with solid tumors. Increasing clinical evidence add to the antagonistic pleiotropy of cellular senescence as differential prognostic outcomes, ranging from improved to impaired outcome, are demonstrated. In a simplified model we propose that the prognostic implications of OIS as well as TIS are highly context-dependent and primarily depend on the senescence burden, the secretion and the composition of the SASP and/or duration of SASP presence, thereby providing a rationale for the differential outcomes of OIS as well TIS observed within the same cancer type as well as between different types of cancer discussed in this review. However, (pre)clinical research is warranted to provide adequate evidence to further support this model, and to better comprehend when and how senescent cancer cells give rise to a beneficial or detrimental outcome.

The detection of cellular senescence in cancer patients can be achieved by various methods and using various markers. Despite clear algorithms to accurately assess and quantify senescent cells in vitro and in vivo [1, 44], a plethora of different senescence markers, single or combined with other markers, at different translational levels are currently used to demonstrate the presence of cellular senescence (Table 1). Hence, it is difficult to compare clinical data and to draw reliable conclusions regarding the prognostic implications of cellular senescence, as well as the implementation of emerging senolytics (i.e., targeted removal of senescent cells) [42, 78, 263, 271] and senomorphics that modify/suppress the SASP [32, 263, 272], underlining the need for a uniform and consistent application of recognized and validated markers of cellular senescence at different translational levels. Of note, as AI-based computational pathology is making its way into medicine and clinical practice [273], an AI-based detection of cellular senescence might potentially make multi-marker detection of senescent cells redundant in the near-future. Since the prognostic impact of senescence is mainly mediated by the SASP, extensive profiling of the SASP in specific disease contexts (i.e., organ- and trigger-specific (OIS versus TIS)), as well as the identification of biomarkers representing the senescence burden will be paramount [75]. Especially longitudinal monitoring of senescent cells and their SASP will be of particular interest, as preclinical models are not able to capture the beneficial or detrimental effects exhibited by senescent cells and the SASP over an extended period of time.

Since the TME, containing cancer-associated fibroblasts and infiltrating immune cells, is believed to be a major contributor to therapy resistance and disease progression [274], the interaction of TME with senescent cells as well as the SASP should be investigated more closely. This can be achieved using appropriate preclinical models that precisely recapitulate this complex heterogeneity, such as in vitro 3D culture technologies (e.g., organoids), thereby resembling a more physiological human cancer model [275]. Interestingly, by combining single-cell RNA-sequencing with spatial transcriptomics [276], it is feasible to map the location of distinct cell types and subpopulations in the TME and investigate the interaction of senescent cancer cells with the TME more in-depth.

As an emerging cancer hallmark, the involvement of cellular senescence in cancer is complex and highly context-dependent, exerting potential beneficial and/or detrimental effects. Therefore, senescence must be approached in a nuanced way regarding its repercussions in cancer. Only in this way it is possible to optimally exploit cellular senescence as an anticancer therapeutic strategy.

#### Abbreviations

CDK: Cyclin-dependent kinase; CRC: Colorectal cancer; IL: Interleukin; NK: Natural killer; OIS: Oncogene-induced senescence; SA-β-Gal: Senescence-associated beta-galactosidase; SASP: Senescence-associated secretory phenotype; TIS: Therapy-induced senescence; TME: Tumor microenvironment.

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#### Authors' contributions

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