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INVITED REVIEW

Escape from senescence: molecular basis and therapeutic ramifications

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Abstract

Cellular senescence constitutes a stress response mechanism in reaction to a plethora of stimuli. Senescent cells exhibit cell-cycle arrest and altered function. While cell-cycle withdrawal has been perceived as permanent, recent evidence in cancer research introduced the so-called escape-from-senescence concept. In particular, under certain conditions, senescent cells may resume proliferation, acquiring highly aggressive features. As such, they have been associated with tumour relapse, rendering senescence less effective in inhibiting cancer progression. Thus, conventional cancer treatments, incapable of eliminating senescence, may benefit if revisited to include senolytic agents. To this end, it is anticipated that the assessment of the senescence burden in everyday clinical material by pathologists will play a crucial role in the near future, laying the foundation for more personalised approaches. Here, we provide an overview of the investigations that introduced the escape-from-senescence phenomenon, the identified mechanisms, as well as the major implications for pathology and therapy.

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What is cellular senescence?

First, the reader is urged to consult the glossary (a glossary of terms is provided in supplementary material, File S1) for definitions and explanations of individual terms used here.

Various cellular states reflect reactions of cells to physiological and harmful insults. A central task in pathology is to define how cells respond when challenged by various stress stimuli preserving tissue and organismal homeostasis [1]. Depending on the features of the stressor, cells balance the decision between life (complete recovery) and death when damage exceeds their repair capacity. Between those two extreme settings, other options linked to cell survival can be adopted. For instance, the cell can remain alive but in an arrested state, preventing the generation of damaged offspring cells. That arrested cellular state, which ensures homeostasis by restraining damage, is termed cellular senescence [1–3]. Cellular senescence constitutes a fundamental stress response mechanism against a variety of stimuli, external or internal to the cell (Figure 1) [3]. Exposure of cells to such sublethal stress insults mainly results in the accumulation of DNA damage and elicits the DNA damage response (DDR) pathway, which eventually leads to the establishment of cellular senescence [1,3]. In senescent cells, DNA damage manifests itself in the form of DNA breaks, and activation of the DDR pathway is revealed by the formation of persistent DDR foci [1,3–5].

Cellular senescence can be broadly divided into replicative senescence (RS) and stress-induced premature senescence (SIPS) [3,6]. RS was first described by Hayflick in 1961, when proliferation-competent cells were found to cease replication following successive

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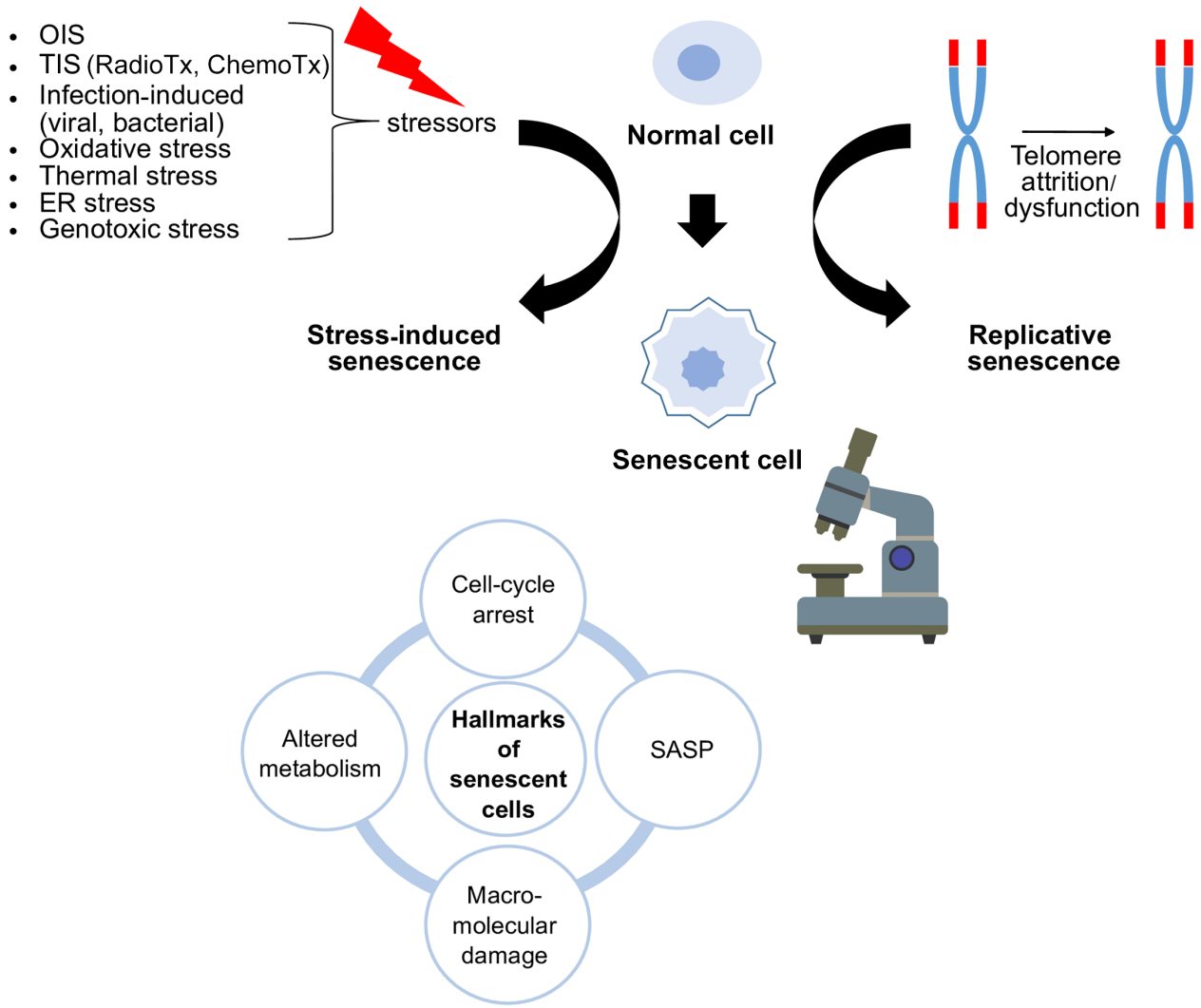

Figure 1. Schematic representation of a non-exhaustive list of known triggering stimuli (stressors) inducing stress-induced senescence. In turn, replicative senescence is activated upon telomere attrition/dysfunction. Irrespective of the triggering stimulus and the type of the senescent programme initiated, senescent cells exhibit four interconnected hallmark features: cell-cycle arrest, macromolecular damage, altered metabolism, and SASP secretion. ER, endoplasmic reticulum; OIS, oncogene-induced senescence; TIS, therapy-induced senescence.

rounds of cell divisions (Figure 1) [7]. This type of senescence, also referred to as primary senescence, is triggered by progressive telomere shortening per cell division as well as upon telomere modifications and/or dysfunction [2,3,8–10]. However, stress-inducing stimuli were later shown to activate senescence programmes prior to reaching the cellular replicative limit (premature types), which led to the identification of SIPS [1,3,11]. A subset of SIPS is driven by oncogenic stress [oncogene-induced senescence (OIS)], preventing the expansion of incipient cancer cells. Oxidative/metabolic stress along with heat and endoplasmic reticulum stress, infectious agents, and chemoradiotherapeutic modalities are other known triggers of SIPS [3,12–17]. As defined in 2019, senescent cells are characterised by a number of interdependent hallmark features [3]. Senescent cell traits dynamically undergo modifications with time, revealing a multifaceted nature which can be chronologically divided into 'early' and 'deep' senescence, depending on the emergence of senescence properties post induction [1,18]. These senescence features include cell-cycle withdrawal, a deregulated metabolic profile, accumulation of macromolecular damage, and acquisition of a unique senescence-associated secretory phenotype (SASP) mainly consisting of pro-inflammatory factors (Figure 1) [3,18–21]. Early senescence pertains to a shift from a transient to a stable cell-cycle arrest where early senescent cells progress to 'deep' or 'full' senescence once SASP production is established [18].

At the molecular level, it is widely accepted that the majority of senescence triggers convey their signalling towards activation of two cardinal cell-cycle regulatory pathways, the p53/p21$^{WAF1/Cip1}$ and p16^{INK4A}/RB signalling axes [1]. Interestingly, cytoplasmic lipofuscin accumulation, a hallmark of senescence, reflects both altered metabolic traits and increased macromolecular

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damage within the senescent environment [3]. The unique secretory properties of senescent cells include the production of inflammatory cytokines, proteases, and a spectrum of molecules that influence the surrounding microenvironment, in an autocrine and paracrine manner, overall mediating senescent cell elimination by phagocytosis (transient or acute senescence) [3,14,15,19–21].

Intriguingly, senescence seems to behave in a bimodal manner, with SASP action being the key modulating factor. When it occurs transiently (acute), senescence appears beneficial as it contains damage (bright side of senescence). As such, cellular senescence plays an important role in tissue homeostasis even during normal development and adult physiology [19–22]. However, on a long-term basis, senescence exerts its detrimental impact on the onset and outcome of a variety of pathologies and on the ageing process [23–27]. Senescent cells can persist either through the development of immune-evading mechanisms or due to a decline in the immune system with age (persistent or chronic senescence). Chronic accumulation of senescent cells and excessive SASP are accompanied by progressive detrimental effects at the tissue and organismal level, revealing a 'dark side' of senescence [1,3,13,18–21]. Such negative effects involve tissue stem cell niche exhaustion, matrix degradation, and mild chronic inflammation, ultimately impairing tissue functionality and laying the foundation for ageing and age-related disorders [18–22,24,28]. Interestingly, in cancer, which is one of the most common age-related diseases, the induction of OIS as an anti-tumour barrier is well established [3,12,13,29]. An intriguing hypothesis has been proposed in connection with the bimodal nature of SASP. According to this hypothesis, upon initial activation, SASP is enriched in components that exert anti-tumour properties, thereby inducing senescence and/or apoptosis in an autonomous manner. However, with time, SASP becomes enriched in factors that promote, in a bystander manner, tumourigenic activities by suppressing immune cell populations [30].

Demystifying senescence cell-cycle re-entry

As already noted, cell-cycle arrest plays a central role in the manifestation of senescence, which was considered for many years to occur in an irreversible manner. Nonetheless, as research in the field progressed and new senescence markers were discovered (see the discussion that follows) a question emerged: Is senescence-associated cell-cycle withdrawal reversible? Transitory proliferation states characterised by cell-cycle arrest and re-entry have been identified in the context of premature senescence as well as cell immortalisation following RS *in vitro* [31,32]. Premature senescence is currently related to three distinct phenomena: senescence reversal, senescence bypass, and senescence escape [31–33]. Before delving deeper into the senescence-escape concept, we clarify the terms and conditions related to cell-cycle re-entry and senescence, which often cause misunderstanding and confusion (see the glossary for further explanation of the terms used). It has been noted that the terms 'reversal', 'bypass', and 'escape' are used interchangeably in the literature to describe either of these cell-cycle re-entry states. A consensus decision should be reached on these definitions to facilitate future research and discussion in this emerging field. *Escape from senescence* is characterised by a reacquisition of replication potency as well as disappearance of lipofuscin aggregates from the cytoplasm. In addition, it is usually preceded by a period of prolonged senescence, whereby a cell is significantly altered at the genomic, transcriptomic, and metabolic levels compared to the pre-senescent state (Figure 2) [33–35]. A phenomenon that is completely irrelevant to senescence is captured by the term *senescence bypass*, during which the senescent state is circumvented upon malignant transformation and the cell cycle is accelerated rather than halted in response to oncogenic stimuli (Figure 2) [36]. Functionally, in senescence escape, gradually acquired genetic alterations are accompanied by aggressive biological cellular traits, such as epithelial-to-mesenchymal transition (EMT), stemness, and drug resistance, while senescence bypass is followed by cellular transformation and the adoption of a malignant phenotype [33,35,37–39]. On the other hand, *senescence reversal* represents a distinct state, where the cell reverts back to its pre-senescent state *shortly* after acquisition of the senescent phenotype (acute/transient senescence) (Figure 2) [40,41]. As such, the structure and function of the cell remain unchanged. The terms 'pseudo-senescence', 'senescence-like', 'short-lived', 'accelerated', and 'uncommitted senescence' have all been used to describe a senescent state amenable to reversion [42]. With regard to the *in vitro* process of cell immortalisation, this is achieved following replicative stress, leading to short intervals of cellular senescence (M1 and M2 stages) [43,44]. Although immortalisation has been described as the outcome of escape from replicative senescence, those cell-cycle transitory phases are distinct from senescence reversal, bypass, and escape; immortalisation properties are not necessarily tumourigenic (no escape), and the genetic landscape is different (no reversal) [44–49].

From a molecular standpoint, senescence reversal represents a case of acute senescence, which is characterised by low $p16^{INK4A}$ levels and a generally unaltered genotype, allowing backtracking to the pre-senescent state [27]. On the other hand, replicative senescence is associated with high $p16^{INK4A}$, which usually also reflects chronic senescence [18–21,24,28]. If a cell has undergone significant genomic, metabolic, and/or epigenetic reorganisation, reversal is not possible. However, cell-cycle re-entry in the form of senescence escape and the emergence of malignant offspring may occur. These transitory cellular state dynamics are not completely understood and require further investigation [27].

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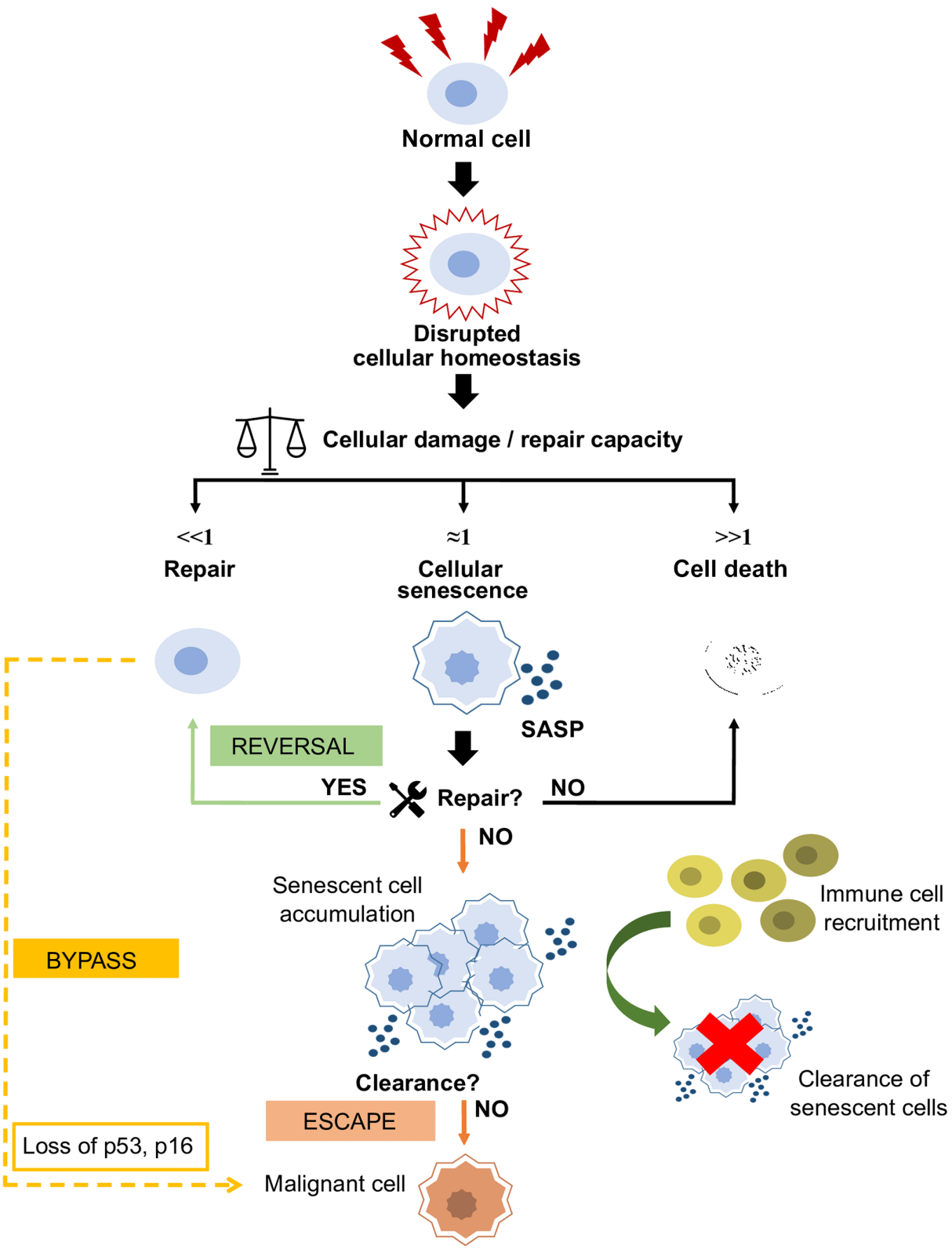

Figure 2. Overview of role of cellular senescence in cellular and organismal homeostasis through transitory cellular states: escape, reversal, and bypass. The stress signal induces imbalance in the cell's homeostatic equilibrium [disrupted cellular homeostasis (red)], in turn triggering the internal repair machinery as a compensatory mechanism. Depending on the features (hallmarks) of the stressor and the capacity for repair (cellular damage to repair capacity ratio), the affected cell may undergo cell death when the damage is irreparable ($\gg$1) or completely withstand the inflicted damage achieving homeostasis ($\ll$1). When the damage is barely withstood (= 1, sublethal), cellular senescence is triggered, and immune cells are chemotactically recruited secondarily to SASP release. When repair is not possible and the damage is critical, the cell dies. Early upon senescence initiation (e.g. low $p16^{INK4A}$ levels; see text for more details), reversal to a pre-senescent state is possible after successful damage resolution (green). In the case of lingering unresolved cellular damage, senescent cells can accumulate especially when their clearance is inhibited (red cross), thereby increasing the chances of senescence escape (orange box) and malignant transformation. Moreover, under low p53 and $p16^{INK4A}$ levels, the cellular senescence state may be bypassed, leading to a malignant cellular phenotype (yellow box).

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The senescence escape phenomenon: mechanistic insights

A number of studies that have emerged in recent decades challenged the dogma of irreversible cell-cycle arrest during senescence and laid the groundwork for the escape-from-senescence concept, a phenomenon with implications in disease progression and therapeutic outcomes (Figure 3, Table 1) [3,33–35]. Although sparse indications for escape from senescence were presented earlier, in the context of therapy-induced senescence (TIS), the first evidence for escape from OIS was presented shortly thereafter (Figure 3, Table 1) [43,78,79]. The oncogenic properties of the replication licencing factors cell division cycle 6 (CDC6) and chromatin licensing and DNA replication factor 1 (CDT1) were elucidated and the escape from the OIS phenomenon was identified through the implementation of an experimental cancer evolution approach [79]. This work, along with earlier pioneering studies, led in 2008 to the proposal of the oncogene-induced DNA damage model for cancer development [12,13,80]. According to this model, activated oncogenes trigger the DDR pathway, which in turn deploys the anti-tumour barriers of apoptosis and senescence, eliminating incipient cancer cells from the earliest stages of cancer development. As DNA damage accumulates, cells' capacity to repair in an error-free manner is exhausted, causing them to shift to an error-prone repair procedure and leading to genomic instability, which eventually breaches anti-tumour barriers and facilitates cancer progression [79]. The model predicted the following aspects: (1) the emergence of genomic instability during cancer development, (2) the manner in which evasion from apoptosis occurs (e.g. frequent *TP53* mutations in tumours), (3) activation of senescence during cancer development, and (4) escape from senescence. As a result, DDR-replication stress, genomic instability, and senescence are now considered hallmarks of cancer [29,81]. Although those predictions were partially supported by various experimental evidence, proof of senescence escape *in vivo* as well as its mechanistic basis were still lacking [12,79,80,82]. An intriguing histopathological observation led us to hypothesise that senescent cells could escape *in vivo*, promoting cancer progression. In particular, in various carcinomas and preneoplastic lesions harbouring p53 defects, we noticed large neoplastic cells paradoxically co-expressing the cell-cycle inhibitor $p21^{WAF1/Cip1}$ and the proliferation marker Ki-67 [34]. This observation implied either that proliferating neoplastic cells tolerated increased $p21^{WAF1/Cip1}$ levels or that prolonged $p21^{WAF1/Cip1}$ activation led to escape from senescence. Based on these findings, it appeared that the traditional tumour suppressor $p21^{WAF1/Cip1}$ exerted pro-tumourigenic properties in a p53-deficient setting. Moreover, in this context, a strong tendency of senescent cells to escape was implied, despite overexpression of a potent senescence mediator. We experimentally recapitulated these histopathological observations and indeed observed senescence escape, reflected by the emergence of proliferating cells with aggressive properties and increased tolerance to genotoxic agents [34]. Mechanistically, chronic $p21^{WAF1/Cip1}$ upregulation was shown to 'saturate' the $CRL4^{CDT2}$ and SCF^{Skp2} ubiquitin ligase complexes, restraining turnover of the replication licensing machinery [34]. Constituent replication stress and DDR activation, in a p53-null setting, resulted eventually in a shift from RAD-51-mediated high-fidelity repair processes to RAD-52-modulated low-fidelity repair, fuelling genomic instability (Figure 4) [83].

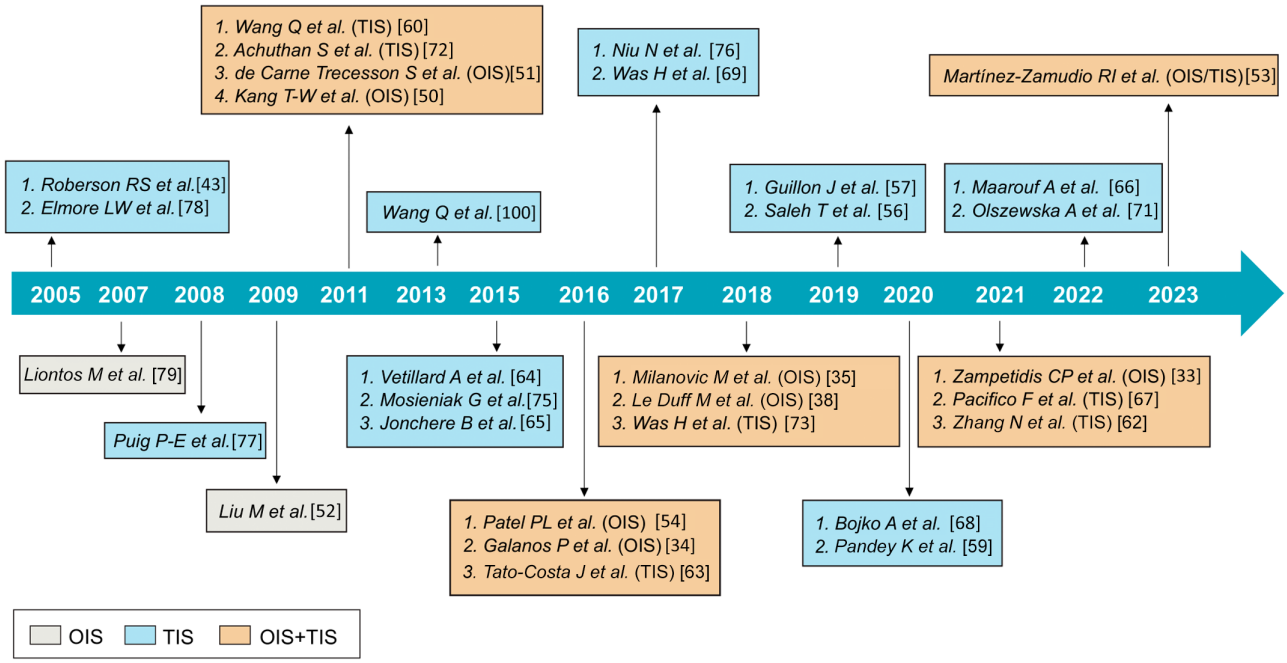

Figure 3. Timeline of studies that emerged in recent decades dealing with escape-from-senescence concept.

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Table 1. Mechanisms of escape from senescence per senescence type and cellular system.

Cell type/cellular system	Mechanism of escape	Reference
Oncogene-induced senescence (OIS)		
Ras-expressing hepatocytes	$p19^{ARF}$ deactivation (spontaneous deficiency and escape)	[50]
HT29 colorectal cell line with absence of p53 and p16 LS174T cells	Downregulation (loss) of $p21^{WAF/Cip1}$ and upregulation of Bcl-xL/MCL1 that repress BH3-only proteins such as BIM or PUMA; Aurora-A-PLK1 pathway and CDC25C spindle checkpoint activator are dysregulated (spontaneous escape)	[51]
MCF10A mammary epithelial cells	$p21^{WAF/Cip1}$ downregulation (setting: tumourigenesis induced by mammary-targeted Ha-Ras or c-Myc) (artificial escape)	[52]
Colorectal cancer	POU2F2, AP1 upregulation leading to $CDKN2A/p16^{INK4a}$ downregulation	[53]
Human diploid dermal fibroblasts (HSF43) BJ cells (ATCC) GM21808 MDA-MB-231 cells MDA-MB-468 cells HCT116 colon cancer cells Fibrosarcoma cells (HT-1080)	De-repression of hTERT expression in a c-Myc-dependent manner through chromatin rearrangement (spontaneous escape)	[54]
Human bronchial epithelial cells	A recurrent chromosomal inversion of BHLHE40 locus, a circadian rhythm-related gene, suffices for driving escape from OIS via subTAD reorganisation	[33]
Colon and breast cancer cells	Upregulated CDK4-EZH2-AP2M1 pathway	[38]
Melanocytes	H3K9me3 histone mark suppression by global H3K9 demethylase activity	[55]
Therapy-induced senescence (TIS)		
HCT116 cells (radiation, PARP inhibitors)	Loss of $p16^{INK4a}$ (spontaneous escape)	[53]
H460 cells (etoposide)	Loss of $p16^{INK4a}$ (spontaneous escape)	[56]
LS174T cells MCF7 cells	$p21^{WAF/Cip1}$ downregulation and resultant CD47 and TSP1 downregulation (spontaneous escape)	[57]
Eμ-Myc transgenic Bcl-2 overexpressing lymphoma cells	P53 knockdown (spontaneous escape)	[35,39,58]
MCF7 cells	CDK2-mediated c-myc phosphorylation (artificial escape)	[59]
LS174T cells MCF7 cells	CyclinD1/CDK4 upregulation (spontaneous escape)	[38]
H1299 human non-small cell lung carcinoma cells (−p16/+pRb/deleted p53)	Upregulation of cyclin-dependent kinase Cdc2/Cdk1 (spontaneous escape)	[43]
p53-null NCI-H1299 cells	Cdc2/Cdk1/survinin upregulation (spontaneous escape)	[60]
MCF-7 cells	Enhanced cdc2 (spontaneous escape)	[61]
MCF7 cells	Bystander effect of SASP, non-cell autonomous activation of Notch signalling (spontaneous escape)	[62]
HCT 116 cells	SASP (paracrine spontaneous escape)	[63]
LS174T and HCT116 cells	Inactivation of Noxa and apoptosis inhibition	[64]
LS174T and HCT116 cells (active p53-p21 axis) HT29 cells (inactive p53)	Mcl-1 signalling upregulation	[65]
MCF-7 and LS174T cells	1. Overexpression of AGR2 (anterior gradient protein 2) 2. Activation of the mTOR/AKT	[66]
MCF7 cells A549 cells	Increase glutamate (spontaneous escape)	[67]
MDA-MB-231 cells MCF7 cells HCT116 cells	Increased autophagy	[68] (spontaneous escape), [69] (artificial escape)
Colon cancer cells	Redox alterations (increased oxidative stress)	[70]
A549 lung cancer cells CT116 colon cancer cells	Hypoxia → cyclin B-mediated activity; downregulation of autophagy enhanced escape	[71]
Eμ-Myc transgenic Bcl-2 overexpressing lymphoma cells	1. Gene expression alterations 2. Loss of restrictive epigenetic changes, i.e. Suv39h1 knockdown	[35,39,58]
MCF7 cells HCT116 cells	Acquisition of stem cell-like traits	[72] (spontaneous escape), [73]
H1299 (p53 and p16 deficient) HCT116 cells Hey cells SKOV3 cells MDA-HGSC-1 cells	Endoreduplication	[74] (artificial escape), [75] (spontaneous escape), [76] (spontaneous escape)
PROb colon cancer cells	DNA endoreduplication and polyploidy (spontaneous escape)	[77]

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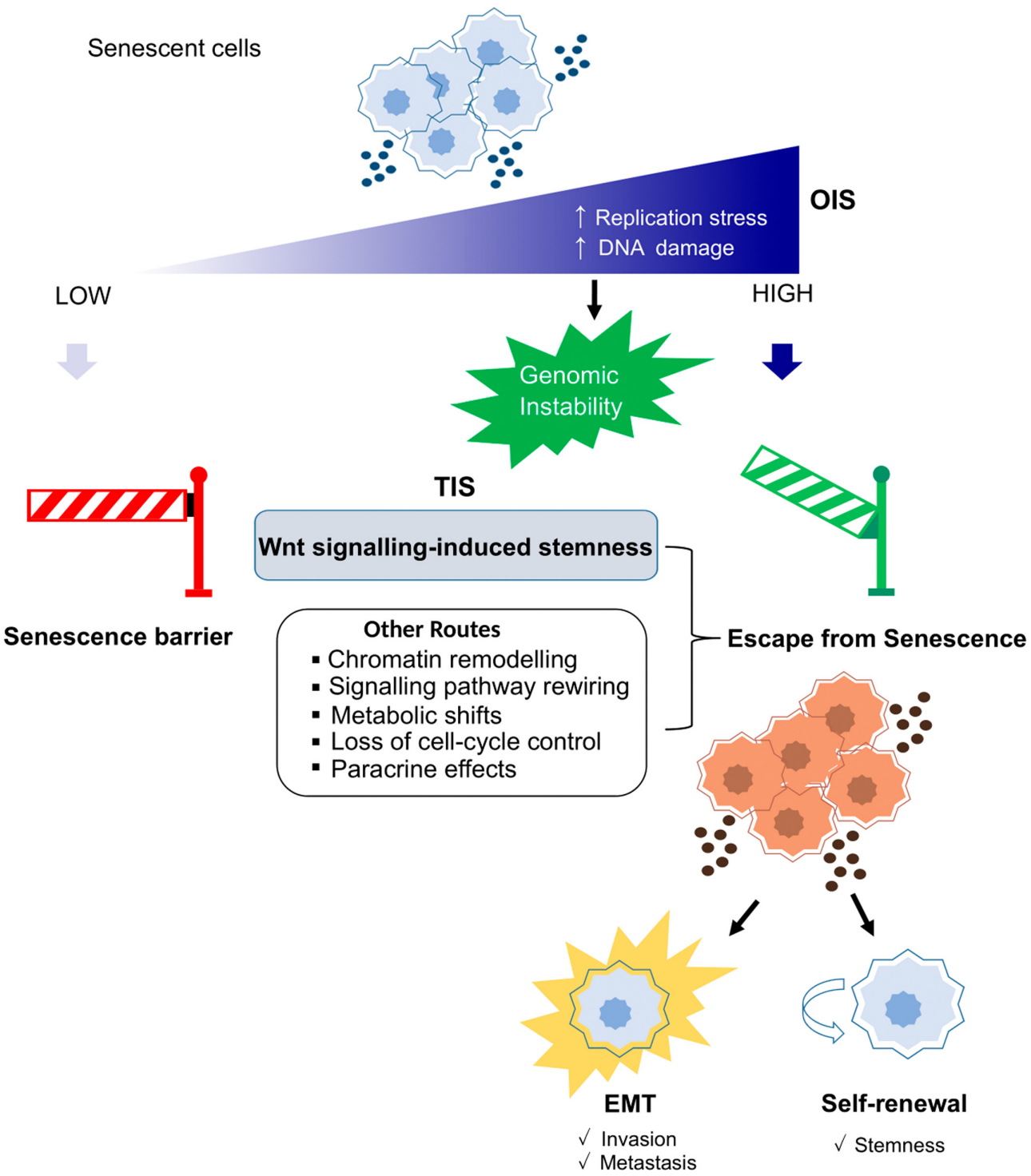

Figure 4. OIS is characterised by progressively increasing levels of replication stress, genomic damage, and eventually genomic instability. The latter acts as a driving force underlying the escape-from-senescence phenomenon. In TIS, acquisition of a Wnt signalling-related stemness phenotype, as well as routes such as metabolic changes, shifts in cell-cycle regulation, and engagement of different signalling pathways, can break the senescence barrier. The resultant escaped cells harbour aggressive biological features reflected by stemness and EMT.

Overall, our observations implied that genomic instability played a role in escape from senescence, but direct evidence was still lacking.

To address how genomic instability may be the driving force underlying escape from senescence, we recapitulated the entire spectrum of epithelial carcinogenesis, challenging human bronchial cells with the potent oncogene CDC6 [33,84]. Oncogene-driven genomic instability initially triggered OIS while cells escaped from senescence over time, similar to the aforementioned trajectories. Moreover, escaped cells displayed EMT coupled with a mixed stem cell signature [33,84]. Interestingly, escaped cells exhibited complete resumption of the cell cycle, were invasive, and generated tumours upon

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injection into nude mice, thereby recapitulating all major features of cancer cells [33,84]. We identified a number of common genetic aberrations in the escaped cells, ruling out stochasticity. Among those, a recurrent inversion on chromosome 3, which harbours the transcription factor BHLHE40, attracted our attention [33]. The basic helix–loop–helix family member E40 (*BHLHE40* also known as *DEC1*) is an essential constituent of the molecular machinery comprising the circadian clock [85–87]. There is substantial evidence linking the circadian clock with the cell-cycle machinery and its disruption has been associated with various pathologies, including cancer [88,89]. The identified inversion was associated with upregulation of *BHLHE40* and an altered expression profile of a plethora of genes that are *BHLHE40* targets, such as mouse double minute 2 homolog (MDM2), the main negative regulator of p53 [33,90]. Notably, *BHLHE40* is well known to regulate thousands of genes encompassing many cell-cycle regulators [33,91]. Overall, these findings imply that *BHLHE40* is essential for establishing escaped phenotypes. This hypothesis was functionally tested by artificially inducing inversion in cells, which subsequently displayed the entire spectrum of escape-from-senescence traits [33,92]. Given that inversion is the outcome of replication stress-induced genomic instability, the latter observation confirmed that genomic instability was indeed the driving force underlying escape from oncogene-induced senescence.

In parallel, studies from other groups provided additional evidence for the escape from OIS and TIS phenomenon (Figures 3 and 4, Table 1) [33,35,38,39,43,50–54,56–60,62–73,75–78,93,94]. Two recent investigations stemmed from the same group. In the first, with the use of a well-known TIS lymphoma model, substantial activation of the Wnt signalling pathway and a distinct stem cell signature in senescent cells were observed [35]. The latter feature was introduced as senescence-associated stemness (SAS) [35]. Genetic silencing of key senescence mediators such as p53 led to the generation of senescence escaped cells that displayed significantly elevated tumour-initiating potential reflected by cell proliferation, colony formation capacity, and tumour formation in mice, in line with our findings [33–35]. Characteristically, a rare and spontaneously occurring senescent escape cell population with similar features was also observed in the lymphoma model, without any genetic intervention, verifying our observations on senescence escape [33–35]. The identification of Wnt signalling as the driving force of newly acquired features in TIS lymphomas was not surprising, given that aberrant Wnt signalling has been associated with the renewal of cancer stem cells, proliferation, differentiation, metastasis, and immune surveillance [35,95,96]. Based on those findings, it was suggested that Wnt-induced SAS may be a general mechanism leading to the generation of highly aggressive and malignant tumour-initiating stem cells in response to cancer therapy.

The second investigation dealt with the rescue of chromatin modifications considered essential for the establishment of senescence, thus promoting escape from senescence [3]. In particular, suppression of the H3K9me3 histone marker by global H3K9 demethylase activity was demonstrated to drive escape from Ras-/Braf-induced senescence (OIS) in melanocytes [55]. The escaped cells displayed cell-cycle re-entry, loss of senescence markers and accelerated melanomagenesis. In line with this, high expression of the demethylases lysine-specific demethylase 1 (LSD1) and Jumonji domain 2C (JMJD2C) was common in melanomas and not in senescent nevi or Braf-senescent melanocytes [55]. Of note, inhibition of LSD1 or JMJD2C restored OIS. Overall, these findings indicated the importance of the suppressive H3K9me3 mark not only in the establishment but also in the maintenance of the senescence phenotype [55]. Moreover, co-operation of LSD1 or JMJD2C with activated Ras/Braf leads to cellular transformation and melanomagenesis, revealing the oncogenic properties of aberrant demethylase expression [55]. At the mechanistic level, H3K9 demethylases were shown to promote E2F target gene expression in Ras-driven cells, and their ability to hinder OIS was found to be dependent exclusively on their catalytic function.

Moreover, other routes are reportedly implicated in senescence escape, denoting the multifaceted nature of this phenomenon (Figures 3 and 4, Table 1). The majority seem to converge on genomic instability acquisition, rendering this feature a critical determinant of the senescence escape phenomenon. These avenues include activation of specific signalling pathways, loss of cell-cycle control, perturbation of metabolic processes including oxidative stress/mitochondrial dysfunction, and polyploidy (Figures 3 and 4, Table 1) [38,47,51,58,67,74,97]. Interestingly, activation of CDK4 in chemotherapy-induced senescence was shown to upregulate the enhancer of zeste homolog 2 (EZH2) methylase, a component of the polycomb PRC2 complex. As a result, AP2M1, a factor that plays an essential role in receptor endocytosis, is induced, modulating the surfaceome of senescent cells in the direction of escape [38]. Inactivation of cardinal cell-cycle inhibitors in senescent cells has been proposed as an additional path that leads to senescence escape [47,51,58,97]. Regarding metabolic traits, increased glutamine transporter SLC1A5 and glutamine synthetase have been shown in cancer cells that have escaped from TIS, indicating augmented glutamine metabolism [98]. Additionally, doxorubicin-treated cancer cells were found to display high levels of glutamate following escape from TIS [98]. Polyploidy is a common trait of senescent cells and has been extensively demonstrated in tumour cells following TIS [61,99]. Polyploidy is linked to chromatin reorganisation, stemness, and drug-resistant properties along with enhanced metabolic energy. As such, polyploidy appears to confer on tumour cells the ability to escape from senescence, promoting a more malignant phenotype and avoiding lethality following anti-cancer interventions [42,94,100,101]. Camptothecin-induced senescent H1299

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cells are often polyploid and escape spontaneously [100]. Such escape was related to overexpression of CDC2 and its downstream effector survivin, conferring increased viability of the escaped cells [60,78,102]. In addition, CDK1 upregulation has been found to promote polyploidy and subsequent escape from TIS, while there is also evidence for senescence escape mediated by autophagic activation [99,100]. Similarly, polyploid features were also evident in cells escaping from OIS during a cancer evolution experiment [79]. Moreover, senescent cells commonly regarded as multinucleated have been proposed to exert an atypical (asymmetrical) budding mitosis, termed neosis, resulting in the generation of clones with proliferative capacity [103]. Whether mother cells are truly senescent remains unclear.

Overall, introducing the escape-from-senescence phenomenon, particularly in the cancer field, creates the basis for revisiting currently applied treatment modalities that target proliferating cells and renders accurate tracing of senescent cells an issue of paramount importance to pathology and therapy.

Detecting senescence escape: a challenging task

To detect escape from senescence, we need to identify senescent cells. This is quite easy *in vitro*, given that one of the top senescence markers, SA-β-gal, is efficient for cell culture analysis [104]. However, at the *in vivo* level and particularly in archival material, SA-β-gal is inefficient [104,105]. This problem was solved with the recently developed GL13 reagent (commercially known as SenTraGor™), which is able to identify senescence in any biological material, including archival material routinely processed in pathology laboratories [106]. GL13 detects lipofuscin, a histological pigment commonly discerned by pathologists. Lipofuscin, the 'dark matter' of cells, was initially observed by Adolph Hannover in 1843, but its relationship to a stress state, like senescence, had evaded notice for many years. Moreover, GL13 was used in a multimarker-based algorithm, adopted by the senescence community, for reliable senescence identification [3,107]. Notably, by applying this marker, cellular senescence was detected in a variety of diseases, so *GL13 introduced cellular senescence to pathology*. In particular, GL13 made it possible to identify the first clinical entity harbouring senescent cells, namely Hodgkin lymphoma [37]. This malignancy attracted attention because it exerts a number of morphological and molecular features that are reminiscent of the senescence phenotype. In particular, it is characterised by large neoplastic cells (Reed-Sternberg cells) that are embedded in an inflammatory milieu, exerting robust paracrine functions and expressing markers of senescent cells [$p21^{WAF1/Cip1}$(+)/$p16^{INK4A}$(+)/Ki-67(−) pattern] [108,109]. Notably, lymphomas with high numbers of senescent cells exhibited resistance to chemotherapy, an observation that could be attributed to the protumourigenic properties of prolonged and/or robust SASP [1,3,109]. The escape-from-senescence phenomenon could also be a mechanism contributing to this outcome (see the next section). Ever since GL13 discovery, the spectrum of pathological entities has been expanded to include benign, preneoplastic, and neoplastic lesions, while senescent cells have also been recognised in various experimental and ageing contexts (Table 2) [37,106,107,110–137]. In particular, in clinical settings, skin pathologies (nevi, seborrheic, and actinic keratoses), lung, pancreatic, urothelial and laryngeal precancerous lesions, various types of carcinomas following treatment, sarcomas, haematological malignancies (Langerhans histiocytosis), and a variety of tissues from aged organs were included (Table 2). Interestingly, while the involvement of senescence in infectious diseases has been hypothesised for many years, during the recent SARS-CoV-2 pandemic it became documented by applying GL13 and the senescence detection algorithm. We demonstrated for the first time in COVID-19 patients that SARS-CoV-2 induces senescence accompanied by SASP [137]. Notably, according to our findings, virally induced senescence constitutes a 'fertile' environment for viral mutagenesis [137]. The fate of virally induced senescent cells and whether they might be involved in the 'long COVID' lung manifestation, resembling end-stage pulmonary fibrosis, awaits further investigation [137].

Taking the foregoing factors into consideration, apart from the deleterious effects of SASP on the tissue microenvironment, the presence of senescent cells represents a niche of cells potentially susceptible to escape. Given that senescence escape has been associated with adverse effects (aside from SASP), increased incidence of senescence in human lesions reflects a potentially unfavourable predictive marker [33,34,138]. Thus, pathologists should be aware of identifying senescent cells in human lesions in everyday clinical practice. In the near future, as senolytics are anticipated to enter into clinical use, this information will be important in designing the most effective therapeutic strategy, thereby reforming conventional therapies [37,138]. These issues are discussed in the following section.

Regarding the detection of escape *in vivo*, this is a very challenging task, and currently available tools may be of value. Cells that are likely to have undergone escape from senescence in tissues would reside in the vicinity of a senescent cell niche (GL13+/Ki-67− pattern), exhibiting a GL13−/Ki-67+ pattern. Moreover, staining with additional markers such as $p21^{WAF1/Cip1}$ and $p16^{INK4A}$ (multimarker algorithm) and assessing morphological traits (for instance a spindle shape, which is suggestive of EMT in epithelial cells) could further complement the discrimination of senescent versus escaped cells in the same region. The 'escaped' nature of these cells can be further characterised by their isolation via microdissection and analysis by molecular means. Such an approach would lead to the putative identification of common alterations/signatures that are informative regarding the origin of escaped cells from their neighbouring senescent cells. In any case, more sophisticated approaches need

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Table 2. Clinical and experimental settings harbouring senescent cells, assessed by GL13 staining.

Tissue/organ/entity	Species	Reference
1. Lymph node (Hodkin lymphomas)	Human	[37]
2. Skin Langerhans histiocytosis		
3. Pancreatic intraepithelial lesions		
Human	Human, C57Bl/6 mice	[106]
1. Liver tissue		
2. Seminal vesicle		
3. Irradiated head and neck carcinomas		
4. Chemotherapy-treated breast tissue		
5. Congenital nevi		
Mouse		
1. K-ras-induced mouse lung adenomas		
2. Bleomycin-induced mouse lungs		
3. Palbociclib treated melanoma xenograft		
Human	Human, mice	[107]
1. Oral fibroma		
2. Bone marrow (megakaryocytes)		
3. Urothelial dysplasia (bladder)		
4. Cartilage and synovium (chronic arthritis)		
Mouse		
Myocardium		
Neuronal tissue	Human	[110]
HeLa cell xenograft tumours	NSG mice	[111]
Thymus gland	Human	[112]
Heart, kidney	Wistar rats	[113]
Pancreatic ductal carcinomas	Human	[114]
Skin (actinic keratosis)	Human, K5-rtTA Mice	[115]
Lung	Human	[116]
Various sarcomas	Human	[117]
Neuronal tissue	Human	[118]
Lung, liver, fatty tissue	Er1F/+ mice	[119]
Skin	Human	[120]
Liver	C57Bl/6 mice	[121]
Breast, lung	Human	[122]
Colon, small intestine	Sv129 and C57BL/6 mice	[123]
Small intestine	Sv129 mice	[124]
Lung, airway mucosa	Human, golden hamster	[125]
Myocardium	NSG-DR1 mice	[126]
Kidney	C57BL/6J mice	[127]
Skin (seborrheic keratosis)	Human	[128]
H460 large-cell carcinoma cell line (LCC)	CD1-nude female mice	[129]
Breast	Human	[130]
Brain (frontal cortex, hippocampus)	Human	[131]
Skin	Human 3D organotypic skin epidermis culture	[132]
Lymphoid tissue (lymph node Hodgkin lymphomas)	Human	[133]
Skin (from ventral neck, upper chest region)	C57BL mice	[134]
Intervertebral disc (cartilage)	Human, Wistar rats	[135]
Lung (NSCL carcinomas ± adjuvant therapy)	Human	[136]
Lung, kidney, liver	Human	[137]

to be developed to tackle this issue. To this end, an attractive strategy is emerging from the generation of patient-derived organoids. This *in vitro* system closely approximates the tissue architecture and physiology *in vivo* and can be exploited to provide information on whether senescence escape took place in a lesion [139,140]. Interestingly, sophisticated multiome means encompassing spatial transcriptomics and single-cell multiomics approaches (10X Genomics platforms) coupled with bioinformatics analysis and artificial intelligence allow simultaneous interrogation of the transcriptome and genome in thousands of individual cells within tissues [141]. As such, these technologies may answer the question of whether cells with aggressive features originated from niches of senescent cells that eventually underwent escape.

Therapeutic implications

Currently applied anti-cancer strategies largely rely on interventions (chemo- or radiotherapy) aiming to eliminate proliferating cancer cells [142]. However, such therapies not only fail to remove non-proliferating senescent cells but can also promote senescence (TIS) in cancer cells that survive [143]. The outcome is the emergence of a senescent cancer cell population that exhibits tolerance against traditional therapies, preparing the way

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for tumour relapse, metastasis, and unfavourable clinical outcomes, potentially through the acquisition of cancer stemness traits [35]. Of note, escaped cells acquire tolerance against senescence-inducing drugs, a feature reminiscent of tumour recurrence following primary response to therapy in cancer patients [142,143]. In agreement with this, and as mentioned earlier, high levels of OIS in Hodgkin and Reed-Sternberg cells prior to treatment is associated with adverse prognosis [37]. Moreover, based on these facts, senescence has been proposed as a driver of cancer dormancy. The latter is characterised by cells that remain alive in an arrested state and can be reactivated to give rise to tumour relapse and metastatic disease [67,74,144,145]. Interestingly, a cellular state termed quiescence, which is distinct from cellular senescence but shares some common features, has been proposed to play a role in cancer dormancy [3,145]. Quiescent cells are, like senescent cells, cell-cycle arrested; however, this arrest is reversible and involves another set of molecular pathways ($p27^{Kip1}$ activation), while secretory properties and macromolecular damage are absent [3].

For all the foregoing reasons, senescent cells should be removed from adult tissues. Interestingly, senescent cells demonstrate resistance to apoptosis, an important feature that has been exploited in developing drugs for their elimination [3,37,146]. These agents, termed senolytics, kill senescent cells by selectively inducing apoptosis [37,146]. The emergence of senolysis, which targets the anti-apoptotic Bcl-2 family of proteins, has opened new avenues for modifying traditional cancer therapies that need to be further investigated [37,146]. In this context, a combinatorial regime targeting both proliferating and senescent cells in neoplasms was suggested as a promising strategy (Figure 5) [138]. In untreated and treated malignancies, where escape from

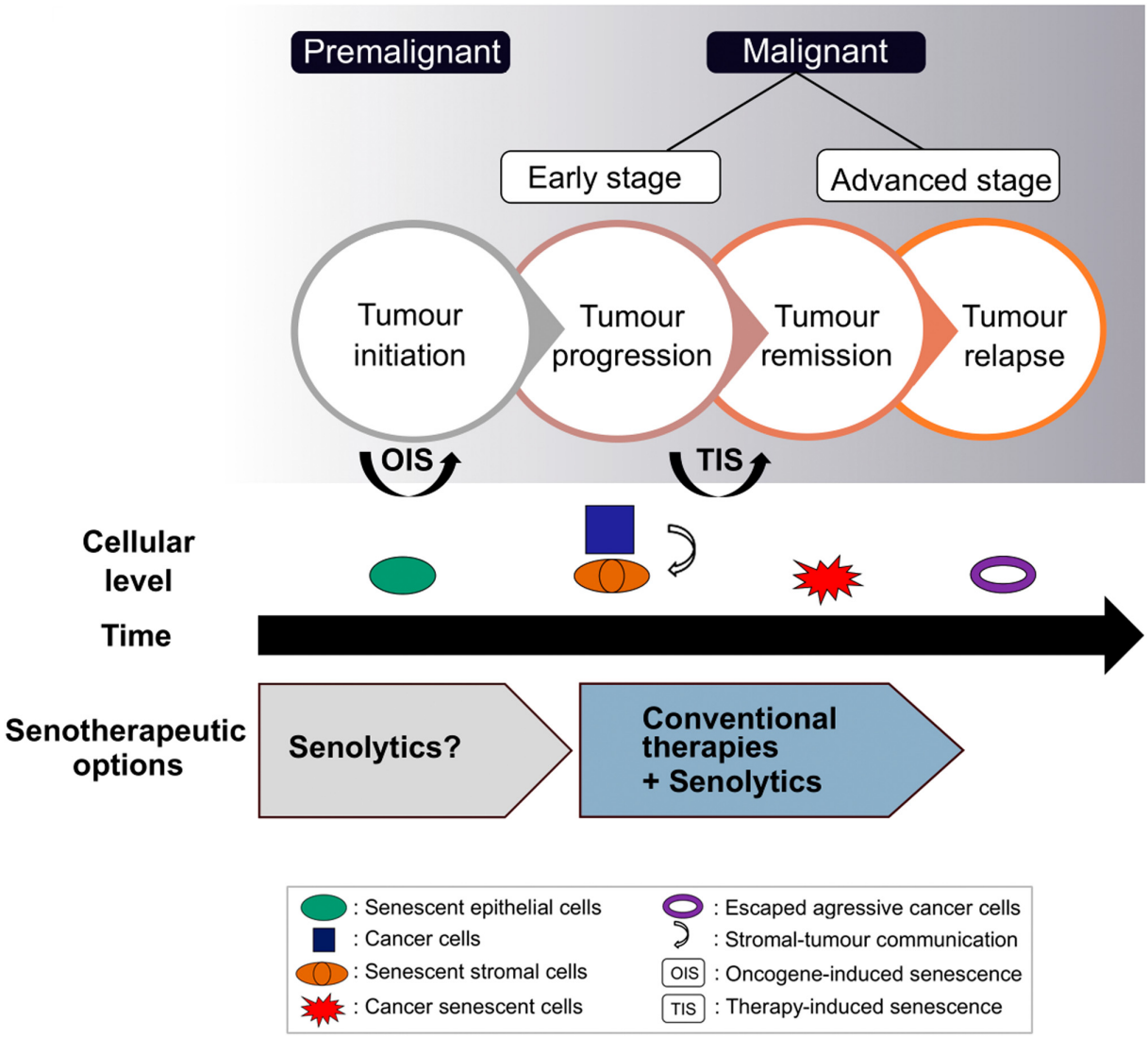

Figure 5. Cellular senescence is involved in the earliest (premalignant) stages of cancer development (OIS) as well as in tumours following treatment (TIS). Moreover, cancer evolution can be fuelled by the presence of senescent cells either in the tumour or the stromal compartment. Tumour progression and tumour relapse after therapy are related to the escape of cancer cells from their senescent state. Senolytics could be implemented across the timeline of cancer's natural history in conjunction with conventional radio/chemotherapeutic modalities for better clinical benefits. Whether it is safe to eliminate senescent cells in premalignant lesions requires thorough investigation and clinical trials.

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senescence may facilitate cancer progression, such interventions might prove extremely beneficial. Additional strategies are also intriguing that propose a two-hit synthetic lethality approach in which senescence is initially provoked in cancer cells (TIS) and then senescent cells are selectively eliminated with senolytics [147]. Our awareness of the mechanisms imposing apoptosis tolerance to senescent cells remains limited. Unveiling the mechanistic insights governing this phenomenon is anticipated to boost and expand the therapeutic potential of senolysis and lead to the discovery of more effective tools in the arsenal against cancer. To that end, additional approaches could be tested, based on reagents that induce ferroptosis, a common type of death in senescent cells [148]. Overall, increasing our knowledge of the escape-from-senescence phenomenon carries significant translational potential to redirect currently applied therapies and to develop revolutionary treatments, improving clinical outcomes for cancer patients [138].

Future perspectives

In this overview of the evidence introducing the concept of senescence escape, we have described how the dogma of permanent cell-cycle withdrawal in senescence has been questioned as attractive translational implications have arisen. Escape from senescence results in transformed cell offspring and detrimental outcomes. Thus, it is essential to distinguish this phenomenon from other types of cell-cycle re-entry that are either related (escape from RS or senescence reversal) or unrelated (senescence bypass) to the senescence phenotype, also clarifying the existing confusion and misconceptions of the field. To this end, and as research in the field is anticipated to expand our awareness, efforts to establish a consensus in the near future seem to be of outmost value.

Although the body of evidence presented so far suggests that escape from senescence is a dire phenomenon, this may not always be the case. It is not uncommon in biomedicine to observe yin-yang phenomena depending on cellular context [149–153]. Escape from senescence and the immune system may well be such a case. Although evidence remains limited, an indication of this stems from *BHLHE40*. Despite its adverse role in the epithelial compartment, a parallel study showed that a subset of regulatory T cells, termed fragile Tregs, harbouring high levels of BHLHE40, suppressed tumourigenesis [33,154,155]. While at first glance this observation appears contentious, it is biologically rational as the organism requires fit immune cells and not senescent or exhausted cells [156,157]. It remains to be shown whether BHLHE40-driven programmes may

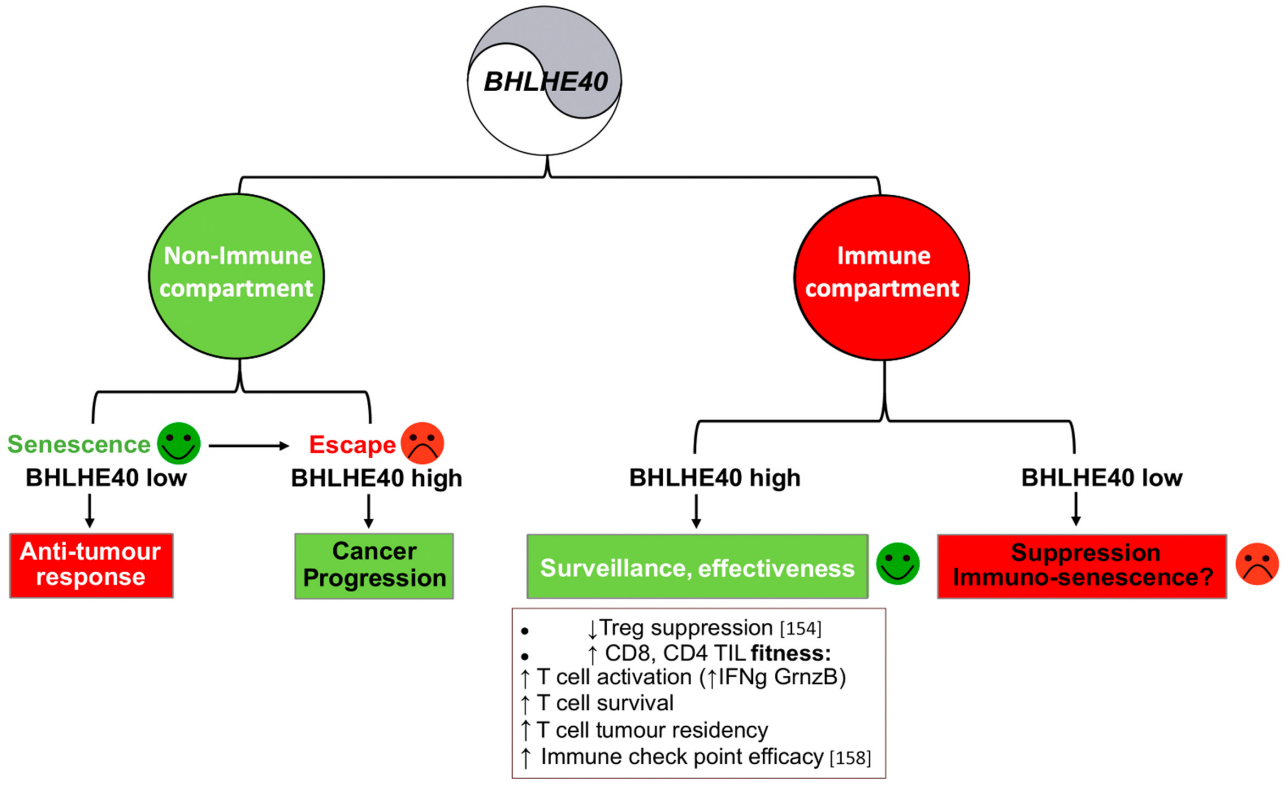

Figure 6. A yin-yang/opposite effect of BHLHE40 transcription factor among different cell types. BHLHE40 levels have been associated with contrasting functions between the epithelial/non-immune and immune compartment. Low BHLHE40 levels are known to favour senescence as an anti-tumour response, while its upregulation may lead to escape towards a tumourigenic phenotype in non-immune cells [158]. In contrast, high BHLHE40 levels stimulate immune cell fitness and effective responses. Following this concept, downregulation of BHLHE40in immune cells might be associated with immune suppression and/or senescence, which warrants investigation.

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bypass/escape T-cell senescence, boosting immune cell fitness (Figure 6) [154,158].

On a different front, genomic instability and chromatin reshuffling are common features of senescent cells [3]. In this context, activation of transposable elements (TEs) has been extensively documented [3,159–161]. TEs are DNA sequences able to change their position within the genome [162,163].We observed a robust upregulation of TE copies during senescence that, however, was drastically reduced upon escape (https://senequest.net/about) [164]. Interestingly, some of these increased copies were close to the transcription start site of genes that constitute the SASP phenotype (*CDKN1*, *CCL8*, *CCL13*, *IFNG*, *IL6*) [3,11,18–21]. Given that the expression of SASP factors is reduced in escaped cells, it would be intriguing to delve deeper into the putative TE–SASP expression relationship and elucidate putative mechanistic aspects, thereby further characterising the escape-from-senescence phenomenon.

Last but not least, the improvement or development of novel detection strategies will allow for an even more accurate assessment of the senescent cell burden in human lesions. This is an imperative task, which will enable the design of the most effective therapeutic strategy in the near future, in the context of precision medicine [165]. Moreover, new methodologies that will allow deeper characterisation of the senescence phenotype, including genetic, epigenetic (e.g. chromatin conformation capture, 3C techniques), and transcriptomic (e.g. single-cell or bulk RNAseq) analyses are anticipated to elucidate aspects with therapeutic implications such as the mechanistic basis of resistance to apoptosis. All in all, the clarification of several important concepts related to senescence escape will revolutionise cancer therapy by utilising personalised approaches in a timely fashion, thereby improving patient outcomes.

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Author contributions

KB performed the literature search and data analysis. KE, RP and VGG wrote the manuscript. AP, KB and KE prepared the figures. VGG supervised the current work. All authors reviewed and approved the final manuscript.

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