

The dynamic nature of senescence in cancer

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Cellular senescence is implicated in physiological and pathological processes spanning development, wound healing, age-related decline in organ functions and cancer. Here, we discuss cell-autonomous and non-cell-autonomous properties of senescence in the context of tumour formation and anticancer therapy, and characterize these properties, such as reprogramming into stemness, tissue remodelling and immune crosstalk, as far more dynamic than suggested by the common view of senescence as an irreversible, static condition.

More than 50 years ago, Leonard Hayflick described the limited proliferative capacity of primary cells propagated in culture¹. This observation of ‘replicative senescence’ as a reflection of aged cells² is linked to progressive telomere shortening every time the cell divides³. Triggers, such as activated oncogenes, cytokines, reactive oxygen species, DNA damage or nucleotide depletion, acutely evoke a phenotypically similar, ‘premature’ type of senescence, positioning stress-induced senescence as a response mechanism with broad implications in health and disease⁴. In contrast to another ultimate cell-cycle exit programme, apoptosis, senescent cells remain viable for an extended period of time and typically exhibit a senescence-associated secretory phenotype (SASP). The SASP, a plethora of largely pro-inflammatory cytokines and chemokines, accounts for the complex crosstalk that senescent cells engage in with neighbouring cells^{4–6}. Hence, the participation of cellular senescence in various physiological and pathological processes may not only be explained by proliferative arrest but also, or even dominantly, by cell–cell interactions and the secretion of factors that affect surrounding cells and compartments⁷. Moreover, senescent cells undergo global epigenetic changes that affect their phenotype and function⁸. In essence, senescent cells may profoundly affect tissue homeostasis, interfere with organ function, instruct other cells in their environment or evoke secondary amplified immune network responses—highly dynamic processes of potential selective advantage for tumour growth.

Detecting, determining and defining senescence

Frequently used markers of senescence include the ‘gold-standard’ senescence-associated β-galactosidase (SA-β-gal) reactivity, lack of the cell-cycle-associated Ki67 protein, high-level expression of the cyclin-dependent kinase (CDK) inhibitor p16^{INK4a} (also known as CDKN2A), lack of DNA replication, as well as focally and globally elevated levels of trimethylated histone H3 lysine 9 (H3K9me3) and induction of SASP factors^{9–12}. However, even a combination of several markers cannot faithfully depict cells as senescent rather than long-term arrested or in an aberrant mode of delayed cell death. Thus, the presence of senescent cells is hard to prove, in particular, *in vivo*, and there is an ongoing search for a ‘senescence signature’, that is, a robust and sensitive panel of markers or gene products defining a cell as being senescent^{13,14}.

An even more puzzling question is what the biological implications of the senescence phenotype actually might be. Senescence is widely understood as an irreversible growth arrest refractory to exogenous mitogens¹¹, but it is not clear to what extent this state is initiated by distinct triggers and events rather than representing a

crude response to overwhelming insults that the cell lacks physiological tools to buffer. Furthermore, it has not been sufficiently investigated how specific the morphological characteristics, such as a flattened cell body, an expanded lysosomal compartment and heterochromatin foci in the nucleus, are to senescence. It is tempting to assume that senescence must be a static end point of a cell’s life cycle, as also implied by signs of terminal differentiation that were detected in senescent cells¹⁵. However, several studies have since demonstrated the reversibility of full-featured senescence, indicating that pre-senescent or early senescent cells are poised to re-enter the cell cycle^{16–19}. Based on the massive epigenetic remodelling underlying the state switch, senescence has also been linked to enhanced plasticity and reprogramming^{18,20–23}, suggesting that the cells may adopt features of less-mature cells or even transdifferentiate. In addition, given the difficulties to robustly determine a cell’s senescent state, it remains to be clarified what distinguishes senescence from dormancy, another reversible arrest condition displaying senescence-reminiscent intrinsic signalling, and where resumed proliferation is linked to stem cell programmes and external, environmental cues^{24,25}. In essence, increasing evidence points towards a much more dynamic nature of the senescent condition. Senescent cells need to constantly renew the H3K9me3 mark, essential for senescence, at distinct target gene promoters due to histone turnover^{18,19}. They degrade heterochromatin and shuttle it to the cytoplasm with consequences that have yet to be investigated^{26–33}. Moreover, they engage in abortive DNA replication attempts, which result in stalled forks^{19,34,35}. Senescent cells also modulate the abundance and composition of their secretome over time^{36,37}, and SASP-related protein synthesis and degradation rewire their metabolism according to senescence-specific bioenergetic demands^{38–40}. Moreover, with surrounding cells operating as signal relays, the SASP may indirectly impinge on the SASP-producing senescent cell itself⁴¹. These complex processes shape a biochemically highly active, continuously changing profile of senescence that is very different from the classical view of a starvation-like resting static condition.

Intrinsic and extrinsic features of senescent cells

Senescence is executed via characteristic changes in the nucleus and the cytoplasm and further communicated across individual cells. Whereas cell-intrinsic signalling accounts for the state switch, the nature of senescence is more global—protecting the organism from potentially harmful cells and engaging in extensive crosstalk with other cells in the environment.

In the nucleus, pro-senescent stresses prompt DNA damage response (DDR) signalling to the cell-cycle blocker p21^{CIP1} (also

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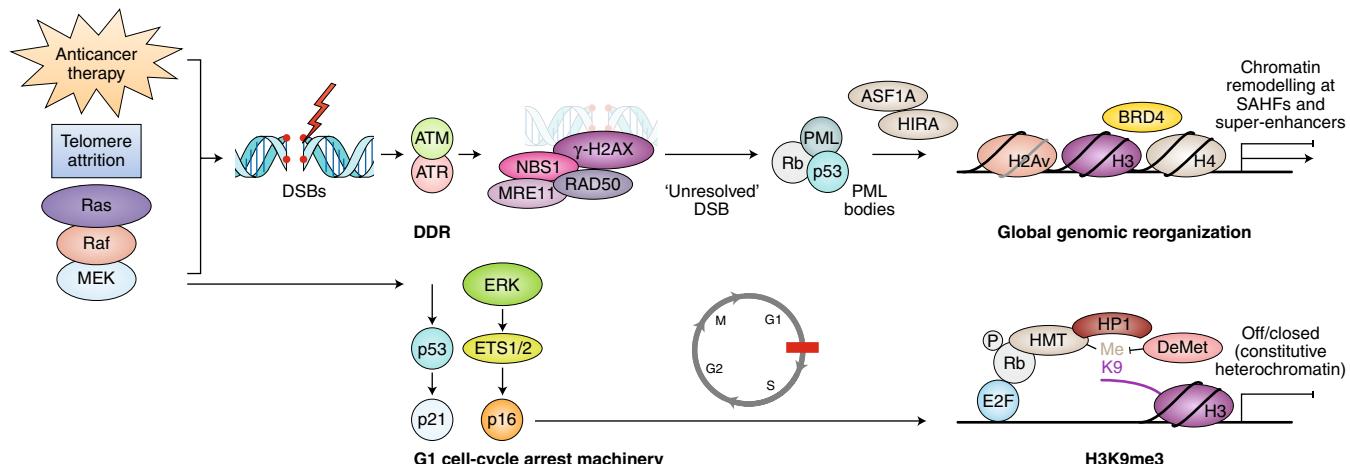


Fig. 1 | Global and focal chromatin remodelling in senescence. Senescence-inducing triggers, such as critically shortened, eroded telomeres, activated Ras-Raf-MEK (mitogen-activated protein kinase kinase) (promoting abortive DNA replication at stalled forks), or cancer drugs, directly or indirectly damage DNA. This evokes a DDR that leads, via the mitogen-activated protein kinase signalling cascade (involving extracellular signal-regulated kinase (ERK) and ETS1/2) and the p53 pathway, to the induction of the G1-phase cell-cycle inhibitors p16^{INK4a} and p21^{CIP1}, respectively^{10,44,109}. Moreover, unresolvable DNA damage sites initiate promyelocytic leukaemia (PML) body formation, further recruiting HIRA and ASF1A chromatin remodelers to establish senescence-associated heterochromatin foci (SAHF), and to eventually globally reorganize the entire genome involving histones H2Av, H3 and H4 and additional remodelling of transcription and super-enhancers through bromodomain-containing protein 4 (BRD4)^{52,53}. Among histone modifications is the transcriptionally repressive H3K9me3 decoration in the vicinity of E2F target genes, mediated by histone methyltransferases (HMTs) bound to G1-phase-typical hypophosphorylated Rb-E2F complexes and potentially antagonized by H3K9-active demethylases (DeMet)^{11,9}. DSBs, double-strand breaks; HP1, heterochromatin protein 1.

known as CDKN1A), and trigger activation of the mitogen-activated protein kinase cascade, which leads to increased expression of the CDK4/6 inhibitor p16^{INK4a} (ref. ⁴²), with both p16^{INK4a} and p21^{CIP1} halting the cell cycle in the G1 phase^{10,34,43,44} (Fig. 1). Moreover, senescence is associated with the induction of p53 and retinoblastoma (Rb) pathways and the related establishment of promyelocytic leukaemia nuclear bodies. This is followed by ASF1A-HIRA chaperone-mediated formation of facultative heterochromatin at specialized domains, resulting in so-called senescence-associated heterochromatin foci as part of a global genomic reorganization^{8,26,45–53}. Importantly, G1-phase-typical Rb-E2F complexes ultimately recruit H3K9 histone methyltransferase activity, mediating the local formation of transcriptionally repressive senescence-associated heterochromatin marks in the vicinity of the promoters of E2F target genes, the products of which drive S-phase entry^{11,35,54,55}. As a consequence, senescent cells, unlike other short-term or long-term arrested cells, become refractory to mitogenic E2F signals¹¹.

Senescence-associated DDR signalling, global genomic reorganization and some non-genotoxic extrinsic stimuli profoundly affect proteostasis and secretion^{26,27,34,36,44,56–59} (Fig. 2). DDR signalling-related cascades, including nuclear factor- κ B, CCAAT/enhancer-binding protein- β (C/EBP- β) and GATA4, drive SASP genes in a mechanistic target of rapamycin (mTOR)-dependent manner^{12,29,39,60–63}. Degradation of the nuclear lamina and depletion of the major structural component lamin B1 (refs ^{50,51}) by autophagy not only promote histone depletion and chromatin remodelling but also result in the formation of microtubule-associated protein light chain 3 (LC3, also known as Atg8)-containing cytoplasmic chromatin fragments^{28,64}. If not eliminated via exosome secretion⁶⁵, these fragments initiate a cyclic GMP-AMP synthase (cGAS) and stimulator of interferon- γ (IFN- γ) genes (STING) response, further enhancing the production of SASP-reminiscent inflammatory cytokines, chemokines and type I IFNs^{30,31,33,66,67}. Of note, one of the most abundant SASP factors, interleukin-6 (IL-6), reinforces senescence in a secretion-independent manner by activating the promoter of the gene encoding the CDK4/6 inhibitor p15^{INK4b} (also known as

CDKN2B) that complements p16^{INK4a} in enforcing the G1 arrest⁶¹. Importantly, SASP factors impose proteostatic stress due to the massive production of prematurely aged, misfolded peptides, which must be eliminated by energy-consuming autophagic degradation³⁹.

Cellular senescence has a major effect on surrounding cells owing to the persistence of viable and secretion-active cells (Fig. 3). Senescent cells communicate with its surroundings through direct cell-cell contacts via ligand-receptor interactions^{36,41,68}, intercellular cytoplasmic channel formation, cell fusion and the senescence-associated export of membrane-encapsulated materials through exosomes and microvesicles. The physiological importance of these interactions remains to be elucidated in detail^{69,70}. However, intense research is focusing on the humoral communication via SASP factors and its biological consequences (Fig. 3). SASP factors can modulate the environment in different ways depending on tissue context, trigger and the state of the SASP-producing and SASP-receiving cells (such as the target cell being in a pre-neoplastic state). The effects will also depend on whether SASP interactions are homotypic between tumour cells or heterologous, for example, from cancer-associated fibroblasts to pre-malignant epithelial cells. Thus, SASP factors may promote the malignant conversion of pre-neoplastic lesions and accelerate tumour progression by maintaining chronic inflammation^{71–73}, serve as immune-modulating damage-associated molecular patterns⁷⁴ or contribute to the robustness of the senescent arrest^{62,63,75,76}.

The SASP-mediated crosstalk between senescent cells and their environment will end when host immune effector cells target and eliminate the arrested cells. Attracted by SASP chemokines and activated by cytokines, cellular components of the innate immune system, such as natural killer cells, macrophages and granulocytes, mediate the clearance of senescent cells^{41,68}. However, a mixed effect was observed in hepatocellular carcinoma, in which C-C chemokine ligand 2, secreted by pre-malignant cancer cells, on the one hand recruited immature myeloid cells, which differentiated into macrophages and cleared these senescent cancer precursors, but on the other hand, promoted growth of established cancer cells through

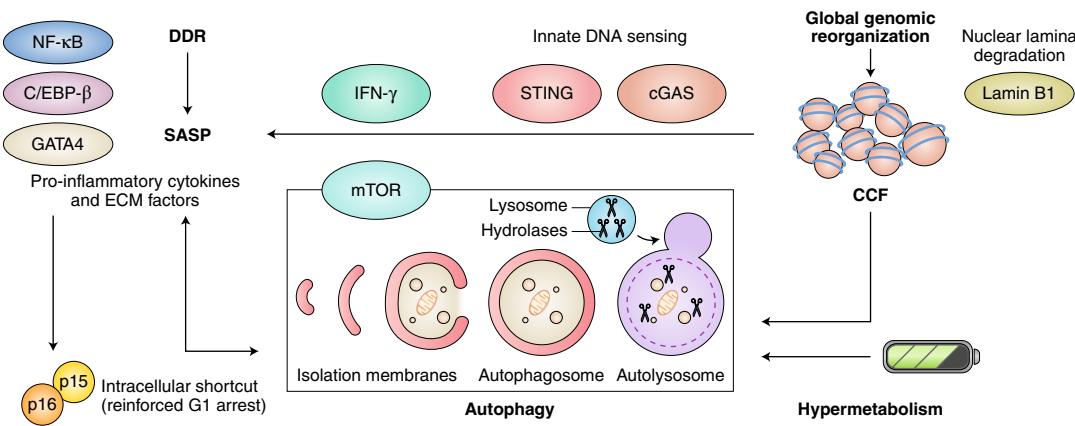


Fig. 2 | Cytoplasmic deregulation of proteostasis and secretion. Pro-inflammatory cytokines and extracellular matrix (ECM) factors, collectively known as the SASP², are produced in an mTOR-dependent manner¹²⁴. The SASP is driven by the DDR through dynamic activation of transcription factors^{29,61}, as well as by cytoplasmic chromatin fragments (CCFs) that are released from the nucleus upon degradation of the nuclear lamina⁵¹ and sensed by the cGAS-STING pathway^{30,31}, leading to IFN- γ activation. Some cytokines reinforce the G1 cell-cycle arrest via direct crosstalk to the *INK4B* promoter⁶¹. Cytoplasmic chromatin fragments and prematurely oxidized and misfolded SASP components evoke proteotoxic stress, which the cells cope with by using ATP-consuming autophagy that is fuelled by hypermetabolic energy provision^{39,125}. NF-κB, nuclear factor-κB.

natural killer cell inhibition⁷⁷. By contrast, activated transforming growth factor-β (TGF-β)-emitting macrophages or T cells, as well as IFN-γ-secreting or tumour necrosis factor-α (TNF-α)-secreting T cells may induce or further reinforce tumour cell senescence^{78–80}.

The reversibility of senescence

The dynamic nature of senescence implies that a senescence-associated cell-cycle arrest is not necessarily terminal. Studies demonstrating resumed proliferation in a senescent cell population suggest that senescence is rather a biological continuum on both the cell-intrinsic and the cell-extrinsic level^{16–19} (Fig. 4). Most of the RNA interference-based or CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9)-based senescence screens are aimed at unveiling genes permitting bypass, whereas less evidence is available in support of true escape from senescence⁸¹. Indeed, acute loss of all three Rb family members allowed Ras-senescent fibroblasts to re-enter the cell cycle, albeit for only a few divisions before death in mitotic catastrophe occurred¹⁷. Cells in replicative senescence with low p16^{INK4a} levels may resume proliferation following acute p53 inactivation¹⁶. These data indicate that there is a state of ‘light’ senescence (representing low p16^{INK4a} levels) that is different from a ‘deep’ senescent state, and determinants of these hypothetical states would need further elucidation. Such factors most likely include the type, strength and duration of the senescence-inducing signal and the (epi)genetic susceptibility of the cell to actually engage in a full-featured senescence programme, with a potential hierarchy among central and auxiliary senescence components yet to be investigated. We found that the H3K9-active demethylases JMJD2C and LSD1 mediate escape from oncogene-induced senescence in fibroblast or melanocyte models¹⁹, and that the H3K9 histone methyltransferase Suv39h1 or p53 is required for senescence, specifically for the maintenance of therapy-induced senescence (TIS) in various malignancies¹⁸. Moreover, single-cell tracking experiments, utilizing a fluorescence-based SA-β-gal assay⁸², caught senescent cells at the transition to actively replicating DNA and dividing. The reversal of truly senescent cells was faithfully demonstrated when cells retaining the senescence marker for a few divisions also exhibited nucleotide incorporation into DNA. Low frequencies of fluorescence-based SA-β-gal or proliferation marker double-positive cells were also detected in unmanipulated tumour cell populations, indicating spontaneous exit from the senescent

cell-cycle block⁸². Importantly, future analyses will characterize the epigenetic and functional status of those ‘revertants’, which are likely to retain some features typical of senescence while having eliminated others, including the control of the cell-cycle arrest. We speculate that the extent and pattern of senescence-associated chromatin remodelling are critical for the stability of the senescent state—the cell-cycle arrest in particular—and a less profound epigenetic impregnation may facilitate what seems to be reversibility. In any case, natural escape is most likely not a full conversion of the senescent back to the pre-senescent cell status.

Senescent features may change over time

Time-resolved analyses of the senescence-associated secretome have generated insights into cell-autonomous and non-cell-autonomous mechanisms of senescence control and subsequent cell fate³⁷. Proteome investigations unveiled a dynamic pattern of the Ras-related SASP, composed of two biochemically distinct and functionally opposing subsets of factors peaking at different phases of the senescence process³⁶ (Fig. 4). The ‘first wave’ is characterized by activated NOTCH1 and TGF-β1 signalling, leading to cell-intrinsic and cell-extrinsic effects. Cell-intrinsic maturation from pre-senescent to full-featured senescence is accompanied by, for instance, TGF-β1-controlled induction of p15^{INK4a}. Target genes of the NOTCH1 intracellular domain, such as *JAG1*, mediate ‘lateral senescence’ through cell–cell contacts to NOTCH receptor-positive neighbour cells, and TGF-β-driven secreted factors, such as connective tissue growth factor, contribute to paracrine senescence^{36,58,78,79}. If NOTCH1-TGF-β activity persists, less pro-inflammatory paracrine activity mediated by TGF-β and lateral induction will spread across the tissue. Lack of NOTCH1-TGF-β instead promotes an immunosuppressive type of senescence that bears the risk of tumour development due to limited immune clearance of senescent cells and their potential stem-like reprogrammed nature (see below^{18,20,23}). Alternatively, a dynamic shift to a ‘second-wave’ secretome characterized largely by C/EBP-β-driven pro-inflammatory cytokines, although favouring host immune-mediated elimination of the senescent cells, may promote the growth of susceptible (pre-)neoplastic bystander cells as a net effect^{36,71}. What signals dictate the switch from a NOTCH1-TGF-β-orchestrated to a C/EBP-β-orchestrated SASP remain elusive. In particular, the role of nuclear factor-κB signalling, which drives

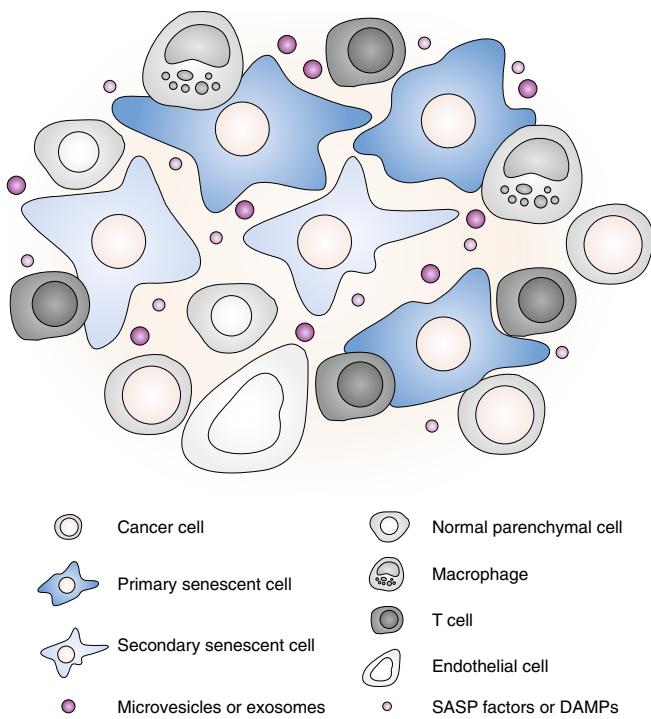


Fig. 3 | Environmental crosstalk following senescence induction by oncogenes or anticancer therapy. Cancer cells damaged above a certain threshold senesce and, in a paracrine manner, evoke secondary senescence in their neighbours that are damaged below this threshold¹⁶. Senescent cells secrete SASP factors and damage-associated molecular patterns (DAMPs)^{12,74} and release membrane-encapsulated cytoplasmic material as microvesicles or exosomes^{65,126}. These components, vesicles and cell-cell contacts account for the complex local (and possibly longer-distance) crosstalk to other (pre-)neoplastic cells in a homotypic manner and to parenchymal cells, stromal cells (fibroblasts and endothelial cells) and host immune cells (macrophages and T cells) in a heterologous manner, launching an avalanche of subsequent intercellular communication and effector functions.

SASP factors largely overlapping with C/EBP- β and engages in a positive-feedback loop with NOTCH1, needs to be elucidated^{83,84}. Moreover, additional lesions in SASP factor-exposed (pre-)neoplastic cells, for instance, at the level of p53 or casein kinase 1- α , have been shown to modulate the SASP as a (para-)inflammatory response, switching it from tumour suppressive to tumour promoting^{85,86}. Importantly, the dynamic complexities, phenotypic variability and functional diversity of the senescent state and its associated cell-mediated and secretome-mediated communication must be studied in natural, heterogeneous cell populations⁸⁷. It will be key to utilize single-cell technologies to spatiotemporally dissect the actual contribution of distinct first-wave versus second-wave type of secretomes, ideally in patient-reminiscent tumour models in which cells have entered senescence in a non-synchronized manner. The ultimate goal is to convert this information into novel SASP-modulating treatment strategies^{88–90}.

A fundamentally reprogrammed state

As alluded to earlier, senescent cells exhibit global changes of their chromatin landscape, leading to epigenetic reprogramming of fundamental cell functions^{8,26,50,51,53,91}. The observation of physiological senescence during embryonic development and tissue

remodelling^{92,93} extended the role of senescence beyond tumour suppression. Consequently, a potential link between senescence-associated reprogramming and self-renewal capacity—two incompatible cell states—gained prime interest in the research community. By dissecting and recapitulating molecular mechanisms underlying tissue repair, the regeneration of damaged tissue could be linked to cellular senescence⁹⁴, with senescent cells promoting the occurrence of stem cell marker-positive cells in their vicinity²³. Senescent cells can even directly enhance reprogramming by ‘Yamanaka factors’ (Oct4, Sox2, Klf4 and Myc) in primed neighbour cells through the SASP. Given the overlap of signalling components of senescence and stemness pathways, among them p16^{INK4a}, p21^{CIP1}, p53, as well as the H3K9me3 mark, and the induction of stemness markers in Ras-senescent keratinocytes²³, we hypothesized a link between senescence-associated reprogramming and cancer stemness¹⁸. Indeed, cells induced to senesce by oncogenes or chemotherapy or cells in a state of replicative senescence exhibit plasticity, express a latent adult tissue stem cell signature and present with stem cell phenotypes. Most importantly, when senescent cancer cells were allowed to escape following acute inactivation of senescence-essential gene moieties, such as Suv39h1 or p53, they exhibited a much higher tumour-initiation potential than a cell population that had never entered senescence. Strikingly, non-stem leukaemia cells gained de novo leukaemia-initiating capacity after passing through temporary senescence as the pivotal reprogramming state¹⁸. The key stemness-associated Wnt signalling pathway was enhanced in senescent cells due to epigenetic activation and remained constitutively active in a small but stable subset of the cancer cells at the post-senescent state, indicating their lasting role as tumour-reinitiating cells *in vitro* and *in vivo* (Fig. 4).

Evasion from senescence has been postulated as a pivotal step in the pre-neoplastic phase of many cancer types, such as the nevus-to-melanoma or the colon adenoma-to-carcinoma sequence^{19,95,96}. However, it remains difficult to pinpoint whether tumour formation occurred as a consequence of bypass or true escape⁹⁷. This difference is important because latent stemness features may be acquired only during senescence, but not while this state is circumvented. Given that senescence and its regulators (such as INK4a/ARF-, CIP1- or TP53-encoded gene products) serve as a barrier to reprogramming to pluripotent stem cells^{98–103}, the epigenomic similarity of senescent cells to cancer cells¹⁰⁴ and the stem-like nature of senescent cancer cells¹⁸, senescence-associated reprogramming might reflect an epigenetically engraved bivalent cell condition in which terminal arrest and latent stemness co-exist. (Pre-)malignant cells may enter oncogene-induced senescence or TIS as a host-protective cellular shield, under which a silent stemness programme epigenetically co-evolves. Such a profoundly remodelled chromatin state could co-control full-featured senescence and stemness capacity, and the expression of genes essential for senescence is reduced, allowing the cell to overcome a Waddington landscape-like epigenetic barrier towards a stem cell state^{8,18,105}. Such bivalent fates may exclude stressed, damaged cells from propagating, while promoting tissue replenishment from post-senescent stem cells at a time which harmful stresses no longer apply. This might be a particularly relevant back-up mechanism when paracrine senescence has spread to tissue borders or insulator structures^{60,61,76,106}. Converting senescent cells into tissue-reinitiating stem cells supports wound healing and tissue repair, although the precision, fidelity and accuracy of this regenerative switch may decline during ageing, and becomes particularly harmful when hijacked by cancer cells^{18,23,94,107}. The bivalent state of stressed cells was also recently illustrated by the identification of a dual, seemingly opposing role for mTOR in reprogramming-related senescence¹⁰⁸. Further investigations are needed to better understand the control of these distinct functionalities in the context of senescence depth, epigenetic reprogramming and the continuous presence or absence of underlying senescence triggers.

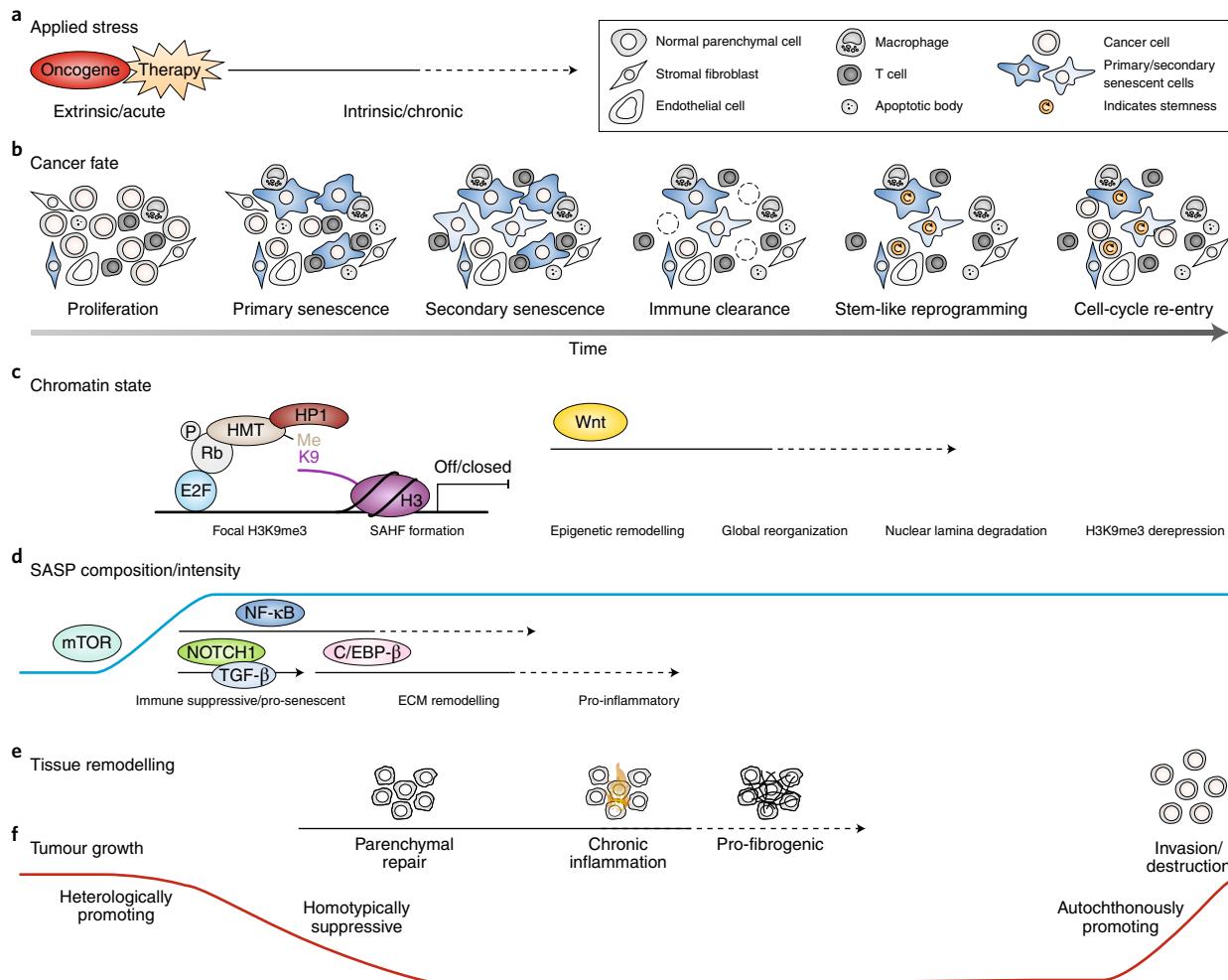


Fig. 4 | The *in vivo* dynamics of various aspects of senescence in cancer control and progression. **a**, Stresses, such as oncogenic activation or anticancer therapy, acutely, and potentially chronically, impinge on tumour cells and their tissue environment^{10,109,110}. Chronic intrinsic changes include activated oncogenes and DNA-damaged but unrepairable telomeres³⁵. **b**, Oncogene-sensitized pre-tumour cells enter primary senescence, which may further spread to adjacent tumour cells as paracrine or secondary senescence via SASP production^{12,76}. Subsequently, innate immune cells, such as macrophages and natural killer cells, attracted by SASP factors clear some senescent cells (indicated by the unfilled dotted circle)^{41,68}, whereas remaining senescent cells with latent stem-like reprogramming may convert into tumour-reinitiating cells following spontaneous cell-cycle re-entry^{18,19}. **c**, Epigenetic changes comprise H3K9me3-governed repression of S-phase-promoting E2F target genes¹¹, global genomic reorganization that includes the formation of senescence-associated heterochromatin foci (SAHF)^{46,52}, degradation of the nuclear lamina^{50,51}, H3K4me3/acetylated HeK27-related remodelling of Wnt stem signalling¹⁸ and, eventually, the derepression of the H3K9me3 mark to permit cell-cycle re-entry¹⁹. **d**, The composition and strength of the SASP, driven by distinct signalling cascades at various points in time after senescence induction, account for early pro-senescence immunosuppression that can evolve into immunogenic, pro-inflammatory effects^{36,61,68,76}. **e**, Early-phase senescence takes part in tissue repair and regeneration⁹⁴, whereas chronic senescence contributes to inflammation and fibrosis¹²⁷, ultimately leading to tissue destruction. **f**, Accordingly, senescent bystander cells, such as in early-phase wound healing, may promote the heterologous growth of pre-tumour cells, whereas cancer cell senescence is tumour-suppressive^{95,109–111}, unless cell-autonomous and non-cell-autonomous reprogramming of senescent cancer cells and their occasional cell-cycle re-entry unleashes the progression of a tumour cell population with enhanced self-renewal capacity^{18,20,23}. Of note, timing is not proportional in scale and is not vertically time matched. Furthermore, the order of some events remains an issue of controversy and ongoing research activities.

Eliminating senescent cells as anticancer strategy

Despite the general consensus that senescence plays a primarily and immediately tumour-suppressive role in many settings^{95,109–112}, the long-term implications of senescent cells are potentially detrimental. Such undesirable biological features that senescent cells may exhibit over time include neoplastic conversion, SASP-related chronic inflammation and cell-cycle re-entry of stemness-reprogrammed senescent cancer cells^{18,71,72} (Fig. 4). Thus, elimination of senescent cells seems to be a reasonable therapeutic strategy. An elegant demonstration in this regard was provided by van Deursen and

colleagues, who generated the ‘INK-ATTAC’ transgenic mouse, in which cells expressing high levels of the senescence indicator p16^{INK4a} are susceptible to pharmacological elimination¹¹³. Continuous exposure to the respective agent not only attenuated multiple ageing-associated disorders in various organ systems but also extended healthy lifespan¹¹⁴. However, the effect of this strategy on natural, age-related tumour development is less clear: whereas neoplastic precursors might have been slowed in their progression, the spectrum and incidence of tumours at the end of life were not affected by drug treatment. Campisi and colleagues generated the

p16-3MR mouse that renders p16^{INK4a}-high cells vulnerable to the pro-drug ganciclovir via *Ink4a*-driven expression of herpes simplex virus thymidine kinase and confirmed the beneficial role of senescent cells in wound healing, as well as the detrimental contribution of chemotherapy-induced senescence in normal tissues^{94,115}. Moreover, both transgenic mouse models demonstrated a positive effect of senescent cell removal on bone-related pathologies, as the *in vivo* application of ‘senolytics’ compounds with the ability to selectively eliminate senescent cells in non-engineered settings, recapitulated the INK-ATTAC-governed or p16-3MR-governed phenotypes^{116,117}.

Indeed, there is now an intense hunt for small-molecule senolytics: among potential compounds are the tyrosine kinase inhibitors ruxolitinib and dasatinib, blockers of the anti-apoptotic Bcl2 family members, such as navitoclax (ABT263), or the flavonoid quercetin^{118–120}, to name a few. However, their usefulness in cancer treatment is still to be seen. As an *in vivo* demonstration of a senolytic cancer treatment strategy that extended the survival of the tumour-carrying host mice, we presented synthetic lethal metabolic targeting of TIS cancer cells. TIS cells became vulnerable to the inhibition of autophagy or energy-providing pathways due to their enhanced energetic needs to degrade accumulated toxic SASP peptides. Indeed, senolysis extended the survival of mice bearing TIS tumours, but not of those mice with an equally treated but genetically senescence-incapable tumour origin³⁹. Of note, therapeutic exploitation of senescence-associated vulnerabilities does not need to result in senolytic cell death, but could convert senescent cells into a more desirable long-term state, for example, by selectively blunting their SASP production and, hence, attenuating tumour inflammation¹²¹. This, in turn, might come with the risk of losing immunogenicity linked to the senescent state, at least in some settings^{41,68}. For instance, neutralization of Wnt signalling, a key stem cell pathway, did not kill senescent cancer cells, but abrogated their newly acquired, clinically detrimental self-renewal capacity and prolonged the survival of TIS lymphoma-bearing mice¹⁸. Collectively, these data pinpoint reprogramming of senescent cells into a latent stemness mode as the prominent underlying perilous feature and underscore a clinical benefit from the elimination of senescent cells.

Outlook

As there is no simple answer to the defining characteristics of senescence, there is equally no clear answer to whether and when senescence is a beneficial or less desirable response to anticancer therapies. Conventional chemotherapeutic agents, but possibly many other anticancer drugs as well, act to some extent via the induction of senescence. Furthermore, given the frequent defects in apoptosis in primary malignancies, senescence operates in many tumours as an additional safeguard mechanism. With increasing awareness of the complex senescent condition, strategies now emerge that separate good from less desirable aspects, for instance, favouring arrest over secretion^{90,121}. Moreover, and as discussed above, senescence induction is not a binary choice but reflects a continuum in which different senescence-associated cellular changes are not necessarily displayed at similar intensities (Fig. 4). The degree of altered cell functions may vary between different tissues and senescence triggers¹⁸. This phenotypic spectrum includes complex genomic reorganization and is mirrored by highly dynamic, wave-like compositional changes in the content of the senescence-associated secretomes^{8,36,37}. The secondary action of senescent cells on homotypic, non-senescent neighbour cells and their impingement on heterologous, stromal or host immune cells is just the overture of a subsequent avalanche of network effects. It will be a challenge to investigate these, especially in natural settings, where the onset of senescence is not a population-wide synchronized event. Single-cell analyses will help to characterize the phenotypic spectrum of senescent cells

within a population in greater depth, and technically more sophisticated studies are needed to fate-track the transcriptional dynamics of vital individual cells on their way into senescence and, perhaps, their progression to a ‘reversal-like’ state out of senescence.

In essence, the apparent contradiction in achieving a beneficial outcome from inducing or restoring senescence on the one hand and removing senescent cells on the other hand is rather a logical interpretation of an initially helpful, tumour-suppressive response that turns into a potential threatening, pro-inflammatory, mitogenic, stemness-reprogrammed condition that is not irreversible or terminal^{19,122,123}. Accordingly, two-hit synthetic lethal strategies that first evoke senescence and then eliminate senescent cancer cells by targeting the particularly vulnerable Achilles’ heels of the senescent condition hold great promise for conceptually novel anticancer therapies^{18,39,81}.

Received: 26 January 2018; Accepted: 7 November 2018;

Published online: 2 January 2019

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Acknowledgements

This work was supported by grants to C.A.S. from the Deutsche Forschungsgemeinschaft (SCHM 1633/9-1 and SCHM 1633/11-1), by the Helmholtz Alliance ‘Preclinical Comprehensive Cancer Center’ grant (no. HA-305) from the Helmholtz Association and by the Deutsche Krebshilfe (grant no. 110678). This work was further made possible by the Berlin School of Integrative Oncology (BSIO) graduate program, funded within the German Excellence Initiative and the German Cancer Consortium (GCC), to S.L. and C.A.S.

Competing interests

The authors declare no competing interests.

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